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In situ hybridization in *Actinidia* using repeat DNA and genomic probes

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Abstract In situ hybridization has been used to probe chromosome spreads of hexaploid *Actinidia deliciosa* (kiwifruit; 2n = 6x = 174) and tetraploid *A. chinensis* (2n = 4x = 116). When a species-specific repeat sequence, pKIWI516, was used, six hybridization sites were observed in some accessions of tetraploid *A. chinensis* and all of *A. deliciosa*. Southern analysis with the pKIWI516 probe revealed that there are two types of tetraploid *A. chinensis*. Genomic probes from diploid *A. chinensis* (2n = 2x = 58) did not differentiate the genomes of hexaploid *A. deliciosa* and tetraploid *A. chinensis*, irrespective of the presence or absence of blocking DNA. The results indicate that the genomes of polyploid *Actinidia* species are similar but not identical. The origin of *A. deliciosa* is discussed.

Key words *Actinidia* · Kiwifruit · Repeat sequence · In situ hybridization

Introduction

Actinidia chinensis and *A. deliciosa* (kiwifruit) are economically the two most important species in the genus *Actinidia* (Ferguson et al. 1996). It has long been recognized that these two species are closely related and, until recently, they were treated as varieties of one species. Their separation into distinct species (Ferguson 1990) was based on morphology, geographical distribution and ploidy differences: *A. chinensis* was known to be diploid (2n = 2x = 58) and *A. deliciosa* hexaploid (2n = 6x = 174). However, subsequently both diploid and tetraploid (2n = 4x = 116) races of *A. chinensis* were reported (Xiong 1992; Yan et al. 1994).

Meiotic analysis in *Actinidia* has been inconclusive. McNeilage and Considine (1989) observed univalents, bivalents and multivalents at metaphase I (MI) of meiosis in a hybrid between *A. arguta* (4x) and *A. deliciosa* (6x), but precise frequencies of the various configurations were difficult to determine. Xiong (1990) observed MI pairing of 29II + 29I in a hybrid between *A. chinensis* (4x) and *A. eriantha* (2x) but could not distinguish between auto- or allosyndetic pairing. Hybrids have also been produced between *A. chinensis* and *A. deliciosa* and diploid and tetraploid races of *A. chinensis* (Yan et al. 1994). However, in all such hybrids the chromosomes are too small and numerous to allow genome analysis based on chromosome pairing configurations (Yan, unpublished results).

The application of molecular techniques to the study of genomic relationships in *Actinidia* is yielding interesting results, even if these are not always easy to interpret. Restriction fragment length polymorphism (RFLP) studies using both chloroplast and nuclear DNA have confirmed that *A. deliciosa* and diploid *A. chinensis* are indeed closely related (Crowhurst et al. 1990; Cipriani and Morgante 1993). Testolin and Ferguson (unpublished results) analysed isoenzyme polymorphisms at 14 loci in 20 *Actinidia* taxa and found that, among the species studied, *A. chinensis* and *A. deliciosa* were the most closely related. Testolin et al. (1995) speculated that *A. deliciosa* is derived from *A. chinensis* alone to the exclusion of other *Actinidia* species. However, Crowhurst and Gardner (1991) using a cloned repeat sequence (pKIWI516) from *A. deliciosa* found no hybridization with diploid *A. chinensis* but obvious hybridization with *A. chrysantha*, a tetraploid species. They therefore suggested that *A. deliciosa* may be derived from several *Actinidia* species. A phylogenetic

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analysis of DNA sequences derived from the polygalacturonase gene (Atkinson et al. 1996) also suggested that *A. deliciosa* and tetraploid *A. chinensis* races were derived from at least two progenitor *Actinidia* species.

Thus, the genomic composition of *A. deliciosa* remains unresolved and new approaches are required. One such approach involves in situ hybridization (ISH). Both total genomic DNA (GISH) and cloned repetitive DNA sequences have been used in such studies (for review see Jiang and Gill 1994a). Cloned repetitive sequences are usually dispersed amongst the chromosomes of a complement and because of this can provide useful tools for genome analysis (Lapitan et al. 1987). Other repeats, such as the rRNA genes, are precisely located and can provide landmarks for chromosome analysis (Jiang and Gill 1994b; Murray et al. 1992). In an attempt to elucidate the genomic relationships between diploid, tetraploid and hexaploid *Actinidia* taxa we report here the chromosomal distribution of a repeat sequence (pKIWI516) cloned by Crowhurst and Gardner (1991) as well as the results of GISH with several specific probes.

