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Restriction-site variation of PCR-amplified chloroplast DNA regions and its implication for the evolution and taxonomy of *Actinidia*

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Abstract Twenty six restriction sites from five PCR-amplified chloroplast DNA sequences (*rbcL*, *psbA*, *rpoB*, and two spacers flanking the *trnL* gene) were mapped and analysed in 20 *Actinidia* taxa, encompassing all four sections into which the genus is divided. At least three species out of the 20 examined have been found to have originated through natural interspecific hybridisation on the basis of the discrepancy between morphological and biochemical traits and the cpDNA profiles of pairs of species. A widely reticulate evolution has therefore been postulated in *Actinidia*. Wagner and weighted parsimony analysis produced consensus trees that did not match the traditional taxonomy based on morphological characters. The molecular data clearly showed that some taxa, such as *A. rufa* and *A. kolomikta*, occupy a wrong position and most, if not all, of the traditional groups represented by sections and series are weakly supported, since they appear as polyphyletic. *A. chinensis* and *A. deliciosa* were confirmed to be very closely related. Since chloroplast DNA is paternally inherited in *Actinidia*, *A. chinensis* is a paternal progenitor, if not the only one, of *A. deliciosa*, the domesticated kiwifruit.

Key words *Actinidia* · Kiwifruit · Chloroplast DNA · Molecular taxonomy · Parsimony analysis · Phylogeny

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Introduction

Interest in the genus *Actinidia* has increased following the economic exploitation of *A. deliciosa* (A. Chev.) C. -F. Liang et A. R. Ferguson, the kiwifruit, which took place 40 yrs ago in New Zealand.

The genus *Actinidia* belongs to the family *Actinidiaceae*, closely related to the *Theaceae*, two families whose upstream lineage is still controversial (Chase et al. 1993; Cosner et al. 1994). The genus contains more than 60 species and a number of infraspecific taxa that are grouped into four sections on the basis of the type and degree of tomentum and the presence/absence of lenticels on the fruit surface (Liang 1984). Most species occur in the wild only in China, with only a few spreading to the border countries (Ferguson 1990). The genus did not receive much attention until its recent domestication. A number of taxa, which were classified in the past century, are represented solely by specimens in herbaria and have not been found in the wild. The description of some taxa is from a limited number of genotypes and, in a few cases, on only one sex (Liang 1984). The taxonomy of *Actinidia* is still based on morphological characters. The extensive variation of such characters, which occur within wide-spread taxa in areas with largely different climatic conditions, together with the frequent interspecific hybridisation which most likely occurs in the wild, has led to the frequent misidentification of species (Ferguson 1990). As a consequence, the systematics of the genus is far from well established and the inconsistency of the position of some taxa became evident when molecular approaches to the systematics of the genus were developed (Crowhurst et al. 1990; Cipriani and Morgante 1993; Webby et al. 1994, Testolin and Ferguson 1997).

Among the possible molecular approaches, the analysis of chloroplast DNA (cpDNA) has received a great deal of attention because of its informativeness over a wide range of taxonomic levels (Palmer et al. 1988; Crawford 1990; Soltis et al. 1992). Due to its

