Original article

Gynodioecy and mitochondrial DNA polymorphism in natural populations of *Beta vulgaris* ssp *maritima*

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Summary – Gynodioecious populations of *Beta vulgaris* ssp *maritima* were found all along the French coasts; 42% of the populations were gynodioecious. Locally, there were large sex-ratio differences between populations, ranging from 0 to 76% of females. This large variation was even found between populations less than 1 km apart. Molecular analysis of mitochondrial polymorphism revealed a high variability; 11 different mitochondrial types were found. The Nvulg type, which is the most frequent type among the maintainers of male sterility used in sugar beet breeding programs, was also the most frequent type in natural populations. Conversely, the characteristic pattern of the Owen CMS (cytoplasmic male sterility), *ie* the Svulg type, was not found. The mitochondrial variability detected within the populations was large; there were 2.10 different mitotypes on average among the 5 individuals sampled per population. There was a highly significant overall difference in mitotype frequencies between populations ($Fst = 0.466; P < 0.001$), distributed both between ($Fst = 0.138; P < 0.001$) and within regions ($Fst = 0.381; P < 0.001$). Female plants were found with almost all of the different mitotypes. Nevertheless, 2 groups of mitotypes were clearly distinguished: the mitotypes Nvulg, A and B were rarely associated with the female phenotype, whereas female plants were often found among the plants having the mitotypes E and G.

sex-ratio variation / gynodioecy / cytoplasmic male sterility / mtDNA polymorphism / *Beta vulgaris* ssp *maritima*

Résumé – **Gynodioécie et polymorphisme mitochondrial chez *Beta vulgaris* ssp *maritima*.** *Des populations gynodioïques sont rencontrées dans toutes les régions prospectées (42% des populations étudiées). Localement, la fréquence des plantes femelles varie fortement entre les populations, depuis une complète absence jusqu'à 76% de femelles. Cette forte variation se rencontre entre populations proches, distantes de moins d'1 km. L'analyse du polymorphisme de l'ADN mitochondrial a révélé l'existence de 11 types mi-*

tochondriaux. Le mitotype Nvulg, caractéristique des mainteneurs de stérilité mâle utilisés dans les programmes d'amélioration de la betterave sucrière, est le type le plus répandu dans les populations naturelles. En revanche, le type Svulg, rencontré chez les plantes de cytoplasme CMS (cytoplasmic male sterility) Owen, en est complètement absent. La variabilité intrapopulation est forte : en moyenne 2,1 mitotypes parmi les 5 individus échantillonnés par population. La différenciation interpopulations de la fréquence des mitotypes est hautement significative $(Fst = 0,466 ; P < 0,001)$, répartie aussi bien entre régions $(Fst = 0,138; P < 0,001)$ qu'entre les populations dans les régions $(Fst = 0,381 ; P < 0,001)$. Des plantes femelles sont trouvées avec tous les types mitochondriaux. Néanmoins, 2 groupes peuvent être distingués : d'une part les mitotypes Nvulg, A et B qui, quelle que soit la région considérée, sont rarement portés par des plantes femelles, d'autre part les mitotypes E et G qui sont toujours associés à de fortes fréquences de femelles.

variation du sex-ratio / gynodioécie / stérilité mâle cytoplasmique / polymorphisme de l'ADNmt / *Beta vulgaris* ssp *maritima*

INTRODUCTION

Gynodioecy, defined as the occurrence of both male sterile (female) and hermaphroditic plants within natural populations, is observed in a wide array of plant taxonomic groups: 7.5% of the angiosperm species display this breeding system (Delannay, 1978; Kaul, 1988). Male sterility is also a genetic resource widely used for plant breeding due to its convenience for performing controlled crosses and for the commercial production of hybrid cultivars. Consequently, the evaluation of the diversity of this resource, as well as the identification of its genetic and molecular determinants are of great interest.