Materials and methods

Plant material was obtained from HortResearch Orchards at Kumeu (near Auckland) and Te Puke, Bay of Plenty, New Zealand (Table 1). Voucher specimens are being deposited at the Herbarium, Landcare Research, Christchurch, New Zealand (CHR). Most plants used are listed by cultivar name or HortResearch selection number. For selection numbers, the prefix (e.g. AA) denotes the taxon (in this case *A. arguta*), the next two numbers (e.g. 02), the accession in

Table 1 Plant material used in the study, their ploidy and detection of the repeat sequence pKIWI516

Taxon	Cultivar or plant	Ploidy	pKIWI516
A. arguta (Sieb. et Zucc.) Planch. ex Miq.	AA02_01	4x	–
A. chinensis Planch.	CK01_01, CK02_01, CK09_01, CK10_02, CK11_01, CK12_03[a], CK13_04, CK15_03, CK17_02, 37.1.16a[b]	2x	–
	CK04_01, CK07_01, CK07_02, CK08_01, CK08_02, CK28_42.5.1e, CK28_42.5.1f, CK31_01, CK54_01[a]	4x	+
	CK05_01, CK24_01, CK24_02, CK32_01[a]	4x	–
A. chrysantha C. F. Liang	CNO1_04, CNO2_01	4x	+
A. deliciosa (A. Chev.) C. F. Liang et A. R. Ferguson	Hayward, Tomuri, Chieftain, DA02_03[a] DA11_01[a], Qinmei[a], MO17, MO1	6x	+
var *deliciosa*	DA01_03_16_06	3x	+
var *chlorocarpa* (C. F. Liang) C. F. Liang et A. R. Ferguson	DB01_01	6x	+
var *coloris* T. H. Lin et X. Y. Xiong	DD01_35.7.15f	6x	+
A. guilinensis C. F. Liang	GI02_L1L2	2x	–
A. hemsleyana Dunn	HA01_01	2x	–
A. macrosperma C. F. Liang	MA01_01	4x	–
A. melanandra Franch.	ME01_01	4x	–
A. polygama (Sieb. et Zucc.) Maxim.	PC13_01	2x	–
A. setosa (Li) C. F. Liang et A. R. Ferguson	SB01_01, SB02_01[a]	2x	–

[a] Ploidy determined by flow cytometry
[b] 37.1.16a is a selection of the cross between CK01_op and CK15_03. A triploid hybrid between 37.1.16a and a tetraploid *A. chinensis* CK39_01 was also used to make chromosome preparations for GISH

chronological order and, where given, the last two numbers following the subscript dash (e.g. 01), a particular genotype selected from that accession. In a few cases, plants are listed by accession number and orchard position (e.g. 37.1.16a, L1L2). To obtain actively growing roots from the orchard grown plants, semi-woody cuttings were taken and grown in pots.

Root tips were pretreated with a saturated solution of 1,4-dichlorobenzene at room temperature for 4 h and then in water at 4°C for a further 4 h before fixing in ethanol : glacial acetic acid (3 : 1) for 2–4 h at 4°C. Air-dried slides were made (Geber and Schweizer 1988) with digestion for 2.25 h in the enzyme mix. Slides were dried overnight and stored at −20°C until required.

For in situ hybridization, standard techniques (Leitch et al. 1994) with a few minor modifications were used. Genomic DNA was isolated from *Actinidia* leaf material using the cetyl-trimethylammonium bromide procedure (Janssen and Gardner 1993). Plasmid DNA from pKIWI516 (Crowhurst and Gardner 1991) was prepared using a standard alkaline lysis method (Sambrook et al. 1989) and digested with *Hin*dIII and *Xba*I to release the repeat sequence monomer element insert. The insert was purified using the Wizard™ DNA Clean-up System (Promega). Probe DNA (1 μg of genomic DNA or 0.4 μg of pKIWI516 DNA) was labelled with biotin-14-dATP by nick translation as recommended by the manufacturer (Life Technologies). Unincorporated nucleotides were removed by double ethanol precipitation. For GISH with blocking DNA, autoclaved (10 KPa; 5 min) DNA from *A. chrysantha* (at 5× or 10× the concentration of probe DNA) was also added to the hybridization mixture. The probe was initially denatured for 20 min at 80°C, cooled on ice and then both probe plus chromosomes were denatured at 80°C for 5 min before hybridization overnight at 37°C using a thermal cycler (OmniSlide, Hybaid). Sites of in situ hybridization were detected by the alkaline phosphatase method using the BRL DNA detection kit. Slides from ISH with the repeat sequence were counterstained with 2% Giemsa for 2 min, whereas the GISH ones were observed directly after detection without counterstaining. For ISH with the repeat sequence, slides from plants without the repeat sequence were included as controls. Southern analysis was carried out as outlined in Crowhurst and Gardner (1991).

