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Gene flow among different taxonomic units: evidence from nuclear and cytoplasmic markers in *Cedrus* plantation forests

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Abstract Hybridization and introgression are important natural evolutionary processes that can be successfully investigated using molecular markers and open- and controlled-pollinated progeny. In this study, we collected open-pollinated seeds from *Cedrus atlantica, Cedrus libani* and *C. libani* × *C. atlantica* hybrids from three French-plantation forests. We also used pollen from *C. libani* and *Cedrus brevifolia* to pollinate *C. atlantica* trees. The progeny were analyzed using three different types of molecular markers: RAPDs, AFLPs and cpSSRs. Chloroplast DNA was found to be paternally inherited in *Cedrus* from the progeny of controlled-crosses. Heteroplasmy, although possible, could not be undoubtedly detected. There was no indication of strong reproductive isolating barriers among the three Mediterranean *Cedrus* taxa. Gene flow between *C. atlantica* and *C. libani* accounted for 67 to 81% of viable open-pollinated seedlings in two plantation forests. We propose that Mediterranean *Cedrus* taxa should be considered as units of a single collective species comprising two regional groups, North Africa and the Middle East. We recommend the use of cpSSRs for monitoring gene flow between taxa in plantation forests, especially in areas where garden specimens of one species are planted in the vicinity of selected seed-stands and gene-conservation reserves of another species.

Keywords Pollination · Hybridization · RAPD · AFLP · Microsatellite · Taxonomy · Genetic resource · *Cedrus*

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Introduction

Hybridization and introgression between genetically distinct populations are important phenomena for generating novel genetic variation within species and the emergence of new taxa through speciation (Arnold 1997). Hybridization has often been documented to occur naturally in forest ecosystems. It is the result of localized overlapping habitats of otherwise allopatric species or of sympatric species with distinctive habitats. Molecular markers have proven useful to identify natural hybrids when the investigated taxa are morphologically difficult to differentiate (e.g. in *Picea rubens* and *Picea mariana*, Bobola et al. 1996). They have also proven useful to study the direction, structure and extent of interspecific gene flow, and assess the degree of species relatedness when introgression is strong in natural forests (e.g. in oaks, Bacilieri et al. 1996; Dumolin-Lapègue et al. 1999; Belahbib et al. 2001).

Hybridization success can be used to evaluate relatedness between taxa. When both seeds and viable seedlings (and later fertile individuals) are readily obtained from crosses between different taxonomical units, lack of natural reproduction is governed only by extrinsic prezygotic isolating reproductive barriers (e.g. different geographic ranges). If taxa have a common phylogenetic origin, they are then better described as members of a collective species rather than as separate species (see Avise 1994 for a review). A good example is given by the collective species *Pinus nigra* Arnold, whose different geographical sub-units readily hybridize under experi-

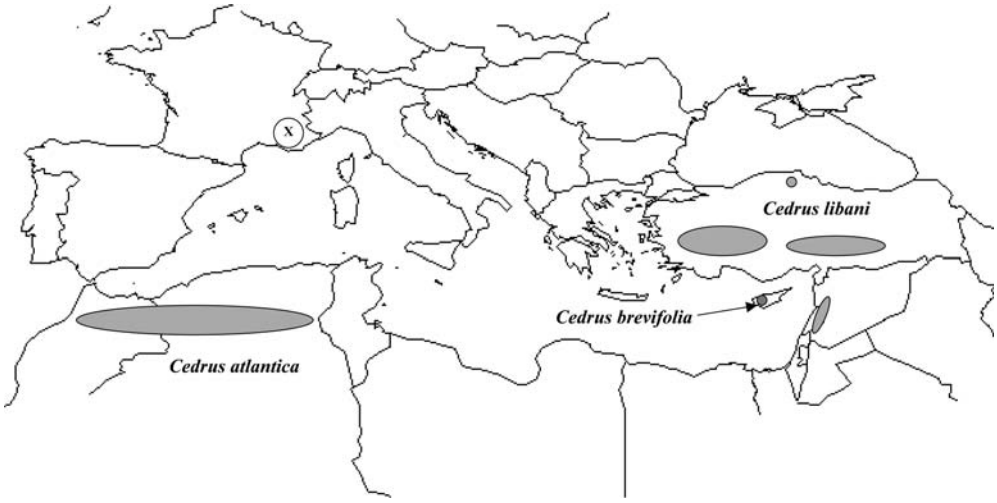

Fig. 1 Simplified natural distribution map of *Cedrus* forests around the Mediterranean and localization (X) of the artificial stands where pollination tests were performed and seeds collected

mental conditions and are described as sub-species (Debazac 1964).