uniparental inheritance, which excludes any recombination, and the low presence of tandem repeats (Sugiura 1992; Powell et al. 1995), cpDNA is highly conserved since it has a lower mutation rate than other cell genomes (Wolfe et al. 1987). Although intraspecific cpDNA variation is far from rare (Harris and Ingram 1991; Soltis et al. 1992), a number of cpDNA regions have been widely reported to be invariant at restriction sites, if not at sequence analysis, within hierarchically low taxa such as species or geographically isolated populations. One of the most widely studied regions of the chloroplast genome is the *rbcL* gene, which encodes the large subunit of ribulose 1,5 biphosphate carboxilase/oxygenase (RUBISCO). Using exclusively *rbcL* sequence data, Chase et al. (1993) explored the phylogenetic relationships of nearly 500 taxa encompassing roughly 265 families of seed plants. Besides *rbcL*, other cpDNA genes, such as *psbA* or *rbcS*, have been found suitable for the same purpose and for the same reasons. Highly conserved genes have some disadvantages. The *rbcL* gene has been found to change too slowly to provide enough mutations for lower-level separation within some families (Steele and Vilgalys 1994) and this led to a search for other useful DNA regions. Regions that evolve faster than *rbcL* and have demonstrated pylogenetic potential are the spacers, which are largely interspersed in the chloroplast genome and for which universal primers can be designed in the flanking coding regions, some open reading frames (ORF) (Steele and Vilgalys 1994), the cpDNA inverted repeat (Downie and Palmer 1992), and the coding regions for the ribosomal RNA molecules (rDNA) and the non-transcribed spacer region (NTS) of the rDNA in the nuclear genome (Clegg and Durbin 1990).

We have previously examined three well conserved regions (*rbcL*, *psbA* and *rpoB*) and two spacers flanking the *trnL* gene and have found that all these regions can be amplified in four species of the genus *Actinidia* by means of primers designed by comparing sequences from other species; we also found that *rbcL* and *psbA* had a low number of mutated restriction sites whereas the spacers were very polymorphic (Cipriani and Morgante 1993). A mixture of regions that evolve at different rates thus appeared a suitable tool for phylogenetic reconstruction within the genus *Actinidia*.

Here we extend the analysis of these sequences to 20 taxa aiming: (1) to verify the consistency of the four sections into which the genus *Actinidia* is split; (2) to assign a more adequate position to some species for which the relationship with others is still controversial; and (3) to study the origin of *A. deliciosa*, the kiwifruit.

Materials and methods

Plant material

Twenty *Actinidia* taxa, encompassing the four sections into which the genus has been split (Liang 1984), where investigated (Table 1). Up to five accessions, each with up to three individuals, where available, were examined for each taxon. *Saurauia serrata*, which belongs to a different genus of the same family, the *Actinidiaceae*, was included as an outgroup for phylogenetic analysis.

Plant material was from the collection of *Actinidia* germplasm held at the University of Udine, Italy, and from the collection held at the HortResearch, Auckland, New Zealand. *S. serrata* was kindly supplied by the Royal Botanic Garden of Edinburgh, Scotland. Taxa classified with some uncertainty, such as *A. cylindrica*, *A. glaucophylla* and *A.* "unknown", which was introduced as *A. arisanensis* but clearly did not match Liang's description, were retained because they were well separated by morphological traits from the other taxa, thus aiming to increase the number of informative characters for the phylogenetic reconstruction.

DNA extraction, PCR amplification and enzyme restriction

Total DNA was extracted from approximately 0.3 g of young leaves with CTAB buffer, separated with chloroform/isoamyl alcohol, centrifuged at 5000 g for 20 min, and precipitated in isopropanol according to the procedure of Doyle and Doyle (1990) as slightly modified by Cipriani and Morgante (1993).

Three pairs of primers were designed to amplify a 1118-bp fragment of the Rubisco large subunit (*rbcL*) sequence of tobacco (Shinozaki et al. 1986), a 991-bp fragment of the photosystem-II D1 protein gene (*psbA*) (Shinozaki et al. 1986) and the *rpoB* gene, following the procedure of Tsumura et al. (1996). Two pairs of primers were designed to amplify the spacers between *trnT* and the 5′ *trnL* exon (*a-b*) and between the *trnL* 3′ exon and *trnF* (*e-f*) respectively, according to Taberlet et al. (1991). See Cipriani and Morgante (1993) for the criteria adopted in selecting both regions and primers.

