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Strict paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in intraspecific crosses of kiwifruit

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Abstract Previous studies have established that chloroplasts are inherited paternally in *Actinidia* interspecific crosses. However, fertilisation problems in interspecific crosses may affect the transmission of organelles. Six female clones, i.e. 'Abbott', 'Bruno', 'Greensill', 'Hayward', 'Jones', 'Monty', and four male clones were used to identify cpDNA polymorphisms within the cultivated kiwifruit species *A. deliciosa*. The restriction patterns by *Hpa*II of a chloroplast fragment amplified by PCR with a pair of universal primers revealed a polymorphism at the intraspecific level. The inheritance of cpDNA in 143 seedlings from three intraspecific crosses in kiwifruit (*Actinidia deliciosa*) was studied. All offspring displayed the restriction pattern of the paternal parent, indicating that maternal inheritance of cpDNA in kiwifruit is rare at best. Strict maternal inheritance of mtDNA was confirmed in the same crosses used to investigate cpDNA transmission. Studies of cytoplasmic inheritance in the *Actinidia* genus represent to date the best documented report of differential organelle inheritance of cpDNA and mtDNA in angiosperms.

Key words *Actinidia deliciosa* · cpDNA · mtDNA · PCR · Universal primers

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Introduction

The *Actinidia* genus, native to China (Li 1952), consists of more than 50 species and forms a polyploid series with the basic chromosome number of 29 (Jie and Beuzenberg 1983; Yan et al. 1997). All members of the *Actinidia* genus are perennial climbing or straggling plants and all appear to be functionally dioecious (McNeilage 1991). Pollen is transferred from male to female flowers both by wind and by insects (Costa et al. 1993). Botanical literature is very confusing on a number of points, and the systematic position of the genus *Actinidia* has not been completely resolved until now. The *Actinidia* genus seems to be closely related to the genera *Clematoclethra* and *Saurauia*, with all three generally considered as constituting the family Actinidiaceae, itself included in the order Theales (Dunn 1911). The other conflicting point concerns the name of the cultivated species. Kiwifruit was originally classified as a variety of *Actinidia chinensis*, i.e. var 'hispida', but differences in morphological traits as well as in chromosome number have recently led Liang and Ferguson (1984) to consider the existence of two distinct species: *A. chinensis* (mainly $2n = 2x = 58$) and *A. deliciosa* ($2n = 6x = 174$). At present, they are the only two *Actinidia* species of economic importance.

The kiwifruit industry is a recent culture based on a single female cultivar of *Actinidia deliciosa*, the 'Hayward' variety. This cultivar was selected in New Zealand in 1930 following one or two generations of breeding following a first introduction of seeds from China in 1904 (Ferguson and Bollard 1990). 'Hayward' is the only cultivar grown to a significant extent both in New Zealand and throughout the world. The reason for this predominance is that 'Hayward' produces fruits of a good size, shape and flavour that can be stored for long periods of time. Nevertheless, breeding programmes have been undertaken to create new cultivars in New Zealand, and to a lesser extent in other producing countries.

In China, fruits of *A. chinensis* were first simply collected from the wild, to be sold locally or for home consumption (Ferguson 1990). Recent research and experimental efforts in China have been directed towards selecting high-quality plants within *A. chinensis* (Ch'ang 1982). An attempt to preserve part of the existing natural resources has also been undertaken at local and national levels (Huang et al. 1997).

An exact knowledge of the genetic diversity of the genus *Actinidia* is required, not only for the development of efficient conservation strategies but also for the design of breeding programmes. Molecular analyses of cytoplasmic DNA are useful tools for evaluating diversity and phylogeny among *Actinidia* species. The determination of chloroplast and mitochondria inheritance is a pre-requisite for the use of organelle DNA molecules in tracing the evolutionary history of plant species. In the past, precise information on organelle genome inheritance in plants has been limited to a few species due to the lack of phenotypic markers. More recently, the development of molecular markers has been of great utility for investigating organelle genome polymorphism and inheritance (Reboud and Zeyl 1994), particularly in species with unknown plastid mutants. With a plastid mutation such as chlorophyll deficiency, a large number of plants can be observed, thereby increasing the chance to detect rare transmission events (Ohba et al. 1971; Hagemann 1992). On the other hand, the use of such phenotypic markers can induce a bias in favour of the wild-type allele, as has been established in *Pelargonium* by Tilney-Bassett and Birky (1981).