Cedrus forests are currently localized in two distinct geographical areas around the world: the Himalayan foothills and the Mediterranean. Around the Mediterranean, *Cedrus atlantica* is found in North Africa, *Cedrus libani* in Turkey, Syria and Lebanon, and *Cedrus brevifolia* in Cyprus (Fig. 1). However, the taxonomical level at which these geographical units should be considered is subject to discussion. All authors agree to consider *Cedrus deodara* as a separate species. However, some authors consider all Mediterranean taxa to be of sub-specific level, separating *C. libani* into two sub-species and thus creating four Mediterranean sub-species (Greuter et al. 1984). Others separate them into two (combining *C. brevifolia* with *C. libani*, Vidakovic 1991) or three different species (Tutin et al. 1964). Recent genetic diversity studies suggested that current Mediterranean *Cedrus* taxa derive from a common phylogenetic group (Scaltsoyiannes 1999; Fady et al. 2000; Bou Dagher-Kharrat 2001). They also suggest that *C. libani* from Turkey and *C. brevifolia* are both more closely related than either is with *C. libani* from Lebanon, and the genetic differentiation of *Cedrus atlantica* from this group is high. Bou Dagher-Kharrat et al. (2001), using karyotype analysis, concluded that *C. brevifolia* should be considered as a subspecies of *C. libani*, and *C. atlantica* as a separate species. Monitoring gene flow and/or performing crosses between the different Mediterranean *Cedrus* taxonomic units are thus likely to be informative on the taxonomic status: the more recent the separation, the closer the genome composition and the easier the crossability.

Finally, experimental hybridization can be used to check the segregation pattern of organelle DNA. Because *Cedrus* belongs to the *Pinaceae*, its cpDNA is expected to be paternally inherited although heteroplasmy (or maternal transmission) is possible (see Wagner 1992 for a review).

In this study, we analyzed the occurrence of hybridization by collecting open-pollinated seeds in plantation forests where both *C. atlantica* and *C. libani* are found. We used the results of controlled-pollination experiments among the three Mediterranean *Cedrus* taxa to confirm the paternal origin of cpDNA in *Cedrus*, making the use of cpSSRs possible to monitor male gene flow in plantation forests through the genotyping of single trees and their open-pollinated progeny. Parent trees and progenies were genotyped using three types of molecular markers: Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and chloroplast microsatellites (cpSSRs).

Materials and methods

In this paper, the word taxon is used to designate a taxonomical unit without reference to its taxonomical level (e.g. species, sub-species or variety).

Open- and controlled-pollinations

Seeds were collected from three plantation forests located in southeastern France (Fig. 1). Natural regeneration occurred regularly and abundantly in all of three forests. Taxonomic status of parent trees was a priori identified using morphological traits derived from the architectural model of Barthélémy et al. (2000).

The St. Michel forest (43°53′35″N; 5°43′54″E) was originally created in 1860 using *C. libani* seeds from Lebanon, and later further planted using *C. atlantica*. Adult trees in this forest were identified as either *C. atlantica, C. libani* or *C. libani* × *C. atlantica* hybrid trees. We randomly collected three open-pollinated cones per tree from the lower part of the crown of one *C. libani* tree and four *C. libani* × *C. atlantica* hybrid trees.

The Luberon (43°48′18″N; 5°16′36″E) forest originated in 1860 from Algerian *C. atlantica* seeds. The Lambert (43°13′07″N; 6°20′16″E) forest was created 40 to 50 years ago from seeds of unknown origin. Adult trees from the Lambert forest were identified as *C. atlantica* types, although four *C. libani* × *C. atlantica* hybrid trees were also found. We randomly collected three open-pollinated cones from the lower part of the crown of two *C. atlantica* trees in Luberon and from one *C. atlantica* tree in Lambert on which controlled-pollinated cones were also obtained (Table 1). Controlled-pollination was performed by either bagging ovulate cones and pulverizing a small amount of pollen into the bag at receptivity, or by mass-pollinating ovulate cones at receptivity

