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Introgression of *Allium fistulosum* L. into *Allium cepa* L.: cytogenetic evidence *

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Summary. A diploid *Allium cepa* plant was recovered from the backcross of an interspecific triploid (2 × *A. cepa* + 1 × *A. fistulosum*) to an *A. cepa* diploid which exhibited both *A. cepa* and *A. fistulosum Adh-1* alleles. Cytogenetic analyses revealed a recombinant sub-telocentric chromosome. The *ADH-1* locus is believed to be on the long arm of the sub-telocentric *A. fistulosum* chromosome 5. Meiosis of the triploid progenitor gives strong evidence that recombination occurred. *A. fistulosum* chromosome 8 has been substituted for *A. cepa* chromosome 1.

Key words: ADH-1 locus – *Allium* – Chromosome substitution – Cytogenetics

Introduction

Alliums are of considerable importance worldwide. Tremendous genetic diversity exists among the over 600 species (McCollum 1976). Several of the more important members of the *Cepa* section, those with hollow leaves, and cultivated as food sources are *A. cepa* L. (common bulb onion), *A. fistulosum* L. (Japanese bunching onion), and *A. schoenoprasum* L. (chives). *Allium cepa* is widely grown and is of economic importance in the United States. One of the aims in the genetic improvement of commercial *A. cepa* cultivars is to breed for resistance to a number of pests to which this crop is susceptible; resistance genes are found in other species. F_1 hybrids of *A. cepa* with other taxa have been obtained (Table 1), but most of them have been with *A. fistulosum;* no hybrids have been reported with *A. schoenoprasum*. *A. fistulosum* is a closely related species possessing traits which would be desirable if introgressed into *A. cepa*. Progeny obtained by sexual backcrossing of *A. cepa* × *A. fistulosum* hybrids have been limited because of the high degree of sterility in the F_1. Recently, Cryder et al. (1987) and Cryder (1988) have reported characteristics of (*A. fistulosum* × *A. cepa*) × *A. cepa* backcross populations.

Sterility of diploid F_1 hybrids poses a major barrier to gene introgression, but transferring traits from one species to another may be accomplished through the use of alien addition lines (Savitsky 1978). Several *Allium* single chromosome addition lines (2n = 17) and hyperploid

Table 1. Interspecific crosses involving *Allium cepa* or *A. fistulosum*

Cross	Reference	
A. cepa × *A. ascalonicum*	El-Gadi and Elkington	1975
A. cepa × *A. fistulosum*	Emsweller and Jones	1935a
	Levan	1936
	Maeda	1937
	Saini and Davis	1967
	El-Gadi and Elkington	1975
	Dolezel et al.	1980
A. cepa × *A. galanthum*	Saini and Davis	1967
	McCollum	1971
	El-Gadi and Elkington	1975
A. cepa × *A. oschaninii*	Saini and Davis	1967
	McCollum	1972
A. cepa × *A. pskemense*	Saini and Davis	1967
	McCollum	1971
A. cepa × *A. royeli*	Saini and Davis	1967
A. fistulosum × *A. ascalonicum*	Cochran	1950
A. fistulosum × *A. cepa*	Corgan and Peffley	1986
	Peters et al.	1984
A. fistulosum × *A. galanthum*	El-Gadi and Elkington	1975

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(2n=20–24) plants were recovered from the cross of an interspecific triploid (2n=3x=24; 2×*A. cepa*+1×*A. fistulosum*)× a diploid (2n=2x=16), *A. cepa* (Peffley et al. 1985). One hyperploid resulting from that cross was DG122, an interspecific triploid. This plant was backcrossed to diploid *A. cepa* cv 'Temprana' and a diploid individual designated DG122-13 was obtained. This BC_1 plant is the subject of this investigation because an earlier analysis (Peffley and Currah 1988) found that DG122-13 was diploid (2n=16) and possessed both *A. cepa* and *A. fistulosum Adh-1* alleles (*Adh-1*^{1}/*Adh-1*^{3}). The *A. fistulosum* allele (*Adh-1*^{3}) may have resulted from either the substitution of an entire *A. fistulosum* chromosome or the introgression of the segment containing the gene of *Adh-1*^{3}.

