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Relationships among *Cichorium* species and related genera as determined by analysis of mitochondrial RFLPs

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Abstract Mitochondrial DNA polymorphism was employed to assess cytoplasmic diversity among cytoypes of the genus *Cichorium* and related genera of the tribe *Lactuceae* (*Asteraceae*). Hybridization patterns of total DNA using six restriction enzymes and five heterologous mtDNA probes were examined. From estimates of mtDNA diversity, *Cichorium spinosum* appeared as an ecotype of *C. intybus* rather than a separate species. Interspecific mtDNA polymorphism in the genus *Cichorium* was higher than that observed in *Cicerbita Crepis*, *Lactuca* and *Tragopogon*. Molecular data seemed to indicate that *Catananche* is very distant from the other genera examined. Intergeneric comparisons allowed the clustering of *Cicerbita*, *Lactuca* and *Cichorium*, genera which belong to different subtribes. However, further molecular investigations on a larger number of genera are needed to clarify the relationships among genera within and between subtribes of the tribe *Lactuceae*.

Key words Mitochondrial DNA · RFLP · *Asteraceae* · *Cichorium*

Introduction

Nuclear-cytoplasmic incompatibility is known to exist, and a common observable phenotype of such incompatibility in plants is cytoplasmic male sterility (CMS). In several of the most extensively studied plant species, the CMS phenotype is associated with rearrangements of specific regions of mitochondrial DNA, resulting in the synthesis of altered or chimaeric proteins (reviewed by Hanson 1991).

By somatic hybridization, new organelle-nuclear genome combinations can be obtained between sexually-incongruent species and useful agronomic traits from a wild species can be introduced into the gene pool of a crop species (Pelletier et al. 1988). Characterization of cytoplasmic diversity can be a tool providing information on whether or not a given species is a reasonable candidate as a cytoplasmic recipient in somatic hybridization (Perl et al. 1991, Derks et al. 1992). Organelle restriction fragment length polymorphisms (RFLPs) have already been used as molecular markers of cytoplasmic diversity. RFLP markers also permit the study of organelle genomes of somatic fusion products (Belliard et al. 1979; Galun et al. 1982).

Plant mitochondrial genomes change rapidly in size and structure but slowly in primary sequence (Palmer and Hebron 1988). Although mtDNA restriction pattern variability has been used as a measure of phylogenetic relationship in several plant species such as coffee (Berthou et al. 1983), wheat (Vedel et al. 1978), and maize (Timothy et al. 1979; Kemble et al. 1983; Wessinger et al. 1983), the large size, complexity, and rearrangements of mitochondrial genomes preclude direct interpretation of restriction enzyme patterns (Sedoroff et al. 1981; Sedoroff 1987). To overcome these limitations, restriction patterns that are characterized by specific mitochondrial gene probes hybridized to Southern blots have been used for *Nicotiana* (Bland et al. 1985), *Lycopersicon* and *Solanum* (McClean and Hanson 1986), *Pennisetum* (Chowdhury and Smith 1988), *Aegilops* and *Triticum* (Graur et al. 1989; Breiman et al. 1991). Studies on interspecific and intraspecific mtDNA variation are relatively limited and, so far, there has been no report for such an analysis in the family *Asteraceae*, which includes *Cichorium* species.

Cytoplasmic male sterility would be a very useful tool in hybrid seed production in *Cichorium intybus*. In order to use mitochondrial diversity in somatic hybridization experiments, information on mtDNA polymorphism in

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the genus *Cichorium* and related genera of the family *Asteraceae* is needed. The genus *Cichorium*, divided into three species in the Flora Europeae (Tutin et al. 1964–1980), *C. endivia*, *C. intybus* and *C. spinosum*, has been placed in the subtribe *Hyoseridinae* of the tribe *Lactuceae*, and can be associated with several genera depending on the morphological characters examined. The subtribe *Hyoseridinae* is a heterogeneous group characterized by its predominantly Mediterranean distribution, and by the pappus and cypsela of its members (Stebbins 1953). A study of pollen morphology and exine stratification confirmed the variability existing within the subtribe *Hyoseridinae* (Blackmore 1981) but did not clarify the relationships between *Cichorium* and the other genera of this subtribe.

