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Mitochondrial genome of *Thymus vulgaris* L. (Labiate) is highly polymorphic between and among natural populations

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Analysis of mitochondrial DNA polymorphism in natural populations of *Thymus vulgaris* revealed the existence of high variability between and among populations. Using southern hybridization with two restriction enzymes analysis and one heterologous probe, 13 mitotypes were detected among three natural populations. All the mitotypes except two were specific to a single population. This high polymorphism was also detected with three other heterologous probes. To analyse this variability further, we studied restriction patterns of purified mtDNA from single individuals sampled from each of three natural populations. The three individuals showed completely different *Eco*RI mtDNA restriction fragment patterns, while *Eco*RI cpDNA restriction fragment patterns were identical for the three individuals. To test the possibility of paternal mtDNA as a potential source of the polymorphism observed, the mode of inheritance of mtDNA was studied and found to be maternal.

Keywords: cytoplasmic DNA, male-sterility, mitochondrial DNA polymorphism, *Thymus vulgaris*.

Introduction

Organelle DNAs are considered as interesting markers of population polymorphism in genetic and ecological studies, mainly because of their uniparental mode of inheritance and important polymorphism. Whereas the chloroplast (cp) DNA has been widely used to characterize genera or species (e.g. Palmer, 1985), the mitochondrial (mt) DNA has proved a powerful tool for distinguishing sub species (e.g. Palmer, 1988). Generally, only one individual per taxon is analysed in those type of studies, accepting the underlying assumption that the variation between individuals or populations of a same taxa is negligible compared with the variation between taxa. However, even though very little has been published on variability of plant cytoplasmic DNA within natural populations, it seems that the above assumption is not justified. In cultivated species, studies have reported the existence of several mtDNA types within a species (*Vicia faba*, Thiellement, 1982; maize, Sisco *et al.*, 1985; sorghum, Bailey-Serres *et al.*, 1986; barley, Holwerda *et al.*, 1986; rice, Kadowaki *et al.*, 1988). For wild species, some studies have shown mtDNA polymorphism between individuals of the same population (Holwerda *et al.*, 1986; *Beta maritima*, Boutin *et al.*, 1987; *Plantago lanceolata*, Rowendal *et al.*, 1987).

As the mode of inheritance of organelle DNA can influence widely the level of polymorphism in natural populations of a species, an analysis of mtDNA inheritance can provide useful information. Chloroplast and mitochondrial DNAs are considered to be maternally transmitted among Angiosperms. However, important exceptions have been reported — *Pelargonium* (Tilney-Basset, 1978), *Oenothera* (Brennicke, 1980), *Medicago sativa* (Schumann & Hancock, 1989), *Brassica napus* (Erickson & Kemble, 1990) and some lines of potato (Grunn, personal communication). In contrast, Gymnosperms display a wide range of organelle transmission modes: mitochondria are paternally inherited in *Pseudotsuga* (Neale *et al.*, 1986) and *Taxus*, maternally inherited in *Ephedra* and mixed transmissions occur in *Picea* (Chesnoy, 1987).

Cytoplasmic male-sterility (CMS) genes have been identified in the mitochondrial genome of several plants (see Breiman & Galum, 1990, for a review) and

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both nuclear and mitochondrial genes combine to give the sterility trait. The existence of cytoplasmic male-sterility is correlated with the existence of a polymorphism for cytoplasmic genes (or more precisely mitochondrial genes). By definition, two different mitochondrial genes are necessary to maintain nucleocytoplasmic male sterility. A better knowledge of the underlying genetic structure, in terms of variations in male-sterile mitotypes of wild species where CMS exist, is thus necessary. In natural populations of gynodioecious species, both female (or male-sterile) and hermaphrodite individuals can be found. *Thymus vulgaris* L. is the most intensively studied gynodioecious species examined so far because of the large variation in female frequencies (from 5 to 95 per cent) found in natural populations (Dommée *et al.*, 1983; Couvet *et al.*, 1986; Gliddon *et al.*, 1987; Belhassen *et al.*, 1989, 1990). The involvement of cytoplasmic genes in the determination of male-sterility in *Thymus vulgaris* has been demonstrated (Belhassen *et al.*, 1991). In the same study, mtDNA hybridization patterns were able to distinguish between the different cytoplasms identified through genetic experiments.

In the present work we analysed the diversity of mitochondrial DNA between and among three populations of the gynodioecious species, *Thymus vulgaris* L.. The complete mitochondrial and chloroplastic restriction patterns of individuals sampled in different natural populations were analysed. The mode of inheritance of mtDNA was followed in six controlled crosses to test the hypothesis of parental mtDNA transmission as a source of polymorphism.

Materials and methods

Natural populations diversity

Three stations were chosen in Languedoc (southern France): La Jasse 3 (LJ3), Montlaur (ML) and Figaret (FT). These stations belong to post-fire populations; hence, their year of inception is known. Characteristics of the stations are given in Table 1. LJ3 is one of our thoroughly studied permanent stations (see Belhassen *et al.*, 1989, where ML and FT were respectively called A and G). At each station, the sex and location of each individual were plotted.

