Homoeologous recombination is recurrent in the nascent synthetic allotetraploid *Arachis ipaënsis* × *Arachis correntina*4x and its derivatives

Ye Chu1, David Bertioli,2,3,4 Chandler M. Levinson,3 H. Thomas Stalker,5 C. Corley Holbrook,6 and Peggy Ozias-Akins1,3,*

1Horticulture Department, University of Georgia, Tifton, GA 31793, USA
2Center for Applied Genetic Technologies, University of Georgia, Athens, GA 30602, USA
3Institute of Plant Breeding, Genetics and Genomics, University of Georgia, Athens, GA 30602, USA
4Department of Crop and Soil Science, University of Georgia, Athens, GA 30602, USA
5Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA
6USDA- Agricultural Research Service, Crop Genetics and Breeding Research Unit, Tifton, GA 31793, USA

*Corresponding author: Rm 107 NESPAL, 2356 Rainwater Road, Tifton, GA 31793, USA. pozias@uga.edu

Abstract

Genome instability in newly synthesized allotetraploids of peanut has breeding implications that have not been fully appreciated. Synthesis of wild species-derived neo-tetraploids offers the opportunity to broaden the gene pool of peanut; however, the dynamics among the newly merged genomes creates predictable and unpredictable variation. Selfed progenies from the neo-tetraploid *Arachis ipaënsis* × *Arachis correntina* (A. ipaënsis × A. correntina)4x and F1 hybrids and F2 progenies from crosses between A. hypogaea × [A. ipaënsis × A. correntina]4x were genotyped by the Axiom 48K SNP array. Homoeologous recombination between the A. ipaënsis and A. correntina derived subgenomes was observed in the S0 generation. Among the S1 progenies, these recombined segments segregated and new events of homoeologous recombination emerged. The genomic regions undergoing homoeologous recombination segregated mostly disomically in the F2 progenies from A. hypogaea × [A. ipaënsis × A. correntina]4x crosses. New homoeologous recombination events also occurred in the F2 population, mostly found on chromosomes 03, 04, 05, and 06. From the breeding perspective, these phenomena offer both possibilities and perils; recombination between genomes increases genetic diversity, but genome instability could lead to instability of traits or even loss of viability within lineages.

Keywords: *Arachis hypogaea* (peanut); homoeologous recombination; synthetic allotetraploid; A. ipaënsis; A. correntina

