Theor Appl Genet (1996) 93: 626–632

P. Lashermes · J. Cros · M. C. Combes · P. Trouslot
F. Anthony · S. Hamon · A. Charrier

Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L.

Received: 4 April 1996 / Accepted: 26 April 1996

Abstract CpDNA variation among 52 tree samples belonging to 25 different taxa of *Coffea* and two species of *Psilanthus* was assessed by RFLP analysis on both the total chloroplast genome and the *atpB-rbcL* intergenic region. Twelve variable characters were distinguished allowing the identification of 12 different plastomes. The low sequence divergence observed might suggest that *Coffea* is a young genus. The results were in contradiction with the present classification into two genera. Additionally, cpDNA inheritance was studied in interspecific hybrids between *C. arabica* and *C. canephora*, and in an intraspecific progeny of *C. canephora*, using PCR-based markers. Both studies showed exclusively maternal inheritance of cpDNA.

Key words Chloroplast DNA · *Coffea* · Inheritance · RFLP

Introduction

Coffee-trees belong to the genus *Coffea* in the family Rubiaceae. The subgenus *Coffea* consists of approximately 100 taxa so far identified (Bridson and Verdcourt 1988). All species are woody, ranging from small shrubs to robust trees, and originate in the inter-tropical forest of Africa and Madagascar. *Coffea arabica* L. (2n=4x=44) and *Coffea canephora* Pierre (2n=2x=22) are the only cultivated species of economic importance. *C. arabica* is a natural allotetraploid and is self-fertile, while other species are diploid and generally self-incompatible (Charrier and Berthaud 1985).

Analysis of chloroplast DNA (cpDNA) variation has proven to be immensely valuable for plant phylogenetic reconstruction (Clegg and Zurawski 1992; Olmstead and Palmer 1994). Before using cpDNA as an evolutionary marker in the genus *Coffea*, different features such as the mode of cpDNA inheritance and the importance of intraspecific variation (Harris and Ingram 1991) need to be considered. In addition, the value of cpDNA for studying genetic relationships between *Coffea* species is related to the type and extent of the observed variation.

Uniparental-maternal is the most common mode of plastid inheritance in angiosperms (Sears 1980), although there is evidence for the inheritance of paternal plastids in many species (Hageman and Schröder 1989; Reboud and Zeyl 1994). Plastid maternal inheritance in *C. arabica* was suggested on the basis of cytological evidence from epifluorescence microscopy (Corriveau and Coleman 1988). Using restriction endonuclease fragment analysis of the cpDNA genome, Berthou et al. (1983) also reported maternal inheritance in an interspecific hybrid between *C. canephora* and *C. arabica*. However, the low sensitivity of such restriction analysis to small amounts of DNA, and the fact that only one hybrid tree was analysed, possibly prevented the detection of heteroplasmy and of low-frequency paternal inheritance.

The purpose of the present study was: (1) to estimate cpDNA genome variation among and within a large set of *Coffea* taxa, and (2) to determine the mode of inheritance of cpDNA. Assessment of cpDNA variation was performed by RFLP (restriction fragment length polymorphism) analysis of both the total chloroplast genome, using heterologous probes, and the *atpB-rbcL* intergenic region. The inheritance of cpDNA was determined in interspecific hybrids between *C. arabica* and *C. canephora* (4x), and in intraspecific progeny of *C. canephora*. For this purpose, we used cpDNA markers based on the polymerase chain reaction (PCR), which is known for its sensitivity and efficiency in detecting rare sequences (Saiki et al. 1988).

Communicated by R. Hagemann

P. Lashermes (✉) · J. Cros · M. C. Combes · P. Trouslot
S. Hamon
ORSTOM, Laboratoire de ressources génétiques
et d'amélioration des plantes tropicales, BP 5045,
F-34032, Montpellier, France

F. Anthony
CATIE, 7170 Turrialba, Costa Rica

A. Charrier
ENSAM, place Viala, F-34060, Montpellier, France

Materials and methods

Plant material

The plant material included in the cpDNA variation analysis was obtained from a field-based collection resulting from several expeditions in Africa and Madagascar (Anthony 1992). The sampling strategy was to maximise the likelihood of detecting variation among accessions. Therefore, *Coffea* species were represented by several samples when more than one genetic group had been revealed by previous agro-morphological studies (Hamon et al. 1995). In total, 50 samples belonging to 25 taxa were analysed. The closely related genus *Psilanthus* was also represented by two species, *P. ebracteolatus* and *P. mannii*, which belong to the subgenera *Afrocoffea* and *Psilanthus*, respectively (Bridson and Verdcourt 1988). The accessions surveyed and their origins are indicated in Table 1.

