

Phylogenetic Relationships in *Actinidia* as Revealed by RAPD Analysis

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ABSTRACT. Phylogenetic relationships within the *Actinidia* were investigated using randomly amplified polymorphic DNA (RAPD) markers. DNAs from 40 taxa, including 31 species encompassing all four sections and four series of the traditional subdivisions within the genus, were amplified using 22 preselected 10-mer oligonucleotide primers. A total 204 DNA bands were scored across the 40 taxa, of which 188 (92%) were polymorphic. A wide range of genetic similarity was observed among the taxa (0.13 to 0.61). The average similarity between varieties of the same species was 0.54, and between different species was 0.28, respectively. Although the phylogenetic analysis revealed a clear indication that section *Leiocarpaceae* was a monophyletic group, subdivisions of the other three traditional sections were poorly supported. The UPGMA phenogram showed that the majority of the species clustered into geographic subgroups in accordance with their natural distribution (the Yangtzi River, southeastern China, southern China and southwestern China). The intrageneric subdivisions of *Actinidia* appeared to be difficult, but some subdivisions could be explained by the geographic distribution of the species, particularly for species of Liang's sections of *Maculatae* and *Stellatae*. The phylogenetic relationships among several species with previous taxonomic uncertainty are also discussed on the basis of the RAPD data. The results of this study supplement our previous understanding of the *Actinidia* taxonomy based solely on morphological characters.

The genus *Actinidia* Lindl. belongs to the family Actinidiaceae and comprises 66 species and 118 taxa according to a recent report (Huang et al., 2000). The best-known species are *A. deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson and *A. chinensis* Planch., from which most commercial kiwifruit varieties have been developed. Since economic potential of *A. deliciosa* was exploited following a single seed introduction into New Zealand from China in 1904 and the first commercial orchard was established in New Zealand in 1930 (Ferguson and Bollard, 1990), an international kiwifruit industry of more than 100,000 hectares with an annual production of one million tons has been developed since the early 1970s (Huang and Ferguson, 2001). A rapid expansion of the industry has brought about an increased interest in broadening the genetic base of the breeding programs, and further exploitation of related species has rekindled an interest in botanists and horticulturists to try to better understand the phylogenetic relationships and taxonomic hierarchy within the genus (Ferguson, 1990a; Li, 1952; Liang, 1984), which is prerequisite to formulating the appropriate germplasm accession management strategy in the kiwifruit repositories.

Actinidia has a remarkably wide geographic distribution in eastern Asia, extending from the equator (tropics) to cold temperate regions as far north as 50° latitude (Ferguson, 1990a; Liang,

1983). In general, however, the native distribution of most taxa of *Actinidia* is centered around the mountains and hills of south central and southeast China with the QinLing mountains forming a northern boundary and the HengDuan mountains forming a western boundary. In addition to four species native to neighboring countries [*A. strigosa* Hook. f. et Thorns. found in Nepal, *A. petelotii* Diels in Vietnam, *A. hypoleuca* Nakai and *A. rufa* (Sieb. et Zucc.) Planch. ex Miq. in Japan], 62 species, about 45 varieties, and seven forms have been found in China (Cui, 1993; Ferguson, 1990a; Liang, 1983). All members of *Actinidia* are dioecious perennial climbing vines characterized by obligate outcrossing. The variation in morphological characters, chemical contents, ploidy levels, isozyme markers, and DNA markers is tremendous among taxa within the genus, as recently discussed by Huang et al. (2000). In particular, the variation in ploidy level includes diploids ($2n = 58$), tetraploids ($2n = 116$), hexaploids ($2n = 174$), and occasional octaploids ($2n = 232$), forming a reticulated intraspecific and interspecific structure within the genus (He et al., 1998; Huang et al., 2000; McNeilage and Considine, 1989; Xiong and Huang, 1988; Yan et al., 1994, 1997).

The taxonomy of *Actinidia* has remained equivocal since Lindley erected the name *Actinidia* in 1836. An early taxonomic treatment by Gilg (1893) split eight species into two groups based on types of inflorescence (solitary and cyme). In the first systematic revision, Dunn (1911) recognized 24 species and established four sections *Vestitae*, *Maculatae*, *Anpulliferae* and *Leiocarpaceae*, based on the degree of pubescence, shape of ovary, and presence or absence of lenticels on the fruit surface. Li (1952), by emphasizing the structure of leaf hairs and by eliminating the ambiguous character of ovary shape in the second revision, divided the section *Vestitae* into two sections *Stellatae* and *Strigosa*, merged the section *Anpulliferae* into section *Leiocarpaceae*, and retained

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Table 1. List of the *Actinidia* taxa sampled in the present study and their original distribution

