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Emergence of gynodioecy in wild beet (*Beta vulgaris* ssp. *maritima* L.): a genealogical approach using chloroplastic nucleotide sequences

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Gynodioecy is a breeding system where both hermaphroditic and female individuals coexist within plant populations. This dimorphism is the result of a genomic interaction between maternally inherited cytoplasmic male sterility (CMS) genes and bi-parentally inherited nuclear male fertility restorers. As opposed to other gynodioecious species, where every cytoplasm seems to be associated with male sterility, wild beet *Beta vulgaris* ssp. *maritima* exhibits a minority of sterilizing cytoplasms among numerous non-sterilizing ones. Many studies on population genetics have explored the molecular diversity of different CMS cytoplasms, but questions remain concerning their evolutionary dynamics. In this paper we report one of the first investigations on phylogenetic relationships between CMS and non-CMS lineages. We investigated the phylogenetic relationships between 35 individuals exhibiting different mitochondrial haplotypes. Relying on the high linkage disequilibrium between chloroplastic and mitochondrial genomes, we chose to analyse the nucleotide sequence diversity of three chloroplastic fragments (*trnK* intron, *trnD–trnT* and *trnL–trnF* intergenic spacers). Nucleotide diversity appeared to be low, suggesting a recent bottleneck during the evolutionary history of *B. vulgaris* ssp. *maritima*. Statistical parsimony analyses revealed a star-like genealogy and showed that sterilizing haplotypes all belong to different lineages derived from an ancestral non-sterilizing cytoplasm. These results suggest a rapid evolution of male sterility in this taxon. The emergence of gynodioecy in wild beet is confronted with theoretical expectations, describing either gynodioecy dynamics as the maintenance of CMS factors through balancing selection or as a constant turnover of new CMSs.

Keywords: *Beta vulgaris* ssp. *maritima*; gynodioecy; statistical parsimony; cpDNA diversity

1. INTRODUCTION

Gynodioecy is a particular breeding system referring to populations where both hermaphroditic and female (i.e. male-sterile) plants coexist (Darwin 1877). It has been considered as a transitory state from hermaphroditism to dioecy (see Barrett 2002 for a recent review), but its rank of second-most-frequent plant breeding system among angiosperm species suggests that it can also be a stable system (Richards 1997). This gender polymorphism can be viewed as the outcome of a genetic conflict between two genomes differing in their transmission: male-sterilizing factors encoded by the mitochondrial genome improve their transmission through resource reallocation, while the nuclear genome 'reacts' by re-establishing male fertility through specific restorer alleles (Cosmides & Tooby 1981; Saumitou-Laprade *et al.* 1994; Schnable & Wise 1998; Budar *et al.* 2003; Hanson & Bentolila 2004). Genetic investigations of natural populations have often shown that the genetic basis of male sterility is complex, involving several cytoplasmic and nuclear loci in epistasis (De Haan *et al.* 1997*a*; Charlesworth & Laporte 1998; Van Damme *et al.* 2004; Bailey & McCauley 2005).

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The dynamics of cytoplasmic male sterility (CMS) factors have been studied with theoretical models that predict either rapid turnover of CMS factors (epidemics) or single-point equilibrium cycles through balancing selection (Frank 1989; Gouyon *et al.* 1991; Bailey *et al.* 2003). Recent investigations of two gynodioecious *Silene* species revealed that an epidemic scenario may explain the low chloroplastic diversity observed in *Silene vulgaris* (Ingvarsson & Taylor 2002), while, in contrast, a negative frequency-dependent selection was invoked to explain the maintenance of ancient mitochondrial divergent haplotypes in *Silene acaulis* (Städler & Delph 2002). The investigation of cytoplasmic diversity in gynodioecious populations has always revealed several haplotypes associated with gender polymorphism (Koelewijn 1995; De Haan *et al.* 1997*b*; Olson & McCauley 2002). The same goes for *Beta vulgaris* ssp. *maritima*, wild sea beet, which exhibits 20 mitochondrial haplotypes (mitotypes) (Desplanque *et al.* 2000), four of which are clearly associated with male-sterility (*E*, *G*, *H* and *Svulg*; Saumitou-Laprade *et al.* 1993; Cuguen *et al.* 1994; Laporte *et al.* 1998; Ducos *et al.* 2001). It must be noted that, in contrast with other gynodioecious species, non-sterilizing mitotypes constitute a large part of the mitochondrial diversity of wild beet. Therefore wild beet is a unique system in which the study of the origin of CMS in a gynodioecious species can prove particularly interesting.