For the determination of cpDNA inheritance, the plant material studied included two interspecific hybrids (Et30×IF181T and Caturra×IF181T) between *C. arabica* (female parent) and *C. canephora*, 15 individual trees resulting from an intraspecific cross in *C. canephora* between DH160-02 (female parent) and IF200, and five doubled haploids derived from the clone IF200 of *C. canephora*. These doubled haploids (DH) were developed using haploid embryos which occur spontaneously in association with polyembryony (Lashermes et al. 1994).

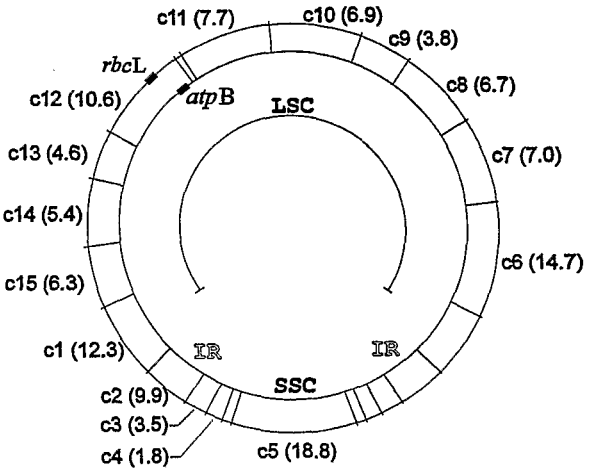

Fig. 1 Location of *Lactuta sativa* cpDNA *Sac*I fragments (Jansen and Palmer 1987) used as probes in this study. The size (kb) of each probe is indicated. The approximate location of the intergenic region *atpB-rbcL* is also represented. *IR* inverted repeat; *LSC* large single-copy region; *SSC* small single-copy region

DNA isolation, Southern-blot hybridisation and detection

Total DNA was isolated from lyophilised leaves as previously reported (Lashermes et al. 1993) except that CTAB was replaced by MATAB (mixed alkyl tri-methyl ammonium bromide) in the extraction buffer. Approximately 10 μg of DNA was digested with each of three restriction endonucleases (*Eco*RI, *Eco*RV and *Dra*I) and samples were fractionated on 1% agarose gels. Following electrophoresis, the restricted DNA was denatured and blotted onto a nylon membrane (Hybond N^+ Amersham) under alkaline conditions, according to the manufacturer's recommendations.

Southern hybridisation of coffee DNA was done with heterologous probes from *Lactuca sativa* consisting of 15 *Sac*I cpDNA clones (Fig. 1). This set of clones represents approximately 95% of the lettuce chloroplast genome (Jansen and Palmer 1987). Blots were prehybridised in 10 ml of 3×SSC, 5×Denhardt's solution, 6% PEG 8000, 0.1% SDS and 100 μg/ml of herring sperm DNA at 65°C for 5–8 h in a hybridisation oven. CpDNA probes were radiolabelled by incorporating $dCT^{32}P$ using random priming. Blots were hybridised in fresh buffer (3×SSC, 2×Denhardt's solution, 6% PEG 8000, 0.1% SDS and 100 μg/ml of herring sperm DNA) at 65°C with 100 ng of the labelled and denatured DNA probe. Hybridisation was allowed to proceed for 16–20 h. Filters were washed two times for 5 min each at room temperature with 2×SSC, 0.1% SDS, followed by two times for 15 min at 65°C with 0.5×SSC, 0.1% SDS in plastic boxes. Blots were exposed to X-ray films with intensifier screens for 4–72 h at –80°C.