Taxon	Accession no.	Ploidy	Origin	Natural distribution of taxa (provinces in China and neighbor country)
Sect. <i>Leiocarpae</i> (Dunn) Li	Abbreviation LEI			
Ser. <i>Lamellatae</i> C.F. Liang	Abbrev. Lam			
<i>A. arguta</i> var. <i>arguta</i> (Sieb. et Zucc.) Planch. et Miq.	2-4-1AA81BJ	4x	Hebei	Heilongjiang, Jilin, Shandong, Shanxi, Hebei, Henan, Shaanxi, Anhui, Zhejiang, Jiangxi, Hubei, Yunnan, Fujian
<i>A. kolomikta</i> Maxim.	2-2-2KA81BJ	4x	Jiling	Heilongjiang, Jilin, Liaoning, Hebei, Shaanxi, Hubei, Sichuan, Yunnan
<i>A. melanandra</i> var. <i>melanandra</i> Franch	1-7-1ME81HB	4x	Xingshan county, Hubei	Sichuan, Yunnan, Guizhou, Gansu, Shaanxi, Henan, Hubei, Hunan, Jiangxi, Anhui, Zhejiang, Fujian
Ser. <i>Solidae</i> C.F. Liang	Abbrev. Sol			
<i>A. polygama</i> (Sieb. et Zucc.) Maxim.	3-15-3PC82SC	4x	Chengdu city, Sichuan	Jilin, Liaoning, Shandong, Gansu, Shaanxi, Henan, Hubei, Hunan, Zhejiang, Sichuan, Yunnan, Guizhou
<i>A. macrosperma</i> var. <i>macrosperma</i> C.F. Liang	2-1-1MA82JX	4x	Wuling county, Jiangxi	Zhejiang, Jiangxi, Jiangsu, Hubei, Anhui, Guangdong
<i>A. macrosperma</i> var. <i>munoides</i> C.F. Liang	2-9-5MB83JX	4x	Jiangxi	Zhejiang, Anhui, Jiangxi, Jiangsu
<i>A. valvata</i> var. <i>valvata</i> Dunn	2-12-1VA83GX	4x	via Guangxi Botanical Garden	Anhui, Jiangsu, Zhejiang, Jiangxi, Hubei, Hunan, Guangdong
Sect. <i>Maculatae</i> Dunn	Abbrev. MAC			
<i>A. callosa</i> var. <i>discolor</i> C.F. Liang	3-13-I CC84FJ	2x	Jianning county, Fujian	Sichuan, Yunnan, Guizhou, Anhui, Zhejiang, Fujian, Jiangxi, Hunan, Guangdong, Guangxi
<i>A. callosa</i> var. <i>henryi</i> Maxim.	3-8-1CF85GX	2x	Guangxi	Shaanxi, Gansu, Sichuan, Yunnan, Guizhou, Anhui, Zhejiang, Jiangxi, Fujian, Hubei, Hunan, Guangdong, Guangxi
<i>A. callosa</i> Lindl. var. <i>strigillosa</i> C.F. Liang	3-14-2CG97GX	4x	Guangxi	Guizhou, Hunan, Guangxi
<i>A. chrysantha</i> C.F. Liang	2-11-2CN81HN	4x	Hunan	Guangxi, Guangdong, Hunan
<i>A. cylindrica</i> var. <i>cylindrica</i> C.F. Liang	3-14-3CR98GX	2x	Guangxi	Guangxi
<i>A. cylindrica</i> var. <i>reticulata</i> C.F. Liang	3-1-1CT83GX	4x	Guangxi	Guangxi
<i>A. glaucophylla</i> var. <i>glaucophylla</i> F. Chun	2-5-5GB98GX	2x	Guangxi	Hunan, Guangdong, Guangxi, Guizhou
<i>A. glaucophylla</i> F. Chun var. <i>rotunda</i> C.F. Liang	3-14-4GE98GX	2x	Guangxi	Guangxi
<i>A. indochinensis</i> Merr.	2-5-i IA98GX	2x	Guangxi	Yunnan, Guangdong, Guangxi
	3-7-3RB83GX	2x	Guangxi	Sichuan, Yunnan, Guizhou, Hubei, Hunan, Guangxi, Jiangxi
<i>A. rufa</i> (Sieb. et Zucc.) Planch. ex Miq.	3-10-2RE90GX	2x	Japan, via Guangxi Botanical Garden	Japan
<i>A. sabifolia</i> Dunn	1-2-2SA98GX	4x	Hunan	Fujian, Hunan, Jiangxi
Sect. <i>Strigosae</i> Li	Abbrev. STR			
<i>A. hemsleyana</i> Dunn	1-6-4HA98GX	2x	Zhejiang	Fujian, Zhejiang, Jiangxi

Table 1. Continued.

Taxon	Accession no.	Ploidy	Origin	Natural distribution of taxa (provinces in China and neighbor country)
<i>A. melliana</i> Hand.-Mazz.	1-6-3MJ98GX	2x	Guangxi	Jiangxi, Hunan, Guangdong, Guangxi
Sect. <i>Stellatae</i> Li	Abbrev. STE			
Ser. <i>Perfectae</i> C.F. Liang	Abbrev. Per			
<i>A. chinensis</i> var. <i>chinensis</i> Planch.	Wuzhi-CKXOJX	4x	Wuling county, Jiangxi	Shaanxi, Henan, Anhui, Jiangsu, Zhejiang, Hubei, Hunan, Jiangxi, Guangdong, Guangxi, Fujian
<i>A. deliciosa</i> var. <i>deliciosa</i> (A. Chev.) C.F. Liang et A.R. Ferguson	SX-1-DA82HB	6x	Xingshan county, Hubei	Gansu, Shaanxi, Henan, Hubei, Hunan, Sichuan, Yunnan, Guizhou, Guangxi
<i>A. deliciosa</i> var. <i>chlorocarpa</i> (C.F. Liang)				
C.F. Liang et A. R. Ferguson	2-10-1DB90GX	4x	Guangxi	Yunnan, Sichuan, Guangxi
<i>A. eriantha</i> var. <i>eriantha</i> Benth	3-1-1EA80JX	2x	Fujian	Guizhou, Hunan, Jiangxi, Fujian, Guangdong, Guangxi
<i>A. eriantha</i> Benth var.				
<i>calvescens</i> C.F. Liang	2-13-5EC98GX	2x	Guangxi	Guangxi
<i>A. farinosa</i> C.F. Liang	2-16-5FA97GX	2x	Guangxi	Guangxi
<i>A. fulvicoma</i> var. <i>fulvicoma</i> Hance	3-12-3FF92JX	2x	Dayu county, Jiangxi	Guangdong, Hunan, Jiangxi, Fujian
<i>A. fulvicoma</i> var. <i>lanata</i> f. <i>lanata</i> (Hemel.) C.F. Liang	2-12-2FG98GX	2x	Guangxi	Jiangxi, Fujian, Hunan, Guangdong, Guangxi, Guizhou
<i>A. latifolia</i> var. <i>latifolia</i> (Gardn. et Champ.) Merr.	3-2-5LC84FJ	2x	Fujian	Anhui, Zhejiang, Jiangxi, Fujian, Hunan, Sichuan, Yunnan, Guizhou, Guangxi, Guangdong, Taiwan
<i>A. liangguangensis</i> C.F. Liang	2-7-5LF97GX	2x	Guangxi	Guangdong, Guangxi, Hunan
<i>A. persicina</i> Huang et Wang	3-4-2PS84FJ	2x	Jianling county, Fujian	Fujian, Zhejiang
<i>A. rufotricha</i> C.Y. Wu var.				
<i>glomerata</i> C.F. Liang	2-8-4RG98GX	2x	Guangxi	Guangxi, Guizhou
<i>A. styracifolia</i> C.F. Liang	3-5-1SF90GX	2x	Hunan	Hunan, Fujian
Ser. <i>Imperfectae</i> C.F. Liang	Abbrev. Imp			
<i>A. grandiflora</i> C.F. Liang	2-6-1GH90GX	4x	Sichuan	Sichuan
<i>A. guilinensis</i> C. F. Liang	3-2-2GI90GX	2x	Guangxi	Guangxi
<i>A. hubeiensis</i> Huang et Sun	1-1-1HU91HB	2x	Ychang, Hubei	Hubei
<i>A. lijianensis</i> C.F. Liang et Y.X. Lu	1-11-1LG90GX	2x	Guangxi	Guangxi
<i>A. zhejiangensis</i> C.F. Liang	2-8-1ZA92ZJ	2x	Qingyuan county, Zhejiang	Zhejiang, Fujian