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The maternal transmission of cytoplasm leads to strong linkage disequilibrium between chloroplastic and mitochondrial variants, as found in *B. vulgaris* (Desplanque *et al.* 2000) and in *S. vulgaris* (Olson & McCauley 2000), despite occasional paternal leakage in this last species (McCauley *et al.* 2005). Considering this documented linkage disequilibrium and the fact that plant mitochondria are subject to frequent rearrangements making direct sequencing difficult, we sequenced three chloroplast fragments and assessed one chloroplastic DNA RFLP variation in representative individuals previously known for their mitochondrial haplotypes. The haplotype genealogy we derived allowed us to propose a scenario for the emergence of CMS in the species, addressing the question of whether the different CMSs belong to a single evolutionary lineage, ultimately leading us to infer the evolutionary dynamics of gynodioecy in beet.

2. MATERIAL AND METHODS

(a) *Plant material*

Wild beet, *B. vulgaris* ssp. *maritima* is a wind-pollinated, short-lived perennial species widely distributed along the western coasts of Europe and around the Mediterranean Basin. In this study, we mainly focused on the chloroplastic diversity of natural accessions of *B. vulgaris* ssp. *maritima* but we also investigated the cpDNA diversity of three representatives of cultivated beet (*B. vulgaris* ssp. *vulgaris*): one on the *Nvulg* mitotype, and two on a specific mitotype named *Svulg* (also called 'Owen CMS', Owen 1945) occurring at very low frequencies in the wild (Arnaud *et al.* 2003; Viard *et al.* 2004).

The 35 individuals included in this study were selected according to their Southern RFLP mitotypes. The number of replicates for each mitotype is related to its occurrence in natural populations (Cuguen *et al.* 1994; Desplanque *et al.* 2000). For instance, mitotypes *Nvulg* and *A* are represented by five and four individuals, respectively, because they are widely found in natural populations, while other rarer mitotypes are represented by only one individual. Replicates were chosen on the basis of their geographical location, each sample coming from a distinct population along the western European coast (see the electronic supplementary material for geographical location and GPS coordinates of the sampled populations, the sexual phenotype of the individuals and their mitotype according to the nomenclature established in Desplanque *et al.* 2000).

(b) *DNA isolation and molecular analysis*

Total genomic DNA was isolated from dried leaf tissue using either the method described in Saumitou-Laprade *et al.* (1993), a miniprep procedure modified from Dellaporta *et al.* (1983) or a DNeasy 96 Plant Kit (QIAGEN, Inc., Valencia, CA, USA) for the more recently collected accessions.

Three cpDNA regions were selected for sequencing: the *trnK* intron (K1K2) including the *matK* gene, the *trnD–trnT* intergenic spacer (DT) and the *trnL–trnF* intergenic spacer (LF). On account of its size (about 2500 bp), the K1K2 region was amplified in three overlapping fragments. The set of primers used was: K1-F (GTTGCCCGGGATTCGAA)/matK1-R (ATTAGGGCATCCCATTAGTA) for the first part of K1K2 (temperature of annealing (T_a) = 50 °C) (modified from Grivet & Petit 2003); matK2-F (CTAGCACAAGAAAGTCGAAG)/matK6b-R (GGATTTCTAACCATCTTGTT) for the second part of K1K2 (T_a = 54 °C) (Grivet & Petit 2002); matK6-F (GATTCTGTTGATACATTCGAG)/K2-R (GAGTACTCGGCTTTTAAGTG) for the third and last part of K1K2 (T_a = 54 °C) (modified from Grivet & Petit 2003); trnD-F (ACCAATTGAACTACAATCCC)/trnT-R (CTACCACTGAGTTAAAAGGG) for DT (T_a = 56.5 °C) (Grivet & Petit 2003); trnL-F (GGTTCAAGTCCCTCTATCCC)/trnF-R (ATTTGAACTGGTGACACGAG) for LF (T_a = 57.5 °C) (Taberlet *et al.* 1991). PCR-amplification was performed in a 25 µL mix containing 25 ng of DNA template, 3 mM of $MgCl_2$, 1.5 µL of Buffer 10X (Perkin–Elmer, Norwalk, CT), 0.2 µM of each primer, 200 µM of each dNTP and 0.625 U μL^{-1} of hot start *Taq* polymerase (AmpliTaq Gold, Perkin–Elmer, Norwalk, CT). PCR mixture underwent the following conditions on a 9700 thermal cycler (Perkin–Elmer, Norwalk, CT): 12 min denaturing at 94 °C, 40 cycles of 30 s denaturing at 94 °C, 45 s annealing at T_a (see above) and from 1 to 2 min extension (depending on the fragment length) at 72 °C and a final extension step at 72 °C for 10 min, after 40 cycles. The PCR products were then purified using a QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, CA, USA) and directly sequenced with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer, Norwalk, CT, USA). Sequence data were obtained on a 3100-Avant Genetic Analyser (Applied Biosystems). An additional chloroplastic polymorphism was used: mitotypes *Svulg* and *T* are known to be associated with a *Hin*dIII restriction site mapped in the *petG–psbE* region (Desplanque *et al.* 2000). To reveal this polymorphic site, amplifications were performed in a total volume of 15 µL according to the method described in Ran & Michaelis (1995). PCR products were restricted with *Hin*dIII and electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining.