PCR amplification and restriction fragment length polymorphism analysis

Two regions of the chloroplast genome, *atpB-rbcL* and *ndhC-trnV*, were amplified from total DNA of different samples via the polymerase chain reaction (PCR) using specific primers. The primers were designed to match the highly conserved coding regions flanking both intergenic fragments. The region *atpB-rbcL* corresponds to the intergenic spacer between the *atpB* (ATP synthetase Beta subunit) and *rbcL* (RuBisCO large subunit) genes, and the first 56 codons of *rbcL* (Spichiger et al. 1993). The nucleotide sequences of PCR primers were A1 (GAAGTAGTAGGATTGATTCTC), A2 (TACAGTTGTCCATGTACCAG) (V. Savolainen, personal communication) and N1 (ACGGTTCGAATCCGTATAGC), N2 (CATATTCGTGAAGCAGAAAC) (G. Second, personal communication) for the amplification of *atpB-rbcL* and *ndhC-trnV* fragments, respectively. Amplifications were performed in a volume of 50 μl containing 10 mM Tris HCl, pH 9.0, 0.1% Triton X-100, 1.5 mM MgCl2, 50 mM KCl, 150 μM each of dATP, dCTP, dGTP, dTTP, 0.5 μM of each primer, 50 ng of total DNA, and 0.5 U of *Taq* polymerase (Promega). Reactions were performed in a PTC-100 thermal cycler (MJ Research). After 5 min heating at 95°C, 35 cycles were run. Each cycle consisted of 1 min at 95°C, 1 min at 58°C and 2 min at 72°C. This was followed by 4 min at 72°C.

Approximately 300 ng of purified *atpB-rbcL* fragments from the 52 samples included in the cpDNA variation analysis was digested with each of four restriction endonucleases (*Rsa*I, *Taq*I, *Hae*III and *Hin*fI) and separated on 3% agarose gel (Metaphor) with a TBE buffer system. The restriction fragments were analysed using the sequence of *C. arabica* published in the EMBL database (ref.: CHCARBCLA; Manen and Savolainen) as a reference.

Regarding cpDNA inheritance analysis, a preliminary study indicated that the *ndhC-trnV* fragment presents a length mutation between the genotypes IF200 and DH160-02 of *C. canephora*, while the *atpB-rbcL* fragment shows base mutations between *C. arabica* (accessions Et30 and Caturra) and *C. canephora* (clone IF181T) which can be identified by digestion with restriction enzymes. Amplified *atpB-rbcL* fragments were digested with both *Alu*I and *Sau3*A. The *ndhC-trnV* fragments and the *atpB-rbcL* digests were separated by electrophoresis in a 1.5% agarose gel.

Results

RFLP analysis of the cpDNA genome

Hybridisation of the 15 *Sac*I lettuce cpDNA probes to Southern blots for each of the three enzymes revealed a total of 169 different restriction fragments among the 52 accessions analysed. Only fragments longer than 0.4 kb were identified, and 64, 43 and 62 restriction fragments were detected in *Eco*RI, *Eco*RV and *Dra*I digests, respectively. After correcting for the fact that adjacent probes hybridise to many of the same fragments, it was estimated that a total of 112

