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Cytoplasmic male sterility in the olive (*Olea europaea* L.)

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Abstract The olive tree is usually hermaphrodite but self-incompatible. In the Western Mediterranean some cultivars are totally male-sterile. Three different male-sterile phenotypes have been recognised. To infer the genetic basis of male sterility we studied its inheritance and cytoplasmic diversity in wild (oleaster) and cultivated Mediterranean olive. In the cross *Olivière*×*Arbequina*, the male-sterile trait was maternally inherited and affected all progenies. We also checked that both chloroplast and mitochondrial DNAs are maternally inherited. RFLP studies on chloroplast and mitochondrial DNAs revealed several cytotypes: two chlorotypes and four mitotypes in cultivars and oleaster (wild or feral Mediterranean olive). Furthermore, a total linkage desequilibrium between the CCK chlorotype and the MCK mitotype in cultivars and oleaster from different regions supports the fact that paternal leakage of organelles was not observed. The male sterility (*ms 2*) displayed by *Olivière*, plus six other cultivars and three oleaster was strictly associated with the CCK chlorotype and the MCK mitotype. These facts suggest that *Olivière* carries cytoplasmic male sterility. Male-fertile and male-sterile oleasters carrying this cytotype showed the presence of restorer alleles. This CMS might be due to a distant cross between olive taxa. The two other male-sterile phenotypes displayed by *Lucques* (*ms 1*) and *Tanche* (*ms 3*) were associated with the ME1 mitotype but we have not demonstrated CMS.

Key words cpDNA · Cytoplasmic male sterility · mtDNA · *Olea europaea* · Inheritance · RFLP

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Introduction

In flowering plants two types of male sterility are distinguished according to their mode of inheritance: nuclear or genic male sterility (gMS) and cytoplasmic male sterility (CMS) (reviewed by Kaul 1988). In most cases, gMS is determined by a single locus and due to a recessive allele. The mode of inheritance of male sterility can be established through crosses and back-crosses. In the case of CMS, the male sterility is inherited through the female parent. This form of male sterility is usually observed among back-crosses and has been shown to be associated with mitochondrial DNA rearrangements (Vedel et al. 1994). For CMS, fertility is usually regained by a dominant nuclear restorer allele able to override the effects of the cytoplasm.

Olive (*Olea europaea* L.) is grown mostly for oil and also for canned fruit. It represents about 5-million ha in Mediterranean Europe and provides an appreciable income for olive growers. All present olive cultivars in the world originated from those improved around the Mediterranean Basin and this tree crop is closely associated with the Mediterranean climate (Zohary and Spiegel-Roy 1975; Besnard et al. 1998). Oleaster is considered as the wild stock for olive and exists both as wild or feral trees. Clonal propagation either by cuttings or grafting has led to the clonal genetic basis of cultivars.

In some species several mechanisms of male sterility coexist, as shown for maize, wheat and sunflower. To determine the mode of inheritance of male sterility from one individual, crosses and back-crosses have to be made with different pairwise combinations of lines to enhance the chance of obtaining a clear-cut segregation for male sterility/male fertility making it possible to propose a model. In olive the situation is complex due to the length of the generation time (about 10 years in orchards) and the variety of pollen abortion stages depending on cultivars. Fortunately, crosses between olive cultivars were already performed 20 years ago, enabling to us to observe progenies, but it appeared unrealistic to plan new crosses.

Table 1 List of cultivars and oleasters studied and their provenance*. Individuals observed for their pollen viability. The country origin of the denominations is in brackets (Fr=France; France; Al=Algeria; Tu=Tunisia; Mo=Morocco; Sp=Spain; Pt=Portugal; It=Italy; Its=Sicily, Italy; Yu=Yugoslavia; Gr=Greece; Tk=Turkey; Is=Israel; Sy=Syria; Eg=Egypt). CBNMP, "Conservatoire Botanique National Méditerranéen de Porquerolles", France; INRA M, "Institut National de Recherche Agronomique", Montpellier, France; IRO P, "Institute for Olive Research", CNR, Perugia, Italy; NYRC, "Newe-Ya'ar Research Center", Ramat Yishay, Israel; OGB C, "Olive Germplasm Bank", Cordoba, Spain. The number in brackets represents the number of trees analysed for cpDNA and mtDNA polymorphism in the wild populations. * cultivars analysed with *Cla*I, *Hind*III, *Xba*I and *Xho*I to screen enzyme/probe couples which reveal polymorphism

