



## Limitation of gene flow by distance in the common yellow jasmine (*Chrysojasminum fruticans*, Oleaceae): implications for the study of its mating strategies

Aurore Puyoou, Hervé Gryta, Anne-Laure Fuchs, Pierrick Blanchard, Laure Civeyrel, Pierre-Olivier Cheptou, Mathilde Dufaÿ, Julia Dupin, Patricia Jargeat, Emilie Lecompte, et al.

### ► To cite this version:

Aurore Puyoou, Hervé Gryta, Anne-Laure Fuchs, Pierrick Blanchard, Laure Civeyrel, et al.. Limitation of gene flow by distance in the common yellow jasmine (*Chrysojasminum fruticans*, Oleaceae): implications for the study of its mating strategies. *Botanical Journal of the Linnean Society*, In press, 10.1093/botlinnean/boad035 . hal-04133393

HAL Id: hal-04133393

<https://hal.science/hal-04133393>

Submitted on 19 Jun 2023

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Journal:	<i>Botanical Journal of the Linnean Society</i>
Manuscript ID	BOTJLS-Jan-2023-4211-ART.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	14-Apr-2023
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Keywords:	cpDNA < Genetics, gene flow < Genetics, genetic differentiation < Genetics, genetic structure < Genetics, microsatellites < Genetics, mitochondria < Genetics, self-incompatibility < Genetics, Mediterranean < Geography

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## ABSTRACT

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The common yellow jasmine (*Chrysojasminum fruticans*; Oleaceae) is a distylous shrub occurring in the wild in southwestern Europe and the Mediterranean Basin. Little is known about the genetics of its populations and such information would be necessary to investigate its spread and mating strategies. Here, the organization of its genetic diversity was investigated among and between 13 populations from southern France, including a 35-years old experimental plot ('CEFE', CNRS Montpellier). Markers (microsatellites and indels) were developed to screen polymorphisms in nuclear, chloroplast, and mitochondrial genomes. Low linkage disequilibrium was observed between chloroplast and mitochondrial haplotypes likely resulting from paternal leaks in their inheritance as reported in other species of tribe Jasmineae. Yet, analyses of 36 progenies issued from parents with distinct chloroplast and/or mitochondrial DNA haplotypes only revealed a maternal contribution. Natural populations of *C. fruticans* are moderately to highly differentiated at the regional scale with a strong isolation-by-distance pattern detected on nuclear data, indicating limited gene flow. An isolated site ('Moulis'), located on the marginal distribution area, was remarkably genetically depauperate and highly differentiated from other populations. Further studies on the variation of mating strategies in *C. fruticans* should consider populations with contrasting patterns of genetic diversity. The artificial 'CEFE' population also offers opportunities for experiments in a closed system.

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ADDITIONAL KEYWORDS: Chloroplast genome – Heterostyly – IBD – Jasmineae – Microsatellite – Mitochondrial genome – Organelle paternal leakage – Population genetics – Self-incompatibility.

## INTRODUCTION

Many ecological and evolutionary processes directly depend on how genetic diversity is structured within and among natural populations. For instance, levels of genetic variation and patterns of gene flow among populations determine both the opportunity for selection and the geographical scale at which selection can act (Slatkin, 1987; Sork, 2016). In plants, this mainly depends on dispersal strategies of pollen and seeds, but also on cross-compatibilities between individuals (Barrett, 2002; Charlesworth, 2006). Beyond the acknowledged usefulness of population genetics descriptors for applied purposes, such as conservation biology, describing the distribution of genetic diversity in a species also constitutes the first step of many basic research studies, in particular those that investigate the ecology and evolution of reproductive systems.

The common yellow jasmine [*Chrysojasminum fruticans* (L.) Banfi; Oleaceae] is a distylous shrub, which is the only native European species of Jasmineae, the sister lineage of the olive tribe or Oleeae (Wallander and Albert, 2000; Dupin *et al.*, 2020). In various members of Oleeae, a homomorphic self-incompatibility (SI) system with only two groups (di-allelic SI: DS) has been discovered. This unusual DS, compared to highly diversified SI (see for instance Bush *et al.*, 2010), asks the question of its maintenance as well as its potential role in the evolution of separate sexes in Oleaceae (Vassiliadis *et al.*, 2000; Saumitou-Laprade *et al.*, 2010; Vernet *et al.*, 2016; Billiard *et al.*, 2015). In addition, a breakdown of the same DS system has been documented in some individuals of one Oleeae species (de Cauwer *et al.*, 2021). No information of the genetic structure is currently available in the common yellow jasmine, and more generally in any species of Jasmineae. Such data has both practical and theoretical importance: (i) studying levels of variation will help in defining molecular markers and identifying populations of potential interest; (ii) measurements of heterozygosity should provide some indirect insight on the mating system and on possible breakdown of the DS; and

(iii) patterns of gene flow through both seed and pollen may help us to infer geographical scale at which evolutionary processes take place.

Our study species (*C. fruticans*) occurs in the wild in southwestern Europe and the Mediterranean Basin (Dommée, Thompson & Cristini, 1992; Olesen *et al.*, 2003; Banfi, 2014). It is generally found on dry and sunny soils of hillsides, often rocky with a slight preference for limestone, leading to a heterogeneous and fragmented habitat. The species is a small rhizomatous semi-evergreen shrub with slightly fragrant yellow flowers, which appear from April to June, and small black berries (0.5 cm in diameter) in the autumn. Individuals of *C. fruticans* either have “pin” flowers (long style and low anthers = longistylous) or “thrum” flowers (short style and high anthers = brevistylous), and reciprocal crosses mostly occur between the two morphs (Dommée, Thompson & Cristini, 1992; Gutián, Gutián & Medrano, 1998). Pollination and seed dispersal are respectively mediated by numerous insects of different orders (Thompson, 2001) and by birds (Puech, 1986). A generalist pollination system that may impede specialization of the plant to particular pollinators has been notably described by Thompson (2001). On the other hand, no study has specifically documented fruit dispersal of *C. fruticans*, but its frequent occurrence in abandoned cultivated areas and firebreaks suggests an efficient spread at the local scale (e.g. Debussche, Escarré & Lepart, 1982). Although pollinated by an insect community and its fruits spread by birds, the dispersal of the common yellow jasmine should be limited by geographic distance due to its occurrence in a fragmented habitat. A strong structure of its genetic diversity among populations is therefore expected, but gene flow measured on the organellar genomes or on the nuclear genome should not be impacted in the same way due to their different transmission modes (Petit *et al.*, 2005).

In this study, we thus aim at describing the population genetics of *Chrysojasminum fruticans* in southern France, in particular the isolation by distance (IBD) and the genetic differentiation between populations. Patterns of genetic diversity on different genomic

compartments were investigated and compared to assess the relative contribution of pollen and seeds in the global gene flow. First, genotyping methods (microsatellites and indels) were developed to screen polymorphisms in nuclear, chloroplast, and mitochondrial genomes, which should show contrasting mutation rates (Wolfe *et al.*, 1987) and could therefore also give complementary information about the impact of demographic processes operating at different historical scales on the species genetic variability and structure. The inheritance of both cytoplasmic organelles was beforehand tested in a few progenies in order to ensure that their polymorphisms can be used to study seed dispersal. A marked genetic differentiation between populations was detected, especially on nuclear data for which a strong IBD was observed confirming a limitation of gene flow, especially for the pollen. The implications of our results for investigating mating strategies in *C. fruticans* were finally briefly discussed.

## MATERIAL AND METHODS

### PLANT SAMPLING AND DNA EXTRACTION

Leaves of a total of 196 individuals ('ramets') from 13 French populations and one Spanish population were collected and preserved in silica gel (Table 1). To avoid sampling clones, we collected ramets that were at least 3 m apart. Floral morph and GPS coordinates of individuals (or populations) were also recorded (Table 1; Supporting Information, Table S1). Although style length varies in both morphs (Thompson & Dommée, 2000), the distinction between brevistylous (B) and longistylous (L) individuals has never been ambiguous on open flowers. In addition, 15 herbarium specimens from the Museum National d'Histoire Naturelle de Paris (MNHN-P) were sampled (Supporting Information, Table S2), representing *Chrysojasminum fruticans* populations throughout its range (from Morocco-Portugal in the West to Iran-Turkmenistan in the East). Nine of those specimens were collected more than a century ago.

Those specimens were used as outgroups to our study and also allowed us to test the utility of our markers over the whole distribution area of *C. fruticans*. All populations sampled in this study are assumed to have a natural origin, with the exception of the one from the “plateforme des terrains d’expérience du LabEx CeMEB” ('CEFE', Centre d’Étude Fonctionnelle et Evolutive de Montpellier), known to have an artificial origin since it was planted ca. 35 years ago by John D. Thompson and Bertrand Dommée, with individuals from different localities near Montpellier.

The DNA of each sample was extracted with the BioSprint DNA Plant Kit (QIAGEN). About 20 mg of material was placed in a 2-mL tube with three tungsten beads and frozen at -80°C for one hour before being crushed using TissueLyser II (QIAGEN). Then, after one hour of incubation at 65°C in 500 µL of 2X CTAB buffer, DNA extraction was performed following the supplier's protocol. Finally, the DNA was resuspended in 130 µL of TE buffer and stored at 4°C.

#### GENOPYING PROCEDURE

To genetically characterize all the sampled *C. fruticans* individuals, we developed 29 nuclear DNA loci (including 23 microsatellites and six indels) and 15 cytoplasmic loci [five mitochondrial DNA (mtDNA) loci (four indels and one microsatellite) and ten chloroplast DNA (cpDNA) loci (two indels and eight microsatellites)] (Supporting Information, Tables S3 to S5). We used shotgun genome sequencing data (*i.e.* HiSeq data on individual 'Celleneuve L11') to random search and assemble microsatellite or indel regions as described in Salmona *et al.* (2020). All assembled nuclear sequences are deposited in GenBank. In contrast, the cytoplasmic DNA loci were developed from length polymorphisms revealed on plastomes and mitogenomes of individuals collected in France (GenBank no MH559274, and OQ124187 to OQ124196).

The primers used to amplify the 44 loci are given in the Supporting Information (Tables S3 to S5). All primers were defined to have the same annealing temperature ( $T_m$ ).

Some markers were amplified alone (simplex), while others were amplified in multiplex (either two markers for nuclear loci, or three or four for cytoplasmic loci). The PCR conditions are those reported by Besnard *et al.* (2011). These amplifications were performed in a total volume of 25  $\mu$ L following the method of Schuelke (2000), which allows the integration of fluorescence on fragments amplified with a dye-labeled M13 primer. Three different fluorochromes were used depending on the markers: 6-FAM, ATTO565 and HEX. The PCR multiplexes and the fluorochrome used for each locus are given in the Supporting Information (Tables S3 to S5). The same PCR program was used for all markers: denaturation of the DNA at 95°C for 2 min followed by 25 cycles with a 30 sec denaturation at 95°C, followed by an annealing at 55°C and then a 90 sec elongation at 72°C. Ten additional cycles with a lowered annealing temperature of 51.5°C allowing integration of the dye-labeled M13 primer into amplified fragments were added and were followed by a final elongation of 20 min at 72°C.

After PCR amplification, the markers were multiplexed (several markers of different sizes were mixed together) in formamide with ROX-500 (Applied Biosystems) used as internal size standard. The amplified fragments at each locus were separated by chromatography on an ABI 3730 capillary sequencer (Applied Biosystems) by GenoScreen (Lille). The chromatograms were then read on GENEIOUS v.9.1.2 (Kearse *et al.*, 2012). To limit reading errors, all loci were read twice independently by AP and GB.

## DEFINING CYTOPLASMIC HAPLOTYPES AND NETWORK RECONSTRUCTION

For chloroplastic and mitochondrial genomes, polymorphisms were revealed at multiple loci (see above). Plastid and mitochondrial DNA profiles (chlorotypes and mitotypes) were defined by the combination of alleles from these loci. Relationships between chlorotypes on one hand, and between mitotypes on the other hand, were established by constructing a reduced-median haplotype network with NETWORK v.10.2 (Bandelt, Forster & Röhl, 1999). All polymorphisms observed were coded as in Gryta *et al.* (2017) [*i.e.* number of repeats for microsatellites, or binary (0/1) for indels].

ON THE INHERITANCE OF ORGANELLES IN *C. FRUTICANS*

As potential for paternal leakage of chloroplasts and mitochondria has been previously reported in several *Jasminum* species (Sodmergen *et al.*, 1998; Zhang, Liu & Sodmergen, 2003), it was necessary to check the inheritance of organelles in *C. fruticans*. As a first source of evidence, we measured the linkage disequilibrium (LD) between chlorotypes and mitotypes. In the case of a strict maternal inheritance, we expect a strong LD (as already shown in beet, *Silene*, and olive; Desplanque *et al.*, 2000; Olson & McCauley, 2000; Besnard *et al.*, 2002). In contrast, the presence of a few paternal leakages may reduce LD between haplotypes of the two organelle genomes. The LD was estimated with Lewontin's normalized  $D'$  (Lewontin, 1964, 1988), and its significance was tested with Fisher's exact tests using ARLEQUIN (Excoffier & Lischer, 2010). The  $D'$  index was calculated (i) separately on each sampled population where polymorphism was observed on both organellar genomes, and (ii) on all French individuals considered as a single population. Then, we also verified the inheritance of organelles in a few progenies (*i.e.* seeds resulting from open pollination) for which parents display different cpDNA and/or mtDNA haplotypes. In the 'CEFE' and 'Moulis' populations, we previously identified 36 progenies issued from such crosses (*i.e.* 33 with a different chlorotype, and 32

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3 with a different mitotype; Supporting Information, Table S6). Genotyping of the chloroplastic  
4 and mitochondrial haplotypes in these progenies allowed us to determine from which parent  
5 cytoplasmic organelles were inherited.  
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10 POPULATION GENETICS ANALYSES  
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13 To avoid bias in population genetics analyses due to repetitions of the same individual, clones  
14 (genets) needed to be excluded. In a population, plants sampled a few meters apart may  
15 correspond to ramets from the same stump, and the probability of finding the same genotype at  
16 29 multi-allelic nuclear loci is theoretically very low (e.g. Baali-Cherif & Besnard, 2005). Thus,  
17 when several close individuals shared the same genotype or a very similar genotype (*i.e.*  
18 differing by a mutation step on a long allele), we considered them as a clone. In this case, only  
19 one ramet was kept for analyses. In order to represent the genetic proximity between all  
20 analyzed individuals, Bruvo *et al.* (2004) genetic distances were first calculated on the nuclear  
21 microsatellite data between each pair of genotypes with the R package *Poppr* (Kamvar, Tabima  
22 & Grünwald, 2014) and a dendrogram was reconstructed according to the unweighted pair  
23 group method with arithmetic mean (UPGMA). For further analyses, we only considered  
24 populations with at least seven genotypes.  
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28 *Populations' genetic diversity*  
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31 Genetic diversity patterns were described for each population. First, observed ( $H_O$ ) and  
32 expected ( $H_E$ ) heterozygosities under the Hardy-Weinberg ideal population model (*i.e.*  
33 population of infinite size, panmictic reproduction, non-overlapping generations), and the Weir  
34 & Cockerham Fixation Index ( $F_{IS}$ , Weir & Cockerham, 1984) were calculated using the  
35 GENETIX software (Belkhir *et al.*, 2004) for each nuclear locus. Allelic richness ( $A_R$ ) was  
36 calculated with the R package HIERFSTAT (Goudet, 2005) to compare the number of alleles  
37 between populations. The presence of null alleles was tested for each population with a 95%-  
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confidence interval using MICRO-CHECKER v.2.2.3 (van Oosterhout *et al.*, 2004). As all loci were successfully amplified, the null allele frequency was estimated according to Brookfield (1996). For cytoplasmic data (cpDNA and mtDNA), haplotypic diversity was calculated from the formula  $D = 1 - \sum p_i^2$  with  $p_i$  the frequency of haplotype  $i$  and was multiplied by  $n / (n - 1)$  to account for differences in sample sizes, as described in Gryta *et al.* (2017). This parameter was estimated independently for chloroplast and mitochondrial data ( $D_{\text{cp}}$  and  $D_{\text{mt}}$ ), and for their combination ( $D_{\text{cp+mt}}$ ).

#### *Genetic structure of populations*

To measure genetic differentiation between populations, the fixation index  $F_{\text{ST}}$  of Weir & Cockerham (1984) was calculated between each pair of populations using GENETIX. These calculations were performed separately for nuclear data (bi-paternally transmitted, and thus dispersed by pollen and seed) and cytoplasmic data (assumed to be maternally transmitted, and dispersed by seed). However, for cytoplasmic data, the main mode of transmission of plastids and mitochondria remains uncertain in Jasmineae (Sodmergen *et al.*, 1998; Zhang, Liu & Sodmergen, 2003). Occasional paternal transmission of one or both cytoplasmic genomes could impact on differentiation patterns between populations. Because of this uncertainty, we estimated the  $F_{\text{ST}}$  of the cytoplasmic data using four different approaches: (i) only on haplotypes of the chloroplast genome, (ii) only on haplotypes of the mitochondrial genome, (iii) considering the chloroplast and mitochondrial genomes as two independent cytoplasmic loci, and (iv) combining cpDNA and mtDNA information to define cytoplasmic haplotypes. Patterns of isolation by distance (IBD) were then compared between each approach. The geographical distribution of cpDNA and mtDNA haplotypes across populations was also mapped using QGIS v.3.0.3 (QGIS Development Team, 2018).