Table 1 Origin of the accessions analysed for cpDNA variation

Taxa		Accession code	Origin
1	*Coffea arabica* L	ET 12-5	Ethiopia
2		Catura amarillo	Brazil (cultivar)
3		Marsabit/3099	Kenya
4		Marsabit/3058	Kenya
5		hibrido de Timor	Timor island
6	*C. bertrandi* Chev.	Bertrandi	Madagascar
7	*C. brevipes* Hiern	Mount Cameroon	Cameroon
8		Mungo	Cameroon
9	*C. canephora* Pierre	IF 444	Côte-d'Ivoire (cultivar)
10		IF 200	Côte-d'Ivoire (cultivar)
11		IF 155	Côte-d'Ivoire (cultivar)
12		IF A25	Côte-d'Ivoire (cultivar)
13		IF 410	Côte-d'Ivoire (cultivar)
14		IF 160	Côte-d'Ivoire (cultivar)
15	*C. congensis* Froehner	03 255	Central African Rep.
16		03 1650	Congo
17		03 429	Central African Rep.
18		03 103	Central African Rep.
19	*C. costatifructa* Bridson	08 111	Tanzania
20	*C. eugenioides* Moore	04 1485	Kenya
21		04 010	Kenya
22		04 005	Kenya
23	*C. farafanganensis* Leroy	Farafanganensis	Madagascar
24	*C. humblotiana* Baillon	OB 080	Comoro islands
25	*C. humilis* Chev.	07 141	Côte-d'Ivoire
26	*C. kapakata* Chev.	intro. Brazil	Angola
27		intro. Tanzania	Angola
28	*C. liberica* Hiern	pop. Koto/EC 05	Cameroon
29	*C. liberica* var. *dewevrei*	pop. N'Dongue/05 797	Central African Rep.
30	(De Wild. & Th. Dur) Lebrun	pop. Balifondo/05 559	Central African Rep.
31	*C. liberica* var. *liberica*	EA1	Côte-d'Ivoire
32	(Hiern) Lebrun	pop. Taï/05 242	Côte-d'Ivoire
33	*C. millotii* Leroy	Millotii	Madagascar
34	*C. perrieri* Drake	Perrieri	Madagascar
35	*C. pervilleana* Drake	Pervilleana	Madagascar
36	*C. pseudozanguebariae* Bridson	08 228	Tanzania
37		08 021	Kenya
38	*C. racemosa* Lour.	intro. Tanzania	Mozambica
39		intro. Portugal	Mozambica
40	*C. salvatrix* Swynn. & Phil.	intro. Tanzania	Botswana
41		intro. Brazil	Mozambica
42	*C. sessiliflora* Bridson	PA 4	Kenya
43		08 161	Tanzania
44	*C. sp.* Mayombe	Mayombe	Congo
45	*C. sp.* Moloundou	OC 210	Congo
46	*C. sp.* N'gongo II	OC 282	Congo
47	*C. sp.* N'koumbala	OC 105	Cameroon
48	*C. sp. X*	Sp X	unknown
49	*C. stenophylla* Don	FB 1	Côte-d'Ivoire
50		FA 21	Côte-d'Ivoire
51	*Psilanthus ebracteolatus* Hiern	OA 153	Côte-d'Ivoire
52	*P. mannii* Hook. f.	OA 009	Côte-d'Ivoire

restriction sites had been assayed. One-hundred-and-four restriction sites were shared by all 52 accessions while eight sites varied among taxa (Table 2). All variable sites are in the large single copy of the chloroplast genome. Digestion with *Eco*RI revealed significantly more polymorphism (six sites) than the *Eco*RV or *Dra*I digests (one site each).

A heterogeneous profile was detected for coffee species originating in Madagascar using the c6 probe on the *Eco*RI digest (Fig. 2). Incomplete digestion was ruled out since repeated assays produced consistent results for all samples. Differences in intensity of hybridisation signals between profiles were constant across experiments and genotypes.

Table 2 Chloroplast DNA restriction fragment length polymorphisms observed among 52 accessions of coffee species

Character	Enzyme		Probe (position[a])	Nature
Total cpDNA	1	*Eco*RI	c6	Restriction-site mutation
	2	*Eco*RI	c6	Restriction-site mutation
	3	*Eco*RI	c6	Restriction-site mutation
	4	*Eco*RI	c7, c8	Restriction-site mutation
	5	*Eco*RI	c11	Restriction-site mutation
	6	*Eco*RI	c13, c14	Restriction-site mutation
	7	*Eco*RI	c6	Restriction-site mutation
	8	*Dra*I	c13	Restriction-site mutation
atpB-rbcL region	9	*Hae*III	(380)	Restriction-site mutation
	10	–	(418–692)	Insertion/deletion (30 bp)
	11	–	(370–418)	Insertion/deletion (30 bp)
	12	–	(130–370)	Insertion/deletion (10 bp)

[a] Position sites referring to the *atpB-rbcL* sequence of *C. arabica* (CHCARBCLA, EMBL database)

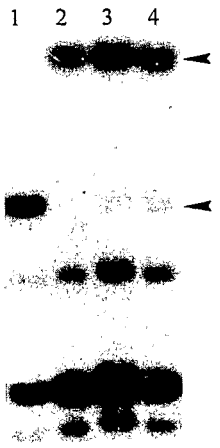

Fig. 2 Autoradiograph showing a heterogeneous profile detected for coffee species originating in Madagascar using a c6 cpDNA probe on an *Eco*RI digest. *Lane 1* is *C. sessiliflora* (42), *lane 2 C. racemosa* (38), *lane 3 C. farafanganensis* (23) and *lane 4 C. millotii* (33). *Arrows* indicate variant fragments