Cultivar	Collection or provenance	Cultivar	Collection or provenance
*Aglandau** (Fr)	INRA M	*Ascolana Tenera* (It)	IRO P
*Blanquetier d'Antibes** (Fr)	INRA M	*Biancollila* (Its)	IRO P
Bouteillan (Fr)	OGB C	*Dolce Agogia* (It)	IRO P
*Cailletier** (Fr)	INRA M	*Frantoio* (It)	IRO P
Capanacce (Fr)	Corsica	*Giarraffa* (Its)	IRO P
*Cayet Bleu** (Fr)	CBNMP	*Leccino* (It)	IRO P
*Cayet Rouge** (Fr)	CBNMP	*Leucocarpa** (It)	IRO P
*CBNMP 9-16** (Fr)	CBNMP	*Moraiolo** (It)	IRO P
*Colombale** (Fr)	CBNMP	*Nocellara del Belice* (Its)	IRO P
*Corniale** (Fr)	Montpellier, Bel Air	*Pendolino* (It)	IRO P
*Courbeil** (Fr)	CBNMP	*San Felice* (It)	IRO P
*Curnet** (Fr)	CBNMP	*Zaituna* (Its)	IRO P
Ghjermana (Fr)	Corsica	*Oblica* (Yu)	OGB C
*Grossane** (Fr)	INRA M	*Amygdalolia** (Gr)	INRA M
*Lucques*** (Fr)	INRA M	*Kalamata* (Gr)	OGB C
*Olivière*** (Fr)	INRA M	*Koroneiki** (Gr)	OGB C
*Picholine*** (Fr)	INRA M	*Vallanolia* (Gr)	OGB C
*Pigale** (Fr)	Juvignac	*Domat* (Tk)	OGB C
*Rougette de Pignan** (Fr)	Pignan	*Sofralik** (Tk)	INRA M
*Sabina** (Fr)	Corsica	*Uslu* (Tk)	OGB C
*Tanche*** (Fr)	INRA M	*Azam** (Sy)	Palmyre
*Verdale de l'Hérault** (Fr)	Montpeyroux	*Kaissy* (Sy)	OGB C
*Zinzala** (Fr)	Corsica	*Zaity** (Sy)	OGB C
*Azeradj** (Al)	Tizi Ouzou	*Barnea* (Is)	NYRC
*Chemlal*** (Al)	INRA M	*Merhavia* (Is)	OGB C
*Chemlal Mechtrass** (Al)	Tizi Ouzou	*Nabali* (Is)	Mohassen
Taksrit (Al)	Tizi Ouzou	*Shimlali* (Is)	NYRC
*Chemlali** (Tu)	CBNMP	*Souri* (Is)	OGB C
Chetoui (Tu)	OGB C	*Toffahi** (Eg)	OGB C
*Zarazi** (Tu)	INRA M		
Picholine Marocaine (Mo)	OGB C	Oleaster populations	Provenance
*Arbequina*** (Sp)	INRAM		
Canivano Blanco (Sp)	OGB C	Izmir (5)	Turkey
Empeltre (Sp)	OGB C	Mont Carmel (18)	Israel
Cornicabra (Sp)	OGB C	Messine (17)	Silicy, Italy
*Lechin de Sevilla** (Sp)	OGB C	Nice, Mont Boron* (21)	France
Picual (Sp)	OGB C	Tizi Ouzou* (12, of which 5 were observed for sexual phenotype)	Kabylie, Algeria
*Sevillenca*** (Sp)	OGB C		
Galega (Pt)	OGB C		

