HETEROCHROMATIC PATTERNS IN ALLIUM

1. THE RELATIONSHIP BETWEEN THE SPECIES OF THE CEPA GROUP AND ITS ALLIES

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SUMMARY

The karyotype of ten taxa in the *cepa* group of *Allium* and two allied species have been investigated using fluorochromes and Giemsa staining techniques. The chromosomes of all species have terminally located heterochromatic segments which show reduced Quinacrine-fluorescence and are deeply stained by the Giemsa technique. Together with morphological similarities these characteristics indicate a close relationship between the alliums of the *cepa* group and the two allied species *A. schoenoprasum* and *A. ledebourianum*. On the basis of chromosome morphology and C-band endowment the hybrid origin of the viviparous forms of the Onion: *A. cepa* var. *viviparum*, *A. aobanum* and *A. wakegi* is confirmed.

1. INTRODUCTION

TECHNIQUES for the linear differentiation of plant and animal chromosomes are now well established tools for cytogenetic research (Vosa, 1975). Several studies on many plant species (Vosa, 1970, 1971, 1973*a, b, c*; Vosa and Marchi, 1972*a, b*; Schweizer, 1973; Marks and Schweizer, 1974) have demonstrated that a pattern of heterochromatic segments can be readily revealed by either specific fluorochromes or various pretreatments followed by Giemsa or orcein staining. However, only the C-type or constitutive heterochromatin bands have been found in plants; no G-type bands have been obtained so far although these are easily demonstrable in most animals (Hsu, 1973; Vosa, 1975).

The present study is concerned with the heterochromatic patterns of the Alliums of the *cepa* group together with some of its allied species using fluorochromes and Giemsa techniques.

2. MATERIALS AND METHODS

Origin of the material:

Allium cepa L.—the common or garden onion; seeds from cultivated plants and from commercial sources.

A. ascalonicum L.—the Shallot of English gardens rarely flowers and it is propagated vegetatively; bulbs from cultivated plants.

A. cepa L. var. *viviparum* (Metzg.) Alef.—the Egyptian or Top Onion; bulbils from several Botanic Gardens and from commercial sources.

A. aobanum Araki—a large viviparous onion from Japan; bulbils received from Professor M. Kurita, Ehime University, Japan.

A. wakegi Araki—a small viviparous type from Japan; bulbils received from Professor Kurita.

A. oschanini O. Fedtsch.—a wild species endemic to the Pamir-Alai mountains ranges of Turkmenistan (Central Asia); seeds collected from the wild and received from Ashkhabad Botanic Garden (Turkmenskoi SSR).

A. galanthum Kar. & Kir.—a wild species found in Central Asia and in Siberia; seeds received from Tashkent Botanic Garden.

A. vavilovii M. Pop. & Vved.—a species resembling *A. cepa* L. in gross morphology and found wild over a large region from Northern Persia to the mountains of Turkmenistan in Central Asia; seeds received from Tashkent and Ashkhabad Botanic Gardens.

A. fistulosum L.—a widely cultivated species not found for certain in the wild but probably derived from some form of *A. altaicum* Pall.; seeds from cultivated plants.

A. altaicum Pall.—a species with a wide distribution in Siberia and in Central Asia; seeds received from Tashkent Botanic Garden.

A. schoenoprasum L.—a widely cultivated species, very polymorphic for size and flower colour, known in Britain as Chives. Seeds from cultivated plants.

A. ledebourianum Roem & Schult.—this represents a wild form of the *schoenoprasum* group found wild in Siberia and in the Northern regions of the Far East; seeds received from Yerevan Botanic Gardens (Armenia SSR).

The sexual species were raised from seeds, the viviparous forms from bulbils. All have $2n = 16$ and were grown in pots under cool greenhouse conditions.

For the cytological preparations the root-tips were pretreated with 0.05 per cent colchicine for 4-5 hours at room temperature and fixed overnight in 1:3 acetic-alcohol. For fluorescence studies fixed root-tips were squashed in 45 per cent acetic acid and the subsequent procedure was as in Vosa (1973a, b). For the Giemsa staining the standard procedure for routine karyotype analysis in this laboratory was used (Vosa, 1973a). In some cases after the barium and SSC treatments, the fluorochrome Hoechst 33258 was used instead of Giemsa. Thus, after SSC incubation and rinsing in distilled water, the material was air-dried and then stained in 0.01 per cent H.33258 in absolute alcohol for 3-5 minutes. This treatment gives intense fluorescence of the H-segments. These are usually much better differentiated than with the Giemsa stain.

