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The chromosomal locations of enzyme-coding genes *Adh*-1 and *Pgm*-1 in *Allium fistulosum* L.*

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Summary. The chromosomal locations of two enzyme-coding genes were investigated using allele dosage effects in two *Allium cepa* alien addition lines possessing different *A. fistulosum* chromosomes as the trisomes. *Adh-1* has been assigned to the *A. fistulosum* sub-telocentric chromosome 5, while *Pgm-1* is on the *A. fistulosum* sub-metacentric chromosome 4. Karyotype data of a short-day cultivar of *A. cepa*, 'Temprana', *A. fistulosum* 'Ishikura Long White', and the relevant chromosomes of the two trisomics are presented.

Key words: *Allium cepa* – *A. fistulosum* – Trisomics – Karyotype – Isozyme

Introduction

The bulb onion, *Allium cepa* L. is one of the most familiar plants used for demonstration and training in cytology (e.g. Sundberg 1981), and has been used extensively for investigating the effects of mutagens on chromosomes (Grant 1982). Most of the research has been concerned with gross chromosomal abnormalities (Hernandez et al. 1986) or with the occurrence of sister chromosome exchanges (SCEs) (Schvartzman and Cortes 1977; Cortes et al. 1983) rather than with the location of genes on chromosomes. Only the location of the nucleolar organizing region (NOR) on the shorter arm of the only sub-telocentric chromosome of the complement was obvious.

Karyotypes of *A. cepa* have been published in varying degrees of detail (El-Gadi and Elkington 1975; Vosa 1976; Stack and Comings 1979; Cortes et al. 1983; Kalkman 1984), and karyotypes of *A. fistulosum* were included in some of these studies. Cortes et al. (1983) published data on *A. cepa* (no cultivar name) and Kalkman (1984) presented karyotype data on the *A. cepa* cvs 'Downing Yellow Globe', 'Lemi' (a Finnish cultivar) and 'Italian Red Torpedo'. Both of these authors used C-banding as an additional means of chromosome identification and presented relative length and short arm/total chromosome length data. Cytologists have tended to treat *A. fistulosum* as a homogeneous botanical species, but it has had a long history of domestication in the Far East where many distinct types have been developed. It is therefore as important to define the *A. fistulosum* cultivar being studied as it has been in *A. cepa*.

A number of morphological marker genes occur in the onion, eg. bulb coat colors (Clarke et al. 1944; El-Shafie and Davis 1967), flower colors (Davis 1960), leaf waxiness and glossiness (Jones and Mann 1963), and chlorophyll deficiency characters (Berninger and Buret 1967). None of these characters has been assigned to specific chromosomes, and little has been published on linkage in onion.

It is possible to map genes using isozymes as markers when the isozymes appear in trisomic dosages and the extra chromosome or trisome is identified. This approach has been applied to the investigation reported here. Our objective was to determine the chromosomal locations of *Adh-1* and *Pgm-1*. Two alien addition lines, DG5 and DG59, were the subject of this investigation. PGM (DG5) and ADH (DG59) isozymes appear in these plants in trisomic dosages with 2 *A. cepa* alleles and 1 *A. fistulosum* allele (Peffley et al. 1985). The plants were generated by the backcrossing of cv. 'Delta Giant', a

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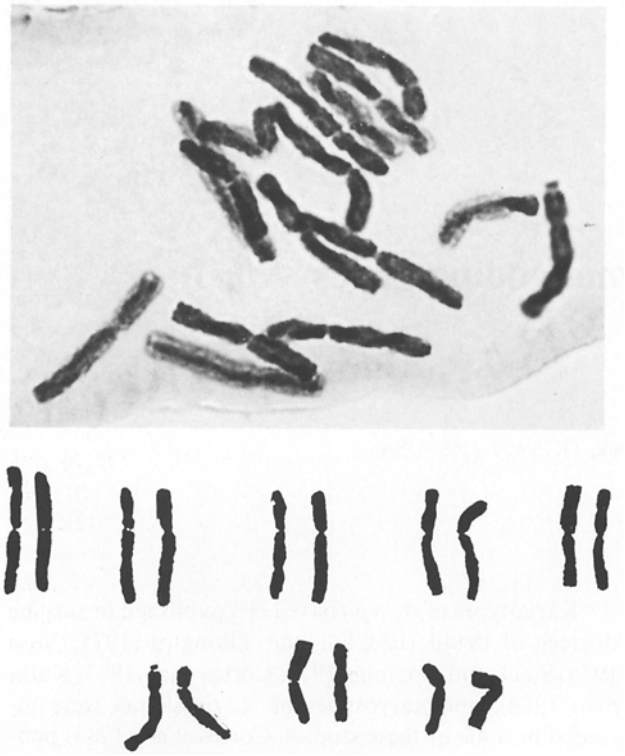