The distribution of gynodioecious populations and the sexual phenotype frequencies vary widely among species. In different species of thyme, all populations studied are gynodioecious, although there is a remarkably large variation in female frequency among populations: between 10 and 95% in *Thymus vulgaris* (Dommée *et al*, 1983; Belhassen *et al*, 1989); between 17 and 87% in *Thymus zygis*; and between 41 and 99% in *Thymus mastichina* (Manicacci, 1993). Conversely, in some other species like plantains, the frequencies of male steriles are lower, between 4 and 22% in *Plantago lanceolata* (Van Damme, 1984), and 5 and 27% in *Plantago coronopus* (Koelewijn, 1993). Finally, in other species including *Armeria maritima*, gynodioecious populations are only found in a restricted part of the distribution area of the species (Baker, 1966; Vekemans *et al*, 1990). Male sterility has been frequently reported in wild beet (Mikami *et al*, 1985; Halldén *et al*, 1988, 1990; Mann *et al*, 1989). Previous studies have focused on the estimation of female frequency variation, as well as on the origin of the maintenance of male steriles at high frequency in natural populations (Boutin *et al*, 1987, 1989; Saumitou-Laprade *et al*, 1993). Highly contrasting frequencies of male steriles (19% and 62%) have been observed in 2 natural populations from a small estuary on the French coast of the English Channel (Boutin *et al*, 1987, 1989).

In almost all of the gynodioecious species studied, male sterility is controlled by nuclear and cytoplasmic interaction (Kheyr-Pour, 1980, 1981; Van Damme, 1983; Kaul, 1988; Belhassen *et al*, 1991; Koelewijn 1993). The sexual phenotype of the plant is the result of the interaction between a sterilising cytoplasmic component and a variable number of nuclear suppressors of male sterility (called restorer genes of male fertility). In cultivated beet, cytoplasmic male sterility (CMS) has long been used for the production of hybrid lines (Owen, 1945). In wild beet, the genetic determination of male sterility is also nucleo-cytoplasmic, but the cytoplasmic factors involved differ from those used in the breeding programs of cultivated sugar beet (Boutin *et al*, 1987).

The existence and the maintenance of females in gynodioecious species have puzzled evolution biologists for a long time (at least since Darwin, 1877), and have attracted considerable attention, both theoretical and empirical. From a theoretical point of view, the existence of females at high frequency in populations is unexpected. As females can be considered as hermaphroditic individuals having lost their male function, they suffer from a 2-fold disadvantage in the transmission of their genes to the next generation. Thus, in order to persist in populations, they must have a compensatory advantage in some components of fitness (see Couvet *et al*, 1990, for a review).

Molecular analyses of several plant species have connected the expression of male sterility with chimeric genes in the mitochondrial genome (Hanson, 1991). Plant mitochondria have an active recombination system. Chimeric genes are believed to be one of the consequences of molecular rearrangements; different open reading frames are joined together and cotranscribed, in addition to standard mitochondrial genes.

In plants, including wild gynodioecious species, the degree of mitochondrial molecular polymorphism is poorly known. Data from the literature suggest a wide range of variation among species. In *Thymus vulgaris*, more than 30 different hybridisation patterns have been found among 200 individuals from 15 populations (Atlan, 1991). At the other extreme, only 3 mitochondrial types have been identified in *Plantago lanceolata*, each correlated to one of the 3 different cytoplasms implicated in male sterility in this species (Rouwendal *et al*, 1987). In *Beta vulgaris* ssp *maritima*, 5 different mitochondrial types have been found within 2 populations (Boutin *et al*, 1987; Saumitou-Laprade *et al*, 1993).

In this study we have tried to answer the following questions:

(i) What is the proportion of gynodioecious populations in *Beta vulgaris* ssp *maritima*, and what is the variation of the frequency of male steriles in natural populations?

(ii) What is the degree of mitochondrial molecular polymorphism and how is it distributed among populations?

(iii) What is the relationship between this polymorphism and the occurrence of male sterility in this species?

MATERIALS AND METHODS

The species

Beta vulgaris ssp *maritima*, wild beet, is a wind-pollinated, short-lived perennial and gynodioecious species. Some of its populations contain hermaphroditic and male sterile individuals (females), characterised by their inability to produce efficient pollen. The species is widely distributed along the coasts of Western Europe and around the Mediterranean Basin. *Beta vulgaris* ssp *maritima* belongs to the same species as cultivated beet (leaf, table, fodder and sugar beets referred to as *Beta vulgaris* ssp *vulgaris*), and represents the wild relative of these taxa.

