

# The effect of different genome and cytoplasm on meiotic pairing in maize newly synthetic polyploids

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**Abstract** Allopolyploidization plays the special role in the evolution of many crops. Moreover, the evolution in early stage of some allopolyploidization events is predicted to be effected by nuclear-cytoplasmic interactions. Maize and teosintes are well model system for study of genetic recombination in allopolyploidization. In order to investigate the effects of genome organization and cytoplasm on genome evolution in newly synthesized allopolyploids (neopolyploids), a series of neopolyploids were produced by reciprocal crosses of maize and *Zea perennis*. By using dual-color genomic in situ hybridization, intra- and intergenomic meiosis pairing of these polyploids were quantified and compared with regard to its genome organization and cytoplasm background. In the four neopolyploids, the stability of maize genome is consistently lower than that of *Z. perennis* genome. Additional, the stability of maize genome is affected by genome ploidy. The cytoplasm, genome composition and their interaction do have the special role in chromosome pairing and the meiosis

behaviors in *Zea* allopolyploids vary significantly and showed non-diploidization. *Z. perennis* cytoplasm may give a relatively relaxed environment for maize genome.

**Keywords** Maize · *Z. perennis* · Allopolyploid · Cytoplasm · Meiosis · Dual-color GISH

## Introduction

Polyploid refers to the presence of three or more chromosome sets in one nucleus, is wide spread in plants and considered to be a major force in plant evolution (Comai 2000; Lavania 2013). Recent studies show that all of flowering plants undertake one or more ancient genome doubling events (Wood et al. 2009; Conant et al. 2014; Woodhousea et al. 2014; Moghe and Shiu 2014). Additional, there are also high frequency of polyploid events among most important crops such as maize, rape, coffee, cotton, wheat and so on (Hilu 1993; Chen 2007; Matsushita et al. 2012). Otherwise, as a result of ancient and recent polyploid events, it is interesting to study what happens during the process of diploidization following neopolyploids (Cheung et al. 2009).

In some neopolyploids, comparing with the parents, genome, which is not a simple accumulation, can change fast or in a long time (Mandakova et al. 2010; Schnable et al. 2012). The increased genes and genome dosages in neopolyploids can result in

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genome instability, such as chromosome imbalance, meiosis chaos, sequence loss and gene mutation (Wendel 2000; Levy and Feldman 2002; Liu and Wendel 2002; Chen 2007; Jackson and Chen 2010). Genome organization and cytoplasm background have a significant influence on genome instability in allopolyploids (Cheung et al. 2009, De Storme and Geelen 2013). During wheat polyploidization process, the genome tends to lose specific low copy sequences from D genome, which shows parental tendency and inherent difference of parent genome stability (Ozkan et al. 2001). Similar phenomenon is observed in synthetic brassica hybrids, comparing with A and C genome, B genome has more stable and less pairing variation (Cui et al. 2012). But for Brassica allotetraploids, which have highly differentiated cytoplasm, the genome coming from the male parent was significantly modified, compared to the genome coming from the female parent (Song et al. 1995), and other experimental results obtain the same conclusion (Cui et al. 2012). A possible explanation is that the maternal enzymatic system may treat the male chromosomes as exogenous intruder (Wang et al. 2010; Hu et al. 2013). The degree of cytoplasmic influence is decided by divergent relationship of parent genome in allopolyploid. *B. juncea* has higher genome change than that in *B. napus* with the explanation is that A and B genomes have higher degrees of divergence than that between A and C genome (Cui et al. 2013). In addition, some other reports find that genome instability of *arabidopsis* is controlled by both genetic and environmental factors (Armstrong et al. 2001). Poor homologous synapsis I (*Phs1*) and penetration and arbuscule morphogenesis I (*Pam1*) also play an important role in genome instability (Ronceret et al. 2009; Lukaszewski and Kopecky 2010; Feddermann et al. 2010). As a whole, the genome instability in the early stage of polyploidization is affected by multiple factors, in order to have a further understanding, more experimental models and more experimental methods should be explored.

Meiosis plays an important role in the life cycle of sexual species (Tiang et al. 2012). It insures the genetic stability of genome, increases the genetic diversification of gamete and enhances the adaptability of offspring (Santos et al. 1983; Bogdanov 2003; Bernstein and Bernstein 2010). Studies on meiosis not only address the question of genetic relationship of

parental genome but also provide important clues on genome evolution in allopolyploids. Previous researchers have shown that crosses are possible between maize and *Z. perennis* (Tang et al. 2005). Therefore, allopolyploids which contain whole sets chromosomes of the two species may be created by artificial pollination (*Zea* allopolyploids). In past years, a series of studies about meiosis of *Zea* allopolyploids have been conducted, and lead to great advances in our understanding of the relationship between maize and *Z. perennis* genome, and a hypothesis has been offered that maize has a tetraploid origin and *Z. perennis* has an octaploid origin (Tito et al. 1991; Naranjo et al. 1994; Poggio et al. 1999; Molina et al. 2012). But, the genome evolution during the process of diploidization following *Zea* allopolyploid is still cryptic. As mentioned above, genome organization and cytoplasm background do influence the stability of parent genome in some allopolyploids. However, the effects on meiosis behavior of *Zea* allopolyploid are still uncertain, as well as, whether inherent difference of genome stability exists between male and female genome in *Zea* allopolyploids.