Polymerase chain reactions were carried out in a 50-μl volume containing 200 ng of genomic DNA, 0.2 μM of each primer, 200 μM of each dNTP, 50 mM KCl, 10 mM TRIS-HCl pH 8.3, 0.01% gelatin, 2.5 mM $MgCl_2$, and 1 U of *Taq* polymerase (Boehringer Mannheim) with the following thermal cycle profile: 95°C for 5 min for one cycle; 80°C for 3 min for one cycle; 94°C for 1 min, 50°C for 1 min, 72°C for 1 min 30 s for 26 cycles; 72°C for 7 min for one cycle. The *Taq* polymerase was added during the 3 min at 80°C. The PCR conditions were slightly modified for *rpoB* gene as follows: $MgCl_2$ concentration was decreased to 1.5 mM, the annealing temperature was increased to 55°C, and the number of cycles was increased to 35.

The amplified *rbcL* was restricted with *Alu*I and *Mse*I; *psbA* was restricted with *Hin*fI and *Rsa*I, following the results of previous studies (Cipriani and Morgante, 1993; Cipriani 1994), and ten restriction endonucleases (*Alu*I, *Hae*III, *Hha*I, *Hin*fI, *Mnl*I, *Mse*I, *Msp*I, *Rsa*I, *Sau*3AI, *Taq*I) were chosen to restrict both spacers and the *rpoB* gene, following the tobacco sequences of these regions (Shinozaki et al. 1986).

The digests were electrophoresed in 3% Metaphor (FMC Bioproducts) agarose gels or 4% agarose gels (3% NuSieve, 1% Seakem, FMC Bioproducts) containing ethidium bromide.

Data analysis

Restriction sites were mapped with the aid of the double digests, and the matrix of binary characters (0 = restriction site absent, 1 = restriction site present) was analysed using the PAUP package version 3.1 (Swofford 1993).

The phylogenetic reconstruction was based on both Wagner and weighted parsimony methods. In the first case, a branch-and-bound search was carried out on unordered characters with the ACCTRAN, TBR, COLLAPSE, and MULPARS options of the program. The weighted parsimony analysis performed a general heuristic search, since branch-and-bound search required too much

Table 1 List of *Actinidia* taxa and populations analysed in the present study arranged according to the classification of Liang, revised by Ferguson (Ferguson 1990). Where the country of origin was unknown, the place of introduction is reported in brackets

Taxon	Population, origin	Ploidy
Sect. *Leiocarpae*		
Ser. *Lamellatae*		
A. arguta (Sieb et Zucc) Planch	# AA05, unknown, Japan	4*x*
	# 61, unknown, (U.S.A.)	4*x*
	# 67, unknown, (U.S.A.)	4*x*
	# 68, unknown, (U.S.A.)	4*x*
	# 66, unknown, (U.S.A.)	6*x*
A. arguta var. *purpurea* Rehd	# L3A2, unknown, (UK)	4*x*
A. rufa (Sieb et Zucc) Planch	# RE01, Niyazaki, Kyushu, Japan	2*x*
A. kolomikta (Maxim et Rupr) Maxim	# 57, unknown, (China)	2*x*
	# 65, unknown, (U.S.A.)	2*x*
Ser. *Solidae*		
A. polygama (Sieb et Zucc) Maxim	# PC03, Japan	2*x*
	# PC07, Beijing, China	2*x*
A. valvata Dunn	# VA01, Lushan, Jiangxi, China	4*x*
Sect. *Maculatae*		
A. cylindrica (?) C-F Liang	# 127, Guilin, Guangxi, China	2*x*
A. glaucophylla (?) F Chun	# 133, Guilin, Guangxi, China	2*x*
A. chrysantha C-F Liang	# CN01, Guilin, Guangxi, China	4*x*
A. indochinensis Merr	# IA01, Guilin, Guangxi, China	2*x*
A. "unknown"	# 152, unknown, Taiwan?[a]	2*x*
Sect. *Strigosae*		
A. hemsleyana Dunn	# HA01, Qiyuan, Fujian, China	2*x*
	# 50, unknown, (Italy)	2*x*
"hairy" *A. hemsleyana* Dunn	# MJ01, Fujian, China	2*x*
	# 126, Guilin, Guangxi, China	2*x*
Sect. *Stellatae*		
A. latifolia (Gardn et Champ) Merr	# LC01, Guilin, Guangxi, China	2*x*
	# 131, Guilin, Guangxi, China	2*x*
A. fulvicoma Hance	# 81, Jiangxi, China	2*x*
A. lanceolata Dunn	# 83, Zhejiang, China	2*x*
A. eriantha Benth	# EA01, Qiyuan, Fujian, China	2*x*
	# EA02, Longshen, Guangxi, China	2*x*
A. chinensis Planch	# CK01, unknown, China	2*x*
	# CK09, Meiling, Jiangxi, China	4*x*
A. deliciosa (A Chev) Liang et Ferguson	# DA01, Yichang, Hubei, China	6*x*
	# DA02, unknown, China	6*x*
(outgroup)		
Saurauia serrata DC	# 156, unknown (UK)	n.c.[a]

