Theor Appl Genet (1997) 94: 465–471

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Mitochondrial DNA variation and crossability of leek (*Allium porrum*) and its wild relatives from the *Allium ampeloprasum* complex

Received: 14 August 1996 / Accepted: 23 August 1996

Abstract Mitochondrial (mt) DNA variation in the cultigens leek, kurrat and prei-anak is limited compared to that of their wild relatives in the *Allium ampeloprasum* complex. The phylogenetic relationships among these cultigens and their wild relatives is quite close, with the majority of the species clustering within one mitochondrial clade. The presence in leek of an extra-mitochondrial genetic element was noted. Analysis of crossability showed that all species were interfertile with leek. It is suggested that the genetic variation present within the *A. ampeloprasum* complex could be exploited in order to broaden the genetic basis of leek.

Key words Leek · *Allium ampeloprasum* complex · Mitochondrial DNA · Domestication · Interspecific hybridization · Breeding

Introduction

The evaluation of cytoplasmic variation has been largely neglected in the assessment of genetic variation by plant breeders. However, after the Southern corn blight incident (Levings 1990), an increasing amount of literature has become available which stresses the importance of nucleo-cytoplasmic interactions, not only in the case of cytoplasmic male sterility (CMS) but also for other agronomically important traits (Young and Virmani 1990; Pollak 1991; Virk and Brar 1993). Consequently, nowadays the identification and conservation of genetically diverse germplasm (nuclear as well as cytoplasmic) is increasingly considered essential for breeding programmes.

In the genus *Allium*, organellar DNA studies have been carried out primarily in the subgenus *Rhizirideum*, which contains such agronomically important species like as onion (*A. cepa*), chives (*A. schoenoprasum*), Chinese chives (*A. tuberosum*) and Japanese bunching onion (*A. fistulosum*). Variation in chloroplast (cp) DNA in the section *Cepa* has been studied by Havey (1991; 1992), and a cpDNA map of onions is available (Katayama et al. 1991). Extensive analyses of mitochondrial (mt) DNA variation in onion (de Courcel et al. 1989; Holford et al. 1991; Havey 1993; Satoh et al 1993; Havey and Bark 1994) and chives (Potz and Tatlioglu 1993) have been carried out.

Conversely, in *Allium*, the other subgenus which comprises agronomically important crops like garlic (*A. sativum*) and leek (*A. porrum*), few studies have been carried out analysing cytoplasmic variation. Only Havey (1991), in a study on phylogenetic relationships among cultivated *Alliums*, has compared garlic and leek on the cpDNA level, while Maass and Klaas (1995) reported on nuclear DNA variation in garlic. However, no studies are known in which mtDNA variation is assessed in the subgenus *Allium*.

Leek and its cultivated relatives are thought to originate from *A. ampeloprasum* (Stearn 1978). The latter species together with *A. commutatum*, *A. bourgeaui* and *A. atroviolaceum*, forms the *A. ampeloprasum* complex (*sensu* von Bothmer 1974) in Greece. The *A. ampeloprasum* complex is composed of a polyploid series (for review see Mathews 1996), whereas for leek and its cultivated relatives only one ploidy level is known ($2n = 4x = 32$). It has recently been proposed that this crop-weed complex belongs to the so-called cotton model (van Raamsdonk 1995). In this model natural polyploidization prior to domestication has occurred,

Communicated by R. Hagemann

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and reproductive isolation is present between the different ploidy levels.

Leek is primarily cultivated in Europe. The major problems confronted by the grower are its susceptiblity to pests and diseases and its lack of uniformity. In general, the exploitation of variation occurring in wild species via interspecific hybridization and subsequent backcrossing has proven to be of great commercial value. For example, in the case of onion, a close relative of leek, disease resistances have been introduced from the wild species into onion (Kofoet et al. 1990, de Vries et al. 1992). However, no study of the feasibility of interspecific hybridization between leek and its wild relatives has yet been published.

The main objectives of the study reported here were to examine mtDNA variability in leek and its wild relatives by restriction analyses and to assess if crossing barriers are present between leek and its wild relatives. This investigation was also intended to be a preliminary study towards the better understanding of the origin, cultivation and domestication of leek.