Identification of mitochondrial genome variation in the genus *Cichorium* and other genera thought to be closely related according to morphological classifications (Bonnier and De Layens 1923; Stebbins 1953; Blackmore 1981) would be a useful reference for plant breeding due to the increasing importance of cultivated chicory as an industrial crop. This study presents the first analysis of mitochondrial DNA polymorphism at the intergeneric and interspecific levels between various members of the family *Asteraceae*.

Materials and methods

Plant materials

Mitochondrial DNA diversity was studied in the subfamily *Cichorioideae* of the family *Asteraceae*. Most of the species tested belong to the genera *Cichorium* and *Lactuca* of the tribe *Lactuceae*. *H. annuus* from the tribe *Heliantheae* was chosen as an outgroup species. The sources of seeds and the chromosome number for each of the taxa included in this study are given in Table 1. Plants from seeds of each accession were grown in a greenhouse.

Plant DNA isolation and Southern blotting

Total DNA was extracted from approximately 3 g of young expanding leaves, taken from one plant, according to Dellaporta et al. (1983). For each cytotype, eight µg of DNA were digested with *BamHI*, *BglI*, *ClaI*, *Hind*III, *Sal*I and *Sst*I (Bethesda Research Laboratories, UK). The 24 species studied in this report were separated into three groups for practical reasons. The first group encompassed 13 plants chosen within the genus *Cichorium*. The second group was formed with

Table 1 Sources of plant materials used for DNA analysis (*MNHN* Museum National d'Histoire Naturelle, Paris, *INRA* Institut National de la Recherche Agronomique, *Obt INRA* cultivar obtained by INRA, *Ctifl* Centre technique interprofessionnel des fruits et legumes, *Clause*, *F. Desprez*, and *Hoquet* are private seed companies)

Taxa	Code	2n	Sources
Cichorioideae			
Catananche caerula L.	CCA	18	MNHN
Cicerbita alpina Wallr (85-263)	CAL	18	MNHN
C. plumieri L. Kirschleger (84-271)	CPL		MNHN
Chondrilla juncea L. (CR 14)	CJU	14	INRA Versailles
Cichorium endivia L. var. *crispa* (FDP 12)	FRI	18	INRA Versailles-Ctifl
C. endivia L. var. *latifolia* (cv Geante maraichere)	GMA	18	INRA Versailles-Ctifl
C. endivia L. wild-type from Israel (431)	ISR	18	F. Desprez
C. intybus L. inbred line ALG	ALG	18	INRA Versailles-Ctifl
C. intybus L. cv Catalogne (DD 403)	CAT	18	Clause
C. intybus L. cv Chioggia (OT 2-8-5-1)	CHI	18	INRA-Ctifl
C. intybus L. cv Flash (white seeds)	FLB	18	Hoquet (84350, Obt INRA)
C. intybus L. cv Flash (ALG cytoplasm)	FLN	18	Hoquet (84350, Obt INRA)
C. intybus L. industrial type (FD1)	FD1	18	F. Desprez
C. intybus L. inbred line Pain de sucre (jupiter)	JUP	18	INRA Versailles-Ctifl
C. intybus L. cv Trevise (TRT 1)	TRE	18	INRA Versailles-Ctifl
C. intybus L. inbred line Verone (8-1-1)	VER	18	INRA Versailles-Ctifl
C. intybus L. wild-type from Turquey (CST 3)	CST	18	INRA Versailles-Ctifl
C. spinosum	SPI	18	INRA Versailles-Ctifl
Crepis biennis L.	CBI	40 +/−	MNHN
C. foetida L.	CFO	10	MNHN
C. setosa Haller	CSE	6/8	MNHN
Lactuca alpina L.	LAL	18	INRA Versailles
L. perennis L. (LS 286)	LPE	18	MNHN
L. saligna L.	LSL	18	MNHN
L. sativa L. cv. Ardente	LSA	18	INRA Versailles
L. serriola L. (LSE 18)	LSE	18	INRA Versailles
L. tatarica L.	LTA	18	INRA Versailles
L. virosa L. (IVT 1398)	LVI	18	MNHN
Sonchus maritimus L. (84-340)	SMA	32	MNHN
Taraxacum officinale Weber (87-17)	TOF		MNHN
Tolpis barbata Adanson	TBA	18	MNHN
Tragopogon crocifolius L. (87-230)	TCR	12	MNHN
T. pratensis L.	TPR	12	MNHN
T. porrifolius L. ssp *australis* Nyman (86-219)	TPO	12	MNHN
Asteroideae			
H. annuus wild-type (521)	HAS	34	INRA Montpellier

