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An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS

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Members of the genus *Camellia* interbreed relatively freely and several natural species hybrids exist. Species introgression into the cultivated germplasm of tea, *Camellia sinensis* L. (O. Kuntz), from related *Camellia* species has been postulated, and it is thought that teas currently under cultivation are not archetypal varieties. Randomly amplified polymorphic DNAs (RAPDs) and organelle-specific polymerase chain reactions were used to establish the affinities among cultivated tea and its wild relatives. The measures of similarity obtained indicated that RAPDs were taxonomically informative in *Camellia*, and the species relationships revealed were generally consistent with those obtained using morphological, compatibility and terpenoid affinities. Species-specific RAPD products and products potentially diagnostic of introgressive hybridization into the cultivated gene pool were identified. The organellar genomes were remarkably conserved, with polymorphism detected in only one of four noncoding regions in the chloroplast and mitochondrial genomes.

Keywords: *Camellia* spp., gene introgression, phenetics, randomly amplified polymorphic DNA, similarity.

Introduction

A fundamental goal of germplasm collection and conservation is the understanding of genetic relationships within and between the species of concern. A good understanding is critical for the effective organization and management of such germplasm collections. In plant breeding programmes, estimates of genetic relationships can be useful for the identification of parents for hybridization, and for reducing the number of accessions needed to maintain a broad range of genetic variability. The genus *Camellia* is composed of over 80 taxa (Sealy, 1958), of which only one, *C. sinensis* L. (O. Kuntz), is currently used commercially as a source of the beverage tea. The potential for economic use of other species as a beverage is, however, real, and several, including *C. taliensis*, *C. grandibractiata*, *C. kwangsiensis*, *C. gymnogyna*, *C. crassicolumna*, *C. tachangensis*, *C. ptilophylla* and *C. irrawadiensis*, are already used in parts of Asia (Chang & Bartholomew, 1984). In addition, seed from *C. japonica* and *C. oleifera* have been widely used for oil extraction in Japan and China, respectively (Sealy, 1958), and most *Camellia* spp. are of great ornamental value.

During the past century, a number of studies have examined the relationships between *Camellia* species in cultivation (Sealy, 1958; Chang & Bartholomew, 1984 and references cited therein; Chuangxing, 1988; Tien-Lu, 1992). In general, morphological characters, phytochemicals and terpenoids have been used. However, it has been argued that these may not reflect the true level of genetic differentiation, as most are subject to large environmental effects. Leaf terpenoids have, nevertheless, proved useful in analysing closely related species (Takeo, 1983; Nagata, 1986; Owuor *et al*., 1987; McDowell *et al*., 1995), but at higher levels of classification they are considered to be less useful owing to convergence

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and reticulate evolution. Ackerman (1973) and Takeda (1990) have evaluated cross-compatibility of several *Camellia* species to determine their phenetic relationships. Because most species within the genus hybridize freely, several species complexes have been formed that have ultimately made evaluation of relationships even more daunting. Several minor taxa have been treated as conspecific with major taxa, although more recently accumulated evidence has shown that these minor taxa have no natural distribution and are derived from hybridization events involving different species (Parks *et al*., 1967; Uemoto *et al*., 1980). By analysing for the presence and distribution of phytochemicals (e.g. eugenol glycoside, sasanquin and fluorescent flavonoid sulphates) in a wide range of *Camellia* species and their hybrids, Parks *et al*. (1981) were able to demonstrate the introgression of *C. japonica* traits into *C. sasanqua* giving hybrids that were treated as a distinct species (*C. hiemalis*) by Chang (1981).

Two of the major monographers of the genus, Sealy (1958) and Chang (Chang & Bartholomew, 1984), have identified two patterns of species relationships with very different arrangements of subgenera and generic sections. The more recent of these studies (Chang & Bartholomew, 1984) has recognized *Camellia* as comprising two interfertile ancestor groups. Ancestor group 1 comprises one distinct subgeneric group, *Camellia*, and a second ill-defined complex group, *Protocamellia*. Ancestor group 2 is represented by two distinct subgenera, *Thea* and *Metacamellia*. The subgenus *Protocamellia* has morphological characteristics of all the other three subgenera but does not merit treatment as a distinct genus (Chang & Bartholomew, 1984). The subgenus *Thea* has eight sections, one of which (section *Thea*) comprises cultivated tea (Chang & Bartholomew, 1984; Chuangxing, 1988). Tien-Lu (1992), however, recognized only seven sections in the subgenus *Thea*. It has been proposed that all *Camellia* have a common ancestor and evolved from the primitive *Protocamellia* in two distinct directions, one for *Thea* and *Metacamellia* and the other for *Camellia* (Chang & Bartholomew, 1984).

Species compatibility studies within the genus have shown that most interspecific (inter- and intrasectional as well as intrageneric) crosses can be made without much difficulty, although some groups are more compatible than others (Ackerman, 1973; Takeda, 1990). This provides opportunities for breeders to exploit the wide germplasm of *Camellia* by looking at other species for traits of interest in their breeding endeavours. Knowledge of genetic relationships between tea and the other related species is, therefore, vital for the efficient selection of parents for interspecific hybridization.