the section *Maculatae*. Thirty-five species and 14 varieties were described in his revision. The most recent revision by Liang (1984) retained Li's division of the four sections but with a modification of further subdividing two series *Lamellatae* and *Solidae* within section *Leiocarpae* and two series *Perfectae* and *Imperfectae* within section *Stellatae* by taking into account stem pith structure and stellate hairs, respectively, in each section. He significantly increased the number of taxa to a total of 51 species, 35 varieties and six forms (Liang, 1984). Since then, there have been many new species published (Huang and Wang, 1995; Jiang, 1995; Shi et al., 1994; Sun and Huang, 1994) and the intrageneric subdivisions have again come into question. Phylogenetic analyses based on 20 to 50 morphological characters as well as microstructures of leaf trichomes resolved all species with a smooth fruit skin as a monophyletic group (section *Leiocarpae*), but subdivision of the other sections was ambiguous (He et al., 2000; Huang et al., 1999; Li et al., 2000). Phylogenetic relationships of a limited number of taxa as revealed by allozyme and cpDNA markers were also unable to clearly subdivide the other

three sections (Cipriani et al., 1998; Testolin and Ferguson, 1997). In general, the taxonomy of the *Actinidia* based on morphological characters is not clearly resolved and the phylogenetic relationships within the genus are difficult to assess because the boundary between intra- and interspecific classification is blurred by the extensive variation of the morphological characters, and the fact that the various transitional forms existing between taxa have probably resulted from natural hybridization between species with sympatric distributions (Ferguson, 1990a). Little is known about the speciation process in *Actinidia*, although the genus has been speculated to be undergoing a fast speciation in central-southwest China, which is well known for its diverse topography. This region has been considered the center of diversity of the genus and is where most of the new species of *Actinidia* are being found (Liang, 1983; Cui, 1993).

A national germplasm repository and breeding program for *Actinidia* was established in 1978 at the Wuhan Institute of Botany (WIB), in Wuhan, Hubei, People's Republic of China. The long-term goal of the program is to develop a comprehensive

conservation *ex situ* repository of all currently known *Actinidia* species and a genetically rich germplasm collection for further development of superior kiwifruit cultivars. A refined understanding of species boundaries and relationships are of great practical importance for the curator to formulate collecting priorities and sampling strategies and for breeders to use the germplasm resources within the *Actinidia* genus. The objectives of this study were to 1) examine intrageneric subdivisions in *Actinidia* for providing accurate information to assist germplasm management of taxonomic entries and accessions in the repository; 2) evaluate the phylogenetic relationships among *Actinidia* species to aid parent selection in currently ongoing interspecific breeding programs; and 3) compare these results with information on the natural distribution of the various species for future expedition plans to be designed and to enhance collecting efficiency.

Materials and Methods

PLANT MATERIALS. All plant materials were collected from the germplasm repository for *Actinidia* at the WIB. Forty taxa of 31 species, encompassing all four sections and four series of the traditional subdivisions within the genus, were investigated in the present study (see Table 1). Where available, three to five plants of each taxon were included for analysis.

DNA EXTRACTION, PCR AMPLIFICATION. Total nucleic acids were isolated from about 2 g of fresh leaf tissue using a modification of the cetyltrimethylammonium bromide (CTAB)-based procedure outlined by Wagner et al. (1987). The RNA component of these individual extracts was removed by incubation in the presence of RNase A as described by Ausubel et al. (1987). Oligonucleotide 10-base primers were obtained from Operon Technologies Inc. (Alameda, Calif.). DNA amplification was based on the protocol reported by Williams et al. (1990). The reaction consisted of the following in 24 μ L total volume: 6.25 ng template DNA, 1 μ L primer DNA (5 μ M stock), 3.6 μ L dNTPs (1 mM stock), 2.4 μ L 10 \times *Taq* DNA polymerase reaction buffer (500

mM KCl, 100 mM Tris-HCl, 1.0% Triton X-100, 15 mM MgCl₂, and 2.0 U *Taq* DNA polymerase. Reactions were loaded in flexible microtitre plates and overlaid with 25 μ L of mineral oil. Microtitre plates were placed in preheated (85 $^{\circ}$ C) MJ Research PTC-100 programmable temperature cyclers (Watertown, Mass.) and covered with mylar film. The DNA samples were immediately amplified using the following thermal profile: 5 s at 95 $^{\circ}$ C; 1 min 55 s at 92 $^{\circ}$ C; followed by 45 cycles of 5 s at 95 $^{\circ}$ C, 55 s at 92 $^{\circ}$ C, 1 min at 35 $^{\circ}$ C, and 2 min at 72 $^{\circ}$ C; followed by 7 min at 72 $^{\circ}$ C. The reactions ended with an indefinite hold at 4 $^{\circ}$ C. Amplification products were electrophoresed in 2% agarose gels and TAE buffer (40 mM Tris base, 20 mM sodium acetate, 2.0 mM EDTA, glacial acetic acid to pH 7.1) for about 3.5 h at 3 V \cdot cm⁻¹ (150 V). A total of 3.0 μ L loading buffer (10 \times TAE, 50% glycerol, and 0.25% bromophenol blue) was added to each reaction prior to electrophoresis. After electrophoresis, the gels were stained with ethidium bromide (0.3 μ g \cdot mL⁻¹) for 45 min, washed in distilled water for 1.0 h, and photographed under ultraviolet light using a Polaroid MP-4 camera and Polaroid 667 instant film (Cambridge, Mass.).

