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RAPDs AND NONCODING CHLOROPLAST DNA REVEAL A SINGLE ORIGIN OF THE CULTIVATED *ALLIUM FISTULOSUM* FROM *A. ALTAICUM* (ALLIACEAE)[1]

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The origin of the crop species *Allium fistulosum* (bunching onion) and its relation to its wild relative *A. altaicum* were surveyed with a restriction fragment length polymorphism (RFLP) analysis of five noncoding cpDNA regions and with a random amplified polymorhic DNA (RAPD) analysis of nuclear DNA. Sixteen accessions of *A. altaicum*, 14 accessions of *A. fistulosum*, representing the morphological variability of the species, and five additional outgroup species from *Allium* section *Cepa* were included in this study. The RFLP analysis detected 14 phylogenetically informative character transformations, whereas RAPD revealed 126 polymorphic fragments. Generalized parsimony, neighbor-joining analysis of genetic distances, and a principal co-ordinate analysis were able to distinguish the two species, but only RAPD data allowed clarification of the interrelationship of the two taxa. The main results of this investigation were: (1) *A. fistulosum* is of monophyletic origin, and (2) *A. fistulosum* originated from an *A. altaicum* progenitor, making *A. altaicum* a paraphyletic species. Compared with *A. altaicum* the cultivated accessions of the bunching onion show less genetic variability, a phenomenon that often occurs in crop species due to the severe genetic bottleneck of domestication. *Allium altaicum* and *A. fistulosum* easily hybridize when grown together, and most garden-grown material is of recent hybrid origin.

Key words: Alliaceae; *Allium altaicum; A. fistulosum;* domestication; noncoding cpDNA; phylogenetic analysis; random amplified polymorphic DNA; restriction fragment length polymorphism analysis.

Allium fistulosum L. (Welsh onion or Japanese bunching onion) is widely cultivated from Siberia to Tropical Asia and shows the largest morphological variability in China, Korea, and Japan (Inden and Asahira, 1990; Haishima, Kato, and Ikehashi, 1993). Two types can be distinguished on the basis of their use as vegetables and are sometimes taxonomically treated as two subspecies: subsp. *fistulosum* with four varieties, grown for its pseudostems, and subsp. *caespitosum* (Makino) Kaz. with three varieties, grown for pseudostems and leaf blades (Kasakova, 1978).

Allium altaicum Pall., the wild bunching onion (called *butun* in Siberia), was discovered in 1735 near Lake Baikal by Steller (1740). About 40 yr later, the species was found again in the Altai mountains and described under its valid name by Pallas (1773). The frost-resistant bulbs of *A. altaicum* are gathered in Siberia and Mongolia and are locally used as condiment and vegetable comparable to the bulb onion (*A. cepa* L.). Due to the great morphological similarity between the two species, *Allium altaicum* is proposed to be the nearest wild relative of the bunching onion and is still common in Southern Siberia and Mongolia (Hanelt, 1985; Friesen, 1987, 1988, 1995).

In spite of their overall similarity, the two taxa are easily distinguished by some morphological characters. *Allium altaicum* possesses a well-developed bulb with a red-brown tunic, whereas *A. fistulosum* forms a poorly developed bulb, normally with a white tunic, with a diameter hardly exceeding that of the pseudostem. The other morphological characters often adduced to differentiate the two species such as length and diameter of pedicels or density of the umbel are not unequivocal differences. Karyotypes of *A. altaicum* and *A. fistulosum* are very similar (Derjagin and Jordansky, 1971; Inada and Endo, 1994). Interspecific hybridization between these two *Allium* species occurs easily and hybrids show a high pollen and seed fertility (Inada and Iwasa, 1983; Nishitani, 1984).

The origin of *A. fistulosum* is uncertain and several different hypotheses have been proposed. De Candole (1883) followed Ledebour (1830) and Regel (1875) in the opinion that the wild species, *A. altaicum*, is the direct progenitor of *A. fistulosum*, which originated in Siberia. Prochanov (1930) and Li (1970) suspected that both taxa originated in northern China from an unknown (extinct) ancestor, so that *A. altaicum* is the sister group to *A. fistulosum* instead of its progenitor. Havey (1992), in a study of maternal phylogenies within *Allium* section *Cepa*, found *A. altaicum* to be paraphyletic after the exclusion of *A. fistulosum*, which together form the sister group to *A. cepa* and *A. vavilovii* M. Pop. et Vved. Conversely, a restriction fragment length polymorphism (RFLP) analysis of the nuclear genome with cDNA probes (Bradeen and Havey, 1995) revealed *A. fistulosum* to be paraphyletic in relation to *A. altaicum* with a weak sister-group relationship of both to *A. galanthum* Kar. et Kir. Van Raamsdonk, Smiech, and Sandbrink (1997) found *A. altaicum* and *A. fistulosum* to be closest relatives in an analysis of mostly morphological characters and proposed the paraphyly of *A. altaicum* with respect to *A. fistulosum* on the basis of random amplified

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polymorphic DNA (RAPD) data. Sister groups to the two taxa were *A. galanthum*/*A. pskemense* B. Fedt. in their morphological analysis and *A. roylei* Stearn in the analysis of the RAPD data. A completely different hypothesis was published recently by Dubouzet, Shinoda, and Murata (1997) who used dot blot hybridization with RAPD probes and found *A. galanthum* to be the sister taxon of *A. fistulosum*. *Allium altaicum* in this analysis is the closest relative of both taxa. Due to small within-species samples in most of these studies no safe conclusions about a monophyletic or polyphyletic origin of *A. fistulosum* could be drawn.

In the framework of a phylogenetic analysis of the entire genus *Allium* we analyzed *A. altaicum* and *A. fistulosum* in more detail to obtain insight into the mode of evolution of the crop species. To do this we used two different molecular marker systems and several accessions of the two taxa representing the known morphological and geographical variation. For the investigation of interspecific relationships we conducted a RFLP analysis of polymerase chain reaction (PCR) amplified DNA fragments (Arnold, Buckner, and Robinson, 1991; Liston, 1992) from rapidly evolving regions of the chloroplast genome. This method allows a rapid screening of large numbers of taxa for phylogenetically informative characters (Mes et al., 1997). It makes use of universal PCR primer sets designed by Taberlet et al. (1991) and Demesure, Sodzi, and Petit (1995) for the amplification of noncoding parts of the chloroplast genome. The primers anneal to highly conserved gene sequences flanking variable spacers and introns. Sequences of noncoding parts of the chloroplast genome are widely used in systematic studies at lower taxonomic ranks due to high levels of sequence variation (e.g., Morton and Clegg, 1993; Hodges and Arnold, 1994; van Ham et al., 1994; Downie, Katz-Downie, and Cho, 1996; Kelchner and Clark, 1997).

For studies in closely related groups the amount of variation found even in noncoding cpDNA might be too small for analyses of large numbers of accessions or for taxa that originated only recently. To circumvent this problem we used RAPD data (Welsh and McClelland, 1990; Williams et al., 1990). RAPD analyses reveal even small genetic differences, since a large part of the nuclear genome will be scanned, as can be seen by mapping studies of segregating markers in a wide variety of plant families (e.g., Rieseberg et al., 1993; Bachmann and Hombergen, 1996; Byrne et al., 1997; Emebiri et al., 1997; Lawson, Lunde, and Mutschler, 1997; Powell et al., 1997; Serquen, Bacher, and Staub, 1997). Another advantage of this method is that it is less expensive and can be performed more rapidly than most other methods (Morell et al., 1995). However, RAPD techniques have some limitations, such as low reproducibility of some bands and the uncertain homology of comigrating fragments in gel electrophoresis (Van de Zande and Bijlsma, 1994; Harris, 1995; Pillay and Kenny, 1995; Rieseberg, 1996). Most of the limitations of RAPD analysis can be overcome by carefully adjusting the reaction and detection conditions (Bachmann, 1997; Colosi and Schaal, 1997; Friesen and Klaas, 1998) and generating large data sets where the phylogenetic signal outweighs the noise produced by nonhomologous fragments.