Molecular markers, such as the restriction fragment length polymorphisms (RFLPs) and randomly amplified polymorphic DNAs (RAPDs), provide an efficient means of estimating genetic relationships (Ecke & Michaelis, 1990; Miller & Tanksley, 1990; Jung *et al*., 1993). Because of their simplicity, RAPDs have been widely used in establishing relationships within and between different species (Adams & Demeke, 1993; Ratnaparkhe *et al*., 1995; Yamagishi, 1995; Hoey *et al*., 1996; Orozco-Castillo *et al*., 1996). In *Camellia sinensis*, RAPDs have been demonstrated to be useful in genotype differentiation within cultivated germplasm (Wachira *et al*., 1995). Variation in chloroplast (cp) and mitochondrial (mt) DNAs has been used to examine relationships among distantly related taxa (Waugh *et al*., 1990; van de Ven *et al*., 1993; Olmstead & Palmer, 1994). These studies have been simplified by the design of consensus primers that can be used in PCR to amplify homologous regions in different species and have proved to be highly informative (Taberlet *et al*., 1991; Demesure *et al*., 1995). Here, we report the use of RAPD markers and PCR amplification of chloroplast and mitochondrial sequences to assess relationships among cultivated tea and allied wild species of the genus *Camellia*. The results obtained have been compared with existing information based on cross-compatibility and morphological markers used mostly in present-day taxonomy.

Materials and methods

Plant material

Twenty *Camellia* species belonging to eight sections of the genus *Camellia* were used in this study (Table 1). These included nine tea cultivars (*C. sinensis*) and two closely related wild species, *C. irrawadiensis* and *C. taliensis*. A total of 28 genotypes were evaluated.

DNA isolation and PCR amplification

DNA was isolated using the modified method of Gawel & Jarret (1991) as described by Orozco-Castillo *et al*. (1994). RAPD reactions were conducted on individual DNA samples as described by Wachira *et al*. (1995). Numerous adjustments were initially examined in order to optimize the RAPD assay to achieve the necessary reproducibility and resolution for all *Camellia* spp. The arbitrary sequence 10-mer primers used were either obtained

Table 1 *Camellia* genotypes studied and their taxonomic affinities*

Subgenus	Section	Species	Source
Camellia	*Camellia*	*C. pitardii*	NRIVOT, Japan
		C. saluensis	NRIVOT, Japan
	Paracamellia	*C. brevistyla*	NRIVOT, Japan
		C. kissi	NRIVOT, Japan
		C. miyagii	NRIVOT, Japan
		C. tenuiflora	NRIVOT, Japan
	Oleifera	*C. oleifera*	NRIVOT, Japan
		C. sasanqua	NRIVOT, Japan
	Furfuracea	*C. furfuracea*	NRIVOT, Japan
Protocamellia	*Archecamellia*	*C. granthamiana*	NRIVOT, Japan
Metacamellia	*Camelliopsis*	*C. assimilis*	NRIVOT, Japan
		C. salicifolia	NRIVOT, Japan
	Theopsis	*C. fraterna*	NRIVOT, Japan
		C. lutchuensis	NRIVOT, Japan
		C. nokoensis	NRIVOT, Japan
		C. rosaeflora	NRIVOT, Japan
		C. tsai	NRIVOT, Japan
Thea	*Thea*	*C. irrawadiensis*	TRFK, Kenya
		C. taliensis	NRIVOT, Japan
		C. sinensis(var. *sinensis*) Yabukita	NRIVOT, Japan
		C. sinensis (var. *assamica*) AK1296	NRIVOT, Japan
		C. sinensis (var. *assamica*) URLC1	PBI, U.K.
		C. sinensis (var. *sinensis*) TJL4	PBI, U.K.
		C. sinensis (var. *assamica*) B6/61	PBI, U.K.
		C. sinensis (var. *assamica*) S15/10	TRFK, Kenya
		C. sinensis (var. *sinensis*) 56/89	TRFK, Kenya
		C. sinensis (var. *sinensis*) K/Purple	TRFK, Kenya
		C. sinensis (var. *assamica*) BB21	TRFK, Kenya

*After Chang (1981).
NRIVOT, National Research Institute of Vegetables, Ornamental Plants and Tea; TRFK, Tea Research Foundation of Kenya; PBI, Plant Breeding International.

from Operon Technologies (Almeda, CA, U.S.A.) or were synthesized at the Scottish Crop Research Institute on an Applied Bio-Systems 392 PCR-mate oligonucleotide synthesizer. The 39 informative primers finally screened and chosen for analysis are listed in Table 2a. Some organelle-specific universal primers listed in Table 2b were used to amplify homologous noncoding regions of the mtDNA and cpDNA. PCRs were performed as for RAPD, but with different annealing temperatures (shown in Table 2b). All PCR products were fractionated in agarose gels and visualized as described previously (Wachira *et al*., 1995). Amplified cp and mtDNA products were screened for insertions, deletions and substitutions by digestion with 11 restriction enzymes. The enzymes used were *Hae*III, *Rsa*I, *Msp*I, *Taq*I, *Ava*I, *Bam*HI, *Eco*RI, *Acc*I, *Hin*fI, *Sma*I and *Dra*I.