Materials and methods

Plant material. Materials from two plants, DG122 and DG122-13, were studied; the generation of these plants is described above.

Mitotic analyses. Roots were collected from greenhouse-grown DG122 and DG122-13 plant material. Chromosome counts were obtained from metaphase cells of root tips that had been pretreated with 1-bromonaphthalene for 24 h, fixed in 3:1 ethanol:glacial acetic acid, and stored in 70% ethanol. Roots were hydrolyzed in 5*N* HCL at room temperature for 10 min, stained with acetocarmine, squashed on the slide, and viewed under 40× magnification. Squashes for karyotyping were photographed under 63× magnification.

Meiotic analyses of greenhouse-grown DG122. Inflorescences were collected and fixed in 1-bromonaphthalene for 5 h, fixed in 6:3:1, ethanol:chloroform:glacial acetic acid, and stored in 70% ethanol until viewing. Meiocytes were analyzed at first prophase, metaphase, anaphase, and telophase in all plant material.

Pollen evaluation. Pollen from six anthers each of DG122 and DG122-13 was placed in a microfuge tube, three drops of acetocarmine were added, and pollen was dispersed by vibrating the tube with a vortex. The solution was micropipetted onto a slide for viewing. A minimum of 100 pollen grains were observed per sample. Three samples from each of six anthers were evaluated.

Phenotypic evaluation of DG122-13. DG122-13 was evaluated for *A. cepa* and *A. fistulosum* characteristics. These included: bulbing shape and maturity; leaf shape and color; scape emergence, height, shape, hollow center; spathe shape, color, and thickness; umbel shape and anthesis; perianth shape, color, texture, and senesence; isozyme alleles: *Adh-1, Idh-1, Pgi-1, Pgm-1.*

Results and discussion

Mitotic analyses

Chromosome counts and structures from root-tip squashes of DG122 and DG122-13 karyotypes are shown in Figs. 1 and 2, respectively. The 24 chromosomes of DG122 separate into eight (*A. cepa*) pairs and eight (*A. fistulosum*) shorter chromosomes. This corresponds with published karyotypes of *A. cepa* and *A. fistulosum* (Kalkman 1984; Vosa 1976; Peffley and Currah 1988) and the report by Jones and Rees (1968) that the total chromosome volume of *A. cepa* is 27% greater than those of *A. fistulosum*. Short arm/total chromosome length and percent chromosome length per cell data were used as the bases for numbering the chromosomes and to identify the most likely homologues in DG122-13 (Table 2). DG122-13 was studied previously (Peffley and Currah 1988) and was found to have a recombinant sub-telocentric chromosome. This sub-telocentric was similar in length and morphology to the *A. fistulosum* sub-telocentric, i.e., shorter in length than the *A. cepa* sub-telocentric chromosome, but possessing a small satellite like the *A. cepa* sub-telocentric and not the larger one found in *A. fistulosum*. This recombinant chromosome could have been recovered if the *A. fistulosum* and *A. cepa* sub-telocentric chromosomes paired and crossing-over occurred. Two recombinant types would be formed: a long sub-telocentric with a large knob and a short sub-telocentric with a small knob. Only the latter was recovered. A similar explanation was made independently by Dr. J. N. de Vries (personal communication). Meiosis in DG122 was investigated in order to confirm the possibility of such a cross-over event. DG122-13 also has an *A. fistulosum* chromosome, believed to be number 8 (Peffley and Currah 1988), substituted for *A. cepa* chromosome 1, the longest and most metacentric (Fig. 2). The homoeology relationship of these chromosomes is being investigated (Mangum and Peffley, in preparation).