TABLE 1. The origin of the investigated accessions (All and K–accession numbers of Genebank collection, Tax–accession numbers of the Department of Taxonomy of the Institute for Plant Genetic and Crop Plant Research, Gatersleben) of *A. fistulosum*, *A. altaicum*, and five outgroup species.

A. altaicum Pall.	Tax1422 (Russia, Tuva), Tax1425 (Russia, Altai, Ust-Koksa), Tax1667 (Mongolia, Ulan-Bator), Tax1678 (Mongolia, Charchira-ul), Tax1691 (Mongolia, NW Delun), Tax2477 (Mongolia, Bajan-Chongor), Tax2746 (Russia, Baikal), Tax2760 (Russia, Tuva, Ersin), Tax2766 (Russia, Tuva, Zagan-Shibety), Tax2776 (Russia, Tuva, Mongun-Taiga), Tax3400 (Russia, Altai), Tax5123 (Mongolia, Selenga), Tax5125 (Mongolia, Selenga), Tax5149 (Russia, Altai), Tax5561 (Russia, Altai), Tax5559 (Russia, Altai)
A. altaicum × *fistulosum*	Tax1503 (Tadjikistan, BG Chorog)
A. fistulosum L.	K0433 (China), All515 (Germany), All740 (Kazakhstan), All742 (Mongolia), All748 (Georgia, Caucasus), K2055 (Korea), K6173 (Japan 'Iwaki'), K6174 (Japan 'Matsumoto Ippanfuto'), K6175 (Japan 'Nogina Negi'), K7803 (Korea), K8481 (Russia), K8778 (Japan 'Yamagawa Nebuka'), K9174 (Japan)
A. galanthum Kar. et Kir.	Tax5009 (Kyrgyzstan)
A. oschaninii O. Fedtsch.	Tax5016 (Kyrgyzstan)
A. praemixtum Vved.	Tax5712 (Uzbekistan, Mogoltau)
A. pskemense B. Fedt.	Tax3333 (Uzbekistan, Pskem Mountain Range)
A. vavilovii M. Pop. et Vved.	Tax5239 (Turkmenistan, Kopetdag)

MATERIALS AND METHODS

Plant materials—A total of 35 accessions of seven species from *Allium* section *Cepa* from the living collection of the Department of Taxonomy and the Genebank of the IPK Gatersleben were investigated: *A. altaicum* (16 accessions), *A. fistulosum* (14 accessions), and one accession each of *A. pskemense*, *A. praemixtum* Vved., *A. vavilovii*, *A. galanthum*, and *A. oschaninii* O. Fedtsch. as closely related outgroup species (Table 1). The material was carefully selected because *A. altaicum* and *A. fistulosum* easily hybridize when grown together. Most of the *A. altaicum* accessions were directly collected as bulbs from natural populations, representing the geographical range of this species (Fig. 1). The *A. fistulosum* accessions used were chosen to reflect the morphological and ecological diversity in this cultivated species as well as differences in the use as vegetable. About ten additional ingroup accessions we used at the beginning of this investigation turned out to be of hybrid origin when morphological characters were carefully examined. All doubtful plants were received from botanical gardens via seed exchange. Accordingly the entire garden material (20 accessions) was excluded from the analysis to avoid confusion of the results by incorrectly determined plants or hybrids. In order to illustrate the behavior of hybrid material, one garden accession, Tax1503 from the Botanical Garden Chorog, was left in the analysis.

Isolation of DNA—DNA was isolated with NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) according to the instructions of the manufacturer. The concentration of the extracted DNA was checked on an agarose gel.

RFLP analysis of cpDNA—For a RFLP analysis of *A. altaicum*, *A. fistulosum*, and five outgroup species the five following noncoding regions of the chloroplast DNA were PCR amplified (Demesure, Sodzi,

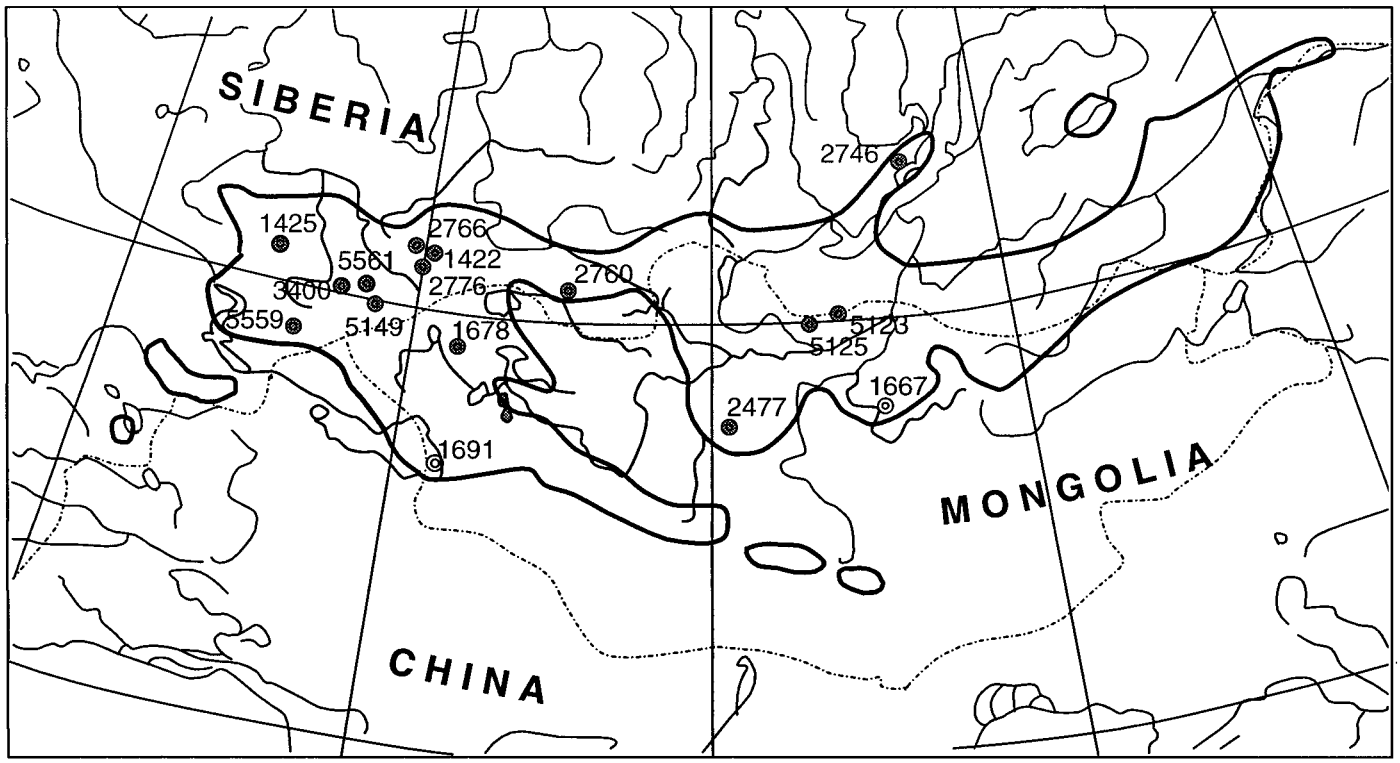

Fig. 1. Distribution map of *A. altaicum*. Numbers indicate the origin of the investigated accessions.

