New Phytologist

Research

Incorrect recombination partner associations contribute to meiotic instability of neo-allopolyploid *Arabidopsis suecica*

Floriane Chéron[1], Valentine Petiot[1], Christophe Lambing[2], Charles White[1] and Heïdi Serra[1]

[1]Genetics, Reproduction and Development Institute (iGReD), CNRS UMR 6293, Inserm U1103, Université Clermont Auvergne, 63000, Clermont-Ferrand, France; [2]Plant Science Department, Rothamsted Research, Harpenden, AL5 2JQ, UK

Author for correspondence:
Heïdi Serra
Email: heidi.serra@uca.fr

Received: *21 November 2023*
Accepted: *29 November 2023*

New Phytologist (2023)
doi: 10.1111/nph.19487

Key words: allopolyploid, *Arabidopsis suecica*, crossover, homoeologous chromosomes, meiosis, pairing, polyploidy, recombination.

Summary

- Combining two or more related homoeologous genomes in a single nucleus, newly formed allopolyploids must rapidly adapt meiosis to restore balanced chromosome segregation, production of euploid gametes and fertility. The poor fertility of such neo-allopolyploids thus strongly selects for the limitation or avoidance of genetic crossover formation between homoeologous chromosomes.
- In this study, we have reproduced the interspecific hybridization between *Arabidopsis thaliana* and *Arabidopsis arenosa* leading to the allotetraploid *Arabidopsis suecica* and have characterized the first allopolyploid meioses.
- First-generation neo-allopolyploid siblings vary considerably in fertility, meiotic behavior and levels of homoeologous recombination. We show that centromere dynamics at early meiosis is altered in synthetic neo-allopolyploids compared with evolved *A. suecica*, with a significant increase in homoeologous centromere interactions at zygotene. At metaphase I, the presence of multivalents involving homoeologous chromosomes confirms that homoeologous recombination occurs in the first-generation synthetic allopolyploid plants and this is associated with a significant reduction in homologous recombination, compared to evolved *A. suecica*.
- Together, these data strongly suggest that the fidelity of recombination partner choice, likely during the DNA invasion step, is strongly impaired during the first meiosis of neo-allopolyploids and requires subsequent adaptation.

Introduction

Polyploidization or whole-genome duplication is one of the driving forces of both diversity and speciation. It has occurred in every eukaryotic kingdom and is particularly prevalent in the evolutionary history of plants, where it has largely contributed to genome evolution (Soltis *et al.*, 2003; Jiao *et al.*, 2011; Van De Peer *et al.*, 2017). Numerous crops of major economic importance are polyploids as having multiple sets of chromosomes can be beneficial for plants. Gene duplication provides a bulwark against the impact of deleterious mutation, improves biomass, development, and fertility of polyploids compared with their progenitors and contributes to promote stress resilience (Comai, 2005; Udall & Wendel, 2006; Bomblies, 2020).

Polyploids can be classified according to their mode of origin. Autopolyploids arise within species and possess more than two homologous genomes, while allopolyploids have a hybrid origin and thus carry two or more sets of related but non-identical homoeologous chromosomes derived from different evolutionary lineages (Ramsey & Schemske, 2002). Depending on the progenitor genomes, the homoeologous chromosomes may differ in number, genomic synteny and DNA sequence (Ramsey & Schemske, 2002; Soares *et al.*, 2021).

When they first arise, newly formed (neo-)allopolyploids face numerous challenges as polyploidization potentially leads to changes in genome structure and regulation, chromatin remodeling, epigenetic changes, and activation of transposable elements (Blasio *et al.*, 2022). A key problem is the restoration of faithful segregation of homologous and homoeologous chromosomes during meiosis, the specialized cell division that underlies gamete formation for sexual reproduction (Ramsey & Schemske, 2002; Comai, 2005; Grandont *et al.*, 2013; Lloyd & Bomblies, 2016). In neo-allopolyploids, related chromosomes can pair, synapse and recombine with multiple partners simultaneously, leading to the formation of complex chromosomal structures called multivalents. Persistence of multivalents and unpaired univalents at the first meiotic division results in unbalanced chromosome segregation and production of aneuploid gametes, severely reducing fertility of neo-allopolyploids (Ramsey & Schemske, 2002; Otto, 2007). These events also lead to genome disorganization in the offspring by homogenizing the two (or more) subgenomes or eliminating the gene contribution of one parent

(Lloyd & Bomblies, 2016). Meiotic instabilities, such as frequent whole-chromosome aneuploidies and/or persistence of multivalents at metaphase I, have indeed been reported in neo-allohexaploid wheat (Zhang *et al.*, 2013) and neo-allotetraploid *Arabidopsis suecica* (Comai *et al.*, 2000, 2003). Resynthesized *Brassica napus* also show evidence for extensive homoeologous exchanges (Szadkowski *et al.*, 2010; Gaebelein *et al.*, 2019) as well as neo-allopolyploid rice (Sun *et al.*, 2017; Li *et al.*, 2019). Early-generation allopolyploids are thus under strong selection to overcome the challenges of controlling meiotic pairing and recombination (Henry *et al.*, 2014).

Established allopolyploids are highly fertile and behave like true diploids during meiosis with bivalents formed between homologous chromosomes and prevented between homoeologous chromosomes, as seen in the absence, or only rare occurrence of multivalents in meiosis (Ramsey & Schemske, 2002). Although the prevention of homoeologous recombination plays a crucial role in meiotic adaptation to allopolyploidization, understanding of the underlying molecular mechanisms remains limited and only a few regulators have been identified and studied.

Striking recent examples come from studies of allohexaploid bread wheat, in which it has been demonstrated that TaZIP4-B2 (*Ph1*, *Pairing homoeologous 1*) promotes early pairing between homologous chromosomes (to the detriment of homoeologous chromosomes) and later prevents recombination intermediates established between homoeologous chromosomes from becoming CO (Martín *et al.*, 2014, 2017, 2021; Rey *et al.*, 2017; Blasio *et al.*, 2022). Also in wheat, the plant-specific DNA mismatch repair protein TaMSH7-3D (*Ph2*, *Pairing homoeologous 2*) has recently been identified as an inhibitor of homoeologous recombination (Serra *et al.*, 2021). In *B. napuns*, gene copy number of a member of the same family (MSH4) has been shown to affect homoeologous recombination frequency (Gonzalo *et al.*, 2019). The *PrBn* (*Pairing regulator in B. napus*) and BnaA9 (*BnaPh1*, *B. napus Pairing homoeologous 1*) loci are also involved in inhibition of the formation of homoeologous crossovers but the causative genes remain unidentified to date (Jenczewski *et al.*, 2003; Higgins *et al.*, 2021). In *A. suecica*, the *BYS* (*Boy named sue*) quantitative trait locus has been described as a potential suppressor of homoeologous recombination, but no candidate gene has yet been identified and the underlying mechanisms remain unknown (Henry *et al.*, 2014).

Arabidopsis suecica is a particularly interesting model species for studies of how newly formed allopolyploids respond and adapt to changes associated with polyploidization, including meiotic challenges. *Arabidopsis suecica* ($2n = 4x = 26$) is a recent allopolyploid formed through the hybridization of *Arabidopsis thaliana* ($2n = 2x = 10$) and *Arabidopsis arenosa* ($2n = 2x$ (4*x*) = 16 (32)) *c.* 16 thousand years ago (Novikova *et al.*, 2017). Its origin is believed to be in the fertilization of an unreduced ovule of diploid *A. thaliana* by a diploid pollen from a diploid or a tetraploid *A. arenosa* plant after the Last Glacial Maximum in Eastern Europe or central Eurasia (Novikova *et al.*, 2017; Nibau *et al.*, 2022). *Arabidopsis suecica* is a self-fertile and fecund plant with 13 chromosome pairs: five initially coming from *A. thaliana* and eight from *A. arenosa*. The ancestral species diverged *c.* 6 million years ago (Hohmann *et al.*, 2015; Mandáková *et al.*, 2017) and recent sequencing of the *A. suecica* genome revealed that the sequence diversity between its two sub-genomes is *c.* 11.6% (Burns *et al.*, 2021). Importantly, the high degree of genetic diversity between *A. suecica* sub-genomes is not enough to ensure proper homologous pairing and suppress recombination between homoeologous chromosomes (Henry *et al.*, 2014).

Elucidating the evolutionary processes of meiotic stabilization of neo-allopolyploids requires characterization of both unevolved (neo) and evolved (natural, established) allopolyploid meiosis. In this work, we mimicked the speciation process of an allopolyploid species by synthesizing neo-allotetraploid *A. suecica* and analyzing the first allopolyploid meiosis (when the progenitor genomes first interact) in order to identify the challenges that neo-allopolyploids must overcome to survive. We show that early prophase I centromere dynamics is altered in neo-synthetic plants and that this is associated with both a reduction in homologous recombination and an increase in homoeologous recombination, suggesting alteration in recombination partner choice. Interestingly, our study highlights considerable variability in fertility and meiotic behavior between newly synthesized allopolyploid siblings.

Materials and Methods

Plant material and growth conditions

The natural *Arabidopsis suecica* accession ASS3 (Burns *et al.*, 2021) used in this study were obtained from the laboratory of Magnus Nordborg (Gregor Mendel Institute, Germany). The first-generation neo-synthetic *A. suecica* were derived from a single cross between the natural autotetraploid *A. thaliana* Wa-1 (from the NASC, N22644) as female recipient and the natural autotetraploid *A. arenosa* Care-1 (from the ABRC, CS3901) as pollen donor. The other first-generation neo-synthetic *A. suecica* studied were the result of a single cross between an autotetraploid *A. thaliana* Ler (from the NASC, N3900) and the autotetraploid *A. arenosa* Care-1 (from the ABRC, CS3901).

Arabidopsis suecica plants were stratified at 4°C during a week and then grown under long-day conditions (16 h : 8 h, light : dark) at 23°C for 4 wk. The plants were then vernalized at 12°C for 4 wk and returned to 23°C to induce flowering.