We analysed the mitochondrial DNA (mtDNA) of all the adult individuals of each station to characterize their mtDNA pattern (i.e. mitotype). Total DNA was extracted according to Dellaporta *et al.* (1983) with the following modifications: the extraction buffer contained 50 mM β-mercaptoethanol, precipitations were carried out at $-70\,^{\circ}C$ for 30 min and nucleic acids solutions were treated three times with a mixture of phenol (1 vol.), chloroform (1 vol) and meta-cresol (0.2 vol) for a complete removal of proteins. Restricted electrophoresed DNAs were transferred onto Zeta-Probes (Bio-Rad) membranes by using vacuum blotting. A radioactivity-labelled mitochondrial probe (nick translation) allowed the visualization of the fragments of mtDNA that hybridized with it. Two enzymes (*Hin*dIII and *Bst*I) and one probe (mitochondrial DNA probe coding for the 18S-5S ribosomal RNA, named '18S-5S', Falconnet *et al.*, 1984) were used to characterize the individuals of LJ3 and FT. For ML, only the enzyme *Hin*dIII with the 18S-5S probe was used. This probe was found to detect the most polymorphism among three other probes that we screened (data not shown).

Organelle DNA analysis

All individuals of *Thymus vulgaris* L. were collected from natural populations of Languedoc (France) and transferred to a greenhouse. Plants were placed under darkness for 4 days before green leaves were harvested. Mitochondrial and chloroplast DNAs were purified from 20 to 50 g of material according to the procedure of Vedel & Quétier (1974) with the following modifications: because of the presence of large amounts of aromatic compounds, the extraction buffer was supplemented with 1.25 per cent (w/v) polyethyleneglycol 6000 and 1 per cent (w/v) soluble polyvynylpyrrolidone and the concentration of β-mercatoethanol was increased to 50 mM to prevent oxidation after grinding. Chloroplasts were too fragile

Table 1 Characteristics of the three studied stations located within 30 km radius of Montpellier, France

Station	Complete name	Year of Birth	Bedrock of the site	Area (m^2)	Sample size (Ind.)
LJ3	La Jasse-β	1978	Marly limestone	10.8	25
FT	Le Figaret-1	1984	Soft limestone	6.0	12
ML	Montlaur	1978	Hard limestone	1.4	15

to undergo a DNAse treatment: they were purified by three cycles of differential centrifugations (Vedel & Quétier, 1974). Mitochondria were DNAse-treated and purified through a 38 per cent Percoll gradient (Neuburger *et al.*, 1982) instead of a sucrose gradient. Organelle DNA was released by the sarcosyl-EDTA-proteinase K procedure and treated by phenol-chloroform before an ethidium bromide-CsCl ultracentrifugation. Restriction endonucleases were used according to the supplier's indications except that spermidine (10 mM final) was added to the restriction buffer to ensure complete digestions. Vertical 0.8 per cent agarose slab gels (20 × 15 × 0.4 cm) were run in TEA buffer at 50 volts for 12 h and stained with ethidium bromide. Negatives of UV flurescent photographs were scanned on a double-beam Mark III CS Joyce-Loebl microdensitometer.

Inheritance study

Plants used to study the inheritance of the mitochondrial DNA were chosen as follows. Starting with a single wild-collected plant (female f1) we selected three daughters (f14, f17 and f24) derived from open pollination. These three females were crossed with the same hermaphrodite (h42) to detect an eventual female effect on the mitochondrial transmission. Simultaneously, female f24 was crossed with three different hermaphrodites (h42, h68 and h74) to study a possible paternal effect on mitochondrial transmission. Furthermore, one female offspring from cross f24 × h68 was back-crossed with h68, and a female offspring from this back-cross (f24′) was crossed with a new hermaphrodite, h132, to study the transmission after several generations. The offspring analysed were all derived through maternal line from the same original female f1, after four generations for the offspring from cross f24′ × h132 (named offspring number 11 and 12 in the result section), and after two generations for all other offspring (named offspring numbers 1–10 in the result section). For each of these crosses, one hermaphrodite and one female were chosen among the progeny for mtDNA analysis to determine any sex effect on the transmission. Total DNA was extracted as described above. Hybridized DNA was immunoenzymatically detected with fast-green of Chromogene plus (Bioprobe) as the final dye. Southern hybridizations were carried out under stringent conditions (42 °C, 50 per cent formamide, 2 × SSC) following the Chemiprobe method (Lebacq *et al.*, 1988); the probe used was a 26 S ribosomal mtDNA clone of wheat (Falconet *et al.*, 1984, 1988).