Here, different allopolyploids between maize and *Z. perennis* can be synthesized in the laboratory from unequivocal progenitors, which can provide a new efficient model for studying the early evolution of neoallopolyploids. In this study, genomic in situ hybridization (GISH) is used to authenticate the pattern of chromosome paring in a series of *Zea* allopolyploid hybrids which have maize and *Z. perennis* cytoplasm, respectively. Thus, this article will put forward a new insight into the influence of genome organization and cytoplasm background on chromosome paring in *Zea* allopolyploids and give a further understanding of the evolution in early stage of allopolyploidization events.

## Materials and methods

### Plant material and crosses

Diploid maize wf9 ( $2n = 20$ ) and tetraploid maize Twf9 ( $2n = 40$ ) derived from wf9 were provided by USDA, and *Z. perennis* (9475,  $2n = 40$ ) was provided by the International Maize and Wheat Improvement Center (CIMMYT). These materials were grown in the experimental field of Sichuan Agricultural University,

Jinghong, China, in 2011. Two pairs of reciprocal crosses were performed by means of hand pollination. In 2012, pre-germination interspecific hybrid seeds were planted in the plastic pots (12 × 12 cm, inner diameter × height) filled with soil and placed in the experimental garden of Sichuan Agricultural University for early identification. Seedlings were transplanted into larger plastic pots (26.5 × 26.5 cm, inner diameter × height) after 5-leaf stage.

### Chromosome and DNA preparation

Root tips collected from young seedlings of interspecific hybrids were pretreated in  $\alpha$ -bromonaphthalene saturated solution for 2.5–3 h at room temperature, and then fixed in Carnoy's solution I (3: 1, ethanol: glacial acetic acid, v/v) for at least 24 h, finally, transferred to 70 % ethanol solution and stored at 4 °C. Young anthers collected from tassels of interspecific hybrids were fixed directly in the Carnoy's solution I for at least 12 h, and then transferred to 70 % ethanol solution and stored at 4 °C.

The treated root tips and anthers were washed in water to remove the ethanol solution, and subsequently transferred to an enzyme solution containing 6 % cellulase (R-10, Yakult, Japan) and 1 % pectinase (Y-23, Yakult, Japan) for 2.5–5.0 h at 37 °C. Root tips and anthers were washed in water again to clean the remaining enzyme solution, and finally squashed onto clean slides in a drop of Carnoy's solution I and flame dried. Preparations showing well-spread metaphase cells and pollen mother cells (PMC) were selected by phase-contrast light microscopy (Olympus BX-41, Japan) and saved at –20 °C for in situ hybridization procedures. Total DNA was extracted from young leaves of wf9 and 9475 using the improved 2 × CTAB method (Fu et al. 2015).

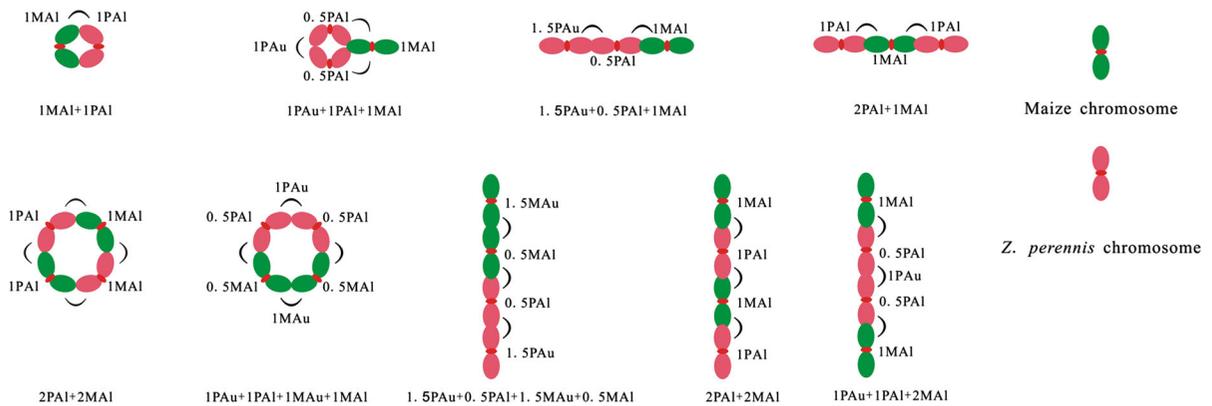
### Genomic in situ hybridization

Wf9 genomic DNA were labeled with DIG-Nick Translation Mix (Roche, Swiss) and 9475 genomic DNA were labeled with BIOTIN-Nick Translation Mix (Roche, Swiss) following the manufacturer's protocols. The prepared slides were dried in air blowing thermostatic oven for 1 h at 60 °C and then pretreated with 50 ml RNase solution (0.1  $\mu$ g/ml RNase in 2 × SSC, Solarbio) in thermostat water

