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A genome-specific repeat sequence from kiwifruit (*Actinidia deliciosa* var. *deliciosa*)

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Summary. Six members of a family of moderately repetitive DNA sequences from kiwifruit (*Actinidia deliciosa* var. *deliciosa*) have been cloned and characterized. The repeat family is composed of elements that have a unit length of 463 bp, are highly methylated, occur in tandem arrays of at least 50 kb in length, and constitute about 0.5% of the kiwifruit genome. Individual elements diverge in nucleotide sequence by up to 5%, which suggests that the repeat sequence is evolving rapidly. Homologous sequences were found in *A. deliciosa* var. *chlorocarpa*. The repeat sequence was not found under low stringency hybridization conditions in the diploid *A. chinensis*, the species most closely related to the hexaploid kiwifruit, or in eight other *Actinidia* species. However, homologous repeats were detected in a tetraploid species, *A. chrysantha*. The results provide the first molecular evidence to suggest that kiwifruit may be an allopolyploid species.

Key words: *Actinidia deliciosa* – Kiwifruit – Genome-specific DNA – Tandemly repeated DNA – Methylation

Introduction

Repetitive DNA is present in almost all eukaryotic species and comprises a high proportion of the genome in most plants (Sorenson 1984; Walbot and Cullis 1985). Repetitive DNA can be dispersed throughout the genome or organized in arrays of tandem repeats. The role of such repetitive DNA sequences remains speculative. Tandemly repeated DNA is often associated with centromeric and telomeric heterochromatin (John and Miklos 1979; Bedbrook et al. 1980) leading to suggestions that it has a role in pairing of chromosomes and speciation (Bostock 1980; Rose and Doolittle 1983). Alternatively, they may be 'junk' DNA, without functional significance and concerned solely with their own survival (Doolittle and Sapienza 1980).

Recently, tandemly repeated DNA sequences have been characterized from many plant genera including *Aegilops* (Rayburn and Gill 1986, 1987), *Arabidopsis* (Martinez-Zapater et al. 1986), *Brassica* (Benslimane et al. 1986; Halldén et al. 1987), *Cucurbita* (Ganal and Hemleben 1986; Leclerc and Siegel 1987), *Linum* (Cullis and Cleary 1986), *Lycopersicon* (Schweizer et al. 1988), *Nicotiana* (Koukalová et al. 1989), *Oryza* (Wu and Wu 1987; Zhao et al. 1989), *Raphanus* (Grellet et al. 1986), *Secale* (Bedbrook et al. 1980; Appels et al. 1986), *Sinapis* (Capesius 1983), *Triticum* (Metzlaff et al. 1986) *Vicia* (Kato et al. 1984) and *Zea* (Dennis and Peacock 1984). Since repetitive DNA evolves rapidly between species (Singer 1982), it has been utilized as a source of probes that are species-specific (*Lycopersicon* and *Solanum*, Schweizer et al. 1988; *Triticum*, Metzlaff et al. 1986) or genome-specific (*Aegilops*, Rayburn and Gill 1986, 1987; *Oryza*, Zhao et al. 1989). Such repetitive DNA sequences can be used as phylogenetic tools to study evolutionary relationships among species (Halldén et al. 1987; Zhao et al. 1989).

We are interested in determining the origin of the hexaploid genome of kiwifruit (*A. deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson var. *deliciosa*), a member of the Actinidiaceae. A close relationship has long been recognized between *A. deliciosa* and *A. chinensis* Planch; indeed, *A. deliciosa* was initially considered to be a subspecies of *A. chinensis* (Chevalier 1941; Liang 1975). Recently, we confirmed this close relationship using RFLP analysis with both nuclear and chloroplast probes (Crowhurst et al. 1990). We would like to determine whether *A. deliciosa* has an autopolyploid origin, with

A. chinensis as a probable progenitor, or an allopolyploid origin.

This paper reports the isolation and cloning of an *A. deliciosa* genome-specific repeat, following reassociation between DNA from *A. deliciosa* and *A. chinensis*. Several members from this tandem repeat family within *A. deliciosa* are characterized, and the distribution of homologous sequences in other *Actinidia* species is discussed with respect to the origins of *A. deliciosa*.

Materials and methods

Plant material

All plant material was isolated from a collection of plants held in the DSIR research orchards at Kumeu and Te Puke. DNA was isolated from *A. arguta* var. *arguta, A. chrysantha, A. eriantha* f. *eriantha, A. indochinensis, A. kolomikta, A. latifolia* var. *latifolia, A. polygama, A. rufa,* and *A valvata*. In addition, DNA was isolated from single plants of *A. deliciosa* var. *chlorocarpa* and of *A. deliciosa* var. *deliciosa* cultivars (female: 'Allison,' 'Bruno,' Elmwood,' 'Gracie,' 'Greensill,' 'Hayward,' 'Jones,' 'Monty,' and male: 'Matua,' M56 and M58). All of the *A. deliciosa* var. *deliciosa* plants were derived from the original 1904 accession of this species into New Zealand. DNA was also isolated from one female plant from the *A. deliciosa* var. *deliciosa* accession CCH1 of 1975. *A. chinensis* leaves and cuttings were obtained from a small population of plants derived from seed obtained from China in 1977 and 1981 (DSIR seed accessions CCH4, CN12, and CN21).