Results

Natural populations diversity

The mtDNA patterns of individuals of the three stations with 18S–5S ribosomal probe (Figs 1–4) and the mitotypes are schematized in Figs 5 and 6. This probe was found to detect the most polymorphisms when compared with the other probes we screened. With 26S ribosomal, *Cox*I, and *Cox*II probes, we still detected important polymorphism amounting to about 70 per cent of that found with the 18S-5S probe (data not shown). The distribution of the different individuals of the populations in the 'mitotype' classes with this last probe is given in Table 2. For population LJ3, three mitotypes were distinguished: with *Hin*dIII, the 'L21' mitotype were composed of three bands of high intensity (around 5.7 kb) and six thinner bands which result from different stoichiometries (Fig. 5), the 'L36' and 'L44' mitotypes were composed of only three bands. In that population, the mitotype classification obtained with the enzyme *Hin*dIII was the same as that obtained with *Bst*I (Table 2). In population FT, *Hin*dIII enabled us to detect five different mtDNA classes (Fig. 5), while *Bst*I distinguished only three mitotypes classes included in the previous ones (Fig. 6). The first enzyme enabled us to subdivide the mitotype 'S1' into

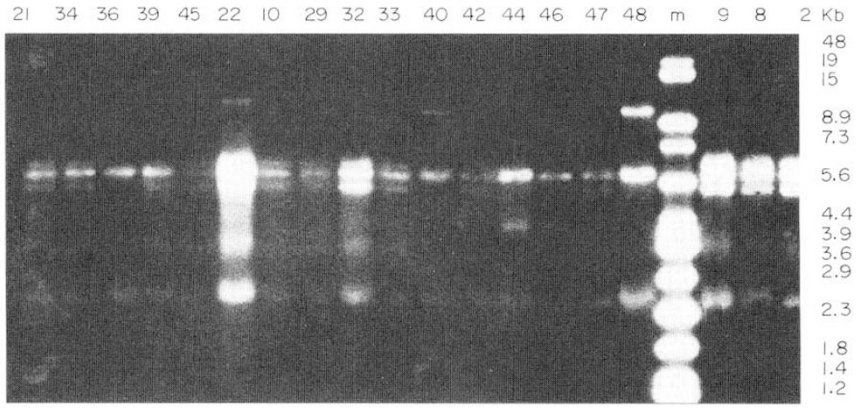

Fig. 1 Southern analysis of mtDNA of LJ3 individuals. Total DNA was digested with *Hin*dIII and hybridized with 18S–5S wheat mt cloned DNA. m is the molecular weight marker, 'Raoul' Appligène (weight indicated on the right).

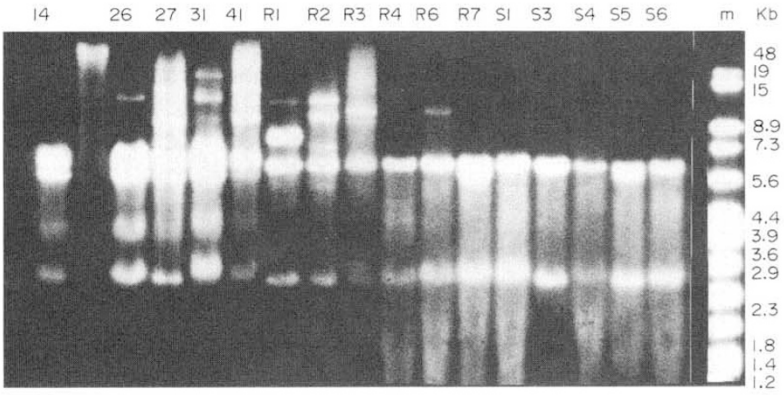

Fig. 2 Southern analysis of mtDNA of LJ3 and FT (name beginning by R or S) individuals. Total DNA was digested with *Hin*dIII and hybridized with 18S–5S wheat mt cloned DNA. m is the molecular weight marker, 'Raoul' Appligène (weight indicated on the right).

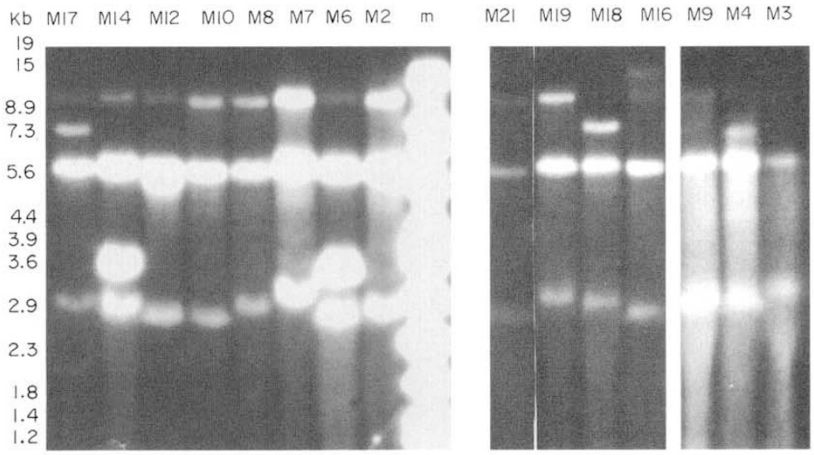

Fig. 3 Southern analysis of mtDNA of ML individuals. Total DNA was digested with *Hin*dIII and hybridized with 18S–5S wheat mt cloned DNA. m is the molecular weight marker, 'Raoul' Appligène (weight indicated on the right).