Materials and methods

Plant material and species crosses

The *A. commutatum* plant material originated from the Aegean Islands (Greece) and was a gift of Dr. R. von Bothmer, Swedish University of Agricultural Sciences, Svalöv, Sweden. The other wild *Allium* material was obtained from various sources. The *A. porrum* cultivars were obtained from the Centre for Genetic Resources (CGN), Wageningen, The Netherlands (Table 1). The *Allium* material was grown in pots in a frost-free glasshouse.

Species crosses between leek and its wild relatives, i.e. *A. ampeloprasum*, *A. commutatum*, *A. bourgeaui* and *A. atroviolaceum*, were carried out in small glasshouses and blow flies were used for pollination. Crosses with *A. ampeloprasum* were performed using a genetic male-sterile *A. ampeloprasum* plant as a female and leek cv 'Carina' as a pollen donor. The other three crosses were carried out using a genetic male-sterile leek plant as the female parent. Seed was only harvested from the male-sterile plants.

Table 1 *Allium* material involved in this study (*cv* cultivar, Centre for Genetic Resources, The Netherlands; *n* number of genotypes analysed)

Accession	*n*	Origin
Leek group		
A. porrum		
cv Romeo	8	CGN
cv Argenta	10	„
cv Arkansas	9	„
cv Porino	9	„
cv St. Victor	1	„
cv Alma-Norda	1	„
cv Violet de St. Victor	1	„
cv Verina	1	„
cv Colonna	1	„
cv Blue de Solaize	1	„
cv St. Victor	1	„
cv Gavia	1	„
cv Corine	1	„
cv Jaune de Poitou	1	„
A. kurrat	1	Egypt, CPRO
Prei-anak	1	Indonesia, CPRO 89077
Wild relatives		
A. ampeloprasum		
Tinos	1	B773, von Bothmer
Korfu	1	HB Graz, Austria 91BG23807
ssp. *babingtonii*	1	HB Dublin, Ireland
Great headed garlic	1	Malta, CPRO 92059
Steep Holms Island	1	HB Kew, UK 1885
A. commutatum		
Tinos	1	B767, von Bothmer
Mikinos	1	B764, „
Kasos	1	B466, „
Paros	1	B072, „
Siros	1	B739, „
Andipos	1	B714, „
Sikinos	1	B574, „
Allonisos	1	B821, „
Rhodos	1	B273, „
Ios	1	B579, „
A. bourgeaui		
ssp. *bourgeaui*	1	HB Berlin, 93BG04312
ssp. *cycladicum*	1	HB Berlin, 93BG04313
A. atroviolaceum	1	IPK Gatersleben, Tax 1372/88
A. pyrenaicum	1	Station Nat. d'Essais, France

Organelle DNA purification, isolation and restriction analyses

The mitochondrial and chloroplast DNA isolation was a modification of the procedure of Wilson and Chourey (1984) and Bookjans et al. (1984) respectively.

Mitochondria purification

For purification of the *Allium* mitochondria 100 g tissue, preferably white pseudo-stems, was blended (4 s) in 500 ml cold (4°C) buffer A [330 m*M* mannitol, 50 m*M* TRIS, 3 m*M* EDTA, 0.1% BSA, 10 m*M* β-mercapto-ethanol (pH 8.0)]. The resulting homogenate was filtrated once through a cheese-cloth and centrifugated (4°C) for 10 min at 1500 *g*. The supernatant containing the mitochondria was centrifuged for 15 min at 12 000 *g* (4°C). The resulting supernatant was poured off and the pellet was resuspended by means of a paint brush in 10 ml buffer A. Then 100 µl of 1 *M* $MgSO_4$ and 1 ml DNAse (4 µg/µl) was added to the solution. The mixture was left for 1 h on ice; 3 volumes of a buffer (4°C) containing 1.25 *M* NaCl, 50 m*M* TRIS and 20 m*M* EDTA (pH 8.0) were then added, and the whole mixture was thoroughly but gently shaken in order to eliminate the DNAse. Next the mitochondria were pelleted for 15 min at 12 000 *g* and resuspended in 50 ml of a TE buffer (50 m*M* TRIS-HCl and 10 m*M* EDTA; pH 8.0). This cleaning procedure was repeated three times. MtDNA was isolated from the pellet.