3. RESULTS

(i) *A. cepa* L. and *A. ascalonicum* L. (fig. 1a and b; plate II, fig. 1)

All the chromosomes possess small distally located H-segments. These may be dot-like or up to about 2.3 micra in length in colchicine metaphase chromosomes. Centromeric heterochromatin is present in all the chromosomes in the form of very thin bands sometimes seen as paired dots in late C-metaphases. Individual chromosome types are polymorphic for the size of the terminal bands. Except for the morphologically distinguishable nucleolar chromosomes (pair 8) the polymorphism is not easily scored owing to the similarity of most of the chromosomes in the complement, especially pairs 3, 4 and 5 (M_3 , M_4 and M_5 of Battaglia, 1957). In addition to the normal nucleolar chromosomes, a number of plants in all the varieties

studied have a second nucleolar pair (pair 6) in which the nucleolar organisers occupy a distal position on the long arm (see also Battaglia, *loc. cit.*). Most plants are heteromorphic with only one of the satellites present. The satellite and the terminal H-segment appear as two separate bands.

FIG. 1

- (a) *A. cepa*. Karyotype of a plant of the var. "Yellow Globe". Note heteromorphism of chromosomes 1, 2, 6 and 8.
 (b) *A. ascalonicum*. Karyotype of a plant from a clone cultivated in Tuscany (Italy). Chromosomes 2, 7 and 8 are heteromorphic.

- (ii) *A. cepa* var. *viviparum* (fig. 2a), *A. aobanum* Araki (fig. 2b) and *A. wakegi* Araki (fig. 2c)

Apart for some small difference in size the chromosomes of these forms are similar to one another. They include 14 submetacentric chromosomes and two rather dissimilar subterminal chromosomes. In *A. aobanum* all the chromosomes except the third largest possess terminal H-segments in the short arm (1, 5, 8, 12 and 16) or in the long arm (6 and 11) or in both arms (2, 4, 7, 9, 10, 13, 14 and 15). Chromosome 15 seems to correspond closely with the nucleolar chromosome of *A. cepa* both in morphology and in banding patterns. The other nucleolar chromosome (16) resembles the corresponding chromosome of *A. fistulosum* but it is shorter and lacks the terminal band in the long arm.

FIG. 2

- (a) *A. cepa* var. *viviparum*. Karyotype of a plant from a clone cultivated in Oxford Botanic Garden.
 (b) *A. aobanum* and (c) *A. wakegi*. Karyotypes of plants cultivated at the Botany School, Oxford and originated in Japan.

(iii) *A. oschanini* O. Fedtsch. (fig. 3a; plate I, fig. 2)

Although the general morphology is similar to *A. cepa*, the band endowment is different. Only chromosome pairs 5, 6 and 7 have terminal bands in both arms; no bands were found in the long arms of pairs 3, 4 and 8 or either arms of pair 2. In addition, pair 4 has a double distal band in the short arm and pair 7 has a thin intercalary and also in the short arm. Very little scorable heteromorphism has been detected in the five-plant sample

FIG. 3

- (a) *A. oschanini*. Karyotype of a homomorphic plant.
 (b) *A. galanthum*. Karyotype of a plant heteromorphic for chromosomes 2, 4 and 7.
 (c) *A. vavilovii*. Karyotype of a plant heteromorphic for chromosomes 3, 4, 6 and 8.

examined. This is probably related to the origin of the seeds which may have been collected from a single, more or less homomorphic plant.

(iv) *A. galanthum* Kar. & Kir. (fig. 3b)

Its gross morphology as well as its chromosome complement and heterochromatin endowment are similar to *A. cepa* with the possible exception of the nucleolar chromosome (pair 8) which is somewhat shorter.

(v) *A. vavilovii* M. Pop. & Vved. (fig. 3c)

Chromosome morphology and position of nucleolar attachments are similar to *A. cepa* except for the second nucleolar chromosome (pair 6) which has not been detected in the six-plant sample studied. As in *A. cepa* all chromosomes have terminal heterochromatin but difference in size between H-segments are more marked than in the former species.