bath for 1 h at 37 °C, subsequently washed twice in 2 × SSC for 5 min each at room temperature. Chromosomal DNA was denatured for 2.5 min by immersing the slides in 70 % deionized formamide (FAD) in 2 × SSC at 70 °C, and then dehydrated quickly through an pre-cooling 70, 95, and 100 % ethanol series for 5 min each at –20 °C, respectively. The hybridization mixture contained 150  $\mu$ l 50 % FAD, 60  $\mu$ l 10 % dextran sulfate (DS), 30  $\mu$ l 2 × SSC, 15  $\mu$ l 0.5 % sodium dodecyl sulfate (SDS), 3  $\mu$ l 10  $\mu$ g/ $\mu$ l salmon sperm DNA (SSDNA) and 18  $\mu$ l labeled probes for six slides. The hybridization mixture was denatured in thermostat for 5 min at 85 °C, and then chilled on ice for 10 min quickly. 45  $\mu$ l denatured hybridization mixture was added on each air dried slide and hybridization was performed in incubator chamber for 18–24 h at 37 °C. After hybridization, slides were washed in 20 % FAD, 2 × SSC and 0.1 × SSC at 42 °C for 15 min each, and then washed in 0.1 % TritonX-100 for 5 min, subsequently washed in 1 × PBS three times for 5 min each at room temperature. The next steps were performed in the dark. After air drying the slides, 50  $\mu$ l antibody diluent which contained anti-digoxigenin-fluorescein (0.6  $\mu$ g/ $\mu$ l in 1 × PBS, Roche) and CY-3 fluorescein (0.6 % in 1 × PBS, sigma) were loaded onto slides, and hybridization was performed in incubator chamber for 1 h at 37 °C. Slides were washed with 1 × PBS three times at room temperature for 5 min each. After drying, chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI), and then observed with fluorescence microscope (Olympus BX-61, Japan). Images were taken with Media Cybernetics CCD 700 (Charge Coupled Device) and Image Pro Plus 6.0 (Media Cybernetics, Inc.). Adobe Photoshop 5.1 was used for image processing.

### Analysis of chromosomes composition

In order to analyze the chromosome pairing with overall level, the chromosome calculation formula of autosyndesis and allosyndesis were carried out referring to the method of Cheng Cui (Cui et al. 2012) and modified slightly (Fig. 1). Additional, the relative chaotic coefficient (RCC) = (chromosomes number of bivalents)/(total chromosomes number—chromosomes number of bivalents).



**Fig. 1** The model diagram of chromosome calculation formula. Maize and *Z. perennis* chromosomes were labeled by green and pink, respectively. MAu represented autosynthesis chromosomes of maize and MAI represented allosynthesis

chromosomes of maize, PAu represented autosynthesis chromosomes of *Z. perennis* and PAI represented allosynthesis chromosomes of *Z. perennis*

**Results**

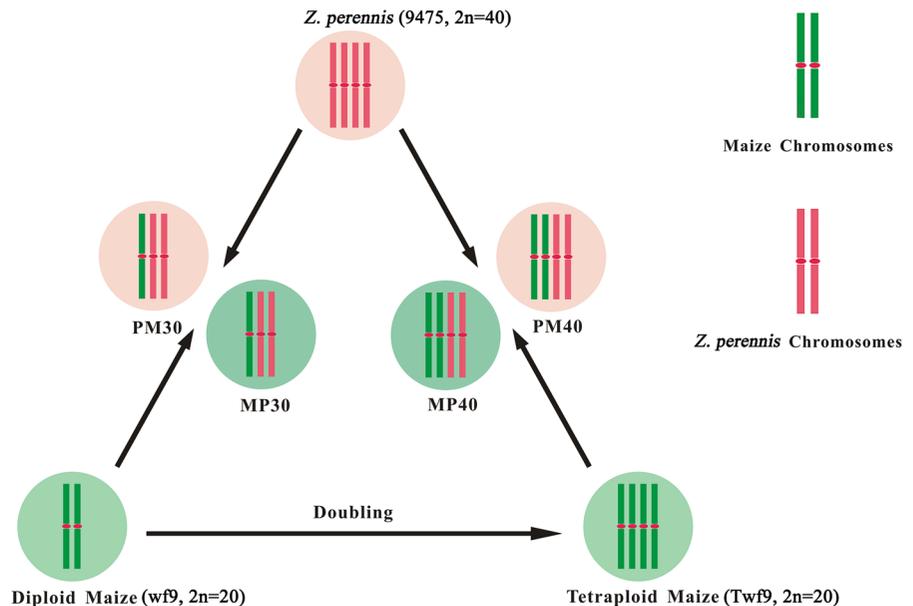
**Materials synthesis and cytology identification**

Two pairs of reciprocal crosses between maize and *Z. perennis* were carried out, and the following allopolyploid hybrids were produced (Fig. 2). Allotriploid MP30 and PM30 were obtained from the reciprocal crosses between wf9 and 9475, which contained the cytoplasm of wf9 and 9475, respectively. While allotetraploid MP40 and PM40 were obtained from

the reciprocal crosses between Twf9 and 9475, which contained the cytoplasm of Twf9 and 9475, respectively.

Generally, genome organization of MP30 and PM30 were stable. However, chromosome number instability occurred in MP40 and PM40, mainly due to aneuploidy individuals and abnormal meiosis behavior existed in tetraploid maize population (unpublished). In order to accurately obtain the allopolyploid hybrids with whole sets of chromosomes from parents, dual-color GISH was used to identify the chromosome

**Fig. 2** Schematic illustration showing the production of allopolyploid hybrids from pair crosses of three parents (*wf9*, *Twf9*, 9475). The light green circle represents maize cytoplasm and the light pink circle represents *Z. perennis* cytoplasm. The maize and *Z. perennis* chromosomes are labeled by green strip and pink strip, respectively, and both of them have red centromere in the middle of chromosome