In order to determine the number of genetic clusters actually present in our sample and to assess the isolation between populations, a Bayesian approach was then used to assign individuals to genetic clusters on the basis of their genotypes. STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000) was used on the nuclear data set. For this analysis, the Spanish population ('La Aliseda') was excluded due to its great distance from all the other studied populations and the absence of intermediate populations between them. The artificial 'CEFE' population was also excluded in order not to bias the analyses (e.g. due to admixture between individuals). This analysis was carried out under the admixture model (mixing of gametes possible at reproduction) for a duration of 200,000 generations after a burn-in period of 50,000 generations. The analysis was performed for a number of clusters ( $K$ ) ranging from 1 to 14 with ten iterations for each value of  $K$ . The most likely number of clusters was determined using the *ad hoc* measure of  $\Delta K$  performed with STRUCTURE HARVESTER (Earl & vonHoldt, 2012). In order to determine the putative origin of the artificial CEFE population, we then assigned each individual from this location to the ten natural French populations (with at least ten genets) using GENECLASS2 (Piry *et al.*, 2004). The standard criterion described by Rannala & Mountain (1997) was used to compute the probabilities with Bayesian statistics. The population assignment simulation algorithm described by Paetkau *et al.* (2004) was used in order to simulate 10,000 genotypes for each population. An arbitrary threshold probability value of 0.05 or greater was then applied to identify the possible origin of CEFE genotypes among natural populations.

#### *Limitation of gene flow by distance and relative contribution of pollen and seeds*

As IBD was expected, Mantel (1967) tests between geographical distances and  $F_{ST}$  values between pairs of populations were performed in southern France on the ten natural populations with at least ten genotypes. These tests were done with 999 permutations using the R package *ade4* (Chessel, Dufour & Thioulouse, 2004) for each genomic compartment in order to compare

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3 patterns according to the origin of the data (nuclear *vs.* cytoplasmic). In addition, the relative  
4 contribution of pollen and seeds in the global gene flow ( $R$ ) was investigated following the  
5 method of Ennos (1994). This approach allows an indirect estimation of pollen and seed flows  
6 from genetic differentiation estimates ( $F_{ST}$ ) on both bi-parentally inherited nuclear markers  
7 ( $F_{STn}$ ) and maternally inherited cytoplasmic markers ( $F_{STc}$ ). The  $R$  estimation is based on a  
8 model of island migration (Wright, 1965), and can be used under several assumptions: (i) a very  
9 low mutation rate compared to migration rate, (ii) a constant and equal size of populations, and  
10 (iii) a constant and equal migration rate between populations, the last two points representing a  
11 drift/migration equilibrium. This equilibrium turns out to be rapidly reached in nature (Slatkin,  
12 1993). However, when the species has a long generation time and/or if perturbations are quite  
13 recent, it might be necessary to check these assumptions. In addition, the precision of estimates  
14 of genetic differentiation used to infer gene flow depends on the sampling of populations (Petit  
15 *et al.*, 2005; Besnard *et al.*, 2021). Although the  $R$  estimate can help comparing the relative  
16 contribution of pollen and seeds in global gene flow among taxa (Ennos, 1994; Petit *et al.*,  
17 2005), it thus still needs to be interpreted with caution.  
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## RESULTS

All loci were successfully amplified in all *Chrysojasminum fruticans* individuals freshly collected in southern France and Spain (whole dataset provided in the Supporting Information, Table S1), and all revealed polymorphisms. A few genets with several ramets were identified in populations 'La Aliseda', 'Amélie-les-Bains', 'Prades-le-Lez', and 'La Turbie'. These genets can cover a relatively large surface (up to about 90 m<sup>2</sup> for an individual of the Spanish population 'La Aliseda'). Only one ramet per genet was subsequently kept for our analyses (for a total of 186 genets). Among the 179 genets sampled in France, 89 were recorded as

brevistylous and 90 as longistylous (Table 1). In addition, most of our markers yielded amplifications with herbarium samples, pointing out their high potential to analyze genetic variation in old specimens collected more than 100 years ago. Cytoplasmic markers revealed the presence of seven chlorotypes and six mitotypes in French populations, most of them being shared with the few analyzed individuals from the Southern Iberian Peninsula to the Eastern Maghreb (e.g. all mitotypes, and chlorotypes 1, 3, 4 and 7; Supporting Information, Table S1). By combining cpDNA and mtDNA information, 20 cytoplasmic haplotypes were identified in southern France (Table 3), where five of the six mitotypes were associated with more than one chlorotype, and five of the seven chlorotypes were associated with more than one mitotype. Consequently, the LD between haplotypes of the two organellar genomes, measured according to Lewontin (1964), was relatively low ( $D' = 0.368$ ,  $p$ -value < 0.001) compared to other plants where such data are available [e.g.  $D' > 0.95$  in *Olea europaea* (Besnard *et al.*, 2002), *Silene vulgaris* (Olson & McCauley, 2000), and *Beta vulgaris* (Desplanque *et al.*, 2000)]. However, relatively strong and significant LD between organellar genomes was found at the population scale (*i.e.* 'La Aliseda' and 'CEFE';  $D' > 0.88$ ,  $p$ -value < 0.005; Supporting Information, Table S7). Overall, a non-strict association between haplotypes of both cytoplasmic genomes indicates that organelles are not always inherited together (implying paternal leaks). Yet, analyses of all 36 progenies only revealed a maternal inheritance of the two organellar genomes (Supporting Information, Table S6), suggesting that paternal leaks may be occasional. Consequently, the structure of organellar DNA diversity should be mostly influenced by seed dispersal. In addition, all 36 crosses involved a pair of brevistylous and longistylous individuals (24 B x L and 12 L x B; Supporting Information, Table S6).

## STRUCTURE OF THE NUCLEAR GENETIC DIVERSITY ON THE WHOLE SAMPLE

The genetic proximity between all individuals of *C. fruticans* is represented by a dendrogram (Supporting Information, Fig. S1). All individuals from French populations were clustered together. Individuals from the same population were generally found in the same group suggesting a strong structure of populations in the South of France. Individuals from the Spanish population 'La Aliseda' formed the closest cluster to the French populations. Individuals from the rest of the Mediterranean were more distant from French and Spanish populations, except for individuals from Tunisia and Portugal. This analysis also showed that individuals from Iran and Turkmenistan are very similar to each other and genetically the most distant from all other individuals analyzed.

## GENETIC DIVERSITY OF POPULATIONS ANALYSES

Most nuclear loci revealed polymorphism within populations, even the di-allelic indel markers (Supporting Information, Table S8). Nevertheless, a few loci were not variable in some populations, especially in 'Moulis' (eight loci) and 'La Aliseda' (six loci). Among populations, most loci showed no excess of homozygosity or significant deviation from expected heterozygosities at the Hardy-Weinberg equilibrium, suggesting that the species reproduces mainly via outcrossing. For all populations, the global  $F_{IS}$  value was thus not significantly different from 0. The MICRO-CHECKER analysis detected probable presence of null allele in only five populations for one locus each (Cf-TA-1 for 'Bages', Cf-ATT2 for 'CEFE', Cf-GAA19 for 'Moulis', Cf-GAA22 for 'St-Maurice-Navacelles', and Cf-GAA4 for 'Puycelsi', with respectively the followings Brookfield estimates of null allele frequencies: 0.019, 0.215, 0.093, 0.079, and 0.154). Considering the multiple tests performed (*i.e.* 29 loci x 12 populations), such observations may be fortuitous and all loci should have a very low frequency of null alleles. Finally, the average expected heterozygosity and allelic richness (calculated for seven diploid

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3 individuals) showed that 'Moulis' ( $H_E = 0.35$ ;  $A_R = 2.36$ ) is genetically impoverished compared  
4 to all other populations ( $0.41 < H_E < 0.56$ ;  $2.84 < A_R < 3.89$ ; Table 2). The highest level of  
5  
6 nuclear diversity was revealed in 'St-Maurice-Navacelles'.  
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10 Most populations showed polymorphisms on both chloroplast and mitochondrial  
11 genomes (Fig. 2; Supporting Information, Table S8), with a maximum of five mitotypes in 'St-  
12 Maurice-Navacelles' and four chlorotypes in 'CEFE'. The combination of both genomes  
13 allowed us to detect from two to eight cytoplasmic haplotypes per population, with a haplotypic  
14 diversity ( $D_{cp+mt}$ ) varying from 0.19 ('Celleneuve') to 0.91 ('St-Maurice-Navacelles').  
15 Conversely to the nuclear genome, the 'Moulis' population did not show a particularly reduced  
16 diversity of cytoplasmic haplotypes ( $D_{cp+mt} = 0.50$ ), although its chlorotype diversity was  
17 among the lowest ( $D_{cp} = 0.11$ ; Table 2).  
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## POPULATION STRUCTURE

The  $F_{ST}$  values calculated from the nuclear data supported a significant genetic differentiation  
between most pairs of populations (Supporting Information, Table S9). The dendrogram  
reconstructed from these  $F_{ST}$  (Supporting Information, Fig. S2) showed that geographically  
close populations were also genetically close, except in the case of 'Moulis', which was found  
here genetically more differentiated from the other French populations than the Spanish  
population was. Also, the artificial population of 'CEFE' (Montpellier) whose individuals  
originate from neighboring populations was found to be close to the other populations sampled  
near Montpellier ('Celleneuve' and 'Prades-le-Lez').

An analysis performed with STRUCTURE on nuclear data from ten natural French  
populations (with at least ten genotypes) revealed the presence of several genetic clusters. Peaks  
in the graphical representation of  $\Delta K$  (Fig. 1A) allowed the identification of population  
structuring at two levels:  $K = 2$  and 10. For  $K = 2$ , 'Moulis' was distinguished from other

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2 populations (Fig. 1B), whereas the ten populations defined *a priori* were relatively well  
3 supported at  $K = 10$  (Fig. 1C). The assignment of ‘CEFE’ individuals to these natural  
4 populations confirmed the putative origin of this artificial population from collects around  
5 Montpellier, since successful assignments (27% of genets) only concerned the three locations  
6 collected in Hérault ('St-Maurice-de-Navacelles', 'Celleneuve', and 'Prades-le-Lez'; Supporting  
7 Information, Table S10).

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9 Genetic relationships between cytoplasmic haplotypes assessed by reduced-median  
10 network analyses (Fig. 2) showed three slightly differentiated lineages of chlorotypes (*i.e.* 1-2,  
11 3-6, and 7) and no clear genetic structure for mitotypes. The geographical distribution of these  
12 chlorotypes and mitotypes (Fig. 2) did not show any clear pattern of geographical structuring.  
13 However,  $F_{ST}$  values calculated from these cytoplasmic data were relatively high and most of  
14 them were significantly different from 0 (Supporting Information, Table S8).

### 15 ISOLATION BY DISTANCE AND CONTRIBUTION OF POLLEN AND SEEDS TO GLOBAL GENE FLOW

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17 Strong IBD was observed for the nuclear data (Mantel test,  $r = 0.528$ ,  $p$ -value = 0.004; Fig. 3A).  
18 In contrast, the cytoplasmic data did not show any isolation pattern by distance (Fig. 3B),  
19 regardless of how the  $F_{ST}$  values were calculated (*i.e.* based on cpDNA, mtDNA, or when  
20 combining information of both organellar genomes; Supporting Information, Fig. S3). Based  
21 on the estimates of global genetic differentiation between populations for nuclear markers  
22 ( $F_{STn} = 0.17$ ,  $F_{IS} = 0.17$ ) and cytoplasmic genomes ( $F_{STc} = 0.50$ ), the Ennos’s index ( $R = 3.618$ )  
23 showed that the contribution of the pollen to global gene flow is somewhat greater than that of  
24 seeds. However, this index was relatively low compared to many other plants (median  $R = \text{ca.}$   
25 17; Petit *et al.*, 2005).  
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## DISCUSSION

6 This work represents the first population genetics study of *Chrysojasminum fruticans*. We  
7 mainly sampled populations in southern France and recorded floral morphs on all individuals.  
8 Genetic variation was then detected in the three genome compartments allowing revealing  
9 contrasting levels of diversity among populations as well as a strong genetic structure resulting  
10 from limited gene flow. It also brought information on the relative contribution of seeds and  
11 pollen in the global gene flow. Our results will have various methodological implications when  
12 developing genetic studies on this species, especially to study its reproductive and dispersal  
13 strategies.

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26 First, our sampling in southern France revealed that both floral morphs have a balanced  
27 frequency among all populations (89 B vs. 90 L), and the few analyzed progenies (36) also  
28 confirmed that crosses should mainly occur between these two morphs (Dommée, Thompson  
29 & Cristini, 1992; Gutián, Gutián & Medrano, 1998). These preliminary observations are  
30 consistent with a diallelic self-incompatibility system but a wider sampling is still necessary,  
31 especially to measure how mating patterns vary among populations and floral morphs (for  
32 instance, to test if cross-compatibility or selfing rate vary among morphs in relation with stigma  
33 height or any other traits; Thompson & Dommée, 1993, 2000).

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44 Then, a set of markers was developed for investigating variation of the three genomic  
45 compartments in *C. fruticans*. Such tools may have applications in different fields from  
46 population genetics and phylogeography to parentage analyses. Here, chloroplast and  
47 mitochondrial markers showed great potential for investigating the inheritance of both  
48 organellar genomes by either investigating the linkage disequilibrium between chloroplast and  
49 mitochondrial haplotypes, or directly by checking their inheritance in progenies. The low  
50 linkage disequilibrium on the whole sample supports a non-strict maternal inheritance in  
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3 *C. fruticans* as already documented in other Jasmineae (Sodmergen *et al.*, 1998; Zhang, Liu &  
4 Sodmergen, 2003), but our preliminary progeny analyses indicate that paternal leakage should  
5 be rare. Consequently, we used organellar DNA polymorphism for investigating seed dispersal.  
6 Furthermore, nuclear microsatellite markers may be useful in assisting the identification of  
7 genets in populations and in determining fine population structure to further study demographic  
8 processes at a local scale. Here, they revealed shared genotypes by close ramets indicating the  
9 presence of clones in the investigated populations due to vegetative growth. The shoots of a  
10 single stump can cover up to several tens of m<sup>2</sup> (as already observed in olive trees; Baali-Cherif  
11 & Besnard, 2005), indicating *a priori* a long persistence of certain individuals. Based on our  
12 observations, avoiding multiple sampling of the same genotype in population genetic studies  
13 may therefore require collecting ramets at least ten meters apart.  
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16 Once the clones were removed, our population analysis showed a strong structure of the  
17 genetic diversity in *C. fruticans* (*i.e.* high differentiation between pairs of populations) on both  
18 nuclear and cytoplasmic data. In addition, strong IBD is observed on the nuclear data, but not  
19 on the cytoplasmic data (Fig. 3). The presence of a very strong IBD is common in insect-  
20 pollinated species with limited pollen and seed dispersal (*e.g.* Jump *et al.*, 2009). In *C. fruticans*,  
21 the pollen is sticky and should be mostly spread by various insects (Thompson, 2001) at the  
22 local scale. In contrast, fleshy fruits can be spread by birds (Puech, 1986), and the longest  
23 dispersal events may thus occur at a larger distance for seeds than for pollen, especially in a  
24 very fragmented landscape (*e.g.* García-Verdugo *et al.*, 2010; Trapnell *et al.*, 2022). The relative  
25 contribution of pollen and seeds to global gene flow reflects this expectation, with an *R* value  
26 inferior to 5 as frequently observed in most insect-pollinated and fleshy fruited species (Oddou-  
27 Muratorio *et al.*, 2001; Petit *et al.*, 2005). Furthermore, random long-distance dispersal by seeds  
28 through the landscape would lead to a patchy structure of organellar lineages in space (Petit *et*  
29 *al.*, 1997), and could explain the absence of IBD on both maternally inherited genomes. In  
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addition, the plants mainly produced fruits in autumn (Puech, 1986) when migratory birds are travelling southwards, and a latitudinal pattern could thus be expected. It is noticeable that organellar haplotypes observed in southern France are shared with populations from the Southern Iberian Peninsula to the Eastern Maghreb, suggesting that seeds can indeed travel long distances with birds and could have even crossed the Mediterranean Sea, as already shown in another Mediterranean shrub, *Pistacia lentiscus* L. (Martinez-Lopez *et al.*, 2020). Nonetheless, it is possible that cytoplasmic haplotypes identified here have diverged a long time ago, and more variable loci would reveal some differentiation between such distant populations. It is thus presently too speculative to interpret our diversity patterns in the context of Mediterranean micro-refugia (*i.e.* south-eastern Pyrenees, southern Cevennes, Mont Ventoux, eastern Provence, Alpes Maritimes; Médail & Diadema, 2009), and a denser and wider sampling of the studied area would also be required for this purpose.