Raw data of sequences were read, verified and aligned using the SeqScape v. 2.0 (Applied Biosystems) and BioEdit v. 5.09 (Hall 1999) softwares. Singletons (polymorphism present in only one individual) were confirmed with a second PCR amplification followed by independent sequencing.

(c) *Sequence analysis and haplotype network construction*

DnaSP v. 4.10 (Rozas *et al.* 2003) software was used to compute estimates of the nucleotide diversity (π) (Nei 1987).

Since the chloroplast genome constitutes a single linkage unit, the three cpDNA regions and the substitution affecting the *Hin*dIII restriction site in the *petG–psbE* region were concatenated to build a unique haplotype for each individual.

Relationships among the cpDNA haplotypes were estimated by a network construction using statistical parsimony (Templeton *et al.* 1992). In order to avoid reticulation due to possible homoplasy of microsatellite length polymorphism (poly-A), only the substitutions and the insertions/deletions (indels) were used to draw the topology of a first network using TCS v. 1.13 software (Clement *et al.* 2000). Subsequently, information provided by the microsatellite length polymorphism was added onto the network, assuming that these characters would only affect the length of the branches. Only the final network resulting from this two-step procedure is presented below.

3. RESULTS

(a) *Characteristics of sequences and nucleotide polymorphism*

The three fragments, K1K2, DT and LF yielded final sequence alignments of 2490, 986 and 384 bp, respectively. Among the 35 individuals we analysed, sequencing revealed 10 polymorphic sites in K1K2, eight in DT and five in LF, including substitutions, indels and microsatellite length variations. These polymorphic sites are compiled in table 1. The overall nucleotide diversity on the sequenced sample gave an estimated $\pi = 0.0009 \pm 0.00007$.

The sequences of the consensus haplotype (corresponding to individuals Nv_1, Nv_5 and *C*, see table 1) have been registered in the GenBank nucleotide sequence database under accession numbers DQ116790, DQ116791 and DQ116792 for K1K2, DT and LF, respectively.

A particular polymorphic site was found in DT. It consists of the replacement of a trinucleotide motif 'TTT' by its complementary motif 'AAA' (position DT117, see table 1). These three nucleotides are flanked with a 21 bp length inverted repeat sequence forming the stem of a stabilized hairpin that may have facilitated the mini inversion (Kelchner & Wendel 1996).

Contrary to what we expected, only two poly-A tracts, K39 and DT219 (out of the five found in the chloroplastic fragments) exhibited homoplasy.

With regard to the mutation detected in the *petG–psbE* region by PCR–RFLP, only the three individuals exhibiting mitotype *Svulg* shared the additional *Hin*dIII restriction site as well as the two representatives of mitotype *T*.

(b) *Haplotype genealogy*

By combining the data of the three sequenced regions (representing a total of 3860 bp) and the PCR–RFLP polymorphism of the *petG–psbE* region, we obtained a total of 25 cpDNA haplotypes among the 35 individuals, corresponding to 20 mitotypes. Statistical parsimony applied to this dataset led to the construction of the network displayed in figure 1. It is characterized by a star-like topology with short branches due to a limited number of mutation steps separating the haplotypes. At first glance, most of the haplotypes are organized around a core haplotype associated with mitotypes Nv_1, Nv_5 and *C* and a second sub-network organized around the cluster of haplotypes A_3 and *K*, whereas the haplotypes *L* and *D* are in intermediate positions between the two sub-networks. Individuals sharing the same mitotype display the same (or closely related) cpDNA haplotype in the network, confirming a congruent evolutionary history of both genomes. Sterilizing cytoplasms belong to separate lineages and derive independently from the core haplotype Nv_1, Nv_5 and *C*.