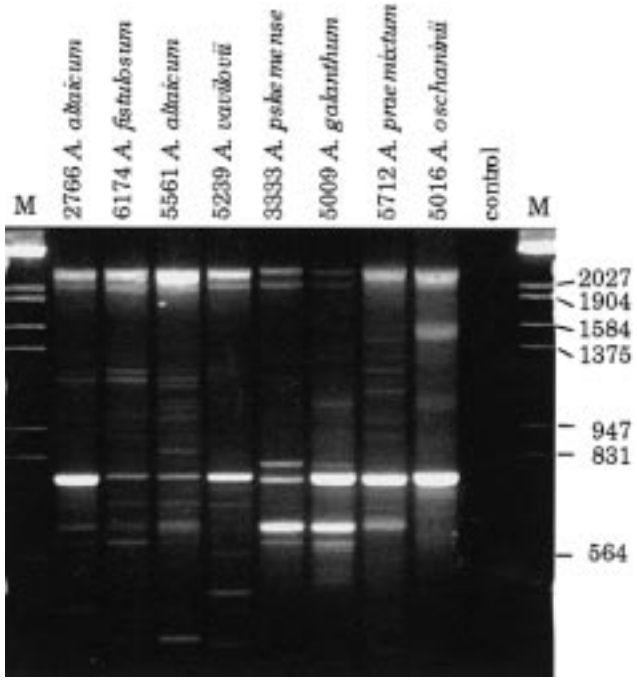

Fig. 2. Ethidium-bromide-stained agarose gel of a RAPD reaction with primer AC19 of *A. altaicum*, *A. fistulosum*, and five outgroup taxa from *Allium* sect. *Cepa*. Only prominent bands were scored for the phenetic analysis.

and Petit, 1995; Tsumura et al., 1995) in 50-μL reaction volumes and digested with restriction enzymes: *psa*A-*trn*S and *trn*C-*trn*D were digested with *Alu*I, *Ava*I, *Ban*II, *Bgl*II, *Dde*I, *Dra*I, *Hind*III, *Hinf*I, *Msp*I, *Nsi*I, and *Tru*I; *trn*K and *trn*T-*trn*D with *Alu*I, *Bgl*II, *Cla*I, *Dde*I, *Dra*I, *Eco*RI, *Hae*III, *Msp*I, and *Ssp*I; *psb*C-*trn*S with *Hae*III and *Hind*III. Phylogenetically informative restriction site mutations and length variations were scored and compiled into a binary data matrix. When restriction site homology was unclear, the restriction sites were mapped by double digests. Generalized parsimony analysis was performed with PAUP 3.1.1 (Swofford, 1993) using the heuristic search option, MULPARS, ACCTRAN, TBR branch-swapping, 200 random addition sequences, and a stepmatrix with a gain/loss ratio of 1.3:1 for restriction sites (Albert, Mishler, and Chase, 1992). Indels and inversions were not weighted. A bootstrap analysis was performed with 500 resamples. *Allium oschaninii*, most distantly related to the ingroup (Havey, 1992; Bradeen and Havey, 1995; Pollner, unpublished data), was specified as the outgroup taxon.

RAPD analysis—Two RAPD analyses were conducted: one including the five outgroup species together with two accessions of *A. altaicum* and one of *A. fistulosum* and a second including all accessions of *A. altaicum* and *A. fistulosum* together with the outgroup *A. oschaninii*. Amplification was carried out in 25-μL reaction volume, using ten arbitrary ten-basepair (bp) primers (A04, AA17, AB04, AC19, C05, G02, G13, G19, D01, and D03) obtained from Operon Technologies, Alameda, California. The amplification conditions were optimized according to Friesen and Klaas (1998). One-third of the reaction mixtures was separated on 1.5% agarose gels in 0.5x TBE, followed by staining with ethidium bromide (Sambrook, Fritsch, and Maniatis, 1989). Clearly visible RAPD bands (Fig. 2) were scored manually for presence (1) or absence (0) from enlarged photographs of the gels. Differing band intensities were not taken into account to avoid errors introduced by competition among priming sites during the initial rounds of PCR (Bachmann, 1997). Only bands reproducible in two independent amplification

TABLE 2. Phylogenetic informative character transformations found by a RFLP analysis in five noncoding regions of the chloroplast genome.

1	*psa*A-*trn*S		All515, All740, All742, All748, K0433, K2055, K6173, K6174, K6175, K7803, K8481, K8778, K9174	20-bp insertion
2	*psa*A-*trn*S	*Bgl*II	Tax3333, Tax5016, Tax5712	site loss
3	*psa*A-trnS	*Dde*I	Tax3333, Tax5016, Tax5712	site gain
4	*psa*A-*trn*S	*Dra*I	Tax3333, Tax5016, Tax5712	site gain
5	*psb*C-*trn*S		Tax1994, Tax5009, Tax5016, Tax5239, Tax5712	25-bp deletion
6	*trn*C-*trn*D		Tax1425, Tax1503, Tax1691, Tax3400, Tax5561	100-bp deletion
7	*trn*C-*trn*D	*Alu*I	Tax3333, Tax5016	site gain
8	*trn*C-*trn*D	*Dde*I	Tax3333, Tax5016, Tax5712	site loss
9	*trn*C-*trn*D	*Dra*I	Tax3333, Tax5016, Tax5712	site gain
10	*trn*C-*trn*D	*Hind*III	Tax3333, Tax5016, Tax5712	site gain
11	*trn*C-*trn*D	*Hinf*I	Tax3333, Tax5016, Tax5712	site gain
12	*trn*K-*trn*K	*Dde*I	Tax3333, Tax5016, Tax5712	site gain
13	*trn*K-*trn*K	*Eco*RI	Tax3333, Tax5712	site gain
14	*trn*T-*trn*D	*Eco*RI	Tax5009, Tax5016, Tax5239	inversion >20 bp

reactions were included in the data analyses. From the resulting 1/0 data matrices pairwise genetic distances were calculated using the Jaccard coefficient. Finally, phenograms were prepared with the neighbor-joining cluster analysis (Saitou and Nei, 1987) of the genetic distances. The neighbor-joining method was used because it is less sensitive for unequal mutation rates on different branches than UPGMA (Swofford et al., 1996). The genetic distance matrix of the *A. altaicum*/*A. fistulosum* group was also subjected to a principal co-ordinate analysis (PCA). From the distances, new independent axial co-ordinates, which represent most of the variability of the original data, were calculated. The taxa were then plotted as points in a three-dimensional continuous space defined by the first three co-ordinates. These calculations were done in the NTSYS-PC program, version 1.8 (F. J. Rohlf, 1993; distributed by Exeter Software, New York).

DNA sequencing—To characterize a 20-bp cpDNA length mutation differentiating *A. altaicum* and *A. fistulosum* in the RFLP analysis of the *psa*A–*trn*S (GGA) intergenic spacer, DNA of five accessions (including *A. fistulosum* All515 with an additional 20-bp length mutation) was examined by sequencing. RFLP mapping of the indel revealed a position ~350 bp downstream from primer trnS. To get insight into the sequence composition of the spacer flanking the indel region without sequencing the entire length of the spacer by primer walking we used an adapter-mediated sequencing strategy. DNA of a 50-μL PCR reaction was incubated with 12 U *Eco*RI in 1x KGB buffer (McClelland et al., 1988) at 37°C. After 1 h 3 units T4 DNA Ligase, 1 μL 10 mmol/L ATP, 10 pmol EcoRI-adapter, fitting the *Eco*RI ends without restoring the restriction site (Vos et al., 1995), were added, and the tube was filled to a final reaction volume of 100 μL with water, resulting in a 0.5x KGB buffer concentration, which is sufficient for both enzymes. After 3 h at 37°C the enzymes were inactivated by heating the reaction mixture to 90°C for 10 min. The DNA fragments were electrophoretically separated in a 1.5% agarose gel, and the larger fragment (2.2 kb), containing the indel, was eluted in 100 μL TE with a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The fragment was reamplified with the primers Eco+0 (corresponding to the EcoRI-adapter; Vos et al., 1995) and trnS and again gel purified.