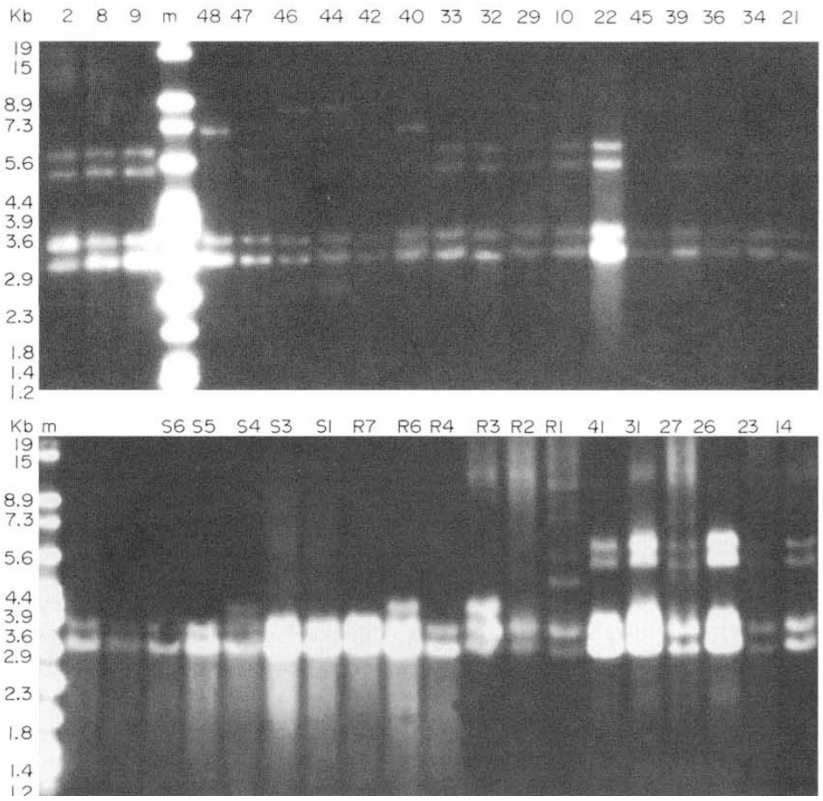

Fig. 4 Southern analysis of mtDNA of LJ3 and FT (name beginning by R or S) individuals. Total DNA was digested with *Bst*I and hybridized with 18S–5S wheat mt cloned DNA. m is the molecular weight marker, 'Raoul' Appligène (weight indicated on the right).

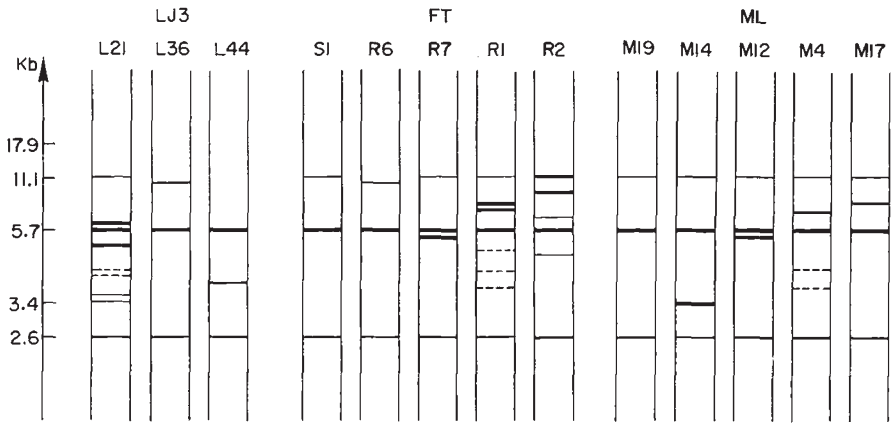

Fig. 5 Pictures of the mt DNA pattern of the 'mitotypes' of the three natural populations (LJ3, FT and ML) with *Hin*dIII enzyme and 18S–5S probe.

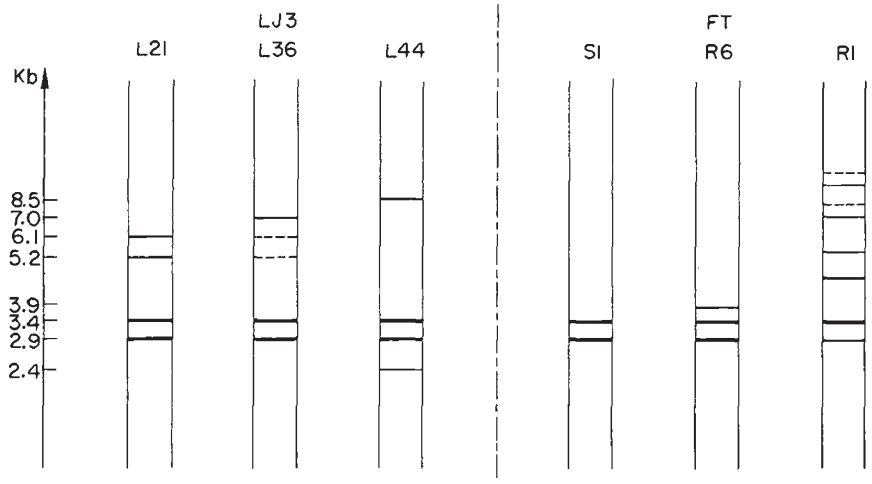

Fig. 6 Pictures of the mt DNA pattern of the 'mitotypes' of two (LJ3 and FT) of the three natural populations with *Bst*I enzyme and 18S–5S probe.

mitotypes 'S1', 'R7' and 'R2' (Table 2), which indicate that *Hin*dIII allows us to detect smaller differences than *Bst*I. For population ML, five mitotypes were found with *Hin*dIII (Fig. 5). Our method of analysis of mtDNA allows us to distinguish 13 mitotypes within those three stations. Two of the mitotypes were not specific to one station (S1 = M19 and R7 = M12). It is interesting however, to note that the enzyme *Bst*I distinguished 'L36' and 'R6' (Fig. 6).