Finally, in a context of gene flow limitation, small marginal populations are expected to diverge rapidly. In our study, the 'Moulis' population is highly differentiated from the others and is genetically impoverished compared to other populations, supporting the fact that it is highly isolated. This population is relatively large (several hundred thousand individuals over several hundred ha), and its low diversity may be explained by a relatively recent expansion from a few founders. However, the necessity that recent populations were initially founded from at least two compatible individuals could be a constraint, and self-compatible individuals may be temporarily favored on the front of colonization to ensure reproduction (Baker, 1955; Pannell & Barrett, 1998). Although no significant inbreeding index was observed in all populations indicating that the species is mainly out-crossing, preliminary paternity analyses on seeds collected in 'Moulis' from two mother plants in two following years showed a high rate of self-fertilization (ca. 70%) on a brevistyloous individual (unpublished data), suggesting that this population is indeed not completely allogamous as similarly reported in some populations of

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3 *Ligustrum vulgare* L. (de Cauwer *et al.*, 2021). Thus, effective selfing events could have also  
4 contributed to genetic diversity erosion of the 'Moulis' population.  
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## CONCLUSION AND PERSPECTIVES

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13 Our results have several implications for further studies on the variation of mating strategies in  
14 *Chrysojasminum fruticans*. Variable levels of genetic diversity among unconnected populations  
15 imply contrasting demographic histories with possibly varying loads of deleterious mutations  
16 (e.g. Liu *et al.*, 2022). In a distylous species, if the selfing rate is higher in one morph than in  
17 the other, questions arise about the allocation of resources and their evolutionary consequences  
18 on the reproductive strategies (e.g. possible transition to cryptic dioecy). For this reason, it  
19 seems relevant to study mating of the common yellow jasmine in various contexts. One could  
20 first compare the rates of self-fertilization (by paternity analysis on seeds) on the two floral  
21 morphs in several populations, and in case of marked differences, study the associated  
22 inbreeding depression. If one morph is much more self-pollinating in some populations, it  
23 would also be very relevant to follow the investment of each morph in reproduction and  
24 vegetative growth (Dommée, Thompson & Cristini, 1992).  
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Finally, the artificial population on the experimental field of the 'CEFE' Montpellier  
(with approximately 200-300 mature individuals in a hedge of ca. 250 m<sup>2</sup>) may also offer some  
opportunities for studying mating in a semi-natural context. Our genetic data supported that  
individuals have mostly originated from different localities near Montpellier. Its high  
accessibility may allow long-term follow up of individuals (e.g. measure of their investment in  
sexual functions over years). Due to the limitation of gene flow by distance, this population can  
be considered as strongly isolated from natural stands (at several kilometers). It will be fully  
characterized with our markers in order to investigate pollen and seed dispersal, as well as

mating between morphs (Besnard *et al.*, 2020). Microsatellite analyses should allow investigating pollen gene flow via paternity analyses of seeds, while recruitment over last decades could be investigated via reconstructing pedigrees (e.g. Bacles, Lowe & Ennos, 2006; Bittencourt & Sebben, 2007). Finally, cytoplasmic variation in the population could be useful to investigate the inheritance of organelles and estimate the rate of paternal leaks in known crosses.

#### ACKNOWLEDGEMENTS

We thank Myriam Gaudeul (MNHN-P), Alex Baumel, Elodie Chapuis, Nicolas Juillet, Gérard Largier (CBN-PMP), Elwen Le Bras, Frédéric Médail, James Molina (CBN-Med), and John Thompson, for their help in plant sampling. The 'CEFE' population is managed by the Platform "Terrains d'Expériences" of the LabEx CeMEB (ANR-10-LABX-04-01). We also thank two anonymous referees for their constructive comments.

#### FUNDING

This research was funded by the Region Occitanie of France, through the BIODIVOC programme (grant REPOL). J.D. and G.B. received support from the H2020 Marie Skłodowska-Curie Actions (grant 842234-FRUITFUL), and A-L.F., P-O.C. and G.B. from the European Union's Horizon 2020 project GEN4OLIVE (H2020-SFS-2020-1; G.A. No. 101000427). P.B., L.C., P.J., H.G. and G.B. are members of the EDB laboratory, which is supported by the Laboratoire d'Excellence (LABEX) CEBA (grant ANR-10-LABX-25-01) and LABEX TULIP (grant ANR-10-LABX-0041), both managed by the Agence Nationale de la Recherche in France.

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DATA AVAILABILITY

Genotype data generated in this study are provided in the Supporting Information (Table S1).

Thirteen herbarium specimens were collected in Amélie-les-Bains, Bages, CEFE, Moulis, Moussoulens, and Puycelsi, and deposited at the Montpellier herbarium (MPU; HG-2023-001 to -005, and GB-2023-001 to -008).

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3 **Table 1.** List of sampled populations, with their location and geographic coordinates. All  
4 populations except one ('La Aliseda', Spain) are located in France. Nr and Ng refer to the  
5 number of collected 'ramets' and of recorded genotypes (after excluding clones), respectively.  
6 For each French locality, the number of brevistyloous (B) and longistyloous (L) genotypes is given  
7 in bracket (for more details see Supporting Information, Table S1). Lat. = Latitude; Long. =  
8 Longitude.  
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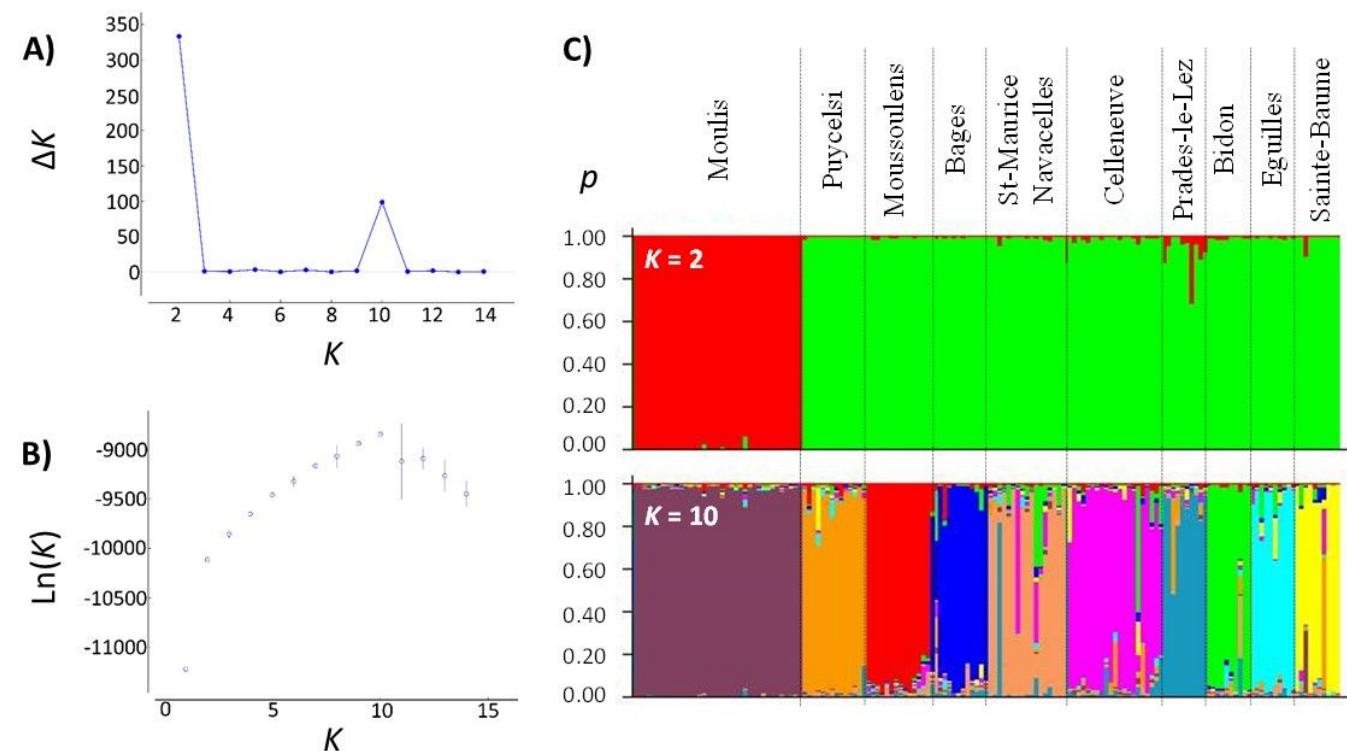
Locality	Department (or province)	Lat., Long.	Nr	Ng (B/L)
La Aliseda	Jaén	38.335, -3.569	10	7
Amélie-les-Bains	Pyrénées Orientales	42.478, 2.666	9	5 (3/2)
Bages	Aude	43.132, 2.985	12	12 (8/4)
Bidon	Ardèche	44.370, 4.532	10	10 (5/5)
CEFE (Montpellier)	Hérault	43.638, 3.862	15	15 (6/9)
Celleneuve	Hérault	43.611, 3.821	21	21 (10/11)
Eguilles	Bouches du Rhône	43.601, 5.2669	10	10 (5/5)
Moulis	Ariège	42.979, 1.084	37	37 (18/19)
Moussoulens	Aude	43.286, 2.249	15	15 (8/7)
Prades-le-Lez	Hérault	43.693, 3.861	11	10 (5/5)
Puycelsi	Tarn	44.015, 1.676	14	14 (8/6)
Sainte-Baume	Bouches du Rhône	43.327, 5.657	10	10 (4/6)
St-Maurice-Navacelles	Hérault	43.891, 3.510	17	17 (8/9)
La Turbie	Alpes Maritimes	43.747, 7.411	5	3 (1/2)

**Table 2.** Summary of genetic diversity parameters estimated within populations with at least seven individuals. Expected and observed heterozygosities were calculated using GENETIX (Belkhir *et al.*, 2004), the allelic richness ( $A_R$ ) with the R package HIERFSTAT (Goudet, 2005), and the haplotypic diversities ( $D$ ) according to Gryta *et al.* (2017). Parameters estimated per locus are given in the Supporting Information (Table S8).  $A_R$  = Allelic richness,  $H_E$  = Expected heterozygosity under Hardy-Weinberg,  $H_O$  = Observed heterozygosity,  $D_{cp}$  = Chloroplast haplotype diversity,  $D_{mt}$  = Mitochondrial haplotype diversity,  $D_{cp+mt}$  = Cytoplasmic haplotype diversity.

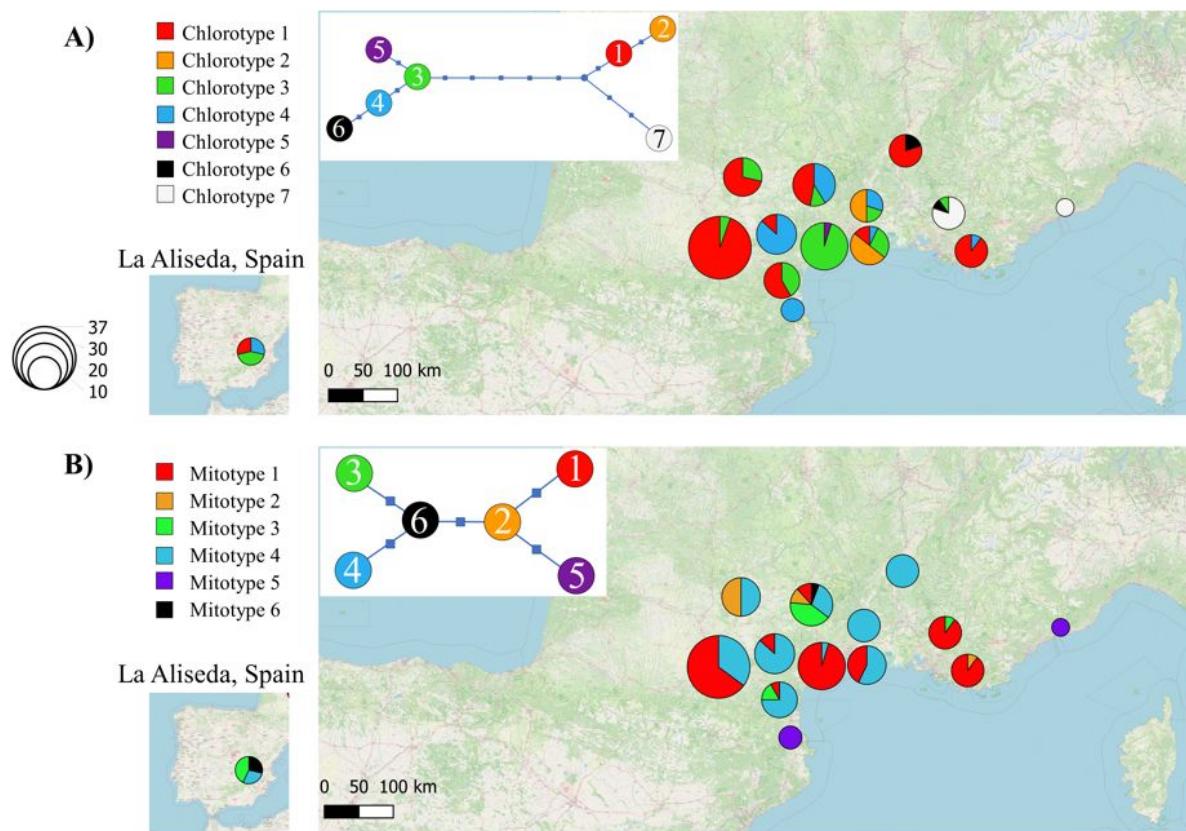
Population	Nuclear DNA			Cytoplasmic DNA		
	$A_R$	$H_E$	$H_O$	$D_{cp}$	$D_{mt}$	$D_{cp+mt}$
La Aliseda	3.17	0.42	0.45	0.76	0.76	0.76
Bages	3.38	0.50	0.49	0.53	0.44	0.65
Celleneuve	3.32	0.47	0.47	0.10	0.10	0.19
CEFE	3.78	0.55	0.57	0.70	0.53	0.70
Prades-le-Lez	3.21	0.47	0.51	0.69	0.00	0.69
St-Maurice-de-Navacelles	3.89	0.56	0.58	0.63	0.76	0.91
Moussoulens	3.32	0.50	0.54	0.75	0.75	0.83
Puycelsi	3.36	0.50	0.46	0.44	0.54	0.74
Bidon	3.29	0.49	0.58	0.36	0.00	0.36
Sainte-Baume	2.84	0.41	0.46	0.20	0.20	0.20
Eguilles	3.19	0.47	0.52	0.38	0.20	0.38
Moulis	2.36	0.35	0.37	0.11	0.47	0.50

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3 **Table 3.** Association between mitotypes and chlorotypes on all *C. fruticans* individuals  
4 collected in southern France (179 individuals). Occurrence number of each chlorotype-mitotype  
5 combination is reported. Based on these data, the LD between haplotypes of the two organellar  
6 genomes, measured according to Lewontin (1964), was relatively low, but still highly  
7 significant ( $D' = 0.368$ ,  $p$ -value < 0.001).  
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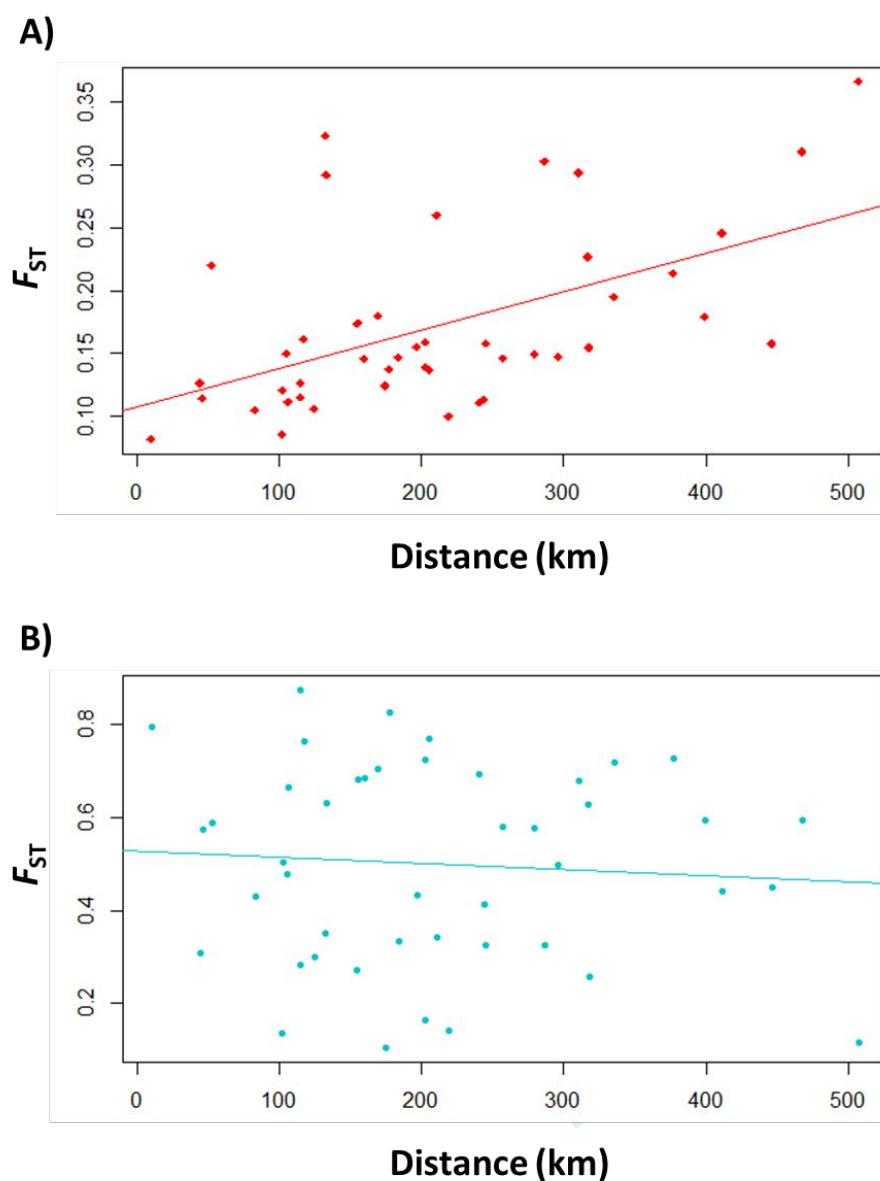
Mitotype no	Chlorotype no							<i>Total</i>
	1	2	3	4	5	6	7	
1	36		26	3	1	8		74
2	6		1	3				10
3	2		4	3			1	10
4	37	12	10	15			2	76
5				1				1
6				5		3		8
<b>Total</b>	<b>81</b>	<b>12</b>	<b>41</b>	<b>30</b>	<b>1</b>	<b>11</b>	<b>3</b>	<b>179</b>