Olive is allogamous, hermaphrodite, and usually wind-pollinated. Most of its cultivars are self-incompatible – a trait descended from oleaster – but some, such as *Olivière*, *Chemlal*, *Lucques* are totally male-sterile. Under cultivation this obviously implies using another variety as pollinator: *Azeradj*, *Cayon* and *Picholine* for *Chemlal*, *Lucques*, and *Olivière*, respectively. Several male-sterile phenotypes, differing in the stage of pollen abortion, have been reported in some of the most common varieties (Villemur et al. 1984); however, CMS has not been reported as yet. It is suspected that male sterility was selected because it provides some advantages: vigour, higher potential for production probably because of different resource allocation, and longer pollen reception of the flowers.

We therefore carried out a comprehensive study to determine whether male sterility in olive is genic or cytoplasmic. We analysed inheritance of the male-sterile phenotype and chloroplast and mitochondrial DNAs based on the *Olivière*×*Arbequina* cross. We report the results of chloroplast and mitochondrial DNA polymorphism analyses combined with pollen observation in olive cultivars and oleasters. We discuss the origin of this male sterility and its interest in the management of genetic resources.

Materials and methods

List of cultivars and oleaster trees

We characterised 68 cultivars and 73 oleaster trees for cpDNA and mtDNA polymorphisms (Table 1). Several cultivars have been previously described as male-sterile: *Chemlal* (Kabylie, Algeria), *Olivière*, *Lucques*, *Tanche* (France), *Zarazi* (Tunisia) (Villemur et al. 1984) and *Sevillenca* (Spain) (Rallo, personal communication). In addition, 110 progenies of the *Olivière*×*Arbequina* cross (female parent x male parent) were characterised for both chlorotype and mitotype in order to determine the mode of inheritance of cytoplasmic DNAs in olive. Out these 110 18-years old trees, 72 have started flowering. *Olivière*× *Picholine* (ten trees) and *Lucques*×open pollinated (18 trees) progenies were also observed for male phenotype.

Pollen observations

The observation of stamens and pollen bagging was performed on all trees mentioned (Table 1). The cultivars from the Cordoba (OGB C) and Perugia (IRO P) collections – representing economically important cultivars in the Mediterranean Basin – have been reported as being male-fertile except for *Sevillenca* (Rallo, personal communication). Observations on 26 oleasters were performed in the populations from Nice and Kabylie only. At the beginning of blossoming, flowers were examined for anther dehiscence and the presence or absence of pollen. In the latter case, we analyzed the content of anthers. Pollen sacs were crushed in a drop of acetocarmine or in Alexander solution. Microspore observation was made at a magnification of ×400.

DNA preparation

DNA preparation was carried out according to a modified method described by Saghai-Maroof et al. (1984). Five grams of leaves were ground in liquid nitrogen and the powder was mixed with 20 ml of 2× CTAB buffer (100 mM Tris-HCI pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB) and 200 mg of sodium disulphite. This mix was incubated at 65°C for 1 h. Ten milliliters of chloroform/isoamylic alcohol (IAA) (24/1) were added and shaken for 20 min. The solution was decanted by centrifugation at 4500 g for 20 min. The supernatant was recovered and the operation repeated twice. The supernatant was then mixed with 20 ml of isopropanol and stored at –20°C overnight. The DNA pellet was recovered, washed with an ethanol solution (ethanol 76%, 10 mM ammonium acetate), dried, and removed in 0.5 ml of 1× TE (10 mM Tris-HCl pH 8, 1 mM EDTA).

DNA preparation for RFLP

The DNA solution was transferred to an ultra-centrifugation tube (Kontron). Ten micrograms of ethidium bromide and Triton (1% final concentration) were added. Caesium chloride solution was also added to obtain a final refractive index of η=1.397. This solution was centrifuged at 200 000 g for 24 h. The DNA band was recovered with a syringe. The volume was adjusted to 5 ml with water. DNA was precipitated by the addition of ammonium acetate (final concentration 0.2 M) and cold ethanol (10 ml). The DNA pellet was recovered and washed with 75% ethanol. The DNA pellet was then dried and dissolved in 0.3 ml of 1× TE.