Pollen viability test

Pollen viability was assessed using Alexander staining (Alexander, 1969). Briefly, anthers from unopen flower buds were collected and dissected on a slide with 10 µl of Alexander solution (1% Malachite green, 1% acid fuchsin, 1% orange G). Living (fertile) pollen grains are stained in purple while non-viable (sterile, aborted) pollen grains remain green. About 500 pollen grains per anther and four anthers from different inflorescences were analyzed per plant using a Zeiss AxioImager.Z1 epifluorescence microscope (Carl Zeiss AG) in bright-field illumination using a ×20 objective. Statistical analysis was performed using Kruskal–Wallis test with PRISM v.9 (GraphPad Software, San Diego, CA).

Meiotic chromosome spreads

Meiotic chromosome spreading was performed according to the method described by Ross *et al.* (1996). *Arabidopsis suecica* flower buds were fixed in 100% ethanol : acetic acid 3 : 1 (v/v) for 3 × 30 min and stored at −20°C until use. The flower buds were rehydrated 2 × 5 min in water and rinsed 2 × 5 min in 10 mM citrate buffer pH 4.5. Then, the buds were digested in an enzyme solution (0.3% (m/v) cellulase (Sigma), 0.3% (m/v) pectolyase (Sigma) and 0.3% (m/v) cytohelicase (Sigma) in citrate buffer) for 3 h at 37°C in a humid chamber. The enzyme solution was then replaced with citrate buffer to stop the reaction. Each bud was transferred to a clean slide, crushed and 20 µl of 60% acetic acid was added to the slide. The slide was incubated on a 45°C heating block for 1 min, taking care to stir the drop to allow the chromosomes to spread. Finally, the chromosomes were fixed by adding 200 µl of Carnoy solution to the slide. The slides were then rinsed 2 min with sterile water, incubated with 4% formaldehyde in 1× PBS for 10 min and rinsed with sterile water for 5 min. After drying, the slides were mounted in VECTASHIELD medium containing 1.5 µg ml^{-1} DAPI (Vector Laboratories Inc., Burlingame, CA, USA). Pollen mother cells at metaphase I stage were imaged on a Zeiss LSM 800 Airyscan microscope with ×60 objective and a Zeiss DAPI filter. For each cell, the number of univalents (unpaired chromosome), rod bivalents (pair of chromosomes linked by a unique chiasma), ring bivalents (pair of chromosomes linked by at least two chiasmata), trivalents (three chromosomes linked by two chiasmata) and quadrivalents (four chromosomes linked by three or four chiasmata) were counted independently by two people. Frequency of chiasmata (the cytological manifestation of meiotic crossovers) was then calculated according to the criterion described in Parra-Nunez *et al.* (2020). Statistical analysis was performed using Kruskal–Wallis test with the GraphPad Prism v.9 software.

Fluorescence *in situ* hybridization

The centromeric probes (containing copies of the 180 bp centromeric repeat) specific to each sub-genome, *A. arenosa* and *A. thaliana*, were prepared by amplifying respectively, the pGEMT-AaCEN with dUTP-DIG and pCW640 with dUTP-Cy3 using T7 and M13 primers.

Meiotic chromosome spreads were denaturated for 30 min at 60°C and the slides were incubated with 100 µl of RNase (100 µg ml^{-1} in 2× SSC) for 1 h at 37°C in a humid chamber. Pre-hybridization washes were twice 2× SSC, once 1× PBS, once 4% formaldehyde in 1× PBS, twice 1× PBS and once 70%, 90% and 100% ethanol dehydration series. Slides were incubated with 20 µl of the hybridization solution containing 1 µl of each probe, HB50 (50% formamide, 2× SSC, 50 mM sodium phosphate) and 20% Dextran Sulfate (in HB50) at 80°C for 2 min followed by an overnight incubation at 37°C in a moist chamber. Post-hybridization washes were performed at 42°C once in 2× SSC, twice in SF50 and once in 2× SSC. One hundred microliter of blocking solution (5% BSA, 0.2% Tween 20, 4× SSC) was added on the slides and incubated at 37°C for 30 min in a humid chamber. Slides were washed twice with 4T and once with TNT before mouse anti-digoxygenine antibody (in TNB) was added on the slides and incubated at 37°C in a dark moist chamber for 30 min. Three washes with 4T were performed before adding the Alexa 488 goat anti-mouse antibody (in TNB) and incubated at 37°C in a dark moist chamber for 30 min. Then, the slides were washed twice in TNT and once in sterile water at room temperature. Finally, the slides were dried and mounted in VECTASHIELD medium containing DAPI (1.5 µg ml^{-1}; Vector Laboratories Inc.).

All observations were made with a Zeiss AxioImager.Z1 epifluorescence microscope with a ×100 Plan Apochromat objective with oil immersion and appropriate filters for DAPI, DsRed and GFP. For each pollen mother cell, the number of magenta foci staining the centromeres of the *A. thaliana* sub-genome (AtCEN) and green foci staining the centromeres of the *A. arenosa* sub-genome (AaCEN) were quantified independently. The number of partially or fully overlapping green-magenta foci were defined as interactions between centromeres of both sub-genomes (AtCEN-AaCEN). Statistical analysis was performed using unpaired two-tailed Mann–Whitney test adjusted for multiple comparisons with Bonferroni correction.

MLH1 immunolabelling

Immunostaining of MLH1 was performed on meiotic chromosome spreads at diakinesis stage as described by Chelysheva *et al.* (2010). Briefly, the slides were incubated 10 min in 100% ethanol, washed with PBST, incubated in the hot tri-sodium solution (pH 7) for 45 s and washed again in PBST. The slides were then incubated with 50 µl of the solution containing the antibodies α-MLH1 (rabbit, 1 : 200 dilution, gift from Mathilde Grelon (INRAe Versailles)) and α-ASY1 (guinea pig, 1 : 250 dilution, gift from Eugenio Sanchez-Moran (University of Birmingham)) in a moist chamber at 4°C for 2 d. The slides were washed three times in PBST and then incubated 30 min with secondary antibodies (Cy3 donkey anti-rabbit and Alexa 488 goat anti-rabbit used at 1 : 100 dilution) at 37°C in a humid chamber. Three PBST washes were performed before airdried and mounted the slides in VECTASHIELD medium containing DAPI (1.5 µg ml^{-1}; Vector Laboratories Inc.).

All observations were made with a Zeiss AxioImager.Z1 epifluorescence microscope with a ×100 Apochromat plane objective with oil immersion and appropriate filters for DAPI, DsRed and GFP. For each cell at diakinesis stage, the MLH1 foci overlapping heterochromatic regions (marked with DAPI) were quantified. Statistical significance was assessed using unpaired two-tailed *t*-test.

Results

Neo-allopolyploid *A. suecica* exhibit reduced fertility compared with their natural counterparts

Eleven synthetic *A. suecica* siblings were produced from a single cross between two natural autotetraploids, *A. thaliana* accession Warschau (Wa-1; ♀) and *A. arenosa* accession Care-1 (♂). The

newly synthesized allopolyploids (Syn) carry the same chromosome composition as the natural *A. suecica* (Nat) but exhibit reduced seed set per silique (Fig. 1a,b; Supporting Information Dataset S1). Interestingly, silique lengths and seed numbers per silique are highly variable along floral stems of most of the synthetic plants (Fig. 1a), as well as among sister plants (from 0 to 16.3 ± 2.3 seeds per silique in average in synthetic plants 8 and 5, respectively) (Fig. 1b). Quantification of pollen grain viability