Results

Chromosome numbers in *A. chinensis* and *A. deliciosa*

The ploidy of the plants studied is given in Table 1. Plants of *A. chinensis* were either diploid, 2n = 2x = 58 (Fig. 1a) or tetraploid, 2n = 4x = 116 (Fig. 1b). A plant derived from the cross between diploid *A. chinensis* and tetraploid *A. chinensis* was found to be a triploid (Fig. 1c). All *A. deliciosa* selections were hexaploid, 2n = 6x = 174 (Fig. 1d) except for the polyhaploid *A. deliciosa* selection DA01_03_16_06, which was triploid. Meiosis has not yet been studied in such polyhaploids. Chromosomes of all *Actinidia* species were small, of similar size and lacked distinguishing morphological features.

Southern analysis using the repeat sequence pKIWI516

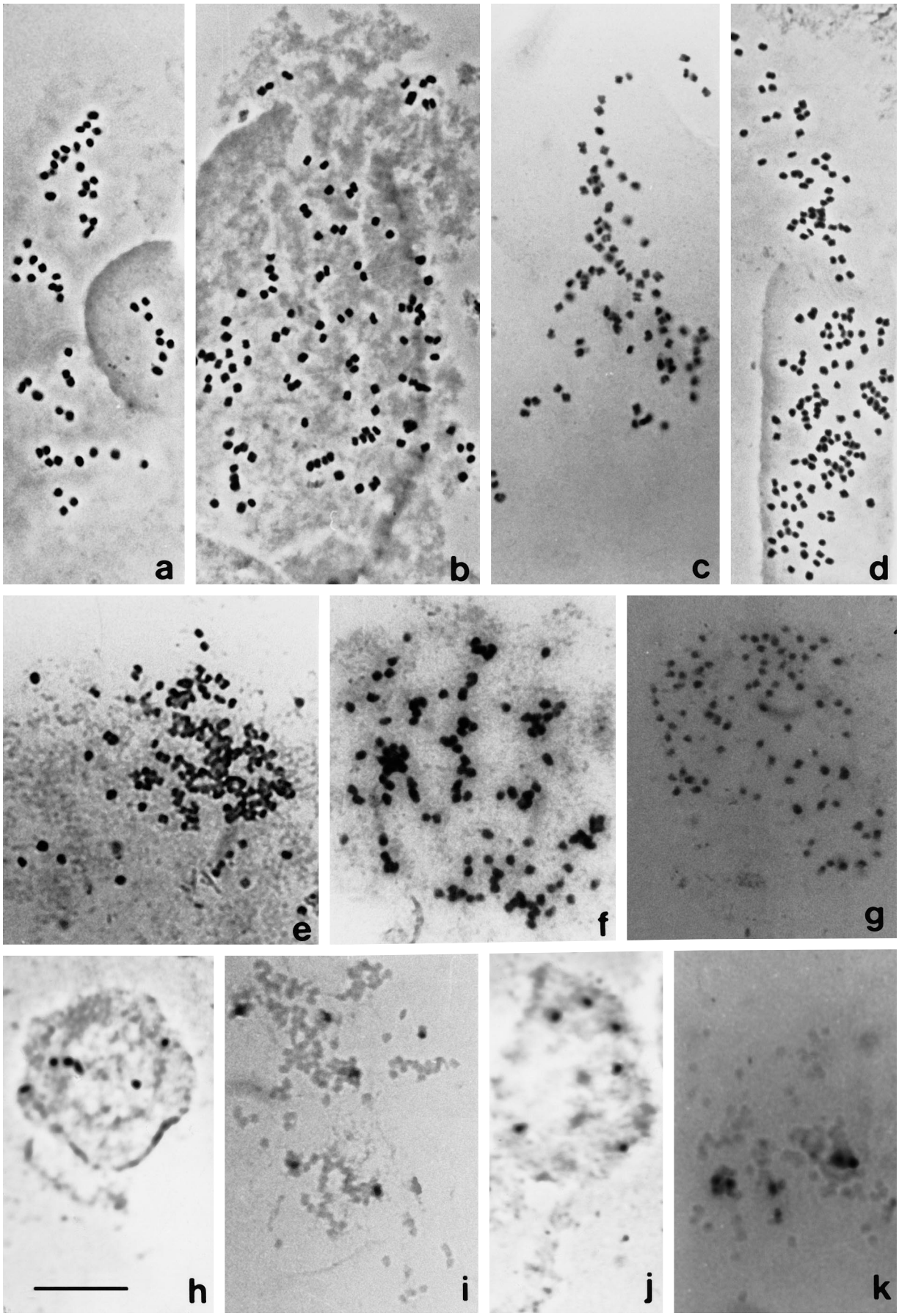
The repeat sequence, pKIWI516, was detected in all 11 *A. deliciosa* genotypes tested, including plants of the varieties *A. deliciosa* var *deliciosa*, *A. deliciosa* var *chlorocarpa* and *A. deliciosa* var *coloris* (Fig. 2, Table 1). A plant from a new introduction (CN02_01) of *A. chrysantha* was checked against the initial introduction (CN01_04), and both of them were positive to the repeat (Fig. 2). The repeat sequence was not detected in plants from 11 different diploid *A. chinensis* accessions but was detected in 9 out of the 13 tetraploid *A. chinensis* plants tested (Fig. 2, Table 1). Five other newly introduced diploid and tetraploid *Actinidia* taxa were also examined, and all of them were negative (Table 1). At least 2 plants from 5 different accessions of *A. deliciosa* and tetraploid *A. chinensis* were investigated, and in every case no intra-accession variation was observed.