species from the genera *Catananche*, *Chondrilla*, *Lactuca*, *Sonchus*, *Taraxacum* and *Tolpis*. In group 3, species from the genera *Crepis*, *Helianthus* and *Tragopogon* were included. In analyses concerning groups 2 and 3, *Cichorium intybus* (inbred line ALG) was added as a control group. Digested DNAs were separated by electrophoresis (0.8% agarose gel), blotted and UV-cross linked to Hybond-N filters (Amersham, UK) (Khandjian 1987). *Hin*dIII-digested lambda DNA was used as a molecular marker for DNA fragment size calculations. Prehybridization and hybridization were performed according to standard procedures at 42 °C with 50% formamide (Maniatis et al. 1982). The ^{32}P-labelled probes (specific activity of 1×10^9 cpm/μg) were synthesized using random oligonucleotide primers and ^{32}P-dCTP as described by Feinberg and Volgelstein (1983). The membranes were washed twice for 5 min in 2 × SSC (standard saline citrate) at room temperature, twice for 5 min in 0.2 × SSC, 0.1% SDS (sodium dodecylsulphate) at 65 °C. Filters were exposed to Kodak X-ARS film overnight to 1 week with one Quanta III intensifying screen at − 80 °C for 30 min, followed by neutralization in 0.1 × SSC, 0.1 SDS (sodium dodecylsulphate), 0.2M Tris-HCl (pH 7.5) for 30 min.

Characteristics of DNA probes

Each DNA sample was hybridized with five specific mtDNA probes. We used a 1-kb *Eco*RI-*Sst*I fragment harboring the ATPase subunit alpha (*atp*A) gene from wheat (B. Lejeune, Orsay), a 950-bp *Hin*dIII-*Pvu*II fragment which contains the ATPase subunit 9 (*atp*9) gene from sunflower (H. Recipon, Orsay), a 680-bp *Hin*dIII-*Eco*RI fragment containing part of the coding sequence of the apocytochrome b (*cob*) gene (3′ terminal sequence) from maize (Dawson et al. 1984), a 1.3-kb *Hin*dIII-*Eco*RI fragment which contains the cytochrome oxidase subunit I (*cox*I) gene from wheat (B. Lejeune, Orsay), and a 600-bp *Sal*I-*Eco*RI fragment which harbors the exon 2 of the cytochrome oxidase subunit II (*coxII*) gene from wheat (B. Lejeune, Orsay).

Data analysis

Each hybridizing fragment detected by Southern analysis was treated as a unit. The presence or absence of each fragment was scored respectively as 1 and 0. Those cases in which the presence or absence of a fragment was questionable were scored as − 1. The matrix tables (genotypes × fragments) containing the values 1, 0 and − 1 were computed using the programme package "Restsite v. 1.1", developed by Miller (1990), on an IBM-compatible PC. Indices of relatedness (F-values) between cytotypes were estimated using the shared fragments method according to Nei and Li (1979). The F-values were calculated from the RFLP data using: $F_{xy} = 2n_{xy}/(n_x + n_y)$, where n_x was the number of mtDNA fragment analysed in cytotype x, n_y in cytotype y and n_{xy} the number of shared fragments between cytotypes x and y. Phenograms were constructed on the distance matrix files via the Unweighted Pair-Group Method Analysis (UPGMA) (Sneath and Sokal 1973).

Results

Mitochondrial DNA variation in the genus *Cichorium*

In the genus *Cichorium*, a very low degree of mtDNA variation was detected among species and genotypes; most enzyme-probe combinations displayed uniform hybridization patterns. The *cob* probe did not detect any differences among all the chicories tested, while the *atp*A, *atp*9, *cox*I and *cox*II probes differentiated the *C. endivia* genotypes from those of *C. intybus*. An example

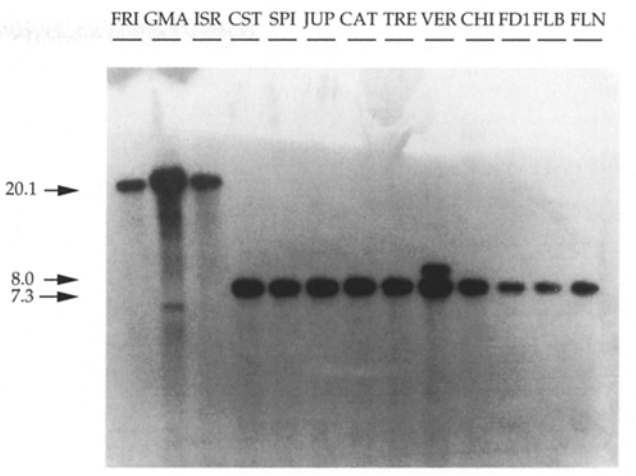

Fig. 1 Autoradiograph of total DNA from *Cichorium* cytotypes restricted with *Sal*I and hybridized with the *atp9* gene from sunflower labelled and used as probe. The approximate size of the fragments is indicated in kilobases (kb)