Table 2. Chromosome morphology of DG122-13[a]

Chromosome	Arm ratio (SA/SA+LA)	Relative chromosome length (%)
1	0.488±0.01	8.48±0.75
2	0.478±0.01	4.13±0.17
3	0.404±0.02	7.51±0.38
4	0.400±0.01	7.11±0.19
5	0.393±0.02	6.54±0.34
6	0.389±0.02	6.35±0.98
7	0.401±0.04	7.03±0.57
8	0.387±0.03	7.02±0.42
9	0.480±0.01	7.03±0.19
10	0.472±0.01	6.47±0.18
11	0.273±0.04	6.31±0.47
12	0.239±0.04	4.64±0.26
13	0.447±0.05	5.67±0.47
14	0.441±0.04	5.57±0.11
15	0.469±0.02	5.09±0.27
16	0.432±0.04	5.03±0.28

[a] Means derived from five karyotypes

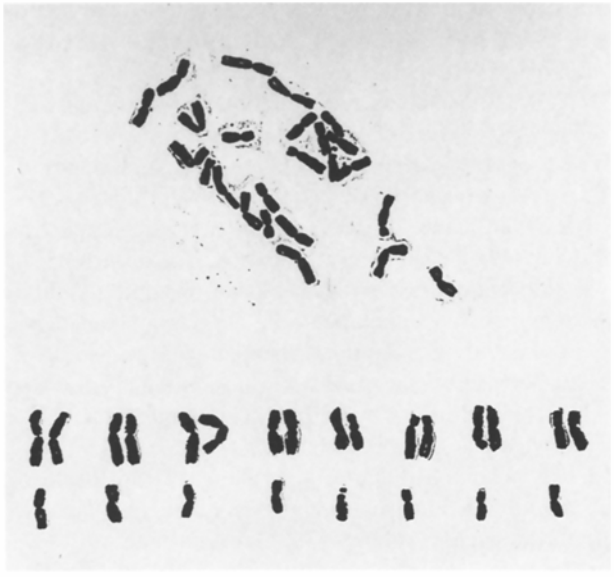

Fig. 1. Karyotype of DG122

Fig. 2. Karyotype of DG122-13. *Af 8* substituted for *Ac* 1 (*small arrow*); recombinant sub-telocentric similar in length to *Af*, large satellite similar to *A. cepa* (*large arrow*)

Meiotic studies

Diakinesis, metaphase I, anaphase I, and telophase I were studied in DG122. Data were recorded on meiocytes where all 24 chromosomes could be accounted for (Table 3). The most common meiotic configurations were eight bivalents (presumably *A. cepa*) and eight univalents (presumably *A. fistulosum*). Heteromorphic bivalents and long univalents were observed. The *A. cepa* sub-telocentric was associated with its *A. fistulosum* homoeologue, leaving as a univalent the unpaired *A. cepa* sub-telocentric (Fig. 3), thus providing clear support that recombination between the *A. cepa* and *A. fistulosum* genomes does occur. As few as five and as many as ten bivalents were observed. The additional bivalents are loosely associated and are of the same shape as those photographed by Emsweller and Jones (1935b, plate 2c); we conclude that these are paired *A. fistulosum* chromosomes (Fig. 3). One bivalent was visible into anaphase (Fig. 4).

A small segment was associated with a heteromorphic bivalent in diplotene, diakinesis, early anaphase, and late anaphase (Fig. 5). If the segment is counted as a chromosome (in Fig. 5D the segment appears to have a median centromere) the cell would contain 25 chromosomes; otherwise, it would be classed as a non-centric fragment and 24 chromosomes scored in the cell. The origin of this segment is unexplained, but possibilities are hyperploidy or a supernumerary, B, chromosome. Painter and Muller (1929) discussed hyperploidy produced from part of a chromosome: a simple translocation with appropriate crossing-over or a deletion of the chromosome's middle part, which would leave a small piece from one end attached to a small fragment which bears the spindle fiber. Surviving segments have been observed in *Drosophila* (Painter and Muller 1929) and vary genetically and cytologically. Supernumerary, B, chromosomes have been reported in *A. cepa* (Noda 1953) but are reminiscent of the behavior of this segment. B chromosomes have been