Fig. 1. Karyotype of *Allium cepa* cv. 'Temprana'

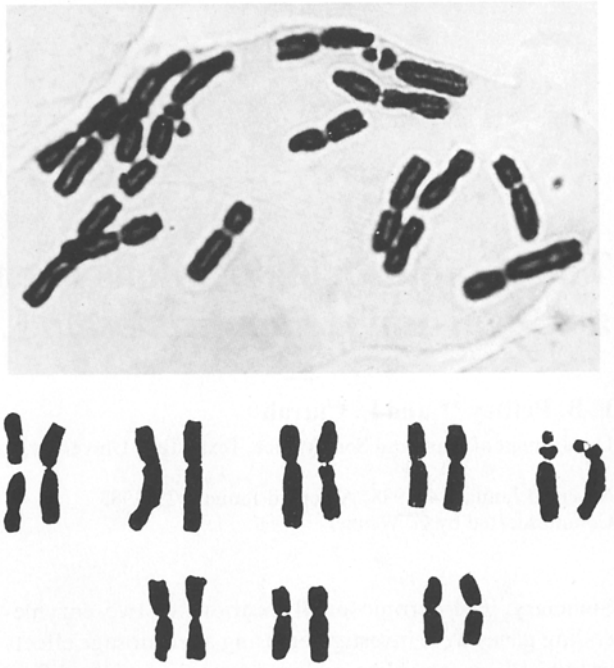

Fig. 2. Karyotype of *A. fistulosum* cv. 'Ishikura Long White'

triploid shallot (Perkins et al. 1958) which has the constitution *A. cepa* (2×)+*A. fistulosum* (×), to *A. cepa* cv. 'Temprana' (2×).

Karyotypes of *A. cepa* cv. 'Temprana' and *A. fistulosum* cv. 'Ishikura Long White' aided in distinguishing the two *A. fistulosum* chromosomes of the trisomics.

Materials and methods

Plant materials used in this study had been previously assayed for alcohol dehydrogenase (ADH), glycerate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) (Peffley et al. 1985). Roots for cytological studies were taken from field-grown plants forced with their basal plates suspended over water-filled plastic beakers. After 5–7 days, fast-growing roots were cut at approximately 1 cm from the tip, kept in iced, distilled water for 24 h, then fixed in 1 : 3 acetic acid-ethanol before hydrolyzing at 58 °C for 10 min in 1N HCl. Roots were kept in 70% alcohol in a refrigerator until squashing on slides in ferric aceto-carmine.

Photographic prints of mitotic metaphases were measured using a ZIDAS image analyzer[1] to obtain short and long arm lengths for each chromosome in the complement. Short arm to total (SA + LA) length ratios were calculated for each chromosome, and the lengths of the individual chromosomes were calculated as percentages of the sum of all chromosome lengths for the cell. Each chromosome's two coordinates, arm ratio and percentage length, were used to plot its position on a two-dimensional diagram, and from this the most likely homologous pairs were selected. Mean lengths and ratios were calculated for each of the 8 pairs of *A. cepa* and *A. fistulosum* chromosomes. Trisomics were C-banded using a technique similar to that of Kalkman (1984).

[1] Mention of a brand name or trademark does not imply endorsement by Texas Tech University

Results and discussion

Karyotypes

Data on chromosome morphology of *A. cepa* cv. 'Temprana' and *A. fistulosum* cv. 'Ishikura Long White' are presented in Table 1, and sample karyotypes are shown in Figs. 1 and 2, respectively. We use short arm/total chromosome length data (Table 1) as the basis for the identification of the trisomes.

Alcohol dehydrogenase (ADH)

The alien chromosome of DG59 (Fig. 3) is unmistakeably the short sub-telocentric chromosome of *A. fistulosum* with a characteristic large satellite on the short arm (Fig. 2). The relative chromosome length in the trisomic (2n = 17) complement was $3.9 \pm 0.11\%$, and the short arm/total chromosome length ratio was 0.256 ± 0.009 (means of 9 cells). Because the short arm of this chromosome consists mainly of heterochromatin and the nucleolar organizing region (the area which does not stain with acetocarmine), there is a higher probability that the *Adh-1* locus is borne on the long arm of this chromosome.