Plant material

Two sampling schemes were used to determine the distribution of gynodioecy. First, 93 natural populations were sampled in 1989 during a collecting mission in collaboration with INRA (Prof H Laby) and USDA (Dr DL Doney). The populations sampled were located on the French Mediterranean, Atlantic, English Channel and North Sea coasts, and the coasts of Belgium, the south Netherlands and south-eastern England. Some inland populations located in south-western France (Gers and Lot-et-Garonne) were also sampled. Individual open pollinated progenies were collected in each population and sown in the experimental garden of the University of Lille. The sexual phenotype of a average of 8.9 individuals per population (SD = 7.0) was determined. A population was considered as gynodioecious if at least one male sterile plant was observed among the offspring. This method yields a conservative estimate of the proportion of gynodioecious populations as some populations can be incorrectly scored as non-gynodioecious due to the small sample size.

Secondly, during June 1990, additional samples were collected to estimate the local female frequency variation. The coasts between Le Hourdel (Baie de Somme) in France and Burghsluis (Zeeland) in the Netherlands were investigated. Populations of wild beet in this region are predominantly found in the estuaries of coastal rivers. Thirty-three populations from 10 estuaries were sampled. In each population, the sexual phenotype of almost all the flowering individuals was scored.

Molecular analysis

Thirty-eight of the total of 93 populations were randomly selected for molecular analysis of mitochondrial polymorphism; 5 individuals per population were analysed. Their sexual phenotype was determined to establish the relationship between mitochondrial polymorphism and male sterility.

Restriction fragment length polymorphism (RFLP) of mitochondrial DNA was analysed as follows. Total DNA was isolated from fresh leaves, digested with EcoRI, subjected to electrophoresis and transferred to nylon membranes using a vacuum Southern transfer apparatus. Several mitochondrial probes labelled with digoxigenin were hybridised. Hybridisation profiles were visualised by immunological detection and chemiluminescence (Saumitou-Laprade *et al*, 1993). In a previous study

(Saumitou-Laprade *et al*, 1993), 9 mitochondrial probes were used to test for polymorphism, and 2 were shown to be highly discriminative. These 2 probes were used in this study: a heterologous probe from maize, ATPase subunit 6 (Dewey *et al*, 1985); and a non-coding mitochondrial sequence from sugar beet, pBv4 (Saumitou-Laprade *et al*, 1993). Neither of these 2 probes distinguished between *Beta maritima* N2 and *Beta vulgaris* Nvulg mitochondria, although their EcoRI restriction profiles are clearly different (Saumitou-Laprade *et al*, 1993). Thus, the 12.5 kb EcoRI fragment of mtDNA in the Nvulg restriction pattern and absent from the N2 pattern was used as a differential probe. This fragment called 'Nvulg/N2' was isolated from an EcoRI digest of mtDNA by 0.8% agarose gel electrophoresis and was purified using the Qiaex procedure (Diagen). No cloning step was used, and 1 μg of this DNA was labelled with digoxigenin and used as a probe in the hybridisations.

Genetic data analysis

Differences in mitotype frequencies among populations within regions and between regions were analysed using F-statistics for haploid data (Excoffier *et al*, 1992).

RESULTS

Gynodioecy in wild beet populations

Gynodioecious populations were found in all the regions sampled (fig 1). In 42% of the populations, at least 1 male sterile was observed among the plants characterised for their sexual phenotype. The proportion of gynodioecious populations varied between the different regions: from 23% along the coast of the Channel to 71% among the populations from the Mediterranean coast (table I). Within the north region, the variation of male sterile frequencies was large (table II). Male steriles were not found in all of the estuaries. Female frequency varied widely between populations (from 0 to 76%); in some cases the local variation was large between populations less than 1 km apart.

Table I. Frequencies of gynodioecious and non-gynodioecious populations within the different regions sampled.