(vi) *A. fistulosum* L. (fig. 4a)

All chromosomes possess terminal H-segments which are somewhat larger than those found in *A. cepa*. The satellites on the short arm of pair 8 are always much larger than in the onion (cf. Battaglia, 1957) and are completely heterochromatic.

FIG. 4

- (a) *A. fistulosum*. Karyotype of a plant from a clone cultivated in Oxfordshire. Chromosomes 3 and 4 are heteromorphic.
 (b) *A. altaicum*. Karyotype of a plant heteromorphic for chromosomes 1 and 6.

Plate I

FIG. 1.—*A. altaicum*. Incomplete mitotic metaphase. Note heteromorphic nucleolar chromosomes (arrows).

FIG. 2.—*A. oschanini*. Incomplete mitotic metaphase. Note nucleolar chromosome (No. 8) and the intercalary band in chromosome 4 (arrows).

FIG. 1.—*A. cepa*. Incomplete mitotic metaphase. Note heteromorphic nucleolar chromosomes (arrows).

FIG. 2.—Nucleolar chromosomes in *A. cepa* stained (a) with Hoechst 33258 after denaturation/reannealing and (b) with Quinacrine without pretreatment.
 c, d and e—Nucleolar chromosomes in *A. galanthum* (c) and *A. cepa* (d and e) from the same plant. Note heteromorphism.
 f, g and h—Polymorphic nucleolar chromosomes in *A. altaicum*.

(vii) *A. altaicum* Pall. (fig. 4b; plate I, fig. 1)

Chromosome morphology as well as band endowment agree with *A. fistulosum*.

(viii) *A. schoenoprasum* L. (fig. 5a)

The polymorphism of this species includes diploid, triploid and tetraploid forms. The total length of the chromosome complement is much shorter and according to Jones and Rees (1968) its nuclei contain about half the DNA of *A. cepa* (16.9:33.5 picograms, see also Rees and Jones, 1972). Except in pairs 1, 5 and 8 where H-segments are found only in the short arm, all chromosomes possess distally located heterochromatin in both arms. This is particularly prominent in the short arm of pair 1 and in the nucleolar pair where the small satellite is completely heterochromatic. Chromosome pair 3 has a second heterochromatic band in the short arm.

FIG. 5

- (a) *A. schoenoprasum*. Karyotype of a homomorphic plant cultivated in Oxford Botanic Garden.
 (b) *A. ledebourianum*. Karyotype of a plant heteromorphic for chromosomes 1 and 4.

(ix) *A. ledebourianum* Roem & Schult. (fig. 5b)

The chromosome complement is very similar to that of *A. schoenoprasum* but some plants are heteromorphic for the second band in pair 3 and there is heteromorphism for the presence or absence of H-bands in the long arms of pairs 1, 5 and 8.

4. DISCUSSION

The patterns described for *A. cepa*, *A. ascalonicum* (as *A. cepa* var. Shallot), *A. galanthum* and *A. fistulosum* are similar to those reported by El-Gadi and Elkington (1975) but there are differences in details in that these workers did not record:

- (a) centromeric heterochromatin, which is easily detected in most preparations;
- (b) the deeply staining satellites of the nucleolar chromosomes, which are especially prominent in *A. fistulosum*;
- (c) inter-plant variation.

Kurita (1958, 1963) using a combined staining-hot hydrolysis technique together with oxyquinolin pretreatment, demonstrated the presence of terminal heterochromatin in *A. cepa* and *A. aobanum*. The H-pattern is very similar to that reported here.

The present study of several cultivars of *A. cepa* has confirmed the widespread polymorphism in the nucleolar chromosomes (plate II, figs. d and a; text-fig. 1a) which can be detected in Feulgen-stained preparations (Battaglia, 1957) and revealed variation in the size of the terminal bands in all other autosomes. These polymorphisms apply also to *A. ascalonicum*. In this last species chromosome morphology and band size variation are practically indistinguishable from those in *A. cepa*. This is a confirmation of the suggestion of De Candolle (1886) that *A. ascalonicum* is but a variant of the garden onion (*A. cepa*).