Chloroplast purification

The chloroplasts were prepared from well developed leaves (green parts). Approximately 50 g of leaves were crushed by mortar and pestle in 3–5 volumes of a buffer (4°C) containing 1.25 *M* NaCl, 50 m*M* TRIS, 20 m*M* EDTA, 0.1% BSA and 10 m*M* β-mercapto-ethanol (pH 8.0–8.3). After filtration through four layers of cheese cloth the filtrate was centrifuged for 15 min at 1500 *g* (4°C). The

pellet was resuspended in 50 ml of the aforementioned buffer and centrifuged once again for 15 min at 1500 *g* (4°C). The resuspension and centrifugation procedure was repeated three times. CpDNA was isolated from the pellet.

Cp- and mtDNA isolation

The purified chloroplasts and mitochondria were resuspended in 600 µl of a buffer (4°C) containing 100 m*M* TRIS, 50 m*M* EDTA, 100 m*M* NaCl and 10 mM *β*-mercapto-ethanol (pH 8.0). The mixture was transferred to an Eppendorf tube, SDS was added to a final concentration of 1% (w/v) and the mixture was incubated for 45–60 min at 65°C. The DNA was purified by two phenol extractions and one phenol-chloroform extraction. Afterwards, 20% SDS (1/20 volume) and 200 µl 5 *M* KAc was added to the solution, and the mixture was held for 30 min at −20°C, then centrifuged and the supernatant transferred to 400 µl isopropanol and 40 µl 5 *M* NH_4Ac. Once again the mixture was kept for 30 min at −20°C afterwhich the DNA was pelleted. The DNA was washed twice with a cold 70% EtOH solution and vacuum dried. Finally, the DNA was resuspended overnight at ambient temperature in 2 µl TE (10 m*M* TRIS and 1 m*M* EDTA; pH 8.0).

Restriction analyses

Two to three micrograms organeller DNA was completely digested (enzymes used: *Eco*RI, *Bam*HI and *Xho*I) according to the manufacturer's (Life Technologies) protocol. Subsequently the restriction fragments were separated by electrophoresis on 0.8% (w/v) agarose gels, stained with ethidium bromide and viewed under UV light. Lambda DNA restricted with *Bst*EII (New England Biolabs) was used as a molecular weight standard.

Phylogenetic analyses

Insight was obtained into the phylogenetic relationships of the *A. ampeloprasum* complex by carring out Wagner parsimony analyses using PAUP (version 3.0; Swofford 1991). Consensus trees were based on all most parsimonious trees of equal length. *A. pyrenaicum* was chosen as an outgroup because this species is a member of the subgenus *Allium* but with a geographic distribution quite distinct from that of the *A. ampeloprasum* complex.

Results

Mitochondrial DNA isolation

Even when the mtDNA isolation protocol is used, it is still possible that mtDNA is contaminated with cpDNA. Therefore we compared the cpDNA *Eco*RI restriction patterns with the mtDNA *Eco*RI restriction patterns of leek. Figure 1 shows that contamination of the mtDNA with cpDNA cannot be excluded because in a number of cases fragments with identical sizes were observed (Fig. 1). However, the cp bands observed within the range of scorable organellar DNA fragments (6–20 kB) did not interfer to any large extent with the mt fragments found in this range (Fig. 1).

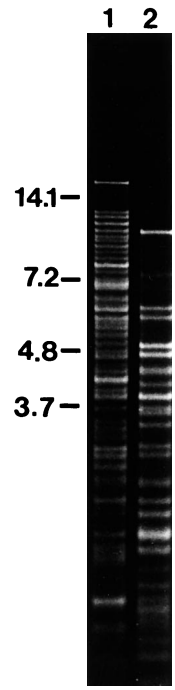

Fig. 1 *Eco*RI restriction patterns of mtDNA (*lane 1*) and cpDNA (*lane 2*) of leek (*A. porrum*). Fragment length sizes (kb) are indicated on the *left*

