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Genome affinity in *Rubus* (Rosaceae) inferred from meiotic chromosome pairing of sixteen wild and cultivated bramble resources

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Abstract

To illustrate the mode of polyploid origin in *Rubus*, meiotic processes of pollen mother cells and pollen viability in sixteen wild and cultivated bramble resources with various ploidy levels were investigated in this study. In diploids, formation of seven bivalents was the most predominant meiotic association at metaphase I, except for wild *R. ellipticus* var. *obcordatus* and red raspberry cultivars (*R. idaeus*) displaying a few univalents, but in black raspberry cultivar (*R. occidentalis*) both univalent and multivalents were recorded. Meiotic pairing in tetraploids *R. lambertianus* var. *glaber*, blackberry hybrids 'Shawnee' and 'Arapaho' recorded 13.64, 7.41 and 12.33 mean bivalents per cell, respectively. Various chromosome configurations were observed in hexaploid blackberry 'Ollalie' with mean of 12.80 bivalents and 3.85 quadrivalents. Besides predominant bivalents, univalents and multivalents were accounted in significant proportions among heptaploid blackberry cultivars. Abnormal chromosomal behaviours including chromatid bridges, lagging chromosomes, unequal segregation, micronuclei, and polyads were also frequently observed. The frequency of meiotic irregularities significantly increased with increasing ploidy levels, which has influence on the percentage of sterile pollen grains. Based on meiotic pairing and abnormalities, wild *R. lambertianus* var. *glaber* and blackberry 'Arapaho' were supposed to be well-established allotetraploids through diploidization process; blackberry 'Shawnee' and 'Ollalie' to be segmental allopolyploids with certain variations, while 'Black Butte', 'Boysen' and 'Kotata' to be alloheptaploids *via* intra-specific hybridization accumulating stable chromosomal variations. In addition, structural variations such as chromosomal inversion and translocation play an important role in the evolutionary history of *Rubus*.

Keywords: *Rubus* L., raspberry, blackberry, chromosome pairing, meiotic abnormality, allopolyploid

Introduction

Rubus is a large taxonomically challenging genus with ubiquitous polyploidy, apomixis and frequent hybridization (Thompson 1997). Focke (1910, 1911, 1914) established the widely adopted *Rubus* taxonomy that contained 12 subgenera with three largest being *Idaeobatus*, *Malachobatus*, and *Rubus* genera. Based on comprehensive investigations, 40 per cent of approximately 400 *Rubus* species are polyploids (Thompson 1997; Naruhashi et al. 2002; Wang et al. 2008a). Those *Rubus* plants used for horticultural plantations are generally named bramble or edible *Rubus* (Gui and Hu 2002). According to Jennings (1988), and Qu and Sun (1990), brambles are composed of Raspberry, Blackberry, and Dewberry. The raspberry cultivars consist of red (*R. idaeus* spp.), black (*R. occidentalis* L.), yellow (*R. xanthocarpus* Bureau et Franch.) and purple raspberry; the latter is *via* hybridization between *R. idaeus* var. *strigosus* Maxim and black raspberry (Weber 2007). Mainly, blackberry (*R. allegheniensis* Porter.) cultivars originate from multiple intra-specific hybridization events, making it hard to identify their primitive ancestral species (Hall 1990; Thompson 1995; Daubeny 1996; Krewer et al. 2001). Taxonomically, raspberry and blackberry are assigned into predominant diploid subgenera *Idaeobatus*, *Chamaemorus* and *Cylactis*, and polyploid subgenera *Rubus*, respectively (Jennings and McNicol 1991; Daubeny 1996). To date, the alternative possibilities of auto- or allo-polyploid origin these species have not been well investigated.

Chromosomes remain important not simply

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because they carry the genes, but because their behaviour determines the mechanism of inheritance (Sumner 2003). Genome affinity is usually determined by chromosome pairing behaviour. Meiotic pairing of chromosomes has been investigated earlier in several *Rubus* species, including diploid *R. parvifolius*, *R. coreanus*, *R.* × *nikaii* and *R.* × *hiraseanus*; triploid hybrid *R.* × *tawadanus*; tetraploid *R. sieboldii*, *R. assamensis*, *R. setchuenensis*; and octoploid *R. buergeri* (Iwatsubo and Naruhashi 1991, 1993, 1996, 1998; Iwatsubo et al. 1996; Chen et al. 2015). These studies are helpful to clarify the origins of commercially important brambles to analyze their potentialities as breeding materials and to shed light on genomic relationships in the genus *Rubus*. Meiosis in hybrids could be studied by light microscopy and the degree of differentiation between hybridizing taxa estimated by analyses of chromosome pairing behaviour and meiotic abnormalities (Rieseberg et al. 2000).

The species used in the study are endemically distributed in Southwest China and hold great potential for *Rubus* breeding. The cultivars considered here were mainly produced by hybridization between different parents, including various ploidy levels ($2n = 2x$, $4x$, $6x$, $7x = 14$, 28, 42, 49) (Thompson 1995; Wang and Zhang 2003; Wang et al. 2008a, b). The objectives of the present study were (i) to analyze the chromosome pairing behaviours and chromosomal abnormalities during meiosis of pollen mother cells (PMCs) within sixteen wild and cultivated brambles at different ploidy levels, (ii) to understand genome affinity of these polyploids from subgen. *Malachobatus* and *Rubus*, and (iii) to illustrate the possible role of chromosomal variation in the evolutionary history of *Rubus* genus.