Data analyses

RAPD bands were scored for presence (1) or absence (0). Only data from intensely stained unambiguous polymorphic bands were used for statistical analysis. Bands of similar size but with intensity differences were not included in the analysis. Measures of similarity (Nei & Li, 1979) and principal coordinate analyses were derived with the GENSTAT 5 (1987) statistical package.

Results and discussion

RAPDs

Of 45 decamer primers screened on a subset of four *Camellia sinensis* genotypes, 39 consistently produced the same multiband fingerprints. These

Table 2a Primers used for the detection of polymorphism in *Camellia* species and their sequences

Primer	Sequence	Primer	Sequence
SC10-01	5′-GGTAGCAGTC-3′	OPR-05	5′-GACCTAGTGG-3′
SC10-12	5′-GTTTCCGGTG-3′	OPR-20	5′-ACGGCAAGGA-3′
SC10-19	5′-CGTCCGTCAG-3′	OPU-15	5′-ACGGGCCAGT-3′
SC10-97	5′-TCCGGCTTTC-3′	OPU-20	5′-ACAGCCCCCA-3′
OPA-09	5′-GGGTAACGCC-3′	OPB-03	5′-CTCCCTGCAA-3′
OPB-10	5′-CTGCTGGGAC-3′	OPV-14	5′-AGATCCCGCC-3′
OPD-08	5′-GTGTGCCCCA-3′	OPV-15	5′-CAGTGCCGGT-3′
OPG-09	5′-CTGACGTCAC-3′	OPV-20	5′-CAGCATGGTC-3′
OPH-09	5′-TGTAGCTGGG-3′	OPW-01	5′-CTCAGTGTCC-3′
OPI-04	5′-CCGCCTAGTC-3′	OPW-02	5′-ACCCCGTCAA-3′
OPJ-05	5′-CTCCATGGGG-3′	OPW-04	5′-CAGAAGCGGA-3′
OPJ-07	5′-CCTCTCGACA-3′	OPW-06	5′-AGGCCCGATG-3′
OPK-15	5′-CTCCTGCCAA-3′	OPW-11	5′-CTGATGCGTG-3′
OPL-01	5′-GGCATGACCT-3′	OPW-14	5′-CTGCTGAGCA-3′
OPM-14	5′-AGGGTCGTTC-3′	OPW-18	5′-TTCAGGGCAC-3′
OPN-03	5′-GGTACTCCCC-3′	OPX-08	5′-CAGGGGTGGA-3′
OPQ-10	5′-TGTGCCCGAA-3′	OPX-09	5′-GGTCTGGTTG-3′
OPQ-12	5′-AGTAGGGCAC-3′	OPX-17	5′-GACACGGACC-3′
OPQ-15	5′-GGGTAACGTG-3′	OPY-06	5′-AAGGCTCACC-3′
OPR-03	5′-ACACAGAGGG-3′		

Table 2b Primer sequences used to amplify specific chloroplast and mitochondrial regions of the *Camellia* genome

Region amplified	Primer name	Sequence	Annealing temperature (°C)
−V7 region mt rDNA	Mt-P1V7 Mt-P2V7	5′-TATGAACAACAAACCTGTCTTTAACGGGATGG-3′ 5′-GCGGACTTGACGTCATCCCCACCTTCCTCCAG-3′	53
cp-*trn* L (UAA) intron	Intron 1 Intron 2	5′-CGAAATCGGTAGACGCTACG-3′ 5′-GGGGATAGAGGGACTTGAAC-3′	54
cp-*trn* L (UAA)–*trn* F (GAA) Intergenic region	Rus 7 Rus 8	5′-GGTTCAACTCCCTCTATCCC-3′ 5′-AATTGAACTGGTGACACGAG-3′	54
cp-*trn* T (UGU)–*trn* L (UAA) Intergenic region	Inter A Inter B	5′-CATTACAAATGCCATCGTCT-3′ 5′-TCTACCGATTTCGCCATATC-3′	54

were then used to screen the 28 genotypes listed in Table 1. Consistently well-amplified products ranged in size from 0.3 to 2.3 kb. Figure 1 shows a typical example of the polymorphism generated. A total of 197 unambiguous polymorphic bands were scored in the 28 genotypes. We could not exclude the possibility that different-sized DNA fragments contained homologous DNA sequences or that similar-sized products were nonhomologous. In addition, we did not attempt to confirm that the amplified fragments were exclusively generated from nuclear DNA. Measures of similarity based on single-linkage cluster analysis and principal coordinate analysis were used to examine the data. These measures have been strongly recommended for RAPDs, as the use of numerous random characters results in errors of homology or mis-scoring being accounted for as 'noise' in the analyses. Genetic similarity within the nine cultivated genotypes of *Camellia sinensis* ranged from 0.48 to 0.82 (results not shown). The tea accessions studied therefore exhibit extensive RAPD variation, which is consistent with an earlier study (Wachira *et al*., 1995). Within the wild *Camellia* germplasm, the similarity coefficients ranged