RFLP procedure

Three micrograms of total DNA was restricted with 24 U of each restriction enzyme (Boehringer) according to recommendations. Restriction fragments were electrophoresed in 1% agarose gel and transferred onto Hybond N+ nylon membrane (Amersham) with a transblotter. Probes were labelled by random priming using 74 mBq [^{32}P]dCTP (111 TBq/mmol). The membranes were hybridised in a 7% SDS, 0.25 M NaPi and 1 mM EDTA solution at 65°C for 18 h and then rinsed in a 1% SDS and 40 mM NaPi solution three times at 65°C for 30 min. Autoradiograms were obtained at –80°C for a sufficient time depending on the labelling intensity with Hyperfilm MP (Amersham).

Chloroplast DNA polymorphisms

To reveal chloroplast DNA polymorphism, we used five restriction enzymes: *Cla*I, *Eco*RI, *Hind*III, *Xba*I and *Xho*I, following the method already described (Forcioli et al. 1994), except that we employed chloroplast DNA (1 µg) from *Phillyrea media* L. (provided by P. Saumitou-Laprade, Lille University) as a probe. This probe was hybridised with olive total DNA to reveal chloroplast DNA polymorphism. *Phillyrea* is related to *Olea*. These two genera belong to the family *Oleaceae* and to the tribe *Oleeae*. Moreover, cpDNA evolves slowly, and thus, we expected a full cross-hybridisation between *Phillyrea* cpDNA and olive cpDNA.

Mitochondrial polymorphism

Four restriction enzymes: *Cla*I, *Hind*III, *Xba*I and *Xho*I, and five mitochondrial probes were used in pairwise combinations to look for polymorphism: *cox3* from *Oenothera* (Hiesel et al. 1987), *atp9* from maize (Dewey et al. 1985*)*, *nad6* from wheat (Haouazine et al. 1993), *rpl2* from tobacco (Vitard et al. 1992) and *26S* from *Oenothera* (Manna and Brennicke 1985). Nei and Li (1979) distances (D_{ij}) between each mitotype were computed: $D_{ij}=n_{ij}/n_i+n_j$, where n_{ij} is the number of common bands in individuals i and j, n_i and n_j are the number bands in individuals i and j respectively.

Results

Male-sterile phenotypes in olive tree

Among the cultivated trees observed for sexual phenotype, the male-sterile individuals include the following: *Blanquetier d'Antibes*, *CBNMP 9–16* (undetermined cultivar), *Chemlal*, *Courbeil*, *Curnet*, *Lucques*, *Olivière*, *Tanche* and *Zarazi*. Among the 26 observed oleasters, three are male-sterile: one from Mont Boron and two from Kabylie. The progenies of the *Olivière*×*Arbequina* (72 individuals) and the *Olivière*×*Picholine* (ten individuals) crosses were all male-sterile. In contrast, we observed 18 open-pollinated offspring of *Lucques* which were male-fertile except for two individuals (*L134* and *L450*).

Male-sterile olive trees were grouped according to the stage of pollen abortion and the presence of residue microspores either still grouped in tetrads or as isolated microspores in the mature anthers (Table 2). The phenotype *ms 2* is the more frequent and is displayed by both cultivars and oleasters. Individuals of this phenotype originated in different areas (France, Algeria and Tunisia). In contrast, the other phenotypes were displayed by only one cultivar (phenotype *ms 1*: *Lucques* and its progenies; phenotype *ms 3*: *Tanche*).

cpDNA polymorphism

Among all the cultivars and oleasters studied we observed two chlorotypes. One group of cultivars and ol-

Table 2 List of male-sterile olives observed in this study or reported by other authors and pollen abortion phenotype. 'Unknown' means that no detailed report is available from the literature

