Chromosomal structure of populations of *Scilla autumnalis* in the Iberian Peninsula

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Four cytological races of *Scilla autumnalis*, Liliaceae, were found in a study of 31 populations from Spain and Portugal. Two are diploids with $2n = 14$, designated AA and B^7B^7, which differ by 70 per cent in DNA content, one is an allotetraploid AAB^7B^7 and one an autotetraploid of the B^7 genome. Races AA and AAB^7B^7 are Iberian endemics, B^7B^7 is widespread throughout the Mediterranean, while the autotetraploid is the common European race. In Iberia, the races are parapatric and distribution is not related to climatic conditions. The populations are chromosomally heterogeneous. Polymorphisms include B-chromosomes (five types) and supernumerary segments. Large euchromatic segments occur on the homoeologues A1 and B1 in both diploids and the allotetraploid, converting these acrocentrics to metacentrics. A wide spectrum of non-polymorphic numerical and structural variants was also found. The chromosomal structure of this species complex is discussed.

Keywords: B-chromosome, cytotype geography, polyploid complex, population structure, supernumerary segment.

Introduction

One of the most chromosomally variable plant species is *Scilla autumnalis* L., an autumn-flowering bulb of the family Liliaceae. The variation encountered in this widespread species is of many types: polytypic, defining chromosome races, polymorphic, reaching high frequencies within populations, and mutational, affecting one or a few individuals within a single population. The polytypic variation involves an aneuploid series of four races at the diploid level, from $2n = 10$ to $2n = 14$, and a polyploid complex built from these races up to the hexaploid level ($2n = 6x = 42$). So far 10 distinct races have been identified across the range of the species (Battaglia, 1957; Ainsworth *et al.*, 1983; Guillén & Ruiz Rejón, 1984; S. Taylor, unpublished results). Within these races, three types of polymorphisms have been detected: B-chromosomes, supernumerary segments and pericentric inversions. In addition, translocations, inversions, and aneuploids (in the polyploids) occur sporadically to give a remarkably high level of chromosomal variation within natural populations of this species (Ainsworth *et al.*, 1983; Guillén & Ruiz Rejón, 1984; Ruiz Rejón *et al.*, 1980, S. Taylor, unpublished results).

In this paper we present the results of our analyses of populations of *S. autumnalis* in a particularly interesting region, the Iberian Peninsula. All three classes of chromosomal variation have previously been recorded in this area. Detailed studies of the geographical distribution of cytological races, the nature of polymorphisms, and the mutational load in populations are reported here which should give insights into the origin, maintenance and significance of the extensive chromosome variation in this highly complex species.

Materials and methods

Bulbs were collected from 31 populations in the centre, south and west of the Iberian Peninsula. Nineteen populations were from Spain and 12 from Portugal (see Table 1).

Actively growing root tips were used for mitotic analysis. Roots were pretreated either with 0.05 per cent colchicine for 3 h or with 2 mM hydroxyquinoline for 4–5 h, and then fixed in 3:1 alcohol:acetic acid. Staining was carried out either with 2 per cent acetic orcein, or by the Feulgen reaction enhanced by squashing in 2 per cent lactopropionic orcein. Meiotic

preparations were made both from buds fixed in 3:1 alcohol:acetic acid and stained with 1 per cent aceto-carmine, and from unfixed anthers squashed in 2 per cent lactopropionic orcein as stain fixative.

Results

The chromosome races

Four cytological races have been found in the populations from Spain and Portugal. Two are diploids with $2n = 14$, designated AA and B^7B^7 (Ainsworth *et al.*, 1983) and two are tetraploids, the allotetraploid derivative AAB^7B^7 with $2n = 28$ and the autotetraploid $B^7B^7B^7B^7$. The AA and AAB^7B^7 races are known only from Iberia. The B^7B^7 diploid is widely distributed throughout the Mediterranean area, and the $B^7B^7B^7B^7$ is the characteristic race of mainland Europe and England.

The AA diploid. The seven chromosomes of the A genome are individually recognizable at mitotic metaphase (Fig. 1a). They range from 5.3 to 7.7 μm in

length, with a total haploid complement length of 43 μm. Chromosomes 1–3, 5 and 6 are submetacentric with arm ratios about 1:2; chromosome 3 also carries a nucleolar-organizer region adjacent to the centromere in the long arm. Chromosome 4 is distinctly more acrocentric with an arm ratio of 1:4.3 while chromosome 7 is nearly metacentric.