? Some uncertainty, [a] n.c. not checked

time for calculation, and used an asymmetrical stepmatrix, where restriction site gain was weighted 2:1 over loss. ACCTRAN, TBR, COLLAPSE, and MULPARS options were adopted as above. An "Ancestral state unknown" statement was also forced on the search. A strict consensus tree was constructed from each of the two resultant sets of most parsimonious trees using the CONTREE option.

Results

The five sets of primers gave amplified products in all accessions, including *S. serrata*. No polymorphism was found among accessions within taxa. Two-hundred and forty four fragments, 146 of which polymorphic, were found by analysing less than 3% of the cpDNA genome, which has a size of about 160 kb in *Actinidia* (Hudson and Gardner 1988).

Twenty six restriction sites were mapped: five on each of the two spacers, nine in the *rpoB* gene, four in *rbcL* and three in the *psbA* region (Table 2). Three deletions were also found in the *a*-*b* spacer and one in the *e*-*f* spacer, but these characters were not mapped and were not used in the subsequent phylogenetic analysis. The list of the characters processed, and their state in the different species, is reported in Table 3. Seventeen restriction-site mutations occurred in two or more species and were therefore phylogenetically informative; nine were autapomorphic, that is distinctive to a single species but, although interesting because they separated individual taxa, they were not retained by the program for phylogenetic reconstruction.

The Wagner parsimony analysis yielded 11 equally parsimonious trees with a length of 40 steps, a consistency index of 0.65 (0.55 excluding un-informative

Table 2 Restriction-site mutations found at five chloroplast DNA regions and used for phylogenetic reconstruction in *Actinidia*. The numbers in parentheses indicate fragments not observed on the gel and whose presence was postulated on the basis of the sequence length

Character	cpDNA region	Enzyme	Mutation (bp)	
			0	1
1	*e-f*	*Hin*fI	170	85 + 85
2	*e-f*	*Taq*I	450	220 + 230
3	*e-f*	*Taq*I	450	380 + 70
4	*e-f*	*Mse*I	290	180 + 70 + (40)
5	*e-f*	*Mse*I	290	250 + (40)
6	*a-b*	*Hin*fI	390	310 + 70 + (10)
7	*a-b*	*Hin*fI	390	330 + 60
8	*a-b*	*Hin*fI	130	100 + (30)
9	*a-b*	*Hin*fI	85	55 + (30)
10	*a-b*	*Rsa*I	700	500 + 200
11	*rpoB*	*Sau*3AI	554	423 + 131
12	*rpoB*	*Sau*3AI	423	266 + 157
13	*rpoB*	*Sau*3AI	423	246 + 157 + (20)
14	*rpoB*	*Alu*I	224	160 + 64
15	*rpoB*	*Hin*fI	378	279 + 102
16	*rpoB*	*Hin*fI	381	216 + 165
17	*rpoB*	*Taq*I	690	520 + 170
18	*rpoB*	*Hha*I	1245	860 + 385
19	*rpoB*	*Msp*I	620	540 + 80
20	*rbcL*	*Alu*I	650	600 + 50
21	*rbcL*	*Alu*I	787	653 + 134
22	*rbcL*	*Mse*I	290	160 + 130
23	*rbcL*	*Mse*I	420	385 + (35)
24	*psbA*	*Hin*fI	532	340 + 192
25	*psbA*	*Hin*fI	290	260 + (30)
26	*psbA*	*Rsa*I	458	344 + 114

characters), a homoplasy index of 0.35, and a retention index of 0.63. Figure 1 shows the strict consensus tree generated from the most-parsimonious trees along with restriction-site gains/losses.