of these results in shown in Fig. 1. The mtDNA of the *C. intybus* Verone plant showed four differences from the mtDNA of the other *C. intybus* plants. Two differences were specific to the Verone plant and the two others were also found in *C. endivia*. The mtDNA of the wild chicory isolated from Turkey (CST) exhibited three differences from the mtDNA of the other *C. intybus* genotypes.

From these data, mtDNA polymorphism was not detected between the two cultivated *C. endivia*, var. *crispa* and var. *latifolia*, or between the *C. intybus* industrial type (FD1), cv Catalogne, cv Chioggia, cv Trevise, cv Pain de sucre and *C. spinosum*.

RFLP analysis in the genus *Cichorium*

F-values, estimates of relatedness between cytotypes, ranged from 1.000 (e.g., Trevise vs Catalogne) to 0.727 (*C. endivia* cv Geante maraichere vs the wild chicory from Turkey) (Table 2). Within *C. intybus*, the proportion of shared fragments was high. Only the mtDNA of the Verone and CST cytotypes displayed an appreciable level of variation. For *C. endivia* cytotypes, the F-values ranged from 1.000 to 0.985. Lower values (averaging 0.799) were obtained when the *C. endivia* and *C. intybus* cytotypes were compared.

The phenogram obtained by the UPGMA method (Sneath and Sokal 1973) displays two important features (Fig. 2). Firstly, *C. endivia* cytotypes are separated from those of *C. intybus*. Secondly, it appears that the *C. spinosum* cytotype is enclosed within the *C. intybus* cytotypes.

Mitochondrial DNA variation in other genera

Notable variation of hybridization profiles differentiated the 22 species chosen using the *atp*A probe, what-

Table 2 Proportion of shared mtDNA fragments between 13 cytotypes of the genus *Cichorium* (Codes for genotypes are included in Table 1. The number of shared fragments between pairs of cytotypes appears in the right portion of the matrix. The lower left portion of the matrix indicates the F-values calculated from the RFLP data using $F_{xy} = 2n_{xy}/(n_x + n_y)$ where n_{xy} is the number of common RFLPs for two cytotypes x and y, and n_x, n_y are the numbers of total RFLP markers for cytoplasms x and y respectively (Nei and Li 1979). On the diagonal is indicated the total number of hybridized fragment per genotype obtained from the five probe × six enzyme combinations)

Genotypes	CAT	CHI	CST	FD1	FLB	FLN	FRI	GMA	ISR	JUP	SPI	TRE	VER
CAT	*33*	33	31	33	32	33	26	26	27	33	33	33	33
CHI	1.000	*33*	31	33	32	33	26	26	27	33	33	33	33
CST	0.953	0.953	*32*	31	30	31	24	24	25	31	31	31	31
FD1	1.000	1.000	0.953	*33*	32	33	26	26	27	33	33	33	33
FLB	0.969	0.969	0.923	0.969	*33*	33	27	27	28	32	32	32	33
FLN	0.985	0.985	0.939	0.985	0.985	*34*	27	27	28	33	33	33	34
FRI	0.776	0.776	0.727	0.776	0.805	0.794	*34*	34	34	26	26	26	28
GMA	0.776	0.776	0.727	0.776	0.805	0.794	1.000	*34*	34	26	26	26	28
ISR	0.794	0.794	0.746	0.794	0.823	0.811	0.985	0.985	*35*	27	27	27	29
JUP	1.000	1.000	0.953	1.000	0.969	0.985	0.776	0.776	0.794	*33*	33	33	33
SPI	1.000	1.000	0.953	1.000	0.969	0.985	0.776	0.776	0.794	1.000	*33*	33	33
TRE	1.000	1.000	0.953	1.000	0.969	0.985	0.776	0.776	0.794	1.000	1.000	*33*	33
VER	0.942	0.942	0.898	0.942	0.942	0.957	0.788	0.788	0.805	0.942	0.942	0.942	*37*