The B^7B^7 diploid. The B^7 genome has conspicuously shorter chromosomes than the A genome, ranging from 3.2 to 5.3 μm in length, and in addition the chromatids are of narrower diameter (Fig. 1b). The total haploid length is 29.3 μm, 68 per cent of the A genome, while the chromosome volume is only 55 per cent of the A genome (13.8 versus 24.7 μm^3). Genomes A and B^7, however, are remarkably similar in appearance, each B^7 chromosome having a counterpart in the A genome. The B^7 chromosomes have, therefore, been numbered by Ainsworth *et al.* (1983) with reference to their A genome presumptive homoeologues rather than on the basis of their overall length. This introduces some changes in the rank order, for example chromosome 4 of the B^7 genome is the second
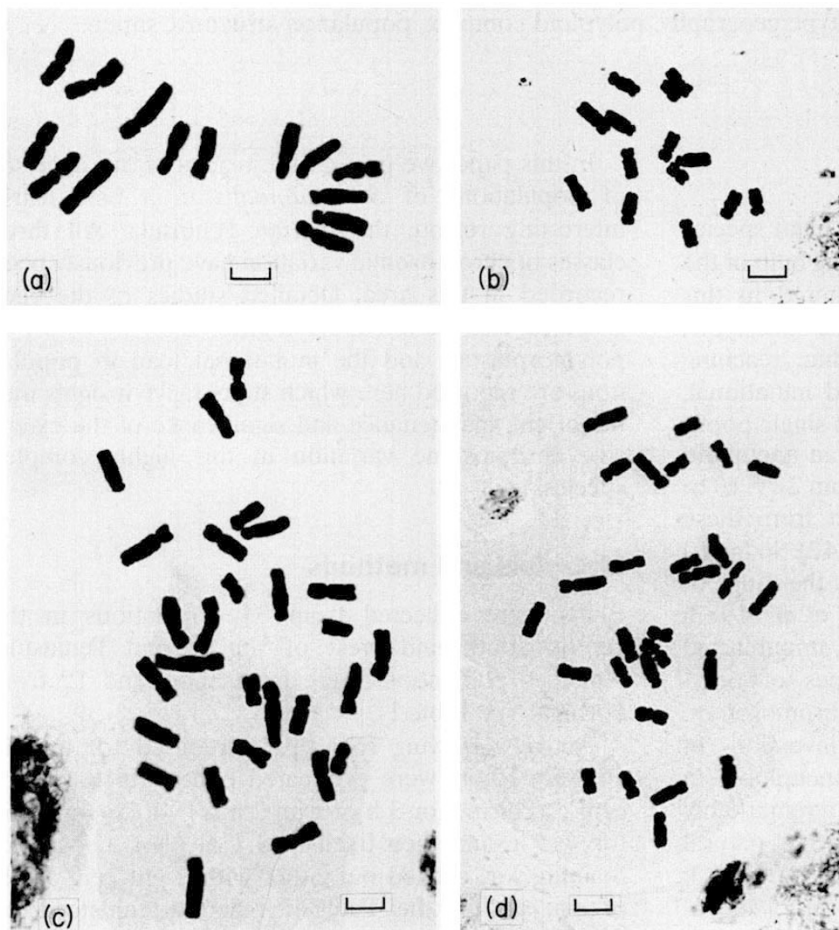

Fig. 1. Chromosome complements of the four races of *Scilla autumnalis* in the Iberian Peninsula. (a) AA (b) B^7B^7 (c) AAB^7B^7 (d) $B^7B^7B^7B^7$. Bar represents 5 μm in all figures.

largest in the complement, but homoeology has been demonstrated at meiosis for two of the pairs (4 and 7; Jenkins *et al.*, 1988) by analysis of pairing. The absolute differences in length between the A and B^7 homoeologues are rather constant (1.4–2.4 μm), from 10 to 17 per cent of the total difference between the genomes. The precise quantity, however, is not correlated with the overall length of an individual chromosome, hence the changes in rank order between genomes.

The AAB^{7}B^7 tetraploid. The tetraploid AAB^{7}B^7 recognizably contains both diploid genomes (Fig. 1c), which suggests that little structural rearrangement has occurred since the polyploidization event. The tetraploid, however, displays only one pair of nucleolar-organizer chromosomes, on the B3 chromosome. The A organizers are not expressed in the presence of B^7-derived NORs. During meiosis, the tetraploid is a strict bivalent-former, which indicates allopolyploidy. This pairing precision breaks down, however, in unbalanced hybrids with AB^{7}B^7 or AB^{7}B^{7}B^7 genome combinations, in which some chiasma formation between homoeologues takes place (Jenkins *et al.*, 1988; White *et al.*, 1988).

The B^{7}B^{7}B^{7}B^7 tetraploid. The autotetraploid race carries four identical copies of the haploid B^7 complement with four expressed nucleolar-organizer regions (Fig. 1d). At metaphase-I of meiosis up to four quadrivalents have been observed in a single PMC, although the majority of chromosomes are involved in bivalent associations.

Distribution of the chromosome races. Six populations of the AA race have been found. The race is characteristic of the south eastern part of Spain, from the province of Sevilla to Granada. Five of the populations have been found in this region. Remarkably, the other AA population was collected in Portugal at Peniche, Estremadura, about 480 km to the north-west (Fig. 2). This population was growing in soil pockets on a limestone shelf within 20 m of the sea. Two other coastal populations from Portugal growing in similar habitats (Cabo Mondego and Foz de Arelho), as well as all the adjacent inland populations, were allotetraploid. The B^{7}B^7 diploids have been located so far in two regions: near Madrid on the central plateau, and near Badajoz in southwestern Spain adjacent to the Portuguese border. One central population in Madrid province has been studied, and three near Badajoz.