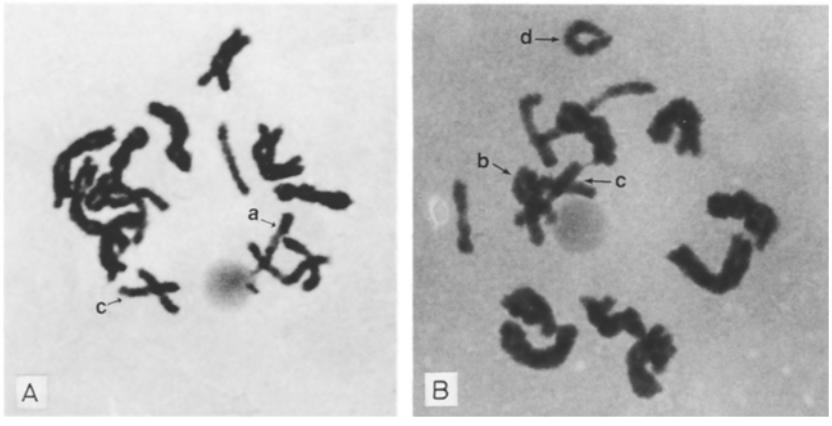

Fig. 3A and B. DG122 diakinesis with ten bivalents and four univalents. *A. cepa* sub-telocentric univalent (a); *A. cepa* sub-telocentric associated with *A. fistulosum* homoeologue (b); loosely associated *A. fistulosum* chromosomes (c); isochromosome ring univalent (d)

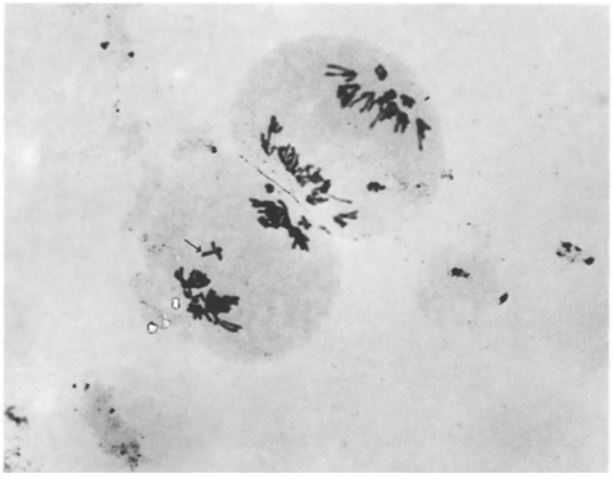

Fig. 4. DG122 late anaphase with loosely associated *A. fistulosum* chromosomes lagging

Table 3. Meiotic analysis of interspecific triploid DG122

Chromosomal configurations[a]	No. of cells observed	% of total cells
Diakinesis/Metaphase I		
10II + 4I	2	4.7
9II + 6I	2	4.7
8II + 8I	22	51.2
8II + 5I + 1III	3	7.0
7II + 7I + 1III	5	11.7
7II + 6I + 1IV	1	2.3
6II + 6I + 2III	3	7.0
6II + 1III + 1IV + 5I	1	2.3
6II + 4I + 2IV	1	2.3
5II + 6I + 2IV	2	4.7
5II + 1I + 1III + 2V	1	2.3
No. of cells observed	43	
Anaphase I		
Without bridge		
no laggard	75	48.7
with laggard	61	39.6
With bridge	11	7.1
Double bridge	3	2.0
Triple bridge	4	2.6
No. of cells observed	154	
Telophase I		
Without micronuclei	53	53.0
With micronuclei		
one	32	32.0
one with one bridge	4	4.0
two	9	9.0
two with one bridge	1	1.0
three	1	1.0
total		47.0
No. of cells observed	100	

[a] I univalent; II bivalent; III trivalent; IV quadrivalent; V pentavalent

reported paired with A genomes in many species (for review, see Macguire 1988). A miniscule univalent was observed in late metaphase (Fig. 5C).