The in-group taxa were divided into two clades supported by nine characters, six of which without any further change downstream. One clade is formed by the *A. arguta* complex and includes the botanical varieties *arguta* and *purpurea*. The second clade encompasses all remaining taxa.

We also attempted a generalised parsimony reconstruction using weighted characters. This is a recent PAUP implementation, available in the 3.1 version, which allows a so called stepmatrix to be defined, where transformation between character states can be asymmetric. In our analysis, site-gain was given a weight of 2 and site-loss of 1. The transformation costs were chosen within the range suggested by Albert et al. (1992). The weighted analysis yielded 14 most-parsimonious trees. The consensus tree (data not shown) showed most of the features of the consensus tree generated from Wagner characters. The only relevant difference was the position of the *A. chinensis*/*A. deliciosa* complex as these taxa were now clustered, together with the *A. arguta* complex, *Saurauia* and *A. kolomikta*, in the first clade.

Table 3 Data matrix of cpDNA restriction-site mutation characters in *Actinidia* and *S. serrata* (outgroup). The characters are listed in Table 2

Taxon	Character number
	00000000011111111112222222 12345678901234567890123456
S. serrata (outgroup)	00001000011001110101101010
A. arguta	00011100010100110100000010
A. arguta var. *purpurea*	00011100010100110100000010
A. rufa	00000000111100110110100110
A. kolomikta	10000000001100010110110110
A. polygama	00000000111100110110111110
A. valvata	00100000111100110110111110
A. cylindrica	00000010111110110010110100
A. glaucophylla	00000010111110110010110110
A. chrysantha	00000001101100111110100110
A. indochinensis	00000000111100110110110110
A. hemsleyana	00000000111100110110110111
"hairy" *A. hemselyana*	00000000111100110110110111
A. "unknown"	00000000111100110110100110
A. fulvicoma	00000010111110110010110110
A. latifolia	01000000111100110110100110
A. lanceolata	00000000111100110110110110
A. eriantha	01000000111100110110100110
A. chinensis 2*x*	00000000011100100110110110
A. chinensis 4*x*	00001000011100100110110110
A. deliciosa	00001000011100100110110110

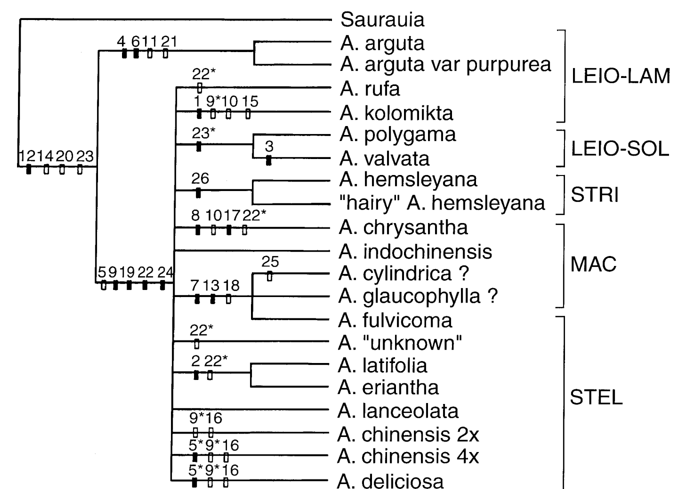

Fig. 1 Strict consensus tree of 11 equally most-parsimonious trees of the *Actinidia* chloroplast genome obtained with the Wagner method of analysis. *Numbers* along branches indicate the restriction-site gain (*filled rectangle*)/loss (*open rectangle*). Character codes are those of Table 2. *Asterisks* indicate homoplasy. *LEIO* = section *Leiocarpae*, LAM = series *Lamellatae*, *SOL* = series *Solidae*, *MAC* = section *Maculatae*, *STRI* = section *Strigosae*, STEL = section *Stellatae*

