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Meiotic chromosome pairing behaviour of natural tetraploids and induced autotetraploids of *Actinidia chinensis*

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Abstract

Key message **Non-preferential chromosome pairing was identified in tetraploid *Actinidia chinensis* and a higher mean multivalent frequency in pollen mother cells was found in colchine-induced tetraploids of *A. chinensis* compared with naturally occurring tetraploids.**

Abstract Diploid and tetraploid *Actinidia chinensis* are used for the development of kiwifruit cultivars. Diploid germplasm can be exploited in a tetraploid breeding programme via unreduced (2*n*) gametes and chemical-induced chromosome doubling of diploid cultivars and selections. Meiotic chromosome behaviour in diploid *A. chinensis* 'Hort16A' and colchicine-induced tetraploids from 'Hort16A' was analysed and compared with that in a diploid male and tetraploid males of *A. chinensis* raised from seeds sourced from the wild in China. Both naturally occurring and induced tetraploids formed multivalents, but colchicine-induced tetraploids showed a higher mean multivalent frequency in the pollen mother cells. Lagging chromosomes at anaphase I and II were observed at low frequencies in the colchicine-induced tetraploids. To investigate whether preferential or non-preferential chromosome pairing occurs in tetraploid *A. chinensis*, the inheritance of microsatellite alleles was analysed in the tetraploid progeny of crosses between *A. chinensis* (4*x*) and *A. arguta* (4*x*). The frequencies of inherited microsatellite allelic combinations in the hybrids suggested that non-preferential chromosome pairing had occurred in the tetraploid *A. chinensis* parent.

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Introduction

Actinidia (kiwifruit) species have only recently been introduced into cultivation and the cultivars grown commercially either have been selected from the wild or are at most only several generations removed from the wild. Consequently, there is considerable potential for the development of new hybrids and cultivars, although the rate of progress is hampered by their long generation times, a dioecious breeding system, high heterozygosity, and high chromosome numbers. A range of ploidy levels of *A. chinensis* occur naturally in China (Zhang 1983; Xiong and Huang 1988; Li et al. 2010) and current breeding efforts involve diploids and tetraploids. Diploid germplasm can be used in the tetraploid *A. chinensis* breeding programmes only if unreduced (2*n*) gametes are produced by diploids and are identified in crosses in which the diploids used for previous crosses were found to produce unreduced gametes (Yan et al. 1997), or if polyploidy can be induced in diploids (Wu et al. 2011).

We have doubled the chromosome number of the normally diploid ($2n = 2x = 58$) cultivar *A. chinensis*

'Hort16A' using in vitro colchicine treatment of somatic tissue, and have regenerated autotetraploid plants (Wu et al. 2011). We have also doubled chromosome numbers and regenerated plants from a number of elite, diploid selections of red-fleshed kiwifruit of this species. In all cases there was a significant increase in fruit size, with the colchicine-induced tetraploids producing fruit that were on average 50–60 % heavier than those of their progenitor diploids (Wu et al. 2012). Fruit quality of the induced tetraploids was also altered, as they had lower dry matter content and matured earlier than fruit of their diploid progenitors (Wu et al. 2013). A conspicuous feature of the tetraploid fruit was their reduced number of full developed, black seeds (Wu et al. 2009; J.-H. Wu, unpublished). Both fruit size and dry matter content are strongly correlated with full developed seed number within the same genotypes in the closely related species *A. deliciosa* (Hopping and Hacking 1983; Gonzalez et al. 1998; Nardozza et al. 2010). More recently in *A. chinensis*, it was also found that fruit size of 'Hort16A' was related to the number of seeds (Goodwin et al. 2013) and even to fertilized ovules without full development (Seal et al. 2013). It is important to know whether irregularities at meiosis might contribute to the reduction in seed number. Recently, the effects of 2*n* gametes on sex ratios in *A. deliciosa* (6*x*) × *A. chinensis* (2*x*) and *A. deliciosa* (6*x*) × *A. eriantha* (2*x*) hybrids (Seal et al. 2012), and the meiotic chromosome pairing in *A. deliciosa* (6*x*) × *A. eriantha* (2*x*) hybrids (Mertten et al. 2012), have been evaluated. A better understanding of the relationships between diploid and tetraploid *A. chinensis* and the nature of meiotic chromosome pairing in tetraploid *A. chinensis* (i.e. whether preferential or non-preferential pairing) would also assist in genetic mapping, the design of breeding strategies for tetraploid *A. chinensis*, and *Actinidia* genetics and genomics studies through ploidy manipulation (Wu 2012).