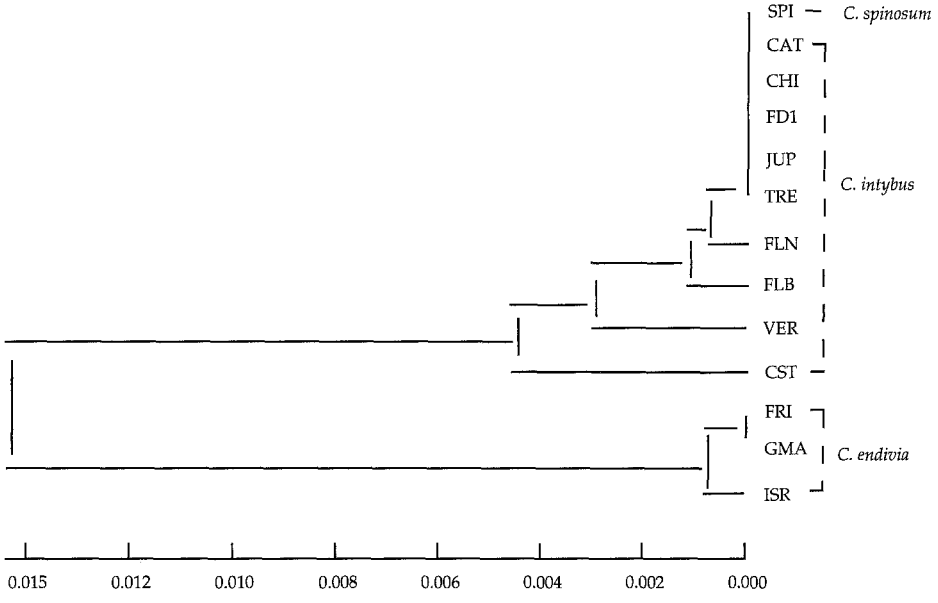

Fig. 2 UPGMA dendrogram (Sneath and Sokal 1973) of relationships among cytotypes of the genus *Cichorium* based on mitochondrial DNA polymorphisms

ever the enzyme tested in this study. For the *atp*9 probe, a lower polymorphism was usually detected whatever the enzyme used, expect for *Catananche caerula* which appeared to have a mtDNA distinct from those of the other species for three out of the six enzymes employed. Mitochondrial DNA polymorphism among species was also detected with the probe/enzyme combination *cob*/*Bam*HI or *Cla*I. From the hybridization patterns obtained with the *cox*I probe combined with the *Bam*HI, *Bgl*I, *Cla*I, and *Hind*III restriction endonucleases, three species were distinguished from the others, *C. caerula*, *Chondrilla juncea* and *Taraxacum officinale*. Hybridization patterns with the *cox*II probe exhibited little difference with either *Sal*I or *Bam*HI, but showed higher polymorphism with *Bgl*I.

Interspecific RFLP analysis in other genera

Mitochondrial DNA variation between *Cicerbita alpina* and *C. plumieri* species (Table 3) was relatively low (0.866). Among the *Crepis* species examined (Table 4), *C. foetida* and *C. setosa* appeared as the most-related species (0.892). Mitochondrial DNA of the different *Lactuca* species showed low polymorphism. F-values ranged from 1.000 between, e.g., *L. alpina* and *L. virosa* to 0.756 between *L. perennis* and *L. sativa* (Table 3). *L. saligna*, *L. perennis* and *L. tatarica* were the most-distant species in this genus. Among the *Tragopogon* species analysed (Table 4), *T. porrifolius* and *T. pratensis* appeared as the most-related species (0.955), *T. crocifolius* and *T. pratensis* as the most remote (0.742).

Table 3 Proportion of shared mtDNA fragments between species of several genera of the tribe *Lactuceae* (*Asteraceae*) (Codes for species are included in Table 1. The number of shared fragments between pairs of cytotypes appears in the right portion of the matrix. The lower left portion of the matrix indicates the F-values calculated from the RFLP data using $F_{xy} = n_{xy}/(n_x + n_y)$ where n_{xy} is the number of common RFLP for two cytotypes x and y, and n_x, n_y are the numbers of total RFLP markers for cytoplasms x and y respectively (Nei and Li 1979). On the diagonal is indicated the total number of hybridized fragments per species obtained from the five probe × six enzyme combinations)