The spatial distribution of mitotypes in natural populations of *Thymus vulgaris* L. was plotted for each individuals and is given in Fig. 7 for LJ3, the mitotype 'L21' was found only in female individuals and was highly dominant within the station (80 per cent of the individuals). The mitotype 'L36' was only found in three hermaphrodites (12 per cent of the individuals). Females and hermaphrodites had statistically different mitotypes in LJ3 (Fisher's exact test, $P < 0.0004$). It was not the case for ML and FT (Fisher's exact test, $P > 0.1$)). Furthermore, 'L21' mitotype was found to be spatially grouped in a patch of female (Fig. 7). The frequency of the 'L21' mitotype was significantly higher inside the patch of females than outside of it (Fisher's exact test, $P = 0.04$).

Organelle DNA analysis

The isolation of restrictable organelle DNA from thyme required severe modifications to the experimental procedure (see Materials and methods). The cpDNA isolated from three individuals belonging to different natural populations gave identical *Eco*RI patterns (data not shown). Chloroplastic membranes seemed very fragile and the DNAse step could not be achieved, even at low concentrations. Twenty-six bands are present, whose sizes range from 17 to 1.2 kb. Three bands, at 12.8, 3.8 and 3.1 kb respectively, display a double stochiometry, checked on microdensitometer tracings. The long inverted repeat carrying the ribosomal operon, present in the chloroplast genome of all tested families of higher plants except some *Leguminosae* (Palmer, 1985) and conifers seems, there-

Table 2 Distribution of the individuals in the classes of mitochondrial DNA pattern with *Hin*dIII and *Bst*I enzymes and 18S-5S probe. Bold numbers are names of the classes

La Jasse 3		Figaret		Montlaur
*Hin*dIII 18S–5S	*Bst*I 18S–5S	*Hin*dIII 18S–5S	*Bst*I 18S–5S	*Hin*dIII 18S–5S
L21	**L21**	**S1**	**S1**	**M19**
L21	L21	S1	S1	M19
L2	L2	S3	S3	M2
L8	L8	S6	S6	M3
L9	L9	R4	R4	M7
L10	L10	**R7**	R7	M8
L14	L14	R7	S5	M9
L22	L22	S5	R2	M10
L23	L23	**R2**		M16
L26	L26	R2		M21
L27	L27			
L29	L29	**R6**	**R6**	
L31	L31	R6	R6	**M17**
L32	L32	S4	S4	M17
L33	L33	R3	R3	M18
L34	L34			
L39	L39	**R1**	**R1**	**M14**
L41	L41	R1	R1	M14
L42	L42			M6
L45	L45			
L47	L47			**M4**
L36	**L36**			M4
L36	L36			**M12**
L40	L40			M12
L48	L48			
L44	**L44**			
L44	L44			
L46	L46			

fore, to be present also in the labiate *Thymus vulgaris* L. All the other bands are present in a normal stoichiometry. The total length of the chloroplast genome has been calculated at about 140 kb and is in the range of values reported for the chloroplast genomes of higher plants carrying inverted long repeats.

In contrast to cpDNA, the mitochondrial DNA isolated from the same three individuals gave three different *Eco*RI patterns (Fig. 8). Owing to the very low amounts of purified mtDNAs, they were run to independent gels, as soon as obtained. The three patterns are markedly different from each other. There are 27–30 visible bands, with several ones in higher stochiometry and others in faint relative amounts. The sum of the molecular weights of the different bands are presented in Table 3; when the stochiometry is taken into account, genome sizes of 153, 165 and 185 kb, respectively, are found for the three individuals. These sizes range among the lowest values reported for higher plants. Nevertheless, the sizes we obtained are certainly underestimated. As an example, the first estimation of mitochondrial genome for *Brassicaceae* (180 kb; Lebacq & Vedel, 1981) was improved after cloning each band (210 kb; Palmer, 1988). A complete molecular cloning is required to ascertain exact genome sizes. However, it should be kept in mind that the three plants of *Thymus vulgaris* L analysed showed different mtDNA patterns. The DNA extraction must therefore be performed individually and, as samples cannot be pooled, the available mtDNA is limited to the small amount that can be obtained from a single individual.

Inheritance study

The inheritance of mitochondrial DNA in *Thymus vulgaris* L was therefore studied by using total cellular DNA isolation and Southern hybridizations. First attempts showed that the combination of *Bst*I as the restriction nuclease and the wheat mtDNA clone carrying the 26S rRNA gene (Falconet *et al.*, 1984, 1988) as the probe was convenient for RFLP identifications. We first checked that the three hermaphrodites used as fathers, h42, h74 and h132, had different mtDNA patterns from each other and that these were also different from that displayed by the female f24 (Fig. 9). The mtDNA patterns of the 11 offspring are shown in Fig. 10. The results summarized in Table 4 show that, except in one case, the mtDNA hybridization pattern of the offspring are all identical and are identical to that of female f24, whatever the father, the sexual phenotype of the offspring or the number of generations of successive crosses. The offspring that showed a different hybridization pattern was offspring 12 (derived from cross f24′ × h132). The same results were observed with 18S-5S ribosomal mtDNA probe (data not shown).