Chromosome pairing behaviour during meiosis in polyploids can range from completely random pairing of chromosomes (non-preferential pairing) where each chromosome has more than one potential partner, which may result in the formation of multivalents, to preferential pairing where chromosomes have partners with which they are more likely to pair during meiosis. To describe this range of pairing behaviour, Sybenga (1994) introduced the preferential pairing parameter ρ (range 0.0–0.66), where 0 is completely non-preferential pairing and 0.66 is completely preferential pairing (a chromosome always pairs with its specific partner). To gain a better understanding of meiotic chromosome pairing behaviour in *A. chinensis*, we have observed meiosis in colchicine-induced tetraploids of 'Hort16A' and in diploid and tetraploid male plants of *A. chinensis* raised from seeds collected from the wild in China. To determine whether preferential or non-preferential pairing occurs in tetraploid *A. chinensis*, we used microsatellite markers to track the inheritance of alleles in offspring from *A. chinensis* (4*x*) × *A. arguta* (4*x*) crosses.

Materials and methods

Plants

The plants studied consisted of two vines of diploid ($2n = 2x = 58$) *A. chinensis* 'Hort16A', and six colchicine-induced tetraploid ($2n = 4x = 116$) regenerants from it. A diploid ($2n = 2x = 58$) male (M1) and two tetraploid ($2n = 4x = 116$) males (M2 and M3) of *A. chinensis* raised from seeds collected from the wild in China were used for comparison. In addition, 87 vines from among six families of *A. chinensis* (4*x*) × *A. arguta* (4*x*) hybrids and their parents were used for analysis of microsatellite inheritance (Table 1).

Autotetraploids induced from 'Hort16A' were planted in the Plant & Food Research orchard at Kerikeri with two different genotypes of tetraploid *A. chinensis* males from the Plant & Food Research germplasm collection and a diploid *A. chinensis* male cultivar ('Meteor') in equal number to the three males at a ratio of one male:eight female plants (Wu et al. 2012, 2013).

Sampling and chromosome analysis

The procedures for flower bud induction and the preparation of meiotic chromosomes were similar to those described by Wu and Mooney (2002) and Wu et al. (2012). Dormant canes were collected and held in sealed plastic bags at 4 °C for 5 weeks to satisfy winter chilling requirements for the induction of flowering. The canes were then cut into five node lengths and placed in water in 0.5-L jars in the greenhouse to allow buds to break dormancy and produce new shoots with flower buds. Once flower buds appeared to be at about the right stage of development, single buds were taken at 1000 and 1500 hours to check the

Table 1 *Actinidia chinensis* × *A. arguta* hybrid families used to analyse microsatellite inheritance

Family	Female parent	Male parent	Number of individuals
K808	T02.13-03-03b	AA07_01	36
K809	T02.13-03-03b	AA13_01	3
K810	T02.13-03-03b	AA23	6
K811	T02.13-03-03b	AA24	16
K812	T02.13-03-03b	AA25	10
K813	T02.13-03-03b	AA26	16