Fifty nanograms dsDNA and primers Eco+0 and trnS were used in cycle sequencing reactions with the Rhodamine Dye Terminator Kit (Applied Biosystems Inc., Foster City, California), according to the recommendations of the manufacturer. Sequence detection was performed on an ABI 377 DNA sequencer. Two additional *Allium* specific sequencing primers (Eco-381: 5′-CTTTATCCATAGAATATAGTAKGTAGGC-3′ and trnS-580: 5′-TACGAATAATTCCGACAACTTCAGG-3′) were developed and used as internal primers to sequence the entire 2.2-kb fragment in four accessions (two *A. altaicum* and two *A. fistulosum*). The overlapping sequences of the analyzed taxa were combined in consensus sequences and manually aligned.

RESULTS

PCR-RFLP of cpDNA—The analysis of noncoding cpDNA revealed 14 phylogenetically informative characters (Table 2). All accessions of *A. fistulosum* are characterized by a putative 20-bp insertion in the *psa*A-*trn*S region, suggesting a monophyletic origin of *A. fistulosum*. The restriction site pattern of the hybrid Tax1503 in the *trn*C-*trn*D region is identical with that of four *A. altaicum*, sharing a 100-bp deletion containing a *Bgl*II restriction site in the other accessions. The ingroup accessions are characterized by an inversion in the *trn*T-*trn*D spacer, shared with the *A. praemixtum*/*A. pskemense* clade, and a 25-bp insertion in region *psb*C-*trn*S, unique to *A. altaicum* and *A. fistulosum*. The analysis using generalized parsimony resulted in two most parsimonious trees (16 steps length, consistency index = 0.875, retention index = 0.949). The consensus tree of a bootstrap analysis is shown in Fig. 3. In this tree the clade consisting of *A. vavilovii*/*A. galanthum* together with *A. altaicum*/*A. fistulosum* (100%) as well as the monophyly of the *A. altaicum*/*A. fistulosum* clade (79%) is supported by the bootstrap analysis. The branch uniting all *A. fistulosum* accessions forms a polytomy with the accessions of *A. altaicum* so that one cannot decide whether the two taxa are sister groups or whether *A. altaicum* is the paraphyletic progenitor of *A. fistulosum*.

RAPD—With ten arbitrary primers a total of 128 polymorphic bands were scored for the analysis of *A. altaicum*/*A. fistulosum* together with five outgroup species. Neighbor-joining analysis of pairwise Jaccard distances resulted in the unrooted phenogram shown in Fig. 4, which again reveals the close relationship of the two ingroup taxa. For the detailed analysis of the *A. altaicum* and *A. fistulosum* accessions 126 polymorphic bands were scored and Jaccard distances were calculated. The pairwise similarity between accessions of *A. fistulosum* ranged from 70 to 92% and between accessions of *A. altaicum* ranged from 35 to 76%. Thus, not surprisingly, the wild species, *A. altaicum*, shows more genetic vari-

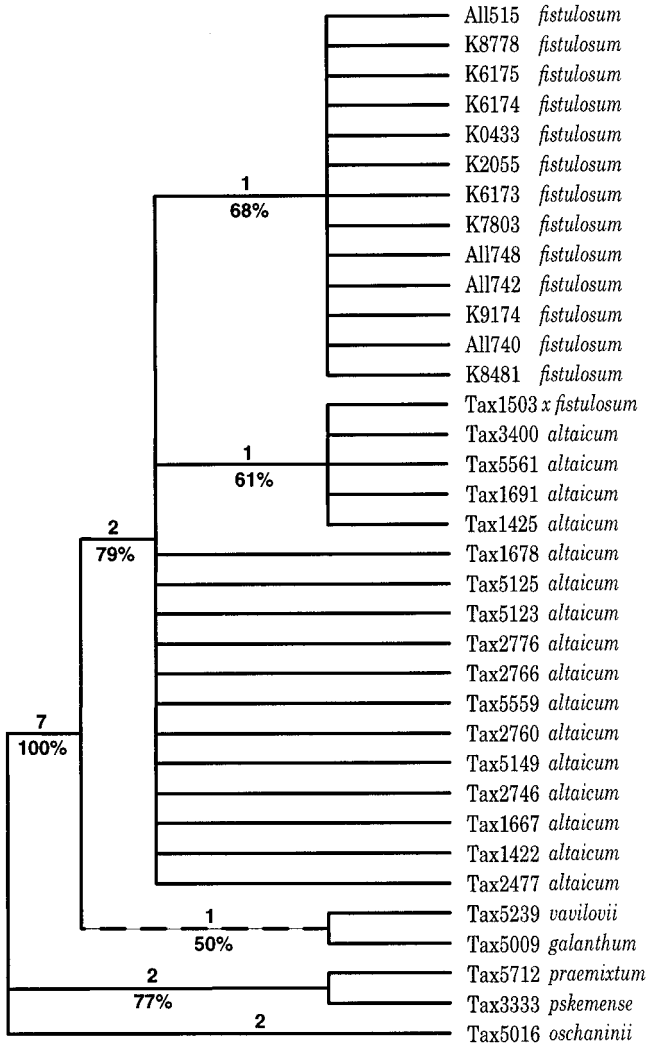

Fig. 3. Consensus tree of a bootstrap analysis (500 resamples) with generalized parsimony of 14 cpDNA RFLP characters. Digits above the branches indicate the number of character transformations supporting the clade. Below the branches bootstrap values (%) are given. The dashed branch collapses in the consensus tree of the two shortest trees found by the generalized parsimony analysis.

ability than the cultivated *A. fistulosum*. Among *A. altaicum* we found three accessions (Tax 5559, Tax 5561, and Tax 5149) with high pairwise diversities in relation to the remaining accession of *A. altaicum*. In the dendrogram (Fig. 5) these taxa are the sister group to all other accessions of *A. altaicum* and *A. fistulosum* and occur in a very distinct position in the three-dimensional representation of the principal co-ordinate analysis (Fig. 6). All *A. fistulosum* accessions form a monophyletic unit, which is sister group to the *A. altaicum* accessions Tax1667 and Tax1691, making *A. altaicum* paraphyletic. The putative hybrid Tax1503, morphologically resembling *A. fistulosum* with an *A. altaicum* chloroplast type, here occurs together with *A. fistulosum*. Analyses with *A. oschaninii* replaced by one of the other outgroup species did not alter the general topology within the ingroup and are therefore not shown.

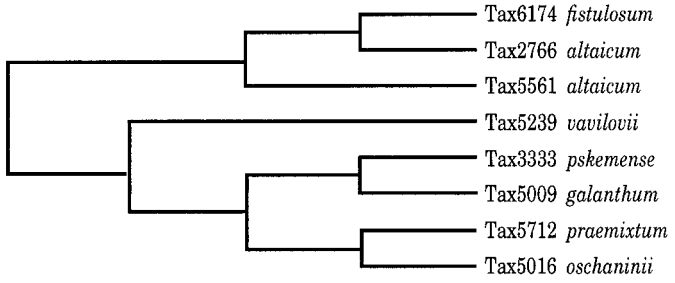

Fig. 4. Phenogram of a neighbor-joining analysis of seven species of *Allium* sect. *Cepa* based on 128 polymorphic RAPD bands. As in Fig. 3, *A. altaicum* and *A. fistulosum* are clearly separated from the remaining species in the analysis.