Materials and methods

Plant materials

Six wild brambles namely, diploid *R. ellipticus* var. *obcordatus*, *R. coreanus*, *R. corchorifolius*, *R. amabilis*, *R. niveus* from *Idaeobatus*, tetraploid *R. lambertianus* var. *glaber* from *Malachobatus* were chosen for this study (Table 1). Ten varieties from raspberry and blackberry were also used here. The wild brambles are widely distributed in Ya'an city, Sichuan Province, and were collected from year 2004 to 2008. Their typical morphologies were shown in Fig. 1. More than three plants were collected from the site for each species and grown at the Teaching and Scientific Research Base of the University. The cultivars were introduced from Canada, Australia, and America, etc., including four raspberry and of six blackberry. Voucher specimens of all these samples were deposited in the herbarium of the College of Horticulture of the University.

Meiotic analysis

Floral buds of 2.0 to 3.7 mm diameter were collected (March to August in year 2006 to 2008) and fixed in modified Carnoy's II solution (absolute ethanol: glacial acetic acid: chloroform = 5 : 3 : 2, v/v) at 25 I for 4 h. After fixation, buds were transferred to 70^{o}C% ethanol and stored in a 4^{o}C refrigerator. Pollen mother cells at diakinesis or metaphase I were squashed and stained with 1.5% carbolic acid fuchsin solution. Photomicrographs were taken with an Olympus BX-51 (Japan) camera system. The frequency of mean pairing (c-value: the mean frequency with which two related chromosome arms pair) was calculated according to Alonso and Kimber (1981). All available phases of meiosis were analyzed. Abnormalities during meiosis, such as unaligned chromosomes at metaphase I, chromatid bridges and laggard chromosomes at anaphase and telophase I and II, were calculated. Ten to thirty individuals for each accession were accessed, with a total of over 40 cells observed for each meiotic stage except that for tetrads, 500 cells were recorded.

Pollen fertility

For the estimation of fertility, pollen grains were examined after staining in 1 % acetocarmine solution for 3 min. Pollen viability was estimated based on stainability. At least 2000 pollen grains from different floral buds were used for the detection. Fully stained pollen grains were considered fertile (Marks 1954).

Results

Chromosome pairing at metaphase I

Chromosome pairing behaviours at metaphase I stage and the respective genome configurations were shown in Table 1 and Figs. 2 and 3. In the diploid wild *R. ellipticus* var. *obcordatus*, chromosome pairing configuration was 0.08 I+6.96 II per cell (Table 1; Figs. 2a, b). Chromosomes paired to form 7 bivalents in all examined PMCs in diploids wild species *R. coreanus*, *R. corchorifolius*, *R. amabilis* and *R. niveus*, revealing a regular meiosis (Figs. 2c-f). In cultivated red raspberry 'Chilcotin', 'Dinkum' and 'Nova', five bivalents and four univalents occurred in ~ 9 % of the MI cells (Table 1; Fig. 2g-j). Multivalents were detected only in cultivated black raspberry 'Bristol' with mean

Fig. 1. Typical morphology of six wild bramble resources. a = *R. ellipticus* var. *obcordatus*; b = *R. coreanus*; c = *R. corchorifolius*; d = *R. amabilis*; e = *R. niveus* and f = *R. lambertianus* var. *glaber*

configuration of 0.67 I +6.33II+0.12III+0.10IV per cell (Table 1; Figs. 2k-o). The chiasmata per cell ranged from 8.90 to 13.63 and per chromosome between 0.64 to 0.97 (Table 1).

In tetraploid wild *R. lambertianus* var. *glaber*, meiotic pairing was characterized with 0.26 I+13.64 II+0.04 III +0.15 IV per cell (Table 1; Fig. 3a, b). For blackberry 'Shawnee', the most frequent type of chromosome configurations was 10II+2IV (Fig. 3c), followed by 1I+10II+1III+1IV, 12II+1IV, and 6II+4IV (Figs. 3d-f). The average association observed was 0.37 I+7.41II+0.23III+2.91IV per cell (Table 1). The mean configuration per cell of blackberry 'Arapaho' was 0.54 I+12.33II+0.24III+0.52IV with the most

Table 1. Origin and meiotic configurations at metaphase I in pollen mother cells of sixteen bramble (*Rubus*) resources