**Figure 1.** Results of the STRUCTURE analysis on ten French populations. A Bayesian approach was used to assign individuals to populations on the basis of their genotypes with STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000). The analysis was performed for a number of clusters ( $K$ ) ranging from 1 to 14 with ten iterations for each value of  $K$ . The most likely numbers of clusters were determined using the *ad hoc* measure of  $\Delta K$  performed with STRUCTURE HARVESTER (Earl & vonHoldt, 2012). **(A)** Absolute values of the second-order rate of change of the likelihood distribution divided by the s.d. of the likelihoods ( $\Delta K$ ); **(B)** Mean log likelihood [ $\text{Ln}(K) \pm \text{s.d.}$ ] averaged over the ten iterations for each  $K$  value; **(C)** Barplots of the population structure for  $K = 2$  and 10. Each genetic cluster is represented by a color. Each vertical bar corresponds to an individual with their percentage assignment ( $p$ ) to the clusters. For  $K = 2$ , the population of 'Moulis' (in red) differs from the nine others (in green). At  $K = 10$ , the clusters correspond fairly well to the populations defined *a priori*.



**Figure 2.** Distribution of (A) chloroplastic and (B) mitochondrial haplotypes in southwestern France. The haplotypes found in a Spanish population ('La Aliseda', Jaén Province) are also indicated. In the left-hand, top corner of each map, the reduced-median haplotype network (reconstructed with NETWORK v.10.2; Bandelt, Forster & Röhl, 1999) is given for each organellar genome.



**Figure 3.** Genetic differentiation between populations as a function of geographical distance for (A) nuclear and (B) cytoplasmic data. Tests of Mantel (1967) were done with 999 permutations using the R package *ade4* (Chessel, Dufour & Thioulouse, 2004). Genetic distances ( $F_{ST}$ ) and geographical distances between population pairs show a significant relationship (Mantel test,  $r = 0.528$ ,  $p$ -value = 0.003) for nuclear data, but not for cytoplasmic data ( $r = -0.052$ ,  $p$ -value > 0.05).

## Supporting information

### Limitation of gene flow by distance in the common yellow jasmine (*Chrysanthemum fruticans*, Oleaceae): implications for the study of its mating strategies

Aurore Puyoou *et al.*

#### SI Appendix includes:

**Table S1.** Plant sampling and complete genotyping dataset - (see excel file)

**Table S2.** List of herbarium specimens of the MNHN-P included in our study

**Table S3.** Characteristics of the 23 nuclear microsatellite loci developed in the present study

**Table S4.** Characteristics of the six nuclear indel loci developed in the present study

**Table S5.** Characteristics of chloroplastic and mitochondrial loci developed in the present study

**Table S6.** Genotyping of 36 progenies issued from parents with distinct chloroplast and/or mitochondrial DNA haplotypes in the 'CEFE' and 'Moulis' populations

**Table S7.** Measure of the LD between organellar genomes at the population scale

**Table S8.** Genetic diversity parameters estimated on both nuclear and cytoplasmic loci within populations with at least seven individuals

**Table S9.**  $F_{ST}$  values calculated between each pair of populations

**Table S10.** Results of the assignment test comparing nuclear SSR genotypes of individuals of the CEFÉ collection to nuclear SSR genotypes of natural populations

**Figure S1.** Dendrogram representing genetic similarity between all individuals based on genetic distances of Bruvo *et al.* (2004) and reconstructed with the unweighted average linkage clustering criterion.

**Figure S2.** Dendrogram representing genetic similarity between populations based on pairwise  $F_{ST}$  estimated from nuclear data and reconstructed on the unweighted average linkage clustering criterion

**Figure S3.** Cytoplasmic genetic differentiation between populations as a function of geographical distance according to different  $F_{ST}$  estimation methods

## References

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3 **Table S2.** List of herbarium specimens of the MNHN-P (Muséum National d'Histoire Naturelle  
4 de Paris) included in our study. For each specimen, the collector name (with a collector number  
5 when available) and the herbarium inventory number (in brackets) are given. Samples can be  
6 viewed at the [MNHN website](#). The geographic origin (locality and country) and date of  
7 collection are also indicated (“00” means that day or month are not indicated on the label).  
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MNHN-P specimen	Locality, country	Date of collection
J. Lewalle 9060 (P03426484)	Oued Cherrad, Morocco	1979-05-01
J.M. Losa Quintana 17304 (P03883854)	Granada, Spain	1994-05-29
E. Bourgeau (P04074654)	Algarve, Portugal	1853-05-30
E. Reverchon 1182 (P04074663)	Granada, Spain	1900-06-00
A. Faure (P01194578)	Oran, Algeria	1940-03-17
A. Letourneau (P03426428)	Ghardimaou, Tunisia	1884-03-31
J. Bourcart (P04074655)	Pishkupije, Albania	1923-06-00
V. Stibrny (P04074662)	Philippopolin, Bulgaria	1893-06-00
B. Balansa (P03869118)	Smyrne, Turkey	1854-05-05
A.H. Brotherus 787 (P03869098)	Gori, Georgia	1881-07-00
R. Gombault 4458 (P03869122)	Zahlé, Lebanon	1930-06-09
A. Callier 252 (P03869093)	Soudak, Crimea	1896-00-00
R. Gombault 4456 (P03869103)	Radjou, Syria	1934-04-15
G. Remaudière 202 (P04254353)	South Sari, Iran	1966-00-00
P. Sintenis 1838 (P03869104)	Kisil-Arwat, Turkmenistan	1901-05-25

**Table S3.** Characteristics of the 23 nuclear microsatellite (SSR) loci developed in the present study. Name, putative homolog in olive [with SSR gene location: coding sequence (cds), intron, or untranscribed region (UTR)], GenBank accession nos, SSR motif (number of repeats based on the sequenced allele in *C. fruticans*), forward and reverse primers, fluorochrome used (Flu), PCR multiplex (Mx; locus amplified in simplex when no indication), allele size range, and number of alleles revealed (Na). \* a M13 tail (TGTAAAACGACGGCCAGT) was added on the 5' extremity of each forward primer. \*\* Two loci are amplified with GAA20 primers (allele intensity of locus B is lower; allele size range indicates they may occasionally overlap).

Locus ID	Putative homolog in olive (encoded protein) – marker location in gene	GenBank no	SSR Motif	Forward primer*	Reverse primer	Flu	Mx	Allele size	Na
CF-ATT-1	LOC111367380 (Kinesin prot.) – 3' UTR	OQ124178	(ATT) <sub>9-10</sub>	TGTGGACTCATTTTATTCCCTC	TACACTCCTTAGACGGTGGA	HEX	-	130-158	7
CF-ATT-2	No gene homology	OQ124179	(AAT) <sub>9</sub>	TGTGTCACGCTGGTTTTTC	CCCGATGTGTTTGGATACA	HEX	-	188-203	5
CF-ATT-3	LOC111367396 (F-box prot.) – 3' UTR	OQ127180	(AAT) <sub>11-12</sub>	CCCACATTGACTCCTCATC	GAATACGATTATAAGAACCTGTTC	HEX	-	143-160	8
Cf-GAA-1	LOC111372306 (Translation initiation factor) – cds	OU230738	(GAA) <sub>15</sub>	TTGGTTTGACAAAGGATGCT	CAGATGGATTAAACATATTCCAC	FAM	Dx-I	154-193	7
CF-GAA-2	LOC111405212 (Unknown prot.) – 3' UTR	OU230739	(GAA) <sub>9-10</sub>	GAAGTGAGACAATCAGACACC	CCTCAATGACTTGCCTTGGT	AT550	204-213	4	
Cf-GAA-3	No gene homology	OU230740	(GAA) <sub>13-14</sub>	TCAGTAGTATGTGCTAATTGATG	AAGGTTCTACCCAATGGTATCT	FAM	Dx-II	205-232	9
Cf-GAA-4	No gene homology	OQ124164	(GAA) <sub>13-15</sub>	CGGTGAAAGGGTAGCAGATA	ATAGGAATGCTCGAAGTATCCT	FAM	Dx-II	247-307	19
Cf-GAA-5	LOC111387121 (Beta-1,3-galactosyl transferase) – 5' UTR	OQ124165	(GAA) <sub>6</sub>	CACCAAGTCGACCAACTGT	GCCTGTCTTCACTCTTCTC	HEX	Dx-III	161-170	6
Cf-GAA-6	No gene homology	OQ124166	(GAA) <sub>7-17</sub>	GCTTCCTCRAACCCAATCATG	GAACAYTGATGTTGCCCTTG	AT565	-	104-161	11
CF-GAA-7	LOC111411240 (ATP-dependent 6-phosphofructo kinase) – 5' UTR	OQ124167	(GAA) <sub>13-14</sub>	ACCTCAGTTTCAGTTGAAAGCT	CTTTTCCGAATTGAAATTGGTAG	HEX	Dx-III	235-250	5
Cf-GAA-9	LOC111395981 (Unknown prot.) - intron	OQ124168	(GAA) <sub>17-18</sub> (GAT) <sub>6</sub> GAA(GAT) <sub>7</sub>	ATTTGGTATGGTCTCTGTTG	TTTCTCAAATCAGCCATTTCGA	FAM	-	250-289	13
CF-GAA-11	LOC111378787 (Fasciclin arabino galactan prot.) – cds?	OQ124169	(AAG) <sub>11</sub>	TGTGCTTGGAAACAGCCTCCT	AATGCCATGGATTCAGATCT	FAM	Dx-I	117-132	6
Cf-GAA-14	No gene homology	OQ124170	(GAA) <sub>25-26</sub>	AATAACGACCAACACCGATCAA	GGAGTTTCACCTATTCTCCT	FAM	-	101-182	23
Cf-GAA-16	No gene homology	OQ124112	(AAG) <sub>18-20</sub>	AAGCCCCAATCCATTATCTAAC	AAGCTAATATCCACAACTCAAAGC	FAM	-	162-243	23
CF-GAA-17	LOC111374342 (60S ribosomal prot.) – 5' UTR	OQ124172	(GAA) <sub>18-23</sub>	CACATGCTTGAGTTGACAAG	CTGGGAGCACAAATTGTTCC	FAM	-	206-257	14
CF-GAA-18	LOC111402076 (Histone acetyl transferase) – cds	OQ124173	(GAA) <sub>21-23</sub>	AAGACGGACACCAATGCACT	AGCTTTCGGCAAGTAAGGA	AT565	-	154-214	16
CF-GAA-19	No gene homology	OQ124174	(TAA) <sub>12-13</sub> (GAA) <sub>27-30</sub>	ATAAGATGCATATGCATCTACCTA	ATCGCTAAAGGTCTTCTTCCT	AT565	-	191-284	25
CF-GAA-20	No gene homology	OQ124175	(GAA) <sub>24-25</sub>	GACCTGAGATTCCAGATCGA	GACAAAGTCTGAAAGCTCG	HEX	-	112-151	15
- (B)**	-	-	-	-	-	HEX	-	94-115	8
CF-GAA-21	No gene homology	OQ124176	(GAA) <sub>11-26</sub> (TAA) <sub>8-9</sub>	TTCTTCGTTGATGCAACAAATC	AACCAAGAAACTTGTGTGGACT	HEX	-	204-357	36
CF-GAA-22	No gene homology	OQ124177	(GAA) <sub>26-32</sub>	TGCTCTAGAGCTATAGTTG	AAGCAAGATATTGAGGAATTCAAG	AT565	-	131-230	32
Cf-ATA-1	No gene homology	OQ808969	(ATA) <sub>12-13</sub>	CTTGTGGTTACGACAGTGT	TAACCTCTAACCAAACCTCATAGT	FAM	-	223-241	7
CF-TA-1	LOC111402738 (Transcription factor) – 5' UTR	OQ808968	(TA) <sub>11-12</sub>	GGACTTCTTCTTGTAGTTGATG	AAACCAAGATGGAATTACCTCA	AT565	-	213-223	3

**Table S4.** Characteristics of the six nuclear indel loci developed in the present study. Name, putative homolog in olive [with indel gene location: coding sequence (cds), intron, or untranscribed region (UTR)], GenBank accession nos, polymorphic motif, forward and reverse primers, fluorochrome used (Flu), PCR multiplex (Mx; locus amplified in simplex when no indication), allele size range, and number of alleles revealed (Na). \* a M13 tail (TGTAAAACGACGGCCAGT) was added on the 5' extremity of each forward primer.

Locus ID	Putative homolog in olive (encoded protein) - marker location in gene	GenBank no	Polymorphic motif	Forward primer*	Reverse primer	Flu	Mx	Size range	Na
Cf-ind-1	LOC111410094 (Fructose-bisphosphate aldolase) – intron	00Q124181	Indel 25 bp	GACCCTCATTCAAGTATTTCCA	CATTAGGACAGTCGTAACATGT	FAM	Dx-IV	136-161	2
Cf-ind-2	LOC111367381 (Chaperone-like protein) – 5' UTR	00Q124182	(AT) <sub>5-6</sub> + indel 8 bp	GGAATCTATCATCAAACACTATAT C	CTAATCATTTCTTGATTGCGGT	HEX	-	274-285	6
Cf-ind-3	LOC111392924 (Trans-resveratrol di-O-methyl transferase) – Intron	00Q124183	Indel 2 bp	CAGGGATCTTCAAATCCGA	AGCTAGCGTGTATCTTAGT	AT565	Dx-V	121-123	2
Cf-ind-4	LOC111406052 (Pollen allergen Ole e 1) – 5'UTR	00Q124184	Indel 7 bp	CTTCCCGAACAAACGATACTC	ATTGAGAGACTGGACGAACG	AT565	Dx-V	168-175	2
Cf-ind-5	LOC111367387 (GATA transcription factor) - 3' part	00Q124185	Indel 20 bp	ATGGCATGGGTTGAGTAGC	CTTTTAAAGTCTAGCTAGAGAGA	FAM	-	101-121	2
Cf-ind-6	LOC111367389 (Cytochrome P450) – intron	00Q124186	Indels 6+37 bp	AGCTCACTTGTGTCGTCTCT	AGCTTCGCAATAGTATTGGAC	FAM	Dx-IV	150-200	3

**Table S5.** Characteristics of chloroplastic and mitochondrial loci developed in the present study. Name, indel length or microsatellite motif (number of repeats based on the sequenced allele in *C. fruticans*), forward and reverse primers, fluorochrome used (Flu), PCR multiplex (Mx), allele size range, and number of alleles revealed (Na). \* a M13 tail (TGTAAAACGACGCCAGT) was added on the 5' extremity of each forward primer.