ISH with the repeat sequence pKIWI516

ISH with the repeat sequence pKIWI516 gave clear and unambiguous results. In both *A. deliciosa* ('Hayward', MO17 and 'Tomuri') and tetraploid *A. chinensis* (CK07_01 and CK08_01) there were 6 discrete hybridization sites on different chromosomes (Fig. 1i, k) and also 6 distinct sites in interphase nuclei (Fig. 1 h, j). After ISH with the repeat sequence, the signals were dark purple-blue in colour, the background was clear and the chromosome edges were well-defined. No signal was detected on control slides made from tetraploid *A. chinensis* (CK05_01), in which the repeat sequence could not be detected by Southern hybridization (Table 1).

GISH using DNA probes from diploid *A. chinensis*

Genomic probes from 2 plants of diploid *A. chinensis* (CK01_01 and CK13_04) were separately hybridized in situ onto denatured chromosome spreads of *A. deliciosa* cultivars 'Hayward' and 'Tomuri' and of the tetraploid *A. chinensis* selections CK07_01 and CK08_01. Hybridization sites were detected on all the chromosomes in both species (Fig. 1e, f) although there appeared to be some variation in signal intensity between different chromosomes of a complement. The triploid *A. chinensis* hybrid (Table 1) was also probed with its maternal diploid genomic DNA, and again all chromosomes showed positive hybridization sites (Fig. 1g). When these experiments were repeated with the addition of denatured genomic blocking DNA from *A. chrysantha*, CN01_04 at concentrations 5 or 10 times that of the probe, no hybridization was detectable. This species was chosen for this purpose as previous studies (Crowhurst and Gardner 1991) had suggested that *A. chrysantha* was a genome donor to *A. deliciosa.*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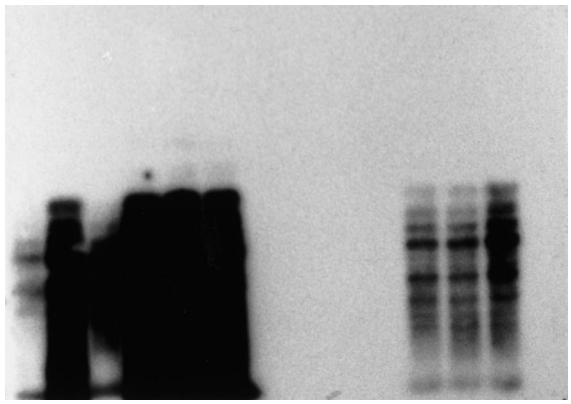

