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THE SPATIAL GENETIC STRUCTURE OF CYTOPLASMIC (CPDNA) AND NUCLEAR (ALLOZYME) MARKERS WITHIN AND AMONG POPULATIONS OF THE GYNODIOECIOUS *THYMUS VULGARIS* (LABIATAE) IN SOUTHERN FRANCE[1]

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Recent advances in molecular biology have allowed the development of techniques to contrast spatial differentiation in nuclear and cytoplasmic genes and thus provide important data on relative levels of gene flow by pollen and seed in higher plants. In this paper, we compare the spatial structure of nuclear (allozymes) and cytoplasmic (cpDNA) genes among populations of the gynodioecious *Thymus vulgaris* in southern France. Based on a combination of three restriction enzymes (CfoI, EcoRV, and PstI), eight chlorotypes (combination of three restriction enzyme patterns revealed by Southern hybridization of *Beta vulgaris* cpDNA) were identified in the 13 studied populations. One chlorotype was particularly abundant and was detected in nearly all populations. Only one chlorotype was specific to a single population. Up to four different chlorotypes were observed in some populations. An F_{ST} of 0.238 ($P < 0.002$) for cpDNA haplotypes indicates spatial structure of cytoplasmic genes among the studied populations. Similar patterns were found within a single young population (CAB) structured in patches and surrounded by a continuous cover of *T. vulgaris* where the F_{ST} is 0.546 ($P < 0.002$). No significant correlation between sex and chlorotype nor between cpDNA diversity and female frequency was detected. Allozyme markers showed markedly less spatial structure ($F_{ST} = 0.021$ among populations and 0.019 in the CAB population, $P < 0.001$). This difference between cpDNA and nuclear allozyme markers suggests that pollen dispersal is more important than seed dispersal both among and within populations.

Key words: allozymes; cpDNA; gene flow; gynodioecy; Labiatae; population structure; *Thymus vulgaris.*

Documenting spatial patterns of genetic variation is a central concern of plant evolutionary biology. Quantification of how neutral markers vary in space provides key information on how gene flow influences natural levels of genetic variation. As a result of much work on this issue, it is now well known that highly localized patterns of population differentiation occur in a large number of plant species (Schaal, 1975; Holwerda, Jana, and Crosby, 1986; Hamrick and Godt, 1989; Strauss, Hong, and Hipkins, 1993; McCauley, 1994).

The examination of patterns of spatial variation in plant populations has been dominated by two main approaches. First, documenting patterns of isozyme variation in natural populations has provided important data on population structure and gene flow in a wide range of species (Govindaraju, 1988; Barrett and Shore, 1989; Hamrick and Godt, 1989). Second, and more recently, the application of methods based on the characterization of nucleotide sequence variation has permitted more extensive and powerful analysis of spatial variation in both nuclear and cytoplasmic genomes. These two approaches have opened the way toward comparisons among nuclear (isozyme, nuclear DNA) and cytoplasmic (chloroplast and mitochondrial DNA) patterns of variation and, since the latter are generally uniparentally (maternally) inherited in angiosperms (Palmer, 1987), toward estimates of the relative importance of the pollen and seed components of gene flow. The finding that chloroplast DNA (cpDNA) is variable within species (Sytsma and Schaal, 1985; Lumaret, Bowman and Dyer, 1989; Soltis, Soltis, and Ness, 1989; Soltis et al., 1989; Saumitou-Laprade et al., 1991; Fenster and Ritland, 1992) has greatly facilitated these comparisons.

In contrast to allozyme markers, maternal inheritance of the cytoplasmic genome indicates that its transmission should not be affected by variation in the mating system (Palmer, 1987). In fact, patterns of differentiation in cytoplasmic genes should resemble those for nuclear genes in selfing species. In those species where seed dispersal is low compared to pollen dispersal (Campbell, 1991; Parra, Vargas, and Eguiarte, 1993), genetic differentiation among populations for maternally inherited cytoplasmic markers can be predicted to be higher than differentiation of nuclear markers (Birky, 1988). In fact, McCauley

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(1995) has found that G_{ST} values based on cpDNA variation may, as a result of different relative rates of transmission (nuclear genes are transmitted in pollen and seed, organelle genes in seeds only) and reduced effective population size, show higher levels of population differentiation than nuclear genes. Finally, Ennos (1994) reported how estimates of pollen flow/seed flow ratios can be obtained by combining studies of nuclear and cytoplasmic markers. Such ratios are often high but also very variable depending on the mating system of the study organism. However, the generality of such trends remains unknown due to a dearth of studies comparing the population genetic structure of nuclear and cytoplasmic markers on individuals in plant species.