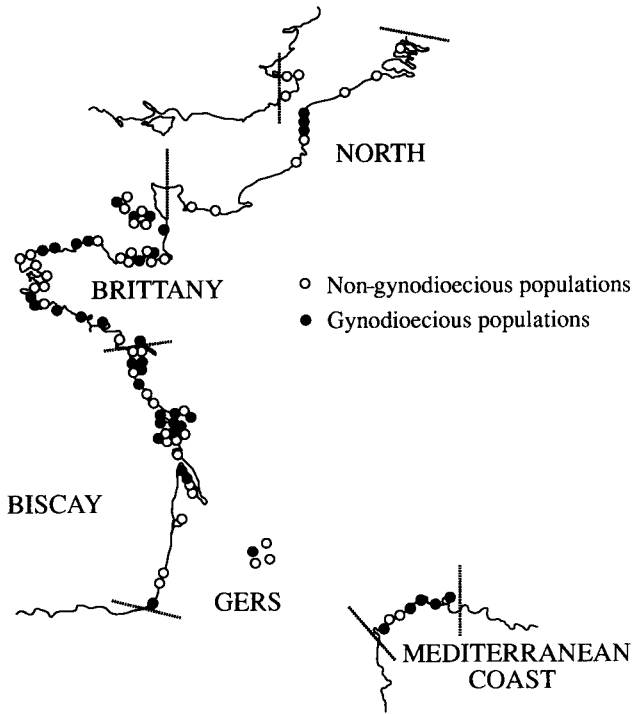| Region	No of populations	Proportion of gynodioecious populations
North	13	0.23
Brittany	36	0.42
Biscay	33	0.45
Gers	4	0.25
Mediterranean	7	0.71
Total	93	0.42

Fig 1. Distribution of gynodioecious and non-gynodioecious populations in the studied area.

Table II. Frequencies of female plants in populations located along the continental coast of the Channel.

Sites	No of populations per site	No of flowering plants sexed	Mean proportion of female plants	Within-site range of the proportion of female plants
Burghsluis	1	46	0	–
Zwin	1	53	0	–
Zeebrugge	1	15	0	–
Nieuwpoort	1	94	0	0
Slack	5	396	0.096	0–0.750
Wimereux	7	1 266	0.147	0–0.765
Canche	11	1 143	0.479	0.083–0.660
Authie	3	596	0.037	0–0.240
Pointe d'Offoy	1	193	0	–
Le Hourdel	2	320	0	0
Total	33	4 122	0.192	–

Polymorphism of the mitochondrial DNA

Five different variants were revealed by the pBv4 probe; 4 of these have been reported previously (Saumitou-Laprade *et al*, 1993) and a new pattern characterised by a single 4.4 kb band was found in this study. Seven different variants were identified by the Nvulg/N2 probe. The variants 1, 2, 3, 4, and 6 are presented in figure 2. Only 2 hybridisation profiles, both previously identified (Saumitou-Laprade *et al*, 1993), were detected with the atp6 probe. The combination of the different variants obtained with each probe was used to define mitochondrial types or mitotypes, designated A to J. Eleven different mitotypes were observed in this study (table III and fig 3): 5 were previously identified by Saumitou-Laprade *et al*, 1993, and the H type had been found among inland adventice beet by Boudry *et al* (1993).
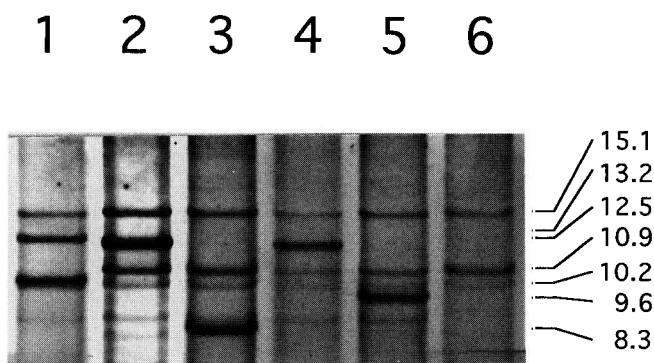

Fig 2. Southern blot analysis of EcoRI-digested total DNA with the Nvulg/N2 probe. Lanes 1, 3, 5, 6: *Beta vulgaris* ssp *maritima*; lanes 2, 4: *Beta vulgaris* ssp *vulgaris*. Lane 1: mtDNA type H; lane 2, 4: mtDNA type Nvulg (O type); lane 3: mtDNA type B (N3); lane 5: mtDNA type C (N2); lane 6: mtDNA type E (S). Molecular weights were estimated by comparison with lambda DNA digested by EcoRI and HindIII.

Table III. mtDNA polymorphism revealed by hybridisation of 3 mitochondrial probes to EcoRI digested total DNA. The different patterns revealed by each probe are designated by numbers. The designations in brackets refer to Saumitou-Laprade *et al* 1993.