Analyses of cytoplasmic transmission within the *Actinidia* genus have been recently assessed. Surprisingly, the independent inheritance of chloroplast and mitochondria has been observed within the genus *Actinidia*: chloroplast and mitochondria appear to be paternally and maternally inherited, respectively (Cipriani et al. 1995; Testolin and Cipriani 1997). In gymnosperms, following the early discovery of Ohba et al. (1971) of the predominantly paternal inheritance of chloroplasts in *Cryptomeria japonica*, there have been many other reports of the paternal inheritance of chloroplasts. But to our knowledge, *Actinidia* is the only genus of angiosperms where a purely paternal mode of plastid inheritance has been reported. Considering the importance of the finding of Testolin and Cipriani with respect to plastid genetics in angiosperms, it seemed desirable to confirm this unusual result in other *Actinidia* crosses and to increase the overall sample size.

Cipriani et al. (1995) investigated the mode of inheritance of mitochondria in the genus *Actinidia* in both interspecific and intraspecific controlled crosses. On the other hand, Testolin and Cipriani (1997) determined the mode of inheritance of chloroplasts only for interspecific hybrids, as it seemed difficult to detect intraspecific polymorphism among the *A. deliciosa* clones. The study presented here was undertaken to confirm the paternal inheritance of chloroplast DNA (cpDNA) within the *A. deliciosa* cultivated species. We therefore analysed four intraspecific crosses of *A. deliciosa* involving a total of 150 offspring using polymerase chain reaction (PCR) amplification of non-coding chloroplast regions and subsequent restriction fragment analysis. The same crosses were used to confirm the maternal inheritance of mitochondrial DNA (mtDNA).

Materials and methods

Parental plants

The present study involved several female and male clones, all belonging to the species *A. deliciosa*. Six female clones selected in New Zealand and introduced into France (INRA Bordeaux) from New Zealand in 1970 were evaluated, i.e. 'Abbott', 'Bruno', 'Greensill', 'Hayward', 'Jones' and 'Monty' (for precise descriptions, see Jie and Thorp 1986). Four male clones, expected to be different from each other, are referred to as M1, M2, M3 and M4 in the text. M1 is the Italian rootstock selection 'D uno', and M2 is a New Zealander pollinator selection named 'Tomuri' (Chalak and Legave 1997). Two other unnamed male clones, M3 and M4, were used.

Sexual crosses

Dioecy is a general although not absolute feature in the genus *Actinidia* (Ferguson 1990). Female plants have pistillate flowers with sterile pollen grains, and male plants have staminate flowers with rudimentary pistils (Polito and Grant 1984). The only cases of hermaphrodism reported until now in *A. deliciosa* species have been the isolated occurrence of male plants used as pollinators in the orchards and bearing small fruits (McNeilage 1991). Hermaphrodite flowers have never been reported in *A. deliciosa* female cultivars. Thus self-fertilisation is prevented, and there is no need of emasculation in crossing experiments. The controlled crosses were performed at the INRA research station of Bordeaux and San Giuliano (France) in 1990 and 1992, respectively. Before flowering, both male and female flowers were bagged to exclude pollen transport by honeybees or wind. At male anther dehiscence, pollination was achieved by brushing the stigma of the female flowers with the anthers of the male flowers.

DNA extraction

Young leaves or buds were harvested in the spring and immediately frozen. Total genomic DNA was extracted and purified according to a modified CTAB procedure (Saghai-Maroof et al. 1984). Frozen tissue was ground into powder with a mechanical mill, incubated in tubes containing 9 ml of extraction buffer (100 m*M* TRIS pH 8.0, 1.4 *M* NaCl, 20 m*M* EDTA, 2% hexadecyltrimethylammonium bromide, 0.1% 2-mercaptoethanol) and agitated gently at 60°C for 60 min. The tubes were cooled before adding 4 ml of chloroform/octanol, 24 : 1 (v/v). After centrifugation at 5000 *g* for 10 min, the upper aqueous phase was treated with 2.5 U of ribonucleases A for 30 min. The final DNA stock solution was stored at − 20°C and diluted 10–100 times before use.