Species	ALG	CAL	CCA	CJU	CPL	LAL	LPE	LSA	LSE	LSL	LTA	LVI	SMA	TBA	TOF
ALG	*29*	20	7	15	19	21	20	21	20	20	20	21	15	19	13
CAL	0.689	*29*	11	17	26	25	25	26	25	26	28	25	16	21	16
CCA	0.241	0.379	*29*	9	11	11	12	12	11	12	11	11	9	10	21
CJU	0.508	0.576	0.305	*30*	18	19	20	18	19	18	17	19	20	16	16
CPL	0.633	0.866	0.366	0.590	*31*	27	28	27	27	25	25	27	18	21	18
LAL	0.636	0.757	0.333	0.567	0.794	*37*	29	36	35	27	27	37	19	20	16
LPE	0.606	0.757	0.363	0.597	0.823	0.783	*37*	28	29	27	27	29	18	22	19
LSA	0.636	0.787	0.363	0.537	0.794	0.972	0.756	*37*	34	28	28	36	19	20	18
LSE	0.606	0.757	0.333	0.567	0.794	0.945	0.783	0.918	*37*	27	27	35	19	21	18
LSL	0.677	0.881	0.406	0.600	0.819	0.805	0.805	0.835	0.805	*30*	28	27	17	22	17
LTA	0.677	0.949	0.372	0.566	0.819	0.805	0.805	0.835	0.805	0.933	*30*	27	16	21	16
LVI	0.636	0.757	0.333	0.567	0.794	1.000	0.783	0.972	0.945	0.805	0.805	*37*	19	20	18
SMA	0.508	0.542	0.305	0.666	0.590	0.567	0.537	0.567	0.567	0.566	0.533	0.567	*30*	15	19
TBA	0.622	0.688	0.327	0.516	0.666	0.579	0.637	0.579	0.608	0.709	0.677	0.579	0.483	*32*	14
TOF	0.426	0.524	0.327	0.677	0.507	0.521	0.463	0.550	0.521	0.548	0.516	0.521	0.612	0.437	*32*

Table 4 Proportion of shared mtDNA fragments between species of several genera of the *Asteraceae* (Codes for species are included in Table 1. The number of shared fragments between pairs of cytotypes appears in right portion of the matrix. The lower left portion of the matrix indicates the F-values calculated from the RFLP data using $F_{xy} = 2\ n_{xy}/(n_x + n_y)$ where n_{xy} is the number of common RFLPs for two cytotypes x and y, and n_x, n_y are the numbers of total RFLP markers for cytoplasms x and y respectively (Nei and Li 1979). On the diagonal is indicated the total number of hybridized fragments per species obtained from the five probe × six enzyme combinations)

Species	ALG	CBI	CFO	CSE	HAS	TCR	TPO	TPR
ALG	*30*	16	13	13	5	14	14	15
CBI	0.561	*29*	23	23	8	14	13	14
CFO	0.406	0.754	*34*	29	8	13	12	12
CSE	0.426	0.793	0.892	*31*	9	12	10	11
HAS	0.156	0.262	0.235	0.276	*35*	7	5	6
TCR	0.417	0.437	0.366	0.352	0.197	*36*	28	26
TPO	0.437	0.426	0.352	0.307	0.147	0.788	*34*	32
TPR	0.476	0.466	0.358	0.343	0.179	0.742	0.955	*33*

Mitochondrial DNA diversity between species of the genus *Cicerbita* was higher than that between *C. alpina* and some *Lactuca* species such as *L. tatarica* or *L. saligna* (Table 3). The interspecific F-values within the genus *Tragopogon* were similar to those within the genus *Crepis*. Notably, mitochondrial DNA polymorphism between the *C. intybus* cytotypes and *C. endivia* ones was higher than interspecific comparisons within the genera *Cicerbita*, *Crepis*, *Lactuca* and *Tragopogon*.

Relationships between the genus *Cichorium* and the other genera examined

The genera *Cichorium* and *Tolpis* seemed to be close to *Cicerbita* and *Lactuca* which appeared very related (Table 3). The mitochondrial DNA polymorphism between the genera *Cicerbita* and *Lactuca* was equivalent to that seen in interspecific comparisons within the genus *Cichorium*. By contrast, the *Cichorium* reference cytotype appeared quite remote from the genera *Crepis* and *Tragopogon* (Table 4) and very distant from *Taraxacum* and *Catananche* (Table 3).