Probes	Nvulg	Mitochondrial DNA types										Total No of
		A (N1)	B (N3)	C (N2)	D	E (S)	F	G (R)	H	I	J	patterns
atp6	2	2	2	2	2	1	1	2	2	2	2	2
pBv4	4	2	3	4	5	2	3	1	3	4	3	5
Nvulg/N2	1	1	2	3	1	4	2	5	6	8	3	7

The Nvulg type, which is the most frequent type among the maintainers of male sterility used in sugar-beet breeding programmes, is also the most frequent type in
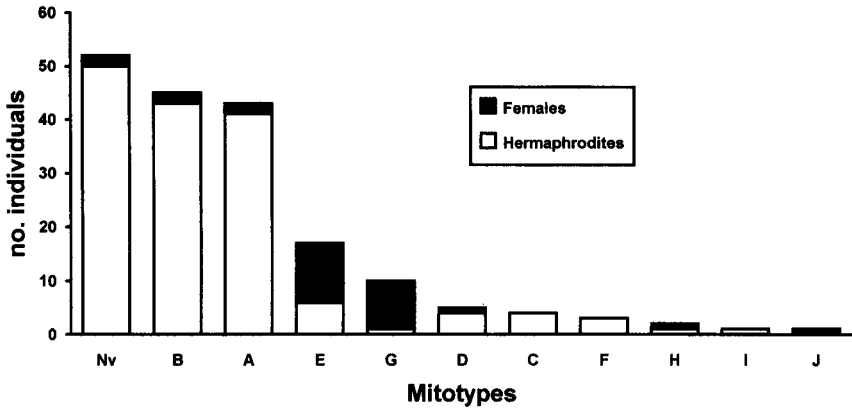
Fig 3. Distribution of female (■) and hermaphrodite (□) individuals among the different mitotypes.

natural populations. Conversely, the characteristic pattern of the CMS Owen, the Svulg mitotype, was completely absent from our sample.

There was no clear qualitative difference between the regions; the most frequent types (Nvulg, A, B, E) were found in nearly every region studied (table IV). On the other hand, some rare mitotypes were restricted to only one region, for example, only 5 individuals all from a single population in the Basque region were type D.

Table IV. Regional distribution of the mitochondrial types and association with male sterility. The number of each mtDNA type in each region is indicated together with the female frequency within each mitochondrial type (in italics).

Regions	Mitochondrial DNA types										
	Nvulg	A	B	C	D	E	F	G	H	I	J
North	18	24	5	3	—	7	3	—	—	—	—
	0.00	*0.00*	*0.00*	*0.00*	—	*0.57*	*0.00*	—	—	—	—
Brittany	3	10	21	1	—	4	—	10	—	—	—
	0.00	*0.00*	*0.09*	*0.00*	—	*0.75*	—	*0.90*	—	—	—
Biscay	11	3	19	—	5	1	—	—	1	1	—
	0.00	*0.00*	*0.00*	—	*0.20*	*1.00*	—	—	*1.00*	*0.00*	—
Gers	16	—	—	—	—	3	—	—	1	—	—
	0.00	—	—	—	—	*0.66*	—	—	*0.00*	—	—
Mediterranean	4	6	—	—	—	2	—	—	—	—	1
	0.50	*0.33*	—	—	—	*0.50*	—	—	—	—	*1.00*
Total No	52	43	45	4	5	17	3	10	2	1	1
Mean female frequency	0.04	0.05	0.04	0.00	0.20	0.65	0.00	0.90	0.50	0.00	1.00

There was a large variability of mitotypes within the populations; 2.10 different mitotypes on average (SE = 0.88) were found among the 5 individuals sampled per population. Nevertheless, there were highly significant overall differences in mitotype frequencies between populations ($Fst = 0.466$; $P < 0.001$). The mitotype frequencies varied significantly both between regions ($Fst = 0.138$; $P < 0.001$) and between the populations within the regions ($Fst = 0.381$; $P < 0.001$).

Male sterility and mitochondrial polymorphism

Over the whole studied geographical area, there was no strict qualitative association between the sexual phenotype and the mitotype of the plants (fig 3). Female plants did not always have the same mitotype and indeed displayed almost all the different mitotypes. Nevertheless, 2 groups of mitotypes can be clearly distinguished according to the occurrence of male sterility: the mitotypes Nvulg, A and B were rarely associated with the female phenotype, whereas female plants were often found among the plants of mitotypes E and G. The sample size did not allow analysis of the relationship between the other rare mitotypes and male sterility.