Restriction fragment length polymorphism (RFLP) of organelle genome

The template for PCR amplification consisted of 4 ng of genomic DNA. The reaction buffer (25 µl) contained 75 m*M* TRIS-HCl,

Table 1 Description of the primers and restriction enzymes used in this study

Primer 1	Primer 2	References	Abbreviation
Chloroplast primers			
*psb*C [psII 44-kDa protein]	*trn*S [tRNA-Ser (UGA)]	Demesure et al. 1995	CS
*trn*D [tRNA-Asp (GUC)]	*trn*T [tRNA-Thr (GGU)]	Demesure et al. 1995	DT
*trn*T [tRNA-Thr (GGU)]	*psb*C [psII 44-kDa protein]	Dumolin-Lapègue et al. 1997	TC
Mitochondrial primers			
*nad*1 exon B	*nad*1 exon C	Demesure et al. 1995	*nad*1-B/C

1.8 m*M* $MgCl_2$, 5 µg of BSA, 20 m*M* $(NH_4)_2SO_4$, 0.01% (w/v) Tween 20, 0.1 m*M* of each of the four dNTP, 0.2 µ*M* of each primer and 0.4 U of *Taq* polymerase (Goldstar, Eurogentec). A description of the primers is given in Table 1. These pairs of primers have been chosen because they successfully amplified cytoplasmic DNA of a wide range of plant species belonging to angiosperms and gymnosperms (Demesure et al. 1995). The amplification was carried out in a 96-well Perkin Elmer GeneAmp PCR System 9600 (details of the amplification conditions are given in Demesure et al. 1995 and Dumolin-Lapègue et al. 1997). The amplified fragments were digested by a single restriction enzyme (*Hpa*II or *Hin*fI) according to the manufacturer's recommendation. The restriction fragments were separated by electrophoresis on 8% polyacrylamide gels in a 1 × TBE buffer, stained in ethidium bromide and visualised by UV light. The 1-kb ladder of GibcoBRL (Life Technologies) was used as the molecular-weight marker.

Random amplified polymorphic DNA (RAPD)

The arbitrary sequence 10-mer primers used were obtained from Operon Technologies (Almeda, Calif.). The primer finally chosen for parentage analysis was OPQ-09 (5′-GGCTAACCGA-3′). The PCR reaction mixture (18 µl) contained 20 m*M* TRIS-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 3.6 µg of BSA, 100 µ*M* each of dATP, dTTP dCTP, and dGTP, 0.7 U of *Taq* DNA Polymerase (Life Technologies™), 1 ng of primer and 30 ng of template DNA. PCR was performed in the same DNA thermocycler as above using 1 cycle of 5 min at 95°C; 45 cycles of 10 s at 95°C, 15 s at 37°C, 2 min at 72°C; and 1 cycle of 4 min at 72°C. The fragments generated by amplification were separated according to size on 1.5% agarose gels in a 1 × TAE buffer, stained and visualised in the same way as the polyacrylamide gels.

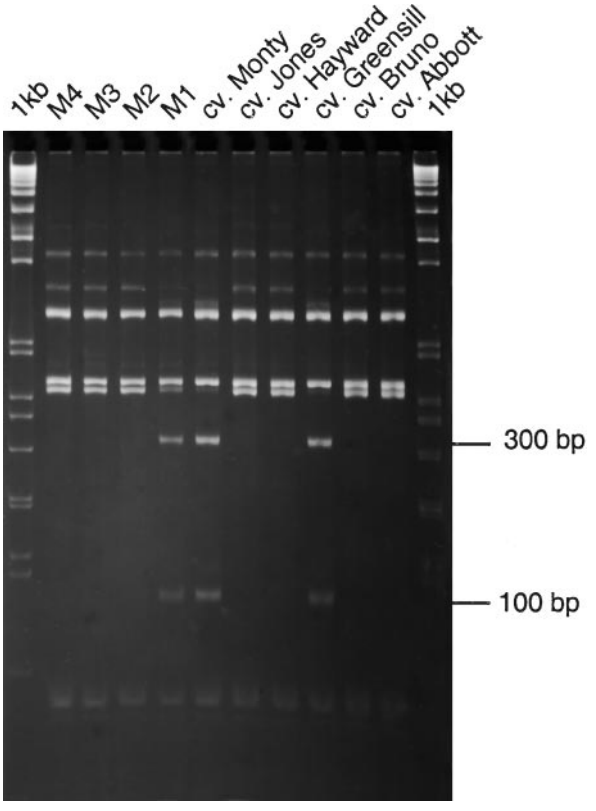

Fig. 1 Chloroplast DNA polymorphism detected at the intraspecific level with CS primers and the *Hpa*II restriction enzyme