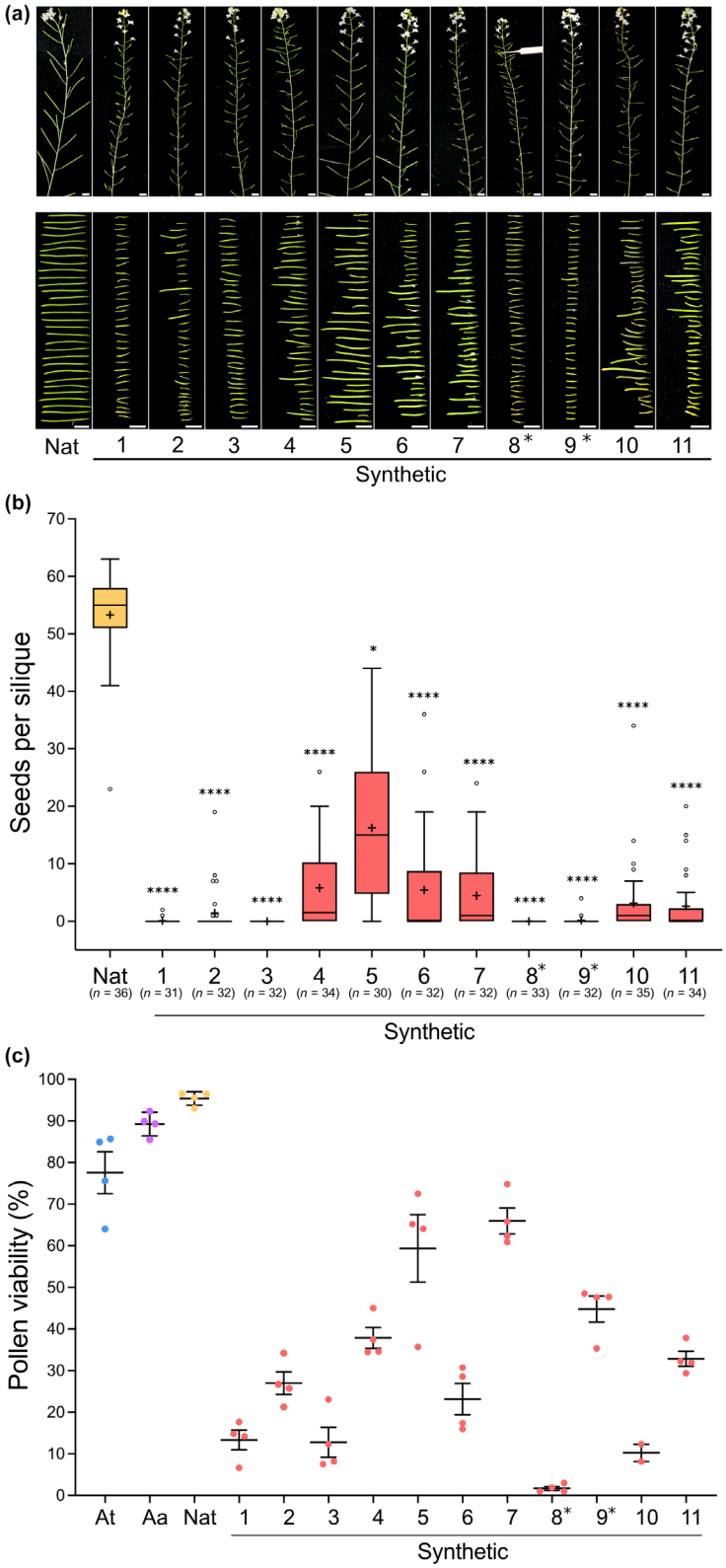

Fig. 1 Fertility of neo-synthetic *Arabidopsis suecica* is strongly reduced compared to evolved *A. suecica*. (a) Phenotype of the primary inflorescence and *c*. 30 siliques of self-pollinated natural plant (Nat, ecotype ASS3) and 11 first generation neo-synthetic siblings originated from a cross between *Arabidopsis thaliana*, ecotype Wa-1 and *Arabidopsis arenosa*, ecotype Care-1 (bars, 1 cm). The asterisks indicate aneuploid plants. (b) Number of seeds per silique counted in *c*. 30 siliques for the indicated genotypes. Tukey's box plots with horizontal middle lines indicating the median values and crosses, the mean values. Whiskers correspond to the lowest and highest values within 1.5 times the interquartile range. The asterisks indicate aneuploid plants (*, $P < 0.05$; ****, $P < 0.0001$, Kruskal–Wallis test). (c) Pollen viability assessed using Alexander staining in the indicated genotypes. Each point represents an inflorescence from which at least 500 pollen grains were analyzed. Bars show mean ± SE. The asterisks indicate aneuploid plants. At, *A. thaliana*, ecotype Wa-1; Aa, *A. arenosa*, ecotype Care-1 ($P < 0.0001$, Kruskal–Wallis test with comparison of each synthetic plant with the natural plant).

using Alexander staining showed that all synthetic plants produce a significantly lower proportion of viable pollen grains than the natural *A. suecica* (Fig. 1c), indicating that self-incompatibility alone is not responsible for the observed reductions in fertility. The neo-allopolyploid siblings moreover exhibit a high heterogeneity in their ability to produce viable pollen (from 1.7 ± 0.5 to $66.0 \pm 3.1\%$ of pollen viability in synthetic plants 8 and 7, respectively).

These data are consistent with a previous report of reduced pollen viability in synthetic *A. suecica* generated from a different cross (autotetraploid *A. thaliana* accession *Landsberg erecta* (Ler) crossed with the natural autotetraploid *A. arenosa* accession Care-1), compared to natural *A. suecica* (*c.* 30% vs 80% of viable pollen, respectively) (Henry *et al.*, 2014). We reproduced the same cross in our lab to evaluate (1) the potential variability in fertility of the offspring siblings and (2) if the specific accessions of the progenitors influence fertility of the offspring. Quantification of seed set per silique revealed that half of the analyzed synthetic plants are sterile, and the rest exhibiting severe reductions in fertility compared to the natural *A. suecica* (at least to 10-fold), with a high variability between plants (Fig. S1). Synthetic *A. suecica* from this cross (*A. thaliana* Ler × *A. arenosa* Care-1) are, on average, less fertile than those generated from the cross between *A. thaliana* Wa-1 and *A. arenosa* Care-1 (Figs 1b, S1) confirming that the genetic background of the progenitors (at least the female parent) impacts fitness of neo-allopolyploids.

Meiotic stability is altered in neo-allopolyploid *A. suecica*

To assess whether the reduced fertility of neo-allopolyploids is associated with meiotic instability, we performed DAPI-stained chromosome spreads of *A. suecica* pollen mother cells at meiotic metaphase I. In agreement with the recent report from Nibau *et al.* (2022), natural *A. suecica* show largely diploid-like meiosis with 13 bivalents in 92.6% of meiocytes and 7.4% with 12 bivalents and a pair of univalents (Fig. 2; Datasets S1, S2). Severe genetic instability is however observed in synthetic *A. suecica*, with 9/11 euploid and 2/11 aneuploid plants (Plant 8 has $26 - 1$ chromosomes and plant 9, $26 + 1$ chromosomes). Compared with natural allopolyploid controls, all synthetic plants exhibit reduced and highly variable proportions of meiocytes with 13 bivalents (from 5% to 77%) (Fig. 2b). We assessed the fraction of meiotic nuclei exhibiting univalents and multivalent chromosome associations in synthetic *A. suecica* and show that frequencies of these meiotic abnormalities vary considerably between siblings (Fig. 2c,d). The presence of univalents (lacking chiasmata) in a high proportion of meioses indicates loss of the obligate chiasma and an inability to maintain crossover assurance in many synthetic plants. The presence of such univalents and multivalents concords with the increased risk of chromosome mis-segregation and the formation of aneuploid and/or inviable gametes (Ramsey & Schemske, 2002). While it is not possible at this stage to confirm that it is the only contributing factor, the meiotic instability observed in neo-allopolyploid *A. suecica* is clearly a major contributor to the reduced fertility of these plants. In agreement with this, we find a positive correlation between meiotic stability (estimated by the proportion of meiocytes with 13 bivalents) and pollen viability ($r = 0.74$, $P = 0.0064$) (Fig. S2).

Homoeologous recombination occurs in neo-allopolyploid *A. suecica* at variable rates

Multivalent chromosome associations at meiotic metaphase I result from the formation of crossovers between non-homologous chromosomes, and potentially between homoeologous chromosomes. To evaluate the extent of homoeologous recombination in neo-allopolyploid *A. suecica*, we performed fluorescence *in situ* hybridization (FISH) using probes able to distinguish the centromeric repeat sequences of *A. thaliana* (At) and *A. arenosa* (Aa) sub-genomes. We searched for allosyndetic chromosome associations (i.e. involving chromosomes from both related sub-genomes) in synthetic *A. suecica* meiocytes at diakinesis and metaphase I and clearly identified At-Aa bivalents and multivalents, that is exhibiting both centromeric signals (Figs 3a, S3; Datasets S1, S3–S5). Interestingly, connections between bivalents were observed at diakinesis that do not persist at metaphase I (Fig. S3), as remarked previously by (Nibau *et al.*, 2022). We have thus limited our analysis of chromosome associations to metaphase I and define three categories of meioses: (I) meioses with no visible allosyndetic associations; (II) meioses showing one or more allosyndetic associations (i.e. bivalent pair and/or multivalent formed with chromosomes from *A. thaliana* and *A. arenosa* sub-genomes) and (III) meioses exhibiting complex structures that are difficult to interpret (Fig. 3a). We quantified numbers of each category in natural *A. suecica* and synthetic plants 1 and 2. Unlike natural *A. suecica* meioses, > 40% of meiocytes from synthetic plants 1 and 2 exhibit clear allosyndetic association(s) (category II) and/or complex chromosome aberrations, that very probably also contain allosyndetic associations (category III) (Fig. 3b). The presence of these chromosome associations indicates that reciprocal exchanges of genetic material occur between homoeologous chromosomes in neo-allopolyploid *A. suecica*, and very likely, at variable frequencies between siblings.

As the complexity of the category III images does not allow accurate discrimination between chromosome configurations, these were quantified in meiocytes of category I and II from both natural and synthetic (plant 1) *A. suecica* (Table 1). This analysis confirms that allosyndetic multivalents (At-Aa) are more frequent in meiocytes of synthetic compared with natural *A. suecica* plants (0.22 ± 0.08 vs 0.02 ± 0.02 At-Aa multivalents per meiosis, respectively; $P = 0.0089$) and revealed that autosyndetic multivalents (associations between more than two chromosomes from the same sub-genome) also occur at low frequency in meiocytes from synthetic plants (Table 1). This implies that recombination between homoeologous chromosomes, but also between non-homologous chromosomes is not efficiently inhibited in neo-allopolyploids.

Genome-wide meiotic crossover frequency is reduced in neo-allopolyploid *A. suecica*

Careful comparison of meiotic chromosome spreads from natural and synthetic *A. suecica* brings to light an interesting difference in