DATA ANALYSIS. One hundred and forty-four RAPD primers (Opcon Technologies, kits A-C, J and U 01–10) were used for initial screening against eight randomly chosen species to identify RAPD markers. Each sample was amplified at least two times to verify reproducibility. Twenty-two primers that amplified a total of 188 reproducible polymorphic bands were then selected and used in the study. Of the 188 polymorphic markers, 156 markers that showed no polymorphism within taxa were then identified and chosen for the Cluster analysis to reveal phylogenetic relationships between the taxa, and the remaining 32 markers were discarded to avoid intrataxon variation that might confound the analysis of inter-taxon relationships. RAPD markers were designated by the manufacturer primer code corresponding to the 10-mer oligonucleotide primer responsible for their amplification, followed by a four digit number indicating the product size in base pairs (Table 2). RAPD marker phenotypes were

Table 2. The primers used and sizes of 156 intertaxa polymorphic DNA fragments in *Actinidia*

Primer code	Size of amplified polymorphic DNA fragments (bp)
OPA-07	1600, 1300, 1159, 1126, 1093, 1000, 900, 805, 700, 650, 514, 448, 400, 350, 300, 280
OPA-11	1680, 1250, 1200, 1093, 1000, 850, 820, 805, 640, 550, 448
OPA-17	1450, 1159, 1020, 805, 700, 550
OPB-08	1800, 1700, 950, 513, 456
OPC-04	1250, 1159, 900, 805, 750, 730, 550, 514
OPD-03	1800, 1690, 950, 700, 514, 490
OPD-16	1850, 1650, 1200, 1000, 950, 900, 850, 700, 550
OPE-16	1650, 1300, 1159, 1093, 1000, 950, 805, 700, 580, 264
OPE-17	1250, 730, 650
OPE-20	1450, 1300, 1000, 680, 500, 300
OPF-16	1500, 1300, 1000, 650, 550, 530, 339
OPF-17	1700, 700
OPF-20	1300, 1000, 550, 468, 339
OPG-04	1800, 1500, 1159, 1093, 1000, 820, 650, 550, 455
OPG-06	2140, 1159, 1000, 950, 900, 805, 700, 650, 600, 550, 514
OPG-07	2000, 900, 805, 780, 514
OPG-14	1159, 900, 700, 600, 490, 420
OPG-15	1650, 1300, 1093, 900, 790, 480, 460
OPG-18	1700, 1550, 1300, 1200, 1093, 900, 850, 700, 514, 280
OPI-07	1000, 900, 650, 600, 450
OPU-02	1600, 900, 805
OPU-06	1350, 1250, 1050, 750, 700, 500

scored as 1 (band present) or 0 (band absent), respectively. NTSYS-pc (v 1.8) was used to compute Jaccard's coefficients of similarity and to construct a phenogram using the unweighted pair group method with arithmetic averages (UPGMA) (Rohlf, 1994).

Results

RAPD VARIATIONS AND GENETIC SIMILARITY AMONG *Actinidia* TAXA. A total 204 DNA bands, amplified by 22 different IO-mer oligonucleotide primers, were scored across the samples. An average of 9.3 DNA bands were amplified per sample/primer combination. Primer OPF-17 amplified as few as two bands, whereas primer OPA-07 amplified as many as 18 bands. The approximate size of the amplified fragments ranged from 280 to 2140 bp (Table 2). Of the 204 DNA bands scored, 188 (92%) were polymorphic. Across all samples and primers, an average of 8.6 polymorphic bands were amplified per sample/primer combination.

A wide range of genetic similarity was observed among the taxa. The highest genetic similarity (0.61) was observed between *A. persicina* and *A. zhejiangensis*, while the lowest genetic similarity (0.13) was between the species *A. fulvicoma* var. *lanata* and *A. sabiifolia*. In general, the average similarity between varieties of the same species was 0.54, and between different species was 0.28, respectively (data not shown).

PHYLOGENETIC ANALYSIS. The phenogram generated by UPGMA clustering analysis revealed two notable observations:

1) different varieties within a species tended to cluster closely with one another; and 2) species within section *Leiocarpae* formed a distinct cluster, supporting the traditional grouping of all the species with smooth skinned fruit as a monophyletic group, but subdivisions within the other three traditional sections were poorly supported (Fig. 1).

To examine genetic relationships among the taxa, two cut-off points ($D_1=0.52$, $D_2=0.32$) were determined according to Xu and Li's (1983) method of grouping taxa at different genetic similarity levels (Fig. 1). In general, these two cut-off points showed the separation of species and groups, respectively. At the 0.52 cutoff point, most of the species were separated with only a few exceptions. The genetic similarity between *A. latifolia* and *A. guilinensis* was more suggestive of a varietal-level relationship than a species-level relationship. This is consistent with their morphological similarity and the fact that *A. guilinensis* was once misidentified as a unique genotype of *A. latifolia* (Liang, 1988). Similarly, *A. zhejiangensis* and *A. persicina* formed a close cluster and had similarity measures more suggestive of a varietal-level relationship than a species-level relationship. A fairly high level of genetic similarity was also observed between the two kiwifruit species, *A. deliciosa* and *A. chinensis*, from which all of the commercial cultivars have been developed. On the other hand, an unexpectedly low genetic similarity was observed among three varieties of *A. callosa* and between two varieties of *A. fulvicoma*, suggesting that a high degree of genetic variation probably exists within the species. The varieties of *A. callosa* had rather low levels of genetic similarity among one another, var.

henryi and var. *discolor* were clustered at a lower genetic similarity than that suggestive of a species-level relationship, while var. *strigillosa* formed a cluster with *A. grandiflora*. Two varieties of *A. fulvicoma* appear to be distantly related to each other.