Phenotype	Pollen degeneration phenotype	List of varieties
ms 1	Only large tetrads, no microspore	*Lucques* *Lucques* progenies "*L134* and *L450*"
ms 2	Mix of tetrads and microspores (sometimes only microspores), aborted pollen	Cultivars: *Olivière*; *Blanquetier d'Antibes*; *CBNMP 9–16; Chemlal*; *Courbeil*; *Curnet*; *Hamra* (Ouksili 1988); *Zarazi* Oleaster: "*Kabylie F3 and F5*"; "*Mont Boron 24*" Progenies: *Olivière×Arbequina* (72); *Olivière×Picholine* (10)
ms 3	Degeneration of pollen mother cells associated with early degeneration of the tapetum, presence of a few viable pollen (=partial male sterility due to rare normal stamen development).	*Tanche*
–	Unknown	*Aaroun* (A. Ouksili, personal communication) *Cerasola* (Baldini and Guccione 1952) *Sevillenca* (L. Rallo, personal communication)

easters including *Olivière* and *Chemlal* carried the chlorotype CCK whereas all the other cultivars and oleasters carried another type called CNC (Fig. 1A). This chloroplast polymorphism between CNC and CCK was due to an extra 2.8 kb *Xba*I fragment in CCK (not shown) and to a shift of approximately 300 bp for an *Eco*RI fragment in CCK compared to CNC (Fig. 1A).

mtDNA polymorphism

A preliminary screening of polymorphisms was performed on a sub-sample of DNAs from 26 Mediterranean olive trees (18 cultivars and eight oleaster trees chosen for their different geographical origin) using *Cla*I, *Hind*III, *Xba*I and *Xho*I and the five probes. We revealed 60 fragments of which 34 were polymorphic enabling us to recognise four mitotypes. These mitotypes could be distinguished using two probe/enzyme couples only: *atp9*/*Hind*III (ME2-MCK) and *atp9*/*Xba*I (MCK-MOM-ME1). The remainder of the sample was therefore analysed using these two pairwise combinations.

The genetic distances computed between the four mitotypes showed that MCK was clearly the more distinctive type as compared to the three others (Table 3). The mitotype MCK carried two fragments hybridising with the *cox3* probe instead of one in the three other mitotypes (Fig. 1B, lanes MCK=*Olivière* and ME1= *Arbequina*). MCK differed from ME1, ME2 and MOM whatever the four restriction enzymes and the five probes were employed. MOM differed from ME1 and ME2 by four different enzyme/probe combinations whereas ME2 differed from ME1 by the lack of a single 1.2-kb *Hind*III fragment and by the presence of a 0.6-kb fragment revealed with *atp9* as a probe.