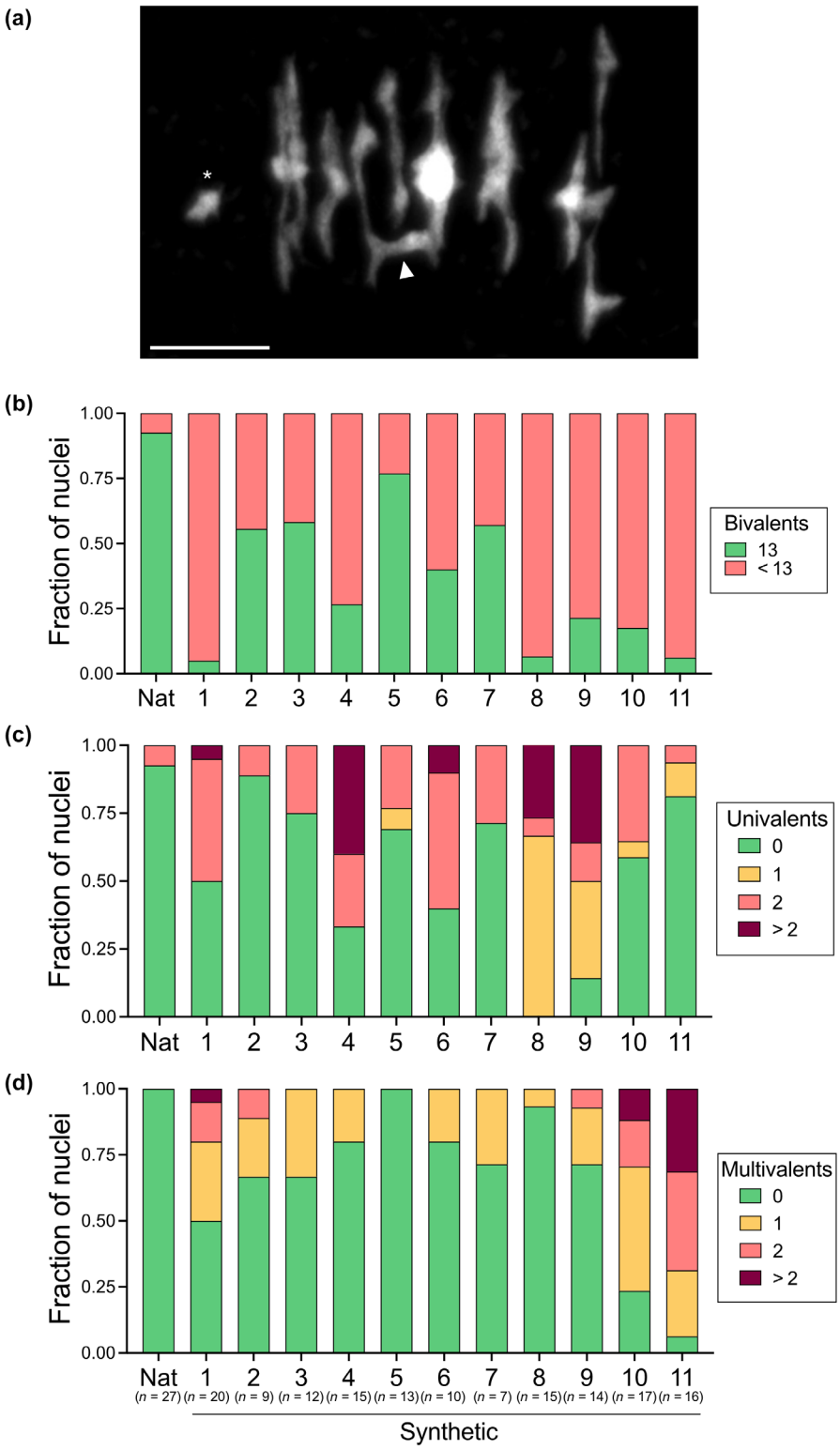

Fig. 2 Abnormal meiotic chromosome configurations are frequent in neo-synthetic *Arabidopsis suecica*. (a) Representative meiotic chromosome spread of male meiocyte at metaphase I from neo-synthetic plant 11 showing univalents (*) and multivalents (triangle). DNA is stained with DAPI (bar, 5 μm). (b) Fraction of nuclei with 13 bivalents at metaphase I in natural (Nat, ecotype ASS3) and neo-synthetic *A. suecica* siblings originated from a cross between *Arabidopsis thaliana*, ecotype Wa-1 and *Arabidopsis arenosa*, ecotype Care-1. (c) Fraction of nuclei with univalents at metaphase I in the indicated genotypes. (d) Fraction of nuclei with multivalents at metaphase I in the indicated genotypes.

bivalent shapes at metaphase I between the two populations (less ring vs rod bivalents in the synthetic plants). As bivalent shape is directly influenced by chiasma number and location, this implies a difference in chiasmata (the cytological manifestation of meiotic crossovers) between these plants. To verify this, we assessed total chiasma frequencies per meiosis by scoring frequencies of univalents, rod and ring bivalents as well as multivalents at meiotic metaphase I in the natural and synthetic *A. suecica* (see the Material and Methods section). While natural *A. suecica* exhibit 20.37 ± 0.39 chiasmata/meiocyte, mean chiasma numbers were reduced in all synthetic plants (from 14.30 ± 0.36 in plant 6 to 17.92 ± 0.45 in plant 3, with a mean chiasma number of 16.39),

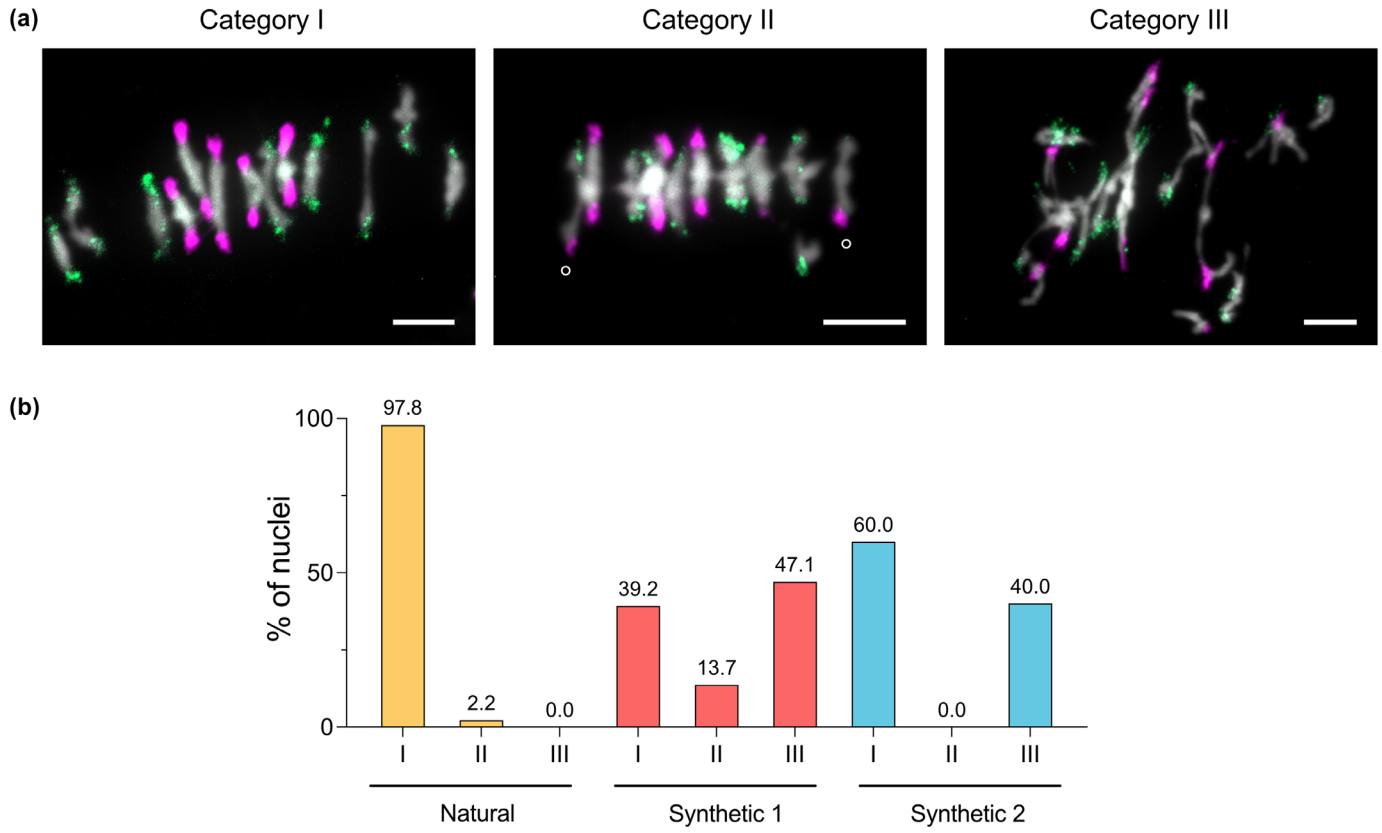

Fig. 3 Evidence of homoeologous exchanges and complex meiotic aberrations in neo-synthetic *Arabidopsis suecica*. (a) Fluorescent *in situ* hybridization of centromeric repeats on neo-synthetic plant 1 meiocytes. Probes specific to *Arabidopsis thaliana* (AtCEN, magenta) and *Arabidopsis arenosa* (AaCEN, green) were hybridized to male meiotic chromosome spreads at metaphase I stained with DAPI (white). Representative images of the three metaphase I categories are represented. Category I, meiosis with no allosyndetic associations; Category II, meiosis showing one or more allosyndetic associations (i.e. bivalent pair (o) and/or multivalent formed with chromosomes from *A. thaliana* and *A. arenosa* sub-genomes); Category III, meiosis exhibiting complex structures that are difficult to interpret (bars, 5 μm). (b) Percentage of nuclei of each category in natural *A. suecica* and neo-synthetic plants 1 and 2.

notwithstanding the increased number of multivalents (Fig. 4; Datasets S1, S2). This corresponds to a statistically significant reduction in mean chiasma frequency per chromosome pair from 1.57 to 1.26 in natural vs synthetic *A. suecica*, respectively ($P < 0.0001$, unpaired two-tailed Mann–Whitney test).

To test whether this reduced chiasma frequency in neo-allopolyploid *A. suecica* is associated with a decrease in Class I crossovers (CO), we performed immunolocalization of MLH1 in evolved and synthetic *A. suecica* meiocytes at the diakinesis stage (Fig. 5). Natural *A. suecica* show 20.28 ± 0.47 MLH1 foci per meiocyte, fully consistent with the chiasma counts (Fig. 4) and a previous report (Nibau *et al.*, 2022). MLH1 foci numbers were significantly lower in synthetic *A. suecica* with a mean of 18.20 ± 0.44 foci per meiocyte ($P = 0.0023$, unpaired *t*-test) (Fig. 5; Dataset S1). This result thus confirms that Class I CO frequency is reduced in neo-allopolyploid *A. suecica* compared with their evolved counterpart. A reduction in class II CO cannot be excluded but the low frequency of this class of CO makes analysis difficult without markers.

The presence of CO between homoeologues in the first meiosis following allopolyploidization is thus accompanied by an overall reduction in numbers of chiasmata and Class I CO, and therefore by reduced numbers of inter-homologous chiasmata. These data strongly suggest that recombination partner choice (homologues vs homoeologues) is perturbed in neo-allopolyploid *A. suecica*.

Meiotic prophase I centromere dynamics is altered in neo-allopolyploid *A. suecica*

Meiotic crossovers observed as chiasmata at diakinesis and metaphase I result from recombination processes initiated at leptotene that are associated with changes in structure and movements of chromosomes (Alleva & Smolikove, 2017). To gain further insight into early meiotic chromosome dynamics in *A. suecica* and determine whether the defects observed in synthetic plants result from problems during earlier stages of meiosis, sub-stages of prophase I were analyzed using FISH with sub-genome-specific centromeric probes (Fig. 6; Datasets S1, S3–S5). In pre-meiotic interphase, centromeres are dispersed and unpaired as shown by the 10 magenta foci staining the centromeres of *A. thaliana* sub-genome (AtCEN) and the 16 green foci staining the centromeres of *A. arenosa* sub-genome (AaCEN) (Fig. 6a,b). The number of fluorescent signals is reduced by more than two-fold at zygotene indicating that centromeres cluster at early meiosis in allopolyploid *A. suecica*, as observed in diploid *A. thaliana*

Table 1 Chromosome configurations at metaphase I in natural plant and neo-synthetic plant 1 *Arabidopsis suecica*.