Introduction

Peanut (*Arachis hypogaea*) is an important crop valued for its high oil and protein content. Originating from South America, peanut is widely grown in the warmer regions of the world yielding a total of 46 million tons in 2018 (http://www.fao.org/faostat). Cultivated peanut is an allotetraploid with homoeologous subgenomes (AABB; 2n = 4x = 40) sharing greater than 90% DNA sequence similarity (Bertioli et al. 2016). Its origin was via the formation of a hybrid between two diploid wild species, *Arachis ipaënsis* (BB; 2n = 2x = 20) and *Arachis duranensis* (AA; 2n = 2x = 20) followed by a single, or very few, natural polyploidization events less than 10,000 years ago (Bertioli et al. 2016). This recent polyploid origin created a strong genetic bottleneck and isolated cultivated peanut from its diploid wild relatives (Krapovickas et al. 2007). The narrow genetic base of cultivated peanut has resulted in limited resistance to many pathogens and diseases. For instance, only moderate levels of resistance to root-knot nematode (*Meloidogyne arenaria*) and late leaf spot (caused by *Nothopassalora personata*) were identified among over 1,000 peanut plant introductions in the US (Holbrook and Noe 1992; Holbrook and Anderson 1995). Screening over 10,000 plant introductions for rust (caused by *Puccinia arachidis*) yielded only 14 moderately rust resistant lines mostly collected from Peru (Subrahmanyam et al. 1985, 1989). In contrast, the wild relatives of peanut harbor strong resistance or immunity to many diseases and pests (Stalker 2017). There are 31 species in the section *Arachis* with only two of them being tetraploid (2n = 4x = 40), i.e., *A. hypogaea* and *A. monticola*. The other 29 species are diploids (2n = 2x = 20 or 2n = 2x = 18). High levels of genetic diversity of the diploid species compared to cultivated peanut is well-known (Kochert et al. 1996, Moretzsohn et al. 2004). An early well-documented introgression event resulted in introduction to the peanut crop of near immunity to root-knot nematode from the diploid species *Arachis cardenasii* (Simpson et al. 1993, 2003; Holbrook et al. 2008a; Nagy et al. 2010). This introgression used a hybridization scheme known as the tetraploid route (albeit in a complex three-way cross; Simpson 1991). First, an A genome hybrid was made by crossing *A. cardeñasii* with *A. diogo*. Then, the B genome (sensu latu) species *A. batizocoi* was crossed with the A genome hybrid to create a sterile AB hybrid. The AB hybrid was treated with colchicine to...
double the chromosome number and restore fertility thereby gaining sexual compatibility with cultivated peanut. Subsequently, efforts have focused on the tetraploid route in the simpler form, starting with one A and one B genome species (Favero et al. 2006, Stalker 2017; Leal-Bertioli et al. 2018). Recent introgressions from the tetraploid route include a new strong resistance to root-knot nematode from A. stenosperma (Ballen-Taborda et al. 2019) and improved pod and seed characteristics from A. ipaënsis and A. duranensis (Fonceka et al. 2009, 2012). Besides peanut, introgression from wild relatives brought in significant benefits to other crops such as improved seed yield in Brassica napus (Qian et al. 2005), fiber quality in cotton (Zhang et al. 2014), and disease resistance in wheat (Ali et al. 2016; Rahmatov et al. 2016).

Following interspecific hybridization and chromosome duplication, active homoeologous recombination can occur between the two distinct types of chromosomes as a consequence of bivalent and tetravalent formation during meiosis (Soltis and Soltis 1999). While bivalent pairing of chromosomes maintains the status of two separate subgenomes, the formation of multivalents leads to the breakdown of their separate identities (Stebbins 1947). The pairing of homoeologous chromosomes can result in replacement of a segment by a copy of the paired homoeologous region through both meiotic crossover and noncrossover (Youds and Boulton 2011). Pioneering cytogenetic studies showed that meiotic chromosomes in peanut consisted of 20 chromosome bivalents in 88–98% of cells; the remainder of cells harbored mostly bivalents together with one or a few univalents, trivalents, or quadrivalents (Husted 1936; Smartt et al. 1978). This clearly indicated the likelihood of genetic recombination between subgenomes. However, in spite of these findings, almost all genetic studies using DNA markers have assumed only recombination within subgenomes. Only more recently has recombination studies using DNA markers have assumed only recombination following interspecific hybridization and chromosome duplication, active homoeologous recombination can occur between the two distinct types of chromosomes as a consequence of bivalent and tetravalent formation during meiosis (Soltis and Soltis 1947). The pairing of homoeologous chromosomes can result in replacement of a segment by a copy of the paired homoeologous region through both meiotic crossover and noncrossover (Youds and Boulton 2011). Pioneering cytogenetic studies showed that meiotic chromosomes in peanut consisted of 20 chromosome bivalents in 88–98% of cells; the remainder of cells harbored mostly bivalents together with one or a few univalents, trivalents, or quadrivalents (Husted 1936; Smartt et al. 1978). This clearly indicated the likelihood of genetic recombination between subgenomes. However, in spite of these findings, almost all genetic studies using DNA markers have assumed only recombination within subgenomes. Only more recently has recombination

### Materials and methods

#### Genetic materials

Interspecific hybrids were made between the two diploid species A. ipaënsis K30076 (Ipa; female) and A. correntina 9530 (Cor; male) at North Carolina State University. Multiple plants of each diploid species were used for crossing. Cuttings from the sterile interspecific hybrids were established and shipped to the University of Georgia Tifton Campus for colchicine treatment. Sterility is expected in interspecific hybrids, and the hybrid nature of putative F1s was confirmed by the presence of greater than 80% aborted pollen grains using the Alexander blue staining method (Alexander 1969).