For different accessions, the *Eco*RI single digest was used to sequentially align the various fragments in order to estimate the total length of the coffee cpDNA genome. The length calculated by the summation of the sizes of the different restriction fragments reached 170 kb. Since the set of probes used represent only 95% of the lettuce cpDNA, and as small restriction fragments could be undetected, the size of the total coffee chloroplast genome was estimated as roughly 175 kb. The 112 restriction sites surveyed therefore represent approximately 0.4% of the coffee cpDNA.

RFLP analysis of the *atpB-rbcL* region

PCR was used to amplify *atpB-rbcL* intergenic spacers from the 52 accessions studied. A single band was obtained which varied in length from approximately 930 to 960 bp, depending on the accession. Restriction fragments were analysed following digestion with each of the four restriction enzymes employed (*Rsa*I, *Taq*I, *Hae*III and *Hin*fI). Using the *C. arabica* sequence as a reference, three short insertion/deletions and one restriction-site change were identified and unambiguously located (Table 2).

Chloroplast DNA variation in the genus *Coffea*

Combinations of the eight restriction-site changes revealed by RFLP analysis on the chloroplast genome, and the four variable characters detected on the *atpB-rbcL* intergenic fragments, produced 12 different cpDNA plastome types among the 52 coffee-tree samples analyzed (Table 3). No intraspecific variation was detected within any of the 12 species for which more than one individual tree was studied. The distinct plastome types identified are shared by accessions from either one, two, three or four different species. *Coffea* species belonging to the different plastomes always originate from the same area (West Africa, Central Africa, East Africa or Madagascar), except for the type-B plastome including *C. costatifructa* and *C. kapakata* which are found in East and Central Africa, respectively. Maximum divergence was observed between plastome D, which encompasses species originating from West Africa (*C. brevipes*, *C. canephora*, *C. congensis*) and plastome K, which includes *C. humblotiana* found in the Mascarenes. Plastomes D and K diverged for 5 of the 12 polymorphic characters (four of the eight restriction-site changes on total cpDNA). The two species belonging to the genus *Psilanthus*, *P. ebracteolatus* and *P. mannii*, did not differ from *Coffea* species, and were included in two relatively divergent plastomes.

Inheritance of cpDNA

The amplified *atpB-rbcL* fragments of two interspecific hybrids between *C. arabica*, used as female parent, and *C. canephora* (4x) were analysed (Fig. 3). Only the cpDNA patterns of arabica parents, either Et30 or Caturra, were observed, indicating strictly maternal inheritance. The *ndhC-trnV* fragment phenotypes were determined for 15 individual trees derived from the cross between DH160-02 and IF200 (Fig. 4). In all trees examined, a 1100-bp fragment identical in size to the fragment of the female parent DH160-02 was amplified, suggesting that each hybrid received its chloroplast genome from its maternal parent. In the five doubled haploids derived from the clone IF200

Table 3 Distribution of the species analysed among the different plastomes identified. For each plastome, the state of the 12 polymorphic characters encoded in the coffee chloroplast DNA is indicated

Plastome type	Character states												Taxa
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0	0	0	1	0	0	1	0	0	0	0	0	*C. arabica, C. eugenioides, C. sp.* Moloundou
B	0	0	0	1	0	0	1	0	0	0	0	1	*P. mannii, C. costatifructa, C. kapakata*
C	1	0	0	1	0	0	1	0	0	0	0	1	*C. liberica, C. sp. X*
D	1	0	1	0	0	0	1	0	0	0	0	1	*C. canephora, C. congensis, C. brevipes*
E	0	0	0	1	1	0	1	0	0	0	0	1	*C. stenophylla, C. humilis*
F	0	0	0	1	0	0	1	1	0	0	0	1	*C. sp.* Mayombe, *C. sp.* N'gongo II
G	0	0	1	0	0	0	1	0	0	0	0	1	*C. sp.* N'koumbala
H	0	0	0	1	0	0	0	0	1	0	0	1	*C. pseudozanguebariae*
I	0	1	0	1	0	0	0	0	0	0	0	1	*C. salvatrix, C. sessiliflora*
J	0	0	0	1	0	0	0	0	0	0	1	1	*C. racemosa*
K	0	0[a]	0	1	0	1	1	0	0	1	0	1	*C. humblotiana*
L	0	0[a]	0	1	0	1	1	0	0	0	0	1	*C. millotti, C. farafanganensis, C. pervilleana, C. bertrandi, P. ebracteolatus*