		Univalents			Bivalents			Multivalents			
	N	At	Aa	Total	At	Aa	At-Aa	At	Aa	At-Aa	Total
Nat	46	0	0	**0**	4.98 ± 0.02 (4–5)	7.98 ± 0.02 (7–8)	0	**12.96** ± 0.04 (11–13)	0	0.02 ± 0.02 (0–1)	**0.02** ± 0.02 (0–1)
Syn	27	0.07 ± 0.07 (0–2)	0.56 ± 0.17 (0–2)	**0.63** ± 0.18 (0–2)	4.22 ± 0.19 (1–5)	6.96 ± 0.20 (5–8)	0.22 ± 0.12 (0–2)	**11.41** ± 0.27 (8–13)	0.19 ± 0.09 (0–2)	0.22 ± 0.08 (0–1)	**0.59** ± 0.12 (0–2)
P-value			0.0002	< 0.0001	< 0.0001	< 0.0001		< 0.0001		0.0089	< 0.0001

Counts include meiocytes from categories I and II but not from category III as they do not allow accurate discrimination and quantification of chromosome configurations. Mean ± SE (range) of numbers of the given class of structure per meiotic nucleus. *N*, number of cells examined. At– univalent, bivalent or multivalent stained with *Arabidopsis thaliana* centromeric probe. Aa, univalent, bivalent or multivalent stained with *Arabidopsis arenosa* centromeric probe; At-Aa, bivalent or multivalent stained with both centromeric probes, that is composed by chromosomes from both sub-genomes. Mean numbers of univalents, bivalents and multivalents are in bold. Mann–Whitney tests were performed to test for statistically significant differences between natural (Nat) and synthetic (Syn) *A. suecica* for each chromosome configuration.

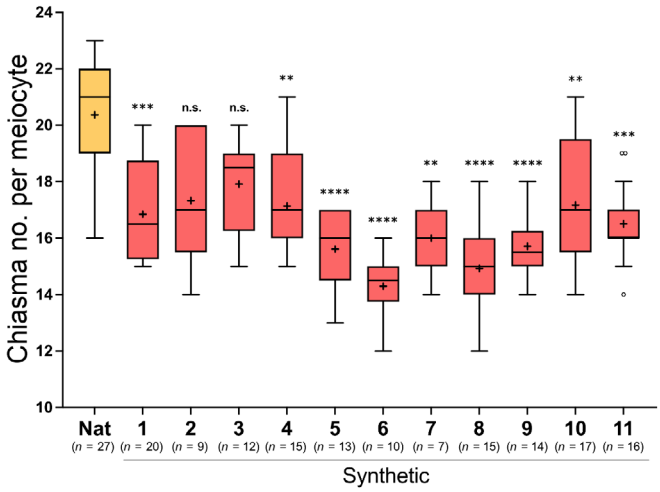

Fig. 4 Chiasma frequency is reduced in neo-synthetic compared with natural *Arabidopsis suecica*. Chiasma number per meiocyte was estimated based on bivalent and multivalent shapes from metaphase I chromosome spreads of the natural (Nat, ecotype ASS3) and each neo-synthetic *A. suecica* originated from a cross between *Arabidopsis thaliana*, ecotype Wa-1 and *Arabidopsis arenosa*, ecotype Care-1. Tukey's box plots with horizontal middle lines indicating the median values and crosses, the mean values (ns, not significantly different; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$, Kruskal–Wallis test). Whiskers correspond to the lowest and highest values within 1.5 times the interquartile range.

(Armstrong *et al.*, 2001; Da Ines & White, 2015). Interestingly, synthetic *A. suecica* meiocytes exhibit on average more AtCEN and AaCEN foci than natural *A. suecica* meiocytes at this stage (Figs 6b, S4). Thus, the interactions between centromeres from the same sub-genome (AtCEN-AtCEN and AaCEN-AaCEN) are delayed, less frequent and/or less stable in synthetic *A. suecica* meiosis than in evolved *A. suecica*.

To assess the interactions between centromeres from *A. thaliana* and *A. arenosa* sub-genomes at early meiosis, we quantified the number of AtCEN-AaCEN overlapping foci (Fig. 6c). The number of AtCEN-AaCEN interactions clearly increases in zygotene compared with pre-meiotic stages (Fig. 6c), indicating that centromere clustering at the onset of meiosis also involves homoeologous chromosomes. Interestingly, AtCEN-AaCEN interactions are more frequent in synthetic compared to natural *A. suecica* meiocytes (3.77 ± 0.24 (Syn1); 2.44 ± 0.27 (Syn2) vs 1.56 ± 0.17 (Nat)) (Fig. 6c) revealing that the zygotene interactions between homoeologous centromeres are significantly more numerous and/or more stable in neo-allopolyploid *A. suecica*. Intriguingly, this zygotene excess of inter-homoeologue centromeric interactions is then corrected as no differences are observed between Nat and Syn plants at pachytene and diplotene (Fig. 6c).

Taken together, these data demonstrate that centromere dynamics at the onset of meiosis is perturbed in neo-allopolyploid *A. suecica*, with a substantial increase in homoeologous associations and a reduction in true homologous associations.

Discussion

Allopolyploidy represents a tremendous evolutionary opportunity for immediate species formation but is associated with an array of

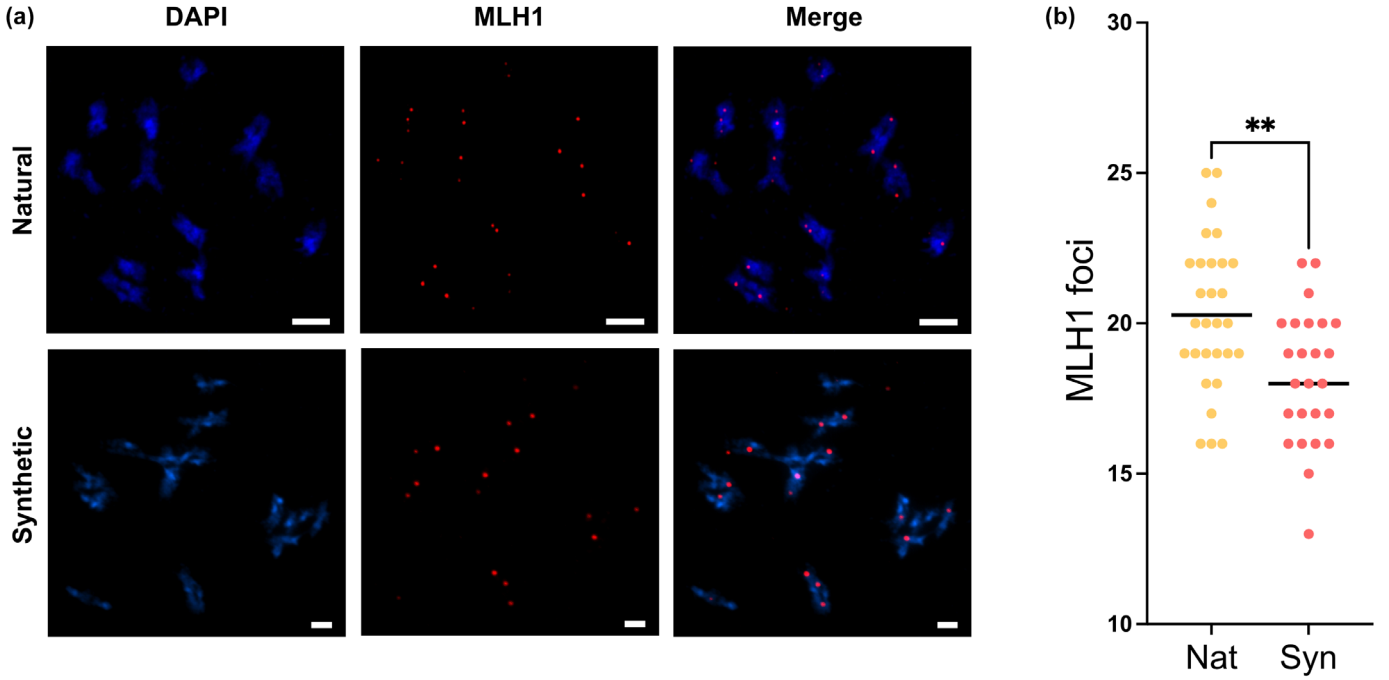

Fig. 5 Genome-wide class I crossover frequency is significantly reduced in neo-synthetic plants compared to the natural *Arabidopsis suecica*. (a) Immunolocalization of the protein MLH1 (red) in male meiocytes at diakinesis stage in natural *A. suecica* and in the neo-synthetic plant 10. DNA was stained with DAPI (blue) (bars, 5 μm). (b) Quantification of MLH1 foci per meiocyte in natural *A. suecica* and the neo-synthetic plant 10. Bars indicate the mean. Statistical significance was assessed using an unpaired two-tailed *t*-test (**, $P < 0.01$).

challenges triggered by the combination of diverged parental sub-genomes within a single nucleus. Recombination between homoeologous chromosomes leads to difficulties with balanced chromosomal sorting and to rearrangements between homoeologues. Importantly, the karyotypic changes resulting from extensive homoeologous exchanges during the first allopolyploid meioses can strongly impact chromosome pairing, meiotic stability and fertility of the following generations (Pontes *et al.*, 2004; Szadkowski *et al.*, 2010; Henry *et al.*, 2014). To better understand the processes involved, we analyzed meiotic chromosome behavior and chromosome sorting in neo-allopolyploid *A. suecica* plants of the first generation, when true homologous and homoeologous chromosomes first meet.