Mitochondrial DNA variation within and between leek cultivars

The within-cultivar mtDNA variation was assessed by analyses of the *Eco*RI, *Bam*HI and *Xho*I (only the data of *Eco*RI are shown) restriction profiles of 8–10 plants of 4 commercially available leek cultivars (Table 2). In all 4 cultivars 2 mitotypes can be distinguished (Fig. 2). *Eco*RI restriction yields 1 mitotype (type I) with a double band at approximately 12 kb and 18 kb, whereas in the other mitotype (type II) only single bands are present at these positions. In addition, type II carries a 6-kb (approximately) band which is absent in type I. Both mitotype I and II are equally distributed among the cultivars analysed (contingency chi-square test: $\chi_3 = 7.256$, $0.05 < P < 0.1$; two-tailed). Restriction analyses with *Eco*RI of 9 different leek cultivars showed essentially the same variation in mtDNA patterns except that in cv 'Blue de Solaize' a new pattern was observed, in which the type is essentially type I but with a band present at approximately 6 kb (Fig. 3).

Mitochondrial DNA variation in close relatives of leek

The *Eco*RI restriction profiles of close relatives of leek, kurrat (a leek grown in Egypt) and prei-anak (a leek grown in Indonesia), proved to be of mitotype I (Fig. 4). *A. ampeloprasum* material from Steep Holms Island (UK; Fig. 4) and Ireland (data not shown) possessed unique *Eco*RI restriction patterns. The other 2 wild *A. ampeloprasum* accessions, 1 from the Greek island Tinos (Fig. 5) and the other from Malta (data not

Table 2 The occurrence of two mitotypes observed in 4 leek (*Allium porrum*) cultivars

Cultivar	Mitotype	
	I	II
Romeo	2	6
Argenta	5	5
Arkansas	7	2
Porino	2	7
Total	16	20

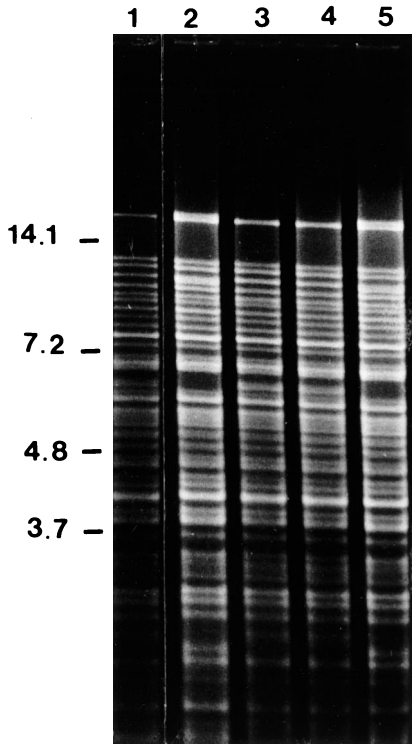

Fig. 2 *Eco*RI restriction patterns of mtDNAs from 5 individuals from leek (*A. porrum*) cultivar 'Porino'. Fragment length sizes (kb) are indicated on the *left*

shown), also possessed distinct *Eco*RI restriction patterns. Compared to leek an enormous amount of mtDNA variation was observed in *A. commutatum*, which was collected from the Aegean islands. No fewer than 8 unique mitotypes in a total of 10 accessions were observed (Fig. 5). The 2 mitotypes of the *A. bourgeaui* accessions also proved to be dissimilar (data not shown).

Extra-mitochondrial DNA

In undigested DNA of leek an extra band at approximately 1.3 kb can be observed after gel electrophoresis, however, no extra fragment was found in *A. commutatum* (Fig. 6).

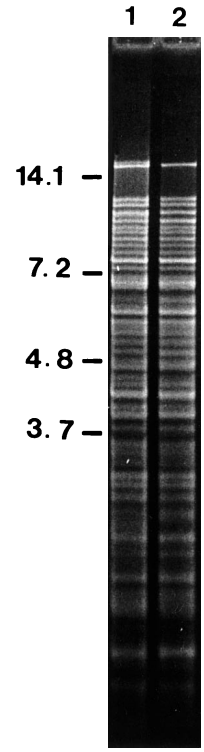

Fig. 3 *Eco*RI restriction patterns of mtDNAs from 2 leek (*A. porrum*) cultivars. *Lane 1* 'Blue de Solaize' and *lane 2* 'St. Victor'. Fragment length sizes (kb) are indicated on the *left*