Discussion

The present work has shown significant variability in the mitochondrial DNA of the labiate *Thymus vulgaris* individuals, within and among natural populations. In such a case, it is clear that it would be very misleading to sample one individual in order to characterize all the species. Few studies have shown the existence of mtDNA polymorphism in natural populations and at such a level (11 mitotypes for 51 individuals sampled in three populations). In wild barley, Holwerda *et al.* (1986) found some variability among Mediterranean poplations. Intrapopulation analysis of mtDNA has been carried out on wild beet (*Beta maritima*, Boutin *et*

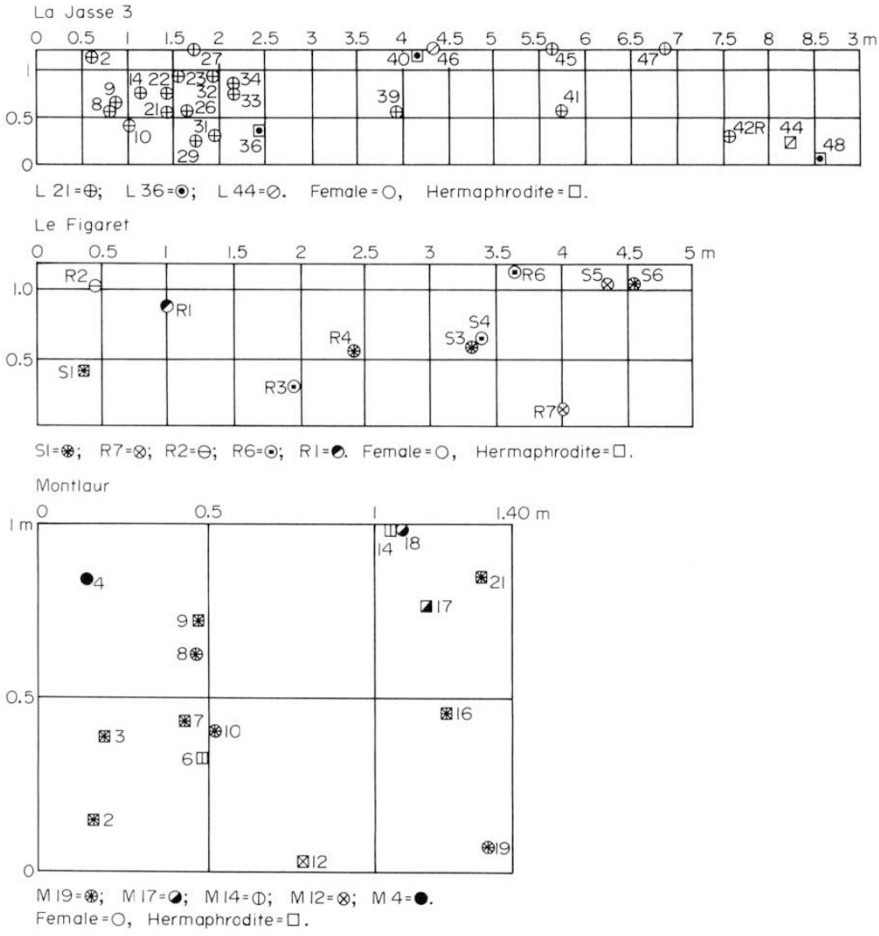

Fig. 7 Spatial distribution of individuals in their natural populations, with indications of their sexual phenotype and their mitotype. Distance are given in metres. Each symbol inside the circle (for female) or square (for hermaphrodite) represents a mitotype as described in Table 2.

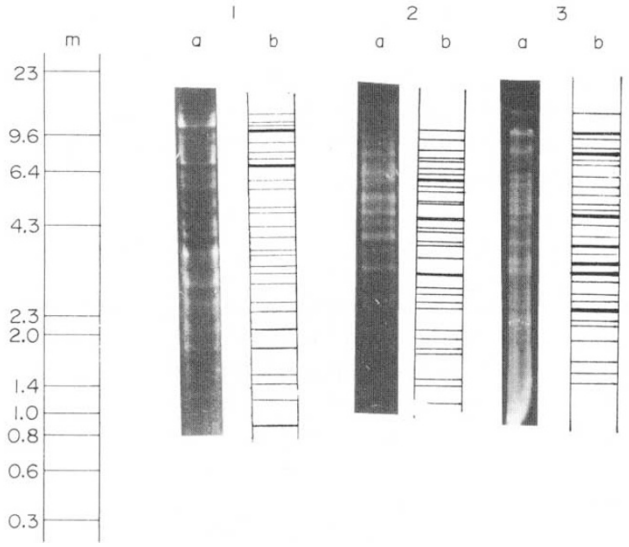

Fig. 8 *Eco*RI restriction patterns of *Thymus vulgaris* L. mtDNA, for three individuals 1, 2 and 3. Lane a: UV fluorescence pictures of BET-stained gel. Lane b: corresponding restriction diagrams. M: lambda *Hin*dIII marker DNA.