stage of meiosis. Pollen mother cells (PMCs) from one or two anthers of fresh flower buds were stained with aceto-carmine and, if found to contain the appropriate meiotic stages (metaphase I to telophase II), the remaining anthers were pre-treated with saturated *p*-dichlorobenzene for 3.5 h at room temperature, then fixed in Carnoy's solution (ethanol:glacial acetic acid, 3:1 v/v) overnight at 4 °C, and stored in 70 % ethanol at 4 °C before use. Although female plants of *Actinidia* are pollen sterile, they show regular meiotic behaviour before their pollen development fails (Ferguson 1984). Anthers were hydrolysed with 20 µl 1 M HCl at 37 °C for 45 min before they were treated with 20 µl of enzymatic solution of 5 % cellulase Onozuka R-10 (Yakult Honsha Co. Ltd) and 1 % pectolyase Y-23 (Seishin Pharm Ltd) in 0.01 M citrate buffer, pH 4.6, at 37 °C for 4 h. Giemsa (5 %) (BDH) in 0.067 M phosphate buffer, pH 6.8, was used for staining. At least 25 PMCs at the appropriate stage from each plant were observed and the frequencies of chromosome configurations at metaphase I and of maloriented or lagging chromosomes at anaphase I and II were calculated.

Microsatellite amplification

DNA was isolated from young leaves of the seven parents and 87 F_1 progeny. Leaves were ground to a fine powder in liquid nitrogen and then DNA was extracted using a DNeasy Plant Mini Kit (Qiagen™) following the manufacturer's instructions. The final eluate volume was 100 µl.

Fifty-four microsatellite markers generated from the *Actinidia* EST database of Plant & Food Research were selected for initial screening of the seven parents of the *A. chinensis* × *A. arguta* hybrids (Supplementary Table 1). The 10-µl PCR reaction mixtures each contained 1 × PCR buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 5 mM $MgCl_2$, 0.2 mM of each dNTP, 4.5 pmol of each primer, 0.7 units of Platinum *Taq* polymerase (Invitrogen) and 5 µl of genomic DNA (about 12.5 ng). Polymerase chain reaction (PCR) was performed in a Techne™ TC-412 thermal cycler with a single cycle of 94 °C for 3 min preceding 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s (annealing temperatures of primers are shown in Supplementary Table 1), and elongation at 72 °C for 1 min. PCR reactions were carried out individually before preparation of three colour multiplexes of products labelled with 6FAM, VIC or NED (Filter Set D) for analysis. The allelic content of each genotype was determined by capillary electrophoresis in an ABI Prism® 3100 Genetic Analyzer (Filter Set D, ROX™ GS300HD size standard) and analysed with GeneMapper™ Software Version 3.0 (Applied Biosystems). Following the initial screen, microsatellite markers were selected for further analysis if they met the following criteria:

1. They amplified three or four alleles in the *A. chinensis* parent.
2. All the alleles they amplified from one or more of the *A. arguta* parents were different in size from those of the *A. chinensis* parent, allowing *A. chinensis* and *A. arguta* alleles in the hybrid offspring to be identified and distinguished.

Selected markers were then screened across the parents and F_1 hybrid offspring of the appropriate *A. chinensis* × *A. arguta* families using the method described above.

Segregation analysis

To understand the chromosome pairing that had occurred during meiosis in tetraploid *A. chinensis,* the microsatellite allelic content of polymorphic loci was determined for *A. chinensis* (4*x*), *A. arguta* (4*x*) and their F_1 hybrids (*A. chinensis* × *A. arguta*). To distinguish whether preferential or non-preferential pairing occurred in the *A. chinensis* parent, we had first to determine the allelic content and frequencies of alleles in the parents, and then we could compare the frequencies of observed gamete allele classes with the expected gamete allele classes. There would be four expected gamete allele classes from *A. chinensis* in the F_1 hybrid progeny under strictly preferential pairing (A preferentially pairs with B) (ac, ad, bc, bd) and six expected classes under non-preferential pairing models (ab, ac, ad, bc, bd, cd). The allelic content and frequencies of alleles in the F_1 hybrids were used to determine dosage and allelic content of the *A. chinensis* parent. Assuming no segregation distortion, the expected frequencies of alleles in gametes depend on the dosage of the allele in the tetraploid parent. Under a non-preferential pairing model, a microsatellite allele present as one copy would be expected in half the gametes; if present as two, copies would be expected in five out of six of the gametes; and if present as three or four, copies would be expected in all the gametes. Under a preferential pairing model, an allele present as one copy would be expected in 50 % of gametes; if present as two, copies would be expected either in 75 % of gametes if the two copies were in different pairs, or in 100 % of gametes if the two copies were in the same pair; or if present in three or four, copies would be expected in all gametes. Parental genotypes were reconstructed based on the allelic content and dosage. Some loci contained fewer than the expected four alleles owing to the presence of null alleles.