Isolation of DNA

DNA was isolated from young leaves collected from shoots immediately after bud burst, either from orchard-grown plants or from water-cultured canes (Crowhurst et al. 1990). Total DNA was isolated using a modification of the cetyltrimethylammonium bromide procedure of Taylor and Powell (1983). Following the initial chloroform extraction (Taylor and Powell 1983), nucleic acids were precipitated by addition of an equal volume of isopropanol. Precipitated nucleic acids were recovered by spooling. High-molecular-weight DNA was then purified away from polysaccharides by sucrose gradient centrifugation (Maniatis et al. 1982). DNA prepared with this method was greater than 100 kb in length as estimated from electrophoresis in 0.35% (w/v) agarose.

Cloning of repetitive DNA sequences

DNA from *A. chinensis* was randomly sheared by sonication using a MSE Soniprep model 150 with a 15 s pulse, which produced fragments with an average size of 1,000 bp. Formamide-Phenol Emulsion Reassociation (F-PERT) reactions were carried out as described by Casna et al. (1986), using 250 µg of sheared *A. chinensis* DNA with 1 µg of *Mbo*I-cleaved *A. deliciosa* var. *deliciosa* DNA. Reassociations were incubated for 24 h on a gel-rocker at 60 rpm (the minimum setting required to maintain an emulsion) and an amplitude of 7.5 cm. Reassociation reactions were purified as described elsewhere (Casna et al. 1986).

Reassociated DNA was ligated into alkaline phosphatase-treated, *Bam*HI-cleaved pUC19 and used to transform *E. coli* DH5α cells made competent by a Ca^{2+}/Mn^{2+} treatment (D. Alexander, personal communication). DH5α cells were grown overnight at 30 °C in 2XL broth (2% bacto-tryptone, 1% yeast extract, 0.1% NaCl, 0.2% glucose, pH 7.0). Fifty-milliliter cultures of 2XL broth were inoculated with 0.5 ml of the overnight growth and shaken at 140 rpm, 30 °C, until the A600 reached 0.45–0.55. The cells were chilled in ice water for 2 h and then centrifuged in 50-ml polypropylene centrifuge tubes at 2,500 g for 10 min at 4 °C.

Cells were resuspended gently in 5 ml of ice-cold buffer (100 m*M* $CaCl_2$, 70 m*M* $MnCl_2$, 40 m*M* NaOAc, freshly made, adjusted to pH 5.5 using 0.1 *M* glacial acetic acid, and filter sterilized). Suspended cells were diluted to 40 ml with the same buffer, incubated on ice water for 45 min, centrifuged at 1,800 *g* for 5 min, and resuspended very gently in 5-ml buffer. Eighty percent glycerol was added drop-wise to a final concentration of 15% (v/v). Cells were stored at −70 °C before transformation. DNA (0.05–10 ng in 100 µl) was added to 200 µl cells, incubated on ice for 30 min, heat-treated at 37 °C for 5 min, diluted with 3.7 ml of prewarmed (37 °C) 2XL broth, and incubated at 37 °C on a rotary shaker for 100 min before plating.

Lambda clones hybridizing to the 128-bp F-PERT repetitive element (pKIWI514) were isolated from a kiwifruit genomic library constructed in λgem11 (kindly provided by C. van Dolleweerd) and purified as described in Maniatis et al. (1982).

DNA hybridization

Restriction enzyme digestions were carried out using the conditions specified by the supplier (Bethesda Research Laboratories, except *Hgi*AI from New England Biolabs). Reactions contained either 5 µg of total genomic DNA or 1–2 µg of λ clone DNA and 10 units of restriction enzyme per microgram DNA.

Digested DNA was resolved on 0.7% (w/v) agarose in TBE buffer (Maniatis et al. 1982). DNA was transferred to Hybond-N+ (Amersham) nylon membrane by alkaline blotting using conditions recommended by the supplier.

Initial screening of F-PERT recombinant clones for genome-specific repeats was performed using ^{32}P-dCTP-labelled, total genomic DNA from *A. deliciosa* var. *deliciosa* and *A. chinensis* as hybridization probes. Plasmid DNAs were digested with *Pvu*II (which excises the insert plus 322 bp of adjacent pUC19 vector sequences), electrophoresed in 1.0% (w/v) agarose, and transferred to Hybond-N+ membrane for screening.

Genomic DNA (300 ng) or repetitive-sequence DNA purified from gels (50–150 ng) was radiolabelled with ^{32}P-dCTP (New England Nuclear) using a Multiprime DNA Labeling Kit (Amersham), and hybridized overnight at 65 °C in 1 *M* NaCl, 1% (w/v) SDS, and 10% (w/v) dextran sulphate (Sigma). Anchor skim milk powder at 1% (w/v) was used as a nonspecific blocking agent. The final wash was performed in 0.2 × SSC, 1% (w/v) SDS at 65 °C. Filters were exposed to Cronex 4 (DuPont) X-ray film at −70 °C.