Confirmation of the assignment of the *Adh-1* locus to the sub-telocentric *A. fistulosum* chromosome was ob-

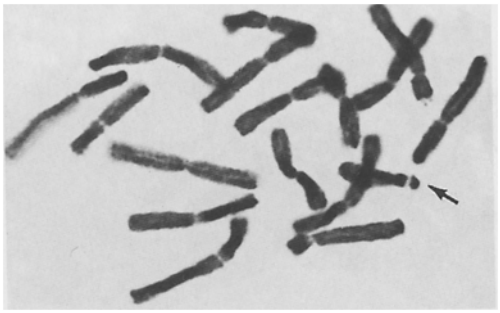

Fig. 3. Trisomic DG59. *Arrow* indicates *A. fistulosum* sub-telocentric chromosome which carries Adh isozyme locus

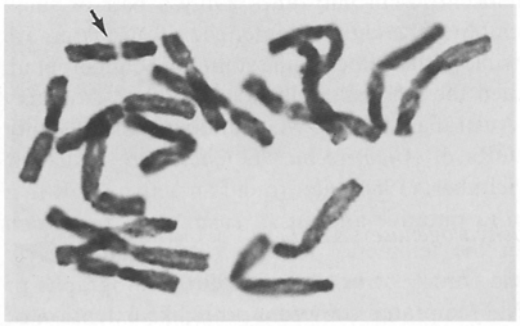

Fig. 4. Diploid DG122-13. *Arrows* indicate sub-telocentric chromosome which carries Adh isozyme and a very small meta-centric chromosome

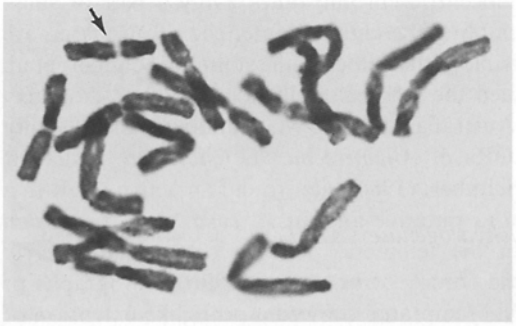

Fig. 5. Trisomic DG5. *Arrow* indicates *A. fistulosum* sub-metacentric chromosome which carries Pgm isozyme locus

tained when a diploid plant, DG122-13, which was recovered from 'Delta Giant' backcross progeny, was found also to carry an *A. fistulosum Adh-1* allele. This plant had two sub-telocentric chromosomes which differed considerably in length (Fig. 4). But neither subtelocentric had a large satellite. Hence, this plant has one each of the NOR chromosomes from *A. cepa* and *A. fistulosum*. This plant also carries a very small metacentric chromosome, and from its size relative to the sub-telocentric chromosome (i.e. number 5) in DG122-13, we identify it as chromosome 8 of *A. fistulosum* (Fig. 2). Chromosome 8 does not code for any loci tested since the only *A. fistulosum* allele DG122-13 possessed was ADH, which was determined in DG59 to reside on the small sub-telocentric. These loci were also tested in another trisomic derived from the same 'Delta Giant' backcross to 'Temprana' (Orozco-Castillo 1986) and are not located on chromosome 7 (Fig. 2). Hence, both of the smallest pairs of *A. fistulosum* chromosomes (i.e. 7 and 8) may be eliminated as carriers of these genes.

Phosphoglucomutase (PGM)