The expected allelic combinations under the non-preferential and preferential pairing models were modified for each microsatellite depending on the dosage and combination of alleles in the *A. chinensis* parent. The maximum expected allelic combinations were six classes under the

non-preferential and four classes using the preferential pairing model. Observed F_1 progeny numbers in allelic combinations were tested by Pearson's Chi-squared tests and multinomial likelihood ratio tests, using the natural logarithm of the ratio between these two probabilities multiplied by negative two [−2 × Sum(In(LR))] and incorporating William's correction as the test statistics of goodness of fit to the expected non-preferential and preferential pairing segregation ratios for each microsatellite. If the null hypothesis is true, the likelihood ratio test and the Pearson's Chi-squared test both converge to a Chi-squared distribution with $k-1$ *df*, the Pearson's Chi-squared test from below and the likelihood ratio test from above. For the markers where we could identify all four different alleles (ABCD) in the *A. chinensis* parent, we could estimate the preferential pairing parameter (ρ) (Sybenga 1994) following the method of Wu et al. (2001) as applied by Curole and Hedgecock (2005). To obtain the maximum estimate of preferential pairing for each marker, we identified the pair of alleles that were observed together in the lowest number of progeny (lowest frequency) and assumed that these two alleles preferentially paired. In the following equations, the two alleles that preferentially pair are denoted as alleles A and B, i.e. A preferentially pairs with B, and C with D. We used the frequency of progeny that contained each of the possible allelic combinations to solve for (ρ), using the following equations. As no evidence of double reduction was observed, we estimated α as 0, so we could use the frequency of progeny with each allelic combination to solve Eqs. (2) and (3) to obtain an average estimate for (ρ):

$$f(\mathrm{AA})+f(\mathrm{BB})+f(\mathrm{CC})+f(\mathrm{DD})=\alpha\left(\frac{2}{3}-\frac{3}{2}\rho^2\right) \quad (1)$$

$$f(\mathrm{AB})+f(\mathrm{CD})=\frac{1}{9}-\frac{1}{3}\rho+\frac{1}{4}\rho^2+\frac{1}{3}\left(\frac{2}{3}-\frac{3}{2}\rho^2-\alpha\left(\frac{2}{3}-\frac{3}{2}\rho^2\right)\right) \quad (2)$$

$$f(\mathrm{AC})+f(\mathrm{AD})+f(\mathrm{BC})+f(\mathrm{BD})=\frac{2}{9}-\frac{1}{3}\rho+\frac{5}{4}\rho^2+\frac{2}{3}\left(\frac{2}{3}-\frac{3}{2}\rho^2-\alpha\left(\frac{2}{3}-\frac{3}{2}\rho^2\right)\right) \quad (3)$$

where, $f(\mathrm{AB})$ is the number of progeny that contains alleles A and B together, divided by the total number of progeny.

Results

Meiotic pairing analysis

The results of the meiotic chromosome analysis are summarized in Table 2 and representative PMC meioses are shown in Fig. 1. The small size of *Actinidia* chromosomes made meiotic analysis difficult, but it was generally possible to differentiate the meiotic configurations into univalents, bivalents and quadrivalents. The two vines of diploid 'Hort16A' showed a clear preponderance of bivalents, with a low frequency of univalents (4 %) and in one, V1, a low frequency of quadrivalents. All the induced autotetraploids from 'Hort16A' had broadly similar frequencies of meiotic configurations, with the exception of RS1, which had a lower mean quadrivalent frequency and narrower range of quadrivalents than the others; however, all the induced autotetraploids from 'Hort16A' showed much higher frequencies of multivalency than their progenitor diploid 'Hort16A'. No univalents were observed in any of

Table 2 Meiotic chromosome pairing and frequency of lagging chromosomes at anaphase I in diploid *Actinidia chinensis* 'Hort16A' and its colchicine-induced autotetraploids, and in diploid and tetraploid *A. chinensis* male plants raised from seeds collected from the wild in China