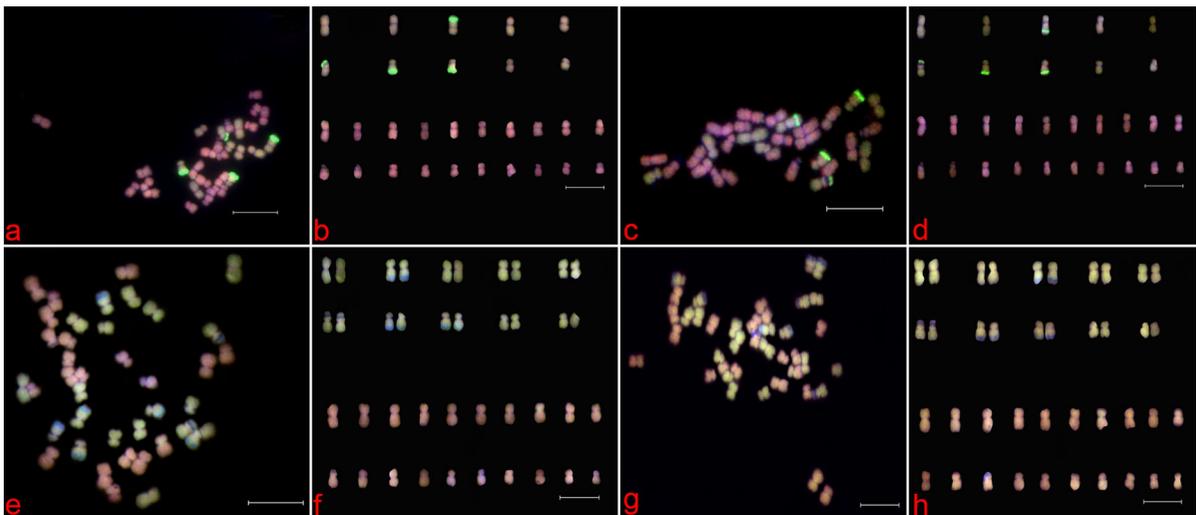
number and chromosome constitution. Allotriploids (MP30, PM30) with one set of maize (10) chromosomes and one set of *Z. perennis* (20) chromosomes, and allotetraploids (MP40, PM40) with two sets of maize (20) chromosomes and one set of *Z. perennis* (20) chromosomes were selected for further meiotic research (Fig. 3), in addition, there were no chromosome recombination detected in the interspecific hybrids.

#### Chromosome pairing analysis in synthetic allotriploids

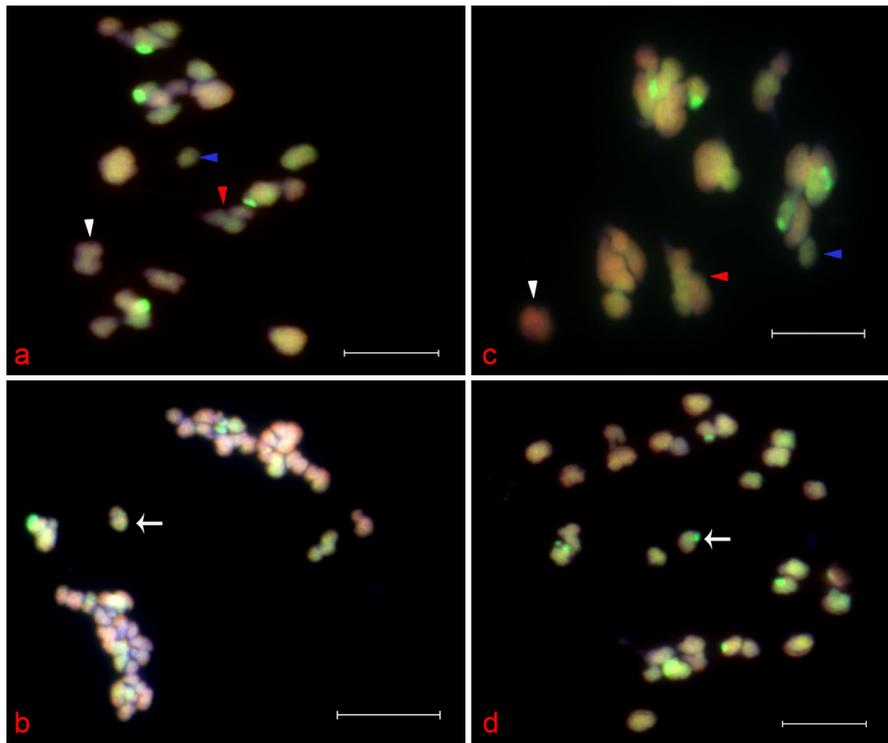
In order to study the chromosome origin of different configuration and analyze the meiotic behavior, especially the differential chromosome pairing affected by genome composition and cytoplasm, dual-color GISH was carried out (Figs. 4, 5). Both allotriploids (MP30, PM30) with 30 chromosomes showed irregular meiosis (Fig. 4a, c), lagging chromosomes were observed frequently in meiosis anaphase I (Fig. 4b, d). The most frequent meiotic configuration of MP30 was  $5\text{III} + 5\text{II} + 5\text{I}$  (16.9 %), with an average of  $4.56\text{I} + 5.44\text{II} + 4.73\text{III} + 0.07\text{IV}$ , while the most frequent meiotic configuration of PM30 was  $4\text{I} + 6\text{II} + 5\text{III}$  (8.47 %), with an average of  $4.04\text{I} + 6.13\text{II} + 4.31\text{III} + 0.20\text{IV}$  (Table 1S). The results were not consistent to previous research (Molina et al. 2012). Most univalents in MP30 and

PM30 belonged to the chromosomes from maize ( $\text{I}^{\text{M}}$ ), with the frequent number of five, and most bivalents were formed by autosyndetic pairings between homologous chromosomes from *Z. perennis* ( $\text{II}^{\text{PP}}$ ), with the frequent number of five; most trivalents in MP30 and PM30 were formed by allosyndetic pairings consisted of one maize chromosome and two *Z. perennis* chromosomes ( $\text{III}^{\text{MPP}}$ ), with the frequent number of five (Fig. 4a, c; Table 1S). The results suggested that five chromosomes of maize in allotriploids were homologous to ten chromosomes of *Z. perennis*.