Identifying the alien chromosome of DG5 (Fig. 5) was more difficult. This chromosome was either slightly smaller than, or overlapping in size with the 2 smallest pairs of *A. cepa* chromosomes (Fig. 1), and it closely resembled them in arm length ratio. Using two-dimensional diagrams, it was decided on the basis of position similarity in position which were most likely to be the two *A. cepa* chromosome pairs, and the remaining one was designated as the alien. The mean arm length ratio for the alien chromosome in 12 karyotypes was 0.435 ± 0.007, while its length as a proportion of the total chromosome length (where $2n = 17$) was $4.38 \pm 0.12\%$. An additional identification tool for the length of the alien chromosome was the ratio of the length of the alien chromosomes to the mean lengths of the 2 longest and most distinctive metacentrics of each cell, chromosome 1 of *A. cepa*. This ratio gave a mean of 0.609 ± 0.020. A similar calculation for the *A. fistulosum* sub-telocentric of DG59 gave a ratio of 0.538 ± 0.019. Using these figures and the karyotype obtained for *A. fistulosum*, it appears that the chromosome carrying *Pgm* is the sub-metacentric chromosome 4 of *A. fistulosum*. In cv. 'Ishikura Long White', this chromosome is slightly longer than the subtelocentric (6.09% of the total complement for no. 4, 5.79% for the subtelocentric) and has an arm ratio of 0.428 (Table 1). This corresponds closely with 0.435 in the alien chromosome of DG5. Chromosomes 3 and 2 of *A. fistulosum* which have arm ratios of 0.409 and 0.373 respectively are less likely candidates because their arm ratios differ more widely from that of the DG5 alien chromosome, and they are substantially longer than chromosome 4 (percentage lengths in cv. 'Ishikura Long White' are 6.91 and 7.42%, respectively, for chromosomes 3 and 2 in our data). Figure 6 shows a C-banded preparation of DG5 in which the 5 smallest chromosomes are visible. The one with very large telomeric bands is assumed to be the alien chromosome: C-bands of *A. fistulosum* are wide in comparison with those of *A. cepa* (Vosa 1976).

Our karyotypes of *A. cepa* and *A. fistulosum* showed some interesting features more easily identified on the two-dimensional diagrams (Figs. 7 and 8) than from the

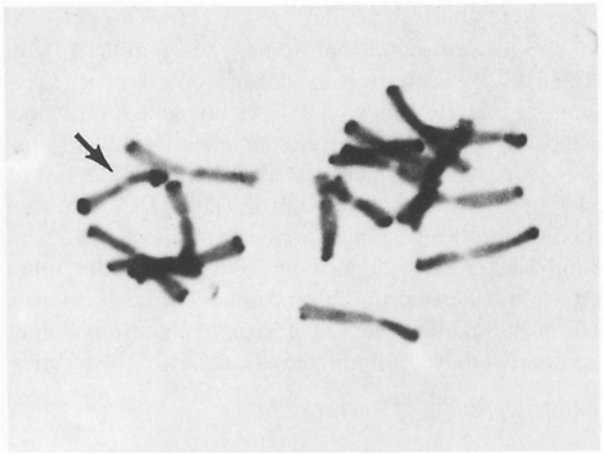

Fig. 6. Giemsa stained preparation of trisomic DG5 metaphase showing wide telomeric bands in the alien chromosome (*arrowed*)

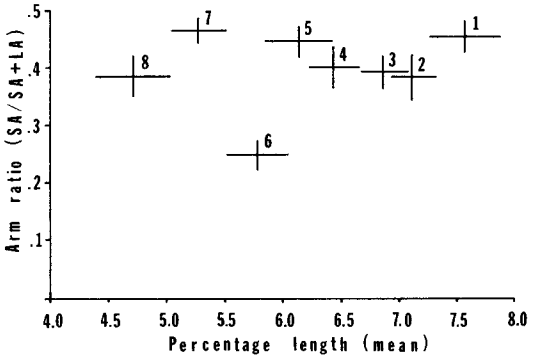

Fig. 7. 2-dimensional representation of *A. cepa* cv. 'Temprana' karyotype with standard deviations. Means of 7 cells

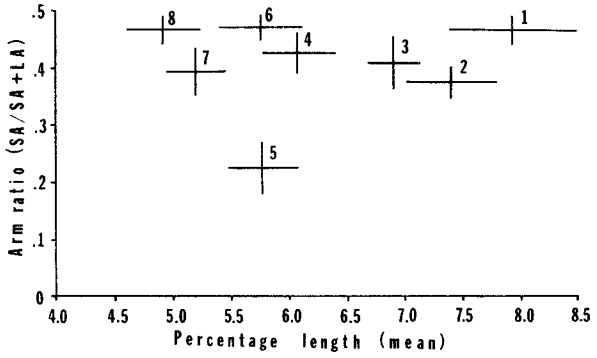

Fig. 8. 2-dimensional representation of *A. fistulosum* cv. 'Ishikura Long White' karyotype with standard deviations. Means of 9 cells

tables. A change has taken place in relative positions of the medium-sized metacentric chromosome and the sub-telocentric, which are clearly nos. 5 and 6 in *A. cepa* but which are virtually identical in length in *A. fistulosum* where the sub-telocentric is only very slightly shorter