The 0.32 cutoff point grouped most taxa into eight distinct clusters that appear to reflect the geographic distribution of the species, while three remaining taxa *A. melliana*, *A. indochinensis* and *A. sabiifolia* were revealed as individual species (Fig. 1). Cluster I consisted of *A. latifolia* and *A. guilinensis*. If *A. guilinensis* were treated as a variety of *A. latifolia*, this group could be a single species. Cluster II included *A. fulvicoma*, *A. eriantha* and *A. styracifolia*. *A. fulvicoma* was considered by Li (1952) to be closely related to *A. eriantha*. The natural distribution of the group is centered in Fujian and Guangdong in southeastern China and extends to Guangxi, Guizhou in southwestern China. Cluster III comprised *A. farinosa*, *A. rufotricha*, *A. glaucophylla*, *A. lianguangensis*, *A. cylindrica* and *A. chrysantha*. These six species have overlapping distri-

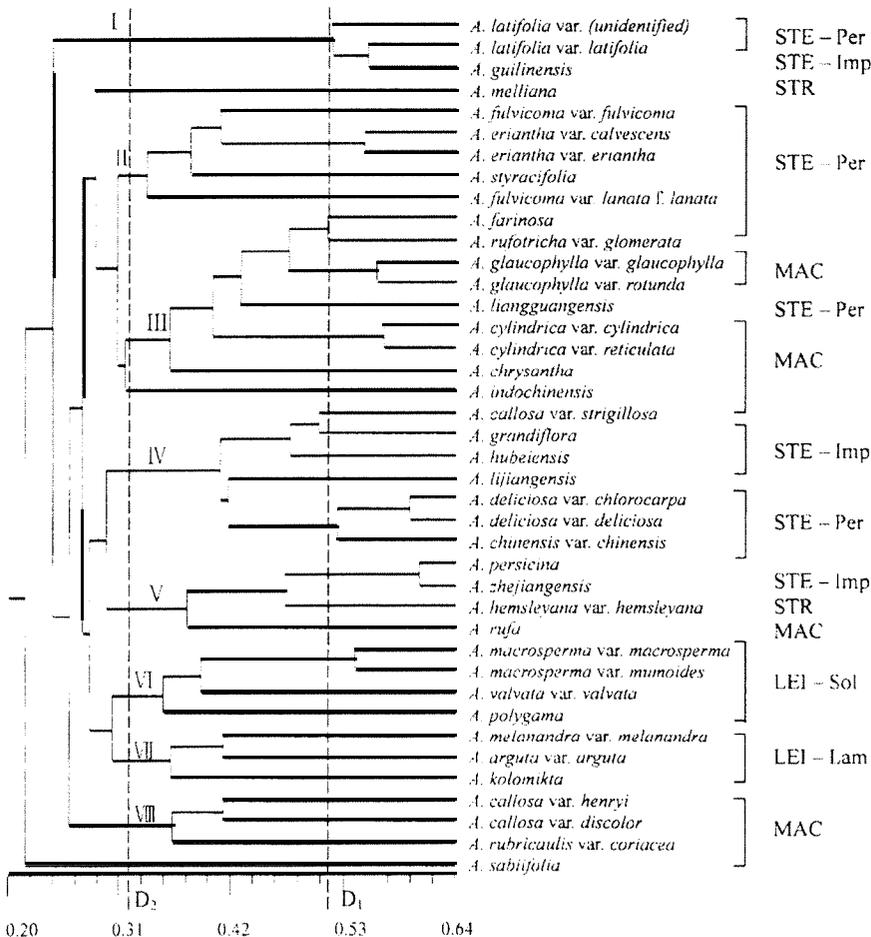


Fig. 1. UPGMA phenogram based on the similarity (Jaccard's coefficient) matrix calculated from RAPD data of 40 *Actinidia* taxa. The dotted lines indicate the two cut-off points. $D_1 = 0.52$ and $D_2 = 0.32$. The traditional subdivisions are labeled as abbreviation listed in Table 1. Numbers on the phenogram label the seven major clusters. Cophenetic correlation coefficient is 0.76.

butions centering in Guangxi and Guizhou in southwest China and spreading over Guangdong and Hunan in southern China. Cluster IV was the kiwifruit complex of *A. deliciosa* and *A. chinensis* plus *A. lijiangensis*, *A. hubeiensis*, *A. grandiflora*, and *A. callosa* var. *strigillosa*. The natural distribution of the group is centered in Hubei and spreads over the middle reach of the Yangtzi river in central China, except for *A. lijiangensis* that is primarily located in Guangxi. However, the genetic similarity between *A. lijiangensis* and other species of the group was reflected well in the phenogram. Cluster V consisted of *A. persicina*, *A. zhejiangensis*, *A. hemsleyana*, and *A. rufa*. Species in this group are distributed primarily in Zhejiang, Fujian and Jiangai in eastern China, except for *A. rufa*, which is native to Japan. Cluster VI was the monophyletic group represented by species only in series *Solidae* section *Leiocarpace*, while cluster VII was the monophyletic group consisting of only species in the series *Lamellatae* section *Leiocarpace*. These two clusters (VI and VII) further formed a larger group reflecting the genetic relationship of section *Leiocarpace* in accordance with the traditional taxonomy. All these species are naturally distributed in northern China. Cluster VIII included two varieties of *A. callosa*, var. *henryi* and var. *discolor* and *A. rubricaulis* var. *coriacea*, suggesting a genetic affinity between *A. callosa* and *A. rubricaulis* var. *coriacea*. In fact, *A. rubricaulis* var. *coriacea* was once treated as a variety belonging to *A. callosa*. It was later revised as an independent species *A. coriacea* (Dunn, 1911; Li, 1952), but in 1984, Liang reclassified it as variety belonging to *A. rubricaulis*.