Results

Organelle polymorphism within *A. deliciosa*

Polymorphic patterns for mtDNA were revealed among *A. deliciosa* clones using the *nad*1-B/C-*Hin*fI combination (data not shown). This polymorphism appears to be due to an insertion/deletion event estimated to be 30 bp in size. The male clone M1 and all the female cultivars could be distinguished from the remaining three male clones. Polymorphism for cpDNA was detected among *A. deliciosa* clones using the CS-*Hpa*II combination. 'Abbott', 'Bruno', 'Hayward', 'Jones', M2, M3 and M4 showed the same haplotype, which differed from that of 'Greensill', 'Monty' and M1 (Fig. 1). This polymorphism seems to be due to an extra *Hpa*II recognition site.

These intraspecific restriction fragment length polymorphisms provided markers for distinguishing the parental cpDNA and mtDNA haplotypes and were used to demonstrate the mode of organelle inheritance. Among all the potentially informative crosses within *A. deliciosa*, only four crosses were available at the INRA Institute, i.e. 'Greensill' × M3, 'Greensill' × M4, 'Hayward' × M1 and 'Hayward' × M2. Interestingly, two crosses among the four were informative with respect to both mitochondria and chloroplast inheritance (Tables 2 and 3).

Table 2 Restriction pattern of mtDNA revealed by *nad*1-B/C primers and the *Hin*fI restriction enzyme among the six parents of the controlled crosses and subsequently used for determining the mode of mtDNA inheritance

Cross	Parents				Progeny		
	Amplified fragment (in bp)		Polymorphic restriction fragments (in bp)		Restriction pattern		
	Female	Male	Female	Male	Maternal	Paternal	Biparental
Greensill × M3	1676	1706	530	560	40	0	0
Greensill × M4	1676	1706	530	560	61	0	0
Hayward × M1	1676	1676	530	530	–	–	–
Hayward × M2	1676	1706	530	560	7	0	0
				Total	108	0	0

Table 3 Restriction pattern of cpDNA revealed by CS primers and the *Hpa*II restriction enzyme among the six parents of the controlled crosses and subsequently used for determining the mode of cpDNA inheritance

Cross	Parents			Progeny		
	Amplified fragment (in bp)	Chloroplastic polymorphic restriction fragments (in bp)		Restriction pattern		
		Female	Male	Maternal	Paternal	Biparental
Greensill × M3	1600	300 + 100	400	0	40	0
Greensill × M4	1600	300 + 100	400	0	61	0
Hayward × M1	1600	400	300 + 100	0	42	0
Hayward × M2	1600	400	400	–	–	–
			Total	0	143	0

Maternal inheritance of mtDNA, in intraspecific crosses of *A. deliciosa*

The informative crosses and the corresponding diagnostic fragments are summarised in Table 2. As seen in Fig. 2, *Hin*fI digests produced a 530-bp band unique to the female clone 'Greensill' and a 560-bp band unique to the male clone M3. That polymorphism allowed the study of mtDNA inheritance in 108 offspring from three crosses. Only the mtDNA patterns of the female clones were observed among the 108 offspring (Fig. 2 and Table 2).

Paternal inheritance of cpDNA in intraspecific crosses of *A. deliciosa*

The polymorphic restriction patterns between the parents are given in Table 3 for the CS-*Hpa*II combination. This polymorphism seems to be due to an extra *Hpa*II recognition site in the female clone 'Greensill' and the male clone M4 that results in a 400-bp fragment being cleaved into a 300-bp and a 100-bp fragment (Fig. 3). As indicated in Fig. 3 and Table 3, all 143 offspring from the three informative crosses exhibited the paternal pattern.