[a] Heterogeneous profile is observed; only the predominant character is taken in consideration

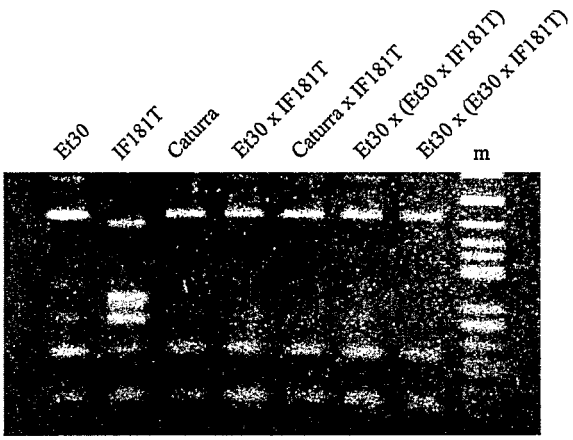

Fig. 3 Electrophoretic pattern of the 960-bp PCR-amplified *atpB-rbcL* fragment from two interspecific hybrids between *C. arabica* (accessions Et30 and Caturra) and *C. canephora* (IF181T) and the parental genotypes, after double digestion with *Alu*I and *Sau*3A

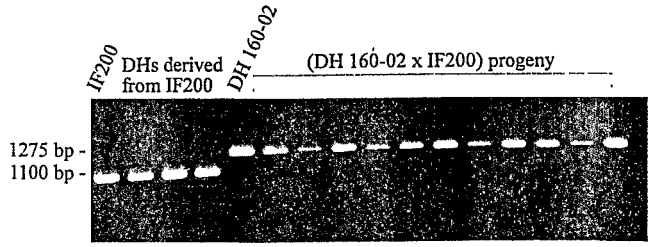

Fig. 4 Electrophoretic pattern of length variation of the PCR-amplified *ndhC-trnV* fragment generated from the clone IF200, doubled haploids derived from IF200, DH160-02, and individual trees resulting from the cross DH160-02×IF200

(Fig. 4), the IF200-specific fragment (1275 bp) was observed as expected.

Discussion

The size of the chloroplast genome of *Coffea* is roughly 175 kb, which is larger than the previous estimate (157 kb) for another species belonging to the Rubiaceae, *Psychotria bacteriophila* (Bremer and Jansen 1991), and the cpDNA size of most land plants (Palmer 1985). An RFLP analysis involving double enzyme digests would be required to construct a physical map of the *Coffea* chloroplast genome. However, single-digest fragments analysis did not show any major structural rearrangements from the other Rubiaceae species analysed using the same set of heterologous probes (Bremer and Jansen 1991). *Coffea* species have the chloroplast genome arrangement typical of most angiosperms examined (Palmer 1985), with one large and one small single copy region (LSC and SSC respectively) separated by an inverted repeat (IR). The large size estimated for coffee seems to be mainly due to the presence of a wide inverted repeat. However, a more accurate estimation of coffee cpDNA size is necessary.

Fifty individual trees representing 25 taxa were assayed to determine the extent and type of cpDNA variation present in the subgenus *Coffea*. Despite a relatively large number of taxa analysed, no intraspecific variation was detected by RFLP analysis on either the total chloroplast genome or the *atpB-rbcL* region, and cpDNA divergence between several species was either reduced or absent. Additional cpDNA analysis involving digestion with numerous restriction enzymes should be used to reveal and study intraspecific variation in coffee species. Another strategy would be to focus cpDNA variation analysis on several

non-coding regions, known to display higher mutational rates than coding regions (Palmer et al. 1988). In this regard, preliminary results obtained with the *ndhC-trnV* fragment in *C. canephora* are promising.