Genotype	Ploidy	Vine or regenerant sampled	Number of pollen mother cells scored	Mean number of configurations per pollen mother cell (range in brackets)			Number of lagging chromosomes
				I	II	IV	
'Hort16A'	2*x*	V1	25	2.11 (0–4)	28.2 (24–29)	0.2 (0–2)	0
	2*x*	V2	30	0	29	0	0
Colchicine-induced tetraploid regenerants from 'Hort16A'	4*x*	RS1	28	0	53.2 (48–51)	2.6 (1–5)	0
	4*x*	RS2	35	0	50 (44–52)	4.2 (3–7)	0
	4*x*	RS3	35	0	49.3 (44–54)	4.4 (2–7)	1–2
	4*x*	RS4	35	0	48.4 (42–50)	4.8 (4–8)	2–4
	4*x*	RS5	26	0	46.8 (42–52)	5.6 (3–8)	0–2
	4*x*	RS6	32	0	47.3 (40–52)	5.4 (3–9)	3–6
Male plants raised from seeds collected from wild in China	2*x*	M1	30	0	29	0	0
	4*x*	M2	25	0	55.2 (52–56)	1.6 (1–3)	0
	4*x*	M3	31	0	54.5 (52–56)	1.8 (1–3)	0

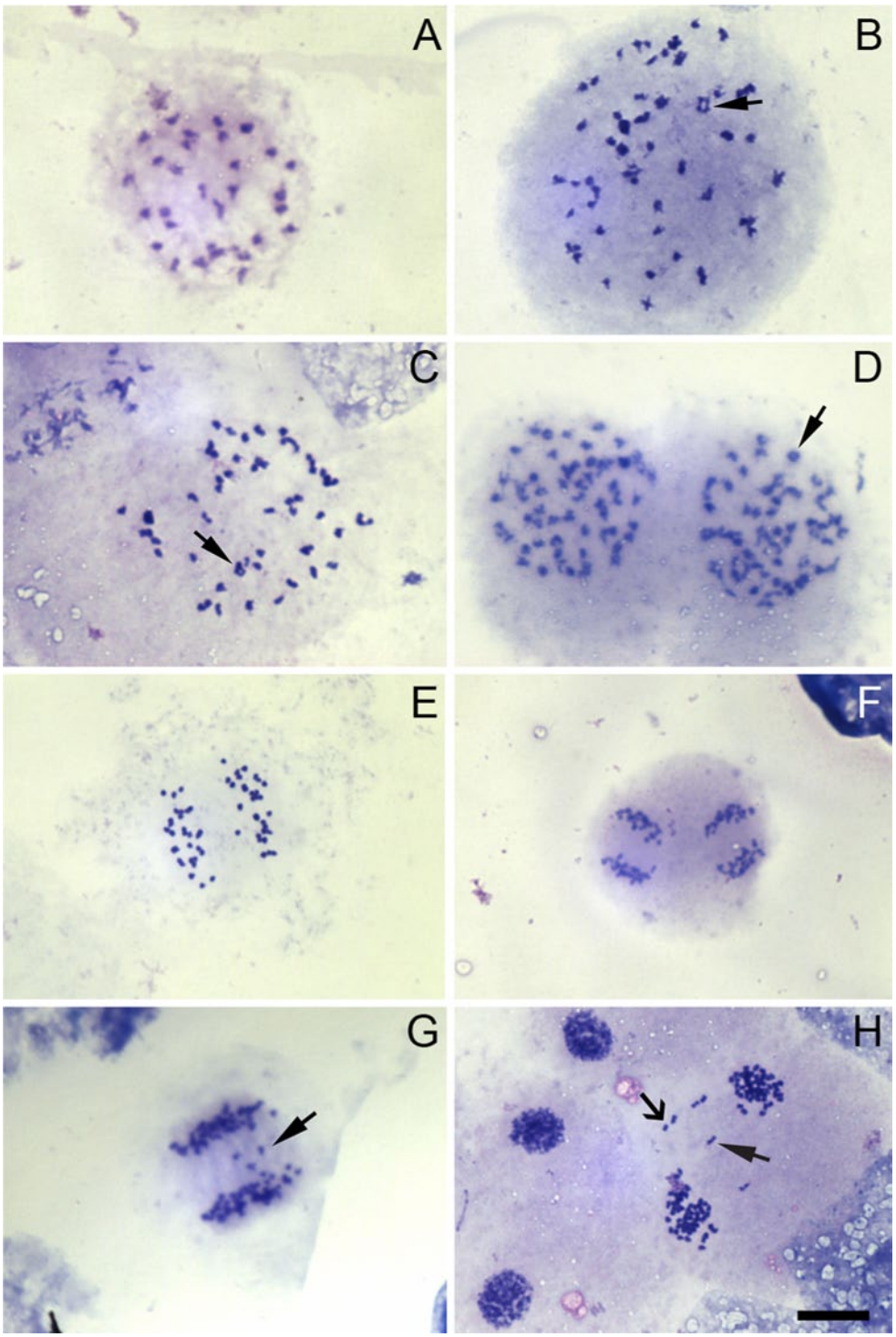

Fig. 1 Pollen mother cells of *Actinidia chinensis* at meiotic metaphase (**a–d**), anaphase I and telophase II (**e–h**). **a** 'Hort16A', $n = 29$; **b–c** colchicine-induced autotetraploid from 'Hort16A', $n = 58$ (an *arrow* indicates a ring as a quadrivalent); **d** tetraploid male, M3, $n = 58$ (an *arrow* indicates a ring as a quadrivalent); **e** diploid male, M1; **f** 'Hort16A'; **g–h** colchicine-induced autotetraploids from diploid 'Hort16A' (an *arrow* in **g** and in **h** indicates a single lagging chromosome; another *arrow* in **h** indicates a pair of lagging chromosomes). *Scale bar* 10 μm in all images

these autotetraploids. The two tetraploid male genotypes, raised from seeds collected from the wild in China, showed relatively low quadrivalent frequencies in comparison to colchicine-induced tetraploid from 'Hort16A', and no univalent formation was detected.

Small numbers (1–6 pairs) of lagging or maloriented chromosomes were seen at anaphase/telophase I and II in a third of the pollen mother cells observed of the regenerants RS3–RS6 (Table 2; Fig. 1). Lagging chromosomes were not seen in the diploid male or in any other tetraploid males.