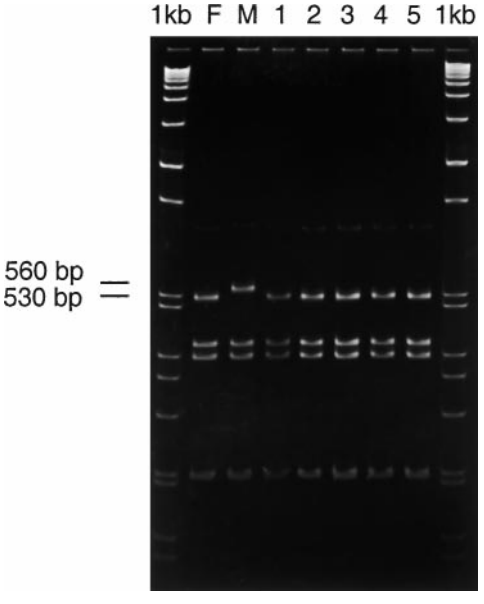

Fig. 2 Maternal inheritance of mtDNA in a progeny from the cross 'Greensill' × M3. The amplified fragment obtained using *nad*1-B/C primers was digested by the *Hin*fI restriction enzyme. The male parent M3 (*M*) differs from the female parent 'Greensill' (*F*) and the progeny (*lanes 1–5*) by a deletion of 30 bp in the first restriction fragment. *1kb* Molecular-weight marker

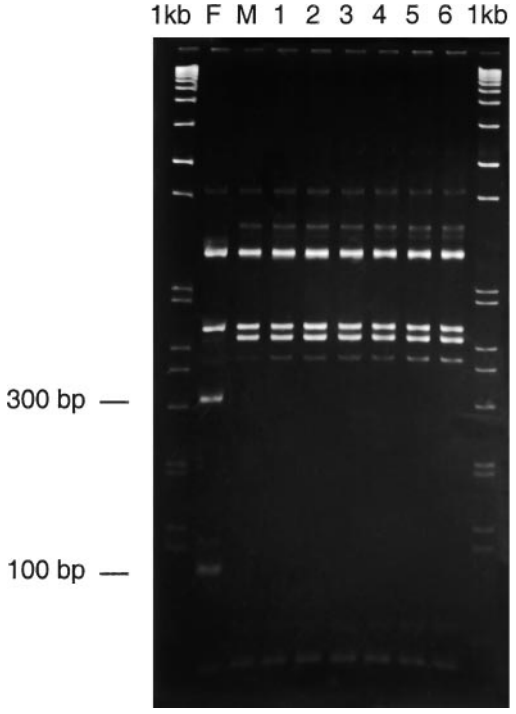

Fig. 3 Paternal inheritance of cpDND in a progeny from the cross 'Greensill' × M4. A 1600-bp cpDNA fragment was amplified by PCR with the CS primers and digested by the *Hpa*II restriction enzyme. The female parent 'Greensill' (*F*) differs from the male parent M4 (*M*) and the progeny (*lanes 1–6*) by two additional bands, 100 bp and 300 bp, expected to result from an extra *Hpa*II restriction site. *1kb* Molecular-weight marker

Occurrence of pollen contamination

Evidence of pollen contamination was detected among the 'Hayward' × M2 non-informative cross using the CS-*Hpa*II combination. Of the 7 offspring analysed, 1 exhibited an unexpected chloroplast pattern of neither paternal nor maternal origin.

RAPD primers were used to amplify total DNA from the three clones used as parents in Corsica, i.e. 'Hayward', M1 and M2, in an attempt to identify which male clone was the parent of the aberrant offspring. Among the 20 primers tested, 1 (OPQ-09) detected clear polymorphisms distinguishing the three parents and was subsequently chosen to conduct a parentage test in both 'Hayward' × M1 and 'Hayward' × M2 progeny. The average length of the amplified bands was calculated to be 1310 bp for the M1-specific major band and 500 bp for the M2-specific major band (Fig. 4). Each RAPD marker segregated in the respective progeny, indicating that the two loci amplified were of nuclear origin. The questionable seedling of the cross 'Hayward' × M2 exhibited the M1-specific major band but not that of M2. On the contrary, all the other offspring patterns were consistent with the respective pedigree. Since the unexpected offspring had also the same cpDNA type as the male M1, contamination by pollen from this clone must have taken place. The occurrence of pollen contamination during artificial pollination may be explained by the fact that the controlled crosses involving the paternal clones M1 and M2 were made the same day by the same person in the same orchard.