DNA sequencing—The alignment of the partial sequences (EMBL database numbers AJ224542 to AJ224546) of the *psa*A-*trn*S intergenic spacer resulted in a matrix of 2170-bp length of which the relevant part is shown in Fig. 7. In addition to the two length mutations detected in the RFLP analysis a poly-A string near the Eco+0 priming site consists of nine and six A in the two *A. altaicum* accessions instead of ten in *A. fistulosum*, necessitating the introduction of one or four gaps. The other parts of the sequences align without gaps. Only 14 sequence positions (0.6 %) were variable of which ten

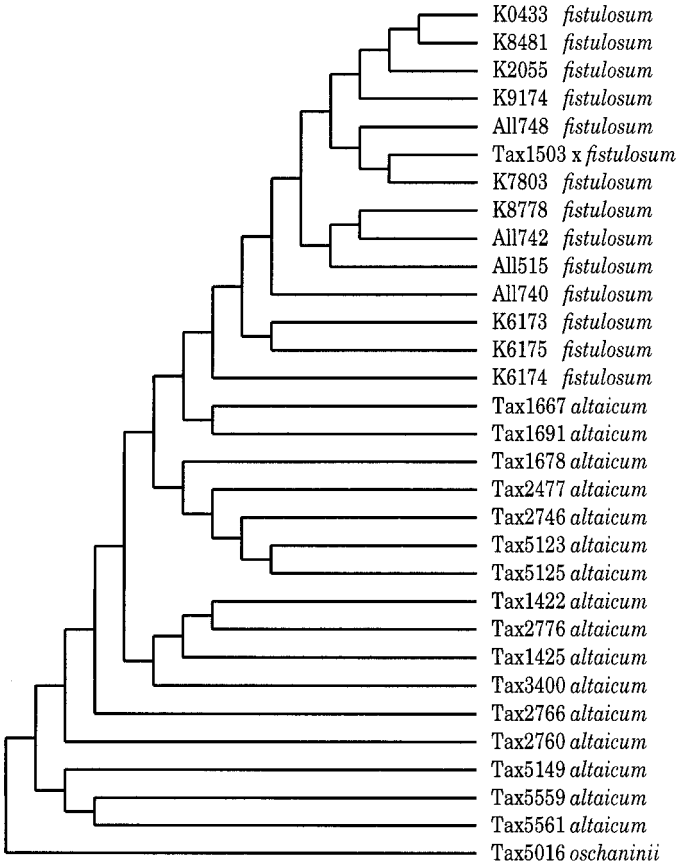

Fig. 5. Neighbor-joining dendrogram of 31 accessions of three species of *Allium* sect. *Cepa* (*A. altaicum*—16 acc., *A. fistulosum*—14 acc., and *A. oschaninii*—1 acc.) based on 126 RAPD bands.

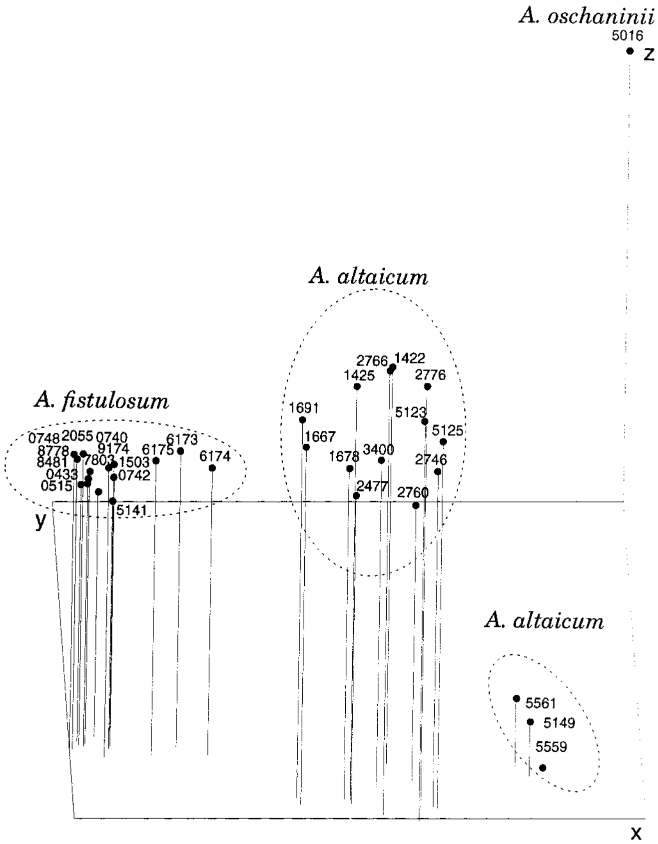

Fig. 6. Plot of the first three principal co-ordinates (accounting for 53% of the observed variation), calculated from the same RAPD distance matrix used for the neighbor-joining analysis in Fig. 5.

were autapomorphic character changes in single accessions. The AT content of the sequenced part (66%) is similar to other noncoding regions of the cpDNA (Shimada and Sugiura, 1991; Downie, Katz-Downie, and Cho, 1996; Kelchner and Clark, 1997). The DNA sequences showed that the length mutation differentiating *A. fistulosum* from the other taxa in the analysis consists of a 19-bp repeat, resulting from a duplication in *A. fistulosum*. The second mutation, autapomorphic for *A. fistulosum* accession All515, is a 21-bp DNA duplication.

DISCUSSION

The main result of this investigation is that *A. fistulosum* originated monophyletically from an *A. altaicum* progenitor as proposed by De Candole (1883), thus contradicting results of Bradeen and Havey (1995) with respect to monophyly and Dubouzet, Shinoda, and Murata (1997) concerning the progenitor of the crop species. Bradeen and Havey (1995) included up to eight accessions of *A. altaicum*/*A. fistulosum* in their analyses, resulting in phenograms that might indicate multiple origins of *A. fistulosum* from *A. altaicum* or vice versa. These results did not allow the authors to draw safe conclusions about the relationship of the two species. Studies of Havey (1992) and van Raamsdonk, Smiech, and Sandbrink (1997) found a close relationship between the two taxa, but due to small sample sizes no decisions about a monophyletic or polyphyletic origin could be made. The sister-group relationship of *A. galanthum* with *A. fistulosum* found by Dubouzet, Shinoda, and Murata (1997) could not be confirmed in our study and was never mentioned in the other analyses cited. Our chloroplast DNA data agree with data from Havey (1992) in showing *A. altaicum* to be the closest relative of *A. fistulosum*, and *A. vavilovii* and *A. galanthum* in a clade together with the former. The analysis of RAPD data (Fig. 4), as well as other studies based on RAPD (van Raamsdonk, Smiech, and Sandbrink, 1997) and nuclear data (Bradeen and Havey, 1995), which might be better comparable to the method used by Dubouzet, Shinoda, and Murata, found only distant relations between *A. galanthum* and *A. fistulosum*. During our survey of the origin of the bunching onion we found a possible explanation for these inconsistencies in different studies of *Allium* section *Cepa*. When we included all available accessions of the two species in a preliminary examination, we obtained nearly unstructured data

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                                                         I                                                      90
A. altaicum TAX1422     TTGTAGTATACAAATGCACGGAAC-------------------TATTACTATTTCATCTAAGTTAGATAACCAAGGACTTTATTTTACTA
A. altaicum TAX2760     TTGTAGTATACAAATGCACGGAAC-------------------TATTACTATTTCATCTAAGTTAGATAACCAAGGACTTTATTTTACTA
A. fistulosum All742    TTGTAGTATACAAATGCACGGAACTATTACTATTTCATCTAAGTATTACTATTTCATCTAAGTTAGATAACCAAGGACTTTATTTTACTA
A. fistulosum K6175     TTGTAGTATACAAATGCACGGAACTATTACTATTTCATCTAAGTATTACTATTTCATCTAAGTTAGATAACCAAGGACTTTATTTTACTA
A. fistulosum All515    TTGTAGTATACAAATGCACGGAACTATTACTATTTCATCTAAGTATTACTATTTCATCTAAGTTAGATAACCAAGGACTTTATTTTACTA
                                             ****                                   ****
                                                                               ****** ******