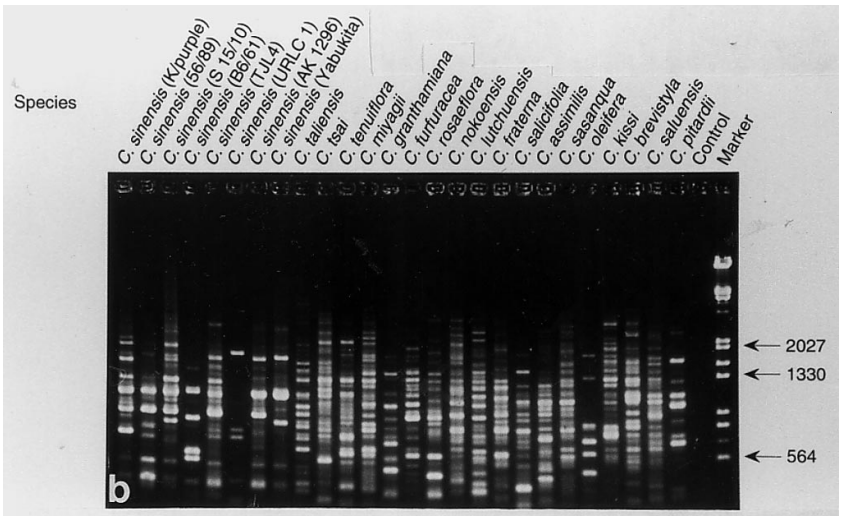

Fig. 1 RAPD profiles of *Camellia* species generated with primer OPV-14 (5′-AGATCCCGCC-3′).

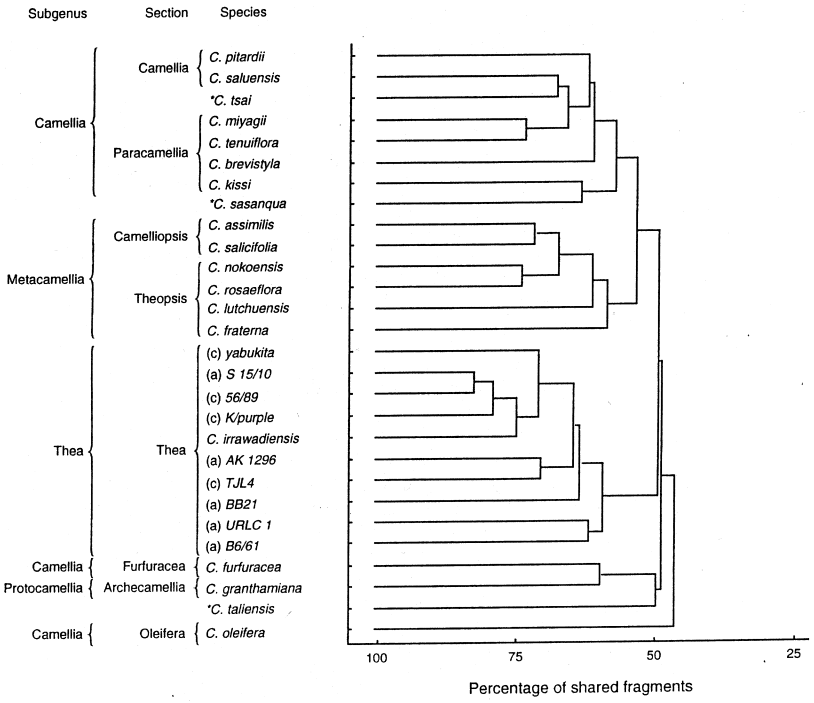

Fig. 2 A dendrogram of the relationships based on RAPD data between 28 taxa of *Camellia* derived from average linkage cluster analysis. *These species did not cluster in their expected groups.

between 0.39 and 0.74 indicating an even greater degree of genetic differentiation.

A dendrogram obtained from individual pairwise comparisons of the 197 RAPD bands among the 28 genotypes generated using average linkage cluster analysis is shown in Fig. 2. Except for a few species (highlighted with asterisks), most cluster within their subgeneric and sectional groups. The short internode branch distances, particularly that separating the subgenus *Thea* from the *Camellia* and *Metacamellia* subgenera, do, however, suggest that these groups may be unstable and that a larger data set is required to increase the overall robustness of the analysis. Given this, it is not surprising that the topology of the dendrogram indicates substantial overlap between different species within the genus. However, *Camellia oleifera* was noticeably discrete, constituting a major branch of the dendrogram. *Camellia furfuracea*, *C. granthamiana* and *C. taliensis* classified in sections *Furfuracea*, *Archecamellia* and