4. DISCUSSION

(a) *Chloroplastic nucleotide diversity in beet and haplotype genealogy*

The number of polymorphic sites enabled us to reach a fine resolution of the haplotype network with a total of 25 haplotypes. This confirmed that the three cpDNA regions we selected (K1K2, DT and LF) can be efficiently used for intraspecific phylogenetic studies. Nevertheless, one should bear in mind that the choice of individuals to be sequenced was based on their previously known mitochondrial haplotypes. Consequently, the nucleotide diversity we found is expected to be an overestimation of the species' cpDNA diversity. But the level of chloroplastic diversity ($\pi = 0.0009$) found in *B. vulgaris* ssp. *maritima* was 4–30 times lower than that observed in other intraspecific studies focusing on identical cpDNA fragments (e.g. Lu *et al.* 2001; Ingvarsson & Taylor 2002; Yamane *et al.* 2003). As a consequence, the restricted polymorphism observed in *B. vulgaris* provides limited power to establish the relative age of the different chloroplastic haplotypes. Nonetheless, chloroplastic DNA variations allowed us to establish a congruent genealogy that mirrors the mitochondrial diversity. Indeed, individuals sharing the same mitochondrial haplotype also shared the same chloroplastic haplotype or were closely clustered, revealing diversity within a single mitochondrial class. Conversely, different mitochondrial haplotypes could share the same chloroplastic haplotype (e.g. *Nvulg* and *C* or *E* and *P*). This result highlights the complementary information brought by the study of both genomes, as already observed in *S. vulgaris* (Olson & McCauley 2000). It also confirms the strong linkage disequilibrium between the two organellar genomes and provides a basis to use the chloroplastic genealogy when studying the emergence of CMS in wild beet.

(b) *Emergence of CMS cytoplasm in beet: single or multiple sterilizing lineages?*

In gynodioecious species, gender is the result of an interaction between maternally inherited CMS genes and bi-parentally inherited nuclear male fertility restorers. As opposed to other gynodioecious species, where every cytoplasm seems to be sterilizing (e.g. *Thymus vulgaris*, Belhassen *et al.* 1991; *Plantago lanceolata*, De Haan *et al.* 1997*b*; *S. vulgaris*, Olson & McCauley 2002; *S. acaulis*, Städler & Delph 2002), non-sterile cytoplasms are frequently found in *B. vulgaris*, with only four different CMSs out of 20 different RFLP mitochondrial haplotypes. As such it is a valuable system for the study of the chronology of events that led to the occurrence of CMSs from non-sterilizing cytoplasms. The dynamics of the breeding system, from the recruitment of restorer alleles to their fixation, is greatly influenced by the way CMSs arise, i.e. in a single lineage through the successive accumulation of sterilizing mitochondrial mutations, or through independent events from distinct lineages.

The coalescent theory predicts that the most common haplotypes are likely to be the oldest, and that most of these haplotypes are interior nodes of the haplotype tree (Crandall & Templeton 1993). In this regard, *Nvulg* is archetypal, as it is the most frequent cytoplasm found in natural populations (Cuguen *et al.* 1994; Desplanque *et al.* 2000), and constitutes the core node of the network (figure 1). All four sterilizing mitotypes are dispersed among several sub-networks, all derived from *Nvulg*. Therefore it appears that the four CMSs do not constitute a single lineage but occurred independently from an ancestral non-sterilizing cytoplasm. It must be noted that the status of *Nvulg* as a non-sterilizing cytoplasm is unambiguous, based on two lines of evidence: (i) a large survey of mitochondrial diversity in wild beet populations has revealed that *Nvulg* is always associated with hermaphrodite plants; (ii) its extensive use in sugar beet

Table 1. Description of haplotypes corresponding to the 35 sequenced individuals. (For *trnK*, *trnD–trnT* and *trnL–trnF* sequenced cpDNA regions, all variable positions are measured from the first sequenced base after the forward primer. For *petG–psbE*, the absence (0) or presence (1) of the *Hin*dIII restriction site is indicated. Dots indicate similarities with the consensus haplotype.)