Diploid individuals of *S. autumnalis* with $2n = 14$ have been recorded from Spain by other authors, but which of the two races these records refer to cannot be ascertained in the absence of illustrations (Battaglia, 1957; Valdes, 1970; Valdes *et al.*, 1978). The locations from which these unresolved diploids were collected are given on Fig. 2.

The 20 populations of the allotetraploid race show a distinctive western distribution in the Iberian Peninsula. They have been found throughout central Portugal, and in Badajoz, Huelva and Sevilla provinces

● AA
○ BB
▲ AABB
■ BBBB

Fig. 2. The distribution of cytological races of *S. autumnalis* in Spain and Portugal. Diploid counts of other authors which may be AA or B^{7}B^7 are indicated by (?).

in southwestern Spain. One collection has been made by the authors in northern Spain (from Tineo in Asturias) and this was also of the tetraploid AAB^7B^7. Plants presumably of this race were also recorded from northern Portugal and near Vigo in north-western Spain by Barros Nevos (1973). The most intensively studied area is south-western Spain. In this region, the allotetraploid and both diploids are found in close proximity. The tetraploids and the B^7B^7 diploids, for example, have been found in populations as little as 10 km apart. So far, no mixed populations have been detected, nor is there any evidence of gene flow between cytological races. The autotetraploid race has so far been found only at Cabo Blanco on Majorca, Islas Baleares. This race, however, is extremely widely distributed (Ainsworth *et al.*, 1983). It occurs on the Mediterranean islands of Corsica and Corfu, throughout Italy, France and England, and in Greece and Turkey. It is not yet known whether it occurs on the mainland Iberian Peninsula itself, but plants with $2n = 28$, which may be of this race, have been found near Pamplona, Navarra. Battaglia (1957) has also recorded $2n = 28$ for plants collected at Arta on Majorca, which are presumably of this autotetraploid race.

The polymorphisms

B-chromosomes. B-chromosomes of Spanish AA diploid populations have been intensively studied previously (Ruiz Rejón *et al.*, 1980; Oliver *et al.*, 1982). In this more extensive investigation B-chromosomes have been found to be a common feature of the three cytological races AA, B^7B^7 and AAB^7B^7.

In the AA race a widespread B-chromosome is found. This B is acrocentric with an arm-ratio of about 1:2 and about half the length of chromosome A7 (Fig. 3a). The B-chromosome has been found in five of the six AA populations sampled, including the isolated population at Peniche in Portugal.

The B has a curious distribution within populations. There is often an excess of individuals with high numbers of Bs despite a low overall B-frequency. The population from Loja (Granada), in which 24 individuals out of 96 carried Bs, is typical. The average B number per plant was only 0.6 but seven plants each with 4 Bs were found. Clearly this distribution deviates from the Poisson expectation.

In the B^7B^7 race, by contrast to the AA race, B-chromosomes have been found only in one population, Rascafria near Madrid (Guillén & Ruiz Rejón, 1984). Interestingly, this B-chromosome is similar in relative size and morphology to that found in the AA

diploids, being acrocentric and about half the length of chromosome B7. It is euchromatic.

Three morphologically distinct B-chromosomes have been found in the AAB^7B^7 populations, one widespread and the others localized in distribution. The common B is a euchromatic element similar in length to chromosome 6 of the B^7 genome but is slightly more acrocentric than the standard autosome B6 (Fig. 3b). Bs of this type have been found in 10 of the 20 populations examined, both in Portugal and south-western Spain (Table 1). Individuals most frequently carry two copies of the B. Plants with one or four Bs are less frequent and plants with three Bs have not been found.

In the Jerez de la Caballeros/Fregenal de la Sierra population a slightly smaller but more acrocentric B has been found in three plants (Fig. 3c). A simple hypothesis is that this B is a derivative of the standard B which has undergone a short-arm deletion; the long arm of the B is of standard length. The final B-type is metacentric and slightly smaller than chromosome 7 of the B^7 genome (Fig. 3d). Its relationship to the standard B is unknown. The B^m has been found in two populations from Huelva.