Taxa	Origin (accession; altitude/m)/(source; ancestry)	2*n*	No. of observed cells	Chromosome configuration						Chiasmata/ cell	c-value
				I	II			III	IV		
					Ring	Rod	Total				
R. ellipticus var. *obcordatus*	*R01-2*; 700	14	47	0.08 (0-2)	6.07 (4-7)	0.89 (0-3)	6.96 (6-7)	-	-	13.02 (11-14)	0.93
R. coreanus	*R01-4*; 680	14	40	-	6.63 (5-7)	0.37 (0-2)	7.00 (7)	-	-	13.63 (13-14)	0.97
R. corchorifolius	*R01-6*; 750	14	42	-	6.35 (5-7)	0.65 (0-2)	7.00 (7)	-	-	13.35 (13-14)	0.95
R. amabilis	*R01-14*; 1250	14	40	-	6.31 (4-7)	0.69 (0-3)	7.00 (7)	-	-	13.31 (13-14)	0.95
R. niveus	*R01-1*; 700	14	41	-	6.42 (5-7)	0.58 (0-2)	7.00 (7)	-	-	13.42 (13-14)	0.96
R. idaeus spp (Red raspberry)											
cv 'Chilcotin'	Canada; -	14	41	0.29 (0-4)	5.33 (4-7)	1.52 (0-4)	6.85 (5-7)	-	-	12.19 (8-14)	0.87
cv 'Dinkum'	Australia; -	14	41	0.15 (0-4)	5.67 (4-7)	1.21 (0-3)	6.88 (5-7)	-	-	12.55 (8-14)	0.90
cv 'Nova'	Canada; Southland × Boyne*	14	44	0.28 (0-4)	4.89 (4-7)	1.97 (0-4)	6.86 (5-7)	-	-	11.76 (8-14)	0.84
R. occidentalis (Black raspberry)											
cv 'Bristol'	America; -	14	45	0.67 (0-4)	2.04 (1-6)	4.29 (1-6)	6.33 (5-7)	0.12 (0-1)	0.10 (0-1)	8.90 (6-13)	0.64
R. lambertianus var. *glaber*	*R01-8*, 680	28	53	0.26 (0-4)	11.69 (10-12)	2.01 (0-6)	13.64 (12-14)	0.04 (0-1)	0.15 (0-1)	25.18 (20-28)	0.90
Hybrid (Blackberry)											
cv 'Shawnee'	America; Cherokee × Ar.586 (Thornfree × Brazos)**	28	43	0.37 (0-4)	3.82 (2-7)	3.59 (4-10)	7.41 (6-12)	0.23 (0-2)	2.91 (1-4)	20.42 (15-26)	0.72
cv 'Arapaho'	America; Ark.631 (Ark.550 × Cherokee) × Ark.883 (Ark.593 × Ark.650)[b]	28	42	0.54 (0-4)	8.78 (3-12)	3.55 (2-9)	12.33 (12-14)	0.24 (0-1)	0.52 (0-1)	23.15 (13-26)	0.83
cv 'Ollalie'	America; Black Logan × Young**	42	41	0.20 (0-2)	11.39 (3-18)	1.41 (2-9)	12.80 (11-21)	0.27 (0-2)	3.85 (0-4)	30.45 (14-39)	0.72
cv 'Black Butte'	America; -	49	45	8.17 (0-17)	7.99 (0-14)	10.01 (8-18)	18.00 (15-21)	1.16 (0-4)	0.35 (0-1)	29.36 (14-38)	0.70
cv 'Boysen'	New Zealand; *R. ursinus***	49	45	5.28 (0-9)	7.77 (2-16)	8.04 (6-18)	15.81 (7-21)	2.57 (0-3)	1.00 (0-4)	31.72 (15-39)	0.76
cv 'Kotata'	-; Osc.743 (Pacific × Boysen) × Osc.877 (Jenner-1 × Eldorado)**	49 (0-9)	42 (2-15)	5.22 (5-18)	7.11 (7-21)	8.13 (0-4)	15.24 (0-4)	1.87 (16-38)	1.67	31.09	0.74

* = Wang and Zhang 2003; ** = Thompson 1995; 2n = Somatioc chromosome number

I = Univalent, II = Bivalent, III = Trivalent, IV = Quadrivalent; – = No data; c-value = Chiasmata / chromosome.

Blackberry hybrids possessed quite complex origin, such as 'Shawnee': Cherokee × AR.586 (Thornfree × Brazos); Cherokee: Darrow × Brazos, Darrow: [Eldorado (*R. allegheniensis* × *R. argutus*) × Brewer (*R. pergratus* × *R. frondosus*)] × Hedrick, Brazos: Lawton (*R. allegheniensis* × *R. frondosus*) × Nessberry; Thornfree: (Brainerd × Merton) × (Merton × Eldorado)

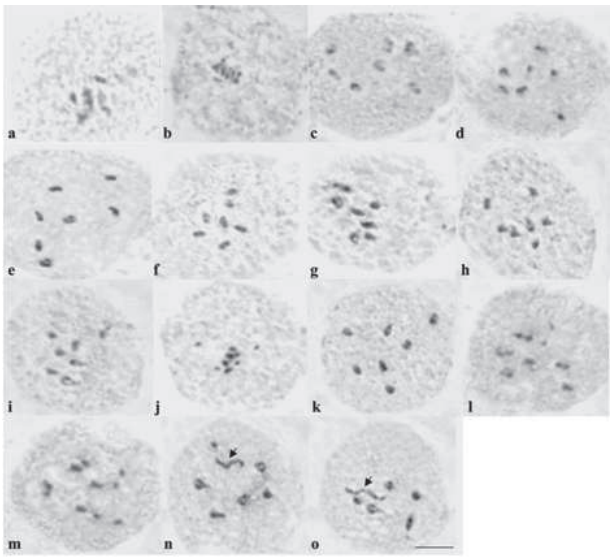

Fig. 2. Meiotic chromosome pairing at metaphase I in nine diploids. I, II, III and IV represent univalent, bivalent, trivalent, and quadrivalent. a-b *R. ellipticus* var. *obcordatus*, a = 7II, b = 2I+6II; c = *R. coreanus*, 7II; d = *R. corchorifolius*, 7II; e = *R. amabilis*, 7II; f = *R. niveus*, 7II; g = 'Chilcotin', 7II; h = 'Nova', 7II; i-j : 'Dinkum', i = 7II, j = 4I+5II; k-o: 'Bristol', k = 7II, l = 2I+6II, m = 4I+5II, n = 1I+5II+1III (arrowed) and o = 5II+1IV (arrowed)