Dot-blot analysis of copy number was carried out using serial dilutions (4 µg to 1.95 ng) of total genomic DNA isolated from the *A deliciosa* var. *deliciosa* cultivars 'Bruno', 'Hayward,' 'Matua,' or 'Monty.' Restriction enzymes *Xba*I and *Hin*dIII were used to cleave the repetitive DNA insert from pKIWI516. This resulted in a fragment containing an additional 6 bp of vector sequence to the left and 12 bp to the right of the repeat sequence. However, pUC19 vector sequences have been shown not to hybridize to *Actinidia* DNA (data not shown). pKIWI516 insert was purified from low-gelling-temperature agarose and serially diluted (170 ng to 80 pg). Human genomic DNA (which does not hybridize to the probe) was added prior to dilution to adjust the total DNA concentration to 4 µg. DNA dilutions were transferred using a BioRad Biodot 84R dot blotting apparatus onto HyBond-N+ membrane in the presence of 0.4 *M* NaOH, 0.6 *M* NaCl. Filters were neutralized in 0.5 *M* TRIS,

0.6 *M* NaCl, dried and then probed with ^{32}P-dCTP-labelled insert from pKIWI516 as described above. The final wash was performed in 2 × SSC, 1% (w/v) SDS at 60 °C, allowing a base pair mismatch of approximately 26%.

Southern hybridizations of genomic and λ DNAs were carried out using an Enhanced Chemiluminescence (ECL) Gene Detection System (Amersham). Purified pKIWI516 repeat element sequence (1 μg) was labelled with peroxidase according to the ECL protocols. Hybridizations were carried out overnight at 42 °C in ECL hybridization buffer containing 0.5 *M* NaCl and either 1% (w/v) milk powder or 5% (w/v) blocking agent (Amersham). Chemiluminescence was detected by exposure of either Hyperfilm-ECL or Cronex 4 (DuPont) X-ray film to filters for periods ranging between 10 and 60 s.

DNA sequence analysis

Repeat sequences cloned into pUC19 were used as templates for sequencing reactions by the dideoxynucleotide termination method (Sanger et al. 1977). A Sequenase Version II sequencing kit (USB) was used with ^{35}S-dATP and either the Universal Primer or Reverse Primer (Promega). Reaction products were fractionated on 6% (w/v) acrylamide buffer gradient gels. Analysis of sequence data was made using the computer programs of Devereux et al. (1984).

Results

We used the F-PERT technique (Casna et al. 1986) to isolate DNA sequences present in the hexaploid *A. deliciosa* var. *deliciosa* but absent from *A. chinensis*. This technique involves the reassociation of two genomes and utilizes a cloning strategy that results in the generation of a plasmid library enriched in sequences specific to one of the genomes.

Characterization of 128-bp, genome-specific repeat element

A series of *Mbo*I clones in pUC19 were generated after the F-PERT procedure. *Pvu*II digests of 48 randomly chosen clones showed that insert DNA ranged in size from 120 to 560 bp, with an average size of 350 bp. In Southern hybridizations, 2 of the 48 clones (pKIWI514 and p503-44) hybridized to total genomic DNA from *A. deliciosa* but not from *A. chinensis*. Sequence analysis of the two clones showed that they contained identical 128-bp inserts; only pKIWI514 was used for further analysis.

Southern hybridizations were then carried out using pKIWI514 as a probe against several species of *Actinidia* (data not shown). These results confirmed that the pKIWI514 sequence was present in *A. deliciosa* but not in *A. chinensis* (or in several other *Actinidia* species). In addition, they suggested that pKIWI514 was part of a larger repeat of around 460 bp and that this repeat was arranged tandemly in the genome. We therefore sought to isolate the complete repeat sequence.

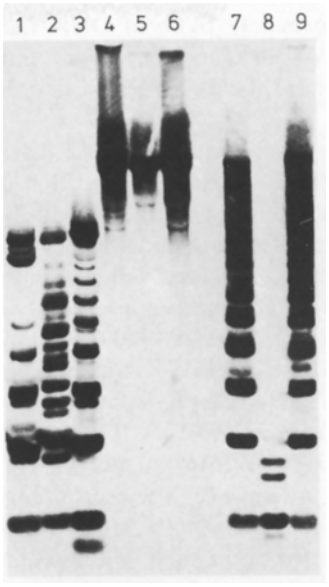

Fig. 1. Organization of pKIWI514 homologous repeat sequences in three λ clones. Hybridization of probe (pKIWI514) to restriction fragments was detected by the enhanced chemiluminescence gene detection system (ECL) using a 10-s exposure to Hyperfilm-ECL (Amersham). Two micrograms of DNA of λKIWI500 (*lanes 1, 4,* and *7*), λKIWI501 (*lanes 2, 5,* and *8*), and λKIWI501 (*lanes 3, 6,* and *9*) was digested with *Cla*I (*lanes 1* to *3*), *Dra*I (*lanes 4* to *6*), and *Hgi*AI (*lanes 7* to *9*). Digests were size-fractionated in 1% (w/v) agarose. Mobility and size of selected molecular weight fragments (BRL 1kb ladder, plus undigested λ DNA) are shown to the left in kb

Characterization of A deliciosa genomic, monomeric repeat elements

Thirty thousand plaques were screened from an amplified kiwifruit genomic library. Four plaques were found to hybridize to ^{32}P-labelled pKIWI514 insert DNA. Three of these plaques (λKIWI500, λKIWI501, and λKIWI502) were purified and characterized.