Discussion

The data set based on fragment presence/absence is seldom used for phylogenetic purposes. The fragment

analysis takes into account both mutations and deletions so that the same event could be computed two or more times. Moreover, because of the non-homology between fragments of similar size, in the absence of a map reconstruction, two fragments of equal length could come from different parts of the sequence and the number of informative characters could therefore be underestimated. There is evidence that the latter risk is negligible with short sequences. Bremer (1991) reported that, among 944 fragments produced during a study on the *Rubiaceae*, not a single fragment was non-homologous between taxa after mapping. We did not use fragment-occurrence data for the phylogenetic reconstruction in order to avoid the risk of supporting clades with non-informative characters; nevertheless, we resort to these data to confirm the proximity of species having the same restriction-site profile.

Although the number of cleavage sites mapped could be considered low for a phylogenetic reconstruction, we expected a low level of homoplasy as reported for other taxonomic reconstructions carried out at low hierarchical levels (Jansen et al. 1992). Homoplastic characters, that is characters with multiple changes resulting from parallel or convergent evolution and from character-state reversals, indeed occurred often and this increased the number of equally parsimonious trees produced by the cladistic analysis. The conflicts between trees resulted in few clades supported in the strict consensus tree.

The parsimony analysis therefore gave insufficient information about the evolution of the *Actinidias*, but some results of taxonomic relevance can be noted.

Evidence for reticulation

We have found several pairs of species that are placed far away from each other on morphological grounds (Liang 1984) but very closely related in terms of the cpDNA analysis. For instance, *A. eriantha* and *A. latifolia* are morphologically clearly distinct (Li 1952; Liang 1984); they also appear quite distinct by flavonoid (Webby et al. 1994) and isozyme analysis (Testolin and Ferguson 1997) but they do not show differences in chloroplast DNA restriction sites (Fig. 1) and they have most cpDNA fragments (data not shown).

Liang placed *A. fulvicoma* and *A. glaucophylla* in different sections according to morphological features (Liang 1984). Our plants of *A. glaucophylla* have been only tentatively classified but the uncertainty was due solely to the weak separation of this from other very closely related taxa all having traits typical of the section *Maculatae*. In our analysis *A. fulvicoma* and *A. glaucophylla* share all cpDNA restriction-site states (Fig. 1) and most of the cpDNA fragments (data not shown).

A third case is the pair *A. rufa* and *A.* "unknown". The latter taxon was introduced into the repository as *A. arisanensis* but it does not match the features of *A. arisanensis* described by Liang (1984). We are not sure what species it is, but from morphological traits it should most likely be placed in the *Stellatae*, and in any case it is clearly different from *A. rufa* with which it still shares all cpDNA restriction-site states (Fig. 1) and almost all cpDNA fragments (data not shown).

Further cases of less-evident incongruity between cpDNA and morphological and biochemical data could be discussed (Testolin et al. 1997). The degree of similarity based on morphological traits and biochemical markers, such as flavonoids and isozymes, relies mainly on the expression of genes carried by the nuclear genome and thus traces a biparental lineage, whereas that traced by cpDNA data is uniparental, and paternal in *Actinidia* where chloroplasts are inherited exclusively from the male parent (Cipriani et al. 1995; Testolin et al. 1997). The most likely explanation for the cases reported above is that one species of each pair is a natural hybrid having the second species, or another species with a similar plastome, as the male parent. If three or more of the 20 taxa examined are indeed natural interspecific hybrids, a reticulate evolution must be postulated for *Actinidia*. The occurrence of natural hybridisation has already been suggested (Ferguson 1990) and we can also support this hypothesis because we carried out many interspecific controlled crosses and have found that most species cross easily with each other even if they belong to different sections (unpublished data).