al., 1987) and plantain (*Plantago lanceolata*, Rowendal, 1987). In both species, two different mitotypes were found within a single natural population. For *Beta maritima*, recent work reveals the presence of the same two mitotypes in another population (Saumitou-Laprade, personal communication). The last two species mentioned are gynodioecious but frequencies of females in natural populations were found to be much lower than for *Thymus vulgaris*. The prediction of the existence of mtDNA polymorphism within natural populations of gynodioecious species, inferred from theoretical studies modelling the maintenance of females through nucleocytoplasmic interactions (Delannay *et al.*, 1981; Charlesworth, 1981; Gouyon & Couvet, 1985; Frank, 1988) was found to be well supported in *Thymus vulgaris*. In three natural populations of *Thymus vulgaris*, we detected 13 mitotypes with two restriction enzymes and one ribosomal probe. This probe was found to detect the most polymorphisms when compared with three other probes we screened, which still reveal important variability

Table 3 Molecular complexity of each mitochondrial genome shown in Fig. 2

1 Kb	2 Kb	3 Kb
12.6	10.8	13.2
11.4	9.2	**10.3***
10.9	8.3	9.9
10.2*	8.0	9.3
8.8	7.9	**8.7***
7.9	7.7	8.0
7.3	7.6	7.4
6.8*	**7.5***	6.7
5.9	7.4	6.2
5.5	7.1	5.8
4.9	6.5	5.5
4.7	6.3	5.2
4.3	**5.1***	**5.0***
4.0	4.8	4.6
3.7	4.8	4.0
3.6	4.5	**3.8***
3.3	4.4	3.6
3.2	4.0	**3.5***
2.9	**3.4***	**3.3***
2.6	3.1	3.0
2.4	3.0	2.8
2.1	2.8	2.7
1.9	2.6	**2.6***
1.6	2.3	2.4
1.5	2.2	2.3
1.3	2.0	2.1
0.9	1.9	1.9
	1.6	1.8
	1.5	1.6
	1.3	
153 Kb	165 Kb	185 Kb

mtDNA bands occuring in a double stochiometry.

amounting to about 70 per cent of that found with the 18S-5S probe. All the mitotypes except two were specific to the station (Table 2) and none of the mitotypes was found in all stations. Almost all the mitotypes were composed of bands with two different stochiometries (large or thin) and some of them displayed a third very low stochiometry (Figs 5 and 6). Those last bands could be interpreted as sublimons resulting from recombination events (Small *et al.*, 1989).

In the absence of genetic evidence obtained through controlled crosses, the existence of molecular differences does not imply a functional difference for male sterility genes. There are probably less functional types than molecular types. However, the relationship between sexual phenotype and mitochondrial type in

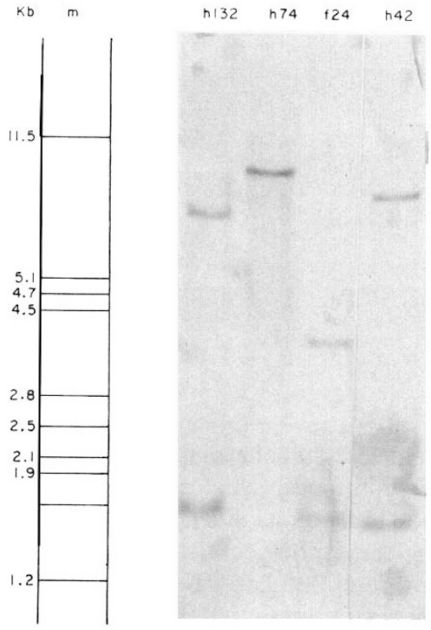

Fig. 9 Southern hybridizations of total DNA of female F24 and the three hermaphrodites h132, h74, h42 used as parents in inheritance study. Total DNA was digested with *Bst*I and hybridized to sulfonated 26S rDNA wheat mt cloned DNA. Females F17 and F14 showed the same hybridization pattern as female F24 (not shown). M: lambda *Pst*1 marker DNA.

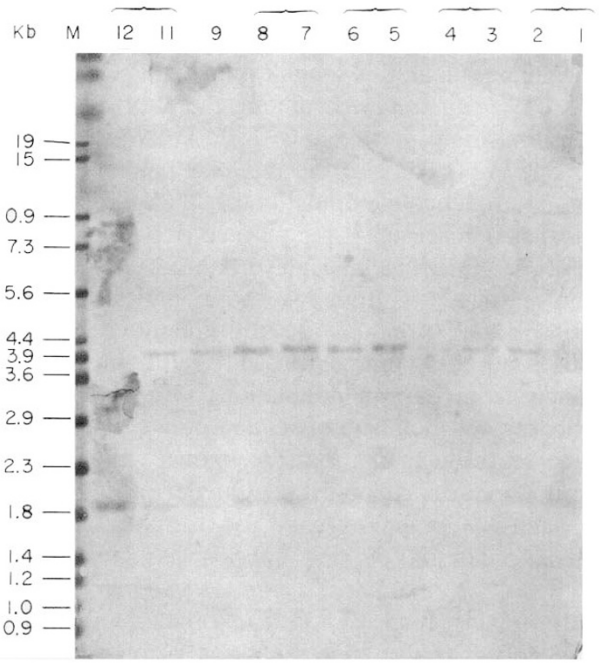

Fig. 10 Southern blot hybridizations of total DNA of the 11 offsprings. Same legend as Fig. 9. M: Raoul marker (Appligène, France). Brackets indicate offspring from the same parent.