Oleifera, respectively, formed a clade, which joined the subgeneric groups *Camellia*, *Metacamellia* and *Thea* at 50 per cent similarity. *Camellia taliensis* was, however, the most dissimilar member of this group. Species of section *Thea* separated from the *Metacamellia*/*Camellia* subgeneric subgroups at 53 per cent similarity. *Camellia irrawadiensis*, a wild tea not traditionally cultivated for the production of leaf tea, was clustered (76 per cent similarity) with *C. sinensis* var. *sinensis* accession K/Purple, a commercial tea clone that contains the purple/brick red anthocyanin plant pigments characteristic of *C. irrawadiensis*. Cultivar K/Purple is most probably a natural hybrid between *C. sinensis* var. *sinensis* and *C. irrawadiensis* (our personal observations). Some species hybrids, at least those involving tea and *C. irrawadiensis*, are known to exist in the wild in parts of Burma and some may have found their way into the cultivated germplasm undetected because of their superficial morphological similarity (Wight & Barua, 1957; Wood & Barua, 1958).

Intersectional crosses undertaken by Ackerman (1973) revealed that three species of section *Paracamellia* hybridized readily among themselves, indicating a close relationship, which was typical of ecospecies. Section *Paracamellia*, however, has one of the greatest distributional areas in the genus *Camellia* and would be expected to be associated with greater diversity. RAPD markers indeed revealed extensive polymorphism within the group. Ackerman's (1973) work, however, involved the three species, *C. kissi*, *C. oleifera* and *C. sasanqua*, whose taxonomy has since been revised (Chang & Bartholomew, 1984). In the present study, *C. kissi* grouped closely with *C. sasanqua* (see Fig. 1), although both are now classified in sections *Paracamellia* and *Oleifera*, respectively. Although species of section *Theopsis* also grouped closely, crosses within the section are often difficult, reflecting behaviour typical of cenospecies and suggesting the existence of genetic isolation barriers usually associated with more distant crosses (Ackerman, 1973). This is strange because the group has a very narrow distribution (Chang & Bartholomew, 1984).

To evaluate the relationships between sectional and subgeneric groups, grouped data analysis was performed. Similarity values (Nei & Li, 1979) ranged from 62.3 per cent between sections *Theopsis* and *Camelliopsis* to 46.4 per cent between sections *Thea* and *Archecamellia*. The similarities between the other sectional groups were almost equal (50–58.9 per cent). An UPGMA dendrogram derived from the grouped data analysis shows section *Thea* as discrete, constituting a major branch of the dendrogram (Fig. 3). Separation of species of section *Thea* from the rest of the members of genus *Camellia* has also been demonstrated by studies on flavanol patterns (Nagata, 1986). Only members of *Thea* in the entire genus contain galloyled catechins, such as (−)-epicatechin gallate and (−)-epigallocatechin gallate. Similarly, only this group contains caffeine (although *C. kissi* contains trace amounts). The amino acid, theanine, is also only present in the leaves of section *Thea*.

Even given the potentially low resolution of the dendrogram (revealed as short internode branches), sections *Camellia* and *Paracamellia* as well as sections *Theopsis* and *Camelliopsis* cluster into separate groups. This result is presented graphically in the form of a principal coordinate plot (PCO) in Fig. 4 in which the first two principal components accounted for 35.2 per cent of the total variation. All the sections generally group according to their supposedly related taxa. Sections *Theopsis* and *Camelliopsis* (the latter also known as *Eriandra*) clustered to form the subgenus, *Metacamellia*. In the translation of Chang's monograph, Chang & Bartholomew (1984) noted that species of these two closely related sections shared many highly derived characteristics. Results presented here also suggest that the subgenus *Thea* has strong affinity to the subgenus *Metacamellia* and more so to section *Theopsis* than *Camelliopsis*, an observation also noted by Chang & Bartholomew (1984). Section *Archecamellia* was the most distant section from *Thea* and probably the most ancestral. Based on analysis of morphological

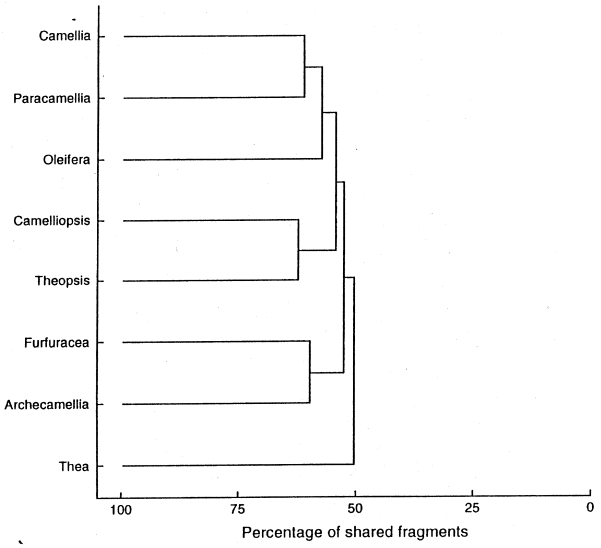

Fig. 3 A dendrogram of eight sections of genus *Camellia* derived by average linkage cluster analysis.