Using the UPGMA phenogram (Fig. 3), three groups of genera can be proposed. One includes *Cicerbita*, *Cichorium*, *Lactuca* and *Tolpis*. The second is formed from *Chondrilla*, *Taraxacum* and *Sonchus*. Lastly, the genus *Catananche* stands apart.

The intergeneric comparison with the outgroup genus, *Helianthus*, confirmed that this genus is quite remote from the other three genera examined, *Cichorium*, *Crepis*, and *Tragopogon* (Table 4).

Discussion

We thought it more convenient to analyse mitochondrial genome variation by probing total DNA with

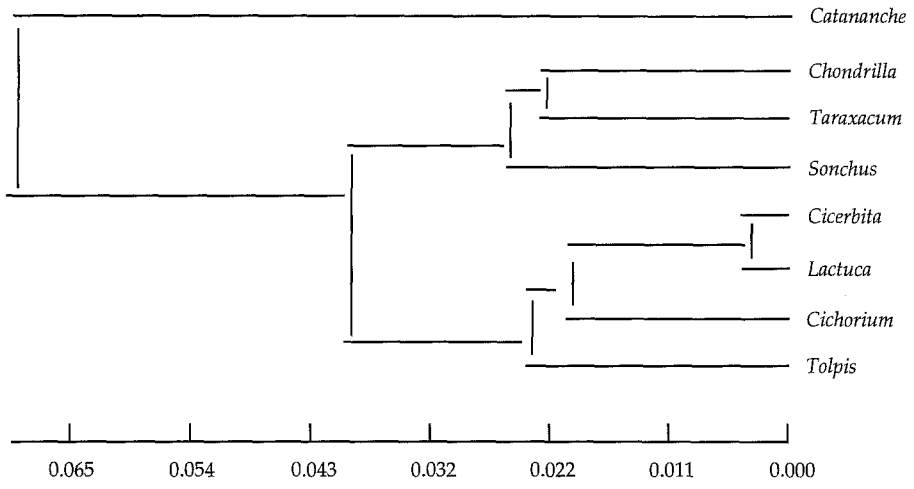

Fig. 3 UPGMA dendrogram (Sneath and Sokal 1973) of relationships among genera of the tribe *Lactuceae* (*Asteraceae*) based on mitochondrial DNA polymorphisms

mtDNA-specific probes than to purify mtDNA from each species and then analyse them. The restriction endonucleases used here were shown to detect mtDNA polymorphism in restriction fragment patterns between two inbred chicory lines (Bellamy 1992). Mitochondrial genomes of higher plants are so complex that it is diffcult to follow all changes affecting the position of visible bands. The high degree of sequence conservation detected among mtDNA coding regions of higher plants (Palmer and Hebron 1988) facilitated the study of mtDNA variation by using heterologous mtDNA probes, on Southern blots of either mtDNA or total DNA, from species in which mitochondrial genes have not yet been isolated. The use of heterologous probes has been shown to work effectively across subclasses of angiosperms. However, using heterologous mtDNA probes on Southern blots of total DNA cannot exclude the hybridization of the probes with non-mitochondrial DNA sequences (Stern and Palmer 1984). The *coxII* gene, normally present in mtDNA, has been found (Nugent and Palmer 1991) in the nuclear genome of five legumes (mung bean, soybean, common bean, pea and cowpea).

RFLP analysis

The study reported here was made on 23 species, members of the subfamily *Cichorioideae*, and on one species of the subfamily *Asteroideae*. The choice of the different species can be explained by several features. Firstly, our main objective was to evaluate the cytoplasmic variability within the genus *Cichorium* and in genera thought to be close to it. Secondly, we wanted to identify groups of genera which harbor approximately the same level of mitochondrial DNA polymorphism. Therefore, *Helianthus* was taken as an outgroup.

F-values based on mtDNA fragment polymorphisms have been used to estimate relatedness in studies of species relationships in *Triticum* and *Aegilops* (Terachi and Tsunewaki 1992), *Daucus* (DeBonte et al. 1984; Ichikawa et al. 1989), *Pennisetum* (Chowdhury and Smith 1988), and *Phaseolus* (Khairallah et al. 1991). Interspecific mtDNA variations in the genera *Cicerbita*, *Cichorium*, *Crepis*, *Lactuca* and *Tragopogon* were quite similar (0.88). However, the F-values obtained between genera ranged from 0.241 (*Catananche* vs *Cichorium*) to 0.934 (*Cicerbita* vs *Lactuca*). By including the outgroup genus, the proportion of shared fragments decreased to 0.156 (*Cichorium* vs *Helianthus*). These results showed that an important level of mitochondrial DNA poly. morphism seems to exist between genera of the tribe *Lactuceae*, which consists of more than 70 genera and 2 300 species displaying rather morphologically-diverse characters and both annual and perennial growth habits. The intergeneric F-values appear concordant with this morphological diversity.