In MP30 and PM30, the average number of  $\text{I}^{\text{M}}$  (3.79, 2.73) was higher than that of  $\text{I}^{\text{P}}$  (0.77, 1.31), while the average number of  $\text{II}^{\text{MM}}$  (0.25, 0.58) was lower than that of  $\text{II}^{\text{PP}}$  (4.34, 4.24) (Table 1). In order to further analyze the meiosis behavior, chromosome pairing within each genome (autosyndesis) and between different genomes (allosyndesis) were quantified and compared. The rates for autosyndesis within maize genome (abbreviated as maize autosyndesis) in MP30 and PM30 were 0.07 and 0.17 respectively, while the rates for autosyndesis within *Z. perennis* genome (abbreviated as *Z. perennis* autosyndesis) were 0.71 and 0.69, which were much higher than the former. On the other hand, the rate for allosyndesis between maize and *Z. perennis* genome (abbreviated as maize allosyndesis) in MP30 (0.56) was identical to that in PM30, while the rates for allosyndesis between maize and *Z. perennis* genome (abbreviated as *Z.*



**Fig. 3** GISH analysis of chromosomes composition of allopolyploid hybrids. *a* (*b*), *c* (*d*), *e* (*f*) and *g* (*h*) represent MP30, PM30, MP40, and PM40, respectively. Yellow signals are from maize genome and pink signals are from *Z. perennis* genome. All bars 10  $\mu\text{m}$



**Fig. 4** GISH analysis of PMCs in MP30 and PM30. *a* and *b* represent PMCs of MP30 at diakinesis and anaphase I, *c* and *d* represent PMCs of PM30 at diakinesis and anaphase I. *Blue triangle* represents maize univalent, *white triangle* represents autosyndetic bivalent of *Z. perennis* genome, *red triangle*

represents allosyndetic trivalent which consisted of one maize chromosome and two *Z. perennis* chromosomes, *white arrow* represents lagging chromosome. *Yellow signals* are from maize genome and *pink signals* are from *Z. perennis* genome. All bars 10  $\mu$ m

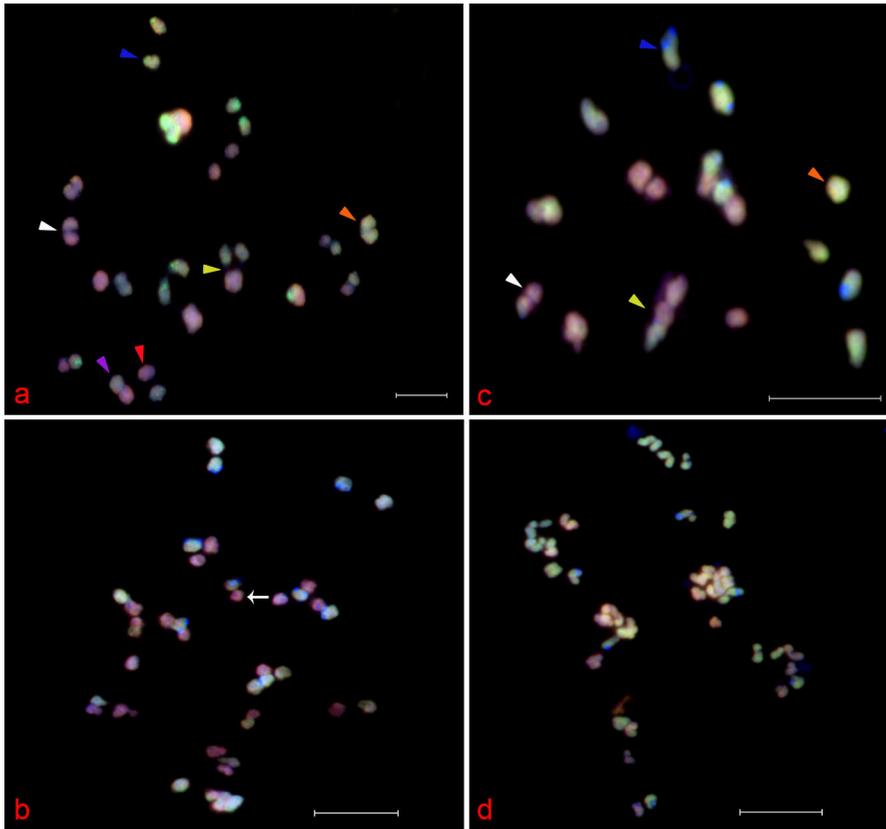
*perennis* allosyndesis) in MP30 and PM30 were 0.25 and 0.26, respectively, which were lower than the former (Table 2). The results suggested that maize genome in allotriploids had lower ability of chromosome pairing than that of *Z. perennis* genome. The RCC of maize genome in MP30 and PM30 (9.00, 9.30) were much higher than that of *Z. perennis* chromosome (1.56, 1.86) (Table 1), probably due to the different genome ploidy level of two parents.