Discussion

We studied the inheritance of chloroplast and mitochondrial genomes in *A. deliciosa* using restriction polymorphisms of PCR-amplified cpDNA and mtDNA fragments. The use of PCR to detect intraspecific organelle DNA polymorphisms and to investigate their mode of inheritance in plants is now possible thanks to the availability of 'universal primers' (Demesure et al. 1995; Taberlet et al. 1991). 'Universal primers' have been designed to match conserved sequences, such as tRNA genes or DNA coding regions, and then amplify mostly DNA noncoding regions. Such noncoding regions of organelle DNA are expected to evolve more rapidly than coding regions and thus to reveal more polymorphism. Genetic analysis using PCR amplifications is fast and reliable and requires very small amounts of DNA. The strict uniparental inheritance pattern observed in our study confirms that none of the DNA fragments amplified was of nuclear origin.

Two pairs of primers were used, the first one to investigate the mode of inheritance of cpDNA in *A. deliciosa*, the second one to confirm the previous finding of maternal inheritance of mtDNA previously pointed out in 32 *A. deliciosa* offspring from two crosses (Testolin and Cipriani 1997). For cpDNA, the CS pair of primers selected amplifies a DNA region between the protein gene *ps*II and the tRNA-Ser(UGA) gene (Demesure et al. 1995). The restriction pattern for the CS fragment with the *Hpa*II enzyme revealed an informative polymorphism at the intraspecific level. To our knowledge, this is the first time that intraspecific cpDNA polymorphism has been reported within the *A. deliciosa* species. For mtDNA, the pair of primers selected amplifies an intron of the *nad*1 gene located between exon B and C (Demesure et al. 1995). Testolin and Cipriani (1997) have already reported a mtDNA polymorphism within the amplified DNA fragment *nad*1 at this taxonomic level.

Paternal inheritance of cpDNA in the *Actinidia* genus had been demonstrated previous to this study using interspecific hybrids (Cipriani et al. 1995; Testolin and Cipriani 1997). However, an abnormal nuclear background may affect the inheritance of the organelles, as has been demonstrated in *Festuca* × *Lolium* intergeneric hybrids by Kiang et al. (1994). In the present study, with the exception of 1 offspring probably sired by contaminating pollen, the restriction pattern of

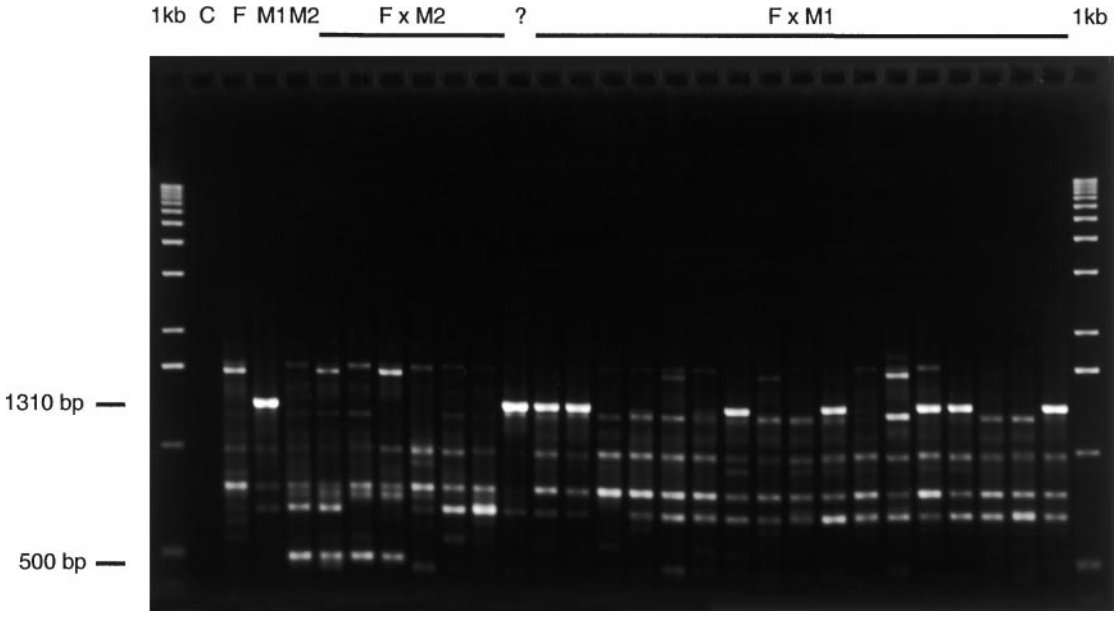

Fig. 4 RAPDs produced with primer OPQ-09 from DNA of the female parent 'Hayward' (*F*), the male parents (*M1* and *M2*), the controlled hybrids ($F \times M1$ and $F \times M2$) and the questionable hybrid (*?*). The PCR product bands referred to in the text are indicated with the approximate base pair lengths. *1kb* Molecular-weight marker, *C* control PCR without template DNA