In a previous study of isozyme variation in the gynodioecious *Thymus vulgaris* L. (characterized by coexistence of female and hermaphroditic plants), we reported, in a single region ~200 km^2 in southern France, that population differentiation is very slight (F_{ST} = 0.038) compared to average within-population differentiation (F_{IS} = 0.110) (Tarayre and Thompson, 1997). Mitochondrial DNA in the same study region shows high variation within and among populations: 11 mitotypes were identified in 51 individuals sampled in three populations by Belhassen et al. (1993) and 53 mitotypes in 86 plants from 13 populations studied by M. Tarayre (unpublished data). Young populations often have a very high (~90%) percentage of female plants (Dommée, Guillerm, and Valdeyron, 1983) structured in patches. In a study of four such populations, mitotypes were often found to be specific to a single patch of individuals within a population (Manicacci et al., 1996). Furthermore, in *T. vulgaris* populations, a mitotype was rarely observed in more than one population, even though the populations were only a few kilometres apart (Belhassen et al., 1993). This marked variability of mtDNA is most likely to be due to intragenomic rearrangements during recombination events, which are a common feature of higher plant mitochondrial genomes (Lonsdale et al., 1988; Palmer and Herbon, 1988). In *T. vulgaris*, the high variability of mtDNA (mainly due to rearrangements during recombination events of the mtDNA genome) poses problems for its use as a marker in studies of population structure and gene flow. Contrary to mtDNA in plants, cpDNA does not generally undergo rearrangement during recombination events and any diversity will be due to point insertion/deletion mutations. Hence the cpDNA genome may be sufficiently conserved and stable to permit phylogenetic studies (Soltis, Soltis, and Milligan, 1992). When sufficient infraspecific variability exists, this genome may also be a useful marker in population genetic studies. For *T. vulgaris*, Belhassen et al. (1993) found that cpDNA inheritance appears to be maternal, as reported in five other Labiates (Corriveau and Coleman, 1988). This maternal transmission may thus provide information on historical seed migration events among and within populations.

There has been no study of cpDNA variation and no comparative work on spatial variation in nuclear and cytoplasmic markers in *T. vulgaris*. The purpose of this work is twofold. First, we quantify cpDNA genetic diversity within and among populations. Second, we compare spatial variation in allozyme markers (nuclear markers inherited from both parents) quantified in a previous paper (Tarayre and Thompson, 1997) with this cpDNA variation in order to contrast gene flow by pollen and seed dispersal. This investigation was conducted on two spatial scales: (1) among populations (ranging from 250 m to 15 km apart) and (2) among patches (ranging from ~10 m^2 to 120 m^2 apart) surrounded by a continuous cover within a young population (<10 yr old) in which relatively recent migration events could be detected.

MATERIALS AND METHODS

Study species—*Thymus vulgaris* L. is a perennial species, common in disturbed habitats in southern Europe. It is a gynodioecious species, i.e., populations are composed of both female (male-sterile) and hermaphroditic plants (Darwin, 1877), the later being self-compatible (Valdeyron, Dommée, and Vernet, 1977; Assouad et al., 1978). In southern France, female frequency varies from 5 to 95%, depending on the age of the population and has a mean value ~60% (Dommée, Assouad, and Valdeyron, 1978; Belhassen et al., 1989). The genes responsible for the male sterility phenotype are cytoplasmic, while the restorer genes of male fertility are nuclear, i.e., the determination of sex is nucleocytoplasmic (Couvet, Bonnemaison, and Gouyon, 1986; Belhassen et al., 1991). The inheritance of mtDNA and cpDNA appears to be maternally inherited (Belhassen et al., 1991, 1993). The length of the total cpDNA in *T. vulgaris* has been estimated to be ~140 kb, from the EcoRI restriction pattern of the entire genome (Belhassen et al., 1993) and is in the range of those plant chloroplastic genomes that carry long inverted repeats (Palmer, 1985).

cpDNA variation—Five plants were sampled in each of 12 populations (A, C, G, VL, T, U, FH, FB, FT, FG, FL, and FF), which show marked variation in female frequency (from 26 to 79%) in and around the St-Martin-de-Londres basin (~20 km north of Montpellier in southern France). In addition, we sampled 31 plants in one population located in the St-Martin-de-Londres basin at the Cabane-La-Plaine population (CAB), in which the spatial distribution of mtDNA was studied by Manicacci et al. (1996), and which, in 1989, contained several well-defined patches of females. These patches now overlap, but female frequencies remain high (>80%). For this study, we sampled 11, five, and five individuals in the three patches (CA, CC, and CG), respectively, and ten plants in the extensive, more continuous cover of *T. vulgaris* (CO) that surrounds the patches. In order to quantify any correlation between cpDNA diversity and female frequency, the sexual phenotype of almost all the individuals was noted and the proportion of female plants in each population recorded.

Characterization of chloroplast genetic information was performed by restriction fragment length polymorphism (RFLP) analysis using complete *Beta vulgaris* cpDNA as a probe hybridized on restricted total DNA extracts from *T. vulgaris*. Total DNA of all the *T. vulgaris* plants was extracted according to Dellaporta, Wood, and Hicks (1983) and Saumitou-Laprade et al. (1993). One microgram of DNA was digested by restriction endonucleases and the different fragments separated by electrophoresis on 0.8% agarose gels in TAE buffer (40 mmol/L Tris, 20 mmol/L NaAcetate, 2 mmol/L EDTA) and transferred to a nylon membrane (Biodyne A from Pall Filtrations Technik GmBH) using the vacuum-blot system of Pharmacia. After transfer the DNA was UV cross-linked (1.2 J/cm^2) to the nylon membranes.

The complete cpDNA from *Beta vulgaris* was labeled with digoxigenin-d-UTP (DNA labeling and Detection Kit Non radioactive, Boehringer, Mannheim, Germany). The probe was hybridized overnight at 68°C and variation in restriction fragment length was revealed by immunological detection and chemiluminescence using CSPD from Tropix (as described in Saumitou-Laprade et al., 1993).

Total DNA was digested with several restriction endonucleases and Spermidine (2 mmol/L) was added to the reaction buffer to facilitate

digestion. Among eight restriction enzymes (BglI, CfoI, EcoRI, EcoRV, HaeIII, HindIII, PstI, and XhoI), three were used in a single digest with the following concentration of restriction enzyme, i.e., CfoI (6 U/μg), EcoRV (8 U/μg), and PstI (8 U/μg) (U = Unit of restriction enzyme). These were selected because they showed clear banding patterns and variations in the high molecular mass (from 23.2 to 9.3 kb). For the cpDNA probe, the entire chloroplastic genome of *Beta vulgaris* was used as a probe (Forcioli et al., 1994).