Locus ID	GenBank no	Polymorphic motif	Forward primer*	Reverse primer	Flu	Mx	Size range	Na
- Plastome	MH559274							
CP1		(T) <sub>13-14</sub>	cp17-For (olive; Besnard et al., 2011)	CGATCAGGGTTCTTTTGATCAC	HEX	cpII	176-177	2
CP2		(C) <sub>9-12</sub> WWW(G) <sub>7-8</sub>	GGAGTTGGCTCTATTAACTAG	ATGATAGTGTGCGAGTCATC	HEX	cpI	116-120	3
CP3		(A) <sub>8-9</sub>	GGATTTATCGACATCAAGTCG	CCGTACAAGGAATTCCGTAG	HEX	cpII	166-167	2
CP4		(TAATAATA) <sub>1-2</sub> + (A) <sub>8</sub> (TA) <sub>10</sub>	CAGATGTATCAGAACTTTTCGAT	TTGGTCGACTAGATATCGTACA	HEX	cpIII	349-357	2
CP5		(A) <sub>9-10</sub> TTC(A) <sub>8-10</sub>	GCATGATGACAAGAACATTCTATCT	ATAGGAACGAAATAACTCGAAGA	HEX	cpI	126-129	4
CP6		(T) <sub>9</sub> +(T) <sub>9-10</sub>	cp45-For (olive; Besnard et al., 2011)	GTTTCAAACCGAACGGGCTC	HEX	cpII	136-137	2
CP7		(A) <sub>11-12</sub>	cp46-For (olive; Besnard et al., 2011)	cp46-Rev (olive; Besnard et al., 2011)	HEX	cpI	108-109	2
CP8		(A) <sub>9-10</sub>	CTTGAGCCACACCTACATTG	GAATCTATGGAGGGCCAT	HEX	cpIV	90-91	2
CP9		(T) <sub>14-15</sub>	GGAGAAAAAGAGGCATTACCT	AATCACGTGTCTTCCTCGTAAG	HEX	cpIV	110-111	2
CP10		Indel 8 bp	TTGGAACAAATTACTCTAATGCGT	CGGAGGATGATTCCAAGGA	HEX	cpIV	236-245	2
- Mitogenome	OQ124187-96							
MT1		Indel 8 bp	TGCATAAGGACTGTAAGCT	CGATAGCAGACATCTCCATC	HEX	mtI	301-309	2
MT2		(A) <sub>9-10</sub>	CTACCTGCTCTACCGAAGA	AGGGAAACAAGCAACGGATGA	HEX	mtI	316-317	2
MT3		Indel 8 bp	GTATAGTGGACACGAGCA	TCTGTACACTCCTCTCATGC	HEX	mtI	327-335	2
MT4		Indel 8 bp	GTACTATCCTATTGGTCAAATCA	CGTATACAGGATTTCACTATTAGA	HEX	mtII	231239	2
MT5		Indel 8 bp	TAGTAATAGGCTCAACTACAAGT	ACCTTCCTATTGGCCAAGT	HEX	mtII	217-225	2

**Table S6.** Genotyping of 36 progenies issued from parents with distinct chloroplast and/or mitochondrial DNA haplotypes in the 'CEFE' and 'Moulis' populations. In these two locations, a dense sampling was performed to characterize the genotype of most putative parents in the population (data available under request). Floral morph (L = Longistylous; B = Brevistylous) of each parent is also recorded. Father of each progeny was identified from nuclear microsatellite data with CERVUS (Marshall *et al.*, 1998). Mitochondrial and chloroplast haplotypes were characterized on all parents and progenies, and highlighted in colour when different between both parents. A maternal contribution was systematically revealed in all progenies for both genomes. MD = Missing data. All crosses were between L and B (12), or B and L (24).

Individual	Floral morph	GAA20_B												GAA20												GAA21												GAA18												GAA22												GAA19												GAA16												GAA17												GAA1												GAA11												GAA3												GAA4												GAA2												GAA9												GAA5												GAA7												GAA14												ATA3												GAA6												ATA1												Indel1												Indel6												TA-1												ATA-1												MT1												MT2												MT3												MT4												MT5												mtDNA haplotype												cpDNA haplotype											
		GAA20	GAA20	GAA21	GAA21	GAA18	GAA18	GAA22	GAA22	GAA19	GAA19	GAA16	GAA16	GAA17	GAA17	GAA1	GAA1	GAA11	GAA11	GAA3	GAA3	GAA4	GAA4	GAA2	GAA2	GAA9	GAA9	GAA5	GAA5	GAA7	GAA7	GAA14	GAA14	ATA3	ATA3	GAA6	GAA6	ATA1	ATA1	Indel1	Indel1	Indel6	Indel6	TA-1	TA-1	ATA-1	ATA-1	MT1	MT1	MT2	MT2	MT3	MT3	MT4	MT4	MT5	MT5	CP1	CP1	CP2	CP2	CP3	CP3	CP4	CP4	CP5	CP5	CP6	CP6	CP7	CP7	CP8	CP8	CP9	CP9	CP10	CP10																																																																																																																																																																																																																																																																																																								
Mother	CEFE_L1B	L	106:106	121:139	210:385	175:190	170:230	212:227	162:162	236:236	169:169	123:126	214:220	253:283	204:204	277:277	163:170	241:244	134:149	151:157	104:143	156:156	136:136	150:150	221:222	226:226	301	317	335	239	225	4	1	177	120	166	357	127	137	109	90	110	245	2																																																																																																																																																																																																																																																																																																																																									
Father	CEFE_7	B	106:106	115:145	225:255	190:199	173:179	212:221	162:162	239:239	169:172	126:126	214:217	268:307	204:204	274:277	163:170	241:244	119:155	151:151	104:104	136:136	150:150	213:213	229:229	309	316	327	239	225	1	1	176	119	167	349	128	136	108	90	110	245	3																																																																																																																																																																																																																																																																																																																																										
Progeny (2021)	CEFE_L1Bx4A		106:106	115:121	MD	190:199	MD	212:212	162:162	236:239	169:169	MD	214:220	253:307	204:204	274:277	163:163	241:241	119:149	151:151	MD	136:156	MD	MD	MD	226:229	301	317	335	239	225	4	1	177	120	166	357	127	137	109	90	110	245	2																																																																																																																																																																																																																																																																																																																																									
Mother	CEFE_L2	L	106:106	115:136	210:210	196:196	161:191	233:239	162:198	251:251	169:169	126:126	211:214	262:268	204:204	259:277	163:163	235:241	137:137	151:157	104:143	136:136	161:161	150:150	213:221	229:232	309	316	327	239	225	1	1	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																									
Father	CEFE_S6	B	106:106	115:136	210:210	196:202	179:201	221:236	162:162	236:251	169:169	126:126	214:214	253:253	204:204	274:274	163:163	235:244	134:137	151:157	MD	136:156	MD	MD	MD	229:229	309	316	327	239	225	1	2	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																									
Progeny (2021)	CEFE_L2x3		106:106	118:136	MD	196:202	191:203	236:239	162:162	251:251	169:169	MD	MD	MD	MD	MD	163:170	241:241	134:137	151:151	MD	136:156	MD	MD	MD	229:229	309	316	327	239	225	1	2	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																									
Progeny (2021)	CEFE_L2x7		106:106	115:115	MD	193:196	MD	239:287	162:162	251:251	163:169	MD	MD	MD	MD	MD	204:204	MD	229:235	309	316	327	239	225	1	1	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																																
Mother	CEFE_L2	L	106:106	115:136	210:210	196:196	161:191	233:239	162:198	251:251	169:169	126:126	211:214	262:268	204:204	274:274	163:163	235:241	137:137	151:157	104:143	136:136	161:161	150:150	213:221	229:232	309	316	327	239	225	1	1	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																									
Father	CEFE_S2	B	103:103	118:188	210:210	193:196	182:188	221:239	162:162	236:251	163:169	126:126	211:214	262:268	204:204	265:277	163:170	235:241	137:137	151:157	104:104	156:156	130:130	232:232	301	317	335	239	225	4	4	177	120	166	357	127	137	109	90	110	245	2																																																																																																																																																																																																																																																																																																																																											
Progeny (2021)	CEFE_L5x10		106:106	115:118	MD	190:199	MD	218:202	162:162	251:251	169:169	MD	MD	MD	MD	MD	204:204	MD	229:232	309	316	327	239	225	1	1	177	119	166	349	128	136	108	90	110	245	3																																																																																																																																																																																																																																																																																																																																																
Mother	CEFE_L5	L	106:106	115:142	204:294	190:199	188:227	218:218	235:239	166:169	126:126	211:214	268:280	204:204	265:277	163:170	235:241	140:140	154:157	104:104	136:136	161:161	150:194	213:221	232:232	309	316	327	239	225	1	1	177	119	166	357	127	137	109	90	110	245	1																																																																																																																																																																																																																																																																																																																																										
Father	CEFE_S5	B	103:103	118:188	210:210	193:196	182:188	221:239	162:162	236:251	163:169	126:126	211:214	263:268	204:204	259:280	163:170	241:244	119:134	151:157	104:104	156:156	130:130	232:232	301	317	335	239	225	4	4	177	120	166	357	127	137	109	90	110	245	2																																																																																																																																																																																																																																																																																																																																											
Progeny (2021)	CEFE_L6x149s2		MD	112:115	222:228	196:199	MD	215:239	162:162	251:251	169:172	MD	301	317	335	239	225	1	1	176	119	167	348	128	136	108	90	110	245	3																																																																																																																																																																																																																																																																																																																																																							
Mother	CEFE_S2	B	103:103	118:188	210:210	193:196	182:188	221:239	162:162	236:251	163:169	126:126	211:214	263:268	204:204	277:277	163:170	235:238	149:152	151:157	104:104	156:156	130:130	229:229	309	316	327	239	225	1	1																																																																																																																																																																																																																																																																																																																																																						

Table S6, end.

Individual	GAA20_B	GAA20	GAA21	GAA18	GAA22	GAA19	GAA16	GAA17	GAA11	GAA3	GAA4	GAA2	GAA9	GAA7	GAA14	ATT3	GAA6	ATT1	Indel1	Indel6	TA-1	ATA-1	mtDNA haplotype	cpDNA haplotype			
Mother	CEFE_S4	B	106:106	115:121	210:288	196:202	173:185	212:221	162:168	236:251	169:172	126:126	214:220	253:268	204:204	274:280	163:163	241:244	152:155	151:151	104:104	156:156	161:161	150:194	221:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_136	L	106:106	118:121	210:210	175:184	170:200	209:215	168:198	236:251	169:169	126:126	211:220	286:301	213:213	274:277	163:170	241:241	134:137	151:151	104:104	156:156	MD	150:150	221:221	226:235	309 316 327 239 225 1 177 119 166 357 127 137 109 90 110 245 1
Progeny (2021)	CEFE_S43A		106:106	118:121	MD	175:202	MD	212:215	162:198	236:251	169:172	MD	MD	268:286	204:213	MD	229:235	301 317 335 239 225 4 15 177 120 166 357 127 137 109 90 110 245 2 17									
Progeny (2021)	CEFE_S43B		MD	115:121	MD	175:202	MD	MD	162:198	251:251	169:172	MD	MD	268:286	204:213	MD	MD	241:244	134:152	151:157	MD	156:156	MD	MD	235:235	301 317 335 239 225 4 16 177 120 166 357 127 137 109 90 110 245 2 18	
Progeny (2021)	CEFE_S46		MD	115:121	MD	175:196	MD	MD	168:198	236:251	169:169	MD	301 317 335 239 225 4 17 177 120 166 357 127 137 109 90 110 245 2 19														
Progeny (2021)	CEFE_S48		106:106	121:121	MD	184:202	MD	209:212	162:168	251:251	169:172	MD	301 317 335 239 225 4 18 177 120 166 357 127 137 109 90 110 245 2 20														
Mother	CEFE_S4	B	106:106	115:121	210:288	196:202	173:185	212:221	162:168	236:251	169:172	126:126	214:220	253:268	204:204	274:280	163:163	241:244	152:155	151:151	104:104	156:156	161:161	150:194	221:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_109	L	103:106	118:121	210:210	175:196	170:200	209:221	168:198	236:236	169:169	126:126	211:224	253:301	204:204	274:277	170:170	241:244	134:152	148:151	104:104	136:156	MD	150:150	221:221	226:232	309 316 327 239 225 1 177 119 166 357 127 137 109 90 110 245 1
Progeny (2021)	CEFE_S44		106:106	115:118	210:210	175:202	MD	209:221	162:198	236:251	169:172	MD	232:235	301 317 335 239 225 4 19 177 120 166 357 127 137 109 90 110 245 2 21													
Mother	CEFE_S4	B	106:106	115:121	210:288	196:202	173:185	212:221	162:168	236:251	169:172	126:126	214:220	253:268	204:204	274:280	163:163	241:244	152:155	151:151	104:104	156:156	161:161	150:194	221:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_L6	L	103:109	121:139	288:288	190:202	167:200	221:257	162:207	215:254	163:169	126:126	214:214	253:253	204:204	277:277	163:163	235:241	149:152	148:154	104:143	156:156	136:161	150:150	221:221	226:235	309 316 327 239 225 1 176 119 167 349 128 136 108 90 110 245 3
Progeny (2021)	CEFE_S45		103:106	121:121	MD	190:202	MD	212:257	162:207	215:236	169:172	MD	MD	301 317 335 239 225 4 20 177 120 166 357 127 137 109 90 110 245 2 22													
Mother	CEFE_S6	B	106:106	118:136	210:291	199:202	179:203	221:236	162:162	236:251	169:169	126:126	214:214	253:253	204:204	277:280	170:170	241:244	134:152	151:151	104:104	156:156	136:161	150:194	213:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_L5	L	106:106	115:142	204:294	190:199	188:227	218:257	198:198	215:239	166:169	126:126	211:214	268:280	204:204	265:277	163:170	235:241	140:142	154:154	104:104	136:136	161:161	150:194	213:221	229:239	309 316 327 239 225 1 176 119 167 349 128 136 108 90 110 245 3
Progeny (2021)	CEFE_S61A		106:106	115:136	MD	199:199	MD	162:198	215:236	169:169	MD	MD	301 317 335 239 225 4 21 177 120 166 357 127 137 109 90 110 245 2 23														
Progeny (2021)	CEFE_S62		MD	115:136	MD	199:199	MD	218:221	162:198	215:236	166:169	MD	301 317 335 239 225 4 22 177 120 166 357 127 137 109 90 110 245 2 24														
Progeny (2021)	CEFE_S63		MD	136:142	MD	199:202	MD	MD	162:198	215:236	166:166	MD	301 317 335 239 225 4 23 177 120 166 357 127 137 109 90 110 245 2 25														
Progeny (2021)	CEFE_S66		106:106	136:142	MD	199:199	179:188	MD	162:198	236:239	169:169	MD	MD	301 317 335 239 225 4 24 177 120 166 357 127 137 109 90 110 245 2 26													
Mother	CEFE_S6	B	106:106	118:136	210:291	199:202	179:203	221:236	162:162	236:251	169:169	126:126	214:214	253:253	204:204	277:280	170:170	241:244	134:152	151:151	104:104	156:156	136:161	150:194	213:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_136	L	106:106	118:121	210:210	175:184	170:200	209:215	168:198	236:251	169:169	126:126	211:220	286:301	213:213	274:277	163:170	241:241	134:157	151:151	104:104	156:156	161:161	150:150	221:221	226:235	309 316 327 239 225 1 177 119 166 357 127 137 109 90 110 245 1
Progeny (2021)	CEFE_S67		106:106	121:136	MD	175:199	170:203	209:236	162:198	236:251	169:169	MD	MD	226:229	301 317 335 239 225 4 25 177 120 166 357 127 137 109 90 110 245 2 27												
Mother	CEFE_S6	B	106:106	118:136	210:288	199:202	179:203	221:236	162:162	236:251	169:169	126:126	214:214	253:253	204:204	277:280	170:170	241:244	134:152	151:151	104:104	156:156	136:161	150:194	213:221	229:235	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_149	L	106:105	115:142	288:288	190:199	173:206	215:236	162:162	251:254	169:172	126:126	220:220	253:268	204:204	274:277	163:163	241:241	134:152	151:151	104:104	156:156	160:160	150:150	221:221	232:235	309 316 327 239 225 1 176 119 167 349 128 136 108 90 110 245 3
Progeny (2021)	CEFE_S68		MD	118:142	MD	199:202	MD	162:162	251:254	169:172	MD	MD	301 317 335 239 225 4 26 177 120 166 357 127 137 109 90 110 245 2 28														
Progeny (2021)	CEFE_S68		106:106	136:142	MD	199:202	173:179	MD	162:162	236:254	169:172	MD	MD	301 317 335 239 225 4 27 177 120 166 357 127 137 109 90 110 245 2 29													
Mother	CEFE_119	B	106:106	121:142	210:288	190:202	179:194	215:236	162:162	236:251	169:172	126:126	211:217	268:268	204:204	259:280	163:170	238:241	152:155	151:151	104:104	136:156	136:161	150:150	213:221	229:232	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_122	L	103:106	121:142	210:221	175:196	170:200	209:221	168:198	236:236	169:169	126:126	211:214	253:301	204:204	274:277	170:170	241:244	134:152	148:151	104:104	136:156	160:160	150:150	221:221	226:232	309 316 327 239 225 1 177 119 166 357 127 137 109 90 110 245 1
Progeny (2022)	CEFE_119-1A		106:106	115:142	210:288	190:190	164:194	215:221	162:198	239:251	169:172	126:126	211:214	253:268	204:204	259:277	163:170	241:241	137:152	151:151	104:104	136:161	150:150	221:221	229:232	301 317 335 239 225 4 28 177 120 166 357 127 137 109 90 110 245 2 30	
Mother	CEFE_119	B	106:106	121:142	210:288	190:202	179:194	215:236	162:162	236:251	169:172	126:126	211:217	268:268	204:204	259:280	163:170	238:241	152:155	151:151	104:104	136:156	136:161	150:150	213:221	229:232	301 317 335 239 225 4 177 120 166 357 127 137 109 90 110 245 2
Father	CEFE_109	L	103:106	118:121	210:210	175:184	170:200	209:215	168:198	236:236	169:169	126:126	211:214	253:301	204:204	274:277	170:170	241:244	134:152	148:151	104:104	136:156	160:160	150:150	221:221	226:232	309 316 327 239 225 1 177 119 166 357 127 137 109 90 110 245 1
Progeny (2022)	CEFE_119-1B		103:106	121:121	210:210	196:202	179:200	221:236	162:168	236:236	169:169	126:126	211:214	253:268	204:204	259:274	163:170	238:244	134:152	148:151	104:104	136:156	136:161	150:150	213:221	226:226	301 317 335 239 225 4 29 177 120 166 357 1