PCR-amplified cpDNA or mtDNA fragments revealed a unique pattern among all the *A. deliciosa* offspring from a given cross. Our data confirm the predominantly if not strictly maternal transmission of mitochondria previously observed within *A. deliciosa* by Testolin and Cipriani (1997) and provide evidence for a predominantly if not strictly paternal transmission of the chloroplast at the same taxonomic level. In the present study, the mode of inheritance of mtDNA and cpDNA was established from 143 and 108 offspring respectively, each involving three intraspecific crosses in kiwifruit.

Organelle inheritance studies are often documented by the study of progeny derived from controlled crosses. Here, as in many other reports, all progeny appear to inherit a particular organelle type from the same parent. Due to the restricted number of offspring that can be studied, rare cases of leakage from the other parent may easily be overlooked. For instance, a drug resistance marker has been used to show that very rare biparental chloroplast inheritance does occur in *Nicotiana* (Medgyesy et al. 1986), a taxa which was previously considered to have exclusively uniparental-maternal chloroplast inheritance (Maliga et al. 1975; Tilney-Bassett 1978). In conifers, although a largely uniparental-paternal mode of inheritance has been confirmed for cpDNA in many species, occasional offspring with maternal or biparental cpDNA genotypes have been observed, for example in *Pinus banksiana* (Wagner et al. 1989) and *Calocedrus decurrens* (Neale et al. 1991).

Because rare cases of biparental transmission can have disproportionate evolutionary importance (Dumolin-Lapègue et al. 1998), it is important to distinguish between two alternative modes of organelle inheritance: a strict uniparental inheritance, in which the organelle is always derived from the same parent, versus a predominantly uniparental inheritance in which the organelle is occasionally inherited from the other parent. We used the binomial model to evaluate the maximum rate of transmission (P) from the other parent (Milligan 1992) by the following formula:

$$P = 1 - (1 - \beta)^{1/N}$$

where N is the total number of progeny, and β the power of the test.

For this study, we conservatively selected a low probability ($1 - \beta = 0.01$) of falsely accepting the strict uniparental inheritance hypothesis. Considering the absence of maternal cpDNA types among the N = 143 offspring analysed, the maximal rate of maternal transmission of cpDNA that may have been overlooked with these sample sizes is 3.2% (Fig. 5). If we take into account the previous results of Cipriani et al. (1995) and Testolin and Cipriani (1997), based on 56 and 63 interspecific hybrids, respectively, the total number of offspring examined for cpDNA inheritance within the *Actinidia* genus reaches 262, and the corresponding rate drops to 1.7% (Table 4). For levels of 0.5% and 1% of maternal chloroplast leakage, the probabilities of observing at least 1 offspring containing maternally derived chloroplasts in an array of 262 offspring are 73% and 93%, respectively, and the

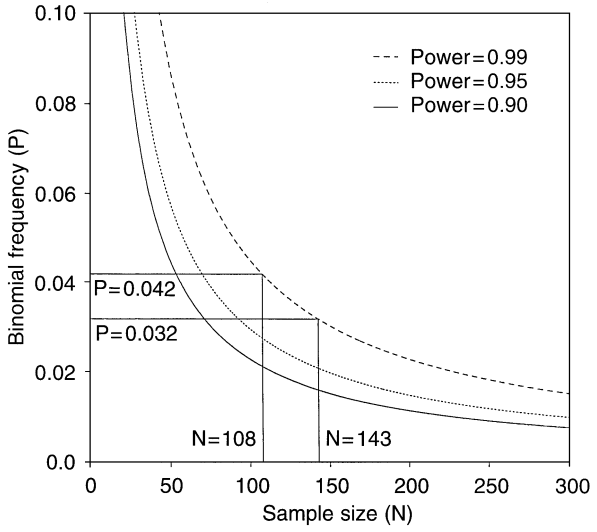

Fig. 5 Relationship between probability of atypical events of organelle transmission (P) and size of the progeny examined (N) for three different powers. This binomial model corresponds to the case where no progeny containing maternally derived chloroplasts or paternally derived mitochondria are found in the progeny sample

true rate of maternal transmission of chloroplasts is therefore probably much lower than 1.7%.