The chromosomes of *A. fistulosum* and *A. altaicum* are similar in size and centromeric index and the total length of these complements is about 20 per cent shorter than that of *A. cepa*. This difference in chromosome size is validated by the work of Jones and Rees (1968) who found that the DNA content of *A. fistulosum* was 30 per cent less than that of *A. cepa*.

On the basis of karyotype, *A. cepa* var. *viviparum* (see also Bozzini, 1964) and the vegetatively propagated entities from Japan, namely *A. aobanum* Araki and *A. wakegi* Araki, together with various viviparous forms of the onion, must be accorded a hybrid origin. Indeed, they are probably all derived from an ancient garden hybrid between some form of *A. cepa* and *A. fistulosum* (see Kurita, 1958; 1963). A confirmation of this hypothesis is provided by morphological studies which show that such viviparous forms are intermediate between these two species.

According to the taxonomic literature, the two other species investigated, *A. schoenoprasum* L. and *A. ledebourianum* Roem. & Schult., belong to the section Rhizirideum. However, taxonomic studies have shown that the genus *Allium* and its sections as conceived until now represent a very unnatural assemblage of species (see Traub, 1968). The criteria of separation between the sections are in many cases small and may not be valid. For example, the main difference between section *Phyllodolon* (*A. fistulosum*, etc.) and section *Cepa* (*A. cepa*, etc.) is the presence or absence of small nectaries at the base of the ovary.

All species studied have fistulose leaves and differ overall only in size from one another: *A. schoenoprasum*, the Chives, resembles a much reduced *A. fistulosum*. But the most important common character in this group of plants and the one which defines their relationship is the position and type of

constitutive heterochromatin. In all these species heterochromatin is distally located, not sensitive to cold and shows reduced fluorescence when stained with Quinacrine (Vosa, 1970).

In all the species where relevant studies are available the position of the bands seems to be independent of chiasma localisation which is proximal in *A. fistulosum* and *A. altaicum* and random in *A. cepa*, *A. vavilovi*, *A. oschanini* and *A. ascalonicum*. The report by Wagenaar (1969) and observations in most of the species in the present study, on the end-to-end association of the chromosomes throughout interphase and early prophase, may be related to a probable primary site of meiotic pairing (see also Stacks and Clarke, 1973) but it does not appear to be connected with chiasma localisation.

There are no significant differences between species with regard to the amount of constitutive heterochromatin as a proportion of metaphase chromosome length. This proportion does not exceed 10-12 per cent and therefore is not likely to be related to differences in nuclear DNA between the species.

Other *Allium* species previously investigated include *A. pulchellum* (as *A. carinatum*, Vosa, 1971) and *A. flavum* (Vosa, 1973c). These species are distinct from the group used in the present study and their heterochromatic pattern includes intercalary and distally located enhanced and reduced fluorescence bands. These latter are very prominent in *A. flavum* where the proportion of enhanced to reduced fluorescence is more or less equal. Preliminary results in other allied species such as *A. stamineum*, *A. fuscum*, *A. paniculatum*, *A. oleraceum* and *A. pulchellum* has revealed the same situation. In *A. stamineum* the proportion of enhanced to reduced fluorescence bands is biased towards the latter.

The various techniques for the linear differentiation of chromosomes provide a very useful tool for assessing taxonomic and phylogenetic relationships. Most of the investigations so far have been based mainly on the location, quantity and size of the heterochromatic bands thus revealed. Experiments intended to assess the chemical "quality" of the bands and the mechanism of their formation whether obtained by fluorochroming, cold or by other differentiating techniques, will give further and more precise information about species affinities. Already several studies in fluorochrome specificity have given useful indications about the chemical nature of the various band types. Thus, Weisblum (1973a, b), Weisblum and de Haseth (1972), Weisblum and Haenssler (1974) and Pachmann and Rigler (1972) have demonstrated that enhanced fluorescence is associated with highly repetitive sequences of AT-base nucleotides whereas repetitive GC sequence actually quench Quinacrine fluorescence. Base composition and degree of repetitiveness together with the types of base interspersions govern fluorescence intensity. It is interesting to note the differences between the Q-reduced fluorescence of the H-bands in all the species studied here, which convalidates their close relationship, and the presence of enhanced as well as reduced fluorescence in the other *Allium* species where comparable studies have been made (Vosa, 1970, 1971, 1973c).

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