Allozyme variation—To quantify allozyme variation within and among the same populations, ~30 plants were randomly sampled in ten of the above populations. In the Cabane-La-Plaine population, ten plants in each of the CA, CC, and CG patches and 20 plants in the continuous cover of *T. vulgaris* around the patches were sampled.

For protein extraction, 0.5 g of leaf material per plant was ground in a Tris-HCl buffer (Yacine and Lumaret, 1988). Four polymorphic, non-duplicated loci were resolved and used in the analysis of population genetic diversity and structure. These loci are two glutamate oxaloacetate transaminases loci (GOT-1 and GOT-2, E.C.2.6.1.1), one leucine aminopeptidase locus (LAP-1, E.C.3.4.1.1), and one phosphoglucomutase locus (PGM-2, E.C.2.7.5.1). The enzyme staining procedure for these loci has been well developed by various authors, i.e., GOT-1 and GOT-2 by Lumaret (1981), LAP-1 by Scandalios (1969), and PGM-2 by Second (1982). An analysis of maternal parent progeny from open-pollinated parents indicated that the control of these four loci follows a Mendelian pattern (M. Tarayre, unpublished data).

Data analysis—Cytoplasmic DNA polymorphism was quantified per restriction enzyme (CfoI, EcoRV, and PstI) and also by the combination of these three enzymes. Each variant pattern was scored and assigned a unique number code designation corresponding to one haplotype.

Nei's (1987) genetic diversity statistics of $H = 1 - \Sigma\ pi^2$, where pi is the frequency of the *i*th allele, were calculated in each population (H_S) and over all populations (H_T). These two indices were evaluated for both allozyme and cpDNA markers and because of their different pattern of transmission, biparental and uniparental, respectively, the expectations are given by $2n(1 - \Sigma pi^2)/(2n - 1)$ for allozymes and by $n(1 - \Sigma pi^2)/(n - 1)$ for the cpDNA.

Wright's (1951) *F* statistics index of population differentiation (F_{ST}) were estimated following the formula of Weir and Cockerham (1984). *F* statistics were calculated using the Fstat computer program adapted for both diploid and haploid data (Goudet, 1995). Means and standard deviations for both markers were calculated by jackknifing across populations.

Assuming a migration-drift equilibrium and the absence of selection on the genetic markers used, the number of migrants (*M*) per generation among populations can be estimated from the F_{ST} values as follows. For biparentaly inherited genes (allozymes in our study) $M = (1/F_{ST(b)} - 1)/4$ (Wright, 1969), where $F_{ST(b)}$ is population differentiation for the biparentally inherited genes. For maternally inherited genes (cpDNA in our study) $M = (1/F_{ST(m)} - 1)/2$ (Birky, Fuerst, and Maruyama, 1989), where $F_{ST(m)}$ is population differentiation for maternally inherited genes.

Following Ennos (1994) an estimate of the pollen/seed flow ratio (*P*/*S*) can be derived, comparing these two estimates of the number of migrants as

$$P/S = [A(1 + F_{IS}) - 2C]/C, \text{ where } A = (1/F_{ST(b)} - 1) \text{ and } C = (1/F_{ST(m)} - 1) \quad (1)$$

where F_{IS} represents the extent of inbreeding within populations.

This formula is valid for hermaphroditic species. For dioecious species, $P/S = (A\text{-}4C)/2C$ because in such species $M = 1/(F_{ST(m)} - 1)$ (Birky et al., 1983). For a gynodioecious species, the relationship between the *P*/*S* ratio and the values of *A* and *C* depends on the inheritance of sex, and the frequency of females (V. Laporte and D. Couvet, unpublished data). However, for the range of female frequencies considered in the present study (Table 5), a value higher than 10 for the ratio $(A\text{-}2C)/C$ indicates that the *P*/*S* ratio is higher than 1 in every case. We thus used Eq. 1 in our analyses.

TABLE 1. Restriction patterns (presence/absence = +/−) of the cpDNA genome as revealed by hybridization with the *Beta vulgaris* cpDNA probe for the three polymorphic restriction enzymes (CfoI, EcoRV, and PstI) found among 13 populations of *Thymus vulgaris* in the St-Martin-de-Londres region in southern France. Only variable fragments are indicated in this table. Total number of analyzed plants = 86. Profile frequencies are shown in parentheses below restriction pattern abbreviations.

CfoI					EcoRV			PstI				
Fragment size (kb)	Restriction pattern				Fragment size (kb)	Restriction pattern		Fragment size (kb)	Restriction pattern			
	C1 (0.88)	C2 (0.08)	C3 (0.02)	C4 (0.02)		E1 (0.94)	E2 (0.06)		P1 (0.79)	P2 (0.14)	P3 (0.13)	P4 (0.04)
7.3	−	−	+	−	23.2	+	+	19.8	−	−	+	−
6.8	+	−	−	+	21.5	+	−	16.2	−	−	+	−
6.3	+	+	+	−	18.4	−	+	15.7	−	+	−	−
								11.9	−	−	−	+
								10.0	+	+	−	+
								9.3	+	+	+	−

RESULTS

cpDNA variation—Table 1 illustrates the different restriction patterns obtained by southern hybridization with the heterologous cpDNA probe for each of the three restriction enzymes (CfoI, EcoRV, and PstI) from the 86 sampled plants over all populations. For each restriction enzyme, the different profiles were determined on the basis of presence and absence of the identified restriction fragments. Shared fragments were not considered. The distinct restriction patterns identified concern a mutation only detected by one restriction enzyme for each identified chlorotype. This suggests that the occurrence of the different variants is mainly due to restriction site mutations. Indeed, after hybridization with the entire chloroplast genome of *Beta vulgaris*, certain relatively large complementary restriction fragments were not observed compared to another profile for the same restriction enzyme.