We show that both seed set and pollen viability are strongly reduced (and highly variable) in neo-allopolyploid compared with evolved *A. suecica* (Fig. 1). While the effects on pollen viability would not be affected by potential self-incompatibility in the synthetic plants, this could be so for seed set – and could point to an explanation for the apparent discrepancy between relatively high pollen viability and low seed sets of some Syn plants (Durvasula *et al.*, 2017; Novikova *et al.*, 2017). We also report the presence of allosyndetic chromosomal associations in neo-allopolyploid meioses (Fig. 3), revealing that recombination between homoeologous chromosomes occurs in synthetic *A. suecica*. Together with previous reports in *A. suecica* and other neo-allopolyploids, these data highlight considerable variations of the frequency of these events between species, accessions and individuals (Comai *et al.*, 2000; Szadkowski *et al.*, 2010; Henry *et al.*, 2014; Sun *et al.*, 2017; Gaebelein *et al.*, 2019; Li *et al.*, 2019; Zhang *et al.*, 2020). We show that the presence of homoeologous recombination in neo-allopolyploid *A. suecica* is associated with a significant reduction in genome-wide crossover frequency, seen both in the decrease in chiasma frequency and Class I CO (MLH1 foci) (Figs 4, 5). Recombination partner choice thus appears to be severely altered in synthetic *A. suecica* with the formation of CO between homoeologous chromosomes appearing to be to the detriment of homologous CO.

Meiotic CO formation is initiated at leptotene by the induction of numerous DNA double-strand breaks and the generation of 3′ single-stranded DNA overhangs, onto which RAD51 and/or DMC1 are polymerized (Brown & Bishop, 2015; Emmenecker *et al.*, 2023). During leptotene/zygotene, this presynaptic nucleoprotein filament catalyzes homology search and invasion of a double-stranded DNA template which will be used for repair (Mercier *et al.*, 2015; Wang & Copenhaver, 2018). In most studied species, DNA invasion is accompanied by chromosome pairing at zygotene and establishment of the synaptonemal complex with a zipper-like effect, leading to full chromosome synapsis at pachytene (Brown & Bishop, 2015; Zickler & Kleckner, 2015; Emmenecker *et al.*, 2023). The joint recombination intermediates are processed to yield non-CO or CO products and chromatid cohesion holds homologous chromosome pairs together until anaphase (Hunter, 2015; Mercier *et al.*, 2015; Wang & Copenhaver, 2018). The mechanisms of recognition and choice of the recombination partner (donor chromatid) in this process are not well understood. Discrimination between homologous vs more diverged homoeologous sequences can potentially be made (1) during the initial strand invasion where the presence of

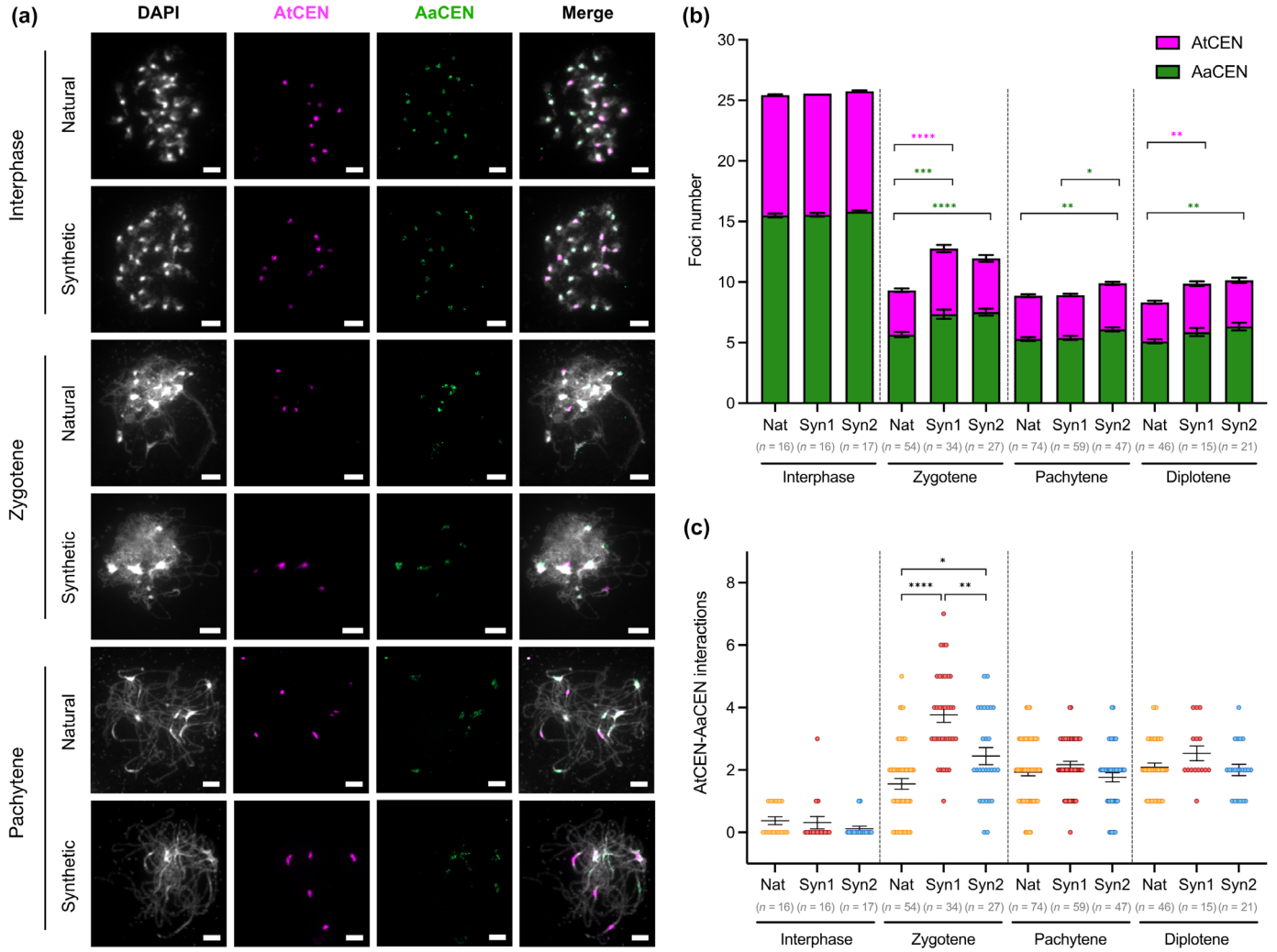

Fig. 6 Comparison of centromere dynamics during meiotic prophase I in natural and neo-synthetic *Arabidopsis suecica*. (a) Fluorescent *in situ* hybridization of centromeric repeats to natural and neo-synthetic plant 1 meiocytes. Probes specific to *Arabidopsis thaliana* (AtCEN, magenta) and *Arabidopsis arenosa* (AaCEN, green) were hybridized to male meiotic chromosome spreads stained with DAPI (white) at the labeled stages (bars, 5 μm). (b) Stacked bar showing the quantification of centromeric foci of *A. thaliana* (AtCEN, magenta) and *A. arenosa* (AaCEN, green) sub-genomes in natural (Nat), neo-synthetic plant 1 (Syn1) and plant 2 (Syn2) at the labeled stages. Bars show mean ± SE. Statistical significance was assessed using unpaired two-tailed Mann–Whitney tests adjusted for multiple comparisons with Bonferroni correction (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). (c) Quantification of overlapping foci between centromeres from *A. thaliana* and *A. arenosa* sub-genomes in natural (Nat), neo-synthetic plant 1 (Syn1) and plant 2 (Syn2) at the labeled stages. Bars show mean ± SE. Statistical significance was assessed using unpaired two-tailed Mann–Whitney tests adjusted for multiple comparisons with the Bonferroni correction (*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$).

mismatches in heteroduplex DNA can promote heteroduplex rejection (i.e. dissociation of invading strand DNA) and/or (2) during CO maturation by preventing recombination intermediates from becoming CO. In established allohexaploid bread wheat, the prevention of recombination between homoeologous chromosomes is thought to be regulated at both levels (Martín *et al.*, 2017; Serra *et al.*, 2021). In *A. suecica*, the presence of interactions between chromosomes from both sub-genomes at zygotene (as seen by centromere interactions; Fig. 6a,c) suggests that homology search and strand invasion could be initiated between both homologous and homoeologous chromosomes, possibly leading to the formation of synaptic multivalents. This is consistent with the few synaptic multivalents reported in *B. napus*, *Aegilops* species, wheat and *Festuca* polyploids (Hobolth, 1981; Thomas & Thomas, 1993; Cuñado *et al.*, 1996; Grandont *et al.*, 2013). Interestingly, the interactions (and possibly pairing) between homoeologous centromeres are more frequent (up to more than twofold) in neo-synthetic compared to evolved *A. suecica* plants at zygotene (Fig. 6c), and this is associated with a reduction in the interactions between centromeres from the same sub-genomes (including homologous interactions) (Fig. 6b). These observations could reflect an abnormal stabilization of DNA strand invasion and exchange within homoeologous sequences, leading to the formation of numerous synaptic multivalents, and consequently to allosyndetic multivalents at diakinesis and metaphase I (Figs 3, S3). In established *A. suecica*, efficient discrimination between homologous vs homoeologous sequences during the strand invasion step could however limit

formation of homoeologous CO and consequently, ensure accurate chromosome segregation.

It has been proposed that meiotic chromosome recognition and pairing are facilitated by initial interactions between particular chromosomal regions such as telomeres, centromeres or specific pairing centers (Da Ines & White, 2015; Zickler & Kleckner, 2015; Zhang & Han, 2017). These initial contacts are thought to trigger chromosome movements that promote homology search and pairing of homologous chromosomes, which can then be stabilized by homologous recombination and synaptonemal complex assembly (Da Ines & White, 2015). In polyploid wheat and rice, centromeres associate and then form homologous pairs prior to the onset of meiosis suggesting that pre-meiotic centromere associations could mediate the sorting of homologous and homoeologous chromosomes in these polyploids (Martinez-Perez *et al.*, 1999, 2000, 2001; Prieto *et al.*, 2004; Barea *et al.*, 2022). In agreement with data as previously described (Comai *et al.*, 2003), we do not observe pre-meiotic centromere association in allopolyploid *A. suecica*, but centromere dynamics comparable to diploid *A. thaliana* and *Brachipodium distachyon*, where centromeres cluster at the leptotene-zygotene transition prior to chromosome arm synapsis (Fig. 6; Wen *et al.*, 2012; Da Ines & White, 2015). Pre-meiotic centromere interactions are thus not a general requirement in allopolyploid species, but perhaps facilitate chromosome sorting in species with higher chromosome numbers.