Supernumerary segments. A characteristic feature of the races of *S. autumnalis* is polymorphism for additional blocks of chromatin (Ainsworth *et al.*, 1983). These supernumerary segments have preferential locations in the complement, with the short arms of chromosomes 1 and 4 most affected. This is the case in both the A and B^7 genomes. They are neither C- nor Q-banding and cannot be observed as interphase chromocentres. In this way they differ qualitatively from the supernumerary segment systems of Orthopterans (John & Miklos, 1979) or the C-band polymorphisms of *Scilla sibirica* (Vosa, 1973), but have a parallel in the widespread segment polymorphism of chromosome 2 of *Muscari comosum* (Ruiz Rejón & Oliver, 1981). In the Iberian populations, euchromatic supernumerary segments have been found in all races except $B^7B^7B^7B^7$, of which only two individuals have yet been examined. In the AA diploids a large segment has been found which converts chromosome 1 into a massive metacentric (ss1A). The segment, which represents about 5 per cent of the haploid complement, has been found only in a single plant from the population at Ronda-Estepa, Sevilla. By contrast, all four populations of B^7B^7 diploids were polymorphic for a segment on chromosome 1 (ss1B). As in the A genome this converts the acrocentric into a metacentric (Fig. 4a). The supernumerary segment was very abundant. In the sample from La Albuera, Badajoz, the proportion of chromosome 1 carrying the segment was 0.65
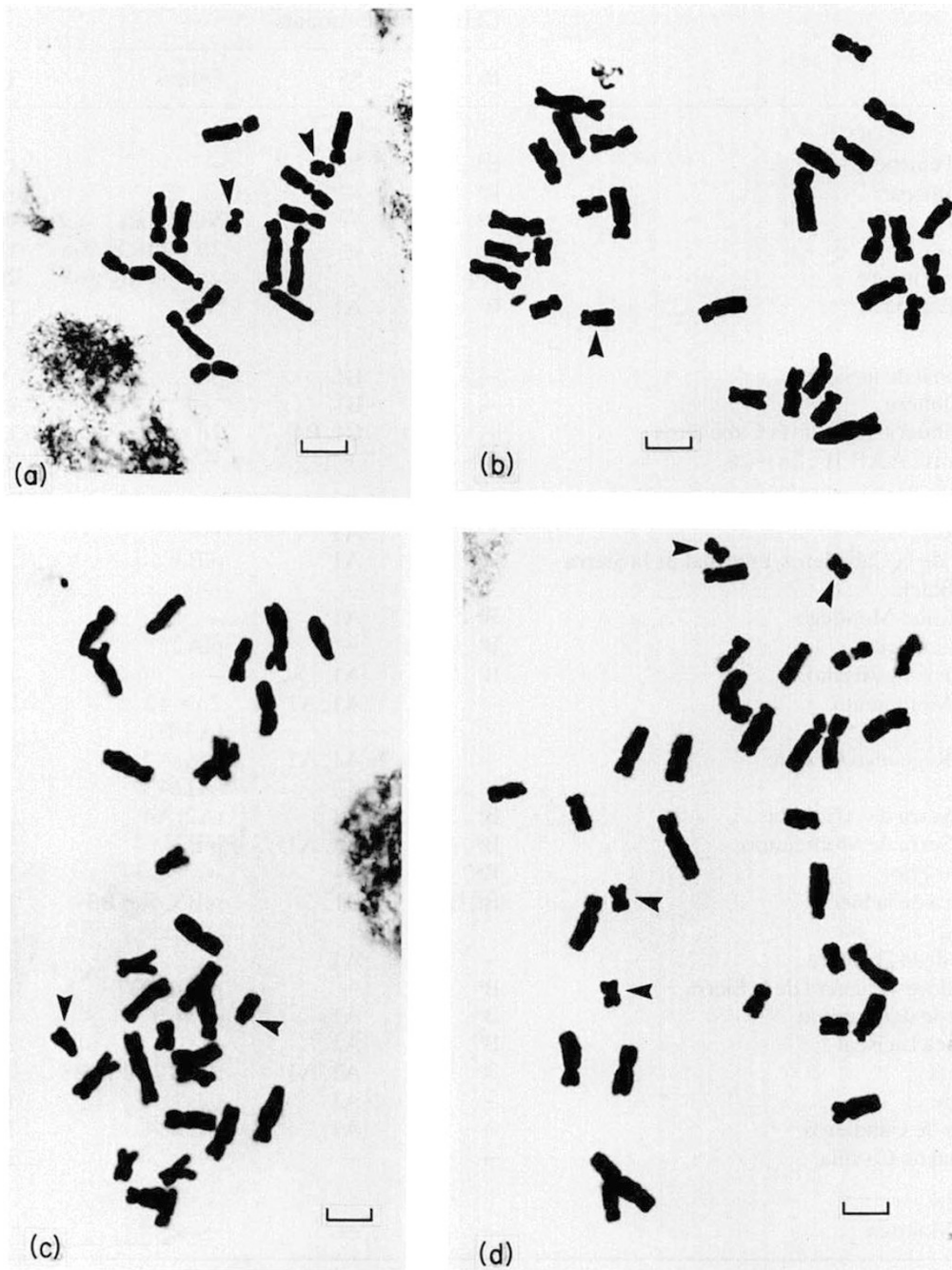
Fig. 3. B-chromosomes in AA and AAB⁷B⁷ races. (a) AA (b–d). Three B-types in AAB⁷B⁷-standard, acrocentric and metacentric.

and all three morphs were found in the population. As well as ss1B, the population from La Albuera/Jerez de la Caballeros also contained a B4 chromosome with a short arm segment (ss4B).

In the allotetraploids, four supernumerary segments involving three chromosomes have been identified. Three are morphologically identical to those described in diploids: those on A1, B1 and B4 (Fig. 4b and c).

Table 1. Chromosome constitutions of 31 populations of *Scilla autumnalis* from the Iberian Peninsula. B-chromosome types (acrocentric B^a, very acrocentric B^{va}, and metacentric B^m) and supernumerary segments (SS) are classified sepatately from the other chromosomal variants.