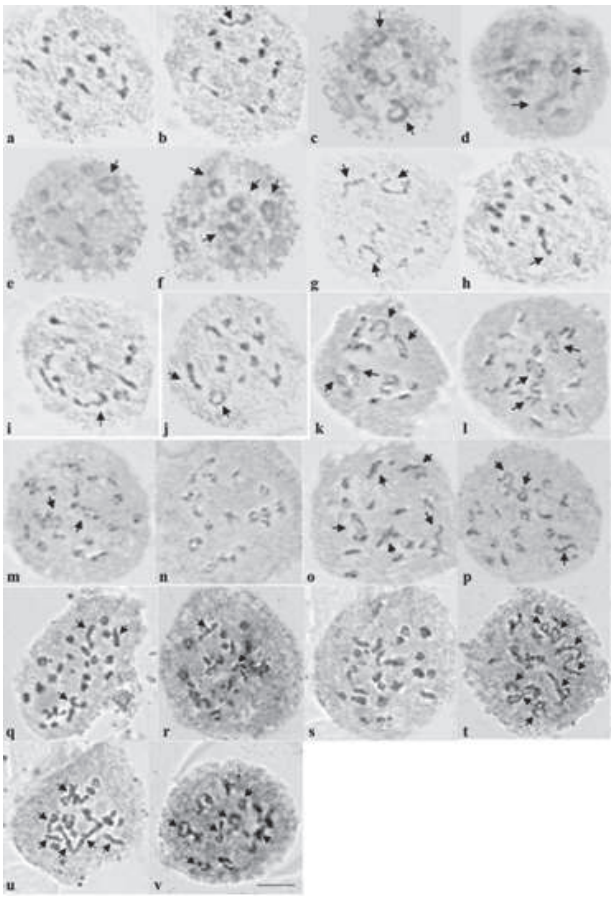

Fig. 3. Meiotic chromosome pairing at metaphase I in tetraploids (a-j), hexaploids (k-p), and heptaploids (q-v). I, II, III, and IV represent univalent, bivalent, trivalent, and quadrivalent. a-b : *R. lambertianus* var. *glaber*, a = 14II, b = 12II+1IV; c-f : 'Shawnee', c = 10II+2IV, d = 1I+10II+1III+1IV, e = 12II+1IV, f = 6II+4IV; g-j : 'Arapaho', g = 9II+2III+1IV, h = 1I+12II+1III, i = 12II+1IV, j = 11II+2III; k-p : 'Ollalie', k = 13II+4IV, l = 15II+3IV, m = 17II+2IV, n = 21II, o = 1I+11II+1III+4IV, p = 2I+15II+2III+1IV; q-r : 'Butte', q = 6I+17II+3III, r = 6I+18II+1III+1IV; s-t : 'Boysen', s = 7I+21II, t = 7I+7II+4III+4IV; u-v : 'Kotata', u = 2I+14II+5III+1IV, v = 14II+3III+3IV. Arrows represent multivalents

frequent type of 9II+2III+1IV (Table 1; Figs. 3g-j). The chiasmata per cell of tetraploids varied from 20.42 to 25.18, with c-values ranging from 0.72 to 0.90 (Table 1).

In hexaploid blackberry 'Ollalie', various chromosome associations, such as 13II+4IV, 15II+3IV, 21II, 1I+11II+1III+4IV, and so on (Fig. 3k-p) were observed. Bivalents and quadrivalents were present in high percentages at meiosis, and the mean configurations recorded were, was 0.20I+12.80II+ 0.27III+3.85IV per cell. Relatively high chiasmata per cell of 30.45 was found in 'Ollalie' with a c-value of 0.72 (Table 1).

Besides predominance presence of bivalents, a medium proportion of univalents and a few multivalents were also detected in PMCs in heptaploid blackberry 'Black Butte', 'Boysen', and 'Kotata'. The mean configurations recorded per cell were, 8.17I+18.00II+ 1.16III+ 0.35IV, 5.28I+15.81II+ 2.57III+1.00IV, and 5.22I+15.24II+1.87III+1.67IV, respectively (Table 1; Figs. 3q-v). The chiasmata per cell ranged from 14 to 39 with an average c-value of 0.73 (Table 1).