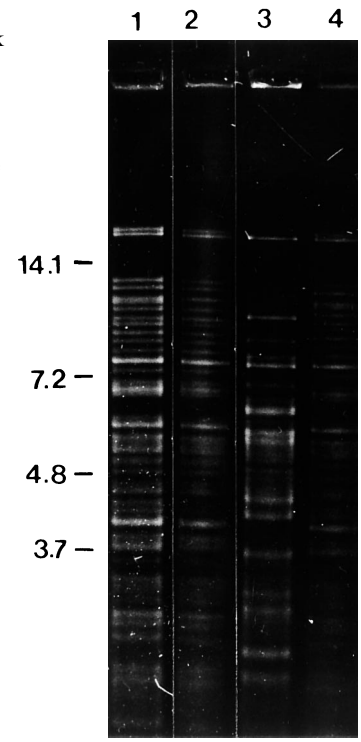

Fig. 4 *Eco*RI restriction patterns of mtDNAs from leek (*A. porrum*) and its relatives. *Lane 1* leek, *lane 2* prei anak, *lane 3 A. ampeloprasum* var '*babingtonii*', *lane 4* kurrat, Fragment length sizes (kb) are indicated on the *left*

Phylogenetic analyses

Twenty-nine *Eco*RI mt bands in the range of 6–20 kb formed the data for the PAUP analysis. PAUP generated a strict consensus of 86 most parsimonious trees

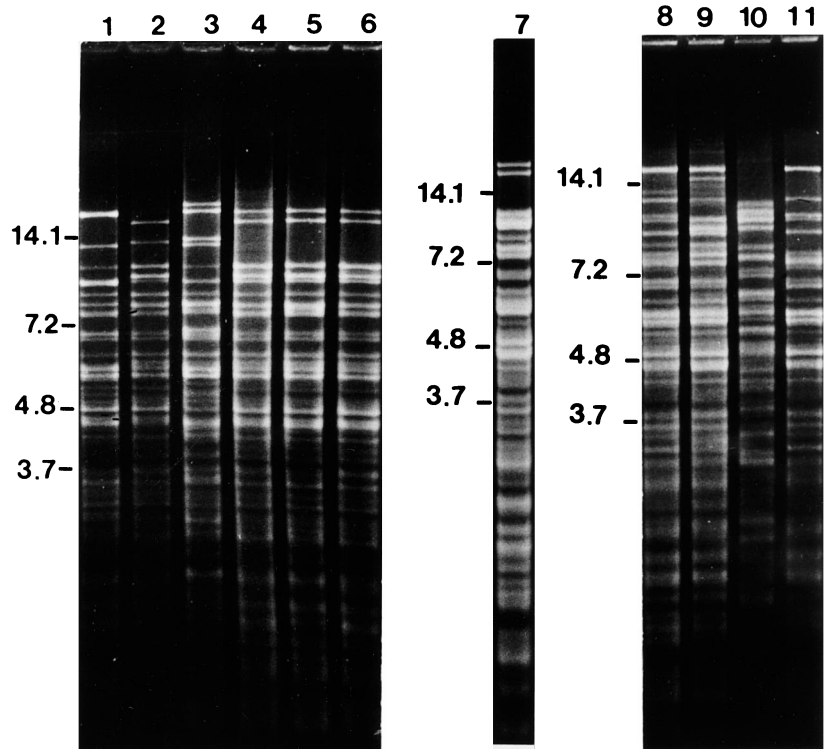

Fig. 5 *Eco*RI restriction patterns of mtDNAs from various accessions of *A. commutatum* and one accession of *A. ampeloprasum* (*lane 10*) originating from different Aegean islands. *Lane 1* Tinos, *lane 2* Mikinos, *lane 3* Kasos, *lane 4* Paros, *lane 5* Siros, *lane 6* Andipos, *lane 7* Sikinos, *lane 8* Allonisos, *lane 9* Rhodes, *lane 10* Tinos, *lane 11* Ios. Fragment length sizes (kb) are indicated on the *left*