	Population location	Bs	SS	Others	Total plants
	AA, $2n = 14$				
1 P	Estremadura, Peniche	B^a	—	—	15
2 S	Granada, Almuñecar	B^a	—	—	10
3 S	Granada, Loja	B^a	—	NOR shift	96
4 S	Granada, Padul	—	—	$2n = 21$	10
5 S	Jaen, Torre del Vinagre	B^a			20
6 S	Sevilla, Roda-Estepa	B^a	A1	t1/7	11
	B^{7}B^7, $2n = 14$				
7 S	Badajoz, Fregenal de la Sierra	—	B1	pi7	9
8 S	Badajoz, La Albuera	—	B1		22
9 S	Badajoz, La Albuera/Jerez de la Cabelleros	—	B1, B4	$2n = 28$	11
10 S	Madrid, Rascafria AAB^{7}B^7, $2n = 28$	B^a	+	+	29
	AAB^{7}B^7, $2n = 28$				
11 S	Asturias, Tineo	—	A1	—	10
12 S	Badajoz, Jerez de la Caballeros/Fregenal de la Sierra	B^{va}	A1	piB3	13
13 P	Beira Litoral, Baleia	—	—	—	16
14 P	Beira Litoral, Cabo Mondego	B^a	A1^s	—	13
15 P	Beira Litoral, Condeixa	B^a	—	piA2	25
16 P	Estremadura, Foz de Arelho	B^a	A1	—	15
17 P	Estremadura, Montejunto	—	A1, A1^s	$2n = 42$ tA3/B1	27
18 P	Estremadura, Reguengo Grande	—	A1, A1^s	tA6/A7 tA1/A3	22
19 P	Estremadura, Serra de Arrabida	B^a	A1	tA2/A4	23
20 P	Estremadura, Serra de Montejunto	B^a	A1, A1^s	piB7	33
21 S	Huelva, Campo Frio	B^a	B4	—	24
22 S	Huelva, Higuera de la Sierra	B^a, B^m	B1	piB3, dup B3 telo A5	14
23 S	Huelva, Rosal de la Frontera	—	A1	—	20
24 S	Huelva, Valdeflores/Higuera de la Sierra	B^a	—	piAl, piB7	14
25 S	Huelva, Valverde del Camino	B^m	A1	piB7	11
26 S	Huelva, Zalamea La Real	B^a	A1	—	20
27 P	Ribatejo, Fatima	B^a	A1, A1^s	tB2/B4, dup B3	26
38 P	Ribatejo, Minde	—	A1	piB7	11
29 P	Ribatejo, Serra de Candieros	—	A1	tA2/B4	16
30 S	Swevilla, Puebal de Cazalla	—	—	—	18
	B^{7}B^{7}B^{7}B^7, $2n = 28$				
31 S	Cabo Blanco, Majorca	—	—	—	2

P = Portugal, S = Spain

The fourth is also located on the short arm of A1. In this case, however, the resultant chromosome is sub-metacentric (ssA1^s) with a short arm intermediate in length between the standard chromosome and the metacentric derivative.

The metacentric ss1A chromosome has been found in 13 of the 20 allotetraploid populations from the whole sampling range. It is usually at low frequency (less than 10 per cent) but at Serra de Candieros it reaches a frequency of 35 per cent. No homozygotes
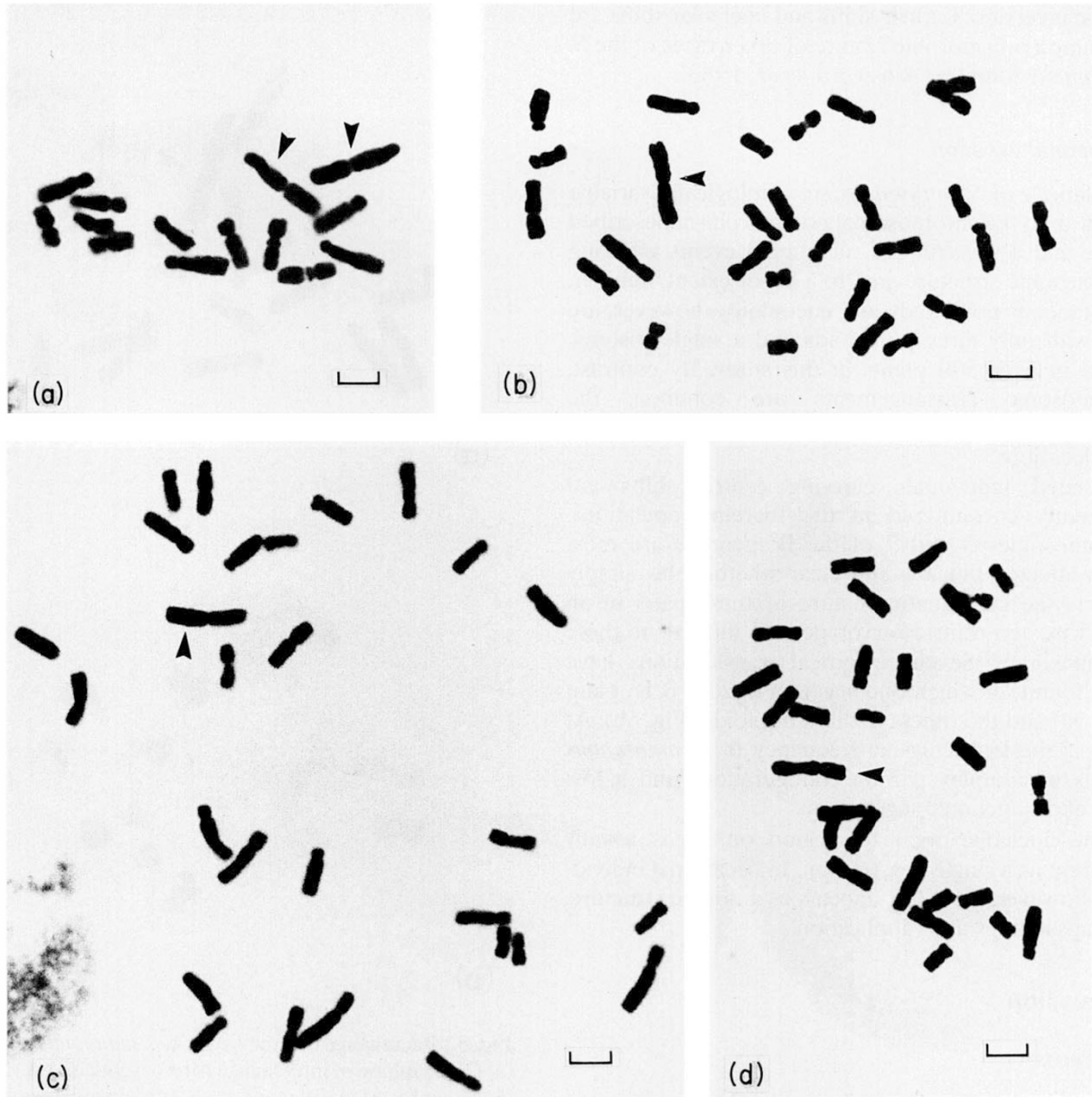
Fig. 4. Supernumerary segments on chromosomes A1 and B1. (a) B^7B^7 ss1 homozygote (b) AAB^7B^7 with ssA1 (c) AAB^7B^7 with ssB1 (d) AAB^7B^7 with short segment ssA1^2.