Variation in meiotic process

Normal meiotic division was observed in wild *R. ellipticus* var. *obcordatus* (Fig. 4). During division I, chromomeres at the zygotene stage (Fig. 4a) formed to seven highly condensed and advanced to well spread bivalents at diakinesis (Fig. 4b), which later became aligned in pairs at the equator of the cell at metaphase I (Fig. 4c, d). Then the paired chromatids synchronously separated from each other at anaphase I (Fig. 4e,

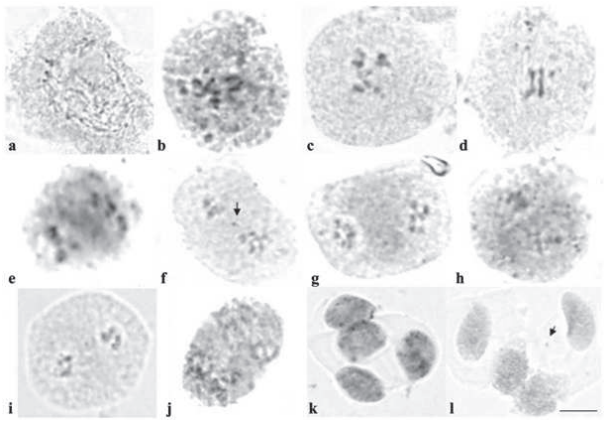

Fig. 4. Normal configurations at meiosis observed in pollen mother cells of diploid *R. ellipticus* var. *obcordatus*. a = Zygotene stage; b = Diakinesis stage; c-d = Metaphase I; e = Anaphase I; f = Anaphase I with fragment (arrowed); g = Telophase I; h = Metaphase II; i = Anaphase II; j = Telophase II; k = Tetrad; l = Tetrad with one micronucleus (arrowed)

f) to form two daughter chromosomes at telophase I with clearly countable chromosome number (2*n* = 14) (Fig. 4g). During division II, the chromosomes of two daughter cells arranged and formed metaphase plate at metaphase II (Fig. 4h). The sister chromatids separated and moved to poles at anaphase II (Fig. 4i) and finally formed four daughter nucleuses at telophase II (Fig. 4j). At the end, cytoplasm was normally segregated into four gamete cells, named tetrad which led to form or develop into pollen grains (Fig. 4k, l).

However, partial chromosome pairing leading to abnormally during meiosis in sixteen wild and cultivated brambles producing univalents, multivalents, lagging chromosome (fragment), chromatid bridges, micronuclei and tetrad of different size, polyads, etc. (Fig. 5; Table 2). In diploids, laggard chromosomes at anaphase I to telophase II appeared more frequently in red raspberry (18.37~28.57%) than that in wild brambles (4.26~17.39%), because paired chromosomes in several cells separated in advance

Table 2. Frequency of laggard chromosomes (fragment) or micronuclei at anaphase and telophase I and II and pollen stainability in sixteen bramble (*Rubus*) resources

Taxa	2*n*	Laggard chromosomes (fragment) at A I-T I		Laggard chromosomes or micronuclei at AII-TII		Pollen stainability (%)
		No. of observed cells	Frequency (%)	No. of observed cells	Frequency (%)	
R. ellipticus var. *obcordatus*	14	92	17.39	94	17.02	95.32
R. coreanus	14	94	4.26	83	6.98	96.78
R. corchorifolius	14	90	4.44	82	7.32	95.49
R. amabilis	14	96	10.42	88	6.82	95.16
R. niveus	14	94	8.51	84	2.38	95.24
R. idaeus spp (Red raspberry)						
cv 'Chilcotin'	14	98	18.37	94	14.89	80.15
cv 'Dinkum'	14	82	26.83	84	14.29	83.17
cv 'Nova'	14	84	28.57	86	16.28	82.55
R. occidentalis (Black raspberry)						
cv 'Bristol'	14	92	54.35	94	61.70	21.57
R. lambertianus var. *glaber*	28	98	8.16	92	13.04	85.35
Hybrid (Blackberry)						
cv 'Shawnee'	28	94	36.17	92	34.78	46.14
cv 'Arapaho'	28	90	26.67	88	25.00	63.55
cv 'Ollalie'	42	86	25.58	92	21.74	48.12
cv 'Black Butte'	49	92	71.74	94	38.30	59.87
cv 'Boysen'	49	96	85.42	98	48.98	46.23
cv 'Kotata'	49	98	83.67	94	53.19	45.19

AI = Anaphase I; TI = Telophase I; AII = Anaphase II; TII = Telophase II

(precocious separation) to form univalents at metaphase I in the former. As predicted, black raspberry 'Bristol' showed the highest frequency of laggard chromosomes or micronuclei (Table 2). A small number of tetrads and pentads of different size were also detected in 'Bristol' (Fig. 5c, d). This might be due to the irregular aligned chromosomes at metaphase I. In tetraploids and hexaploid unequal segregation of chromatids with tetrads and polyads at telophase II were also observed (Figs. 5e-o). Compared with tetraploid 'Arapaho' and hexaploid 'Ollalie', tetraploid 'Shawnee' showed a high degree of abnormalities (Table 2). For heptaploid blackberry cultivars, besides the typical irregularities (Figs. 5p-w), obvious fission of chromosome set was detected in some cells, explained that chromosomes were divided into different regions to get separated with each other. Thus, two possible pathways could produce pentad or hexad of different size at tetrad stage (Fig. 6). These data indicated that abnormal chromosomal behaviours at meiosis in heptaploid accounted for much larger