At least one and as many as three multivalent associations were visible in 39.6% of the meiocytes (Table 3). Studies of *A. cepa* and *A. fistulosum* interspecific hybrids (Emsweller and Jones 1945; Levan 1941; Maeda 1937; Peffley 1986) have reported multivalents with supporting evidence for at least one translocation. The occurrence of two independent pentavalent associations and two independent quadrivalent associations (Fig. 6) gives evidence for at least three reciprocal independent translocation events between *A. cepa* and *A. fistulosum*. The prevalence of structural chromosome differences among related species is widely acknowledged. Speciation events are sometimes associated with karyotypic changes. Translocations are usually strong isolating barriers and may lead to speciation events (White 1978).

Other meiotic abnormalities were observed. A ring univalent, most likely an isochromosome, was observed in several squashes (Figs. 3B and 5B). Lagging chromosomes and bridges were not uncommon in anaphase, with some bridges persisting into telophase.

Pollen evaluation

Of the 534 DG122 pollen grains counted, 61% were viable. DG122-13 had a much reduced viability; only 0.94% of 2,329 grains were viable. The high sterility of

Table 4. Phenotypic classes of DG122-13

Traits	Class	Exhibiting characteristics of		
		A. cepa	*A. fistulosum*	Interspecific
Bulbing	shape	×		
	maturity	×		
Leaf	shape (flat)	×		
	color (green)	×		
Scape	emergence		×	
	height (tall)	×		
	shape (bulbous)	×		
	center	×		
Spathe	shape	×		
	color	×		
	thickness	×		
Umbel	shape	×		
	anthesis (random)	×		
Perianth	shape			×
	color (creamy)			×
	texture (thin)		×	
	senescence (rapid)		×	
Isozymes	*Adh-1*			×
	Idh-1	×		
	Pgi-1	×		
	Pgm-1	×		