fragment	LF					DT								K1K2										*petG–psbE*
polymorphic sites	66	99	105	106–110	118	117–119	219–227	276–279	391	470	690–694	834–841	888	39–51	280	301	677	731	865	1113	1195	1399	2044	*Hin*dIII restriction
consensus haplotype	C	G	G	$(T)_5$	C	TTT	$(A)_9$	$(T)_4$	G	C	$(AATTT)_3$	$(A)_8$	C	$(A)_{12}$	G	A	A	A	A	G	G	A	G	0
A_1	·	T	·	·	·	AAA	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	G	·	·	·	G	·	·
A_2	·	T	·	·	T	AAA	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	·	·	·	·	G	·	·
A_3, K	·	T	·	·	·	AAA	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	·	·	·	·	G	·	·
A_4	·	T	·	·	·	AAA	·	·	·	·	·	$(A)_9$	·	$(A)_{13}$	·	·	·	·	·	·	·	G	·	·
B_1, B_2	·	T	·	·	·	AAA	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	·	·	·	·	G	A	·
C, Nv_1, Nv_5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
D	·	T	·	·	·	AAA	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
E_1	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	G	·	·	·	·	·	·	·	·
E_2, P_2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·
F_1, F_2, O	·	·	T	·	·	·	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	·	·	·	A	·	·	·
G	·	·	T	·	·	·	·	·	·	·	·	·	·	$(A)_{13}$	·	·	·	·	·	·	·	·	·	·
H	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
I_1	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I_2	·	·	T	$(T)_6$	·	·	$(A)_{10}$	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
L	·	T	·	·	·	·	$(A)_{10}$	·	C	·	$(AATTT)_2$	·	·	·	·	·	·	·	·	·	·	·	·	·
M	·	·	·	·	·	·	·	·	·	·	·	·	·	$(A)_{11}$	A	·	·	·	·	A	·	·	·	·
N	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	·	·	·	·
Nv_4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·
Nv_2, Nv_3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·
P_1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	T	·	·	·	·	·
Sv_1, Sv_2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1
Sv_3	·	·	·	·	·	·	$(A)_8$	$(T)_3$	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1
S	·	·	·	·	·	·	·	·	·	T	·	·	·	$(A)_{11}$	A	·	·	·	·	·	·	·	·	·
T_1, T_2	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1
U	·	T	·	·	·	AAA	·	·	·	·	·	$(A)_9$	·	·	·	·	·	·	·	·	·	·	G	·	·