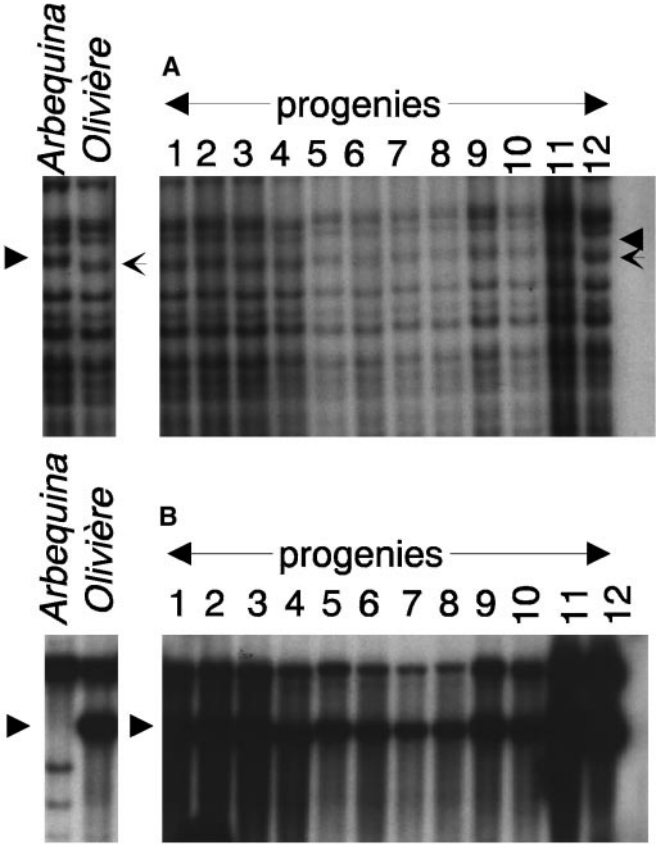

Fig. 1A, B Chloroplast and mitochondrial hybridisation patterns of *Olivière*, *Arbequina* and 12 progenies of the cross *Olivière×Arbequina*. **A** Auto-radiogram of the Southern transfer of *Eco*RI-restricted DNAs hybridised with total chloroplast DNA from *Phillyrea media* as a probe. **B** Autoradiogram of the Southern transfer of *Hind*III-restricted DNAs hybridised with the *cox3* gene (Hiesel et al. 1987) as a probe

Distribution of cytotypes

CCK chloroplast DNA polymorphism (=CCK chlorotype) was found strictly associated with the mitotype MCK. This association was found in seven cultivars and some oleasters (Table 4). The three other mitotypes were associated only with the CNC chlorotype. The resulting classification of the cultivars and oleasters according to both cpDNA and mtDNA types is presented in Table 4. The ME1 mitotype was found in the Eastern Mediterranean trees and in some trees from the West Mediterranean region. The MOM and MCK mitotypes were found only in the west of the Mediterranean Basin. The ME2 mitotype was found in seven cultivars, but not in the oleaster trees.

Inheritance of male sterility and cytoplasmic DNAs in (*Olivière*×*Arbequina*) progenies

Olivière gave the CCK chlorotype whereas *Arbequina* was characterized by the CNC chlorotype. In the 110 progenies checked, all displayed the CCK type, as did the mother parent (Fig. 1A). *Olivière* presented the MCK mitotype whereas *Arbequina* presented the ME1 mitotype. The 110 progenies of the cross *Olivière*×*Arbequina* displayed the MCK type, as did the mother parent (Fig. 1B). Among the 110 progenies, the 72 trees observed were fully male-sterile as was the mother parent.

Table 3 Nei and Li (1979) distances between each mitotype

Mitotype	MCK	MOM	ME1
MOM	0.35		
ME1	0.34	0.08	
ME2	0.37	0.11	0.02

Discussion

Inheritance of cytoplasmic DNAs

In the cross analyzed both the chloroplast and the mitochondrial DNAs were maternally inherited. Maternal inheritance of chloroplast and mitochondrial DNAs has been presumed for species of the *Oleoideae* subfamily (genera *Syringa* and *Osmanthus*) from observations of organelles in sperm cells (Sodmergen et al. 1998). Furthermore, a total linkage desequilibrium was observed between the MCK mitotype and the CCK chlorotype in cultivars and oleasters from two different geographical areas (France, The Maghreb). This suggests that mitochondrial and chloroplast DNAs have always been inherited together and we consequently assumed that paternal leakage events did not occur in olive.

Genetic basis of male sterility in olive

All the progenies of the cross *Olivière*×*Arbequina* displayed the male-sterile phenotype as did the mother parent (*Olivière*). It is associated with the CCK and MCK organelles. This suggests the existence of cytoplasmic male sterility (Fig. 2A) but we cannot exclude the hypothesis of gMS. gMS is generally determined by a single gene. In this case, 100% of male-sterile progenies would involve a homozygous dominant male sterility al-

Table 4 Chlorotypes and mitotypes of cultivars and oleasters. * Male sterility was either verified or reported in the literature. ** Male-sterile individuals were observed in the population