The four site changes observed in the chloroplast genome between the most divergent plastomes (D and K) relative to the 112 restriction sites, representing approximately 672 nucleotides, led to an estimate of maximum overall sequence divergence of 0.6%. Although published studies vary with regard to genome coverage, choice and number of restriction enzymes, sample sizes, and method of evaluation, the amount of cpDNA diversity in *Coffea* can be compared to that in other genera of flowering plants. Genera for which cpDNA sequence diversity has been reported include: *Coreopsis* (0.2%, Crawford et al. 1990), *Tripsacum* (0.36%, Larson and Doebley 1994), *Mitella* (0.5%, Soltis et al. 1990), *Glycine* subg. *Glycine* (1.3%, Doyle et al. 1990), *Gossypium* (2.1%, Wendel and Albert 1992), *Brassica* (2.6%, Palmer et al. 1983), and *Plantago* (3.4%, Wolff and Schaal 1992). Thus, as compared to these genera, *Coffea* exhibits relatively little cpDNA variation.

The few differences observed in this group of *Coffea* chloroplast genomes may be due in part to the relatively long life cycles of coffee-trees, whose generation times have been estimated to be between 20 and 30 years (Berthaud 1986). The result of long life cycles could be a slow accumulation of mutations because of the long "generation time", or reduced nucleotide substitution rates, as has been observed in palms (Wilson et al. 1990) and in *Viguiera* (Schilling and Jansen 1989). In addition, if one assumes that chloroplast genomes evolve at roughly equal rates in all flowering plants, then the low level of cpDNA variation in *Coffea* could suggest that it is a relatively young genus. This interpretation seems reasonable since *Coffea* species hybridise readily with one another and produce relatively fertile hybrids (Charrier 1978; Louarn 1993). Furthermore, the lack of consistent cpDNA variation between the *Coffea* species and the two species, *P. ebracteolatus* and *P. mannii*, of the genus *Psilanthus* does not match the adopted classification into two genera (Leroy 1980; Bridson 1987). Although further study is needed, this result might prompt modifications in the relationship between the two genera.

The inheritance of cpDNA was observed in interspecific hybrids between *C. arabica* and *C. canephora*, and in an intraspecific progeny of *C. canephora*. Both studies showed exclusively maternal inheritance of cpDNA, suggesting that the mode of plastid inheritance in *Coffea* is strictly maternal.

The origin of the heterogeneous profile detected in coffee species from Madagascar remains unclear. The difference in intensity of hybridisation signals between the two profiles was constant and most likely reflects variation in copy number within accessions. This heterogeneity could be interpreted as a consequence of heteroplasmy. However, cpDNA heteroplasmy in plants is more often reported as a result of exceptional biparental cpDNA transmission in crosses between individuals with distinct chloroplast genomes (Lee et al. 1988; Chong et al. 1994; Mason et al. 1994). In the present study, a unique heterogeneous pattern was observed across different species. Other explanations, such as cross homologies between cpDNA and both nuclear and mitochondrial genomes (Timmis and Scott 1983; Stern and Palmer 1984; Sederoff et al. 1986), could be advanced. However, further studies involving isolated cpDNA are required before it can be asserted that such phenomena occur in coffee.

In conclusion, the present results reaffirm the utility of cpDNA variation in systematic studies at the intraspecific level (reviewed in Soltis et al. 1992). However, the low cpDNA variation in *Coffea* requires a study of the most variable regions, such as the intron and intergenic spacer (Gielly and Taberlet 1994), in order to extend the level of resolution of cpDNA. The determination of chloroplast DNA inheritance in coffee-trees was a pre-requisite for use of the chloroplast molecule in tracing the evolutionary history of *Coffea* species. Restriction analysis of PCR-amplified intergenic fragments of chloroplast DNA appeared to be very powerful in examining cpDNA transmission. The use of PCR guarantees high sensitivity and allows the analysis of large sample sets. Such markers could be very useful in further studies on cpDNA gene flow and interspecific hybridisation in natural populations.

Acknowledgements This work was supported in part by the European Community through the International Scientific Co-operation Programme (Contract CI1-CT91-0899). The authors thanks Dr. R. K. Jansen for providing the cloned cpDNA probes from lettuce.

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