The organization of the repeat element in the three λ clones was investigated by Southern analysis, after digestion with *Bam*HI, *Cla*I, *Dra*I, *Eco*RI, *Hgi*AI, *Hin*dIII, *Mlu*I, *Msp*I, *Pst*I, *Sal*I, and *Xba*I. pKIWI514 was found to hybridize to a ladder of fragments with a monomeric size of approximately 460 bp in the *Cla*I and *Hgi*AI digests of clones λKIWI500 and λKIWI502 (Fig. 1). A similar ladder was obtained with *Msp*I (data not shown). The extent of the *Hgi*AI ladder in λKIWI500 and λKIWI502, combined with the lack of restriction sites for *Dra*I, *Eco*RI, *Hin*dIII, *Mlu*I, *Pst*I, *Sal*I, or *Xba*I, suggested that both clones were comprised entirely of repetitive sequence DNA. λKIWI502 possessed a regenerated *Bam*HI site in the cloning site in the right arm, while λKIWI500 regenerated the *Bam*HI site in the left arm, suggesting that λKIWI500 and λKIWI502 arose from independent cloning events. The monomeric repeat unit in λKIWI501 was also found to be 460 bp, but this clone

also contained approximately 14 kb of DNA that did not hybridize to pKIWI514 (data not shown) and that possessed restriction sites for all enzymes used except *Dra*I and *Mlu*I.

Monomeric bands from *Cla*I and *Hgi*AI digests of λKIWI500 and λKIWI502 were isolated and cloned into pUC19 (cleaved with *Acc*I and *Pst*I, respectively). In order to assess the extent of nucleotide sequence divergence heterogeneity among this family of repeats, six of the monomeric clones were sequenced: pKIWI515 (containing a *Cla*I fragment from λKIWI500), pKIWI516, pKIWI517, pKIWI518, pKIWI519 (containing *Hgi*AI fragments from λKIWI500), and pKIWI520 (containing an *Hgi*AI fragment from λKIWI502). Two of the elements from λKIWI500 (pKIWI516 and pKIWI518) were found to be identical in sequence. The repeat length of this repetitive DNA family seems to be highly conserved and was found to be 463 bp in each of the sequenced elements (shown in Fig. 2). A moderate level of sequence variation was found between the elements (0.9–5.0%). Elements from λKIWI500 showed sequence heterogeneity of 0.9–2.2%, whereas greater sequence divergence (3.9–5.0%) was seen between pKIWI520 from λKIWI502 and repetitive elements cloned from λKIWI500.

The consensus repeat sequence was searched for internal homology using the Compare and Dotplot programs (Devereux et al. 1984). There were no regions of significant internal direct repeats greater than 10 bp. However, the consensus sequence contained a palindromic sequence between nucleotides 278 and 296 (Fig. 2). This region was found to be conserved in all sequenced members of the 463-bp repetitive DNA family, but variation was seen in the pivotal nucleotide position (position 287, Fig. 2). A search of GenBank (Version 6.1) revealed that the consensus sequence of the 463-bp repetitive DNA family shares little homology with previously published sequences.