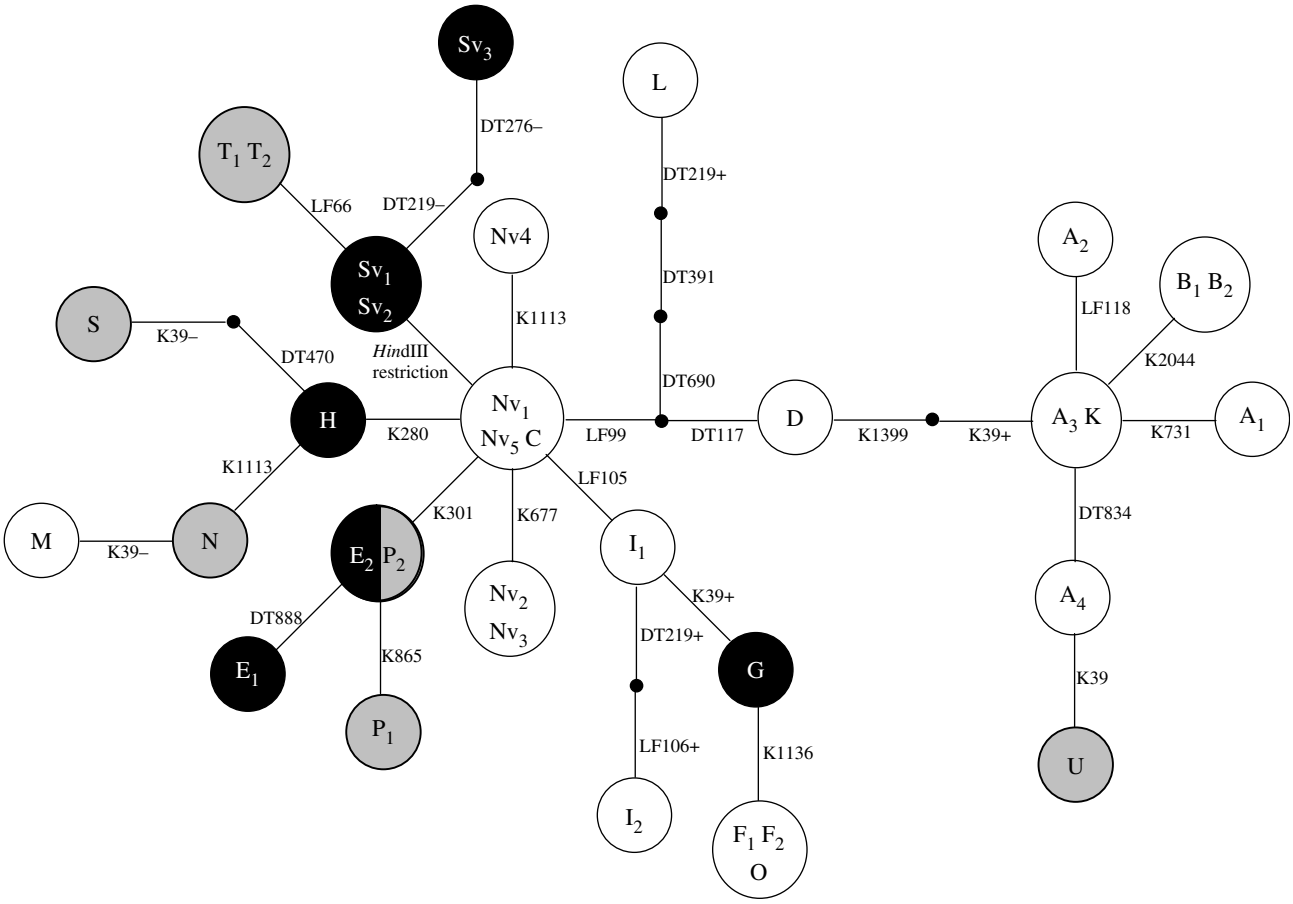

Figure 1. Genealogical relationships between 25 chloroplast haplotypes using statistical parsimony. For each haplotype, names of corresponding individuals are given in the circle. Black dots represent hypothetical haplotypes not found in the sample. Each branch between two haplotypes (sampled or hypothetical) indicates a single mutational step. Each mutation is labelled as follows: code of the fragment (K for K1K2, LF or DT) and position of the mutation on the corresponding sequenced fragment (see table 1). For microsatellite length polymorphism the gain or the loss of an A (or a T) from the consensus sequence is indicated by + or −, respectively. The sexual phenotype associated with each haplotype is visualised by the colour of the circle: black for 'female', white for 'hermaphroditic' and grey for 'unknown'.

plant breeding in maintainer lines (hermaphrodite genotypes that do not restore *Svulg*, a CMS widely used among cultivated lineages) never invalidated this status.

The question remains whether the emergence of independent CMS lineages, as observed in beet, is a general phenomenon in gynodioecious species.

(c) *A joint analysis of mitochondrial and chloroplastic polymorphism for a better understanding of the evolutionary history of the mitochondrial genome*

A closer look at the network reveals that each CMS belongs to a chloroplastic cluster corresponding to individuals sharing the same CMS mitochondrial haplotype (*E* and *Svulg*) as well as other cytoplasms. In the case of *Svulg* and *E*, their closely related cytoplasms, *T* and *P*, respectively, are rare haplotypes and as a consequence the gender they are correlated with has not been determined (grey in figure 1). They might be considered as variants of the related CMS: e.g. a single nucleotide polymorphism in the intron of the mitochondrial gene *cox2*, specific to *Svulg*, was also found in mitotype *T* (P. Touzet, unpublished results). In the case of *G* and *H*, derived chloroplastic haplotypes appeared to be associated with non-sterile mitotypes (*M* for *H*, *F*/*O* for *G*). Nevertheless, it is difficult to date the occurrence of the sterilizing mutations in the mitochondrial genome based on genetic differentiation of chloroplastic haplotypes. On one hand, the non-sterile cytoplasm derived from CMS cytoplasms could be mitochondrial fertile revertants having lost their sterilizing mutations. On the other hand, low cpDNA diversity could give rise to misleading reverting pathways because of either lack of resolution or the recent rise of the CMS lineage. For example, the mitochondrial genome of *G* is characterized by specific variants of two genes: *cox2* and *nad*9 (Ducos *et al.* 2001), which are not found in the mitochondrial genome of *I*, from which the chloroplastic haplotype of *G* is derived, nor in *F*/*O*, which retained the ancestral structure (identical to *Nvulg*) (P. Touzet, unpublished results).