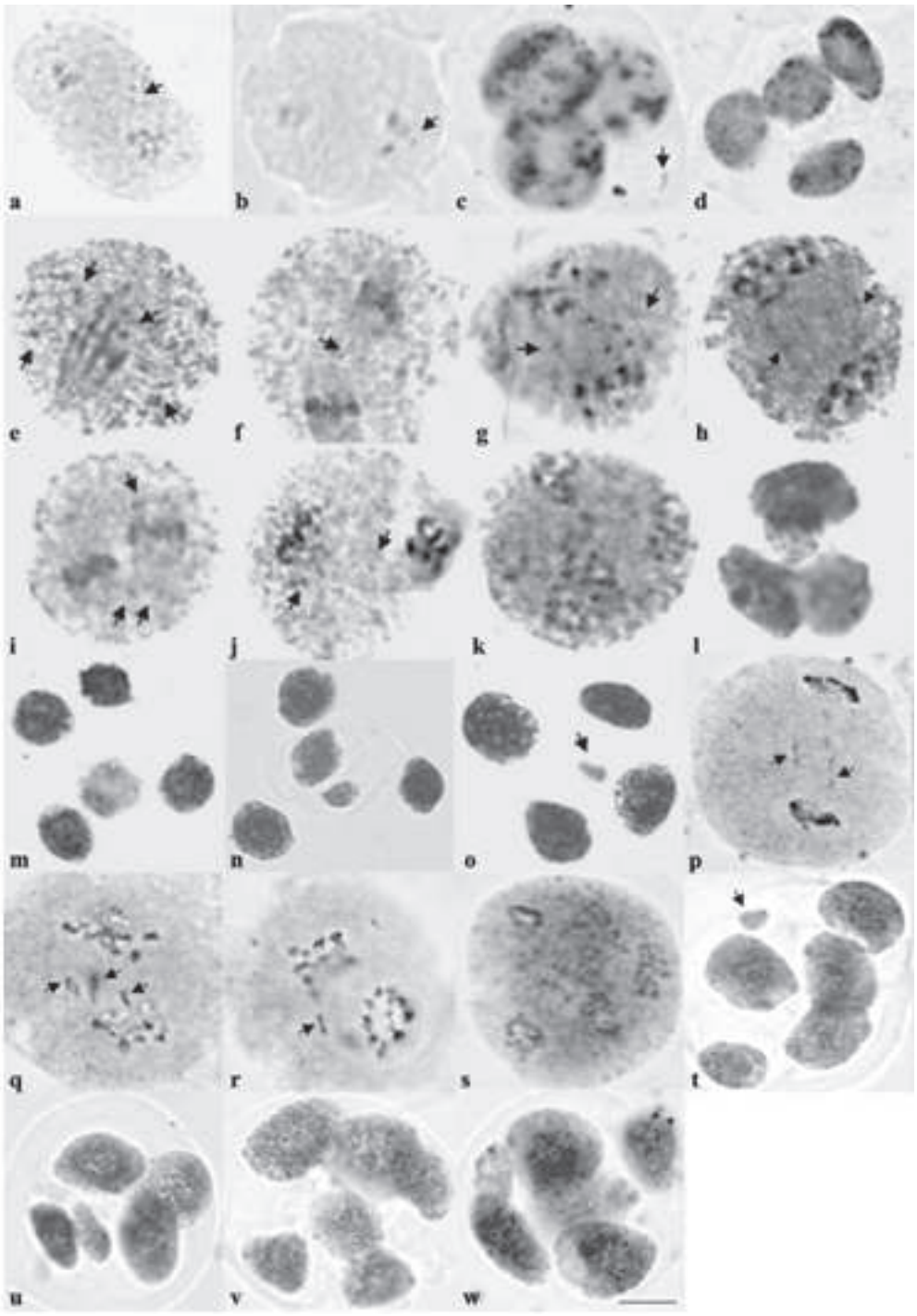

Fig. 5. Meiotic irregularities in diploid (a-d), tetraploid and hexaploid (e-o) and heptaploid (p-w) bramble (*Rubus*) resources. a-b : *R. ellipticus* var. *obcordatus*, a = Telophase I with chromosome fragment, b = Metaphase II with laggard chromosomes; c-d : 'Bristol', c = Triad with micronuclei, d = Pentad; e = Metaphase I with four univalents; f-h : Anaphase I with chromatid bridge or laggard chromosomes; i = Metaphase I with laggard chromosomes; j = Anaphase II with laggard chromosomes; k = Irregular telophase II; l = Tetrad in different size; m = Pentad in most same size; n = Pentad in different size; o = Tetrad with a micronucleus; p = Anaphase I with chromatid bridge; q-r : Anaphase I with laggard chromosomes; s = Irregular telophase II; t = Pentad with a micronucleus and u-w = Polyads