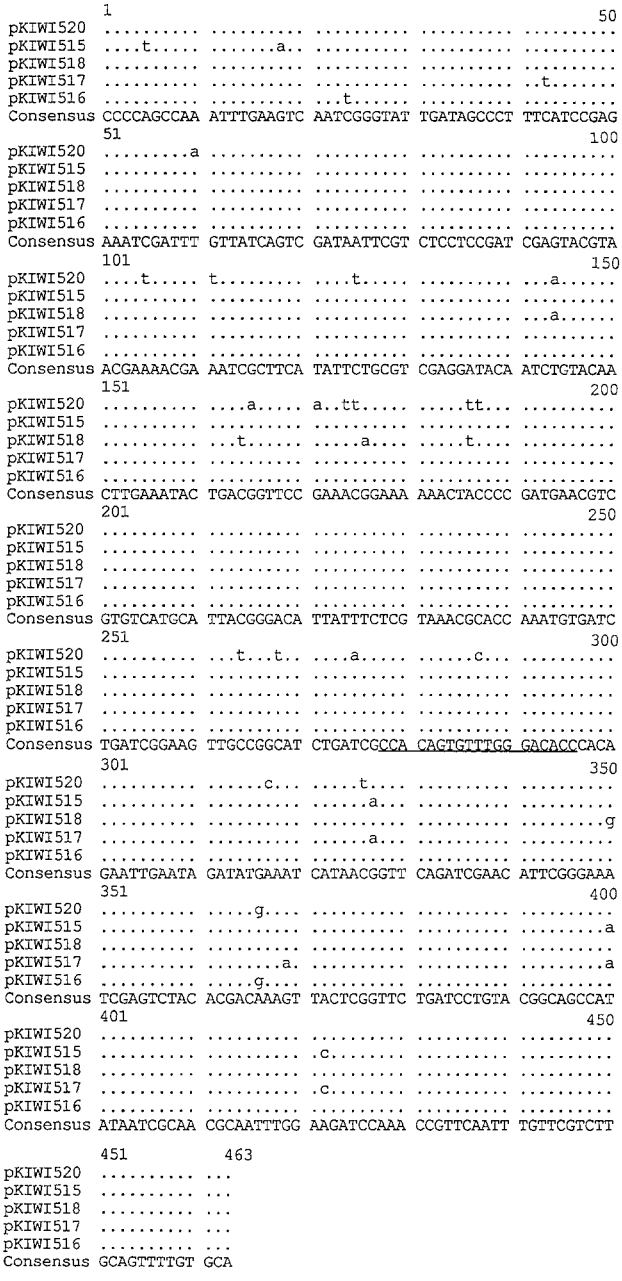

Fig. 2. Nucleotide sequence of five cloned 463-bp, genome-specific monomeric elements from *A. deliciosa*. Nucleotides identical to the consensus sequence are represented by *dots*, differences are noted by the relevant nucleotide. The palindromic sequence is underlined. All of the cloned sequences are aligned at the *Hgi*AI site

Genomic organization of cloned repeat units

To confirm that the repeats are arranged tandemly in the genome, a partial digestion with *Mbo*I was performed (Fig. 3 A). The consensus nucleotide sequence predicts *Mbo*I fragments of 159, 128, 60, 49, 41, 21, and 5 bp. The 159 and 128 bp fragments are seen in the complete digests (Fig. 3 A, lanes 6, 7, and 8). Smaller fragments would not have been retained in this gel, consequently it would not be expected to detect them. Partial digestion (lanes 2, 3, and 4) produced a complex ladder pattern that included the 460-bp unit repeat length and multimers thereof (lane 2, Fig. 3 A). This is taken as evidence of a tandem arrangement of this repeat DNA family in the genome of *A. deliciosa*. The predominant band of approximately 300 bp in lane 5 is presumed to be composed of the 128 and 159 bp *Mbo*I fragments, which are contiguous in tandemly arrayed copies of the genomic monomeric repeat.

The presence of a *Hpa*II/*Msp*I restriction site (CCGG) in the consensus repeat was used to study the methylation of this repeat family in the *A. deliciosa* genome. *Hpa*II will not cleave DNA when the second C residue is 5-methylcytosine, or when either C residue is

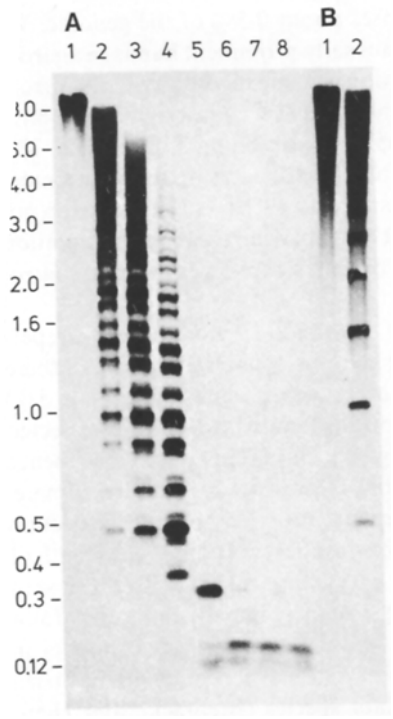

Fig. 3 A and B. Organization of the genome-specific repeat DNA family in the genome of *A. deliciosa*. The figure shows autoradiographs of *A. deliciosa* DNA (4μg/lane) hybridized to pKIWI516. Both gels contained 1% (w/v) agarose. Hybridization of peroxidase-labelled insert from pKIWI516 was detected by enhanced chemiluminescence gene detection system (ECL) using using a 60-s exposure to Hyperfilm-ECL (Amersham) for both **A** and **B**. Molecular weight markers (see Fig. 1) are shown in the *left margin* and are indicated in kb. **A** Southern hybridization of partial *Mbo*I digests of *A. deliciosa* genomic DNA. *Lane 1* contains 1 μg of undigested *A. deliciosa* genomic DNA. *Lanes 2* to *8* correspond to *A. deliciosa* genomic DNA digested for 60 min at 37 °C with 0.15, 0.3, 0.6, 1.25, 2.5, 5.0, and 10.0 units of *Mbo*I per microgram of DNA, respectively. **B** Methylation of the repetive DNA family in the genome of *A. deliciosa*. *Lanes 1* and *2* contain genomic DNA of *A. deliciosa* digested with 10 units *Hpa*II (*lane 1*) or *Msp*I (*lane 2*) per microgram of DNA.

4-methylcytosine. *Msp*I does not cleave DNA when the first C residue is 5-methylcytosine. *A. deliciosa* var. *deliciosa* DNA was digested with an excess of either *Hpa*II or *Msp*I and probed with peroxidase-labelled pKIWI516 fragment. The *Msp*I digest produced a ladder with a base unit length of about 460 bp, while hybridization to the *Hpa*II digest was exclusively to high-molecular-weight DNA, with no indication of a ladder (Fig. 3 B). This difference suggests that the internal C residue in the *Msp*I site of the repeat sequence must be highly methylated. We believe that the outer C residue is also highly methylated, since most of the hybridization in the *Msp*I digest was to multimeric length fragments (Fig. 3 B, lane 2). However, the *Msp*I ladders could also result from incomplete digestion (unlikely, given the amount of enzyme used), or from variability in the DNA sequence of the different tandem copies of the repeat (for example, an *Msp*I site present at nucleotide position 264 in four of the repeat units is absent in pKIWI520; see Fig. 2).