                                                               II                                              180
A. altaicum TAX1422     TGGATTCTTATAACTCGAATCTAATAACT---------------------CAATAAATTTAGATTTGGTTATGACCCAAAAAGGAAAAGG
A. altaicum TAX2760     TGGATTCTTATAACTCGAATCTAATAACT---------------------CAATAAATTTAGATTTGGTTATGACCCAAAAAGGAAAAGG
A. fistulosum All742    TGGATTCTTATAACTCGAATCTAATAACT---------------------CAATAAATTTAGATTTGGTTATGACCCAAAAAGGAAAAGG
A. fistulosum K6175     TGGATTCTTATAACTCGAATCTAATAACT---------------------CAATAAATTTAGATTTGGTTATGACCCAAAAAGGAAAAGG
A. fistulosum All515    TGGATTCTTATAACTCGAATCTAATAACTCAATAAATTTAGATTTGGTTACAATAAATTTAGATTTGGTTATGACCCAAAAAGGAAAAGG
                                               *****                                       *****
                                              <<<<<<|>>>>>> <<<<|>>>>
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Fig. 7. DNA sequence alignment of a part of the *psa*A-*trn*S (GGA) intergenic spacer of the chloroplast genome containing two insertions (dotted), which originated via sequence duplication of a neighboring DNA string (underlined). Asterisks mark inverted repeat sequence motives at the borders of the inserted regions, <|> highlights two mirrored parts of the DNA. Insertion I represents a synapomorphy of *Allium fistulosum*, and insertion II is an autapomorphic mutation of *A. fistulosum* accession All515.

with some *A. altaicum* clustering together with *A. fistulosum* and vice versa, leading to the conclusion that both taxa are polyphyletic. A close investigation of all 'misplaced'' accessions showed that they had come to the living collection via seed exchange from freely interpollinating populations in botanical gardens. Excluding all garden material from the analysis resulted in the strong polarization of the two taxa as shown above, allowing the recognition of *A. fistulosum* as a monophyletic unit within a paraphyletic *A. altaicum*. As an example for a 'wrong'' accession we included Tax1503 (*A. fistulosum* from Botanical Garden, Chorog, Tadjikistan) in this analysis, which contains an *A. altaicum* chloroplast type. F1 hybrids between the two species normally exhibit predominantly morphological characters of the maternal parent (Kokoreva and Jurjeva, 1987) so that pollen-mediated introgression is difficult to detect morphologically. Also, *A. altaicum* and *A. fistulosum* can best be distinguished by subterranean characters, which might easily result in erroneously labelled plants when the two species were grown together and propagation by seeds is not prevented. This facilitates chloroplast capture (as in Tax1503) via repeated backcrosses to one parent and might also explain the postulated sister-group relationship of *A. fistulosum* to *A. galanthum* (Dubouzet, Shinoda, and Murata, 1997) because both species are interfertile (van Raamsdonk, Wietsma, and de Vries, 1992) and garden material was used in the analysis (J. G. Dubouzet, Hokkaido Agricultural Station, Sapporo, personal communication).

Whereas, apart from the study of Dubouzet, Shinoda, and Murata (1997), the close relationship of *A. altaicum* and *A. fistulosum* seems well supported, the nearest relatives of these species are uncertain. Both marker systems used here, as well as the study of Havey (1992), revealed a position of *A. vavilovii* (Figs. 3, 4) close to the ingroup taxa but suggest conflicting hypotheses about the placement of the other species. It was not the aim of this study to analyze the entire section *Cepa*, but additional different topologies in the studies of Bradeen and Havey (1995) and van Raamsdonk, Smiech, and Sandbrink (1997), led us to the conclusion that the phylogeny of *Allium* section *Cepa* is still unresolved.

The monophyletic origin of *A. fistulosum* makes this taxon an additional example for the hypothesis that most cultivated species resulted from single domestication events and subsequently spread into their presently inhabited areas (Zohary, 1996). The monophyly of *A. fistulosum* was revealed by the analysis of noncoding cpDNA and of RAPDs (Figs. 3, 5). A 19-bp insertion in the *psa*A-*trn*S intergenic spacer characterizes all accessions of *A. fistulosum* and clearly distinguishes it from the other taxa in the analysis. DNA sequencing of the mutated region (Fig. 7, insertion I) showed that two partly overlapping inverted repeat sequences flanking the insert allow the formation of two different stem/loop secondary structures: a small hairpin with six paired bases or alternatively a short 4-bp stem with a larger loop. As in other species where inverted repeat sequences flank deletions (von Stein and Hachtel, 1988; van Ham et al., 1994) or inversions (Kelchner and Wendel, 1996; Blattner, unpublished data), they might also be involved in duplication and insertion of the duplicated DNA string adjacent to its pattern in *A. fistulosum*. The second insertion/duplication in the only European accession (All515) of *A. fistulosum* (Fig. 7, insertion II) appears to be comparable to insertion I in the possibility of forming a secondary structure. The two mirrored parts of the sequence might point to an additional mechanism of sequence evolution in noncoding DNA.

Within *A. fistulosum* no differences could be detected by the RFLP analysis of the chloroplast genome fragments, which is not surprising taking into account that only few variable positions (six point mutations representing 0.25%) exist, as shown by sequencing part of the *psa*A-*trn*S spacer. The RAPD data analyses revealed intraspecific variation within *A. fistulosum*. Three accessions from Japan (K6173-6175) exhibit the largest variability (Fig. 6) and occur in a position basal to the remaining accessions analyzed (Fig. 5). Compared with *A. altaicum* the cultivated accessions of *A. fistulosum* show less diversity in the RAPD bands, which agrees with isozyme data (Maass, 1997). This also supports the conclusion that domestication has been localized and has involved a severe genetic bottleneck (Zohary, 1996). New variation arises slowly via mutations or interbreeding with wild relatives, which explains the fact that only one mutation in the chloroplast genome and two RAPD fragments unique to *A. fistulosum* could be found. An analysis of a larger number of accessions of *A. fistulosum*, including an increased number of Japanese and Chinese genotypes, may reveal more intraspecific variation and result in slightly different intraspecific relationships within A. fistulosum. However, even a larger sample of accessions should not substantially alter the result, as the *A. fistulosum* accessions investigated by us are representative of the most divergent morphological groups of the species.

It is interesting that in the RAPD analysis (Figs. 5,6) two of the southernmost accessions of *A. altaicum* (Tax1667 and Tax1691 from Mongolia, Fig. 1), are the closest relatives of the *A. fistulosum* accessions. This result points to a possible origin of *A. fistulosum* in northern China as proposed by Prochanov (1930) for the hypothetical progenitor of both species and would contradict the view of De Candole (1883) who postulated Siberia as the area of domestication of the bunching onion. The inclusion of additional accessions of *A. fistulosum* from China might have elucidated this point. In our analysis Japanese material of the bunching onion (K6174, Fig. 4) is genetically closer to *A. altaicum* than the accession from China (K0433).

Our reconstruction of the origin of *A. fistulosum* raises the question why a bulbless plant has been bred for the use of pseudostems or leaves from a progenitor that possessed a well-developed and widely used bulb. One possible solution to this problem can be seen in the fact that some specimens of *A. altaicum* were found growing in scree and sometimes, if covered with gravel, develop elongated and etiolated pseudostems, which could be a starting point for domestication of a leek-like vegetable.

The results of our work show that relatively fast-evolving cpDNA regions allow phylogenetic analyses in closely related species. The RFLP data permit us in this case to detect species boundaries, while subspecific groupings were not easily resolved by this method (Fig. 3). One

exception was a group of five accessions of *A. altaicum* (Tax1425 to Tax3400, including the hybrid Tax1503, in Fig. 3) that possessed a different chloroplast type with a 100-bp deletion in region *trn*C-*trn*D. The main advantage of the method, i.e., suitability for screening large numbers of accessions very rapidly, works well in comparisons among species. At the population level it may miss most of the small amount of divergence as can be seen from the sequence comparison of the *psa*A-*trn*S spacer. In this case DNA sequencing has the advantage of finding more mutations and permitting the detailed analysis of sequence evolution (base substitution, inversion, or insertion/deletion).