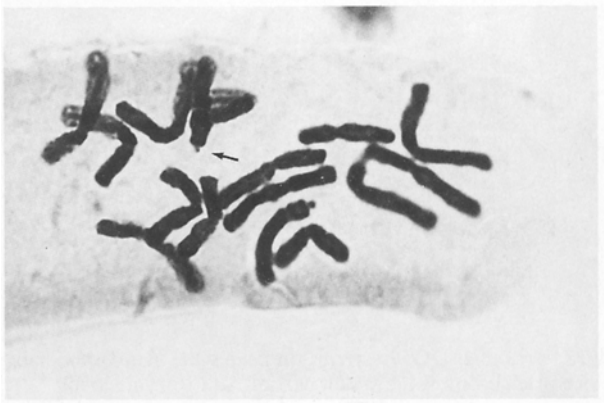

Fig. 9. Metaphase of *A. cepa* cv. 'Temprana' showing a possible nucleolus organizing region (NOR) on the short arm of one chromosome of the smallest pair (*arrowed*)

than no. 5. A similar and more striking change occurs in the relative positions of chromosomes 7 and 8, where 7 is metacentric and 8 submetacentric in *A. cepa*, 7 is submetacentric and 8 is metacentric in *A. fistulosum*. We assume that the homology between these chromosomes of the two species is based more on their arm ratios than on these slight length changes because in some cells the two pairs either overlap in length or their relative lengths may even be completely reversed.

Few karyotypes of short-day onions have been published. These onions have had a long history of separate development from long-day types. The cultivar 'Temprana', a selection from 'New Mexico White Grano' derived earlier from 'Texas Grano' and the older Bermuda types, lacks a well-developed satellite on either of the sub-telocentric chromosomes. Only a pair of small dots, resembling the clubbed antennae of an insect, are usually visible with acetocarmine staining. Schubert et al. (1983) noted the lack of satellites on another short-day cultivar, Australian Brown, when studying the mobility of the NORs of *Allium cepa*, *A. fistulosum*, and their hybrids. Schubert (1984) later found an active NOR in a top onion (a putative ancient *A. cepa* × *A. fistulosum* hybrid) in the telomeric position on a small, nearly metacentric chromosome. Some of our photographs of *A. cepa* cv. 'Temprana' showed a pronglike extension of one of the smaller chromosomes (Fig. 9, arrowed) which may indicate that an NOR has moved in a similar way in this cultivar of *A. cepa*. Schubert and Wobus (1985) noted in plants of top onion that NOR mobility was most pronounced where the original NOR chromosomes had lost their satellites.

Although we did not confirm the identities of the chromosomes by C-banding we found reasonably good agreement between our karyotypes and those of Kalkman (1984) and Cortes et al. (1983) for *A. cepa*. As we were mainly concerned with the five smallest chromosomes of the genome, the fact that we could not accurate-

Table 1. Chromosome morphology of *A. cepa* cv. 'Temprana' and *A. fistulosum* cv. 'Ishikura Long White'

Chromosome	*A. cepa* 'Temprana' (X)[a]		*A. fistulosum* 'ILW' (X)[b]	
	Relative Chromosome Length (%)	Arm Ratio (SA/SA + LA)	Relative Chromosome Length (%)	Arm Ratio (SA/SA + LA)
1	7.57 ± 0.08	0.455 ± 0.008	7.94 ± 0.13	0.463 ± 0.005
2	7.22 ± 0.05	0.386 ± 0.010	7.42 ± 0.09	0.373 ± 0.006
3	6.87 ± 0.05	0.395 ± 0.008	6.91 ± 0.05	0.409 ± 0.011
4	6.43 ± 0.06	0.405 ± 0.010	6.09 ± 0.07	0.428 ± 0.008
5	6.14 ± 0.08	0.449 ± 0.007	5.79 ± 0.07	0.228 ± 0.011
6	5.80 ± 0.07	0.250 ± 0.007	5.75 ± 0.08	0.470 ± 0.005
7	5.27 ± 0.06	0.463 ± 0.006	5.20 ± 0.06	0.393 ± 0.010
8	4.71 ± 0.08	0.388 ± 0.009	4.92 ± 0.07	0.463 ± 0.005