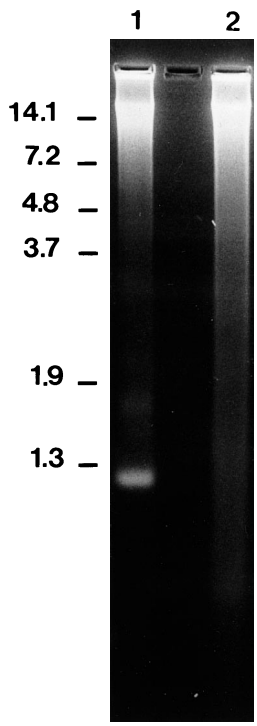

Fig. 6 Extra-mitochondrial DNA in *Allium*. *Lane 1* Leek (*A. porrum*), *lane 2 A. commutatum*. Fragment length sizes (kb) are indicated on the *left*

of 67 steps (CI = 0.418; RI = 0.598). Three clades could be distinguished: clade 1 (the leek clade) consisted of leek and prei-anak, *A. ampeloprasum* from Greece, *A. commutatum* and *A. bourgeaui* ssp. *bourgeaui*; clade 2 (the babingtonii clade) consisted of *A. ampeloprasum* from Holms Island (UK) and from Malta, *A. ampeloprasum* var '*babingtonii*' from Ireland and *A. atroviolaceum*; clade 3 consisted of *A. bourgeaui* ssp. *cycladicum* (Fig. 7).

Crossability

All four species crosses resulted in normal seeds and plants (Table 3).

Discussion

Patterns of mtDNA variation in leek and its wild relatives

Studies specifically aimed at comparing of organellar variation in wild and cultivated forms of a species are scarce. This is the first report on organellar variation in leek and its wild relatives. Only three mitotypes were observed in leek, whereas its wild progenitors, the species belonging to the *A. ampeloprasum* complex, showed a substantial number of mitotypes. The same variation pattern has been observed in cultivated and wild forms of rubber (Luo et al. 1995) and common bean (Khairallah et al. 1992). This may indicate that domestication is accompanied by a reduction in mtDNA variation. However, such a reduction has not been found in other crops. For example, a considerable amount of variation

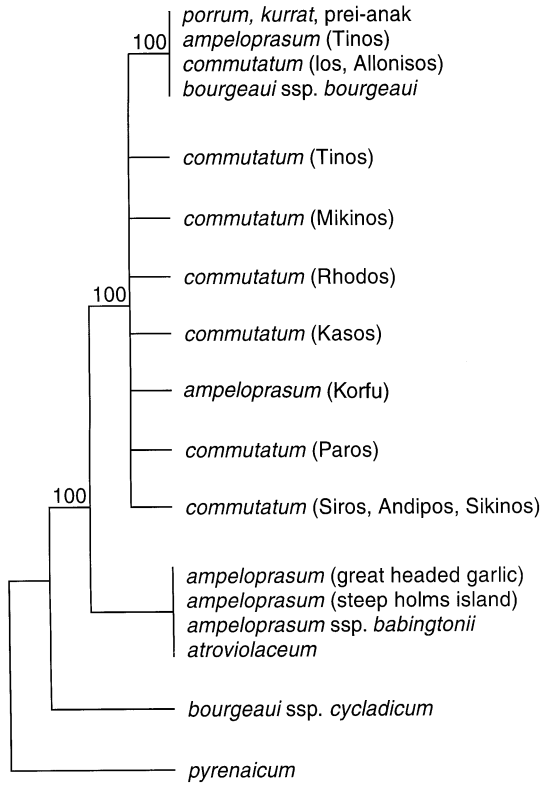

Fig. 7 Consensus tree of the 86 most parsimonious trees from the analysis of the *Eco*RI restriction sites in the mtDNA of leek (*A. porrum*) and its wild relatives from the *A. ampeloprasum* complex. Tree length: 67 steps; consistency index: 0.418

Table 3 Crosses between leek and its wild relatives in the *A. ampeloprasum* complex

Cross		Number of crosses	Number of seeds obtained	Number of plants obtained
♀	♂			
A. ampeloprasum × leek		8	150	35
Leek × *A. commutatum*		3	39	22
Leek × *A. bourgeaui* spp. *cycladicum*		1	22	2
Leek × *A. atroviolaceum*		1	7	5

has been observed in both cultivated and wild species of carrot (Ichikawa et al. 1989) and apple (Ishikawa et al. 1992). On the other hand, little variation has been observed in cultivated and wild forms of barley (Holwerda et al. 1986).