Microsatellite segregation

Fifty-four microsatellite markers were screened for the presence of suitable alleles in the parents of the *A. chinensis* × *A. arguta* hybrids. Eleven markers were selected as having the potential to distinguish between preferential and non-preferential pairing and were used to analyse microsatellite inheritance in the *A. chinensis* × *A. arguta* hybrids; these markers map to 7 of the 29 linkage groups in diploid *A. chinensis*. For eight of these markers, three alleles could be identified in the *A. chinensis* parent; in the other three markers, all four *A. chinensis* alleles could be distinguished. To distinguish between preferential or non-preferential pairing in tetraploid *A. chinensis*, the ability to identify all four chromosomes accurately is important. Selecting microsatellites that amplified three or four alleles in the *A. chinensis* parent assisted in distinguishing between preferential and non-preferential pairing models. The two models could be easily distinguished when the inheritance of all four *A. chinensis* alleles could be tracked in offspring, as six classes of allele combinations are produced from non-preferential and only four from preferential pairing. When three different sized alleles were present, but there were two copies of one allele (AABC), it was difficult to distinguish between the two models because if the same sized alleles are present in different preferential pairs, the same allelic combinations are produced, but at different frequencies. Under the preferential pairing segregation we assume that A pairs with B, and A pairs with C; then for the preferential pairing model, the following allelic combinations are produced: AA, AB, AC and BC, each at 25 % frequency, whereas under the non-preferential pairing segregation, the allelic combinations AA and BC are produced at 16.66 % frequency, and AB and AC are produced at 33.33 % frequency.