Quadrivalents were barely seen in MP30 and PM30, and the average number of  $I^M$  and  $III^{MPP}$  in MP30 was higher than that in PM30, while the average number of  $I^P$ ,  $II^{MM}$ ,  $II^{MP}$  and  $III^{MMP}$  in MP30 was lower than that in PM30, and the average number of other valent types had no difference between MP30 and PM30 (Table 1). As a whole, the rate for maize autosyndesis in MP30 was lower than that in PM30, but there were no significant difference between MP30 and PM30 in the rates for maize allosyndesis, *Z. perennis* autosyndesis and *Z. perennis* allosyndesis

(Table 2). The RCC of maize chromosome in meiosis of MP30 was higher than that of PM30, however there was no significant difference between MP30 and PM30 in the RCC of *Z. perennis* chromosome (Table 1). The results suggested that cytoplasm probably had influence on chromosome pairing in allotriploids, and *Z. perennis* cytoplasm might enhance chromosome pairing and stability of maize genome.

#### Chromosome pairing analysis in synthetic allotetraploids

The allotetraploids (MP40 and PM40) had balanced chromosome numbers of two parent genomes (Fig. 3), and lagging chromosomes were also observed in MP40 (Fig. 5b). However, lagging chromosomes were rarely observed in PM40 (Fig. 5d). The most frequent meiotic configuration of MP40 were  $12II + 4IV$  (15.94 %) and  $8II + 6IV$  (15.94 %), with an average of  $1.17I + 9.97II + 0.13III + 4.62IV$ ,



**Fig. 5** Cytology and GISH analysis of tetraploid hybrids. *a* and *b* represent PMCs of MP40 at diakinesis and anaphase I, *c* and *d* represent PMCs of PM40 at diakinesis and anaphase I. *Blue triangle* represents maize univalent, *red triangle* represents *Z. perennis* univalent, *tangerine triangle* represents autosyndetic bivalent of maize genome, *white triangle* represents autosyndetic bivalent of *Z. perennis* genome, *purple triangle* represents

allosyndetic bivalent which consisted of one maize chromosome and one *Z. perennis* chromosome, *yellow triangle* represents allosyndetic quadrivalent which consisted of two maize chromosomes and two *Z. perennis* chromosomes, *white arrow* represents lagging chromosome. *Yellow signals* are from maize genome and *pink signals* are from *Z. perennis* genome. All bars 10  $\mu$ m

while in PM40, the most frequent meiotic configuration was  $12\text{II} + 4\text{IV}$  (19.70 %), with an average of  $0.56\text{I} + 11.74\text{II} + 0.29\text{III} + 3.78\text{IV}$  (Table 1S). The results were not consistent to previous research (Molina et al. 2012). Most bivalents in MP40 and PM40 were formed by autosyndetic pairings within homologous chromosomes from maize ( $\text{II}^{\text{MM}}$ ) or *Z. perennis* ( $\text{II}^{\text{PP}}$ ), both of them with the frequent number of five; although MP40 and PM40 had the balanced genome, allosyndesis and multivalent still formed. Quadrivalents were common in the two allotetraploids, and most quadrivalents formed by allosyndetic pairings and consisted of two maize chromosomes and two *Z. perennis* chromosomes ( $\text{IV}^{\text{MMPP}}$ ), with the frequent number of five (Fig. 5a, c; Table 1S). The meiosis chromosome configuration

in both allotetraploids also suggested ten chromosomes of maize were homologous to ten chromosomes of *Z. perennis*.

In MP40 and PM40, the average number of  $\text{I}^{\text{M}}$  (0.81, 0.39) was higher than that of  $\text{I}^{\text{P}}$  (0.36, 0.17), while the average number of  $\text{II}^{\text{MM}}$  (4.30) in MP40 was lower than that of  $\text{II}^{\text{PP}}$  (4.71) and the average number of  $\text{II}^{\text{MM}}$  (4.45) in PM40 was a little higher than that of  $\text{II}^{\text{PP}}$  (4.41) (Table 1), probably due to the influence of cytoplasm. The rates for maize autosyndesis in MP40 and PM40 were 0.69 and 0.78 respectively, while the rates for *Z. perennis* autosyndesis were 0.71 and 0.79, and there were no significant difference in maize autosyndesis and *Z. perennis* autosyndesis between MP40 and PM40. The rates for maize allosyndesis in MP40 and PM40 were 0.27 and 0.20 respectively, and

**Table 1** Average number of chromosome associations in PMCs of allopolyploids at diakinesis and metaphase I as revealed by GISH