[a] means derived from 7 karyotypes
[b] means derived from 9 karyotypes

ly separate the larger and very similar chromosomes 2 and 3 (as Kalkman did on the basis of intercalary and telomeric bands) should not affect our conclusions as to the two trisomes' identity. It may be argued that chromosome 4 of *A. fistulosum* may not be homologous with chromosome 4 of *A. cepa*, i.e. any recombination which occurs in interspecific hybrids or hyperploids may not necessarily take place between chromosome 4 of *A. cepa* and chromosome 4 of *A. fistulosum*. Jones and Rees (1968) found that the DNA content and total chromosome volume of *A. cepa* were 27% greater than those of *A. fistulosum*, and at pachytene in interspecific hybrids some bivalents were markedly more asymmetrical than others. While some "homologous" chromosomes differed in length by only 10%, others differed by amounts up to 70%, with unpaired loops and overlaps of this size. Jones and Rees (1968) suggested that amplification of certain parts of the chromosomes may account for the differences. The pronounced length differences at pachytene may however also be evidence of changes in the length order among chromosomes 2, 3 and 4 between the two species. Further studies will be needed to elucidate these points and also to assign *Gdh-1*, *Idh-1* and *Pgi-1* to chromosomes, 1, 2, 3 or 6 of *A. fistulosum*.

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References

Berninger E, Buret P (1967) Étude dés deficients chlorophylliens chez deux espèces cultivées du genre *Allium*: l'oignon *A. cepa* L. et le poireau *A. porrum* L. Ann Amelior Plant 7:175–194

Clarke AE, Jones HA, Little TM (1944) Inheritance of bulb color in the onion. Genetics 29:569–575

Cortes F, Gonzalez-Gil G, Hazen MJ (1983) C-banding and sister chromatid exchanges in three species of the genus *Allium* (*A. cepa*, *A. ascalonicum* and *A. sativum*). Caryologia 36:203–210

Davis EW (1960) Marker genes to facilitate roguing onion-seed fields. Seed World 87:4, 6

El-Gadi A, Elkington TT (1975) Comparison of the Giemsa C-band karyotypes of *Allium cepa*, *A. fistulosum* and *A. galanthum*. Chromosoma 51:19–23

El-Shafie MW, Davis GN (1967) Inheritance of bulb color in the *Allium cepa* L. Hilgardia 38:607–622

Grant WF (1982) Chromosome aberration assays in *Allium*. A report of the U.S. Environmental Protection Agency Gene-Tox program. Mutat Res 99:373–291

Hernandez P, Mingo R, Gonzalez-Fernandez A, Lopez-Saez JF (1986) Relationship of chromosome damage induced by caffeine to growth temperature and ATP level in proliferating cells. Mutat Res 164:327–333

Jones HA, Mann LK (1963) Onions and their allies. Inter-Science, New York, p 169

Jones RN, Rees H (1968) Nuclear DNA variation in *Allium*. Heredity 23:591–605

Kalkman ER (1984) Analysis fo the C-banded karyotype of *Allium cepa* L. Standard system of nomenclature and polymorphism. Genetica 65:141–148

Orozco-Castillo C (1986) Percent solids and other characteristics of progeny resulting from interspecific trisomic onion backcrosses. MS thesis, Texas Technical University 74 pp

Peffley EB, Corgan JN, Horak HG, Tanksley SD (1985) Electrophoretic analysis of *Allium* alien addition lines. Theor Appl Genet 71:176–184

Perkins DY, Kehr AE, Brown RT, Tims EC, Miller JC (1958) Delta Giant, a new long season shallot. Circular No 52, Louisiana SU and A&M Coll Expt Stn

Schubert I (1984) Mobile nucleolus organizing regions (NORs) in *Allium* (*Liliaceae* s. lat.)? – Inferences from the specificity of silver staining. Plant Syst Evol 144:291–305

Schubert I, Wobus U (1985) In situ hybridization confirms jumping nucleolus organizing regions in *Allium*. Chromosoma 92:143–148

Schubert I, Ohle H, Hanelt P (1983) Phytogenetic conclusions from Giemsa banding and NOR staining in top onions (*Liliaceae*). Plant Syst Evol 143:245–256

Schvartzman JB, Cortes F (1977) Sister chromatid exchanges in *Allium cepa*. Chromosoma 62:119–131

Stack SM, Comings DE (1979) The chromosomes and DNA of *Allium cepa*. Chromosoma 70:161–181

Sundberg MD (1981) Making the most of onion root tip mitosis. Am Biol Teacher 43:386–388

Vosa CG (1976) Heterochromatin patterns in *Allium*. I. The relationship between the species of the *cepa* group and its allies. Heredity 36:383–392