For the 86 plants, four, two, and four different restriction profiles were found with the CfoI, EcoRV and PstI enzymes, respectively. Figure 1 illustrates the hybridization patterns obtained for the PstI enzyme. Over all the 13 studied populations, a dominant restriction pattern was identified for each of the three restriction enzymes used, with the frequencies C1 = 0.88, E1 = 0.94, and P1 = 0.79 for the CfoI, EcoRV, and PstI enzymes, respectively (Table 1).

Based on the combination of the three restriction enzymes, eight individual ''chlorotypes'' could be identified (Table 2). Two of these ''chlorotypes'' (I and II) are more widespread than the six others. The frequency of the widespread chlorotypes is 0.59 and 0.14, respectively, whereas the frequency of the remaining chlorotypes varied between 0.08 and 0.02 (Table 2; Fig. 2). The prevalent chlorotype I was found in ten of the 13 studied populations, in five of which (FH, FT, FG, FL, and FF) this chlorotype was carried by all the five sampled individuals. These five populations are situated along a transect running across the St-Martin-de-Londres basin up to the limestone plateaus and can be distinguished by the pre-

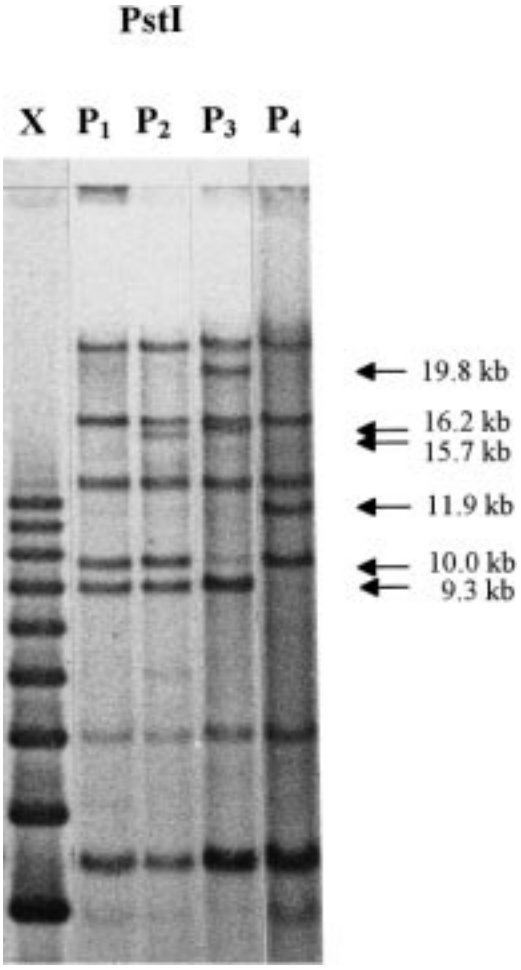

Fig. 1. Example of southern hybridization of *Beta vulgaris* cpDNA to the total DNA digest with PstI restricted enzyme observed in *Thymus vulgaris* survey. Lane X: DNA molecular mass marker X (Boehringer Mannheim) and Lanes P1–P4: the four different restriction patterns.

dominance of different chemotypes. Only the FB population contained more than one chlorotype, including chlorotype I (Fig. 2).

Only chlorotype VIII was specific to a single population (A), the seven others being found in at least two populations (Table 3a). Four different chlorotypes were observed among the five and 31 sampled individuals in T and CAB populations (Fig. 2), respectively. The presence of identical chlorotypes in different populations was not always due to the spatial proximity of such populations, since identical chlorotypes were found in distant populations (Fig. 2).

Four different chlorotypes (I, II, IV, and VII) were identified at the Cabane-La-Plaine (CAB) population (Fig. 2), where chlorotype I was again the predominant type. This chlorotype was, however, absent in the CG patch (Table 3b; Fig. 3). Only one chlorotype was found in the CC and CG patches (I and VII, respectively). In this population, the chlorotype VII was specific to the CG patch and not found in the plants sampled from the continuous cover of plants surrounding the patches. This chlorotype is, however, present in two other populations (G, T) (Table 3a; Fig. 2).

TABLE 2. Description and frequency of the eight chlorotypes using a combination of the different restriction patterns obtained from three restriction enzymes (CfoI, EcoRV, and PstI) on 86 individuals of *Thymus vulgaris* sampled in the St-Martin-de-Londres region in southern France.

CfoI	EcoRV	PstI	Chlorotype	Frequency (N = 86)
C1	E1	P1	I	0.593
C1	E1	P2	II	0.140
C1	E1	P3	III	0.035
C1	E1	P4	IV	0.035
C1	E2	P1	V	0.081
C2	E1	P1	VI	0.023
C3	E1	P1	VII	0.070
C4	E1	P1	VIII	0.023

Spatial structure of cytoplasmic and nuclear markers—At both the among and within population levels, genetic diversity statistics (H_S and H_T) and F_{IS}, F_{IT}, and F_{ST}, values, corresponding to Weir and Cockerham's (1984) "θ", are summarized in Tables 4–6 for both cpDNA and allozyme markers.