Table 1 Description of controlled and open-pollinations performed

Cross no.	Seed tree taxonomic type and code	Type of pollen used	Type of pollination	Location of female tree	Number of seeds collected[a]	Taxonomic type of observed progeny (female × male)	Frequency in % of observed progeny
1	*C. atlantica* Lu01	Unknown	Open	Luberon	30	*C. atlantica*	97
						C. atlantica × *C. libani*	3
2	*C. atlantica* Lu03	Unknown	Open	Luberon	31	*C. atlantica*	97
						C. atlantica × *C. libani*	3
3	*C. atlantica* Lb02	Unknown	Open	Lambert	12	*C. atlantica*	17
						C. atlantica × *C. libani*	83
4	*C. libani* SM	Unknown	Open	St. Michel	30	*C. libani*	33
						C. libani × *C. atlantica*	67
5	*C. libani* × *atlantica* SM01	Unknown	Open	St. Michel	30	Backcross with *atlantica*	97
						Backcross with l*ibani*	3
6	*C. libani* × *atlantica* SM02	*Unknown*	Open	St. Michel	25	Backcross with *atlantica*	92
						Backcross with l*ibani*	8
7	*C. libani* × *atlantica* SM02b	*Unknown*	Open	St. Michel	25	Backcross with *atlantica*	96
						Backcross with l*ibani*	4
8	*C. libani* × *atlantica* SM03	Unknown	Open	St. Michel	30	Backcross with *atlantica*	73
						Backcross with l*ibani*	27
9	*C. atlantica* Lb02	*C. brevifolia*	Controlled	Lambert	6	*C. atlantica*	0
						C. atlantica × *C. brevifolia*	100
10	*C. atlantica* Lu01	*C. brevifolia*	Controlled	Luberon	7	*C. atlantica*	100
						C. atlantica × *C. brevifolia*	0
11	*C. atlantica* Lu03	*C. libani*	Controlled/ mass	Luberon	67	*C. atlantic*	19
						C. atlantica × *C. libani*	81

[a] Figures correspond to total cone yield for seeds collected from controlled pollination. In the case of open-pollinated seeds, it is a random sub-sample from total harvested cone yield

Table 2 Primer combinations used for the RAPD and AFLP analyses

	Name and code	Sequence
RAPD primers used		
	A15	5′-TTCCGAACCC-3′
	A20	5′-GTTGCGATCC-3′
	B12	5′-CCTTGACGCA-3′
	C16	5′-CACACTCCAG-3′
	E9	5′-CCTCACCCGA-3′
	P14	5′-CCAGCCGAAC-3′
	Q5	5′-CCGCGTCTTG-3′
AFLP primer pairs used in the amplification		
*Eco*RI + 3-NNN	E-AAC	5′-GACTGCGTACCAATTC+AAC-3′
*Mse*I + 3-NNN	M-CAT	5′-GATGAGTCCTGAGTAA+CAT-3′
	E-AAC	5′-GACTGCGTACCAATTC+AAC-3′
	M-CAG	5′-GATGAGTCCTGAGTAA+CAG-3′
	E-ACG	5′-GACTGCGTACCAATTC+ACG-3′
	M-CAG	5′-GATGAGTCCTGAGTAA+CAG-3′
	E-ACG	5′-GACTGCGTACCAATTC+ACG-3′
	M-CTT	5′-GATGAGTCCTGAGTAA+CTT-3′
	E-ACG	5′-GACTGCGTACCAATTC+ACG-3′
	M-CTA	5′-GATGAGTCCTGAGTAA+CTA-3′

without bagging. We used two different pollen mixes, one collected from a single *C. libani* tree and the second collected from two *C. brevifolia* trees (Table 1).

DNA analysis

All controlled-pollinated seeds and a subset of randomly chosen open-pollinated seeds from Luberon and Lambert were genotyped using RAPDs, AFLPs and cpSSRs. The randomly chosen open-pollinated seeds from St. Michel were screened using cpSSRs (Table 1). The objectives of RAPD and AFLP analyses were to find specific-band variants present in *C. libani* and *C. brevifolia* pollen parents and populations used as a control, but not present in *C. atlantica* ovulate parents and populations used as a control. We used the two techniques concurrently to avoid risks of improper genotyping linked to possible non-repeatability of both methods (Mac Pherson et al. 1993 for RAPDs, personal observations for AFLPs). An offspring was considered to be a potential hybrid when its profile contained a male-parent band at least once.