In general, crops domesticated according to the tulip model (e.g. apple) and in some cases those representing the chili pepper model (e.g. carrot) show much higher levels of diversity than their wild relatives compared to their counterparts of the cotton model (e.g. leek; van Raamsdonk and van der Maesen 1996). The presence (cotton model) or absence (tulip and chili pepper models) of crossing barriers between wild and domesticated species is a major aspect differentiating the three aforementioned models. Internal isolation barriers especially will hamper plant breeding practice and prevent significant increases of variation (van Raamsdonk 1995).

Relationships within the *A. ampeloprasum* complex

The term *A. ampeloprasum* complex was coined by Von Bothmer (1974). According to Von Bothmer, the complex includes, apart from *A. ampeloprasum*, three other species, namely *A. commutatum*, *A. bourgeaui* and *A. atroviolaceum*. Our results indicate close relationships between the species within the complex because three of the species occur within the 'leek' mt clade. In the 'babingtonii' mt clade 3 non-Greek *A. ampeloprasum* accessions are present which are collected outside Greece, namely Malta, Steep Holms Island (Bristol Channel, UK) and Ireland. These 3 *A. ampeloprasum* accessions are sterile, presumably because of elevated ploidy levels. The *A. ampeloprasum* accession from Malta has been identified as great-headed garlic and has a ploidy level of $2n = 6x = 48$. The *A. ampeloprasum* accession from Steep Holms Island has a ploidy level of $2n = 5x = 40$ (Johnson 1982), and the *A. ampeloprasum* accession of Ireland, known as ssp. *babingtonii*, also has a ploidy level of $2n = 5x = 40$.

The success of species crosses also points at very close relationships between the species in the complex. Moreover, Von Bothmer (1974) observed that on Crete, the only Aegean island on which *A. ampeloprasum*, *A. commutatum* and *A. bourgeaui* occur sympatrically, hybrid swarms are most probably present. He suggested that the only isolation mechanism between the species is an ecological one: they grow in different habitats, namely *A. ampeloprasum* occurs in ruderal places, *A. commutatum* can be found on small islets and inhabits the spray zone and *A. bourgeaui* can be found on steep cliffs.

Domestication of leek and related crops

Not much is known about the domestication of leek. The accepted view is that *A. ampeloprasum* is the wild ancestor of leek (Stearn 1978). However, according to Mathews (1996) it is also possible that other species in the *A. ampeloprasum* complex were involved in the domestication of leek. The results obtained in this study neither contradict nor confirm this point of view. The 'leek' mt clade includes most of the members of the *A. ampeloprasum* complex (only *A. atroviolaceum* is missing) and all members of this group readily cross with leek. However, it is possible that *A. commutatum* is not an ancestor of leek because of the lack of extra-mitochondrial DNA, which is found in leek.

Within the cultivated leek group various subgroups are present. Next to leek and kurrat, pearl onion, great

headed garlic, taree irani, poireau perpetuel and prei-anak can also be distinguished (van der Meer and Hanelt 1990). Leek, kurrat and prei-anak had identical mitotypes, whereas great-headed garlic deviated to a large extent from the others. The similarity in both mt- and cpDNA restriction patterns in leek and kurrat indicate that both species clearly originate from the same gene pool. Prei-anak, a leek indigenous to Indonesia, has also the same organellar variation as leek. This might indicate that prei-anak evolved from an introduction from Europe.

Consequences for leek breeding

The practical consequence of the existence of a rather narrow cytoplasmatic basis of leek is that leek breeders should be encouraged to use species crosses in their breeding programmes to broaden the cytoplasmic (and nuclear) genetic basis of leek. The introduction of more cytoplasmic variants in leek might be very beneficial for the exploitation of nucleo-cytoplasmic interactions. The introduction of (alloplasmic) CMS in leek to enable F_1 hybrid breeding is in this respect of particular importance given the fact that large heterosis effects and a substantial increase in uniformity have been reported for hybrid leek (Smith and Crowther 1995).

Acknowledgements We thank O. Dolstra, A. W. van Heusden, A.P.M. den Nijs and R.E. Voorrips for critically reading this manuscript; Wan Bin, A. Arts and Th. Schouten for the skilful performance of part of the organellar DNA analyses and P. Saat, J. van den Berg and A. Wessels for maintaining the leek collection.

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