For cpDNA, within-population and total diversities were relatively high for the 13 analyzed populations (Table 4), although a large part of the genetic diversity occurred among populations (H_S/H_T = 0.480). In contrast, for allozymes, estimated within population and total genetic diversities, most variation occurs within populations (H_S/H_T = 0.960). We thus observed marked population differentiation for cpDNA (F_{ST} = 0.238, $P < 0.002$), and less for allozymes (F_{ST} = 0.019, $P < 0.001$). The different genetic parameters are relatively similar for three of the four studied loci, although GOT-2 was less polymorphic than the others over all populations considered (Table 5).

Furthermore, both female and hermaphroditic phenotypes carried each chlorotype (Table 3a, b) and no significant correlation was found between cpDNA diversity and female frequency, while a negative correlation was expected with founder effect (r = −0.482, P = 0.09, Table 6). The number of migrants per generation (M) estimated with the F_{ST} values (Wright, 1969) was 1.6 and 11.65 for the cpDNA and allozyme markers, respectively. Furthermore, the high value of the estimator for the ratio of pollen to seed flow (14) indicates that gene flow via pollen is greater than that via seeds among these *T. vulgaris* populations.

Within the CAB population, cpDNA showed some variation within patches, but this variation remained lower than that calculated for allozymes (Table 4). In contrast, the total diversity in the CAB population was greater for cpDNA than for allozymes. For allozymes, the H_S and H_T values were of roughly the same order in the Cabane-La-Plaine population. The patches and the continuous cover of *T. vulgaris* exhibited marked differentiation among themselves for the cpDNA (F_{STcp} = 0.546), and an absence of structure of the allozyme markers (F_{ST} = 0.019). The number of migrants per generation (M) was estimated as 0.42 and 12.91 for cpDNA and allozymes, respectively, among patches and continuous cover combined. Finally, at this small scale of a few metres, the estimator of the ratio pollen/seed flow from Ennos (1994) gives a value of 60, again indicating that pollen migration is greater than seed migration (Table 4).

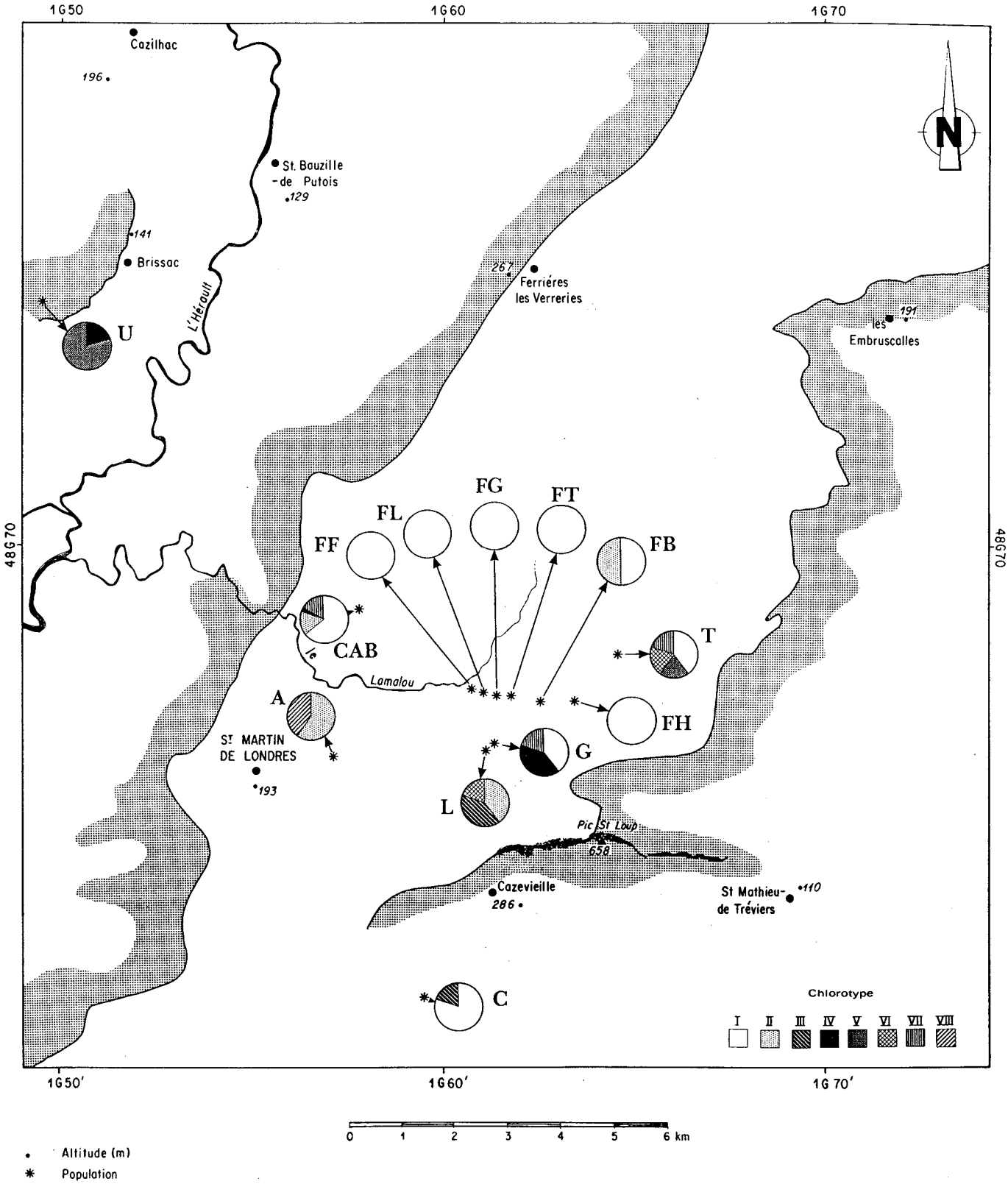

Fig. 2. Distribution of the eight chlorotypes in the 13 populations of *Thymus vulgaris* in the St-Martin-de-Londres region. See Table 5 for identification of population codes.