Seeds were germinated and sown in a nursery prior to molecular identification. Total genomic DNA was extracted from 500 mg of frozen needles ground in liquid nitrogen using the Doyle and Doyle (1990) protocol.

RAPD: we tested 138 Operon-kit primers (Operon Technologies, Inc., Alameda, Calif., U.S.A.) on parent trees from which seeds were obtained after controlled-pollination. PCR, agarose electrophoresis and ethidium-bromide staining protocols were those

described in Lefebvre et al. (1995) modified as follows: PCR buffers were supplemented with bovine serum albumin at 0.2 mg/ml.

AFLP: analyses were carried out using the "AFLP Analysis System I" kit (Life Technologies, Inc.) following the manufacturer's instructions. In all cases *Eco*RI primers were labeled with [γ-^{33}P] ATP (NEN, Belgium) as described by Vos et al. (1995). AFLP primers contained one selective nucleotide for pre-amplification, and three selective nucleotides for amplification. Primers used are listed in Table 2. PCR products were separated on 6% polyacrylamide gels (19:1 acrylamide:bis; 7.5 M urea; 1× TBE buffer). Electrophoresis was performed using a Sequi-Gen GT Nucleic Acid Electrophoresis Cell (Bio-Rad). Gels were pre-run until an adequate temperature (40 °C) was reached. Three microliter samples were mixed with an equal amount of formamide loading buffer, denatured and electrophoresed at 80 W for 3 h 20 min. Gels were then vacuum-dried on a gel drier (Bio-Rad) and exposed to Biomax MR films (Kodak) for 3 days.

cpSSR: seven primers (Vendramin et al. 1996) could be amplified in our *Cedrus* samples. PCR amplifications were performed using a PE model 9700 thermal cycler in a total volume of 25 μl containing 0.2 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.2 μM of each primer, 10× reaction buffer (Pharmacia), 25 ng of template DNA and 1 unit of Pharmacia *Taq* polymerase. The PCR profile was as follows: 5 min denaturation at 95 °C followed by 25 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C and 1 min extension at 72 °C, with a final extension step of 72 °C for 8 min. One of the two PCR primers in each reaction was 5′ Cy5-labelled. PCR products were loaded on a 20 cm-long Reprogel Long Read 6% polyacrylamide gel (Pharmacia) and run on a Pharmacia ALF Express automatic sequencer at 35 W constant power for 80 min. Fragments were sized using the Pharmacia Fragment Manager version 1.2 conversion software. The same gel was loaded three times. Sizing was repeated three times using both external and internal molecular-weight standards (50, 100, 150 and 200 bp).

Results

DNA analyses of parent trees

Taxonomic identification of parent trees was confirmed for the three types of molecular markers used.

RAPD profiles were very similar among taxa. Out of 138, only five primers (A15, A20, B12, E9, Q5) had one band each that was present in *C. libani* and absent in *C. atlantica* parent trees. Only six primers (A20, B12, C16, E9, P14, Q5) had one band each that was present in *C. brevifolia* and absent in *C. atlantica* parent trees (Table 2). The *C. libani* marker bands were found to be polymorphic in six natural populations (data not shown). The two different *C. brevifolia* trees used for pollen collection had identical RAPD phenotypes.

The five AFLP primer pair combinations chosen yielded 16 specific bands usable for hybrid detection. They were always present in *C. libani* or *C. brevifolia* pollen parents and not present in *C. atlantica* ovulate parents. Taxon specificity of bands was confirmed using a range-wide sample of 15 populations and 20 individuals per population (Bou Dagher-Kharrat 2001): the first three primer pairs from Table 2 were monomorphic in the eastern Mediterranean Cedrus group, although the last two were polymorphic with a high frequency of band presence (80%).

Out of the seven amplified cpSSR primers, three were polymorphic among and within taxa: Pt15169, Pt63718, Pt71936. One was polymorphic among taxa: Pt87268. The remaining three were monomorphic: Pt26081, Pt36480, Pt110048. Haplotypes were defined as a set of specific combinations of the observed variants for the four polymorphic fragments. Twenty haplotypes were found and exclusive taxon specificity was confirmed using a 25 population range-wide sample (Fady et al. 2002).