1  
2  
3     **Table S7.** Measure of the LD ( $D'$ ; Lewontin, 1964) between organellar genomes at the  
4     population scale. Significance ( $p$ -value) of the  $D'$  estimate was measured with a Fisher's test.  
5     This test was only done on populations where polymorphism was observed on both organellar  
6     genomes (see Table S1). Ng = Number of genotypes.  
7  
8  
9

Population	Ng	$D'$	$p$ -value
La Aliseda	7	1.000	0.005
Bages	12	1.000	0.048
CEFE	15	0.875	<0.001
Celleneuve	21	1.000	1.000
Eguilles	10	1.000	0.212
Moulis	37	1.000	0.118
Moussoulens	12	1.000	1.000
St-Maurice-de-Navacelles	17	0.558	0.181
Puycelsi	15	0.500	0.073
Sainte-Baume	10	1.000	0.109

**Table S8.** Genetic diversity parameters estimated on both nuclear and cytoplasmic loci within populations with at least seven individuals. The parameters were calculated using GENETIX (Belkhir *et al.*, 2004), except the allelic richness ( $A_R$ ) that was calculated with the R package HIERFSTAT (Goudet, 2005) and the haplotypic diversities ( $D$ ) that were estimated according to Gryta *et al.* (2017).  $N_a$  = Number of alleles,  $A_R$  = Allelic richness,  $H_E$  = Expected heterozygosity under Hardy-Weinberg,  $H_O$  = Observed heterozygosity,  $F_{IS}$  = Fixation index,  $N_h$  = Number of haplotypes,  $D_{cp}$  = Chlorotypic diversity,  $D_{mt}$  = Mitotypic diversity,  $D_{cp+mt}$  = cytoplasmic diversity.  $F_{IS}$  values significantly different from 0 are indicated in bold ( $p$ -value < 0.05 in red, and  $p$ -value < 0.01 in green; without considering multiple testing correction). The detection of probable null alleles with MICRO-CHECKER (Van Oosterhout *et al.*, 2004) is indicated by an asterisk.

		Bages (12)				Bidon (10)				CEFE (15)				Celleneuve (21)				Eguilles (10)				Moulin (37)					
Nuclear compartment	Locus	<i>N<sub>a</sub></i>	<i>A<sub>r</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>r</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>r</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>r</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>r</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	
- Microsatellite (SSR)	GAA1	2	2.00	0.44	0.50	-0.08	4	3.62	0.63	0.50	0.25	4	3.19	0.50	0.40	0.23	2	1.71	0.13	0.14	-0.05	4	3.40	0.59	0.50	0.20	
	GAA2	3	2.98	0.57	0.42	0.31	3	2.92	0.57	0.60	-0.01	3	2.19	0.18	0.20	-0.05	3	2.22	0.25	0.29	-0.12	3	3.00	0.62	0.80	-0.24	
	GAA3	2	1.99	0.33	0.25	0.28	3	2.70	0.40	0.40	0.04	3	2.94	0.50	0.67	-0.30	2	1.82	0.17	0.19	-0.08	1	1.00	0.00	0.00	-	
	GAA4	6	4.94	0.70	0.50	0.21	4	3.68	0.65	0.80	-0.18	8	4.80	0.64	0.67	-0.01	5	3.59	0.65	0.71	-0.08	3	2.68	0.34	0.40	-0.14	
	GAA5	3	2.42	0.23	0.17	0.30	3	2.62	0.27	0.30	-0.08	2	2.00	0.48	0.53	-0.08	2	2.00	0.44	0.38	0.17	2	2.00	0.42	0.60	-0.39	
	GAA6	2	2.00	0.38	0.50	-0.29	2	1.92	0.18	0.20	-0.06	2	2.00	0.39	0.53	-0.33	1	1.00	0.00	0.00	-	3	3.00	0.64	0.80	-0.20	
	GAA7	2	1.94	0.22	0.08	0.65	3	2.92	0.51	0.40	0.26	4	3.64	0.58	0.80	-0.36	2	2.00	0.44	0.38	0.17	3	2.70	0.45	0.60	-0.30	
	GAA9	4	3.41	0.50	0.56	-0.12	5	4.84	0.76	0.80	-0.01	6	5.18	0.75	0.67	0.15	7	5.18	0.76	0.90	-0.17	6	5.10	0.74	0.80	-0.03	
	GAA11	3	2.58	0.43	0.42	0.08	1	1.00	0.00	0.00	-	2	1.86	0.18	0.20	-0.08	3	2.86	0.47	0.38	0.22	2	1.70	0.10	0.10	0.00	
	GAA14	6	4.98	0.74	0.83	-0.08	8	6.92	0.82	0.90	-0.05	8	6.56	0.84	0.80	0.08	9	6.63	0.84	0.90	-0.05	6	5.08	0.72	0.70	0.08	
	GAA16	5	4.00	0.64	0.58	0.14	5	4.10	0.60	0.60	<b>-0.46</b>	5	4.04	0.58	0.60	0.01	4	2.48	0.26	0.29	0.10	3	2.70	0.40	0.40	0.04	
	GAA17	5	4.36	0.64	0.75	-0.13	3	2.92	0.46	0.60	-0.26	7	5.24	0.73	0.73	0.03	5	3.68	0.64	0.62	0.06	6	5.10	0.75	0.80	-0.01	
	GAA18	7	6.27	0.83	0.83	0.04	5	4.69	0.77	0.70	0.13	7	6.11	0.83	0.93	-0.09	7	5.08	0.68	0.67	0.04	6	5.32	0.76	0.70	-0.13	
	GAA19	10	7.17	0.79	0.75	0.18	9	7.62	0.85	0.90	-0.01	15	9.42	0.89	1.00	-0.09	13	8.84	0.89	0.86	0.07	8	6.42	0.74	0.70	0.10	
	GAA20	7	5.73	0.80	0.83	0.01	7	5.78	0.73	0.70	0.09	7	6.36	0.84	0.87	0.00	7	5.01	0.76	0.81	-0.05	5	4.32	0.66	0.70	-0.01	
GAA20_B		2	2.00	0.47	0.58	-0.20	1	1.00	0.00	0.00	-	3	2.46	0.41	0.33	0.21	2	2.00	0.41	0.38	0.09	2	2.00	0.32	0.20	0.42	
	GAA21	10	6.90	0.74	0.75	0.03	4	3.60	0.48	0.50	0.00	10	6.12	0.72	0.73	0.02	15	8.11	0.85	0.90	-0.04	8	6.42	0.74	0.80	-0.04	
	GAA22	12	8.33	0.84	0.83	0.06	10	8.31	0.86	1.00	-0.11	17	10.31	0.92	1.00	-0.06	18	9.56	0.91	0.95	-0.02	7	5.98	0.76	0.90	-0.13	
ATT1		4	3.52	0.58	0.67	-0.11	3	2.98	0.61	0.90	-0.05	2	2.00	0.39	0.27	0.35	3	2.71	0.53	0.43	0.21	4	3.62	0.60	0.60	0.04	
	ATT2	2	2.00	0.47	0.42	0.15	2	1.98	0.26	0.30	-0.13	*2	2.00	0.44	0.13	<b>0.72</b>	2	2.00	0.41	0.38	0.09	2	2.00	0.46	0.50	-0.05	
	ATT3	2	1.58	0.08	0.08	0.00	2	2.00	0.38	0.50	-0.29	4	3.45	0.58	0.60	0.00	2	1.93	0.24	0.29	-0.14	4	3.32	0.35	0.40	-0.11	
	TA1	3*	3.00	0.64	0.33	<b>0.51</b>	3	2.70	0.55	0.40	0.31	2	2.00	0.39	0.53	-0.33	2	2.00	0.47	0.48	0.02	2	1.98	0.26	0.30	-0.13	
	ATA1	4	3.42	0.57	0.50	0.16	2	2.00	0.48	0.60	-0.20	4	3.93	0.73	0.80	-0.07	4	3.12	0.58	0.52	0.12	3	2.70	0.52	0.60	-0.11	
	indel1	2	2.00	0.50	0.50	0.04	2	2.00	0.50	0.90	<b>-0.80</b>	2	2.00	0.46	0.47	0.03	2	1.99	0.39	0.33	0.16	2	2.00	0.46	0.50	-0.05	
	indel2	2	2.00	0.50	0.50	0.04	3	2.70	0.54	0.90	<b>-0.65</b>	2	2.00	0.46	0.47	0.03	2	1.99	0.36	0.29	0.24	2	2.00	0.46	0.50	-0.05	
	indel3	2	1.58	0.08	0.08	0.00	2	1.92	0.18	0.20	-0.06	2	2.00	0.48	0.67	-0.36	2	2.00	0.46	0.62	-0.33	2	1.98	0.26	0.30	-0.13	
	indel4	2	2.00	0.41	0.42	0.04	2	1.92	0.18	0.20	-0.06	2	1.99	0.32	0.40	-0.22	2	1.70	0.13	0.14	-0.05	2	1.92	0.18	0.20	-0.06	
	indel5	1	1.00	0.00	0.00	-	2	2.00	0.50	0.70	-0.37	2	1.99	0.32	0.27	0.20	2	1.93	0.05	0.05	0.00	1	1.00	0.00	0.00	-	
	indel6	2	2.00	0.49	0.50	0.02	2	2.00	0.50	0.90	<b>-0.80</b>	2	1.99	0.32	0.27	0.20	2	1.93	0.34	0.33	0.03	2	2.00	0.32	0.40	-0.20	
	Mean	4.0	<b>3.38</b>	<b>0.50</b>	<b>0.49</b>	<b>0.08</b>	3.6	<b>3.29</b>	<b>0.49</b>	<b>0.58</b>	<b>-1.6</b>	3.8	<b>3.78</b>	<b>0.55</b>	<b>0.57</b>	<b>-0.07</b>	4.0	<b>4.32</b>	<b>0.37</b>	<b>0.47</b>	<b>0.47</b>	4.0	<b>3.19</b>	<b>0.47</b>	<b>0.52</b>	<b>-0.06</b>	
	- Mean SSR	4.6	3.81	0.55	0.53	0.09	4.0	3.60	0.51	0.57	-0.06	5.5	4.25	0.59	0.61	0.00	5.2	3.70	0.51	0.52	0.01	4.0	3.54	0.52	0.57	-0.05	
	- Mean Indel	1.8	1.76	0.33	0.33	0.02	2.2	2.09	0.40	0.63	-0.46	2.0	1.99	0.39	0.42	-0.02	2.0	1.84	0.29	0.29	<b>0.01</b>	1.8	1.82	0.28	0.32	-0.08	
Mitochondrial compartment	Locus	<i>N<sub>h</sub></i>	<i>D<sub>mt</sub></i>																								
	MT1	2		1		2				2				1			2			2			2				
	MT2	2			1			2				2				2			2			2			2		
	MT3	2			1			2				2				2			2			2			2		
	MT4	1			1			1				1				1			1			1			1		
	MT5	2			1			1				1				1			2			1			1		
	mtDNA haplotype	3	<b>0.44</b>		1	<b>0.00</b>		2	<b>0.53</b>		4	<b>0.70</b>		2	<b>0.10</b>		2	<b>0.20</b>		3	<b>0.38</b>		2	<b>0.47</b>			
Plastid compartment	Locus	<i>N<sub>h</sub></i>	<i>D<sub>cpmt</sub></i>																								
	CP1	2		2		2				2				1			2			2			2			2	
	CP2	1			1			2				2				2			1			1			1		
	CP3	2			2			2				2				1			2			2			2		
	CP4	2			2			2				2				1			1			1			1		
	CP5	2			2			3				1				1			3			2			2		
	CP6	2			2			2				1				1			2			2			2		
	CP7	2			2			2				1				1			2			2			2		
	CP8	1			1			1				1				1			2			1			1		
	CP9	1			1			1				1				1			2			1			1		
	CP10	1			2			1				1				1			2			1			1		
	cpDNA haplotype	2	<b>0.53</b>		2	<b>0.36</b>		4	<b>0.70</b>		2	<b>0.10</b>		3	<b>0.38</b>		2	<b>0.20</b>		2	<b>0.47</b>		2	<b>0.11</b>			
Cytosolic compartment		<i>N<sub>h</sub></i>	<i>D<sub>cpmt</sub></i>																								
	Cytoplasmic haplotype	4	<b>0.65</b>		2	<b>0.36</b>		5	<b>0.70</b>		3	<b>0.19</b>		3	<b>0.38</b>		3	<b>0.20</b>		3	<b>0.50</b>		3	<b>0.50</b>		</td	