The copy number of the 463 bp genomic repeat family was estimated by quantitative dot blot hybridization to pKIWI516 insert under conditions that would allow substantial (26%) sequence mismatch. The results (data not shown) suggested that the repeats comprise 0.25–1% of the genomic DNA. Preliminary estimates of the haploid genome size of kiwifruit (1–2 pg) led to a minimum estimate of 5,600 copies of the repeat per haploid genome.

Distribution of similar repetitive DNA elements in other Actinidia species

The distribution of the complete 463 bp repeated sequence in other *Actinidia* species was tested using the pKIWI516 monomer element as a probe. ^{32}P-labelled probe was hybridized at low stringency (up to 23% base pair mismatch) to *Mbo*I or *Hinf*I digests of a number of genomic DNAs (Fig. 4). These included 12 cultivars (both male and female) of *A. deliciosa* var. *deliciosa*, a second variety of *A. deliciosa* (*A. deliciosa* var. *chlorocarpa*), as well as samples of *A. chinensis, A. arguta, A. chrysantha, A. eriantha, A. indochinensis, A. kolomikta, A. latifolia, A. polygama*, and *A. valvata*. Homologous repeat sequences were detected in all 12 cultivars of *A. deliciosa* var. *deliciosa* (Fig. 4 C). Hybridization to *A. deliciosa* var. *chlorocarpa* was identical to that seen in *A. deliciosa* var. *deliciosa* (Fig. 4 B). Of the remaining species, only *A. chrysantha* showed any homology to the repeat, with a single, strongly hybridizing band (ca. 140 bp) detected following overnight autoradiography (Fig. 4 A, lane 3; this lane is underloaded with respect to lane 4). All other lanes were clear after 7-days exposure of the autoradiograph. Two other accessions of *A. chinensis* also showed no hybridization to the pKIWI516 probe, while *A. rufa* had previously been negative using the subclone pKIWI514 (data not shown).

The arrangement of the homologous repetitive DNA sequences within *A. chrysantha* was examined in additional Southern hybridizations (data not shown). Partial *Mbo*I digestion showed that these repetitive elements in *A. chrysantha* are tandemly organized. Both *Msp*I and *Acc*I (which cuts once in the consensus repeat sequence) gave a similar ladder for *A. chrysantha* and *A. deliciosa*, suggesting a monomeric repeat size similar for both species. The complexity of the band patterns in the *Mbo*I partial digests of *A. chrysantha*, coupled to the size of the smallest hybridizing bands in these digests, suggest that the *A. chrysantha* repetitive elements have multiple *Mbo*I restriction sites, but that these are different from those found in the repeat sequence from *A. deliciosa* (compare lanes 3 and 4 in Fig. 4 A).

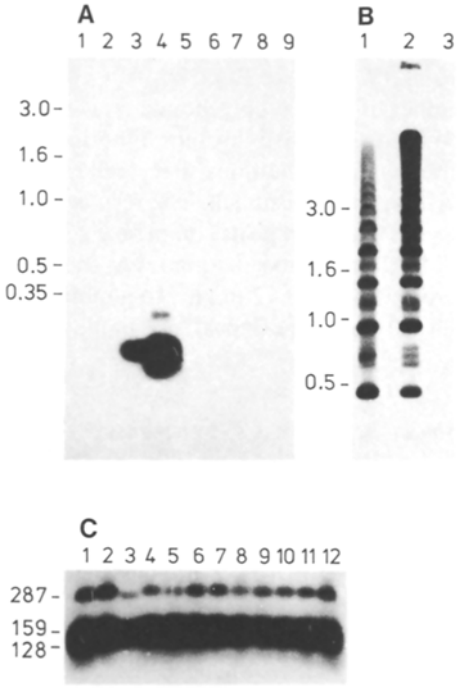

Fig. 4A–C. Distribution of the 463-bp, genome-specific repetitive DNA family in the genus *Actinidia*. The figure shows autoradiographs resulting from the hybridization of insert from pKIWI516 to DNAs from *Atinidia* species and cultivars of *A. deliciosa*. Each lane contains 4 μg of DNA, cleaved with 10 units/μg of restriction enzyme. The probe was labelled with ^{32}P-dCTP and the exposures shown are all for 16 h. Molecular weight markers in **A** and **B** are indicated in kb. The predicted sizes of three hybridizing bands in **C** are shown in bp. **A** Hybridization to nine *Actinidia* species. *Lanes 1* to *9*, respectively, correspond to *Mbo*I-cleaved DNAs of *A. arguta*, *A. chinensis*, *A. chrysantha*, *A. deliciosa*, *A. eriantha*, *A. indochinensis*, *A. kolomikta*, *A. latifolia*, and *A. polygama*. **B** Hybridization to *Hin*fI-cleaved DNA of *A. deliciosa* var. *chlorocarpa* (*lane 1*), *A. deliciosa* var. *deliciosa* (*lane 2*), and *A. valvata* (*lane 3*). **C** Hybridization to 12 cultivars of *A. deliciosa* var. *deliciosa*. *Lanes 1* to *12*, respectively, correspond to *Mbo*I-cleaved DNAs 'Allison,' 'Bruno,' a female seedling of accession CCHl, 'Elmwood,' 'Gracie', 'Greensill,' 'Hayward,' 'Jones,' 'Matua' (male), 'Monty,' M56 (male), and M58 (male).