Open-pollinations

Open-pollinated crosses #1, #2 (Luberon) and #3 (Lambert): these crosses involved *C. atlantica* trees as ovulate parents. RAPD and AFLP analyses indicated that progeny contained low levels (3%) of interspecific *C. atlantica* × *C. libani* hybrid genotypes in Luberon and very high levels (83%) in Lambert. In cross #2, one AFLP and one RAPD *C. libani* band was detected in one seedling, one *C. libani* AFLP band in another and one *C. libani* RAPD band in yet another seedling, although the cpSSR haplotypes of the three seedlings were typical of *C. atlantica*.

Open-pollinated cross #4 (St. Michel): this cross involved a *C. libani* tree as an ovulate parent. Its progeny yielded 67% *C. atlantica* cpSSR haplotypes and 33% *C. libani* cpSSR haplotypes. The first type of seedlings was considered to be either an F1 hybrid or a hybrid derivative of the *C. atlantica* pollen parent descent. The second type of seedling was considered to be typical *C. libani* or a hybrid derivative of the *C. libani* pollen parent descent.

Open-pollinated cross #5 to #8 (St. Michel): these crosses involved hybrid *C. libani* × *C. atlantica* trees as ovulate parents. Their progeny yielded an average of 88% *C. atlantica* cpSSR haplotypes and 12% *C. libani* cpSSR haplotypes. The first type of seedlings was considered to be either backcrossed with *C. atlantica* pollen parents or a hybrid derivative of *C. atlantica* pollen parent descent. The second type of seedling was considered to be backcrossed with *C. libani* pollen parents or a hybrid derivative of *C. libani* pollen parent descent.

Controlled-pollinations

Cross #9 (Lambert): this cross involved a *C. atlantica* tree as ovulate parent and *C. brevifolia* pollen. RAPD and AFLP analyses showed that all six seedlings of the progeny contained *C. brevifolia* marker bands. This was interpreted as being the result of successful hybridization. A single cpSSR haplotype, identical to that of both *C. brevifolia* pollen parents used as pollen donor, was found in all six seedlings.

Cross #10 (Luberon): this cross involved a *C. atlantica* tree as ovulate parent and *C. brevifolia* pollen. The seven seedling progeny did not show any *C. brevifolia* RAPD and AFLP marker bands, indicating the absence of interspecific hybridization. Two different *C. atlantica*

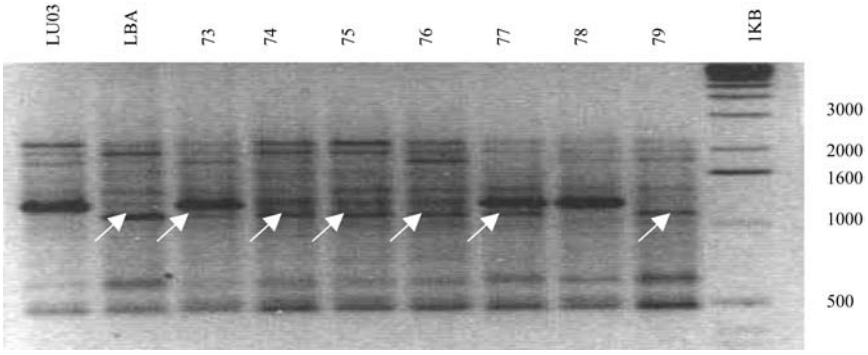

Fig. 2 Part of an RAPD agarose gel (primer B12) showing the progeny of tree *C. atlantica* LU03 pollinated by *C. libani* (LBA). *Arrows* indicate the paternal *C. libani* band used for molecular screening of putative hybrids. The molecular ladder is at the far right and molecular sizes are indicated in base pairs. Sample 78 is not a hybrid although all other progeny are