Table 4 Summary of the results of the mitochondrial inheritance experiment in *Thymus vulgaris*

Father	h42	h42	h42	h42	h74	h132
Mother	f24	f24	f17	f14	f24	f24
Offspring (*n*)	1 2	3 4	5 6	7 8	9 10	11 12
Sex	f h	f h	f h	f h	f h	f h
ADNmt	MM	MM	MM	MM	M–	M?

f = female; h = hermaphrodite; M = Mother; – = mtDNA not successfully isolated; ? = restriction pattern different from the mother and the father.

population LJ3 (females and hermaphrodites had statistically different mitotypes; see Results) indicate that molecular and functional diversity could be related. It would be necessary to confirm with crosses that the 11 mitotypes described here are effectively 11 different male-sterility cytoplasmic genotypes.

In spite of the important difficulties due to the structure of the aromatic species (small leaves, high secondary compounds, etc.), we succeeded in the isolation of minute amounts of chloroplastic and mitochondrial DNAs from *Thymus vulgaris* L. The cpDNA size was estimated at about 140 kb, a value which falls within the range of genome sizes reported for the cpDNA of Angiosperms carrying two copies of the rRNA operon. No polymorphism was detected among the plants analysed. On the contrary, mtDNA was estimated at 153, 165 and 185 kb for the three plants analysed; although based on only one restriction endonuclease and one gel per plant, this rough estimate places the mt genome of *Thymus vulgaris* among the shortest ones reported for Angiosperms (180–2500 kb; Ward *et al.*, 1982). The complexity of the mtDNA patterns indicates an heterogeneous population of DNA molecules. The most striking result is the marked polymorphism, as the three individuals displayed three completely different patterns, with almost no common bands. These individual variations are also illustrated by the previous Southern hybridizations carried out on the 51 individuals from natural populations. The variations are probably not the result of sequence divergence but rather to rearrangements that are known to occur in plant mitochondrial genomes (see Quétier *et al.*, 1985). Such extensive polymorphism for the mtDNA in natural populations of one species has not been reported so far, making *Thymus vulgaris* a choice material in this respect. On the other hand, this extreme polymorphism necessitates a plant by plant study, any pooling of leaves of different individuals being ruled out for mtDNA extraction. This is a serious limitation as one plant gives only enough mtDNA for a single analysis.

In the light of the high level of polymorphism found, the analysis of mtDNA transmission is especially important, because biparental inheritance could give some explanation for the observed variability. This analysis was carried out on 11 offspring that were the grandchildren or the great grandchildren through maternal lineage of the same original female. Despite the number of generations and the fact that all fathers had differing mtDNA patterns from the mothers, all but one offspring present the same mtDNA hybridization pattern, suggesting that the transmission was maternal throughout the studied generations. Offspring no. 12, which presents a mtDNA pattern different from both the other study offspring and its father, could be either a mutation (or a recombination) of the mtDNA, or a contaminant seed. Unfortunately, no further genetic analyses were possible because this individual died before fruiting. The hypothesis of mutation or recombination is probable because of the high variability observed here in mtDNA of *Thymus vulgaris* and because this individual was one of those obtained after four generations. The maternal transmission of mtDNA does not seem to be influenced by factors studied here, such as the paternal mtDNA type, or the sexual phenotype of the offspring (female or hermaphrodite). The method used here does not allow a complete labelling of mtDNA through genetic transmission; indeed, only a part of the mitochondrial genome is studied with the probe 26S, and it is possible that the part of mtDNA not labelled will indicate some non-maternal transmission. However, we choose a restriction enzyme and a probe that reveal polymorphism among the fathers (Fig. 9), and thus the majority of paternally transmitted mtDNA would have been detected. Furthermore, identical results were obtained with the 18S-5S probe (data not shown). In conclusion, maternal inheritance of mtDNA in thyme is observed, whereas paternal transmission is not supported.

As biparental inheritance does not seem to be the cause of the observed polymorphism, it would be interesting to know how such a variability is generated.

The high potential for recombinational rearrangement of mtDNA circles through flip-flop or loop-out (Quétier *et al.*, 1985) could be one candidate mechanism. In this case, it would mean that the recombinational rate of thyme mitochondrial genome is especially high, constantly generating new mitotypes. One alternative explanation could be the maintenance of an ancestral polymorphism through frequency dependent selection. Such frequency dependent selection could result from the reproductive and ecological properties of thyme (A. Atlan *et al.*, unpublished data). Comparisons with other gynodioecious and non-gynodioecious species would reveal whether such high polymorphism is specific to the species *Thymus vulgaris* (or to the genus Thymus) and if it is related to the frequency of cytoplasmic male-sterility within a species.

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