Cichorium species relationships

The F-value between *C. intybus* and *C. spinosum* was extremely high (0.983) suggesting that *C. spinosum* may be an ecotype of *C. intybus* rather than a separate species. Moreover, sexual crosses between these plants were highly successful and the progenies were fertile (Bannerot, unpublished data).

The *C. endivia* wild-type from Israel showed a very low degree of mitochondrial DNA variation when compared to the other *C. endivia* genotypes (0.985) confirming its taxonomic position. Moreover, like the other *C. endivia* genotypes, the wild form from Israel is self-fertile. Achenes of this wild *C. endivia* genotype do not show a pappus. In the Flora Europaea (Tutin et al. 1964–1980) the pappus characteristics (its length and the ratio of pappus length/achene length) constitute the main morphological trait allowing the discrimination of *Cichorium* species. The pappus of *C. intybus* is formed by a crown of short scales and that of *C. endivia* by a crown of long scales. The absence of a pappus on the wild *C.*

endivia achenes implies that this morphological character is not a good criterion for the classification of *Cichorium* species.

From the data obtained on mitochondrial DNA variation, the existence of separate *C. intybus* and *C. endivia* species is confirmed.

Relationships between genera

After morphologica (Stebbins 1953; Jeffrey 1966) and chromosomal (Stebbins et al. 1953) analysis, the genera *Cichorium*, *Crepis* and *Tragopogon* were placed in different subtribes of the tribe *Lactuceae*. Therefore, a high mitochondrial genome polymorphism between these genera was not unexpected (Table 4). The tribe *Lactuceae* is divided into eight subtribes whose composition has been subjected to many changes depending on the character analysed; this applies particularly to the subtribes *Crepidinae* (e.g., *Cicerbita*, *Crepis*, *Lactuca*, *Sonchus*) and *Hyoseridinae* (e.g., *Catananche*, *Cichorium*, *Tolpis*). The lack of application of cladistic principles to morphological traits for the phylogenetic reconstruction in these subtribes might have contributed to the confusion concerning the relationships between genera.

Our analysis of mtDNA polymorphism also revealed the unclear relationships within and between subtribes of the tribe *Lactuceae*. *Catananche* emerged as a very distant genus from the others. This genus, which presents morphological traits belonging to various genera, seems to have an ancestral position. Our data is coherent with the classification made on pollen morphology (Blackmore 1981) indicating that *Catananche* is slightly apart and is related to *Scolymus*. The genus *Scolymus* has been placed in a subtribe by itself and has always been considered as very remote from genera of the subtribe *Hyoseridinae*. From indices of mtDNA relatedness, *Lactuca* seemed to be very close to *Cicerbita*, and *Sonchus* appeared relatively distant from both of them. The relationships between these three genera were recognized in previous treatments (Stebbins 1953; Jeffrey 1966).

Conclusions

Our study is the first to use mtDNA-specific probes on restricted total DNA patterns to assess cytoplasmic diversity among plant species of the tribe *Lactuceae* (*Asteraceae*). In the future, it would be interesting to include other members in this analysis, particularly those belonging to the subtribe *Hyoseridinae*, such as *Arnoseris*, *Hedypnosis* and *Hyoseris*, and to the subtribe *Crepidinae*, such as *Mycelis* and *Prenanthes*, to make major groupings among and within these subtribes. The previous subtribe classifications of the tribe *Lactuceae*, relying on characters derived from anatomical, chromosomal, morphological, palynological and phytochemical studies, have not adequately resolved relationships in these subtribes. Further investigations using powerful DNA-based techniques for systematic and evolutionary studies are needed.

Information about cytoplasmic diversity is of great interest for further studies involving nuclear-cytoplasmic interactions. Identification of mitochondrial genomes sufficiently different from that of *C. intybus* might allow the production via somatic hybridization of a cytoplasmic male-sterility type by disrupting the interaction of nuclear- and cytoplasmically-encoded components of mitochondrial protein complexes.

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