Discussion

INTRAGENETIC SUBDIVISIONS WITHIN *Actinidia*. The current intrageneric subdivisions within *Actinidia* have been challenged by several recent investigations (Cipriani et al., 1998; He et al., 2000; Huang et al., 1999; Li et al., 2000; Testolin and Ferguson, 1997; Webby et al., 1994). Two suggestions for a new revision have been proposed. Based on cluster analysis of 50 morphological characters, Huang et al. (1999) proposed a modified subdivision of the genus into three sections: *Leiocarpace* retaining all species with smooth skinned fruit, *Maculatae* including the species with spotted fruit and, *Vestitae* comprising species with leaf hairs by a further dividing into two series, *Stellatae* for species with stellate leaf hairs and *Strigosae* for species with simple and/or coarse leaf hairs. Li et al. (2000) suggested a subdivision of two subgenera: *Leiocarpace* and *Maculatae*, based on cladistic analysis of 22 morphological characters. However, neither proposal solves the problem that *Maculatae* is very heterogeneous and contains various leaf hair types and various degrees of spotted fruits, making it particularly difficult to delimit species in the *Maculatae* and *Vestitae*. Analyses including a number of different metrics such as general morphology, leaf flavonoid content, isozymes, and cpDNA have all provided evidence for grouping species with smooth skinned fruit as a monophyletic section, but subdivision of the other three traditional sections were ambiguous (Cipriani et al., 1998; He et al., 2000; Huang et al., 1999; Li et al., 2000; Testolin and Ferguson, 1997; Webby et al., 1991). Phylogenetic analysis using RAPDs provides further evidence to support the section *Leiocarpace* as a monophyletic group, but similar to other metrics studied to date, it does not provide any convincing evidence for subdividing the other sections. The UPGMA phenogram clearly showed the section *Maculatae* as a polyphyletic group with most members either standing out as single species or clustered with species of

the section *Stellatae* or section *Strigosae* (Fig. 1). A similar situation was also observed in Liang's *Stellatae*. Liang's section *Strigosae* has been speculated to be an artificial group because of a lack of morphological characters common to the species within it, and because the species all have scattered geographic distribution patterns (Liang, 1983). Two typical species of the *Strigosae* section, *A. melliana* and *A. hemsleyana*, were examined in this study and found not to be closely related. The UPGMA phenogram showed that many species were clustered into geographic subgroups in accordance with their known distributions. About 60% of the species within the genus *Actinidia* are found in southwestern China, where the complicated topography has created a myriad of microclimates influenced by the mountain ranges in the region (Cui, 1993). A geographic distribution pattern associated with speciation has already been suggested (Liang, 1983). The more varied the geographic environments are within a region, the more diverse are the genotypes and taxa (Zao and Liu, 1996). Based on this study and previous data on reticulated ploidy structure (Ferguson, 1990a; Huang et al., 2000), the frequent occurrence of natural hybridization and cross compatibility (Ferguson, 1990b; Wang and Huang unpublished data) and cpDNA analysis (Cipriani et al., 1998), it is reasonable to hypothesize that hybridization is functioning to produce a reticulate evolutionary structure within the *Actinidia*. Liang's section *Leiocarpace* is most likely an ancestral group. The species *A. polygama* and *A. kolomikta* could be considered progenitor species. *A. arguta*, *A. melanandra* and *A. macrosperma* could be more recent derivative species that are still undergoing rapid speciation as the largest numbers of varieties are found within these species (Liang, 1983). It is possible that the spotted fruit species in Liang's section *Maculatae* could be in transition from aprotigenic species with smooth skinned fruit (section *Leiocarpace*) to a pubescent species (most species of section *Stellatae* have more or less a gradation of hairs on the fruit). Meanwhile, overlapped distributions, mutation and natural hybridization may have created a geographically oriented polyphyletic origin to the groups creating an apparent mixed structure to many of the species including Liang's sections *Stellatae* and *Strigosae*. For this reason, a new revision of intrageneric subdivisions of *Actinidia* might need to take into account the geographic distribution of the particular species, especially for species in Liang's *Maculatae* and *Stellatae*.

PHYLOGENETIC RELATIONSHIPS BETWEEN THE SPECIES. The genetic relationships between species revealed by cluster analysis of RAPDs are generally consistent with those described or discussed in traditional taxonomy, but some interesting results and new observations relevant to taxonomy should be noted.

With respect to the affinity between *A. latifolia* and *A. guilinensis*, a close relationship between the species was revealed suggesting a possible varietal-level relationship rather than a species-level relationship. Further, *A. latifolia* appears to harbor a great deal of variation as determined by the low levels of similarity (Fig. 1). This result supports the morphological observations that *A. guilinensis* is quite similar to *A. latifolia* (Cui, 1993; Liang, 1988). *A. latifolia* has a wide geographic range from southeastern to southwestern China, while *A. guilinensis* is narrowly endemic to Guangxi province (Li, 1952; Liang, 1983, 1988). *A. guilinensis* is speculated to have originated from natural hybridization with *A. latifolia* as a parent and may be undergoing rapid speciation (Cui, 1993; Liang, 1988). *A. guilinensis* was classified as a species mostly based on one morphological character that its mature leaf becomes glabrous (Liang, 1988). In fact,

A. guilinensis was once misidentified as a unique genotype of *A. latifolia* (Liang, 1988).

Although *A. deliciosa* var. *chlorocarpa* was recognized as a variety of *A. deliciosa*, its taxonomic status has been questioned (Li et al., 1996). Morphologically, *A. deliciosa* var. *chlorocarpa* is intermediate between *A. deliciosa* and *A. chinensis* for a majority of leaf and fruit characters (Li et al., 1996), but the pubescence on its fruits and stems is extremely similar to that of *A. deliciosa* var. *deliciosa*. The UPGMA phenogram obtained in this study suggests that it is closely related to *A. deliciosa* var. *deliciosa*. A cytogenetic study of *A. deliciosa* var. *chlorocarpa* indicated it was a tetraploid (He et al., 1998). *A. chinensis* has both triploids and tetraploids and has been considered a progenitor of hexaploid *A. deliciosa* (Cipriani et al., 1998; Huang et al., 1997; Testolin and Ferguson, 1997). A very close relationship between *A. deliciosa* var. *deliciosa* and var. *chlorocarpa* was also observed in isozyme analysis (Testolin and Ferguson, 1997). Based on the evidence available, *A. deliciosa* var. *chlorocarpa* has been speculated to be derived from hybridization of hexaploid var. *deliciosa* and diploid *A. chinensis* (He et al., 1998). In addition, the known overlapped geographical distribution of these three taxa also supports their close relationship (Li et al., 1996).