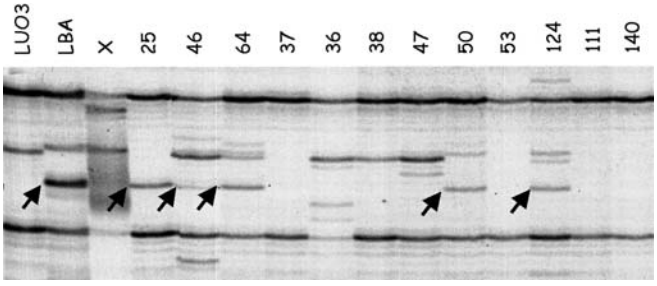

Fig. 3 Part of an AFLP polyacrylamide gel (primer pair ACG / CAG) showing the progeny of tree *C. atlantica* LU03 pollinated by *C. libani* (LBA). *Arrows* indicate the paternal *C. libani* band used for molecular screening of putative hybrids. Lane "x" contains a poorly digested DNA sample. Samples 25, 46, 64, 50 and 124 are hybrids although other progeny are not

cpSSR haplotypes were found, four seedlings carrying the maternal haplotype.

Cross #11 (Luberon): this cross involved a *C. atlantica* tree as ovulate parent and *C. libani* pollen. Hybridization accounted for 81% of the progeny. RAPD and AFLP analyses together showed that 78% of the progeny contained *C. libani* marker bands. This was interpreted as being the result of successful hybridization (Figs. 2 and 3). In two cases (3%), *C. libani* RAPD bands could not be detected in the hybrid progeny, although *C. libani* AFLP bands were, indicating successful hybridization. A single cpSSR haplotype, identical to that of the *C. libani* male parent used as a pollen donor, was found in this hybrid progeny. Two different *C. atlantica* cpSSR haplotypes were found in the 13 (19%) non-hybrid seedlings, one of which was identical to the maternal haplotype in two seedlings.

Discussion

Paternal origin of the chloroplast genome

In controlled-cross #11, two hybrid offspring out of 54 did not carry the paternal RAPD bands expected from the AFLP analysis. This fits the hypothesis of not detecting bi-parentally inherited bands once in a true hybrid when the pollen parent is heterozygotous for all five diagnostic bands, i.e. $(0.5)^5 = 3.1\%$. Otherwise, all genotypes of controlled-crosses #9 and #11 identified as hybrids using RAPDs and AFLPs carried a paternal cpSSR haplotype. These results indicate that the chloroplast genome is paternally inherited in the genus *Cedrus*, as in other members of the Pinaceae (Wagner 1992).

There was no clear evidence of heteroplasmy in our analysis. In open-pollinated cross #2, three samples had *C. atlantica* cpSSR haplotypes but also carried one *C. libani* RAPD band. One of these samples also carried a *C. libani* AFLP band. Although heteroplasmy could explain the occurrence of a maternal haplotype in the progeny, it was considered unlikely as it was not observed once out of 54 times in the controlled-pollinated progeny of the same seed tree (cross #11). We expect some level of non-repeatability (such as DNA degradation, slight changes in PCR, DNA digestion and/or electrophoretic conditions) to be the cause of this discrepancy. Finally, the fact that two *C. atlantica* cpSSR haplotypes were found in the progeny of cross #10 was interpreted as a *C. atlantica* pollen contamination. Although heteroplasmy or maternal apomixis (seed deriving from a non-fecundated female gamete) could explain the occurrence of the maternal haplotype in four out of six seedlings, selfing or pollen from a *C. atlantica* pollen tree carrying the same haplotype as the ovulate tree, seems a more-likely hypothesis.

Open pollination, reproductive isolating barriers and taxonomy

The Luberon forest originates from Algerian *C. atlantica* seeds. *C. atlantica* pollen should thus saturate the pollen cloud at pollen cone maturity in this forest. However, 3% of viable seeds obtained from open-pollinations were made from interspecific hybrids (or hybrid derivatives) carrying *C. libani* marker bands and haplotypes. This *C. libani* pollen could both come from experimental pollen

that unexpectedly escaped from our pillboxes during controlled-pollination procedures or from previously unidentified *C. libani* surrounding trees. The closest possible *C. libani* pollen donors were later identified 4 km away using our molecular markers following a population-wide morphological screening based on the architectural model of Barthélemy et al. (2000). Although their cpSSR haplotypes were identical to that found in the hybrid-type seedlings, such a long-distance pollen-travel makes them unlikely pollen donors. Further, the cpSSR haplotypes found in the hybrid-type seedlings were identical to that of the *C. libani* pollen used in controlled-pollinations, making experimental pollen contamination a more-likely hypothesis. Furthermore, a previous study based on the isozyme analysis of 186 open-pollinated progeny did not show any evidence of hybridization events within this forest (Fallour et al. 2001).