Discussion

In this paper we describe the isolation and characterization of a repetitive DNA family from *A. deliciosa*. Partial restriction digests and characterization of the λ genomic clones showed that the *A. deliciosa*, genome-specific family of repetitive DNA is tandemly organized in the genome. The λ clones contained approximately 15-kb inserts that were composed entirely of the tandem repeat, suggesting that some of the arrays consisted of at least 30 copies. Analysis of ladders produced by restriction digests suggests that some arrays are greater than 50 kb in length. Quantitative dotblots lead to an estimate that the repeat family comprises about 0.5% of the genome. We have not observed unusually prominent bands in agarose gels following restriction enzyme digestion of DNA from *A. deliciosa* or other *Actinidia* species, which would have provided a simple approach to cloning of the repeat. The genome-specific repeat was cloned using the formamide-PERT technique (Casna et al. 1986). However, we do not know how important this approach was to the isolation of the clone, since we did not screen an equivalent control library.

The length of the *A. deliciosa*, genome-specific repetitive DNA family seems to be conserved, although there is a moderate level of sequence variation (up to 5%) among the members of this family, as would be expected for a rapidly evolving sequence. This level of sequence heterogeneity probably represents an underestimate, since further heterogeneity has been noted in restriction enzyme digests (unpublished observations). The length of repeat elements in *A. chrysantha* is similar to those seen in *A. deliciosa* and suggests there may be some constraint or selection pressure on the size of such elements in *Actinidia* species. Conservation of repeat length has been reported in several plant species (Brennicke and Hemleben 1983; Capesius 1983; Dennis and Peacock 1984; Barnes et al. 1985; Ganal and Hemleben 1986). Repeat length in these instances was found to be 170–180 bp or a multimer of this unit size. This size correlates with the nucleosome repeat length (Leber and Hemleben 1979). However, our 463-bp *A. deliciosa* repeat shares little or no homology with such sequences.

A deliciosa is a hexaploid species and is considered to be closely related to the diploid *A. chinensis*. To determine whether *A. deliciosa* is derived from *A. chinensis* via an autopolyploid or an allopolyploid origin, we examined the distribution of this repeat family in other *Actinidia* species. No homology to the repeat family was present in any of the following: *A. arguta, A. chinensis, A. eriantha, A. indochinensis, A. kolomikta, A. latifolia, A. polygama, A. rufa,* or *A. valvata*. These hybridization data suggest that it is unlikely that any of these species contributed the repeat sequence or its precursor to the genome of *A. deliciosa*. However, hybridization was detected to a tetraploid species, *A. chrysantha*, where a homologous repeat of similar size was found. The significance of this homology is not immediately clear. *A. chrysantha* is a species considered to be morphologically and therefore taxonomically distant from *A. deliciosa*. The latest classification of *Actinidia* species (Ferguson 1990) places *A. chrysantha* in the section *Maculatae*, while *A. deliciosa* is in the section *Stellatae*. Moreover, the nature of the polyploidy of *A. chrysantha* is itself unknown. It is possible that *A. chrysantha* and *A. deliciosa* share a common diploid progenitor species or that *A. chrysantha* was itself a progenitor of *A. deliciosa*. These two species have been reported to share overlapping geo-

graphic distributions (Liang 1983) in the Guangxi and Hunan provinces of China. However, there are also a further 40 species of *Actinidia* that we have not tested for the presence of the repeat, and one or more of these may have contributed the repeat to the genome of *A. deliciosa*.

The results presented here provide the first molecular evidence to suggest that *A. deliciosa* may have derived from an allopolyploid origin. The simplest interpretation of our results is that the progenitor species would include *A. chinensis*, plus either *A. chrysantha* or a progenitor of *A. chrysantha*. However, other interpretations that allow for an autopolyploid origin are clearly also possible. For example, the sampling of *A. chinensis* species available in New Zealand is small compared to the large pool of the species in the wild. It remains possible that one of these contains the repeat and is in fact the progenitor of an autopolyploid *A. deliciosa*. Alternatively, *A. chinensis* may have lost the repeat from its genome after the separation of (an autopolyploid) *A. deliciosa*. A number of further experiments could help resolve the issue, including hybridization of this repeat probe to DNA from additional species, isolation of further 'genome-specific' probes, and RFLP analysis of *A. chrysantha* and other candidate genomes.