A. persicina is a recently published species (Huang and Wang, 1995). Based on their study, *A. persicina* appears to be closely related to *A. zhejiangensis*, which is in disagreement with recent conclusion suggesting that it is closely related to *A. grandiflora* and *A. hubeiensis* based on leaf morphology (He et al., 2000). *A. persicina* and *A. zhejiangensis* formed a tight cluster at a genetic similarity about 0.60, which is even higher than the average similarity between varieties (0.53). Plants of the two taxa, growing in WIBs repository, have only slight differences in the degrees of red color in their flowers and brown color in their anthers (Huang, personal observation). Reclassification of *A. persicina* is needed. Another related taxonomic uncertainty involves the positioning of *A. rufa*, a species native to Japan. *A. rufa* was once treated as a variety of *A. arguta*, in section *Leiocarpae* in Li's revision (1952). However, it was recently demonstrated to be more closely associated to *A. hemsleyana* by isozyme analysis (Testolin and Ferguson, 1997). Similarity between *A. rufa* and *A. callosa* var. *henryi* was also suggested based on flavonoid composition (Webby et al., 1994). The RAPD data provide additional support for positioning *A. rufa* in the group consisting of *A. hemsleyana* and *A. zhejiangensis*.

The low level of similarity observed within *A. callosa* reflects well its wide natural distribution and highly variable morphology (Li, 1952; Liang, 1984). In the phylogenetic analysis (Fig. 1), *A. callosa* var. *strigillosa* was not clustered with its species group, but instead it closely clustered with *A. hubeiensis* and *A. grandiflora*, and adjacent to the *A. chinensis*/*A. deliciosa* complex. The possible hybrid origin of *A. hubeiensis* and *A. grandiflora* has been previously suggested based on their morphological similarity and sympatric distributions (Liang, 1984; Sun and Huang, 1994). The RAPD data suggest that *A. callosa* var. *strigillosa* could be a parent of the other taxa, or possibly that *A. hubeiensis*, *A. grandiflora* and *A. callosa* var. *strigillosa* all resulted from a single hybridization event between *A. chinensis* (or *A. deliciosa*) and *A. callosa* and after subsequent speciation processes it differentiated into the present taxa. The specific boundary of *A. callosa* needs to be reconsidered.

Of all the members of the *Actinidia*, *A. fulvicoma* is known to have the most named varieties and forms. Two varieties, *A. fulvicoma* var. *fulvicoma* and var. *lanata*, were examined in the

present study, and found to be highly dissimilar. *A. fulvicoma* exhibits a wide range of morphological variation, and is considered to be a rather heterogeneous species. It is comprised of various forms and many diverse genotypes resulting from natural hybridization, and is still thought to be under rapid speciation (Liang, 1984). A recent phylogenetic analysis based on cpDNA revealed a very close relationship of *A. fulvicoma* with *A. glaucophylla* and *A. cylindrica* (Cipriani et al., 1998). In contrast, however, the RAPD data suggest that it is closely related to *A. eriantha* and *A. styracifolia*. Overlapping distributions and frequent hybridization among these taxa could contribute to their close relationship, and account for the high level of genetic heterogeneity observed within *A. fulvicoma*.

The taxonomic position of *A. kolomikta* has been controversial. It was first placed in the *Ampulliferae* by Dunn (1911) and later subsumed into the section *Leiocarpae* when Li (1952) revised the genus. However, recent evidence based on leaf flavonoids and isozymes indicated that it was quite distinct from any species in the section *Leiocarpae* (Testolin and Ferguson, 1997; Webby et al., 1994). Further evidence for this was provided by phylogenetic analyses based on cpDNA (Cipriani et al., 1998). In contrast to these reports, the RAPD data suggest moderate levels of similarity between *A. kolomikta* and other members in section *Leiocarpae*, and support retaining *A. kolomikta* in the section *Leiocarpae*.

Although there remain unresolved taxonomic relationships within the *Actinidia*, the phylogenetic relationships suggested by the RAPD data presented in this study supplement our current understanding of *Actinidia* taxonomy. A new revision of the intrageneric subdivisions of *Actinidia* based on geographic distribution seems to be a logical step forward if the apparent association between natural distribution and phylogenetic relationship is a result of different hybridization events. RAPD analysis is not usually considered a sufficient analyzing tool to obtain a robust phylogeny of an angiosperm genus such as *Actinidia* that probably has a large number of species derived from hybridization events, and single individuals in each taxon used in this study also imposes limitations on the validity of the conclusions. Additional studies are needed. These studies might attempt to integrate all of the available morphological and molecular data, or collect low copy gene sequence data, in an attempt to obtain further resolution within the genus. Nevertheless, the results presented in this study provide useful information to our ongoing efforts toward the conservation and germplasm management for kiwifruit.