Reassessment of the taxonomic position of species and traditional grouping

We do not attempt any systematic revision, given the few informative molecular characters, the wide interspecific hybridisation postulated above and the unknown polyploid origin of some taxa. Nevertheless the molecular data clearly show that some species have a wrong position and most, if not all, the traditional groups represented by sections and series are weakly supported.

The first clade in the Wagner consensus tree groups together *A. arguta* and *A. arguta* var. *purpurea*, two species of the *A. arguta* complex that are considered the most representative of the section *Leiocarpae*. Moreover the tree shows a pronounced distance from *A. kolomikta* and *A. rufa*, the two further species of the *Leiocarpae* examined which are currently included in that section. Dunn (1911) followed by other botanists (Webby et al. 1994), formerly placed *A. kolomikta* in a distinct section of its own. Treating *A. kolomikta* as a species well separated from the most representative ones in the *Leiocarpae* is supported by the analysis of both leaf flavonoids (Webby et al. 1994) and isozymes (Testolin and Ferguson 1997).

A. rufa is a species from Japan not present in China and for this reason it was not included in Liang's revision of the genus (Liang 1984). Although it has often been associated with the *A. arguta* complex (Ferguson 1990), it is morphologically quite distinct from those taxa (Ferguson, personal communication) and lacks many of the flavonoids found in *A. arguta* and related species (Webby et al. 1994). The isozyme data agree that *A. rufa* should not be grouped with the *A. arguta* complex (Testolin and Ferguson 1997) but do not support a relationship with any other species among those analysed, i.e. approximately those analysed here. Our data leave the same uncertainty about where *A. rufa* should be placed.

Liang (1984) split the section *Leiocarpae* into two series according to the nature of the cane pit which can be either solid or lamellate. *A. polygama* and *A. valvata* belong together with *A. macrosperma* in the series *Solidae* (Fig. 2). The flavonoid and isozyme analysis confirmed that the three species are very similar and quite distinct from the species of any other group (Webby et al. 1994; Testolin and Ferguson 1997) and our data are not in contrast, although the clade is weakly supported by a single homoplastic character (Fig. 1). A solid pit is not unique to the series and some species in the *Maculatae* and the *Stellatae* are also described as having the same feature (Liang 1984). Possession of solid pit alone is therefore not adequate to define this subgrouping.

A third group could be represented by the *A. hemsleyana* complex, which includes *A. hemsleyana* and what we have called 'hairy' *A. hemsleyana*, two taxa which differ on morphological grounds mainly in terms of hair density and distribution, but which showed such appreciable differences in flavonoid composition as to suggest treating them as distinct taxa (Webby et al. 1994). Our data confirm that they could be different, even if closely related, taxa (Fig. 1).

A. hemsleyana is typical of the section *Strigosae*, which is distinguished from the other ones by the coarse, hard, widely diffuse hairs of its representatives. This section is sometimes judged as an artificial group because of the very few characters, apart from the hairiness, common to the species within it (Liang 1983). We examined only two taxa of this section and are unable to answer the question of whether the *Strigosae* is, or is not, an artificial group. In our interspecific hybridisation trials we found that *A. hemsleyana* is the taxon less prone to cross with *Actinidias* from any other section (unpublished data).