Chlorotype	Mitotype	Cultivars	Wild and feral forms
CNC	ME1	*Aglandau, Bouteillan, Cailletier, Cayet Bleu, Cayet Rouge, Colombale, Corniale, Ghjermana, Grossane, Lucques*, Rougette de Pignan, Tanche*, Verdale de l'Hérault, Azeradj, Chemlal Mechtrass, Taksrit, Chemlali, Picholine Marocaine, Arbequina, Canivano Blanco, Cornicabra, Empeltre, Picual, Sevillenca*, Ascolana Tenera, Dolce Agogia, Frantoio, Leccino, Leucocarpa, Moraiolo, Nocellara del Belice, Pendolino, San Felice, Zaituna, Oblica, Kalamata, Koroneiki, Vallanolia, Domat, Sofralik, Uslu, Azam, Kaissy, Barnea, Merhavia, Nabali, Shimlali, Souri*	Izmir (Turkey) Mont Carmel (Israel) Mont Boron (France) (2 trees/21)
	ME2	*Chetoui, Galega, Biancollila, Giarraffa, Amygdalolia, Zaity, Toffahi*	
	MOM	*Capanacce, Picholine, Pigale, Sabina, Zinzala, Lechin de Sevilla*	Tizi Ouzou (3 trees/12) (Algeria) Messine (Italy) Mont Boron (15 trees/21) (France)
CCK	MCK	*Blanquetier d'Antibes*, CBNMP 9–16*, Courbeil*, Curnet*, Olivière*, Chemlal*, Zarazi**	Tizi Ouzou** (9 trees/12 of which 2/3 observed trees are male-sterile (Algeria) Mont Boron** (4 trees/21 of which 1 is male-sterile) (France)

lele (*Ms*/*Ms*) in *Olivière*. This, however, is impossible since it would result from a cross between two male-sterile individuals. Nevertheless, several nuclear genes are sometimes involved in male sterility and could lead to a complex inheritance of this trait. In the context of an epistatic effect of one gene (*Cf*) on an *Ms* gene, this could lead to the fixation of *Ms*/*Ms*. We provide a simple hypothetical example leading to the fixation of a dominant male sterility allele (Fig. 2B) (Lewis and Crowe 1956). A formal rejection of such a genetic determinism would require several backcrosses, and hence at least half a century, in olive. However, the male-sterile phenotype *ms 2* was strictly correlated with the CCK and MCK organelles in seven cultivars and three oleaster trees. Since both cultivars and oleasters were from different geographical origins, such a feature cannot be explained by a nuclear gene determination of male sterility. Maternal transmission of male sterility and the correlation between the male sterile phenotype *ms 2* and a single cytotype CCK/MCK, strongly support the hypothesis of the cytoplasmic inheritance of *cms 2* male sterility.

In wild olive populations from Mont Boron and Kabylie, out of seven observed individuals carrying the MCK/CCK cytotype, four were male-fertile. This argues for the existence of a restoration factor for the MCK mitotype.

In cultivars carrying the ME1 mitotype, several male-sterile phenotypes (*Lucques*, *Tanche*) were found, suggesting a different genetic inheritance of this trait. Moreover, *Lucques* progenies have led to both male-fertile and male sterile trees, but the cross was not controlled. We do not have evidence of CMS associated with the ME1 mitotype.