have yet been found. The acrocentric form ssA1^s, however, is geographically limited and occurs only in five populations all from central Portugal. Chromosome ssA1^2 accompanies ssA1 in four of its five sites and may possibly be a partial deletion of the long supernumerary segment.

The other segments in the allotetraploids (ssB1 and ssB4) have been detected only in single populations, both in Huelva province. At Campo Frio, ssB4 reaches polymorphic proportions.

Other polymorphic variants

Two further polymorphisms were observed, each in a single population. In the AA population from Loja, Granada, a B3 chromosome was found in which the nucleolar organizer region was displaced, probably as a result of paracentric inversion (Guillén & Ruiz Rejón, 1984). In the Condeixa (Beira Litoral) population of the AAB^7B^7 race, an A2 centric shift was observed at a low frequency. This is probably attributable to peri-

centric inversion. Centric shifts and nucleolar shifts are a common polymorphic feature of other races of the *S. autumnalis* complex (Ainsworth *et al.*, 1983).

Mutational variation

Populations of *S. autumnalis* are cytologically variable due both to the chromosomal polymorphism described above and a spectrum of mutational events affecting chromosome structure and, to a lesser extent, number. Spontaneous polyploidy and aneuploidy, however, are rare with only three polyploids and a single trisomic found in over 500 plants in this study. By contrast, chromosome rearrangements are common, the majority of which are centric shifts and reciprocal translocations.

Isolated individuals carrying centric shifts are frequently encountered in the Iberian populations. Chromosomes 3 and 7 of the B^7 genome are most often affected but it is not clear whether this simply reflects the conspicuous nature of these pairs in an otherwise acrocentric karyotype or is intrinsic to these chromosomes. Seven reciprocal translocations have been found, of which one was in a diploid B^{7}B^7 plant (Fig. 5a) and the others in allotetraploids (Fig. 5b). At meiosis the high chiasma frequency of *S. autumnalis* results in complex pairing configurations and a low level of disjunctional segregation.

The nucleolar-organizer region on B3 is usually apparent as a single constriction. In occasional individuals, however, the NOR appears as a double structure, perhaps as a result of duplication.

Discussion

The races

The diploid B^{7}B^7 race is the most widespread in the *Scilla autumnalis* complex. It is a Mediterranean race, stretching from the Spanish–Portuguese border in the west to Israel in the east, a distance of about 2500 km. It is scattered across the whole Mediterranean particularly on the islands such as Sicily, Crete, Malta and Cyprus as well as on the Greek mainland and in parts of north Africa around Tunis and Algiers (Battaglia, 1963; Hong, 1982; Ainsworth *et al.*, 1983; Guillén & Ruiz Rejón, 1984).