Much more difficult to distinguish in both consensus trees are the two remaining Liang sections, the *Maculatae* and the *Stellatae*. We have observed that the *Stellatae* encompasses species such as *A. fulvicoma*, *A. lanceolata*, and *A. latifolia*, which have green spotted fruit very similar to those of the species of the section *Maculatae*; therefore the separation between the two groups is not always clear-cut. Moreover, the species in the *Stellatae* cross easily among themselves and often with species from other sections even if parent species have different ploidy levels (unpublished data). If this happens with controlled crosses, it most likely also occurs in the wild for species that have overlapping geographical distributions. That natural interspecific hybridisation occurs often in the genus *Actinidia* is largely accepted (Ferguson 1990) and above we gave a robust demonstration of this. We could therefore consider the *Maculatae* and the *Stellatae* as heterogeneous sections where different lineages originating through mutations crossed with each other producing a polyphelytic group. Reticulation violates the requirement of cladistic analysis that the relationships being reconstructed are fundamentally hierarchical (Doyle 1992). For this reason we think that phylogeny is hard to infer for these two sections, even in the presence of a large amount of molecular information, if primary species have not been previously sorted out and separated from the natural hybrids.

The *A. chinensis*/*A. deliciosa* complex and the origin of *A. deliciosa*

The diploid and tetraploid races of *A. chinensis* and the hexaploid *A. deliciosa* have similar cpDNA profiles, since they share all but one restriction site (Fig. 1) and nearly all restriction fragments (data not shown). This is no surprise. The two species share so many morphological characters that until recently they were treated as different varieties of the same species *A. chinensis* (Li 1952; Liang 1984; Ferguson 1990).

A. deliciosa forms bivalents at meiosis (McNeilage and Considine 1989) and when crossed to a diploid genotype of *A. chinensis* showed disomic inheritance at ten isozyme loci (Huang et al. 1997). These facts indicate an allopolyploid origin of *A. deliciosa*. Gardner and co-workers using chloroplast and nuclear probes found that *A. chinensis* could be one parent of *A. deliciosa* (Crowhurst et al. 1990). They also found a genome-specific repeat sequence isolated from *A. deliciosa* which did not hybridise with *A. chinensis*, but did with *A. chrysantha* (Crowhurst and Gardner 1991). Subsequently, both leaf flavonoid and isozyme analysis excluded any possibility that *A. chrysantha* had contributed to the kiwifruit genome, whereas *A. chinensis* was confirmed as being very closely related to *A. deliciosa* and a progenitor of the latter species (Webby et al. 1994; Testolin and Ferguson 1997). The data on cpDNA presented here show that there are some molecular differences within the *A. chinensis*/*A. deliciosa* complex and this is probably due to the large geographic area occupied by this complex (Liang 1984; Ferguson 1990). Further evidence of the variability within the *A. chinensis*/*A. deliciosa* complex is given by the Southern blotting carried out with probe pKIWI1516, a repeat cloned from *A. deliciosa*, which

gave a signal in several tetraploid genotypes of *A. chinensis*, though not in all of those assayed, but did not give any signal in nine diploid accessions of *A. chinensis* tested (Yan et al. 1997). If *A. chinensis* and *A. deliciosa* are closely related, since chloroplasts are strictly paternally inherited in *Actinidia* (Cipriani et al. 1995), *A. chinensis* is the paternal, if not the only, progenitor of *A. deliciosa*, the kiwifruit.

Conclusions

The current systematics is based on morphological traits and does not reflect the evolution of the genus since all groups appear polyphyletic from the first molecular analyses.

Many problems make the revision of the systematics of *Actinidia* troublesome: only a limited number of species is available so that some sections are scarcely represented; germplasm exchanged as seed often comes from botanical garden repositories where cross pollination between taxa is not prevented although the interspecific hybridisation even between distant taxa has been definitively ascertained.

Before we proceed with any further phylogenetic reconstruction we need to collect more species from the wild, and also identify the primary species, separating them from the natural interspecific hybrids. This can be done by combining the analysis of morphological traits, or any other analysis based on the nuclear genome, with the analysis of the cpDNA, which in kiwifruit traces the paternal lineage (Cipriani et al. 1995, Testolin et al. 1997). Recently we discovered that mitochondrial DNA is also uniparentally inherited, though in this case through the female parent (Testolin and Cipriani 1997), as occurs in most angiosperms. The transmission of chloroplast and mitochondrial genomes through different parents provides an exceptional opportunity for studying the paternal and maternal genetic lineage in *Actinidia*.

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