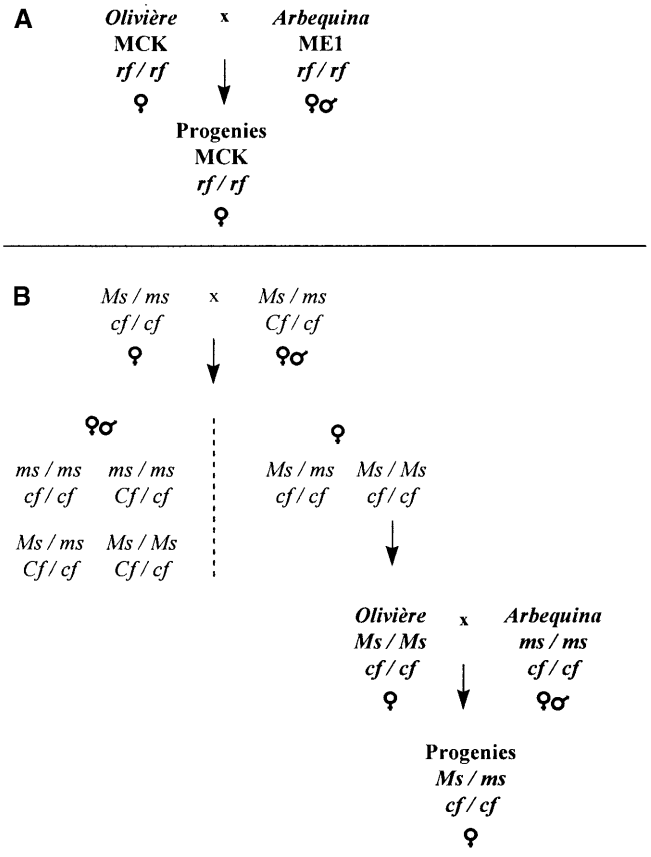

Fig. 2A, B Possible explanations of the male sterility in *Olivière*× *Arbequina* progenies. **A** Cytoplasmic male sterility: the absence of nuclear restoration factor (*Rf*) in *Arbequina* explains the male sterility of all the progenies. **B** Example of a genic male sterility: an epistatic effect between two nuclear genes (*Ms* and *Cf*) is assumed here. *Ms* is a dominant gene of male sterility which is complemented by the *Cf* gene. The homozygosity for the *Ms* gene in *Olivière* associated with the absence of the *Cf* gene in *Arbequina* explains the male sterility of all the progenies of the cross *Olivière*× *Arbequina*. ♀: male-sterile plant; ♀♂: hermaphrodite plant

Origin of cytoplasmic male sterility

In our study, each of the three mitotypes which were not linked with male sterility (ME1, ME2 and MOM) were associated with one chlorotype (CNC). This suggests that mitochondrial DNA rearrangements are more frequent than chloroplast DNA changes, in agreement with observations on other species (Palmer and Herbon 1988). The fourth mitotype, MCK, was the most distant from the three others (genetic distance of Nei and Li (1979): 0.34–0.37) and was associated with a distinctive CCK chlorotype (Table 3). Thus, as observed for other species, such as sunflower (Serror et al. 1990) and sugar beet (Bonavent et al. 1989), CMS associated with a specific cytotype (polymorphisms in both chloroplast and mitochondrial DNAs) has probably resulted from a distant cross. Here the association supported the fact that the MCK/CCK cytoplasm pre-existed in another taxon before a cross with olive. It is likely that distant hybridisation occurred with the human movement of cultivars in the Mediterranean Basin mixing different olive lineages – already suggested by Besnard et al. (1998) based on RAPD data – or because of the existence of a natural hybrid zone between two differentiated Mediterranean taxa. The analysis of the spatial distribution of cytotypes and of nuclear diversity in oleaster trees and cultivars should provide some evidence about these hypotheses.

Selection of male-sterile cultivars

In orchards, male sterility for one cultivar obviously implies the necessity to add another one as pollinator. This raises the question of why male-sterile cultivars are cultivated? *Chemlal* and *Olivière* are the most vigorous among economically important cultivars. Thus, it is probable that the selection of male-sterile cultivars was based on vegetative vigour. In Kabylie (Algeria), the soils assigned to oleiculture are light and often sloping. Vigorous olive cultivars (such as *Chemlal*) are thus required for the implantation of orchards (Loussert and Brousse 1978). Another reason for the selection of such trees is the composition and quality of the oil which are reported to be excellent in *Olivière* and *Chemlal.* MCK points to an independent origin for culti-

vars and it is likely that all cultivars carrying MCK might also display specific traits which were not pollen-transmitted to the other maternal lineages. Thus, we suggest that all male-sterile cultivars have to be conserved in collections to maintain both genic and cytoplasmic diversity.

Conclusion

Our results argue for CMS in olive with a nuclear restorer in oleaster. We suggest that this CMS has an alloplasmic origin. The presence of several mitotypes in the cultivated olive probably reflects its complex origin. In consequence, breeders have to check not only the nuclear diversity but also the cytoplasmic constitution of olive cultivars to classify them in order to preserve their cytoplasmic diversity.

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