Fig. 2 Detection of the repeat sequence pKIWI516 in *Actinidia* taxa. The figure shows an autoradiograph resulting from the hybridization of pKIWI516 to DNAs from *Actinidia* taxa, selections and cultivars. Each lane contains 10 μg DNA, cleaved with *Sau*3AI. The probe was labelled with [^{32}P]dCTP and exposed for 24h. Lanes 1–14, respectively, correspond to *A. chrysantha* CN02_01, *A. deliciosa* var *coloris* DD01_35.7.15f, *A. chrysantha* CN01_04, *A. deliciosa* var *chlorocarpa* DB01_01, *A. deliciosa* var *deliciosa* 'Qinmei', *A. deliciosa* var *deliciosa* 'Hayward', *A. chinensis* CK13_04 (2x), *A. chinensis* CK11_01 (2x), *A. chinensis* CK12_03 (2x), *A. chinensis* CK17_02 (2x), *A. chinensis* CK04_01 (4x), *A. chinensis* CK07_01 (4x), *A. chinensis* CK08_01 (4x) and *A. chinensis* CK24_01 (4x)

Discussion

We have successfully applied in situ hybridization techniques to *Actinidia*, a genus which has large numbers of very small chromosomes. One of the most important reasons for our success with such difficult material was the preparation of high quality spreads: we have used an air-drying method (Geber and Schweizer 1988) which gives high-quality spreads of both chromosomes and nuclei. These preparations appear to be substantially free of cell walls and cytoplasmic debris, thereby enhancing the accessibility of reagents throughout the steps of the procedure. Flame-dried (Yan et al. 1994) and squash preparations failed to give clear, evenly distributed and reproducible results for *Actinidia*. Another important factor was the preservation of chromosome morphology during denaturation. Our use of a programmable thermal cycler for denaturing the chromosome spreads and the probe together greatly improved chromosome integrity, signal intensity and reproducibility.

Crowhurst and Gardner (1991) investigated the distribution of the pKIWI516 repeat by Southern blotting and found strong hybridization signals only with *A. deliciosa* and *A. chrysantha*. Nine other taxa, including diploid *A. chinensis*, gave no signal. In the present study we analysed a further six newly introduced taxa and the newly discovered tetraploids of *A. chinensis* (Yan et al. 1994). *A. deliciosa* var *coloris*, like the other varieties of this species, gave a positive result. In only one species, *A. chinensis*, could the repeat sequence be detected in some genotypes, 9 tetraploid plants from 6 accessions, but not in others, 4 tetraploid plants from 3 different accessions and all 9 diploid accessions. It should be noted that this, the greatest complexity observed, was in the taxon studied in most detail. Only a few plants were tested from each accession, but the results indicate that there is probably no variation within an accession (Table 1).

GISH did not allow us to differentiate between the genomes of *Actinidia* species, which suggests that they are very similar. However, ISH with the repeat sequence gave very interesting results. In both nuclear and chromosome spreads 6 hybridization sites were seen in all *A. deliciosa* plants tested and in those tetraploid *A. chinensis* plants in which the repeat sequence had been detected by Southern blotting. At this stage we do not know whether these sites are confined to one genome or are distributed amongst all the constituent genomes. The fact that the tetraploid and hexaploid have the same number of sites suggests that they may be confined to one genome. Thus, the restricted distribution of the *Actinidia* repeat provides a striking contrast to that of other repeats in genera such as *Lycopersicon* and *Secale* (Lapitan et al. 1989; Jones and Flavell 1982). Many tandem repeats in plants are located in regions of constitutive heterochromatin associated with telomeres and centromeres (Lapitan 1992; Dean and Schmidt 1995) and consequently are distributed amongst all or most of the chromosome complement. Although the small size of the *Actinidia* chromosomes does not allow a very precise location of pKIWI516, it would appear not to be confined to heterochromatic regions at telomeres or centromeres. Crowhurst and Gardner (1991) reported that there is a minimum of 5,600 copies (approximately 0.5%) of the repeat in the haploid *A. deliciosa* genome. Our Southern blots suggest that copy number may vary considerably from one species to another, with a lower copy number in *A. chinensis* and *A. chrysantha* than