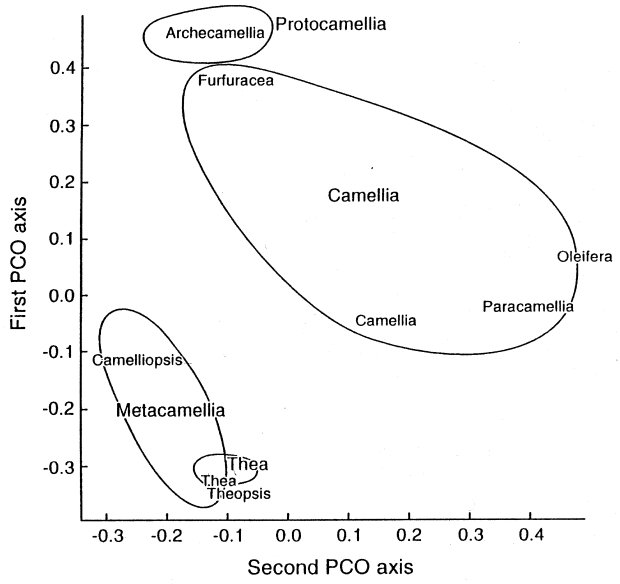

Fig. 4 A principal coordinate plot of eight sections of the genus *Camellia* (based on a reduced similarity matrix). First and second principal components accounted for 35.2 per cent of the total variation.

differentiation and species distribution, Tien-Lu (1992) has suggested that *Thea* evolved from section *Archecamellia* of subgenus *Protocamellia*. Sections *Furfuracea*, *Camellia*, *Paracamellia* and *Oleifera* were grouped to form the large subgenus *Camellia*. The PCO analysis indicates that, although the sectional groups of the subgenus *Camellia* were dispersed, they were clearly distinct from the others and could therefore be grouped. The dispersion may reflect the size and diversity of the subgenus, which is the largest and most widely grown.

RAPDs, therefore, appear to provide useful information on genetic relatedness at the sectional level in *Camellia*. The results presented here indicate that these are in the decreasing order: *Thea* — *Theopsis* — *Camelliopsis* — *Camellia* — *Paracamellia* — *Oleifera* — *Furfuracea* — *Archecamellia*. This is in general agreement with relationships derived by the use of compatibility studies (Takeda, 1990; Ackerman, 1993). However, *Thea* species hybridize more easily with *Camelliopsis* than with *Theopsis* (Ackerman, 1973; Takeda, 1990), which may indicate a closer affinity between these sections.

RAPDs have been widely used in phenetic studies in different species, e.g. *Coffea* (Orozco-Castillo *et al*., 1996), *Juniperus* (Adams & Demeke, 1993), *Pisum* (Hoey *et al*., 1996), *Beta* (Jung *et al*., 1993), *Cajanus* (Ratnaparkhe *et al*., 1995), *Lentil* (Sharma *et al*., 1995) and *Lilium* (Yamagishi, 1995). Overall, the results from these and other studies have conformed closely to those derived from nuclear and chloroplast RFLPs, isozymes, cytogenetic analysis and morphological affinities.

A single RAPD, OPN-03-1400, which was diagnostic of species of section *Thea* used here, was identified (data not shown). Such diagnostic markers are important for strain identification and cultivar characterization and can be used to detect instances of natural interspecific gene introgression. Section-specific RAPD markers have been identified in *Lilium* (Yamagishi, 1995). In such an internally mixed genus like *Camellia*, species-specific markers will be important in determining the flow of genetic material via species introgression. Here, products that are specific to species of other sections and that are only present in tea (*C. sinensis*) at low frequency may be candidates for further analyses of interspecific gene flow in *Camellia*. Such products include bands OPV-03-700 and OPW-18-700, both of which were present in all species of sections *Camelliopsis* and *Theopsis* but only present in two genotypes of section *Thea* (*C. irrawadiensis* and *C. sinensis* accession K/Purple). Further analysis with more species will, however, be required to establish fully the specificity of loci to particular taxa and subsequent interspecific gene flow into tea.

Organellar DNA

Because of their relative resistance to evolutionary change, compared with nuclear DNA, cpDNA and mtDNA sequences have been widely used to investigate interspecific relationships (Jorgensen & Cluster, 1989; Waugh *et al*., 1990; van de Ven *et al*., 1993; Olmstead & Palmer, 1994). Noncoding regions display higher rates of evolution than coding regions and are, therefore, desirable targets for phylogenetic studies. The resolution of many such noncoding regions has been improved by PCR amplification with universal primers (Taberlet *et al*., 1991; Demesure *et al*., 1995) and subsequent direct sequencing. However, the relatively high frequency of insertions/deletions may even, in some cases, make it possible to use the size of PCR product as a genetic marker. The choice of cp/mtDNA regions that maximize phylogenetic information is, however, dependent on the evolutionary timescale of the plant system being studied. The four noncoding regions of the cp and mt genomes amplified with universal primers did not reveal any size polymorphic products in *Camellia*. A 980 bp product was amplified for the *trn*T(UGU) — *trn*L(UAA) intergenic spacer, 410 bp and 200 bp products from the *trn*L(UAA) 3′ exon and