Literature Cited

- Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1987. Current protocols in molecular biology. Wiley, New York.
- Cipriani, G., R. Testolin, and R. Gardner. 1998. Restriction-size variation of PCR-amplified chloroplast DNA regions and its implication for the evolution and taxonomy of *Actinidia*. Theor. Appl. Genet. 96:389-396.
- Cui, Z-X. 1993. *Actinidia* in China. Shandong Scientific Press, Jinan, Shandong, China.
- Dunn, S.T. 1911. A revision of the genus *Actinidia* Lindl. J. Linn. Soc. Lond. Bot. 39:394-410.
- Ferguson, A.R. 1990a. The genus *Actinidia*, p. 15-35. In: I.J. Warrington and G.C. Weston (eds.), Kiwifruit: Science and management. Ray Richards Publ., Auckland, N.Z.
- Ferguson, A.R. 1990b. Kiwifruit (*Actinidia*), p. 603-653. In: J.N. Moore and J.R. Ballington, Jr. (eds.), Genetic resources of temperate fruit and nut crop. vol. 2. Intl. Soc. Hort. Sci., The Netherlands.

- Ferguson, A.R. and E.G. Bollard. 1990. Domestication of the kiwifruit, p. 165–246. In: I.J. Warrington and G.C. Weston (eds.). *Kiwifruit: Science and management*. Ray Richards Publ., Auckland, N.Z.
- Gilg, E. 1893. Dilleniaceae. In: A. Engler and K. Prantl (eds.). *Die natürlichen Pflanzenfamilien*. Verlag Wilhelm Engelmann, Leipzig.
- He, Z-C., S-M. Wang, H. Huang, E-E-Q. Huang. 1998. Study on chromosome numbers of six species and one variety in *Actinidia* Lindl. J. Wuhan Bot. Res. 16:299–301.
- He, Z-C., Y. Zhong, H-T. Liu, X-H. Tang, L. Ye, D-S. Huang, and L-M. Xu. 2000. Quantitative taxonomic analyses of *Actinidia* (Actinidiaceae) in China based on micromorphological characters of foliar trichomes. *Acta Phytotaxonomica Sinica* 38: 121–136.
- Huang, H., F. Dane, Z-R. Wang, Z-W. Jiang, R-H. Huang, and S-M. Wang. 1997. Isozyme inheritance and variation in *Actinidia*. *Heredity* 78:328–336.
- Huang, H., J-Q. Li, P. Lang, and S-M. Wang. 1999. Systematic relationships in *Actinidia* as revealed by cluster analysis of digitized morphological descriptors. *Acta Hort.* 498:71–78.
- Huang, H., J-J. Gong, S-M. Wang, Z-C. He, Z-H. Zhang, and J-Q. Li. 2000. Genetic diversity in the genus *Actinidia*. *Chinese Biodiversity* 8: 1-12.
- Huang, H. and A.R. Ferguson. 2001. Kiwifruit in China. *N.Z. J. Crop. Hort. Sci.* 29:1–14
- Huang, R-H. and S-M. Wang. 1995. *Actinidia persicina*—A new species of *Actinidia*. *J. Wuhan Bot. Res.* 13: 113-115.
- Jiang, H-Z. 1995. Two species of *Actinidia* from Yunnan. *J. S.W. Agr. Univ.* 17:93–96
- Li, H-L. 1952. A taxonomic review of the genus *Actinidia*. *J. Arnold Arb.* 33: 1-6 l.
- Li, J-Q., Q. Cai, and H. Huang. 2000. On the phylogeny of the genus *Actinidia* Lindley. p. 80–86. In: H. Huang (eds.). *Advance in Actinidia research*. Science Press, Beijing, China.
- Li, R-G., M-Y. Liang, J-W. Li, and S-Z. Mao. 1996. Studies on the biological characteristic of genus *Actinidia*. *Guihaia* 16:265–272.
- Liang, C-F. 1983. On the distribution of *Actinidia*. *Guihaia* 3:229–248.
- Liang, C-F. 1984. *Actinidia*, p. 196–268. In: K-M. Feng (eds.). *Flora reipublicae popularis sinicae*. Science Press, Beijing, China.
- Liang, C-F. 198X. New species *Actinidia guilinensis*. *Guihaia* 8:129–131.
- McNeilage, M.A. and J.A. Considine. 1989. Chromosome studies in some *Actinidia* taxa and implications for breeding. *N.Z. J. Bot.* 27:71–81.
- Rohlf, F.J. 1994. NTSYS-pc, numerical taxonomy and multivariate analysis system. Exeter Publ., New York
- Shi, S-D., Q-B. Wang, and Z-S. Zhang. 1994. New taxa of the genus *Actinidia* from Guizhou. *Acta Bot. Yunnanica* 16:335–347.
- Sun, H-M. and R-H. Huang. 1994. A new species of *Actinidia*. *J. Wuhan Bot. Res.* 12:321–323
- Testolin, R. and A.R. Ferguson. 1997. Isozyme polymorphism in the genus *Actinidia* and the origin of the kiwifruit genome. *Syst. Bot.* 22:685–700
- Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroo, S.M. Williams, B.P. Dancik, and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proc. Natl. Acad. Sci. USA* 84:2097–2100.
- Webby, R.F., R.D. Wilson, and A.R. Ferguson. 1994. Leaf flavonoids of *Actinidia*. *Biochem. Syst. Ecol.* 22:277–286.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid. Res.* 18:6531–6535.
- Yan, G., A.R. Ferguson, and M.A. McNeilage. 1994. Ploidy races in *Actinidia chinensis*: Implications for breeding. *Euphytica* 78:175–183.
- Yan, G., J. Yao, A.R. Ferguson, M.A. McNeilage, A.G. Seal, and B.G. Murray. 1997. New reports of chromosome numbers in *Actinidia* (Actinidiaceae). *N.Z. J. Bot.* 35:181–186.
- Xiong, Z-T. and R-H. Huang. 1988. Chromosome numbers of 10 species and 3 varieties in *Actinidia* Lindl. *Acta Phytotaxonomica Sinica* 26:245–247
- Xu, K-R. and D-Z. Li. 1983. A preliminary research on the numerical classification of the genus *Panax* from China. *Acta Phytotaxonomica Sinica* 21:34–43
- Zao, Y-L. and X-P. Liu. 1996. Study on the geographical distribution and floristic characteristics of the genus *Actinidia* Lindl. from Hunan. *Guihaia* 16:9–16