Multinomial likelihood tests of goodness of fit of observed allelic combinations in hybrid progeny to expected non-preferential pairing and preferential pairing segregation ratios are shown in Table 3 and Supplementary Table 2, and Pearson's Chi-squared tests are shown in Supplementary Table 2.

Discussion

The overall regularity of meiosis, with few lagging chromosomes and the absence of trivalents and univalents, suggests that the reduced seed set in the colchicine-induced tetraploids is unlikely to be a consequence of irregular meiotic segregation. Univalent formation is a feature of many induced tetraploids in other species (Katsiotis and Forsberg 1995; Luan et al. 2009) and its contribution to reduced fertility of induced tetraploids has been long recognized (Rees and Jones 1977). The reduced seed set could be partly related to a low availability of diploid pollen from tetraploid males in the orchard where the vines were grown. It is also possible that our observations on meiosis in the pollen mother cells of *A. chinensis* females do not

Table 3 Multinomial likelihood tests of allele combinations from *Actinidia chinensis* for 11 markers with non-preferential and preferential pairing

Marker	Female parental genotype	Linkage Group	Non-preferential		Preferential	
			−2 × Sum[ln(LR)]	*P* value	−2 × Sum[ln(LR)]	*P* value
Ke209	AABC	2	0.543564117	0.909221	7.547989593	0.056338
Ke264	ABCC	8	0.960310209	0.810854	3.547360728	0.314671
Ke367	ABC-	1	8.294565047	0.140731		
Ke417	ABCC	5	5.003232622	0.171561	7.381819137	0.060674
Ke489	ABCD	27	8.068211206	0.152516		
Ke530	ABBC	2	8.056366499	0.044861	17.54778676	0.000545
Ke612	ABBC	13	10.641526080	0.013831	39.43265912	1.41E−08
Ke614	ABBC	1	2.225803530	0.526884	9.547101278	0.022836
Ke639	AABC	2	0.763952754	0.858068	13.96604976	0.002952
Ke672	AABC	1	1.216329927	0.749090	7.88095733	0.048537
Ke673	ABCD	18	2.598335653	0.761618		

accurately mirror meiosis in their megasporocytes, but studying meiosis in megasporocytes is difficult. Although there are striking examples of differences in meiotic behaviour in the male and female germ lines of hermaphrodite plants, the extreme being the chiasmate female and achiasmate male meiosis in *Fritillaria amabilis* (Noda 1968), in most plants the differences are usually only minor. We have therefore limited our studies to meiosis in the pollen mother cells. From our results, different stages of meiosis of pollen mother cells of female kiwifruit plants were still clearly observed, although the nuclei in pollen mother cells degenerate post-meiosis; mature pollen grains of females are devoid of cytoplasm (Ferguson 1984).

A low frequency of multivalent formation has been variously attributed to small chromosome size (Santos et al. 2003), the effects of genes in natural diploid populations that promote bivalent formation (Gill et al. 1993; Corredor et al. 2005), or a low chiasma frequency (Jackson 1982). *Actinidia* chromosomes are small, but it is still possible to discern ring bivalents with two chiasmata from rods with a single chiasma. We have not determined chiasma frequencies, but it is clear that ring bivalents are reasonably common, so that chiasma frequency and small chromosomes are unlikely to contribute to the low multivalent frequency observed. Thus, preferential pairing, the synapsis of chromosomes with greatest similarity, would appear to be the most logical explanation. In highly heterozygous plants such as kiwifruit, induced tetraploids will contain pairs of genetically identical chromosomes that comprise the group of four homologues.

The naturally occurring *Actinidia* tetraploids of wild origin and the colchicine-induced tetraploids differed in the frequency of quadrivalent formation. In most of the colchicine-induced tetraploid plants, the quadrivalent frequency was higher than in the naturally occurring tetraploids. There are two possible explanations for this: one is that there are bivalent-promoting genes in some diploids that reduce quadrivalent formation following chromosome doubling, as demonstrated by Avivi (1976) in *Triticum longissimum*; the other, that there has been selection in the wild plants for reduced quadrivalent formation. Unfortunately, as the autotetraploids were derived from a single individual, alternative markers of chromosome pairing such as microsatellites are not suitable for following chromosome inheritance. Instead, microsatellite markers were used to investigate whether preferential or non-preferential chromosome pairing occurs in a tetraploid of *A. chinensis*. To do this, interspecific hybrid populations between the tetraploid *A. chinensis* and *A. arguta* were used. Interspecific hybrids were selected, as this would increase the diversity of microsatellite alleles present and therefore increase the chance that the alleles from each parent could be clearly identified.