In *Arabidopsis* autotetraploids (that carry four homologous copies of each chromosome), meiotic stabilization is reported to arise largely from an increase in crossover interference that limits crossover numbers per chromosome and consequently, reduces the occurrence of multivalents (Morgan *et al.*, 2021; Bomblies, 2023). Reducing meiotic CO rates is moreover sufficient to decrease multivalent frequency in neo-autotetraploid *Arabidopsis*, although it does not rescue fertility (Gonzalo *et al.*, 2023). In a previous study in allotetraploid hybrids formed between neo- and evolved *A. suecica*, the authors show increased CO rates compared to diploids in a 5 Mb genetic interval on chromosome 3 (Pecinka *et al.*, 2011). Here, we provide a comparison of genome-wide CO frequency in the first neo-allopolyploid meioses vs evolved *A. suecica* meioses and show that CO rates are significantly lower in the neo-synthetic plants (Fig. 4). Thus, while meiotic adaptation is associated with reduced CO formation in autotetraploid *A. arenosa* (Morgan *et al.*, 2021; Bomblies, 2023), meiotic adaptation in allopolyploid *A. suecica* seems instead associated with increased CO formation. A tempting hypothesis would be that, after an initial reduction of meiotic CO to partially stabilize meiosis by limiting homoeologous recombination, a progressive increase of meiotic recombination efficiency in subsequent generations would gradually establish full meiotic adaptation to allopolyploidy – ensuring the obligate crossover required for accurate chromosome segregation and increased genetic diversity among the offspring. We note that this would imply different evolutionary trajectories for auto- and allopolyploid *Arabidopsis* to stabilize their meiosis. An important point to take into account is possible roles of specific progenitors in allopolyploid phenotypes. We note that the ploidy (and the accessions) of *A. suecica* progenitors are still subject to debate (Novikova *et al.*, 2017; Nibau *et al.*, 2022). In this work, we crossed two natural autotetraploid species (*A. thaliana* Wa-1 & *A. arenosa* Care-1) that could have transmitted a low chiasma frequency to the neo-allopolyploids.

Genome sequencing of neo and evolved allopolyploid species revealed that homoeologous rearrangements are larger and globally more frequent in neo-synthetic lines than in natural varieties (Chalhoub *et al.*, 2014; Lloyd *et al.*, 2018; Song *et al.*, 2020; Zhang *et al.*, 2020). For example, natural allopolyploid cotton and *A. suecica* maintain their sub-genomes separate and do not show evidence of bursts of extensive homoeologous exchanges having occurred following their formation (Salmon *et al.*, 2010; Burns *et al.*, 2021). How then can we explain the dramatic meiotic instability seen in neo-allopolyploids? Two interesting observations cast light on this intriguing question. First, we showed that the fitness of neo-synthetic *A. suecica* plants varies according to the genotypes of the progenitors (*A. thaliana* Wa-1 vs Ler; Figs 1, S1). Previous studies have also reported such an effect on the extent of genome rearrangements in neo-synthetic *B. napus*, probably via segregation of genetic variants that affect meiotic stability (Attia & Röbbelen, 1986; Szadkowski *et al.*, 2010; Ferreira de Carvalho *et al.*, 2021). So, it seems that some accessions are more prone to give 'stable' allopolyploid offspring and it is probable that the allopolyploid lineages that survive are those that are, from the outset, less inclined to genome rearrangement with a preference for recombining with homologues over homoeologues. Such an influence of the genetic contribution of the progenitors to 'kickstart' the (partial) fertility of a given neo-allopolyploid could clearly play an important role in the natural context. Second, an unanticipated finding here was the considerable variability between the neo-synthetic siblings regarding fertility, meiotic stability and homoeologous recombination rate – and notably the 'reasonable' fertility of some of these first-generation allopolyploid plants. This observation clearly suggests that the evolved allopolyploids could derive from the more stable neo-allopolyploids within the first generations following the hybridization. Conditions for allopolyploid success could thus initially rely on preexisting genetic determinants which partially limit homoeologous pairing and recombination, to be followed by fine-tuning the adaptation in subsequent generations.

Acknowledgements

We thank Magnus Nordborg (Gregor Mendel Institute, Vienna), Mathilde Grelon (INRAE, IJPB, Versailles) and Eugenio Sanchez-Moran (University of Birmingham) for providing ASS3 seeds, α-MLH1 and α-ASY1 antibodies, respectively. We also thank the platform CLIC (Clermont-Ferrand Imagerie Confocale, Clermont Auvergne University) for confocal microscopy as well as James Higgins (University of Leicester) for access to his epifluorescence microscope. We acknowledge the members of the group for their help and discussions. This work was supported by the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), the Clermont Auvergne University, the ANR JCJC MeioAdapt (ANR-21-CE20-0004-01), the Springboard

Programme for a Sustainable Future and the Bettencourt-Schueller Foundation.

Competing interests

None declared.

Author contributions

FC, CW and HS conceived the research and designed the experiments. FC, VP and HS performed the experiments. CL provided training for MLH1 immunostaining. FC, CW and HS analyzed the data. FC and HS wrote the manuscript with inputs from CW. All authors approved the manuscript.

ORCID

Floriane Chéron https://orcid.org/0009-0006-7756-2695
Christophe Lambing https://orcid.org/0000-0001-5218-4217
Valentine Petiot https://orcid.org/0009-0001-4718-0227
Heïdi Serra https://orcid.org/0000-0002-5457-2050
Charles White https://orcid.org/0000-0001-8152-5797

Data availability

All image data used in this study are available in Datasets S2–S5.

References

Alexander MP. 1969. Differential staining of aborted and nonaborted pollen. *Stain Technology* **44**: 117–122.

Alleva B, Smolikove S. 2017. Moving and stopping: regulation of chromosome movement to promote meiotic chromosome pairing and synapsis. *Nucleus* **8**: 613–624.

Armstrong SJ, Franklin FCH, Jones GH. 2001. Nucleolus-associated telomere clustering and pairing precede meiotic chromosome synapsis in *Arabidopsis thaliana*. *Journal of Cell Science* **114**: 4207–4217.

Attia T, Röbbelen G. 1986. Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. *Canadian Journal of Genetics and Cytology* **28**: 323–329.

Barea L, Redondo-Río Á, Lucena-Marín R, Serrano-Heredia S, Aguilar M, Prieto P. 2022. Homologous chromosome associations in domains before meiosis could facilitate chromosome recognition and pairing in wheat. *Scientific Reports* **12**: 1–11.

Blasio F, Prieto P, Pradillo M, Naranjo T. 2022. Genomic and meiotic changes accompanying polyploidization. *Plants* **11**: 1–32.

Bomblies K. 2020. When everything changes at once: finding a new normal after genome duplication: evolutionary response to polyploidy. *Proceedings of the Royal Society B: Biological Sciences* **287**: 20202154.

Bomblies K. 2023. Learning to tango with four (or more): the molecular basis of adaptation to polyploid meiosis. *Plant Reproduction* **36**: 107–124.

Brown MS, Bishop DK. 2015. DNA strand exchange and RecA homologs in meiosis. *Cold Spring Harbor Perspectives in Biology* **7**: 1–29.

Burns R, Mandáková T, Gunis J, Soto-Jiménez LM, Liu C, Lysak MA, Novikova PY, Nordborg M. 2021. Gradual evolution of allopolyploidy in *Arabidopsis suecica*. *Nature Ecology & Evolution* **5**: 1367–1381.

Chalhoub B, Denoeud F, Liu S, Parkin IAP, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B *et al.* 2014. Plant genetics. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* **345**: 950–953.

Chelysheva L, Grandont L, Vrielynck N, le Guin S, Mercier R, Grelon M. 2010. An easy protocol for studying chromatin and recombination protein dynamics during *Arabidopsis thaliana* meiosis: immunodetection of cohesins, histones and MLH1. *Cytogenetic and Genome Research* **129**: 143–153.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* **6**: 836–846.

Comai L, Tyagi AP, Lysak MA. 2003. FISH analysis of meiosis in Arabidopsis allopolyploids. *Chromosome Research* **11**: 217–226.

Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B. 2000. Phenotypic instability and rapid gene silencing in newly formed Arabidopsis allotetraploids. *Plant Cell* **12**: 1551–1567.

Cuñado N, Callejas S, García MJ, Fernández A, Santos JL. 1996. The pattern of zygotene and pachytene pairing in allotetraploid *Aegilops* species sharing the U genome. *Theoretical and Applied Genetics* **93**: 1152–1155.

Da Ines O, White CI. 2015. Centromere associations in meiotic chromosome pairing. *Annual Review of Genetics* **49**: 95–114.

Durvasula A, Fulgione A, Gutaker RM, Alacakaptan SI, Flood PJ, Neto C, Tsuchimatsu T, Burbano HA, Picó FX, Alonso-Blanco C *et al.* 2017. African genomes illuminate the early history and transition to selfing in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **114**: 5213–5218.

Emmenecker C, Mézard C, Kumar R. 2023. Repair of DNA double-strand breaks in plant meiosis: role of eukaryotic RecA recombinases and their modulators. *Plant Reproduction* **36**: 17–41.

Ferreira de Carvalho J, Stoeckel S, Eber F, Lodé-Taburel M, Gilet MM, Trotoux G, Morice J, Falentin C, Chèvre AM, Rousseau-Gueutin M. 2021. Untangling structural factors driving genome stabilization in nascent *Brassica napus* allopolyploids. *New Phytologist* **230**: 2072–2084.

Gaebelein R, Schiessl SV, Samans B, Batley J, Mason AS. 2019. Inherited allelic variants and novel karyotype changes influence fertility and genome stability in *Brassica* allohexaploids. *New Phytologist* **223**: 965–978.

Gonzalo A, Lucas MO, Charpentier C, Sandmann G, Lloyd A, Jenczewski E. 2019. Reducing MSH4 copy number prevents meiotic crossovers between non-homologous chromosomes in *Brassica napus*. *Nature Communications* **10**: 1–9.

Gonzalo A, Parra-Nunez P, Bachmann AL, Sanchez-Moran E, Bomblies K. 2023. Partial cytological diploidization of neoautotetraploid meiosis by induced cross-over rate reduction. *Proceedings of the National Academy of Sciences, USA* **120**: 2017.

Grandont L, Jenczewski E, Lloyd A. 2013. Meiosis and its deviations in polyploid plants. *Cytogenetic and Genome Research* **140**: 171–184.