In the present study, *Nvulg* and *Svulg* appear to be closely related (only a single substitution separating both chloroplastic genomes), *Svulg* being derived from *Nvulg*. Both mitochondrial genomes have recently been sequenced (Kubo *et al.* 2000; Satoh *et al.* 2004). The comparison of their complete mitochondrial genomes reveals that they do share a high similarity on the coding sequence located in a number of blocks, but that the order and the orientation of these blocks are highly shuffled. In addition, each genome possesses 10% of specific sequences, and *Svulg* has gained a larger size partly due to a large duplication event (Satoh *et al.* 2004).

This is a good illustration of the peculiar and swift evolutionary dynamics in the mitochondrial genome at the intraspecific level (Palmer & Herbon 1988), as is the case in maize (Clifton *et al*. 2004). It also highlights the added value of a chloroplastic genealogy to establish the phylogenetic link between mitochondrial genomes highly divergent in structure but poorly divergent in coding sequences.

(d) *Evolutionary dynamics of gynodioecy in Beta vulgaris*

The dynamics of gynodioecy is still a debated subject (Charlesworth 2002; Jacobs & Wade 2003). Theoretical models have described gynodioecy dynamics either as the maintenance of CMS factors through negative frequency-dependent selection or as a constant turnover of new CMSs arising in populations (epidemic model). The cytoplasmic diversity is expected to be high under balancing selection (with old sterilizing haplotypes), since there would be sufficient time for silent mutations to accumulate between and within haplotypes, but low in the case of epidemic dynamics (*via* recurrent selective sweep).

What can be inferred through the cytoplasmic diversity we observed in this study? The scenario of balancing selection allowing the maintenance of several CMSs could be supported by the following arguments: (i) CMSs are at least old enough to allow for diversification within a given CMS haplotype, as observed in *Svulg* and *E* and (ii) only a limited number of CMSs have been described, suggesting that the emergence of a new CMS is a rare event.

However, we have shown that the overall diversity appeared to be low in *B. vulgaris*. This level of diversity is comparable to what has been found in *S. vulgaris*, another gynodioecious species (Ingvarsson & Taylor 2002). In their study, the authors concluded that low diversity was a signature of epidemic dynamics. This could suggest that epidemic dynamics, rather than balancing selection, govern gynodioecy in beet. However, the star-like organization of haplotype diversity around *Nvulg* may be the signature of a recent bottleneck: independent CMSs having arisen recently. Thus, the diversity of patterns expected under both alternative dynamics might be blunted by historical and demographic events that have influenced the present diversity. Additional data are needed to resolve the question. In this context, we are currently studying the chloroplastic and nuclear diversity within the geographic range of the species, in order to test for a bottleneck event. It should be noted that previous studies have failed to detect any compensation effect in females in beet for CMS *E*, the most frequent CMS (Boutin *et al*. 1988). In addition, as opposed to other gynodioecious species, most of the cytoplasms found in populations are non-sterilizing (Cuguen *et al*. 1994; Forcioli *et al*. 1998; Laporte *et al*. 2001). This may imply that selective forces involved in the maintenance of gynodioecy in beet are mild and consequently may not leave a strong signature, especially if the species has been through a bottleneck. Therefore, it is not yet possible to propose a definitive scenario of the evolutionary dynamics of gynodioecy in beet.

The recent genomic population studies on cytoplasmic sequences have revived an old question that remains unsolved (Ingvarsson & Taylor 2002; Städler & Delph 2002). The development of such studies on a larger number of gynodioecious species might lead us to better comprehend the phenomenon and uncover the relative importance of both alternative dynamics in the wild. The recent cloning of restorer loci in several mono- and dicotyledonous species has revealed that they all belong to the same large gene family, which may be a general reservoir in the recruitment of *de novo* restorer loci (Touzet & Budar 2004). This finding might facilitate the cloning of restorer loci in gynodioecious species in the near future (Wise & Pring 2002), and provide yet another clue to the evolutionary dynamics of this peculiar breeding system.

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