*trn*F(GAA), respectively, and a 600 bp product from the V7 region of mtrDNA. When the amplified products were screened for insertions, deletions and substitutions by digestion with 11 restriction enzymes, no polymorphism was revealed. Only one cpDNA PCR product revealed a single-strand conformation polymorphism (SSCP) (results not shown). This SSCP in the intergenic spacer between the *trnL*(UAA) 3′ exon and *trnF*(GAA) indicated that *C. furfuracea* (section *Furfuracea*), *C. assimilis* (section *Camelliopsis*), *C. nokoensis* and *C. tsaii* (both of section *Theopsis*) shared a common haplotype. The implications of this observation are not clear, although it may indicate possible hybridization between species of the sections involved. SSCP is based upon secondary and tertiary structural conformational differences in single-stranded DNA (Orita *et al*., 1989). The resolution of SSCPs is, however, limited by the size of fragment — the larger it is, the more difficult it is to be resolved effectively. The fragment resolved by SSCP in this study was the smallest. The exact mutational changes responsible for generating the SSCPs were, however, not determined, and it is possible that different events have resulted in a similar SSCP profile. The amplified products will have to be sequenced to determine their precise relationships. A limitation in the use of cpDNA and mtDNA data is that uniparental inheritance effectively eliminates the systematic analysis of taxa derived from hybridization owing to exclusion of information from one of the parental clones. Nevertheless, the lack of size polymorphic markers, PCR-RFLPs and SSCPs from the cp and mtDNA regions amplified using universal primers may lend credence to the theory that all *Camellia* species share a relatively recent and common ancestry.

Conclusions

Results presented here suggest that RAPDs are phenetically informative in *Camellia*, with the species affinities described being generally similar to those obtained using morphological, compatibility and terpenoid data. RAPDs, however, provided a greater degree of resolution at the subsectional and subspecies levels. In similar studies, RAPDs have also provided greater resolution than other technologies (Sharma *et al*., 1995). As RAPDs are abundant, quick to generate, largely reflect unselected genetic alterations, and are not subject to environmental influences, they could provide a useful complement to most traditional and classical systematic characters in *Camellia*.

This study represents only the first step in using DNA-based markers as a tool to implement studies of molecular systematics in this large genus. The inclusion of additional accessions and other species and the use of an increased number of primers may provide a greater resolution of the affinities among these taxa. Direct sequencing of amplified cpDNA and mtDNA products may also be important for recreating phylogenies.

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References

ACKERMAN, W. L. 1973. Species compatibility relationships within the genus *Camellia*. *J. Hered*., **64**, 356–358.

ADAMS, R. P. AND DEMEKE, T. 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). *Taxonomy*, **42**, 553–571.

CHANG, H. T. 1981. A taxonomy of the genus *Camellia*. *Acta Sci. Nat. University of Sunyatseni*, *Monog. Series*, **1**, 1–180.

CHANG, H. T. AND BARTHOLOMEW, B. 1984. *Camellias*. B.T. Batsford, London.

CHUANGXING, Y. 1988. The subdivisions of genus *Camellia* with a discussion on their phylogenetic relationships. *Acta Botanica Yunnanica*, **10**, 61–67 (in Chinese with English summary).

DEMESURE, B., SODZI, N. AND PETIT, R. J. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.*, **4**, 129–131.

ECKE, W. AND MICHAELIS, G. 1990. Comparison of chloroplast and mitochondrial DNA from five morphologically distinct *Beta vulgaris* cultivars: sugar beet, fodder beet, beetroot, foliage beet and Swiss chard. *Theor. Appl. Genet.*, **79**, 440–442.

GAWEL, N. J. AND JARRET, R. L. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Pl. Mol. Biol. Rep.*, **9**, 262–266.

GENSTAT 5 COMMITTEE. 1987. *Genstat 5 Reference Manual*. Clarendon Press, Oxford.

HOEY, B. K., CROWE, K. R., JONES, V. M. AND POLANS, N. O. 1996. A phylogenetic analysis of *Pisum* based on morphological characters and allozyme and RAPD markers. *Theor. Appl. Genet.*, **92**, 92–100.

JORGENSEN, R. A. AND CLUSTER, P. D. 1989. Modes and tempos in the evolution of nuclear ribosomal DNA:

new characters for evolutionary studies and new markers for genetic and population studies. *Ann. Mo. Bot. Gard.*, **75**, 1238–1247.

JUNG, C., PILLEN, K., FRESE, S., FAHR, S. AND MELCHINGER, A. E. 1993. Phylogenetic relationships between cultivated tea and wild species of the genus *Beta* revealed by DNA fingerprinting. *Theor. Appl. Genet.*, **86**, 449–457.