The very high amount of hybrids (or hybrid derivatives) found in open-pollinated cross #3 from Lambert could not originate from experimental pollen contamination, as expected from our Luberon hypothesis. In addition, hybrids carried two different *C. libani* cpSSR haplotypes. The presence of four adult *C. atlantica* × *C. libani* crosses, including one tree immediately adjacent to the tree from which open-pollinated seeds had been collected, could explain the high proportion of hybrid-type seedlings found in this progeny.

The proportion of first-generation hybrids found on the *C. libani* seed tree from St. Michel was of the same magnitude as in Lambert for the *C. atlantica* seed tree. *C. atlantica* haplotypes were in higher proportions than *C. libani* haplotypes for second-generation hybrids or hybrid derivatives. The frequency and spatial distribution of pollen donors from different taxonomic origin, in the vicinity of seed trees, should explain the different genetic composition of the two generations.

Gene flow between different *Cedrus* taxa occurs naturally in plantation forests. Our findings suggest that, when sympatric, there are no strong pre-zygotic reproductive isolating barriers (Avise 1994) among Mediterranean *Cedrus*. In the genus *Abies*, which has similar ecological requirements, crossability is no longer possible between North American and European species (Kormutak 1985) which have been separated since the beginning of the Tertiary, although it is possible within all North American and European taxa. The reason for non-existent or partial reproductive barriers within *Cedrus* taxa may also be because recent (Tertiary and Quaternary) geographic isolation following habitat fragmentation is responsible for their evolution rather than genetic isolation. In fact, fossil remains have been found in Tertiary deposits in Europe and Siberia where *Cedrus* is believed to have been widespread (Gaussen 1964). All hybrid seeds obtained developed into viable healthy seedlings from which our DNA samples were taken. Postzygotic reproductive isolating barriers (Avise 1994) do not seem to exist at the early stage of seedling viability. They do not seem to exist at later developmental stages either, as in St. Michel and Lambert F1 hybrid trees are currently reproducing and yield viable seeds and seedlings. Thus, we clearly demonstrated that hybridization is possible and frequent between *C. atlantica* and *C. libani*, and possible between *C. atlantica* and *C. brevifolia*. Mediterranean *Cedrus* taxa should not be considered as biologically isolated species. Because of obvious phenotypic differences at individual, chromosome and molecular levels (e.g. Barthélemy et al. 2000; Bou Dagher-Kharrat et al. 2001; Fady et al. 2002, respectively), they should not be grouped into one single species either. We propose that they should be merged into one collective species (as suggested by Greuter et al. 1984), and sub-specific taxonomic status given to its two different bio-geographic units, North Africa and the Middle East.

Interspecific gene flow and management of genetic resources

One of the first steps in forest management is the selection of suitable stands for the production of reproductive material and the conservation of adaptive potentials through in situ genetic conservation. In many countries where *Cedrus* forests exist, whether naturally or because of reforestation programs, all three Mediterranean taxa are also planted as garden and park trees. Hybridization may thus occur to a significant extent and needs to be monitored.

Hybridization is one of the ways novel genetic variation can be naturally introduced into populations, thus creating new adaptations and/or new evolutionary lineages (Arnold 1997). Fabre et al. (2001) demonstrated that both *C. libani* × *C. atlantica* and *C. brevifolia* × *C. atlantica* hybrids had an increased resistance to the aphid *Cedrobium laportei* compared to that of *C. atlantica*. Introducing this resistance trait in *C. atlantica* plantation forests might be highly desirable and could be done using natural hybridization. However, hybridization may be a non-desired phenomenon, for example in gene conservation areas, or in seed stands and orchards where harvesting of well-known adaptive quality seeds is done for reforestation purposes.

We have evidence from the St. Michel forest that hybrids produced under natural conditions can develop into mature seed trees. A full appreciation of the impact of interspecific gene flow on gene conservation and forest management would require monitoring of comparative seed production, seedling establishment and viability under diverse experimental and natural conditions. Among the molecular markers we have used, cpSSRs were best suited to unambiguously detect interspecific hybridization. Because they are paternally inherited and differentiated among Mediterranean *Cedrus* taxa, they could be of great interest for monitoring gene flow through pollen.

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