In the Iberian Peninsula, the B^{7}B^7 race has been detected in the central region around Madrid and on the Spanish–Portuguese border. Clearly, to define the distribution more accurately more sampling is required. Particularly lacking are studies of the vast area to the north of Madrid as far as the Pyrenees. Preliminary studies suggest that the area around Pamplona
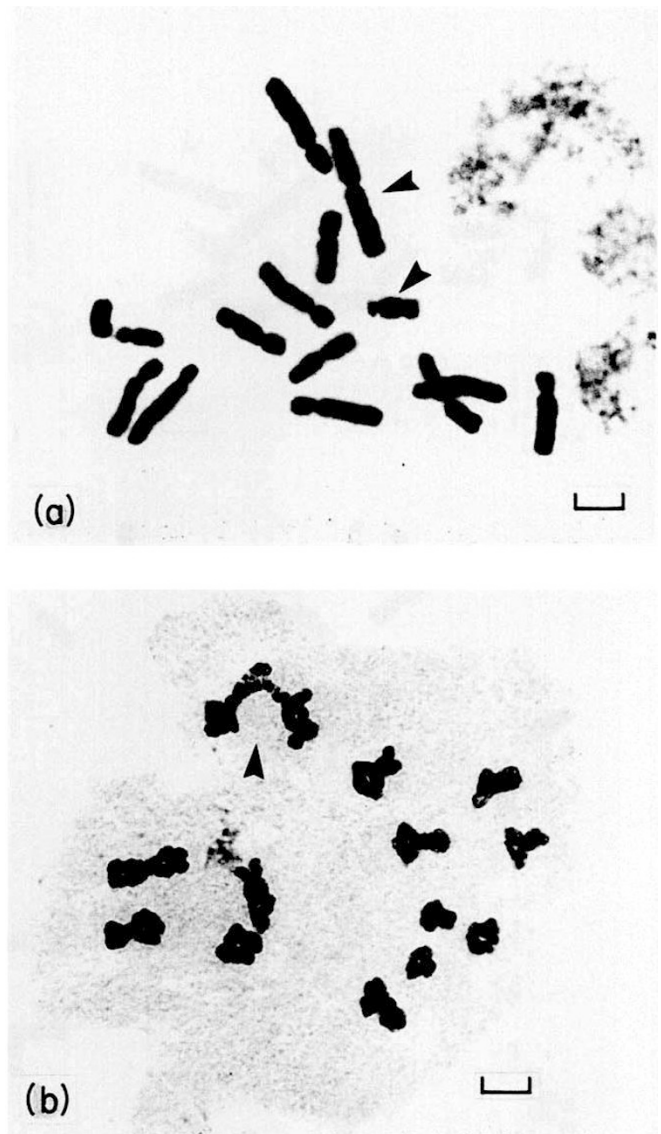

Fig. 5. Interchange heterozygotes in *S. autumnalis*.
(a) C-metaphase of interchange heterozygote in AA race.
(b) Metaphase-I in a plant of AAB^{7}B^7 showing interchange quadrivalent.

near the Pyrenean foothills supports a 28 chromosome race, but whether this represents the AAB^{7}B^7 cytotype or the B^{7}B^{7}B^{7}B^7 race typical of France, Italy and Britain, has not yet been determined. The presence of the B^{7}B^{7}B^{7}B^7 race on Majorca may suggest the latter. Studies in the Orthoptera have demonstrated that the Pyrenean range has acted as a barrier to dispersal and now coincides with racial differentiation (Gosálvez *et al.*, 1988). Similarly, diploid and tetraploid cytotypes of the plant *Plantago media* meet in the central Pyrenees (van Dijk & van Delden, 1990).

The two other cytological races detected on the Iberian Peninsula (AA and AAB^{7}B^7) are endemic. The

AA race is restricted to south-eastern Spain with a single coastal site at Peniche in Portugal. Two other coastal sites of similar habitat, at Foz de Arelho, 15 km to the north, and at Cabo Mondego near Coimbra, 100 km north, were both of the AAB^7B^7 race as was the inland collection from Reguengo Grande 15 km distant. It would be interesting to know whether other populations of constitution AA are scattered across the 500 km which separate Peniche from south-eastern Spain.

The tetraploid race AAB^7B^7 has a distinctly western tendency in Spain and Portugal from the extreme north in Asturias to the extreme south in Sevilla. The tetraploids and the B^7B^7 diploids presumably have a long zone of contact in a north–south band. This has been best defined in south-west Spain in the provinces of Huelva, Badajoz and Sevilla. In this study, populations as close as 10 km have been detected which differ in cytological constitution. Mixed populations may occur in this contact zone and if so it is probable that hybrids will be generated. In experiment there are few barriers to gene flow between these races. Hybrids of constitution ABB are freely formed as pollinators more indiscriminately between individuals of different races. Inter-racial hybrids have twice been detected in natural populations of this complex: between B^7B^7 and B^6B^6 types in western Crete, and between $AAB^7B^7B^7B^7$ and $B^7B^7B^7B^7$ races in England (J. S. Parker, unpublished results).