TABLE 3. Distribution of the different chlorotypes of *T. vulgaris* (a) in the 13 populations, and (b) within the CAB population, in the St-Martin-de-Londres region. The proportion of both sexual phenotypes (hermaphrodite, female) carrying each chlorotype is also reported. n_{cp} represents the total number of different chlorotypes in each population and *N* the sample size.

(a) 13 populations of *T. vulgaris*

	Population														Her-maphro-dites	Females
Chlorotype	A	C	G	VL	T	U	FH	FB	FT	FG	FL	FF	CAB	Total		
I	—	4	2	—	2	—	3	2	4	5	4	5	20	51	0.51	0.49
II	3	—	—	2	—	—	—	2	—	‘—	—	—	5	12	0.50	0.50
III	—	1	—	2	—	—	—	—	—	—	—	—	—	3	0.33	0.67
IV	—	—	2	—	—	1	—	—	—	—	—	—	1	4	0.25	0.75
V	—	—	—	—	1	4	—	—	—	—	—	—	—	5	0.60	0.40
VI	—	—	—	1	1	—	—	—	—	—	—	—	—	2	0.50	0.50
VII	—	—	1	—	1	—	—	—	—	—	—	—	5	7	0.43	0.57
VIII	2	—	—	—	—	—	—	—	—	—	—	—	—	2	0.50	0.50
n_{cp}	2	2	3	3	4	2	1	2	1	1	1	1	4			
N	5	5	5	5	5	5	3	4	4	5	4	5	31	86		

(b) Cabane-La-Plaine (CAB)

	Patches						
Chlorotype	CA	CC	CG	CO[a]	Total	Hermaphrodites	Females
I	9	5	—	6	20	0.45	0.55
II	2	—	—	3	5	0.40	0.60
III	—	—	—	—	—	—	—
IV	—	—	—	1	1	—	1
V	—	—	—	—	—	—	—
VI	—	—	—	—	—	—	—
VII	—	—	5	—	5	0.40	0.60
VIII	—	—	—	—	—	—	—
n_{cp}	2	1	1	3	4		
N	11	5	5	10	31		

[a] Continuous cover.

DISCUSSION

cpDNA variation—In recent years, spatial structure of cpDNA variation has been detected in a range of plant species (see review Soltis, Soltis, and Milligan, 1992). However, very few studies of cpDNA variation have been conducted at the population level, or on a regional scale, most prior studies having been carried out on a wide geographic scale (Milligan, 1991; Fenster and Ritland, 1992; Hooglander, Lumaret, and Bos, 1993; Petit, Kremer, and Wagner, 1993). In the present study, we have demonstrated, at a scale of 200 km^2, high levels of cpDNA diversity within and among *Thymus vulgaris* populations despite the fact that only five plants were sampled in each population. Indeed, four different chlorotypes were observed in a sample size of only five plants in one population (Fig. 2). With a mean number of ~1.8 chlorotypes per population, *T. vulgaris* thus shows similar amounts of cpDNA variability as the gynodioecious *Beta vulgaris* ssp. *maritima* (Forcioli, 1995). Chlorotype I occurred in almost all the studied populations and in 59% of all plants studied (Table 2). Several populations showed only this chlorotype in the sample of five plants (Fig. 2).

Another feature of our results is that even in individual patches sampled in a single recently founded population (<10 yr old) at Cabane-La-Plaine, we were able to identify two chlorotypes in a single patch (Fig. 3). Both of these chlorotypes were also detected in the continuous cover surrounding the patches. In contrast, the CG patch in this population contained a single chlorotype not found in either of the other patches or in the continuous cover of *T. vulgaris*. This chlorotype (VII) was, however, found in two other more distant populations. It is nevertheless likely that this chlorotype exists in the continuous cover of the CAB population but was not detected due to the low sample size. The CG patch thus appears to have been founded by a single maternal lineage because it contains a rare chlorotype. In the CC and the CA patches, which have the predominant chlorotype I, it is difficult to detect colonizing events, which may have occurred several times. A similar argument could be developed for the ‘‘monomorphic’’ FH, FT, FG, FL, and FF populations. The fact that almost all the chlorotypes detected in the recently founded CAB patches are also present in the surrounding continuous cover (CO) of *T. vulgaris* suggests that the colonists involved in a given founding event are drawn primarily from the nearest source, due to the relatively short distances (i.e., few metres) travelled by *T. vulgaris* seeds (Mazzoni and Gouyon, 1985), which have no particular features favoring their dispersal (Assouad, 1972). Thus most recorded colonization events occur in proximity to established populations, as is the case for many species colonizing open areas (Barrett and Shore, 1989; Warwick, 1990; Antonovics et al., 1994). This also means that colonization in species such as *T. vulgaris* is likely to fit the ‘‘propagule pool’’ model of Slatkin (1977), with colonists coming from a single source population.