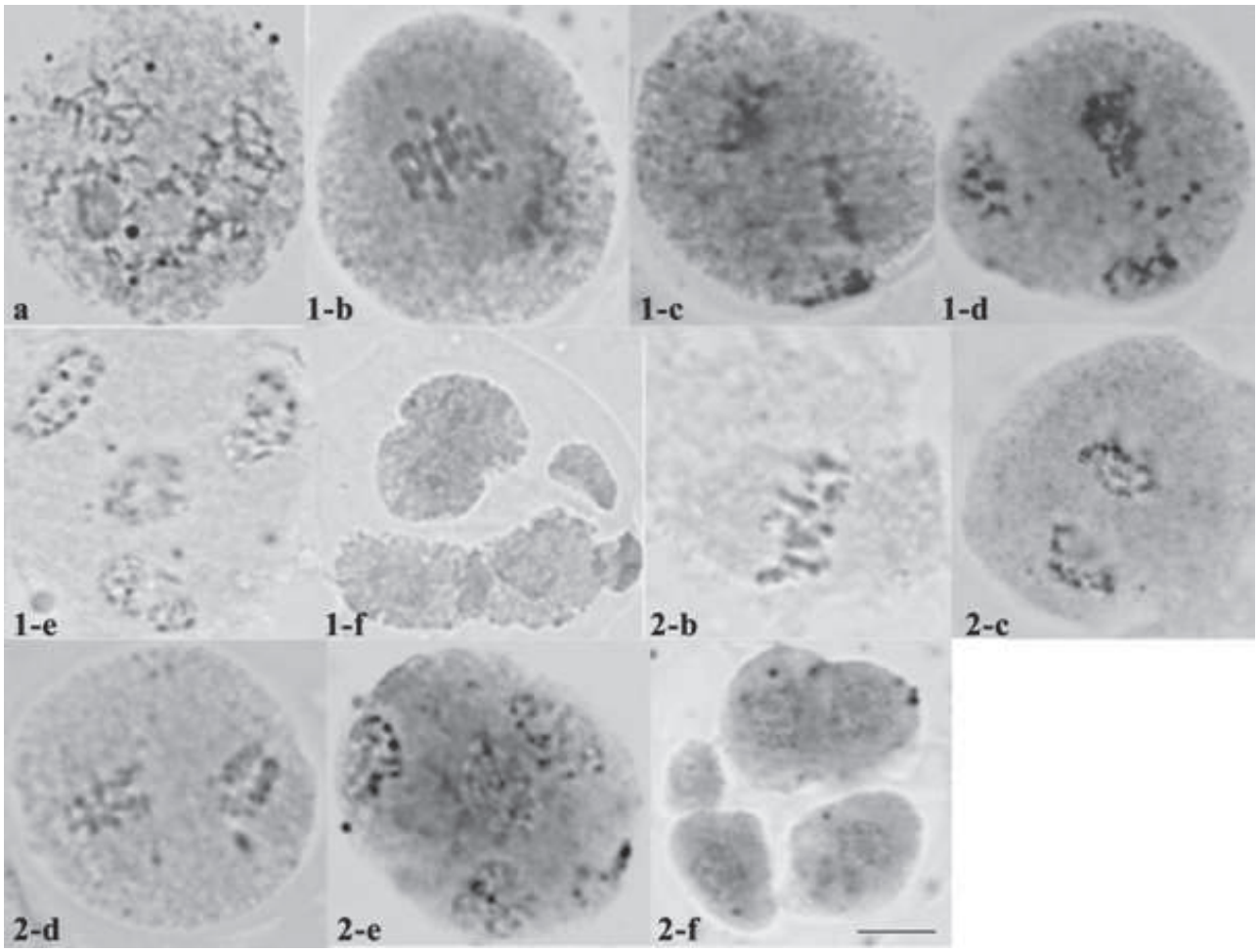

Fig. 6. The possible pathways for the production of two types of polyads in heptaploid blackberry cultivars. 1 : Hexad with different size of microspores produced by meiosis compartment syndrome. 2 : Pentad in different size due to part normal chromosome association. a = Diplophase stage with formation of two areas by chromosomes synapsis; b = Metaphase I; c = Anaphase I; d = Anaphase II; e = Irregular telophase II; f = Polyads

proportion during anaphase I to telophase I (71.74~85.42 %) than those during anaphase II to telophase II (38.30~53.19 %) (Table 2).

Pollen viability

As shown in Table 2, six wild brambles revealed relatively high pollen stainability ranging from 85.35 % to 96.78 %. The pollen viability in the red raspberry was displayed between 80.15 % to 83.17 %, whereas value in black raspberry was low to just 21.57 %. The pollen stainability variations were detected in the blackberry cultivars from 45.19 % to 63.55 %, much lower than wild brambles. The results of pollen viability were consistent with observations in the meiosis.

Discussion

Allopolyploid origin

The meiotic analysis of polyploids is an important element in taxonomic and evolutionary studies of plants. The occurrence of quadrivalents in meiosis may be explained by heterozygosity for chromosome translocations or may reflect homology due to polyploid origin (Stebbins 1971). Multivalent formation is surely expected in young autopolyploids as well as in those allopolyploids originated by somewhat similar species with differentiated chromosome and some kind of genomic similarity, described as segmental allopolyploids (Boff and Schifino-Wittmann 2003). Some degree of homology (partial homology) may exist

between some chromosome of one genome and those of the other genome. Therefore, in such polyploids both bivalents and multivalents are formed, and the former is the main type (Sybenga 1996). For example, there were 78% to 83% of bivalents at metaphase I in segmental allotetraploid *Leucaena* species (Boff and Schifino-Wittmann 2003). On the contrary, quadrivalents counted for considerable percentage from 57% to 81% among various autotetraploid species (Morrison and Rajhathy 1960). As a result, we could speculate the mode of polyploid origin based on the chromosome pairing behaviours during meiosis.