McDOWELL, I., TAYLOR, S. AND GRAY, C. 1995. The phenolic pigment composition of black tea liquours – Part II: Discriminating origin. *J. Sci. Food Agric.*, **69**, 475–480.

MILLER, J. C. AND TANKSLEY, S. D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.*, **80**, 437–448.

NAGATA, T. 1986. Differences in caffeine, flavanols and amino acids contents in leaves of cultivated species and hybrids in the genus *Camellia*. *Jap. Agric. Res. Quart.*, **19**, 276–280.

NEI, M. AND LI, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 5296–5273.

OLMSTEAD, R. G. AND PALMER, J. D. 1994. Chloroplast DNA and systematics – a review of methods and data analysis. *Am. J. Bot*., **81**, 1205–1224.

ORITA, M., IWAHANA, H., KANAZAWA, H., HAYASHI, K. AND SEKIYA, T. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single strand conformation polymorphisms. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 2766–2770.

OROZCO-CASTILLO, C., CHALMERS, K. J., WAUGH, R. AND POWELL, W. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theor. Appl. Genet.*, **87**, 934–940.

OROZCO-CASTILLO, C., CHALMERS, K. J., POWELL, W. AND WAUGH, R. 1996. RAPD and organelle-specific PCR reaffirms taxonomic relationships within the genus *Coffea*. *Pl. Cell Rep.*, **15**, 337–341.

OWUOR, P. O., TAKEO, T., HORITA, H., TSUSHIDA, T. AND MURAI, T. 1987. Differentiation of clonal teas by Terpene Index. *J. Sci. Food Agric.*, **40**, 341–345.

PARKS, C. R., GRIFFITHS, A. AND MONTGOMERY, K. R. 1967. A possible origin of anthocyanin (red) pigmentation in the flowers of *Camellia sasanqua. American Camellia Yearbook*, 229–242.

PARKS, C. R., KONDO, K. AND SWAIN, T. 1981. Phytochemical evidence for the genetic contamination of *Camellia sasanqua*. *Thunberg. Jap. J. Breed.*, **31**, 168–182.

RATNAPARKHE, M. B., GUPTA, V. S., VEN MURPHY, M. R. AND RANJEKAR, P. K. 1995. Genetic fingerprinting of pigeon pea (*Cajanus cajan* (L.) Millsp.) and its wild relatives using RAPD markers. *Theor. Appl. Genet.*, **91**, 893–898.

SEALY, J. 1958. *A Revision of the Genus* Camellia. Royal Horticultural Society, London.

SHARMA, S. K., DAWSON, I. K. AND WAUGH, R. 1995. Relationships among cultivated and wild lentils revealed by RAPD analysis. *Theor. Appl. Genet.*, **91**, 647–654.

TABERLET, P., GIELLY, L., PAUTON, G. AND BOUVET, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.*, **17**, 1105–1109.

TAKEDA, Y. 1990. Cross compatibility of tea (*Camellia sinensis*) and its allied species in the genus *Camellia*. *Jap. Agric. Res. Quart.*, **24**, 111–116.

TAKEO, T. 1983. Effect of clonal specificity of monoterpene alcohol composition of tea shoots on black tea aroma profile. *Jap. Agric. Res. Quart.*, **17**, 120–124.

TIEN-LU, M. 1992. A revision of *Camellia* sec. *Thea*. *Acta Botanica Yunnanica*, **14**, 115–132 (in Chinese with English summary).

UEMOTO, S., TANAKA, T. AND FUJEDA, K. 1980. Cytogenetic studies on the origin of *Camellia vernalis*. I. On the meiotic chromosomes in some related *Camellia* forms in Hirado Island. *J. Jap. Soc. Hort. Sci.*, **48**, 475–482.

VAN DE VEN, W. T. G., DUNCAN, N., RAMSAY, G., PHILLIPS, M., POWELL, W. AND WAUGH, R. 1993. Taxonomic relationships between *V. faba* and its relatives based on nuclear and mitochondrial RFLPs and PCR analysis. *Theor. Appl. Genet.*, **86**, 71–80.

WACHIRA, F. N., WAUGH, R., HACKETT, C. A. AND POWELL, W. 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome*, **38**, 201–210.

WAUGH, R., VAN DE VEN, W. T. G., PHILLIPS, M. S. AND POWELL, W. 1990. Chloroplast DNA diversity in the genus *Rubus* (Rosaceae) revealed by Southern hybridisation. *Pl. Syst. Evol.*, **172**, 65–75.

WIGHT, W. AND BARUA, D. N. 1957. What is tea? *Nature*, **179**, 506–507.

WOOD, D. J. AND BARUA, D. N. 1958. Species hybrids of tea. *Nature*, **181**, 1674–1675.

YAMAGISHI, M. 1995. Detection of section specific random amplified polymorphic DNA (RAPD) markers in *Lilium*. *Theor. Appl. Genet.*, **91**, 830–835.