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References

Appels R, Moran LB, Gustafson JP (1986) Rye heterochromatin. I. Studies on clusters of the major repeating sequence and the identification of a new dispersed repetitive sequence element. Can J Genet Cytol 28:645–657

Barnes SR, James AM, Jamieson G (1985) The organization, nucleotide sequence, and chromosomal distribution of a satellite DNA from *Allium cepa*. Chromosoma 92:185–192

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. Cell 19:545–560

Benslimane AA, Dron M, Hartmann C, Rode A (1986) Small tandemly repeated DNA sequences of higher plants likely originate from a tRNA gene. Nucleic Acids Res 14:8111–8119

Bostock C (1980) A function for satellite DNA? Trends Biochem Sci 5:117–119

Brennicke A, Hemleben V (1983) Sequence analysis of the cloned *Cucumis melo* highly repetitive satellite DNA. Z Naturforsch 38:1062–1065

Capesius I (1983) Sequence of the cryptic satellite DNA from the plant *Sinapis alba*. Biochim Biophys Acta 739:276–280

Casna NJ, Novack DF, Hsu M-T, Ford JP (1986) Genomic analysis, II. Isolation of high-molecular-weight heteroduplex DNA following differential methylase protection and Formamide-PERT hybridization. Nucleic Acids Res 14:7285–7303

Chevalier A (1941) Un *Actinidia* à fruits comestibles intéressant pour la France. (*A. chinensis* Planch. var *deliciosa* Chev.). Rev Bot Appl Agric Trop 21:240–244

Crowhurst RN, Lints R, Atkinson RG, Gardner RC (1990) Restriction fragment length polymorphism in *Actinidia*. Plant Syst Evol 172:193–203

Cullis CA, Cleary W (1986) Rapidly varying DNA sequences in flax. Can J Genet Cytol 28:252–259

Dennis ES, Peacock WJ (1984) Knob heterochromatin homology in maize and its relatives. J Mol Evol 20:341–350

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12:387–395

Doolittle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm, and genome evolution. Nature 284:601–603

Ferguson AR (1990) The genus *Actinidia*. In: Warrington IL, Weston GC (eds) Kiwifruit: science and management. New Zealand Society for Horticultural Science, Ray Richards Publisher, pp 15–35

Ganal M, Hemleben V (1986) Different AT-rich satellite DNAs in *Cucurbita pepo* and *Cucurbita maxima*. Theor Appl Genet 73:129–135

Grellet F, Delcasso D, Panabieres F, Delseny M (1986) Organization and evolution of a higher plant alphoid-like satellite DNA sequence. J Mol Biol 187:495–507

Halldén C, Bryngelsson T, Säll T, Gustafsson M (1987) Distribution and evolution of a tandemly repeated DNA sequence in the family Brassicaceae. J Mol Evol 25:318–323

John B, Miklos GLG (1979) Functional aspects of satellite DNA and heterochromatin. Int Rev Cytol 58:1–114

Kato A, Yakura K, Tanifuji S (1984) Sequence analysis of *Vicia faba* repeated DNA, the FokI repeat element. Nucleic Acids Res 12:6415–6426

Koukalová B, Reich J, Matyásek R, Kuhrová V, Bezděk M (1989) A BamHI family of highly repeated DNA sequences of *Nicotiana tobacum*. Theor Appl Genet 78:77–80

Leber B, Hemleben V (1979) Structure of plant nuclear and ribosomal DNA containing chromatin. Nucleic Acids Res 7:1263–1281

Leclerc RF, Siegel A (1987) Characterization of repetitive elements in several *Cucurbita* species. Plant Mol Biol 8:497–507

Liang C-F (1975) [Classification of *Actinidia chinensis* Planch..]. Acta Phytotaxon Sin 13:32–35 (+plate)

Liang C-F (1983) [On the distribution of *Actinidia*] Guihaia 3:229–248

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Habor Laboratory Press, Cold Spring Harbor/NY

Martinez-Zapater JM, Estelle MA, Somerville CR (1986) A highly repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet 204:417–423

Metzlaff M, Troebner W, Schlegel R, Cullum J (1986) Wheat-specific repetitive DNA sequences – construction and characterization of four different genomic clones. Theor Appl Genet 72:207–210

Rayburn AL, Gill BS (1986) Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4:102–109

Rayburn AL, Gill BS (1987) Molecular analysis of the D-genome of the Triticeae. Theor Appl Genet 73:385–388

Rose MR, Doolittle WF (1983) Molecular biological mechanisms of speciation. Science 220:157–162

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schweizer G, Ganal M, Ninnemann H, Hemleben V (1988) Species-specific DNA sequences for identification of somatic hybrids between *Lycopersicon esculentum* and *Solanum acaule*. Theor Appl Genet 75:679–684

Singer MF (1982) Highly repeated sequences in mammalian genomes. Int Rev Cytol 76:67–112

Sorenson JC (1984) The structure and expression of nuclear genes in higher plants. Adv Genet 22:109–144

Taylor B, Powell A (1983) Isolation of plant DNA and RNA. Focus 4:4–6

Walbot V, Cullis CA (1985) Rapid genomic change in higher plants. Annu Rev Plant Physiol 36:367–396

Wu T, Wu R (1987) A new rice repetitive DNA shows sequence homology to both 5S RNA and tRNA. Nucleic Acids Res 15:5913–5923

Zhao X, Wu T, Xie Y, Wu R (1989) Genome-specific repetitive sequences in the genus *Oryza*. Theor Appl Genet 78:201–209