The results of present study revealed a high degree of bivalent chromosome pairing at metaphase I were observed among polyploid brambles (Table 1). *Rubus lambertianus* var. *glaber* and 'Arapaho' were considered as allotetraploids because bivalent accounted for the most percentages among meiotic associations. A few univalents and multivalents might be due to exchange of reciprocal segments by crossing over between a homologous pair of parental chromosomes. In tetraploid 'Shawnee' and hexaploid 'Ollalie', both predominant bivalents and frequent ring quadrivalents were formed, supporting that they were segmental allopolyploids. Blackberry cultivars often possessed quite complex origin, such as 'Shawnee' and 'Arapaho' from multiple intra-specific hybridization events (Thompson 1995); even the 'Boysen', 'Logan', and 'Young' through hybridization between blackberry and raspberry (Daubeny 1996). Lim et al. (1998) reported that both entire set and translocated chromosomes of raspberry were observed in an aneuoctaploid blackberry cv 'Aurora' ($2n = 8x = 58$) by genomic *in situ* hybridization (GISH) analysis. This mechanism explained why the univalents were frequently present among blackberry cultivars. Therefore, we suggested that three heptaploid blackberry cultivars were allopolyploids originating from intra-specific hybridization due to high frequency of bivalents and univalents.

Variation inferred from meiotic irregularities

Many causes related to cytological disterbances may lead to the occurrence of meiotic irregularities, such as lack of chromosomal homology resulting from hybridization, polyploidy, genetic and also the environmental factors (Pagliarini 2000; Yang et al. 2015). Chromosomal homology an essential event in the meiosis is necessary for chiasmata formation (Souze and Pereira 2011). Here, diploid wild brambles showed normal meiosis and high pollen fertility (>95%), while cultivated raspberry revealed some abnormal chromosome brhaviour and lower pollen fertility (~ 80 %), especially black raspberry (21.57 %) (Table 2). More frequent laggard chromosomes and lower c-values were detected in red raspberry than wild brambles. This suggested that cultivars might accumulate more chromosomal variations than wild during the long-term cultivation history. Interestingly, black raspberry 'Bristol' presented the lowest chiasmata/cell, c-value, and pollen stainability, and the highest percentage of abnormalities (Tables 1 and 2). The results indicated relatively low degree of chromosomal homology between its parents, partially resulting in the presence of univalents at the diakinesis stage. Therefore, we suggested that 'Bristol' was a hybrid between genetically distinct parents. This was consistent with the Jinno's (1958a, b) view that meiotic configurations and irregularities could have influence on the percentage of sterility of pollen grains. However, it seems not completely positively correlated between meiotic configurations and pollen fertility of artificial hybrids. Both of F_1 hybrids between normal and reciprocal cross of *R. minusculus* and *R. corceacanthus* showed highly similar meiotic pairing with most bivalents and rare univalents, but revealed significant differences in pollen fertility (Iwatsubo et al. 1996).

It is determined that the chromosome pairing was affected by different behaviour of chromosomes during meiosis or structural variation in chromosomes. It is revealed that the frequency of meiotic further abnormalities significantly increased with the increasing ploidy level (Table 2). Among tetraploids, wild *R. lambertianus* var. *glaber* showed the most bivalents and the lowest frequency of irregularities, which further demonstrated that it was a well-established allotetraploid evolved through diploidization process, so did tetraploid blackberry 'Arapaho'. As expected, heptaploid blackberry cultivars showed much higher frequency of abnormalities than tetraploid and hexaploid during both meiosis I and II. The irregularities at the end of meiosis I were also responsible for post-meiotic abnormal products such as triads and polyads. Fewer laggard chromosomes were detected in 'Black Butte' than 'Boysen' and 'Kotata', suggesting much lower level of genetic variation in 'Black Butte'. Based on the above results, these cultivars were supposed to be alloheptaploids *via* multiple intra-specific hybridization events with accumulation of stable chromosomal variations. Although, lagging chromosomes were present among heptaploids and

about half of the pollen grains revealed high fertility because of meiosis compartment syndrome (Table 2; Fig. 6). It has also been demonstrated within higher polyploids by Thompson and Maxine (1962).

Based on morphological and chromosomal data, Lu (1983) suggested that evolution in *Rubus* proceeded from woody to herbaceous plants and from compound to simple leaves. In the present study, more abnormal meiotic pairing behaviours and irregularities were detected in the taxa with higher ploidy levels. This indicated that diploid *Rubus* was primitive, and polyploid *Rubus* was advanced, strongly supporting Lu's view. The previous molecular cytogenetics and phylogenetics also provided evidence for this view point (Wang et al. 2015, 2016). As Souza and Pereira (2011) proposed that the formation of chiasmata is a good characteristic, since it ensures the genetic variability in plants, thus having a direct influence on adaptation mechanisms. It is presumed that, structural variations such as chromosomal inversion and translocation due to chiasmata in meiosis, not only provide abundant genetic resources of *Rubus* species, but also play a considerable role in the evolutionary history of genus *Rubus*.

Authors' contribution

Conceptualization of research (XRW); Designing of the experiments (HQF, XRW); Contribution of experimental materials (JZ, TC); Execution of field/ lab experiments and data collection (YW, QC, BS); Analysis of data and interpretation (YW, YL, YZ, HRT); Preparation of manuscript (YW, QC, XRW).

Declaration

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