Microsatellite marker analyses of the *A. chinensis* × *A. arguta* hybrids provided evidence that suggests that non-preferential chromosome pairing had occurred during meiosis in their *A. chinensis* parent. All 11 markers showed patterns of allele segregation that are expected under a non-preferential pairing model, including the three markers where the inheritance of all four alleles could be followed. Preferential pairing seems an unlikely alternative, as although most markers (72 %) also showed patterns of allele segregation that fit with a preferential pairing model, they fit this model only if in every case it was assumed that it was the two alleles of the same size that did not pair together. Also, the ratios of allelic combinations in the progeny were closer to those expected under a non-preferential pairing model, as can be seen by the lower P values obtained when using a preferential pairing model. In addition, in every case when the inheritance of four alleles could be followed (three markers), allele combinations were observed in progeny that were not expected under preferential pairing models. With the three markers where the inheritance of all four alleles could be followed, no progeny contained allelic combinations that would be expected from double reduction events.

Preferential and non-preferential pairing can be viewed as the two extremes of possible pairing within tetraploids; tetraploid taxa may display a mixture of preferential and non-preferential pairing behaviour (Fjellstrom et al. 2001). The degree of preferential or non-preferential pairing is expressed as the preferential pairing factor (ρ) (Sybenga 1988). Strictly non-preferential pairing would have $\rho = 0$ and strictly preferential pairing would have $\rho = 2/3$. For the three markers in which the inheritance of all four alleles could be followed, we obtained estimates of ρ of 0 (Ke673), 0 (Ke489) and 0.18 (Ke367), again suggesting strongly that very little preferential pairing was occurring in the tetraploid *A. chinensis* genotype that was the female parent of the *A. chinensis* × *A. arguta* hybrids.

Although only a low number of multivalents were observed in the two naturally occurring tetraploids of *A. chinensis*, the analysis of microsatellite inheritance indicates that non-preferential chromosome pairing behaviour occurs in the tetraploid *A. chinensis*. A low frequency of multivalents in natural tetraploid *A. chinensis* could be due to the relatively small size of the chromosomes, a low frequency of chiasmata (McNeilage and Considine 1989), or selection of bivalent formation as an adaptive trait by the selection of meiotic pairing control genes that promote bivalent formation. This is consistent with observations of chromosome pairing behaviour in hexaploid *A. deliciosa* (syn. *A. chinensis* var. *deliciosa*), which also shows a predominance of bivalent pairing even though non-preferential chromosome pairing occurs (Mertten et al. 2012), suggesting that this adaptation occurs at multiple ploidy levels

within the *A. chinensis*/*A. deliciosa* complex. There is also evidence in a range of other polyploid genera that a predominance of bivalent pairing occurs even with non-preferential chromosome pairing (Crawford and Smith 1984; Naranjo and Orellana 1984; Soltis and Riesberg 1986; Samuel et al. 1990; Jones 1994; Qu et al. 1998).

The occurrence of non-preferential chromosome pairing in natural tetraploids of *A. chinensis*, the likelihood of the presence of genes within tetraploid breeding populations that promote bivalent formation, and the meiotic chromosome behaviour in colchicine-induced tetraploids suggest that colchicine-induced tetraploids should be easily incorporated into a tetraploid *A. chinensis* breeding programme. Progeny produced by cross between induced and natural tetraploids of *A. chinensis* have been planted, and once the seedlings flower and fruit, they will be used to assess fruit size, quality, and meiotic chromosome behaviour, and to determine whether their fertility and their fruit quality improve, and their fruit size increases.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the experiments complied with current laws of the country in which they were performed.

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