The association between male sterility and mitochondrial polymorphism varied among regions (table IV). In the north region, the female phenotype was closely associated with mitotype E. This mitotype was also found in the other regions where it was also regularly associated with the female phenotype. In Brittany, another highly sterile mitotype, G, was found. Finally, mitotypes Nvulg and A, only rarely associated with the female phenotype in coastal populations from the Basque region to the Netherlands, were closely associated with male sterility in the Mediterranean region.

DISCUSSION

Gynodioecy in wild beet populations

This study shows that the occurrence of female plants in natural populations of *Beta vulgaris* ssp *maritima* is a common characteristic of its breeding system. Indeed, gynodioecious populations were found in every part of the sampled geographical area.

Female frequency differed widely (from 0 to 76%) between populations. This large variation was apparent even on a local scale, between populations less than 1 km apart, in some of the estuaries of the Channel coast. A comparison of our results with those from the literature shows that wild beet belongs to species with moderate to high levels of male sterility (table V).

Since the inaugural paper of Lewis (1941), many population genetic models have examined the conditions for the maintenance of females at high (and variable) frequencies. Initially dealing with nuclear inheritance of male sterility (Lloyd, 1976; Charlesworth and Charlesworth, 1978), they have gradually taken into account a nucleo-cytoplasmic control of the sexual phenotype variation (Charlesworth, 1981; Delannay *et al*, 1981; Frank, 1989; Gouyon *et al*, 1991). In nucleo-cytoplasmic models, male sterility has been seen as a result of an evolutionary conflict between cytoplasmic and nuclear genomes concerning resource allocation to production of

Table V. Variation of female frequency (as a percentage) in various gynodioecious species.

Species	Minimum	Maximum	Authors
Plantago lanceolata	4	22	Van Damme, 1984
Plantago lanceolata	2	31	Krohne *et al*, 1980
Plantago coronopus	5	27	Koelewijn, 1993
Geranium maculatum	0	24	Ägren and Willson, 1991
Nemophila menziezii	0	26	Ganders, 1978
Scandia geniculata	7	25	Webb, 1979
Gingidia spp	0	31	Webb, 1979
Lignocarpa carnolusa	0	41	Webb, 1979
Hebe strictissima	1	34	Delph, 1990
Fuschia excorticata	4	40	Godley, 1955
Bidens spp	9	44	Sun and Ganders, 1986
Cortaderia richardii	0	53	Connor, 1963
Sarcocornia quinqueflora	0	53	Connor, 1984
Cucurbita foetidissima	21	65	Kohn, 1989
Thymus vulgaris	5	95	Dommée *et al*, 1983
Thymus zygis	17	87	Manicacci, 1993
Thymus mastichina	41	99	Manicacci, 1993
Beta maritima	19	62	Boutin *et al*, 1987
Beta maritima	0	76	This work

male gametes (Cosmides and Tooby, 1981). Selfish, sterilising, cytoplasmic genes tend to spread in populations, and are counteracted by nuclear suppressors of male sterility. The nucleo-cytoplasmic conflict generates varying gene frequencies of both cytoplasmic and nuclear genes, with periods of high female frequencies alternating with periods of low frequencies, or even absence, of females (Frank, 1989; Gouyon *et al*, 1991).

The framework provided by these theoretical models suggests explanations for the wide spatial variation of the female frequency observed in many gynodioecious species and especially in wild beet. Restricted gene flow leads to population sub-division and consequently to an asynchrony between different populations in the evolutionary dynamics of the nucleo-cytoplasmic, sex-determining system. Thus, spatial variation of female frequencies could be due to geographically variable cytoplasmic and nuclear gene frequencies. The respective contribution of cytoplasmic and nuclear genes to the sex ratio variation between populations has not been established. In particular, 2 important questions remain to be answered: how many cytoplasms determining male sterility are there in populations, and what is the geographical variation of nuclear restorer genes? Indeed, a low frequency of females in a population can be due either to a low frequency of a cytoplasm conferring male sterility or to a high frequency of restorer genes.

All the identified 'cytoplasmic genes' responsible for male sterility have been found within the mitochondrial genome (Newton, 1988; Hanson *et al*, 1989; Hanson, 1991). The increasing availability of fast and efficient molecular techniques allows specific genetic markers for the mitochondrial genome to be developed. These

markers can be used to characterise the polymorphism of mitochondrial DNA and to analyse the distribution of the cytoplasms in populations.