RAPD analysis detects large amounts of differences even in closely related taxa and, at least if detection conditions are adjusted very carefully, can find reliable phylogenetic signal even above the species level (Fig. 4; van Raamsdonk, Smiech, and Sandbrink, 1997). The neighbor-joining analysis of genetic distances of the RAPD data resulted in a phenogram that closely reflected the results of PCA. In particular, the multivariate analysis of RAPD data with PCA clearly distinguishes three groups in the *A. altaicum*/*A. fistulosum* species complex (Fig. 6). *Allium altaicum* and *A. fistulosum* are separated and three *A. altaicum* accessions from the Altai occur apart from the remaining plants investigated. It is interesting in this respect that the two groups in *A. altaicum* correspond to two life forms of *A. altaicum*. A detailed investigation of *A. altaicum* by Grankina et al. (1986) revealed that this species exhibits two ecologically and morphologically different forms in the Altai mountains. One of these forms, represented in our analyses by three accessions (Tax5149, Tax5559, and Tax5561), possesses abruptly pointed, broad leaves and mostly a single thick stem and is widespread only on rocky terrain in the Altai mountains. The other life form (represented by the remaining accessions of *A. altaicum* in our analysis) shows gradually pointed leaves and usually more than one stem and is common in the entire inhabited area of the species, growing in wetter habitats. Between two populations of the two life forms occurring at a distance of 2 km in the Altai mountains, one of the authors (N. Friesen) found morphologically intermediate individuals, growing in ecologically intermediate habitats. The RAPD differences found between the two groups led us to the conclusion that the obvious morphological difference is not a plastic reaction to environmental conditions but characterizes two phylogenetically distinct populations of *A. altaicum*.

As a result of the inconsistencies between our analysis and previously published studies, which, in our opinion, might mostly be caused by the inclusion of unrecognized hybrid plants, we would recommend using only plants from wild populations or vegetatively propagated material of such plants for studies in groups with high interfertility. Even a careful morphological determination of plants can fail to detect hybridization when backcrosses to one of the parents have occurred. Inconclusive results were obtained not only within *A. altaicum*/*A. fistulosum*, but relationships throughout section *Cepa* are somewhat different in studies published from different working groups. Whereas it is common knowledge that gene trees do not have to be identical with the species tree (Maddison, 1997), so that phylogenetic hypotheses partly depend on the marker systems used, comparable methods should produce congruent results. In this study as well as in the studies of Dubouzet, Shinoda, and Murata (1997) and van Raamsdonk, Smiech, and Sandbrink (1997) RAPD-based markers led to different phylogenies. Apart from methodological problems of RAPD analyses this as well might be a result of the inclusion of concealed hybrids in some of these studies.

LITERATURE CITED

ALBERT, V. A., B. D. MISHLER, AND M. W. CHASE. 1992. Character-state weighting for restriction site data in phylogenetic reconstruction, with an example from chloroplast DNA. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Plant molecular systematics, 369–403. Chapman and Hall, New York, NY.

ARNOLD, M. L., C. M. BUCKNER, AND J. J. ROBINSON. 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. *Proceedings of the National Academy of Sciences, USA* 88: 1398–1402.

BACHMANN, K. 1997. Nuclear DNA markers in plant biosystematic research. *Opera Botanica* 132: 137–148.

———, AND E.-J. HOMBERGEN. 1996. Mapping genes for phenotypic variation in *Microseris* (Lactuceae) with molecular markers. *In* P. D. S. Caligari and D. J. N. Hind [eds.], Compositae: biology and utilization, vol. 2, 23–43. Kew Gardens, London.

BRADEEN, J. M., AND M. J. HAVEY. 1995. Restriction fragment length polymorphisms reveal considerable nuclear divergence within a well-supported maternal clade in *Allium* section *Cepa* (Alliaceae). *American Journal of Botany* 82: 1455–1462.

BYRNE, M., J. C. MURRELL, J. V. OWEN, E. R. WILLIAMS, AND G. F. MORAN. 1997. Mapping of quantitative trait loci influencing frost tolerance in *Eucalyptus nitens*. *Theoretical and Applied Genetics* 95: 975–979.

COLOSI, J. C., AND B. A. SCHAAL. 1997. Wild proso millet (*Panicum miliaceum*) is genetically variable and distinct from crop varieties of proso millet. *Weed Science* 45: 509–518.

DE CANDOLE, A. L. P. P. 1883. Origine des plantes cultives. Bailliere et Co., Paris.

DEMESURE, B., N. SODZI, AND R. J. PETIT. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4: 129–131.

DERJAGIN, G. V., AND A. B. JORDANSKY. 1971. Phenotypic variability of satellited chromosomes I. *Allium cepa* L., *A. fistulosum* L. and *A. altaicum* Pall. *Genetika* 7: 40–56 (in russian).

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K. J. CHO. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpo*C1 intron. *Molecular Phylogenetics and Evolution* 6: 1–18.

DUBOUZET, J. G., K. SHINODA, AND N. MURATA. 1997. Phylogeny of *Allium* L. subgenus *Rhizirideum* (G. Don ex Koch.) Wendelbo according to dot blot hybridization with randomly amplified DNA probes. *Theoretical and Applied Genetics* 95: 1223–1228.

EMEBIRI, L. C., M. E. DEVEY, A. C. MATHESON, AND M. U. SLEE. 1997. Linkage of RAPD markers to NESTUR, a stem growth index in radiata pine seedlings. *Theoretical and Applied Genetics* 95: 119–124.

FRIESEN, N. 1987. The genus *Allium* L. and *Caloscordum* Herbert. *In* L. Malyshev and G. Peshkova [eds.], Flora of Siberia: Araceae-Orchidaceae, vol. 4, 55–97, 177–195. Nauka, Novosibirsk (in Russian).

———. 1988. Alliaceae from Siberia: taxonomy, karyology, chorology. Nauka, Novosibirsk (in Russian).

———. 1995. The genus *Allium* L. in the flora of Mongolia. *Feddes Repertorium* 106: 59–81.

———, AND M. KLAAS. 1998. Origin of some minor vegetatively propagated *Allium* crops studied with RAPD and GISH. *Genetic Resources and Crop Evolution* 45: 511–523.

HAISHIMA, M., J. KATO, AND H. IKEHASHI. 1993. Isozyme polymorphism in native varieties of Japanese bunching onion *Allium fistulosum* L. *Japanese Journal of Breeding* 42: 497–505.

HANELT, P. 1985. Zur Taxonomie, Chorologie and Ökologie der Wildarten von *Allium* L. sect. *Cepa* (Mill.) Prokh. *Flora* 176: 99–116.

HARRIS, S. A. 1995. Systematics and randomly amplified polymorphic DNA in the genus *Leucaena* (Leguminosae, Mimosoideae). *Plant Systematics and Evolution* 197: 195–208.

HAVEY, M. J. 1992. Restriction enzyme analysis of the chloroplast and nuclear 45s ribosomal DNA of *Allium* sections *Cepa* and *Phyllodolon* (Alliaceae). *Plant Systematics and Evolution* 183: 17–31.

HODGES, S. A., AND M. L. ARNOLD. 1994. Columbines: a geographically widespread species flock. *Proceedings of the National Academy of Sciences, USA* 91: 5129–5132.

GRANKINA, V. P., N. FRIESEN, V. M. CHANMINCHUN, N. S. DANILOVA, AND V. A. CHEREMUSHKINA. 1986. Luk altaiskiy, kamennyi, dikij batun - *Allium altaicum* Pall. *In* K. A. Sobolevskaya [ed.], Biologicheskije osobennosti rastenij Sibiri, nuzhdajushijesja v ochrane, 121–140. Hauka, Novosibirsk.