Hybrids of constitution ABB are triploids with a low, but not negligible, fertility. Backcrosses of triploids as egg parents give some offspring in which homoeologues can successfully substitute for each other (H. Vaughan, personal communication) as in *Triticum aestivum*. This emphasizes the close genic similarity of the *Scilla* homoeologues despite their large DNA difference. However, hybrids do suffer a marked decline in fertility. Selection against the hybrids, and thus indirectly against the minority parent, should be severe in natural populations. This should result in a relatively well-defined and even contact zone between races, and the elimination of mixed populations (Barton & Hewitt, 1985). Studies of the dynamics of such hybrid zones in plants have seldom been attempted. Only one study has been made of a zone where polyploid differences define the races, the diploid–autotetraploid boundary in *Plantago media* (van Dijk & van Delden, 1990). This system differs from *Scilla* in that viable triploid hybrids are very rarely formed due to embryo–endosperm incompatibility. Hence the possibility of gene exchange between races is severely curtailed.

The polymorphisms

The common polymorphisms of this species group are B-chromosomes and supernumerary segments. On the basis of morphology, five types of B-chromosomes have been found, one in each diploid and three in the allotetraploid. Bs have previously been recorded in B^7B^7 diploids by Battaglia (1963, 1964) in plants from Sicily and Israel. These Bs were telocentric and acrocentric, respectively, but were of similar size to the submetacentric Bs found in Spain (Guillén & Ruiz Rejón, 1984). Hong (1982) also found telocentric Bs in Aegean plants. In addition, a minute supernumerary chromosome, perhaps a deleted B, was detected in a Sicilian population by Guillén & Ruiz Rejón (1984). B^7B^7 diploids on Crete also contain B-chromosomes (S. Taylor, unpublished results).

Clearly, B-chromosomes are a regular feature of the B^7B^7 race across its whole trans-Mediterranean distribution. The morphological variation observed does not rule out a common origin. Bs can undergo rapid evolution by iso-chromosome formation, deletion and inversion because they are freed from the constraints that operate on members of the standard complement (Bougourd & Parker, 1979; Jones & Rees, 1982). Homology can, of course, most readily be established by meiotic analysis of pairing in plants that carry two or more B-types.

The Bs found in Spanish populations of AA diploids are very interesting as they act as specific derepressors of the *Est-1* structural locus on the standard chromosomes (Ruiz Rejón *et al.*, 1980; Oliver *et al.*, 1982), a unique activity amongst Bs (Jones & Rees, 1982). It would be interesting to test for this behaviour pattern in other B-types and in other ploidy levels in the complex.

The acrocentric B polymorphism in AAB^7B^7 tetraploids is very widespread. It differs somewhat from that in B^7B^7, however, in that it is limited in numbers.

Only plants with 0, 1, 2 and 4 Bs have been found. Individuals with these numbers are not distributed as the terms of a Poisson distribution because there is an excess of plants with 2Bs and a deficiency of 1B plants. This recalls the pattern found in rye and other grasses which is determined by the mechanism of non-disjunction at pollen grain mitosis I (Matthews & Jones, 1982). Clearly, controlled crosses are required to establish whether an accumulation mechanism is in operation. Inbreeding, however, may also lead to a predominance of even numbers of B-chromosomes.

Euchromatic supernumerary segments are regular components of the genetic system of *S. autumnalis*. In a large study of autotetraploid $B^7B^7B^7B^7$ populations, Ainsworth (1982) identified segments on each of the

seven chromosomes but with very unequal frequencies. Chromosomes 1, 3 and 4 were particularly affected. Most of these segments reached polymorphic proportions, and some had wide geographical distributions (Ainsworth *et al.*, 1983).

The segment on the short arm of chromosome 1 in the B^7B^7 race is common in south-western Spain and a morphologically identical chromosome B1 has been found in populations from Corfu, Greece (Ainsworth, 1981). Interestingly, a euchromatic supernumerary segment on the long arm of chromosome 2 in *Muscari comosum* has a similar wide distribution, having been found in Spanish and Aegean populations (Ruiz Rejón & Oliver, 1981).

The supernumerary segment of A1, found commonly in the allotetraploid race, gives a metacentric chromosome that resembles the ssB1 chromosome. There is evidence from chromosome substitutions that the A and B genome chromosomes are homoeologues at the level of structural loci, although they differ in the total DNA amount. The factors which have led to the generation of the segments may be common to the homoeologues in the A and B^7 genomes. That is, the development of a supernumerary segment may depend on loci, or DNA sequences, perhaps located on the short arms of chromosomes A1 and B1 themselves. These supernumerary segments, like that in *Muscari*, are C-band negative and so are unlikely to have arisen by massive repetition of a small family of sequences; the segments account for up to 5 per cent of the haploid genome. Without DNA analysis, however, the nature of these common segments remains obscure; equally obscure is their function, if any. Supernumerary segments can act as cross-over suppressors in *S. autumnalis*, but their effect is limited to the arm on which they are carried. Segments in other organisms may alter the distribution of chiasmata, causing a shift away from the segment as in the genus *Atractomorpha* (Orthoptera) while in other species they act as recombination enhancers over the whole genome (John & Miklos, 1979). The chiasma effect of segments on different chromosomes may be additive (Westerman, 1970).

Heterochromatic supernumerary segments are widespread in dioecious species of the *Rumex acetosa* group (Wilby & Parker, 1988). The segments of *Rumex* and *Scilla* share two characteristics: neither affect overall chiasma frequency, but both show non-Mendelian patterns of inheritance with enhanced transmission in backcrosses (Parker *et al.*, 1988).

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