Henry IM, Dilkes BP, Tyagi A, Gao J, Christensen B, Comai L. 2014. The BOY NAMED SUE quantitative trait locus confers increased meiotic stability to an adapted natural allopolyploid of Arabidopsis. *Plant Cell* **26**: 181–194.

Higgins EE, Howell EC, Armstrong SJ, Parkin IAP. 2021. A major quantitative trait locus on chromosome A9, BnaPh1, controls homoeologous recombination in *Brassica napus*. *New Phytologist* **229**: 3281–3293.

Hobolth P. 1981. Chromosome pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. *Carlsberg Research Communications* **46**: 129–173.

Hohmann N, Wolf EM, Lysak MA, Koch MA. 2015. A time-calibrated road map of brassicaceae species radiation and evolutionary history. *Plant Cell* **27**: 2770–2784.

Hunter N. 2015. Meiotic recombination: the essence of heredity. *Cold Spring Harbor Perspectives in Biology* **7**: a016618.

Jenczewski E, Eber F, Grimaud A, Huet S, Lucas MO, Monod H, Chèvre AM. 2003. PrBn, a major gene controlling homeologous pairing in oilseed rape (*Brassica napus*) haploids. *Genetics* **164**: 645–653.

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS *et al.* 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* **473**: 97–100.

Li N, Xu C, Zhang A, Lv R, Meng X, Lin X, Gong L, Wendel JF, Liu B. 2019. DNA methylation repatterning accompanying hybridization, whole genome doubling and homoeolog exchange in nascent segmental rice allotetraploids. *New Phytologist* **223**: 979–992.

Lloyd A, Blary A, Charif D, Charpentier C, Tran J, Balzergue S, Delannoy E, Rigaill G, Jenczewski E. 2018. Homoeologous exchanges cause extensive

dosage-dependent gene expression changes in an allopolyploid crop. *New Phytologist* **217**: 367–377.

Lloyd A, Bomblies K. 2016. Meiosis in autopolyploid and allopolyploid Arabidopsis. *Current Opinion in Plant Biology* **30**: 116–122.

Mandáková T, Pouch M, Harmanová K, Zhan SH, Mayrose I, Lysak MA. 2017. Multispeed genome diploidization and diversification after an ancient allopolyploidization. *Molecular Ecology* **26**: 6445–6462.

Martín AC, Alabdullah AK, Moore G. 2021. A separation-of-function ZIP4 wheat mutant allows crossover between related chromosomes and is meiotically stable. *Scientific Reports* **11**: 1–13.

Martín AC, Rey MD, Shaw P, Moore G. 2017. Dual effect of the wheat Ph1 locus on chromosome synapsis and crossover. *Chromosoma* **126**: 669–680.

Martín AC, Shaw P, Phillips D, Reader S, Moore G. 2014. Licensing MLH1 sites for crossover during meiosis. *Nature Communications* **5**: 1–5.

Martinez-Perez E, Shaw PJ, Moore G. 2000. Polyploidy induces centromere association. *Journal of Cell Biology* **148**: 233–238.

Martinez-Perez E, Shaw PJ, Moore G. 2001. The Ph1 locus is needed to ensure specific somatic and meiotic centromere association. *Nature* **411**: 204–207.

Martinez-Perez E, Shaw PJ, Reader S, Aragón-Alcaide L, Miller T, Moore G. 1999. Homologous chromosome pairing in wheat. *Journal of Cell Science* **112**: 1761–1769.

Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelon M. 2015. The molecular biology of meiosis in plants. *Annual Review of Plant Biology* **66**: 297–327.

Morgan C, Fozard JA, Hartley M, Henderson IR, Bomblies K, Howard M. 2021. Diffusion-mediated HEI10 coarsening can explain meiotic crossover positioning in Arabidopsis. *Nature Communications* **12**: 4674.

Nibau C, Gonzalo A, Evans A, Sweet-Jones W, Phillips D, Lloyd A. 2022. Meiosis in allopolyploid *Arabidopsis suecica*. *The Plant Journal* **111**: 1110–1122.

Novikova PY, Tsuchimatsu T, Simon S, Nizhynska V, Voronin V, Burns R, Fedorenko OM, Holm S, Säll T, Prat E *et al.* 2017. Genome sequencing reveals the origin of the allotetraploid *Arabidopsis suecica*. *Molecular Biology and Evolution* **34**: 957–968.

Otto SP. 2007. The evolutionary consequences of polyploidy. *Cell* **131**: 452–462.

Parra-Nunez P, Pradillo M, Santos JL. 2020. How to perform an accurate analysis of metaphase I chromosome configurations in autopolyploids of *Arabidopsis thaliana*. *Methods in Molecular Biology* **2061**: 25–36.

Pecinka A, Fang W, Rehmsmeier M, Levy AA, Mittelsten Scheid O. 2011. Polyploidization increases meiotic recombination frequency in Arabidopsis. *BMC Biology* **9**: 24.

Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L, Viegas W, Pikaard CS. 2004. Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. *Proceedings of the National Academy of Sciences, USA* **101**: 18240–18245.

Prieto P, Santos AP, Moore G, Shaw P. 2004. Chromosomes associate premeiotically and in xylem vessel cells via their telomeres and centromeres in diploid rice (*Oryza sativa*). *Chromosoma* **112**: 300–307.

Ramsey J, Schemske DW. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* **33**: 589–639.

Rey MD, Martín AC, Higgins J, Swarbreck D, Uauy C, Shaw P, Moore G. 2017. Exploiting the ZIP4 homologue within the wheat Ph1 locus has identified two lines exhibiting homoeologous crossover in wheat-wild relative hybrids. *Molecular Breeding* **37**: 95.

Ross KJ, Fransz P, Jones GH. 1996. A light microscopic atlas of meiosis in *Arabidopsis thaliana*. *Chromosome Research* **4**: 507–516.

Salmon A, Flagel L, Ying B, Udall JA, Wendel JF. 2010. Homoeologous nonreciprocal recombination in polyploid cotton. *New Phytologist* **186**: 123–134.

Serra H, Svačina R, Baumann U, Whitford R, Sutton T, Bartoš J, Sourdille P. 2021. Ph2 encodes the mismatch repair protein MSH7-3D that inhibits wheat homoeologous recombination. *Nature Communications* **12**: 1–10.

Soares NR, Mollinari M, Oliveira GK, Pereira GS, Vieira MLC. 2021. Meiosis in polyploids and implications for genetic mapping: a review. *Genes* **12**: 1517.

Soltis DE, Soltis PS, Bennett MD, Leitch IJ. 2003. Evolution of genome size in the angiosperms. *American Journal of Botany* **90**: 1596–1603.

Song JM, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R *et al.* 2020. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nature Plants* **6**: 34–45.

Sun Y, Wu Y, Yang C, Sun S, Lin X, Liu L, Xu C, Wendel JF, Gong L, Liu B. 2017. Segmental allotetraploidy generates extensive homoeologous expression rewiring and phenotypic diversity at the population level in rice. *Molecular Ecology* **26**: 5451–5466.

Szadkowski E, Eber F, Huteau V, Lodé M, Huneau C, Belcram H, Coriton O, Manzanares-Dauleux MJ, Delourme R, King GJ *et al.* 2010. The first meiosis of resynthesized *Brassica napus*, a genome blender. *New Phytologist* **186**: 102–112.

Thomas HM, Thomas BJ. 1993. Synaptonemal complex formation in two allohexaploid *Festuca* species and a pentaploid hybrid. *Heredity* **71**: 305–311.

Udall JA, Wendel JF. 2006. Polyploidy and crop improvement. *Crop Science* **46** (Suppl 1): 3–14.

Van De Peer Y, Mizrachi E, Marchal K. 2017. The evolutionary significance of polyploidy. *Nature Reviews Genetics* **18**: 411–424.

Wang Y, Copenhaver GP. 2018. Meiotic recombination: mixing it up in plants. *Annual Review of Plant Biology* **69**: 577–609.

Wen R, Moore G, Shaw PJ. 2012. Centromeres cluster *de novo* at the beginning of meiosis in *Brachypodium distachyon*. *PLoS ONE* **7**: e44681.

Zhang H, Bian Y, Gou X, Zhu B, Xu C, Qi B, Li N, Rustgi S, Zhou H, Han F *et al.* 2013. Persistent whole-chromosome aneuploidy is generally associated with nascent allohexaploid wheat. *Proceedings of the National Academy of Sciences, USA* **110**: 3447–3452.

Zhang J, Han F. 2017. Centromere pairing precedes meiotic chromosome pairing in plants. *Science China Life Sciences* **60**: 1197–1202.

Zhang Z, Gou X, Xun H, Bian Y, Ma X, Li J, Li N, Gong L, Feldman M, Liu B *et al.* 2020. Homoeologous exchanges occur through intragenic recombination generating novel transcripts and proteins in wheat and other polyploids. *Proceedings of the National Academy of Sciences, USA* **117**: 14561–14571.

Zickler D, Kleckner N. 2015. Recombination, pairing, and synapsis of homologs during meiosis. *Cold Spring Harbor Perspectives in Biology* **7**: a016626.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Raw data for fertility and cytogenetic analyses.

Dataset S2 Microscopy images of meiotic metaphases I from natural *A. suecica* and neo-synthetic plants 1 and 2 (Zeiss ZEN).

Dataset S3 FISH images from natural *A. suecica* (Zeiss ZEN).

Dataset ' FISH images from neo-synthetic plant 1 (Zeiss ZEN).

Dataset S5 FISH images from neo-synthetic plant 2 (Zeiss ZEN).

Fig. S1 Fertility of neo-synthetic *Arabidopsis suecica* generated from a cross between *Arabidopsis thaliana*, ecotype Ler and *Arabidopsis arenosa*, ecotype Care-1 is drastically reduced compared with evolved *A. suecica*.

Fig. S2 Positive correlation between pollen viability and meiotic stability in *Arabidopsis suecica*.

Fig. S3 Connections between bivalents at diakinesis in *Arabidopsis suecica*.

Fig. S4 Frequency distributions of AtCEN and AaCEN foci numbers in natural *Arabidopsis suecica* and the neo-synthetic plants 1 (Syn 1) and 2 (Syn 2) at zygotene, pachytene and diplotene stages.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.