Hybrids	I		II		III			IV			RCC		PMCs						
	Total	I	Total	II <sup>MM</sup>	II <sup>PP</sup>	II <sup>MP</sup>	Total	III <sup>MPP</sup>	III <sup>MMP</sup>	Others	Total	IV <sup>PPPP</sup>		IV <sup>MMMM</sup>	IV <sup>MMPP</sup>	Total	wf9	9475	
MP30	4.56	3.79 <sup>a</sup>	0.77 <sup>b</sup>	5.44	0.25 <sup>b</sup>	4.34 <sup>a</sup>	0.85 <sup>b</sup>	4.76	4.55 <sup>a</sup>	0.11 <sup>b</sup>	0.10 <sup>a</sup>	0.07	0.03 <sup>b</sup>	–	0.04 <sup>b</sup>	2.59	9.00 <sup>a</sup>	1.56 <sup>a</sup>	71
PM30	4.04	2.73 <sup>b</sup>	1.31 <sup>a</sup>	6.13	0.58 <sup>a</sup>	4.24 <sup>a</sup>	1.31 <sup>a</sup>	4.31	3.88 <sup>b</sup>	0.31 <sup>a</sup>	0.12 <sup>a</sup>	0.2	0.07 <sup>a</sup>	–	0.14 <sup>a</sup>	2.79	8.30 <sup>b</sup>	1.86 <sup>a</sup>	59
MP40	4.0	1.17	0.81 <sup>A</sup>	9.97	4.30 <sup>B</sup>	4.71 <sup>B</sup>	0.96 <sup>A</sup>	0.13	0.06 <sup>A</sup>	0.03 <sup>A</sup>	0.04 <sup>B</sup>	4.62	0.10 <sup>B</sup>	0.23 <sup>A</sup>	4.29 <sup>A</sup>	1.46	1.60 <sup>A</sup>	1.47 <sup>A</sup>	69
PM40	4.0	0.56	0.39 <sup>B</sup>	11.74	5.45 <sup>A</sup>	5.41 <sup>A</sup>	0.88 <sup>A</sup>	0.29	0.09 <sup>A</sup>	0.06 <sup>A</sup>	0.14 <sup>A</sup>	3.78	0.35 <sup>A</sup>	0.29 <sup>A</sup>	3.14 <sup>B</sup>	0.97	1.05 <sup>B</sup>	1.00 <sup>B</sup>	66

I, II, III and IV represent univalent, bivalent, trivalent and quadrivalent, respectively. I<sup>M</sup> and I<sup>P</sup> indicate valents belonging to maize genome and *Z. perennis* genome. II<sup>MM</sup> and II<sup>PP</sup> indicate bivalents formed by two maize chromosomes and two *Z. perennis* chromosomes, respectively. II<sup>MP</sup> indicates allosyndetic bivalents formed between one maize chromosome and one *Z. perennis* chromosome. III<sup>MPP</sup> indicates allosyndetic trivalents formed between one maize chromosome and two *Z. perennis* chromosomes. III<sup>MMP</sup> indicates allosyndetic trivalents formed between two maize chromosomes and one *Z. perennis* chromosome. III<sup>MMMM</sup> indicates autosyndetic quadrivalents formed by four maize chromosomes. III<sup>PPPP</sup> indicates autosyndetic quadrivalents formed by four *Z. perennis* chromosomes. IV<sup>MMPP</sup> indicates allosyndetic quadrivalents formed between two maize chromosomes and two *Z. perennis* chromosomes

<sup>a,b</sup> Groups significantly different between MP30 and PM30 by  $\chi^2$ -test

<sup>A,B</sup> Groups significantly different between MP40 and PM40 by  $\chi^2$ -test,  $p < 0.05$

the rates for *Z. perennis* allosyndesis were also 0.27 and 0.20, additional, the rates for maize and *Z. perennis* autosyndesis were both higher than that of maize and *Z. perennis* allosyndesis (Table 2). The RCC of maize genome (1.60, 1.05) in PM40 and PM40 was a little higher than that of *Z. perennis* genome (1.47, 1.00) (Table 1), suggested the stability of both genomes were enhanced, especially for maize genome. However, *Z. perennis* genomes in both allotetraploids were also more stable than maize genome.

The average number of I<sup>M</sup>, I<sup>P</sup> and IV<sup>MMPP</sup> in PM40 was lower than that in MP40, while the average number of II<sup>MM</sup>, II<sup>PP</sup> and IV<sup>PPPP</sup> in PM40 was higher than that in MP40 and there were no significant difference on other valent types (Table 1). As a whole, the rates for maize autosyndesis and *Z. perennis* autosyndesis in PM40 were higher than that in MP40, but the rates for maize autosyndesis and *Z. perennis* autosyndesis in PM40 showed an opposite situation (Table 2). Additional, comparing with MP40, the stability of maize and *Z. perennis* genome were both enhanced in PM40, and the average of RCC in PM40 was lower than that in MP40 (Table 1). The results suggested *Z. perennis* cytoplasm in allotetraploids could enhance the stability of both parent genomes and the ability of homologous pairing in both genomes.

## Discussion

Maize, *Z. perennis* and their hybrids is a good model system for allopolyploid study

Allopolyploid, which plays a crucial role in plant speciation, probably undergoes genome change after formed (Mandakova et al. 2010). Interestingly, in the newly synthesized allopolyploid, corresponds to a nature one, genome will change rapidly, if there is no corresponding nature allopolyploid, whereas genome will change slowly (Comai 2000). The process of genome changes can be best researched in the newly synthetic allopolyploids or the nature one with unambiguous source. As one of the main crops in the world, maize and closely related species which called teosintes become a well genetic model in the world (Doebly et al. 2006; Weber et al. 2007; Vigouroux et al. 2008; Van Heerwaarden et al. 2010, 2011; Wills et al. 2013). In this study, reciprocal crosses were successfully made between maize (diploid or