Fig. 1a–d Mitotic metaphase chromosome spreads from root-tip meristem cells of **a** *A. chinensis* CK10_02 (2n = 2x = 58), **b** *A. chinensis* CK08_01 (2n = 4x = 116), **c** a hybrid between diploid (37.1.16a) and tetraploid (CK39_01) plants of *A. chinensis* (2n = 3x = 87), **d** *A. deliciosa* 'Tomuri' (2n = 6x = 174). **e–g** Genomic in situ hybridization to root-tip metaphase chromosomes of **e** hexaploid *A. deliciosa* 'Hayward' probed with biotinylated DNA from diploid *A. chinensis* CK01_01, **f** tetraploid *A. chinensis* CK07_01 probed with biotinylated DNA from diploid *A. chinensis* CK13_04 **g** the triploid hybrid probed with biotinylated DNA from the parental diploid *A. chinensis* 37.1.16a. **h–k** In situ hybridization of biotinylated pKIWI516 probe to **h** interphase nucleus of hexaploid *A. deliciosa* 'Hayward', **i** metaphase chromosomes of hexaploid *A. deliciosa* MO17, **j** interphase nucleus of tetraploid *A. chinensis* CK07_01, **k** metaphase chromosomes of tetraploid *A. chinensis* CK08_01. Bar: 10 μm

A. deliciosa. With our existing techniques, these differences were not detected at the chromosome level.

A close relationship between *A. deliciosa* and diploid and tetraploid forms of *A. chinensis* has been deduced from similarities in morphology (Ferguson 1990) and in flavonoid composition (Webby et al. 1994). Most molecular evidence has confirmed this apparent close relationship, and isozyme analyses and chloroplastic DNA analyses are consistent with *A. deliciosa* having evolved from diploid *A. chinensis* alone without contributions from any other *Actinidia* species (Testolin et al. 1995). According to our present understanding of the *Actinidia* genome, the most likely origin of hexaploid *A. deliciosa* is via tetraploid forms of *A. chinensis* carrying the repeat sequence (Fig. 3) involving the formation of unreduced gametes. Another, less likely, possibility would be a cross between such plants and a diploid (probably diploid *A. chinensis*) followed by somatic doubling. Controlled crosses between diploid and tetraploid forms of *A. chinensis* have produced viable triploid plants (Yan et al. 1994), although somatic doubling has not yet been observed, but this pathway can not be eliminated at this stage.

We have no evidence of the repeat sequence occurring in diploid forms of *A. chinensis*. Such forms may have become extinct or they may have lost the repeat sequence after the evolution of tetraploid forms of *A. chinensis* and of *A. deliciosa*. There is also a major difference between those tetraploids of *A. chinensis* with or without the repeat. This could indicate that tetraploid *A. chinensis* may have multiple origins, as has been shown for polyploids in genera such as *Tragopogon*, *Draba* and *Glycine* (Soltis et al. 1992). Amplification of the repeat sequence at the tetraploid level, with subsequent production of hexaploids, should also be considered. Our results suggest that there are at least two genomes in tetraploid *A. chinensis* and hexaploid *A. deliciosa*, one genome with and the other without the repeat sequence.

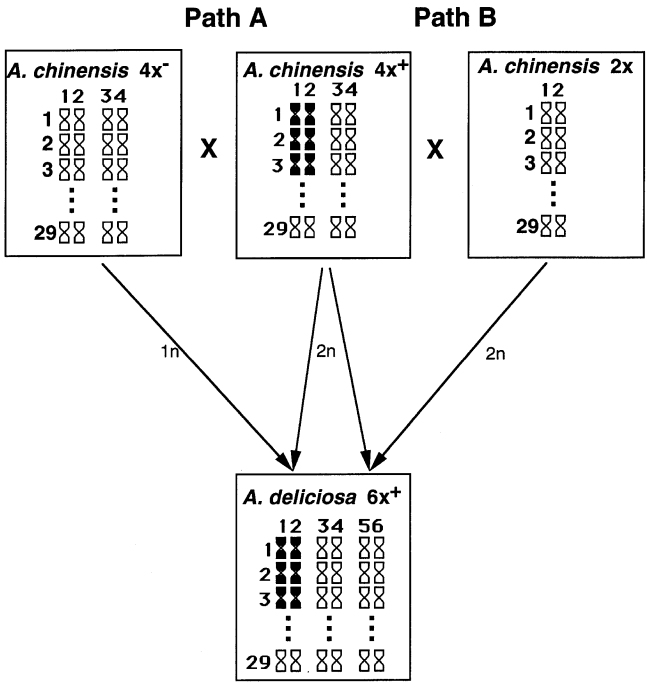

Fig. 3 Possible schemes for the origin of *A. deliciosa*. *A. chinensis* $4x^-$ = tetraploid *A. chinensis* without the repeat sequence; *A. chinensis* $4x^+$ = tetraploid *A. chinensis* with the repeat sequence; *A. chinensis* $2x^-$ = diploid *A. chinensis*; *A. deliciosa* $6x^+$ = hexaploid *A. deliciosa*. *Solid chromosomes*, represent those with the repeat sequence, and the sequence has been arbitrarily assigned to one genome