INADA, I., AND S. IWASA. 1983. Interspecific hybrids between *A. fistulosum, A. schoenoprasum*, and *A. altaicum*. *Abstracts of the Japanese Society for Horticulture Science, Spring Meeting*: 168.

———, AND M. ENDO. 1994. C-banded karyotype analysis of *Allium fistulosum* and *A. altaicum* and their phylogenetic relationship. *Journal of the Japanese Society for Horticultural Science* 63: 593–602.

INDEN, H., AND T. ASAHIRA. 1990. Japanese bunching onion *Allium fistulosum* L. *In* J. L. Brewster and H. D. Rabinowich [eds.], Onion and allied crops, vol. 3, 159–178. CRC Press, Boca Raton, FL.

KASAKOVA, A. A. 1978. *Allium. In* P. M. Zhukovsky [ed.], Flora of cultivated plants, vol. 10. Kolos, Leningrad.

KELCHNER, S. A., AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* 8: 385–397.

———, AND J. F. WENDEL. 1996. Hairpins create minute inversions in non-coding regions of chloroplast DNA. *Current Genetics* 30: 259–262.

KOKOREVA, V. A., AND N. A. JURJEVA. 1987. Reciproknye skreshivanija vidov luka *Allium fistulosum* L. i *A. altaicum* Pall. *In* Progresivnye priemy v tekhnologii selektsii i semenovodstva ovoshnykh kultur, 112–119. Timiryasev Agricultural Academy, Moscow.

LAWSON, D. M., C. F. LUNDE, AND M. A. MUTSCHLER. 1997. Marker-assisted transfer of acylsugar-mediated pest resistance from the wild tomato, *Lycopersicon pennelii*, to the cultivated tomato, *Lycopersicon esculentum*. *Molecular Breeding* 3: 307–317.

LEDEBOUR, C. F. 1830. Flora Altaica, vol. 2. Types et impensis G. Reimeri, Berolini (Berlin).

LI, H.-L. 1970. The origin of cultivated plants in Southeast Asia. *Economic Botany* 24: 3–19.

LISTON, A. 1992. Variation in the chloroplast genes *rpo*C1 and *rpo*C2 of the genus *Astragalus* (Fabaceae): evidence from restriction site mapping of PCR-amplified fragment. *American Journal of Botany* 79: 953–961.

MAASS, H. 1997. Genetic diversity in the top onion, *Allium* × *proliferum* (Alliaceae), analysed by isozymes. *Plant Systematics and Evolution* 208: 35–44.

MADDISON, W. P. 1997. Gene trees in species trees. *Systematic Biology* 46: 523–536.

MCCLELLAND, M., J. HANISH, M. NELSON, AND Y. PATEL. 1988. KGB: a single buffer for all restriction endonucleases. *Nucleic Acids Research* 16: 364.

MES, T. H. M., N. FRIESEN, R. M. FRITSCH, M. KLAAS, AND K. BACHMANN. 1997. Criteria for sampling in *Allium* based on chloroplast DNA PCR-RFLP's. *Systematic Botany* 22: 701–712.

MORELL, M. K., R. PEAKALL, R. APPELS, L. R. PRESTON, AND H. L. LLOYD. 1995. DNA profiling techniques for plant variety identification. *Australian Journal of Experimental Agriculture* 35: 807–819.

MORTON, B. R., AND M. T. CLEGG. 1993. A chloroplast DNA mutational hotspot and gene conversion in a non-coding region near *rbc*L in the grass family Poaceae. *Current Genetics* 24: 357–365.

NISHITANI, S. 1984. Cytogenetical studies on the cultivated species and its wild relatives in the genus *Allium*. *Japanese Journal of Breeding* 34: 274.

PALLAS, P. S. 1773. Reise durch verschiedene Provinzen des Russischen Reichs, vol. 2. Kayserliche Academie der Wissenschaften, St. Petersburg.

PILLAY, M., AND S. T. KENNY. 1995. Anomalies in direct pairwise comparisons of RAPD fragments for genetic analysis. *BioTechniques* 19: 694–698.

POWELL, W., W. T. B. THOMAS, E. BAIRD, P. LAWRENCE, A. BOOTH, B. HARROWER, J. W. MCNICOL, AND R. WAUGH. 1997. Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. *Heredity* 79: 48–59.

PROCHANOV, J. I. 1930. A contribution to the knowledge of the cultivated *Alliums* of China and Japan. *Trudy prikladnoi botaniki, genetiki i selekcii* 24, 123–188 (in Russian with English summary).

REGEL, E. 1875. Alliorum adhuc cognitorum monographia. *Acta Horti Petropolitani* 3,2: 3–256.

RIESEBERG, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. *Molecular Ecology* 5: 99–105.

———, H. CHOI, R. CHAN, AND C. SPORE. 1993. Genomic map of diploid hybrid species. *Heredity* 70: 285–293.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogentic trees. *Molecular Biology and Evolution* 4: 406–425.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SERQUEN, F. C., J. BACHER, AND J. E. STRAUB. 1997. Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. *Molecular Breeding* 3: 257–268.

SHIMADA, H., AND M. SUGIURA. 1991. Fine structural features of the chloroplast genome: comparison of the sequenced chloroplast genomes. *Nucleic Acids Research* 19: 983–995.

STELLER, G. W. 1740. Flora ircutensis, 449 (manuscript, archiv of the Academy of Sciences, St. Petersburg).

SWOFFORD, D. L. 1993. PAUP: Phylogenetic Analysis Using Parsimony, version 3.1.1. Natural History Survey, Champaign, IL.

———, G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference. *In* D. M. Hillis, C. Moritz, B. K. Mable [eds.], Molecular systematics, 2nd ed., 407–514. Sinauer, Sunderland, MA.

TABERLET, P., L. GIELLY, G. PAUOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TSUMURA, Y., K. YOSHIMURA, N. TOMARU, AND K. OHBA. 1995. Molecular phylogeny of conifers using RFLP analysis of PCR-amplified specific chloroplast genes. *Theoretical and Applied Genetics* 91: 1222–1236.

VAN DE ZANDE, L., AND R. BIJLSMA. 1995. Limitations of the RAPD technique in phylogeny reconstruction in *Drosophila*. *Journal of Evolutionary Biology* 8: 645–656.

VAN HAM, R. C. H. J., H. TIHART, T. H. M. MES, AND J. M. SANDBRINK. 1994. Molecular evolution of non-coding regions of the chloroplast genome in the Crassulaceae and related species. *Current Genetics* 25: 558–566.

VAN RAAMSDONK, L. W. D., W. A. WIETSMA, AND J. N. DE VRIES. 1992. Crossing experiments in *Allium* L. section *Cepa*. *Botanical Journal of the Linnean Society* 109: 293–303.

———, M. P. SMIECH, AND J. M. SANDBRINK. 1997. Introgression explains incongruence between nuclear and chloroplast DNA-based phylogenies in *Allium* section *Cepa*. *Botanical Journal of the-Linnean Society* 123: 91–108.

VON STEIN, J., AND W. HACHTEL. 1988. Deletions/insertions, short inverted repeats, sequences resembling *att*-lambda, and frame shift mutated open reading frames involved in chloroplast DNA differences in the genus *Oenothera* and *Munzia*. *Molecular and General Genetics* 213: 513–518.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRIJTERS, J. POT, J. PELEMAN, M. KUIPER, AND M. ZABEAU. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

WELSH, J., AND J. MCCLELLAND. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213–7218.

WILLIAMS, G. K., A. R. KUBELIK, K. L. LIVAK, J. A. RAFALASKI, AND S.V. TINGEY. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

ZOHARY, D. 1996. The mode of domestication of the founder crops of Southwest Asian agriculture. *In* D. R. Harris [ed.], The origins and spread of agriculture and pastoralism in Eurasia, 142–158. University College London Press, London.