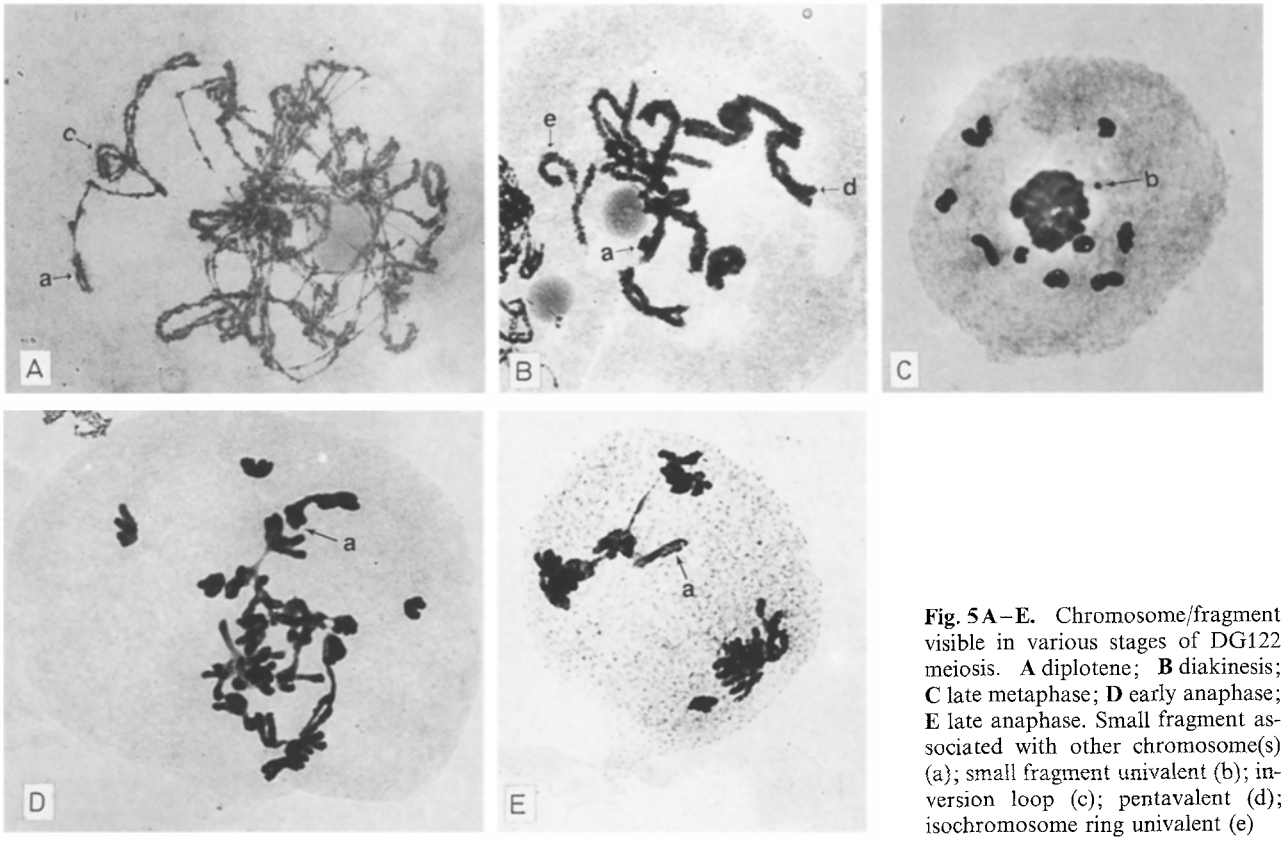

Fig. 5A–E. Chromosome/fragment visible in various stages of DG122 meiosis. **A** diplotene; **B** diakinesis; **C** late metaphase; **D** early anaphase; **E** late anaphase. Small fragment associated with other chromosome(s) (a); small fragment univalent (b); inversion loop (c); pentavalent (d); isochromosome ring univalent (e)

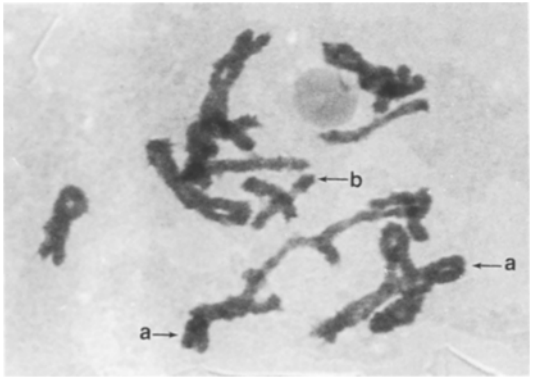

Fig. 6. DG122 diakinesis with six bivalents, four univalents, and two quadrivalents (a); loosely associated *A. fistulosum* chromosomes (b)

DG122-13 pollen may be attributed to a cumulative effect of any of the following events: multiple translocation heterozygosity, loss or substitution of a chromosome, deletion of genetic material.

Phenotypic analyses

DG122-13 phenotype is like *A. cepa* in bulbing and leaf characteristics but has *A. fistulosum* characteristics. Refer to Table 4 for similarities and differences.

Meiosis of DG122 provides evidence for recombination between *A. fistulosum* and *A. cepa* genomes. Investigations of DG122-13 disclosed the recovery of an individual with a recombinant sub-telocentric chromosome, and *A. fistulosum Adh-1* allele, and the phenotype of *A. cepa*. Evidence has been given for introgression of *A. fistulosum* into *A. cepa*, detectable at molecular (isozymic), cytological, and phenotypic levels.

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