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References

Atkinson RG, Cipriani G, Whittaker DJ, Gardner RC (1996) An allopolyploid origin for kiwifruit (*Actinidia deliciosa* var *deliciosa*). Plant Syst Evol (in press)

Cipriani G, Morgante M (1993) Evidence of chloroplast DNA variation in the genus *Actinidia* revealed by restriction analysis of PCR-amplified fragments. J Genet Breed 47:319–326

Crowhurst RN, Gardner RC (1991) A genome-specific repeat sequence from kiwifruit (*Actinidia deliciosa* var *deliciosa*). Theor Appl Genet 81:71–78

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

Dean C, Schmidt R (1995) Plant genomes: a current molecular description. Annu Rev Plant Physiol Plant Mol Biol 46:395–418

Ferguson AR (1990) The genus *Actinidia*. In: Warrington IJ, Weston GC (eds) Kiwifruit: science and management. Ray Richards Publ in association with the New Zealand Society for Horticultural Science, Auckland, New Zealand, pp 15–35

Ferguson AR, Seal AG, McNeilage MA, Fraser LG, Harvey CF, Beatson RA (1996) Kiwifruit. In: Janick J, Moore JN (eds) Fruit breeding, vol 2. John Wiley & Sons, New York London, pp 371–417

Geber G, Schweizer D (1988) Cytochemical heterochromatin differentiation in *Sinapsis alba* (*Cruciferae*) using a simple air-drying technique for producing chromosome spreads. Plant Syst Evol 158:97–106

Janssen B-J, Gardner RC (1993) The use of transient GUS expression to develop an *Agrobacterium*-mediated gene transfer system for kiwifruit. Plant Cell Rep 13:28–31

Jiang J, Gill BS (1994a) Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. Genome 37:717–725

Jiang J, Gill BS (1994b) New 18S.26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma 103:179–185

Jones JDG, Flavell RB (1982) The mapping of highly repeated DNA families and their relationship to C bands in chromosomes of *Secale cereale*. Chromosoma 86:595–612

Lapitan NLV (1992) Organization and evolution of higher plant nuclear genomes. Genome 35:171–181

Lapitan NLV, Gill BS, Sears RG (1987) Genomic and phylogenetic relationships among rye and perennial species in the Triticeae. Crop Sci 27:682–687

Lapitan NLV, Ganal MW, Tanksley SD (1989) Somatic chromosome karyotype of tomato based on in situ hybridization of the TGRI satellite repeat. Genome 32:992–998

Leitch AR, Schwarzacher T, Jackson D, Leitch IJ (1994) In situ hybridization. Bios Scientific Publ, Oxford.

McNeilage MA, Considine JA (1989) Chromosome studies in some *Actinidia* taxa and implications for breeding. N Z J Bot 27:71–78

Murray BG, Bennett MD, Hammett KRW (1992) Secondary constrictions and NORs of *Lathyrus* investigated by silver staining and in-situ hybridization. Heredity 68:473–478

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Soltis PS, Doyle JJ, Soltis DE (1992) Molecular data and polyploid evolution in plants. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular systematics of plants. Chapman and Hall, New York, pp 177–201

Testolin R, Cipriani G, Ferguson AR, Gardner RC (1995) Molecular approaches to systematics of the genus *Actinidia*. In: Abstr 3rd Int Symp Kiwifruit. Thessaloniki, Greece, p 24

Webby RF, Wilson RD, Ferguson AR (1994) Leaf flavonoids in *Actinidia*. Biochem Syst Ecol 22:277–286

Xiong ZT (1990) Observations on the morphology and meiosis of interspecific hybrid triploid in *Actinidia*. Bull Bot Res 10:99–103

Xiong ZT (1992) Chromosome studies on cultivated Chinese gooseberry. Guihaia 12:79–82

Yan G, Ferguson AR, McNeilage MA (1994) Ploidy races in *Actinidia chinensis*. Euphytica 78:175–183