**Table 2** The rates for no pairing, autosyndesis, and allosyndesis in allopolyploids

Hybrids	No pairing	Maize		No pairing	<i>Z. perennis</i>	
		Autosyndesis	Allosyndesis		Autosyndesis	Allosyndesis
MP30	0.37 <sup>a</sup>	0.07 <sup>b</sup>	0.56 <sup>a</sup>	0.04 <sup>a</sup>	0.71 <sup>a</sup>	0.25 <sup>a</sup>
PM30	0.27 <sup>b</sup>	0.17 <sup>a</sup>	0.56 <sup>a</sup>	0.07 <sup>a</sup>	0.68 <sup>a</sup>	0.26 <sup>a</sup>
MP40	0.04 <sup>A</sup>	0.69 <sup>B</sup>	0.27 <sup>A</sup>	0.02 <sup>A</sup>	0.71 <sup>B</sup>	0.27 <sup>A</sup>
PM40	0.02 <sup>B</sup>	0.78 <sup>A</sup>	0.20 <sup>B</sup>	0.01 <sup>B</sup>	0.79 <sup>A</sup>	0.20 <sup>B</sup>

<sup>a,b</sup> Groups significantly different between MP30 and PM30 by  $\chi^2$ -test

<sup>A,B</sup> Groups significantly different between MP40 and PM40 by  $\chi^2$ -test,  $p < 0.05$

tetraploid) and *Z. perennis* which is the polyploid species in teosintes. These materials which contain different ploid and different cytoplasm will be a good model system to study the genome changes, chromosomal behaviors as well as genome affinities, and probably provide a further insight into the genome of maize and *Z. perennis*.

#### Different genome effects on meiotic pairing

In allotriploids, haploid chromosomes of maize could not pair themselves, maize univalents and maize allosyndesis were frequently observed in chromosome synapsis at meiosis I. While in allotetraploids, with diploid chromosomes of maize, there were more bivalents and the rate of maize autosyndesis was improved. Different ploidy levels did influence the meiosis behavior especially in chromosome pairing, and balanced parental chromosome number of allotetraploid may enhance the ability of proper chromosome pairing in meiosis.

Two or more genomes are sharing one nucleus in allopolyploids. For some allopolyploids, new genetic combinations are created which are beyond the addition of parent genomes and show non-Mendelian inheritance (Levy and Feldman 2002). In some allopolyploids, the different genomes from parents may occur divergent changes and different degrees of diploidization (Liu and Wendel 2002), probably due to the discrepant stability of parental genome. In this study, *Z. perennis* genome is more stable than that of maize in four allopolyploids, and possible explanations for our observations include: (a) the phylogenetic analysis shows that *Z. perennis* is more primitive than maize (Wang et al. 2011), therefore, the inherent stability probably exist in *Z. perennis* genome as a result from long-term evolution; (b) recent studies

demonstrate maize genome has tetraploid origin (Gonzalez and Poggio 2011), “A” and “B” genome probably exist partial homology (Molina et al. 2012); (c) for the two allotriploids, the haploid genome of maize can hardly perform the homologous chromosome pairing; (d) different genomes in one nucleus may lead to the disordered expression of *Phs1* and *Pam1*, which play important roles on chromosome pairing (Molina et al. 2012).

Although the genome stability is improved in allotetraploids, allosyndetic valents also exist and the most frequent allosyndetic valent is IV<sup>MMPP</sup>, with the frequent number of five (Table 1S). Additional, the most frequent allosyndetic valent in allotriploids is III<sup>MPP</sup>, with the frequent number of five (Table 1S). All of the results suggest that high level of homology exists between maize and *Z. perennis* and ten chromosomes from maize genome are homologous to twenty chromosomes from *Z. perennis* genome, additional, the homologous chromosomes belong to “A” genome (Gonzalez et al. 2006; Swanson-Wagner et al. 2010; Molina et al. 2012).

#### Nuclear-cytoplasmic interactions effects on meiotic pairing

Cytoplasm is predicted to play a vital role in meiosis behavior (Cifuentes et al. 2010). In this study, although a close relationship exists between maize and *Z. perennis* (Tang et al. 2005), chromosome pairing is significantly different in two allotriploids or two allotetraploids, which reveals parental cytoplasm has distinct contribution for meiosis behavior. Comparing MP30 with PM30, *Z. perennis* cytoplasm in PM30 can reduce the number of maize univalents and improve the maize autosyndetic bivalents, thus, enhancing the stability of meiosis pairing in maize genome.

However, *Z. perennis* cytoplasm has little influence on *Z. perennis* genome and only increases the number of univalents in PM30. Comparing MP40 with PM40, *Z. perennis* cytoplasm in PM40 can reduce the number of univalents and allosyndesis, improve the number of autosyndesis, enhance the stability of meiosis pairing in both maize and *Z. perennis* genome. The results suggest *Z. perennis* cytoplasm may give a relatively relaxed environment especially for maize genome. The possible reasons include: (a) the presence of foreign nuclear genome in cytoplasm from the male parent can result in nuclear–cytoplasm incompatibility (Wang et al. 2010; Cui et al. 2012) and maternal enzymatic system may treat the male chromosomes as exogenous intruders (Hu et al. 2013), thus, gene structure variation, aneuploidy and chromosomes structure variation as well as meiosis confusion can potentially occur, comparing to *Z. perennis* cytoplasm, maize cytoplasm probably has a stronger defense system; (b) *Z. perennis* is more primitive than maize (Wang et al. 2011), and the cytoplasm may have a greater containment than maize; (c) *Z. perennis* cytoplasm probably has a less negative influence on *Phs1* and *Pam1* than maize cytoplasm. Additional, interactions can occur between nuclear and cytoplasm followed by loss of genes from both nuclear genome and plasmon (Rand et al. 2004), which suggests genome stability also may be affected by interactions between different genome composition and cytoplasm (Cui et al. 2012), different results in allotriploids and allotetraploids conform to this speculation.

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