Our results illustrate, in parallel with other recent studies, that cpDNA may show marked variation, even at the population level (Systma and Schaal, 1985; Soltis, Soltis, and Ness, 1989; Soltis et al., 1989; Saumitou-Laprade et

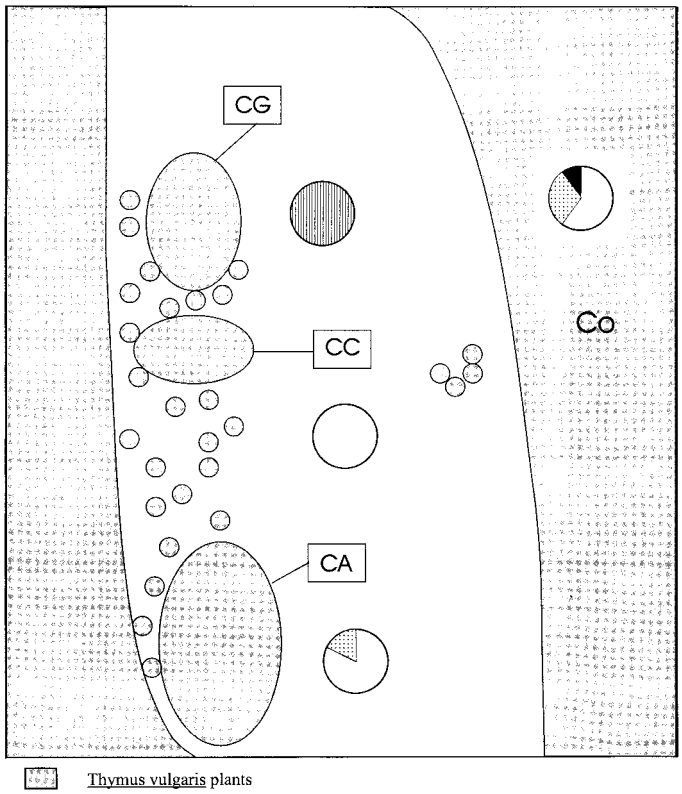

Fig. 3. Distribution of the chlorotypes in the three patches (CA, CC, and CG) and in the continuous cover (CO) of *Thymus vulgaris* in a young population at Cabane-La-Plaine in the St-Martin-de-Londres basin.

al., 1991; Hooglander, Lumaret, and Bos, 1993; McCauley, 1994). In fact, cpDNA variation in *T. vulgaris* is particularly marked given the highly localized geographic scale of the study (ranging from 250 m to 15 km). Over a similar geographic scale, i.e., <25 km between the most widely separated populations, McCauley (1994) found considerable spatial structure in cpDNA in the dioecious *Silene alba* (F_{ST} = 0.674). In the gynodioecious *Beta vulgaris* ssp. *maritima*, marked population structure between regions in France (F_{ST} = 0.43) and within regions (F_{ST} values ranging from 0.34 to 0.75). In contrast, in three Michigan populations of *Trifolium pratense*, which has a unique cpDNA structure, Milligan (1991) recorded 22 different cpDNA genotypes using a single enzyme (HindIII) detecting 11 variants in 39 individuals sampled in a single population. Milligan (1991) found that 7–10 chlorotypes were limited to a single population, hence population differentiation was low (F_{ST} = 0.022). The few investigations of cpDNA diversity in gynodioecious species tend, however, to show that these species contain higher cpDNA variability and greater population differentiation for such markers than other outcrossing species (R. Petit, personal communication). In *Thymus vulgaris*, localized cytoplasmic structure may be high due to a combination of low seed dispersal and the fact that females produce 2–4 times more seed than hermaphrodites. A result of these two features of the biology of *T. vulgaris* is that patches of females with the same cytoplasm may develop. Hence, both cpDNA (this study) and mtDNA (Manicacci et al., 1996) may show marked spatial differentiation.

TABLE 4. Within-population (H_S) and total population (H_T) genetic diversity and F_{ST} values (Weir and Cockerham, 1984) based on a sample of 13 cpDNA and 11 allozyme populations of *Thymus vulgaris* in the St-Martin-de-Londres region. The same parameters were calculated for the Cabane-La-Plaine population among the three patches and the continuous cover of *T. vulgaris* (CO). Pollen flow/seed flow ratios (*P*/*S*) were calculated following Ennos (1994).

Marker	Genetic parameter	Among populations	Cabane-La-Plaine
cpDNA	H_S	0.295	0.197
	H_T	0.614	0.531
	F_{ST} (SD)	0.238 (0.146)	0.546 (0.466)
Allozymes	H_S	0.334	0.293
	H_T	0.348	0.309
	F_{ST} (SD)	0.021 (0.07)	0.019 (0.035)
	P/*S*	13.55	59.41

TABLE 5. Genetic diversity within (H_S) populations and total genetic diversity (H_T) (Nei, 1973). *F* statistics follow Weir and Cockerham (1984) for four polymorphic loci in 11 populations of *Thymus vulgaris.*

Locus	H_S	H_T	H_S/H_T	F_{IS}	F_{IT}	F_{ST}
GOT-1	0.251	0.257	0.976	0.095	0.103	0.010
GOT-2	0.034	0.035	0.979	−0.026	−0.019	0.007
PGM-2	0.644	0.669	0.963	0.056	0.081	0.027
LAP-2	0.405	0.429	0.945	0.080	0.093	0.014
Mean	0.334	0.348	0.966	0.068	0.086	0.019

cpDNA and female frequency—In *T. vulgaris*, none of the eight chlorotypes were consistently associated with a particular sexual phenotype in the 13 different study populations (Table 3). Furthermore, we found no significant correlation between (a) female frequency in each population and chloroplast diversity (r = −0.482, P = 0.09), or (b) female frequency and the diversity of the mitochon-

TABLE 6. Cytoplasmic (cpDNA) and allozyme diversity (H_S) within the 13 *Thymus vulgaris* populations, differing in female frequency, in the St-Martin-de-Londres region.