**Table S8, end.**

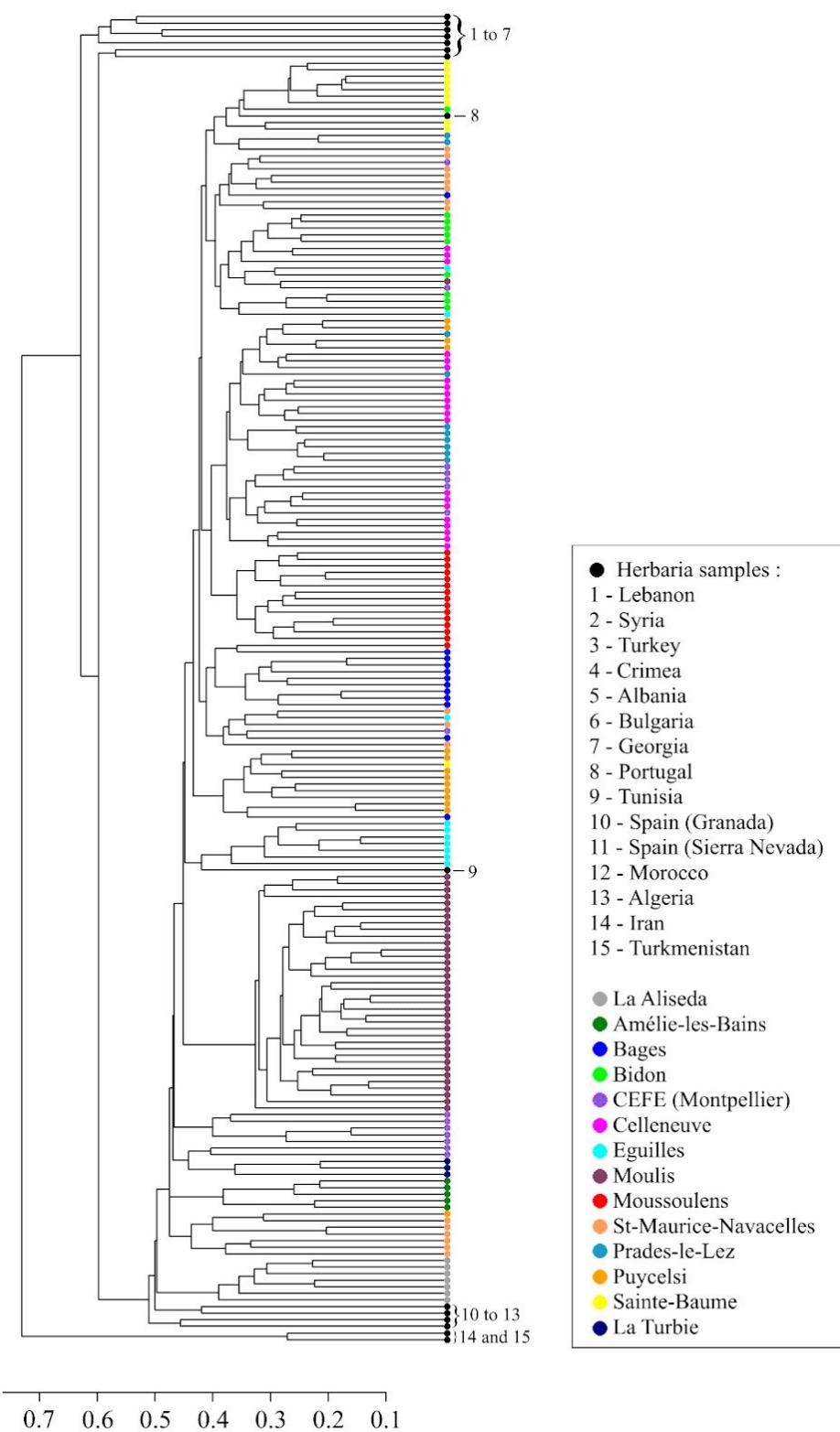
Nuclear compartment	Locus	Moussoulens (15)						St Maurice Navacelles (17)						Prades-Le-Lez (10)						Puycerl (14)						Sainte-Baume (10)						La Aliseda (7)					
		<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>	<i>N<sub>a</sub></i>	<i>A<sub>x</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>o</sub></i>	<i>F<sub>s</sub></i>						
- Microsatellite (SSR)	GAA1	3	2,72	0,48	0,67	-0,36	4	3,07	0,57	0,47	0,20	2	1,92	0,18	0,20	-0,06	4	3,39	0,60	0,43	0,32	3	2,70	0,45	0,60	-0,30	2	2,00	0,49	0,86	-0,71						
	GAA2	2	2,00	0,46	0,47	0,03	3	2,71	0,35	0,35	0,02	3	2,68	0,34	0,40	-0,14	2	2,00	0,44	0,21	0,54	3	2,92	0,54	0,60	-0,06	1	1,00	0,00	0,00	-						
	GAA3	3	2,85	0,46	0,47	0,02	4	3,04	0,42	0,53	-0,22	2	2,00	0,42	0,20	0,56	2	1,50	0,07	0,07	0,00	1	1,00	0,00	0,00	-	2	2,00	0,13	0,14	0,00						
	GAA4	3	2,83	0,42	0,53	-0,22	8	6,05	0,75	0,76	0,01	4	3,82	0,54	0,40	0,30	5*	4,28	0,69	0,43	0,41	4	3,70	0,69	0,60	0,18	4	4,00	0,69	0,71	0,05						
	GAA5	3	2,97	0,62	0,60	0,07	3	2,98	0,59	0,53	0,13	2	2,00	0,50	0,80	-0,57	3	2,26	0,20	0,21	-0,05	2	1,98	0,26	0,30	-0,13	1	1,00	0,00	0,00	-						
	GAA6	1	1,00	0,00	0,00	-	4	2,88	0,31	0,35	-0,11	1	1,00	0,00	0,00	-	1	1,00	0,00	0,00	-	2	1,92	0,18	0,20	-0,06	1	1,00	0,00	0,00	-						
	GAA7	2	1,99	0,32	0,40	-0,22	3	2,89	0,53	0,65	-0,18	2	2,00	0,32	0,40	-0,20	2	2,00	0,41	0,57	-0,37	2	2,00	0,42	0,40	0,10	1	1,00	0,00	0,00	-						
	GAA9	7	5,73	0,81	0,80	0,04	6	4,41	0,61	0,59	0,06	5	4,60	0,68	0,80	-0,13	4	3,39	0,56	0,64	-0,12	5	4,02	0,42	0,50	-0,14	5	5,00	0,78	0,57	0,33						
	GAA11	4	3,46	0,64	0,64	0,12	3	2,41	0,43	0,59	-0,34	3	2,70	0,40	0,50	-0,22	3	2,99	0,66	0,64	0,00	2	1,70	0,10	0,10	0,00	2	2,00	0,41	0,57	-0,33						
	GAA14	6	4,64	0,72	0,73	0,02	11	6,01	0,63	0,71	-0,09	8	6,20	0,74	0,80	-0,04	7	5,72	0,70	0,79	0,01	6	4,72	0,49	0,50	0,03	7	7,00	0,80	0,71	0,18						
	GAA16	5	4,38	0,60	0,73	-0,19	5	3,97	0,67	0,82	-0,21	4	3,70	0,67	0,60	0,15	5	3,89	0,65	0,64	0,05	3	2,70	0,49	0,70	-0,40	4	4,00	0,68	0,86	-0,18						
	GAA17	5	3,93	0,68	0,73	-0,04	7	5,35	0,78	0,76	0,05	5	4,24	0,49	0,50	0,02	6	4,24	0,55	0,64	-0,14	4	3,92	0,70	0,80	-0,09	5	5,00	0,71	0,57	0,27						
	GAA18	9	5,99	0,76	0,80	-0,02	8	5,48	0,77	0,65	0,19	5	4,66	0,73	0,90	-0,18	8	5,76	0,72	0,86	-0,15	4	4,00	0,75	0,90	-0,16	8	8,00	0,77	0,86	-0,04						
	GAA19	7	6,08	0,79	0,67	0,19	13	7,92	0,85	0,88	-0,01	8	7,30	0,85	0,80	0,11	14	8,80	0,85	0,86	0,03	3	2,98	0,59	0,70	-0,15	5	5,00	0,73	0,86	-0,09						
	GAA20	6	4,87	0,70	0,73	-0,01	8	6,40	0,83	0,88	-0,03	6	5,59	0,79	0,90	-0,09	9	7,26	0,85	0,93	-0,05	5	4,38	0,68	0,70	0,02	6	6,00	0,76	0,71	0,13						
	GAA20_B	4	3,45	0,64	0,73	-0,12	3	2,41	0,43	0,35	0,21	2	2,00	0,46	0,70	-0,50	2	1,89	0,19	0,21	-0,08	3	2,70	0,45	0,40	0,15	3	3,00	0,62	0,71	-0,07						
	GAA21	7	4,71	0,53	0,60	-0,09	15	8,39	0,84	0,88	-0,03	8	6,56	0,71	0,80	-0,08	5	3,52	0,37	0,43	-0,12	6	4,50	0,43	0,40	0,11	6	6,00	0,79	0,86	-0,01						
	GAA22	8	6,73	0,84	0,87	0,01	17*	10,04	0,92	0,76	0,20	9	7,34	0,81	0,90	-0,07	12	8,06	0,83	0,79	0,09	8	6,64	0,79	1,00	-0,22	8	8,00	0,85	0,86	0,07						
	ATT1	4	3,72	0,64	0,73	-0,03	4	3,38	0,66	0,76	-0,14	2	2,08	0,48	0,20	0,62	4	3,15	0,38	0,36	0,06	3	2,70	0,45	0,60	-0,30	1	1,00	0,00	0,00	-						
	ATT2	2	2,00	0,39	0,53	-0,33	2	2,00	0,39	0,29	0,27	2	1,98	0,26	0,30	-0,13	2	2,00	0,48	0,21	0,58	2	2,00	0,46	0,50	-0,05	2	2,00	0,24	0,29	0,59						
	ATT3	2	1,47	0,06	0,07	0,00	3	2,94	0,61	0,71	-0,13	2	2,00	0,38	0,50	-0,29	3	2,39	0,25	0,29	-0,10	3	2,70	0,45	0,40	0,15	3	3,00	0,52	0,71	-0,30						
	TA1	3	3,18	0,39	0,50	-0,25	3	2,41	0,52	0,41	0,23	2	1,98	0,26	0,10	0,64	3	2,50	0,48	0,50	0,00	3	2,62	0,27	0,30	-0,08	2	2,00	0,46	0,71	-0,50						
	ATA1	2	1,47	0,06	0,07	0,00	5	3,81	0,67	0,88	-0,28	3	2,98	0,56	0,70	-0,21	4	3,49	0,64	0,64	0,03	3	2,70	0,45	0,30	0,37	3	3,00	0,62	0,29	0,59						
	indel1	2	2,00	0,39	0,53	-0,33	2	1,99	0,33	0,41	-0,23	2	2,00	0,50	0,50	0,04	2	2,00	0,50	0,21	0,59	2	1,70	0,10	0,10	0,00	2	2,00	0,24	0,29	-0,09						
	indel2	3	2,80	0,38	0,47	-0,18	2	1,97	0,29	0,35	-0,19	2	2,00	0,50	0,40	0,25	2	2,00	0,50	0,20	0,59	2	1,70	0,10	0,10	0,00	2	2,00	0,13	0,14	0,00						
	indel3	2	2,71	0,34	0,29	0,19	2	1,81	0,16	0,18	-0,07	2	2,00	0,50	0,70	-0,37	2	2,00	0,50	0,43	0,18	2	2,00	0,42	0,60	-0,39	1	1,00	0,00	0,00	-						
	indel4	2	2,72	0,48	0,64	-0,32	2	2,00	0,47	0,41	0,16	2	2,00	0,38	0,50	-0,29	2	2,00	0,41	0,29	0,33	2	2,00	0,48	0,80	-0,64	1	1,00	0,00	0,00	-						
	indel5	2	1,99	0,36	0,20	0,47	2	2,00	0,44	0,47	-0,10	2	1,92	0,18	0,20	-0,06	2	2,00	0,45	0,43	0,18	2	1,92	0,18	0,20	-0,06	2	2,00	0,41	0,57	-0,33						
	indel6	2	1,99	0,32	0,40	-0,22	2	2,00	0,50	0,47	0,09	2	1,92	0,18	0,20	-0,06	2	2,00	0,46	0,29	0,41	2	1,70	0,10	0,10	0,00	2	2,00	0,46	0,43	0,14						
	Mean	3,8	3,32	0,50	0,54	-0,06	5,3	3,89	0,56	0,58	-0,02	3,5	3,21	0,47	0,51	-0,03	4,2	3,36	0,50	0,46	0,11	3,2	2,84	0,41	0,46	-0,07	3,2	3,17	0,42	0,45	0,00						
	- Mean SSR	4,3	3,57	0,55	0,59	-0,06	6,2	4,39	0,61	0,63	-0,01	3,9	3,53	0,50	0,54	-0,02	4,8	3,72	0,53	0,52	0,04	3,5	3,10	0,45	0,50	-0,04	3,6	3,57	0,61	0,64	0,02						
	- Mean Indel	2,2	2,37	0,38	0,42	-0,07	2,0	1,96	0,36	0,38	-0,06	2,0	1,97	0,37	0,42	-0,08	2,0	2,00	0,48	0,31	0,38	2,0	1,84	0,23	0,32	-0,18	1,7	1,67	0,21	0,24	-0,05						
Mitochondrial compartment	Locus	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>	<i>N<sub>b</sub></i>	<i>D<sub>mt</sub></i>								
	CP1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2						
	CP2	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2						
	CP3	2	2	2</td																																	

**Table S9.**  $F_{ST}$  values calculated between each pair of populations. The parameters were calculated using GENETIX (Belkhir *et al.*, 2004). Values above the diagonal correspond to values obtained with nuclear loci, values below to those obtained with cytoplasmic data (considering chloroplast and mitochondrial data as two different loci). <sup>ns</sup> Values not significantly different from 0 at the 5% threshold, estimated on a total of 1000 permutations. All other values are significantly different from 0.

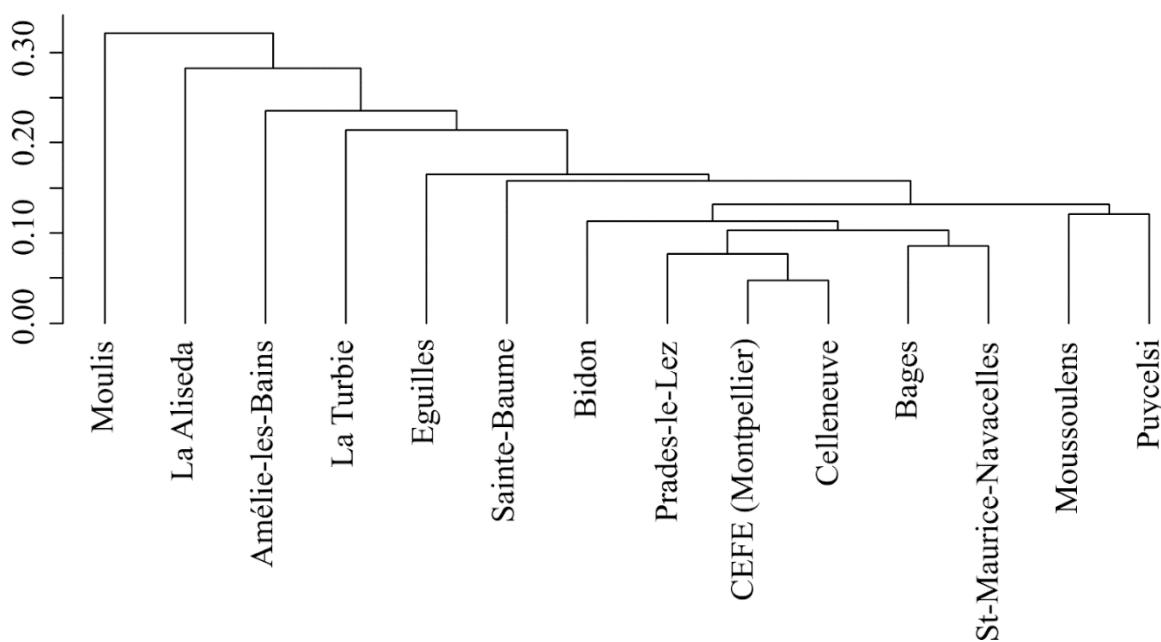
Population	Pop. Code	ALB	BAGE	BIDO	CEFE	CELL	EGUI	MOUL	MOUS	SMN	PLL	PUYC	BAUM	TURB	ALIS
Amelie-Les-Bains	ALB		0,220	0,212	0,194	0,244	0,204	0,433	0,265	0,169	0,269	0,230	0,322	0,260	0,318
Bages	BAGE	0,672		0,100	0,078	0,112	0,146	0,260	0,105	0,086	0,115	0,124	0,147	0,208	0,239
Bidon	BIDO	0,880	0,143 <sup>ns</sup>		0,084	0,126	0,161	0,246	0,149	0,106	0,150	0,155	0,180	0,209	0,298
CEFE	CEFE	0,563	0,179	0,384		0,047	0,107	0,258	0,120	0,070	0,071	0,106	0,105	0,149	0,258
Celleneuve	CELL	0,923	0,664	0,874	0,454		0,146	0,294	0,138	0,114	0,082	0,111	0,137	0,194	0,306
Eguilles	EGUI	0,808	0,579	0,764	0,394	0,684		0,311	0,195	0,155	0,174	0,179	0,220	0,238	0,324
Moulis	MOUL	0,763	0,343	0,442	0,387	0,680	0,593		0,292	0,303	0,227	0,323	0,366	0,445	0,419
Moussoulens	MOUS	0,688	0,431	0,576	0,384	0,825	0,718	0,632		0,174	0,147	0,121	0,214	0,292	0,232
St-Maurice-Navacelles	SMN	0,416	0,136	0,299	0,210	0,575	0,434	0,327	0,272		0,126	0,139	0,158	0,108	0,209
Prades-le-Lez	PLL	0,734	0,284	0,478	0,149	0,795	0,680	0,629	0,333	0,307		0,113	0,159	0,255	0,311
Puycelsi	PUYC	0,659	0,106 <sup>ns</sup>	0,259	0,284	0,693	0,593	0,350	0,503	0,165	0,413		0,158	0,223	0,242
Sainte-Baume	BAUM	0,858	0,499	0,705	0,409	0,770	0,589	0,117 <sup>ns</sup>	0,728	0,327	0,724	0,451		0,265	0,341
La Turbie	TURB	1,000	0,628	0,859	0,530	0,916	0,639	0,749	0,795	0,472	0,736	0,619	0,842		0,310
La Aliseda	ALIS	0,497	0,107 <sup>ns</sup>	0,410	0,177	0,612	0,481	0,464	0,383	-0,022 <sup>ns</sup>	0,308	0,207	0,478		0,485

**Table S10.** Results of the assignment test comparing nuclear SSR genotypes of individuals of the CEFÉ collection to nuclear SSR genotypes of natural populations (with at least ten sampled individuals). We first considered the 15 genotypes initially used in our population genetic study, and then, to increase the power of this analysis, we considered 125 genotypes of the CEFÉ collection (data not shown). The analysis was performed with GENECLASS2 (Piry *et al.*, 2004); in both cases, 27% of individuals (4/15, 34/125) were assigned to at least one putative population (all in Hérault, at maximum 30 km from Montpellier). This result is consistent with our expectations, since the population was initially funded with individuals from different localities around Montpellier, such as Causse-de-la-Selle near St-Maurice-de-Navacelles (J.D. Thompson, pers. com.). An arbitrary threshold probability value of 0.05 or greater was applied to determine the origin of CEFÉ individuals.