Polymorphism of the mitochondrial DNA

We found 11 different mitochondrial types. Five of them have previously been reported (Saumitou-Laprade *et al*, 1993), and a sixth, the H type, identified by Boudry *et al* (1993). The mitotype Nvulg was the most frequent type found in the natural populations investigated. This type is frequent among the maintainers of male sterility used in the breeding programmes of sugar-beet cultivars. Its wide occurrence in natural populations confirms the close relatedness of cultivated and wild beets.

Conversely, the Svulg mitochondrial type, specific to the CMS Owen cytoplasm used in the breeding programs, was not found during this study. However, its occurrence in wild populations has been reported by several authors. It occurs in wild beet in Turkey (Mikami *et al*, 1985; Halldén *et al*, 1988), suggesting that the Owen cytoplasm may have its origin in populations of Beta maritima from this country. Our results suggest that it does not originate from the natural populations in the coastal regions of France. Svulg has been also regularly found in French inland populations of weed beet (Boudry *et al*, 1993), but in this case, its occurrence is likely to be due to the escape of sugar-beet cultivars, introgressed by early flowering types, present in some seed production areas.

There are few reports in the literature of screening natural populations for mtDNA polymorphism. Our study demonstrates a high level of polymorphism, although it is lower than the remarkably large variation found in *Thymus vulgaris* (Atlan, 1991). As the rate of evolution of plant mitochondrial genomes and its variation between species is unclear, further studies are needed to assess the variation of mitochondrial polymorphism in natural populations of gynodioecious and non-gynodioecious species.

Although the variability within populations was large (2.1 mtDNA types on average among the 5 individuals sampled), the differences in the mitotype frequencies between populations was highly significant. This demonstrates that wild-beet seed migration is limited and that there is population subdivision for cytoplasmic genes in this species. Thus, it is likely that populations could be at different stages of the dynamic process suggested by theoretical models. Examination of the association between mitotypes and male sterility gives an insight to this issue.

Male sterility and mitochondrial polymorphism

Female individuals of almost all of the different mitotypes were found. However, high frequencies of male sterility were associated with 2 types: E and G. Type E was found in every region, whereas the G type was only found in populations from Brittany. The relationship between male sterility and mtDNA polymorphism in the populations from the Mediterranean region is unclear and needs further investigation. Thus, our results suggest that only 2 highly sterile cytoplasms are largely responsible for male sterility in the coastal populations of western France. Nevertheless, this has to be confirmed by further genetic analysis using reciprocal

controlled crosses between plants having different mitotypes or having the same mitotype but originating from different regions: the true number of different male sterile cytoplasms could thereby be determined. Indeed, nothing is known about the molecular basis of male sterility in *Beta* and the haplotypes defined by combining the different probes have to be considered as genetic markers in linkage disequilibrium with the male sterile cytoplasms.

Our results confirm that the concept of totally fertile cytoplasm used by plant breeders is an oversimplification and that 'normal' cytoplasms are likely to be 'old' sterile types having almost completely fixed their restorer genes. On the other hand, although male sterility may be found with all the mitotypes, we show a preferential association between male sterility and 2 particular mitotypes across a large geographical area. This suggests that in *Beta vulgaris* ssp *maritima*, the geographical variation of nuclear restorer gene frequencies is limited.

The comparison of our results with the predictions of theoretical models suggests that the dynamics of male sterility in *Beta* populations is slow. The widespread distribution of the types, especially those associated with male sterility, raises 2 questions of particular importance for the management of genetic resources:

1) Why do the sterile types never seem to be fully restored, in any location? Further studies should investigate, using a larger sample size, the spatial variation of restorer genes among populations at different levels, from local neighbourhoods to large geographical regions.

2) Why are most mitotypes found in every region? This could result either from distribution of mitotypes to different regions by migration, in which case all copies of the same mitotype would have a single common ancestor and thus be identical by descent, or from independent recurrent mutations. This question arises because of the latent turbulence of the plant mitochondrial genome. The tempo and mode of evolution of mitochondrial genome within species are poorly known, although this gene is thought to evolve rapidly in structure through homologous recombination, but slowly in sequence (Palmer and Herbon, 1988). However, the frequency of rearrangements within species and, in particular, the generating rate of male sterile cytoplasms are still not known and need further investigation.

ACKNOWLEDGMENTS

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