Population	Female frequency (%)	cpDNA H_S	Allozyme H_S
La Borie (A)	26	0.60	0.367
Versant Linalol (VL)	34	0.80	—
Colline Géraniol (G)	50	0.80	0.334
Les Camps (T)	50	0.90	0.361
Fesq-Linalol (FL)	51	0.00	0.315
Fesq-Fin (FF)	64	0.00	0.310
Fesq-Thymol (FT)	65	0.00	0.319
Fesq-Haut (FH)	68	0.00	0.372
Notre-Dame-du-Suc (U)	72	0.40	0.365
Le Seuil (C)	76	0.40	0.367
Fesq-Géraniol (FG)	76	0.00	0.315
Fesq-Bas (FB)	79	0.67	—
Cabane-de-la-Plaine (CAB)	87	0.55	0.305
Patch A (CA)	86	0.33	0.282
Patch C (CC)	97	0.00	0.285
Patch G (CG)	93	0.00	0.379
Continuous cover (CO)	83	0.60	0.279

drial genome ($r = -0.101$, $P = 0.755$) for the same individuals (M. Tarayre, unpublished data). These two results suggest that the cpDNA types are not related to different functional male sterilities. In contrast to some agronomic species, such as tobacco (Frankel, Scowcroft, and Whitfield, 1979), cotton (Galau and Wilkins, 1989), and sorghum (Chen et al., 1990), where the genetic basis of male fertility is probably related to cpDNA.

Comparison of genetic diversity and population structure using cpDNA and allozymes—In our study of *T. vulgaris,* as in most other studies of cpDNA, diversity was more pronounced among populations than within populations (for review see Forcioli, 1995). In the three young patches (CA, CC, and CG) in the CAB population, the H_S cpDNA values were relatively smaller than in the 12 populations or in the continuous cover of *T. vulgaris* in CAB population (Table 5). This low diversity within patches probably reflects recent colonization by few plants (see Manicacci et al., 1996). In the CAB population, the H_T value may be relatively high because the continuous cover was included. In contrast, for allozymes, the genetic diversity within populations was considerable and even within patches it remained relatively high. This is likely due to high outcrossing occurring between patches and the surrounding continuous cover (see below).

The most interesting feature of the cpDNA differentiation among populations of *T. vulgaris* detected in the present study is that the F_{ST} values for cpDNA (F_{ST} = 0.238, SD = 0.146) is ten times greater than that observed for allozymes (F_{ST} = 0.021, SD = 0.07). Furthermore, the same magnitude of difference between cpDNA and allozyme F_{ST} values was also observed among the three patches and the continuous cover of *T. vulgaris* (F_{ST} = 0.546, SD = 0.466 and F_{ST} = 0.019, SD = 0.035, respectively) in the substructured CAB population. Based on the above F_{ST} values, we were able to estimate the ratio of pollen to seed flow (P/S) based on the formula of Ennos (1994). This indicates that pollen is dispersed 11- to 14-fold more than seeds in *T. vulgaris* in the populations surveyed. In the CAB population, this ratio is greater (P/S = 59.41), perhaps due to low seed dispersal and greater pollen flow between the patches and the continuous cover of *T. vulgaris.* Furthermore, the continuous cover may serve as a unique pollen source for the different patches, this increasing estimates of pollen flow at this site.

The processes operating thus reflect the colonization and extinction of local populations, the development of population structure, concomitant changes in effective population size, and potential differences linked to variation in female frequency among the studied populations. What our data indicate is that gene flow via pollen is higher than that via seed. This is a particularly appealing explanation since *T. vulgaris,* although primarily bee pollinated, is often visited by various Lepidoptera species, which can fly relatively long distances (J.D. Thompson and M. Tarayre, unpublished observations). The few other studies that have been carried out on this issue show a similar trend, despite the range of breeding systems involved (Ennos, 1994; McCauley, 1994).

The different F_{ST} values obtained for nuclear and cytoplasmic markers may also, in combination with differences in gene flow by pollen and seed, be due to differences in the impact of founder events on cytoplasmic and nuclear F_{ST} values. Birky (1988) has shown that the approach to equilibrium following perturbation or founding events should occur at different rates in organelle and nuclear genes. McCauley, Raveill, and Antonovics (1995) have shown in *Silene alba* that recently founded populations share much greater levels of population differentiation than older populations. This structure was related to a mode of colonization in which there is only a limited mixing of individuals from diverse sources. In our study, F_{ST} was higher among patches at the young CAB population than among populations. Hence colonization appears to increase population differentiation in our study region. We also found that, although the correlation between female frequency, which is correlated with population age in this species (Dommée, Assouad, and Valdeyron, 1983; Belhassen et al., 1989), and cpDNA diversity was not significant, it was nevertheless negative and bordered on significance—hence the tendency was in the expected direction. This higher initial diversity among populations may be due to colonization by different sources and limited mixing—hence the patch structure in the CAB population (Fig. 2, plus Manicacci et al., 1996).

Perturbations and founder events in populations of *T. vulgaris* in the region studied may thus be an important component of the genetic structure of natural populations (Belhassen et al., 1991, 1993; Manicacci et al., 1996). Given the rapid colonization by females that often occurs due to greater seed set by females in this species and the fact that in young populations sex determination may be cytoplasmic due to an absence of the appropriate nuclear restorer allele during colonization, local cytoplasmic differentiation resulting from the foundation of new populations may be maintained for long periods of time, and thus observed in older populations. Due to gene flow via pollen, nuclear differentiation may be much less in older populations, as we observed.

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