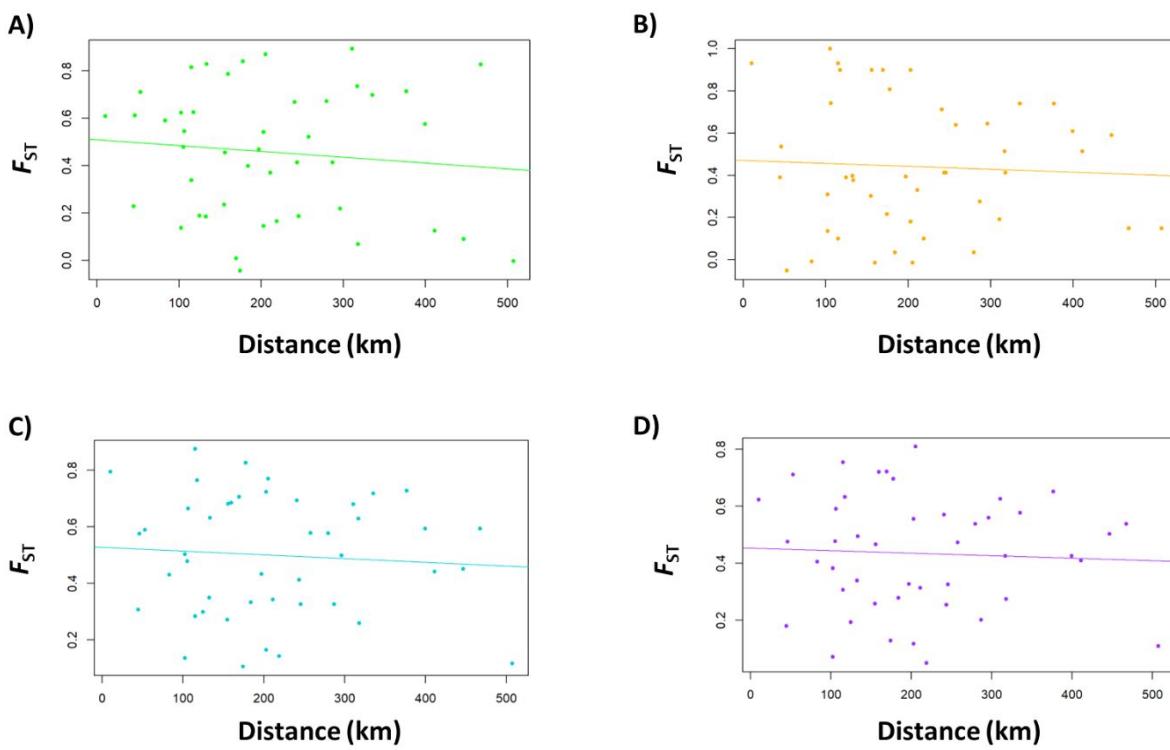
Population	Percent of CEFÉ individuals assigned (15 genets)	Percent of CEFÉ individuals assigned (125 genets)
Bages	0	0
Bidon	0	0
Celleneuve	13.3	5.6
Eguilles	0	0
Moulis	0	0
Moussoulens	0	0
St-Maurice-de-Navacelles	20.0	24.0
Puycelsi	0	0
Prades-le-Lez	0	2.4
Sainte-Baume	0	0



**Figure S1.** Dendrogram representing genetic similarity between all individuals based on genetic distances of Bruvo *et al.* (2004) and reconstructed with the unweighted pair group method with arithmetic mean (UPGMA) using *Poppr* (Kamvar *et al.*, 2014). French individuals are grouped in the same cluster with the individuals from Tunisia and Portugal. Individuals from the same population also tend to cluster together (e.g. 'Moulis', 'La Aliseda').



**Figure S2.** Dendrogram representing genetic similarity between populations based on pairwise  $F_{ST}$  estimated from nuclear data and reconstructed on the unweighted pair group method with arithmetic mean (UPGMA) with Poppr (Kamvar *et al.*, 2014). With the exception of the 'Moulis' population, geographically close populations tend to cluster together in this dendrogram (e.g. 'Prades-le-Lez', 'CEFE' and 'Celleneuve').



**Figure S3.** Cytoplasmic genetic differentiation between populations as a function of geographical distance according to different  $F_{ST}$  estimation methods: **(A)** with chloroplast data alone, **(B)** with mitochondrial data alone, **(C)** with chloroplast and mitochondrial data considered as two independent cytoplasmic loci, and **(D)** by combining chloroplast and mitochondrial information as a single locus for defining cytoplasmic haplotypes. Tests of Mantel (1967) were done with 999 permutations using the R package *ade4* (Chessel *et al.*, 2004). Genetic and geographical distances between population pairs do not show any significant relationship (Mantel test,  $r = 0.005$ ,  $-0.110$ ,  $-0.009$ , and  $-0.064$ , respectively for A, B, C and D;  $p$ -value  $> 0.05$ ) regardless of how the  $F_{ST}$  values are estimated.

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**Table S1.** Plant sampling and complete genotyping dataset. For each sample, we give their code, GPS coordinates (Lat., Long.), date of collect, floral morph (when available), and genetic profiles for 29 nuclear loci, five mitochondrial DNA loci and ten chloroplast DNA loci.

For both mitochondrial and chloroplast genomes, haplotypes were defined (and numbered) by combining the information on their respective loci. Genet duplicates (=identical or nearly identical ramets) are given; ramets that were further excluded for analyses are indicated.

Individual	Lat.	Long.	Date of collect	Floral morph	Clone (excluded ramet)	Genotype matrix																																												
						GAA1	GAA2	GAA3	GAA4	GAA5	GAA6	GAA7	GAA8	GAA9	GAA11	GAA14	GAA16	GAA17	GAA18	GAA19	GAA20_B	GAA20	GAA21	GAA22	GAA23	GAA24	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10														
Amelie_les_Bains_1	42.47787	2.66639	0/5/21	Longistyle		166.169	204.207	214.217	250.250	163.163	104.104	238.241	265.265	126.126	137.137	20.210	248.248	175.181	181.181	212.230	106.106	118.127	207.294	137.137	136.136	188.190	154.154	229.229	213.221	136.136	275.279	219.123	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245
Amelie_les_Bains_2	42.47786	2.66640	2/7/22	Longistyle	= Amelie_les_Bains_1	166.169	204.207	214.217	250.250	163.163	104.104	238.241	265.265	126.126	137.137	210.210	248.248	175.181	181.181	212.230	106.106	118.127	207.297	137.137	136.136	188.190	154.154	229.229	213.221	136.136	275.279	219.123	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245
Amelie_les_Bains_3	42.47786	2.66636	2/7/22	Brévistyle	= Amelie_les_Bains_1	166.169	204.207	214.217	250.250	163.163	104.104	238.241	265.265	126.126	137.137	210.210	248.248	175.181	181.181	212.230	106.106	118.127	207.294	137.137	136.136	188.190	154.154	229.229	213.221	136.136	275.279	219.123	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245
Amelie_les_Bains_4	42.47787	2.66635	2/7/22	Longistyle	= Amelie_les_Bains_1	166.169	204.207	214.217	250.250	163.163	104.104	238.241	265.265	126.126	137.137	210.210	248.248	175.181	181.181	212.230	106.106	118.127	207.294	137.137	136.136	188.190	154.154	229.229	213.221	136.136	275.279	219.123	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245
Amelie_les_Bains_5	42.47787	2.66634	2/7/22	Longistyle	= Amelie_les_Bains_1	166.169	204.207	214.217	250.250	163.163	104.104	238.241	265.265	126.126	137.137	210.210	248.248	175.181	181.181	212.230	106.106	118.127	207.294	137.137	136.136	188.190	154.154	229.229	213.221	136.136	275.279	219.123	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245
Bages_1	43.11845	2.98721	30/3/22	Brévistyle		169.169	204.204	214.214	247.250	163.163	104.146	241.241	265.274	123.126	137.152	162.198	236.242	179.190	194.248	203.106	115.115	210.210	27.209	154.156	188.190	151.151	229.232	213.221	136.161	285.285	126.121	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245	
Bages_2	43.11845	2.98721	30/3/22	Brévistyle		169.169	204.204	214.214	247.268	163.163	104.104	238.241	259.274	126.126	137.137	171.198	236.236	190.199	239.272	203.103	115.127	210.206	27.209	156.156	188.190	151.157	229.232	213.221	136.161	285.285	126.121	101.121	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245	
Bages_3	43.11876	2.98747	30/3/22	Brévistyle		169.169	204.213	214.213	250.250	163.163	104.146	241.241	265.274	123.126	137.137	199.199	218.213	201.104	123.213	217.213	217.212	161.261	236.251	119.152	162.256	236.251	101.101	150.194	309	316	335	231	225	5	176	119	157	349	129	136	108	90	110	245						
Bages_4	43.1280	2.98590	15/4/21	Brévistyle		169.169	204.204	214.217	253.271	163.163	104.104	238.238	258.274	123.126	137.164	198.210	215.257	120.202	227.227	203.103	124.124	210.210	19.292	133.156	188.190	151.157	229.235	213.221	136.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bages_5	43.13362	2.98506	2/1/22	Brévistyle		169.172	204.204	214.213	250.253	163.163	104.104	241.241	265.271	126.126	138.164	162.198	236.256	181.199	194.233	206.106	136.156	190.190	151.151	229.232	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245				
Bages_6	43.13357	2.98489	2/1/22	Brévistyle		169.172	204.204	217.217	253.283	163.170	104.104	241.241	265.274	123.126	137.140	162.263	236.230	190.196	239.239	203.103	136.152	210.222	20.192	133.156	188.190	151.151	229.232	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bages_7	43.13332	2.98456	2/1/22	Longistyle		172.172	207.207	214.213	253.253	163.163	104.146	241.241	265.274	123.126	137.137	171.198	236.236	190.199	239.236	203.103	136.152	210.222	20.192	133.156	188.190	151.151	229.232	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bages_8	43.13329	2.98447	2/1/22	Longistyle		169.172	207.207	214.214	253.251	163.163	104.146	241.241	265.265	126.126	135.164	162.264	236.242	189.199	204.209	203.106	136.151	213.205	23.208	137.159	188.190	151.151	229.235	212.223	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bages_9	43.13252	2.98431	2/1/22	Brévistyle		169.172	204.204	214.214	253.268	163.163	104.146	241.241	265.265	126.126	135.169	162.264	236.242	189.199	204.209	203.106	136.151	213.205	23.208	137.159	188.190	151.151	229.235	212.223	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bages_10	43.13280	2.98430	2/1/22	Brévistyle		169.172	204.204	214.214	253.268	163.163	104.146	241.241	265.265	126.126	135.170	162.265	236.242	189.199	204.209	203.106	136.151	213.205	23.208	137.159	188.190	151.151	229.235	212.223	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245	
Bidon_1	43.36565	5.453107	5/1/2021	Longistyle		169.169	202.213	214.214	253.268	163.163	104.143	241.244	267.247	126.126	137.150	168.198	248.248	196.202	203.202	101.106	156.156	188.190	151.151	229.235	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245				
Bidon_2	43.36571	5.453245	5/1/2021	Brévistyle		169.169	202.213	214.214	253.268	163.163	104.143	241.244	267.247	126.126	137.151	168.198	248.248	196.202	203.202	101.106	156.156	188.190	151.151	229.235	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245				
Bidon_3	43.36571	5.453245	5/1/2021	Brévistyle		169.169	202.213	214.214	253.268	163.163	104.143	241.244	267.247	126.126	137.152	168.198	248.248	196.202	203.202	101.106	156.156	188.190	151.151	229.235	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	127	137	109	90	110	245				
Bidon_4	43.36571	5.453245	5/1/2021	Brévistyle		169.169	202.213	214.214	253.268	163.163	104.143	241.244	267.247	126.126	137.153	168.198	248.248	196.202	203.202	101.106	156.156	188.190	151.151	229.235	212.221	161.161	285.285	121.121	101.121	150.194	301	317	335	231	225	5	177	119	166	357	1									



1	P04074663_Spain	37.95000	-2.450	00/06/1900	-	169:172 204:213 214:214 259:262 163:165 104:161 235:241 277:280 123:123 107:125 162:162 236:239 154:190 224:230 103:103 136:136 210:216 149:149 156:156 188:190 151:154 235:235 0	136:161 277:285 121:121 168:175 121:121 150:150	301 317 335 239 225 4	177 119 166 357 127 137 109 90 110 245 1
2	P04074654_Portugal	37.12000	-8.010	30/05/1853	-	163:163 204:204 214:214 253:253 163:163 104:104 241:241 268:274 123:126 152:152 198:201 215:215 184:196 212:221 106:106 130:133 210:210 140:164 156:158 190:190 151:151 229:232 0	136:161 279:279 121:123 168:175 121:121 150:150	301 317 335 239 225 4	177 119 166 349 127 137 109 90 110 245 7
3	P03426484_Morocco	33.81500	-7.109	5/1/1979	-	166:172 204:213 211:214 262:271 165:170 143:143 235:241 262:265 126:126 122:152 168:168 245:257 154:181 227:251 106:112 115:115 219:228 137:140 133:156 188:188 151:151 229:232 0	136:161 275:279 123:123 175:175 101:121 194:194	309 317 335 239 225 3	177 119 166 349 127 137 109 90 110 245 13
4	P01194578_Algeria	35.70000	-0.700	3/17/1940	-	163:163 204:213 214:217 253:253 163:163 104:104 235:241 265:271 123:126 134:149 162:162 242:251 181:181 215:215 109:112 115:115 213:216 137:188 133:156 188:188 151:157 232:232 0	136:136 275:279 121:121 175:175 121:121 150:194	309 316 335 231 225 5	177 119 166 349 127 137 109 91 111 245 7
5	P03426428_Tunisia	36.43800	8.44300	31/03/1884	-	166:166 204:204 211:214 265:268 163:170 104:143 238:241 277:277 123:126 137:143 162:189 242:245 190:193 236:239 103:106 124:136 216:231 137:161 133:133 188:188 151:157 235:235 0	136:136 275:277 121:121 175:175 0 150:194	309 316 335 239 225 2	177 119 166 349 127 137 109 91 111 245 7
6	P04074655_Albania	40.89500	20.90000	10/03/1883	-	163:193 204:204 211:214 256:268 170:170 137:137 235:241 259:259 123:126 150:151 204:204 230:230 154:190 228:221 103:106 124:136 228:240 140:141 133:133 191:191 140:140 0	136:136 275:275 121:121 168:168 0 150:150	309 317 335 239 225 3	177 119 167 349 127 137 108 90 110 245 10
7	P03426429_Greece	42.20500	14.70000	8/08/1897	-	164:164 204:204 211:214 256:258 123:123 150:150 204:204 230:230 154:190 228:221 103:106 124:136 228:240 140:141 133:133 191:191 140:140 0	136:136 275:275 121:121 168:168 0 150:150	309 317 335 239 225 3	177 119 167 349 127 137 108 90 110 245 10
8	P03869118_Turkey	38.37000	27.00000	05/05/1854	-	163:169 204:204 226:226 0 163:170 104:137 235:235 248:248 154:181 209:242 106:109 112:118 213:249 131:143 133:133 0 151:157 0	136:136 275:275 123:123 168:168 121:121 150:150	309 317 335 239 225 3	177 120 167 349 127 136 108 90 110 245 9
9	P03869093_Crimea	44.84000	34.96000	06/04/1896	-	169:193 207:210 226:232 262:277 161:163 104:104 235:235 268:271 123:126 128:134 204:213 248:248 154:154 248:257 106:109 127:127 255:258 146:146 133:133 190:191 147:149 223:235 0	136:136 275:275 121:121 168:175 121:121 150:150	309 317 335 239 225 3	177 116 167 349 127 137 108 90 110 245 12
10	P03869098_Georgia	41.92000	44.11000	00/07/1881	-	166:193 204:207 211:220 277:277 166:170 104:122 241:241 268:268 123:123 128:149 210:228 239:257 154:181 254:269 106:106 115:142 240:288 152:155 156:156 190:190 149:149 223:223 0	136:136 275:275 121:121 175:175 101:121 150:150	309 317 335 239 225 3	177 116 167 349 127 137 108 90 110 245 12
11	P03869122_Lebanon	33.87000	35.58800	6/9/1930	-	163:163 207:213 211:211 256:268 161:163 107:119 241:241 268:274 123:123 101:140 216:219 215:236 154:154 224:245 106:106 115:115 216:240 131:131 130:133 190:191 154:154 226:238 0	136:161 275:277 121:121 168:168 0 150:200	309 317 335 239 225 3	177 119 167 349 126 137 108 90 110 245 11
12	P03869103_Syria	36.72500	36.65500	4/15/1934	-	163:163 204:213 217:224 268:268 161:163 143:143 241:244 250:271 123:123 101:134 191:192 215:246 181:181 218:239 109:109 130:130 270:291 141:152 133:136 191:191 149:154 226:241 0	136:161 275:285 123:123 175:175 121:121 150:200	309 317 335 239 225 3	177 116 167 349 127 137 108 90 110 245 12
13	P03869104_Turkmenistan	38.91000	56.21000	5/25/1901	-	0 210:210 214:226 256:256 167:170 110:110 250:250 259:271 126:126 116:116 207:243 206:209 154:166 227:227 115:115 136:136 0 140:140 133:133 203:203 149:149 223:223 0	161:161 274:274 123:123 175:175 0 0	309 317 335 239 225 3	176 119 167 349 127 136 108 90 110 245 8
14	P04254353_Iraq	36.45000	53.10000	05/00/1966	-	0 210:210 217:226 256:256 167:167 110:110 250:250 259:271 117:126 116:116 213:225 206:209 166:166 212:212 115:115 112:112 0 137:140 133:136 194:203 149:149 223:223 0	161:161 274:274 123:123 175:175 0 0	309 317 335 239 225 3	176 119 167 349 127 136 108 90 110 245 8