Similarly, the absence of plants characterised by paternal mtDNA types among the N = 108 offspring observed indicates that the true rate of paternal inheritance is lower than 4.2% for the same power of 0.99 (Fig. 5). If we take into account the previous results of Testolin and Cipriani (1997), based on 32 and 102 interspecific and intraspecific hybrids, respectively, the total number of individuals examined within the *Actinidia* genus reaches 242, and the corresponding maximum probability of paternal transmission drops to 1.9% (Table 4).

Altogether, the results provide good evidence for a strong bias towards maternal transmission of mtDNA and paternal transmission of cpDNA within *A. deliciosa*. These results do not agree with the commonly observed uniparental-maternal inheritance of both chloroplast and mitochondrial genomes in angiosperms. Several studies had revealed exceptions to the uniparental-maternal pattern among angiosperms, especially for cpDNA. This genome has been shown to be inherited biparentally, at a high frequency, as in alfalfa (Masoud et al. 1990; Smith 1989) and in *Pelargonium* (Tilney-Bassett and Birky 1981), or at a low frequency, as in *Petunia* (Derepas and Dulieu 1992) and in *Nicotiana* (Medgyesy et al. 1986). But the only case of uniparental-paternal inheritance reported was in the genus *Daucus* (Boblenz et al. 1990), and this was invalidated by Steinborn et al. (1995). With respect to mtDNA, uniparental-paternal inheritance was reported in bananas by Fauré et al. (1994) and in melon by Havey et al. (1998) using 34 and 38 intraspecific hybrids, respectively. This study on kiwifruit represents therefore the most thorough investigation of a case of strict uniparental-paternal inheritance of organelles in angiosperms. The experimental design used in all the chloroplast inheritance studies on kiwifruit involved three intraspecific and eight interspecific crosses and includes 119 intraspecific and 143 interspecific hybrids.

From a cytological point of view, the paternal chloroplast inheritance described in this paper requires the presence of chloroplasts within the pollen grains of *A. deliciosa*. To our knowledge, the only reports on organelle content in pollen grains of the genus *Actinidia* are those of Messina (1993) and Matsunaga et al. (1996). Chloroplasts, as well as mitochondria, are visible in microspore of *A. deliciosa* under transmission electron microscopy (Messina 1993). The cytological observations made by Matsunaga et al. (1996) revealed the presence of large and small fluorescent spots after DAPI staining in the generative cell of the male-derived pollen grain, Consequently, these authors hypothesised

Table 4 Summary of organelle inheritance studies conducted within *Actinidia* genus based upon molecular evidence

Mode of organelle inheritance	Number of crosses (number of progeny) analysed			Total number of progeny	P[a] (%)
	Cipriani et al. (1995)	Testolin and Cipriani (1997)	Present study		
Maternally inherited mitochondria:					
Interspecific crosses	–	4 (102)	–	102	
Intraspecific crosses	–	2 (32)	3 (108)	140	
				242	1.9%
Paternally inherited chloroplast:					
Interspecific crosses	4 (63)	4 (56)	–	119	
Intraspecific crosses	–	–	3 (143)	143	
				262	1.7%

[a] Maximum degree of paternal mitochondrial transmission and maternal chloroplast transmission on the basis of the binomial model curve illustrated in Fig. 5 with a power $\beta = 0.99$

the presence of chloroplasts and mitochondria in the generative cell. The presence of chloroplast nuclei within the generative cell of the male-derived pollen grain of *A. deliciosa* supports the paternal inheritance of the chloroplast previously described. On the other hand, the presence of mitochondria suggest the existence of two separate mechanisms of organelle selection leading to the elimination of paternal mitochondria and the transmission of paternal chloroplast. The mechanisms involved would act during the final stages of pollen development, fertilisation or zygote development. Further experiments have to be performed to find out the mechanisms of paternal chloroplast transmission and maternal mitochondria elimination within the genus *Actinidia*.

Finally, the evolutionary implications of this unusual and contrasted inheritance for the two organelles in this dioecious species should also be investigated. Indeed, among land plants, only conifers, which are usually hermaphrodite species, share this particular mode of inheritance (Birky 1995).

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