For chromosome doubling, 300 cuttings of the diploid hybrid (~20 cm long) were immersed in 0.2% colchicine solution for 12–14 hours at room temperature. The treated cuttings were rinsed under continuously running tap water for 1 hour to remove excess colchicine. The cuttings were shortened by 2 cm from the end of the existing cut. The 18 cm long piece was further cut in half yielding two approximately 9 cm long pieces. Fresh cut ends were dipped in Clonex rooting gel (Growth Technology, Western Way, TA, UK) before being planted in jiffy peat pots (5.7 cm sq. × 5.7 cm H) filled with Promix BX growth medium (Premier Tech Horticulture, Quakertown, PA, USA). The cuttings were kept in seed trays enclosed with a clear cover to reach 100% humidity. The colchicine-treated cuttings were grown under 16/8 hours light/dark condition at room temperature for 3–4 weeks to allow root formation before transplanting to a plastic tub (87 × 55 × 20 cm; LxWxH) filled with Promix growth medium in the greenhouse. Cuttings were periodically checked for the formation of pegs and marked with flags. Pods were harvested 40–50 days after peg identification.

S0 plants of IpaCor4x were used as male parents to cross with two advanced peanut breeding lines 13-2113 [(Tifguard × Florida-07) × C725-19-25] and 13-1014 [(Tifguard × Florida-07) × Georgia-06G]. Florida-07 (Corbet and Tillman 2009), Tifguard (Holbrook et al. 2008a), and Georgia-06G (Branch 2007) are elite peanut cultivars adapted to US southeastern peanut growing regions. C725-19-25 is a high-yielding breeding line with resistance to tomato spotted wilt virus (Holbrook et al. 2008b). Two F2 populations from F1 hybrids (13-1014 × IpaCor4x-S0.2)_F1,4 and (13-1014 × IpaCor4x-S0.5)_F1,4 were grown in the greenhouse for tissue collection and DNA extraction. Each F2 population consisted of 456 individuals.

### Genotyping by SNP array

Genetic materials genotyped by the Axiom Arachis 48 K SNP array (Thermofisher Scientific, Waltham, MA, USA) (Clevenger et al. 2018; Korani et al. 2019) included DNA from two plants of Ipa and two plants of Cor (Supplementary Table S1). These four plants were sister lines to the original parents used to produce the IpaCor4x neo-tetraploid. The diploid hybrids before and after colchicine treatment were included. Six IpaCor4x-S0 and seven IpaCor4x-S1 neo-tetraploid plants were genotyped. All of the S1 plants were progenies from one mother plant i.e., IpaCor4x-S0. Two F1s of the A. hypogaea × IpaCor4x-S0.5 crosses were genotyped together with their A. hypogaea female parents 13-2113 and 13-1014. Two F2 populations (456 lines in each population) descending from (13-1014 × IpaCor4x-S0.2)_F1,4 and (13-1014 × IpaCor4x-S0.5)_F1,4 were genotyped as well. Genomic DNAs were extracted from expanded young leaves by Qiagen Plant DNaseasy kit (Qiagen, Germantown, MD, USA) and quantified by Quant-iT Picogreen dsDNA assay kit (Thermofisher Scientific, Waltham, MA, USA). Genotyping data were analyzed with the Axiom Analysis Suite (Thermofisher Scientific, Waltham, MA, USA). Based on SNP QC matrix developed by the software, SNP markers were grouped in six categories, i.e., PolyhighResolution, NoMinorHom, MonoHighResolution, CallRateBelowThreshold,
Curation of homoeologous recombination events in \textit{IpaCor}^{4X} neo-tetraploid

To study the recombination between the two subgenomes of \textit{IpaCor}^{4X}, diploid parents, \textit{IpaCor}^{2X} hybrids, and all of the \textit{IpaCor}^{4X} \textit{S}_1 and \textit{S}_2 plants were subjected to analysis. To curate the genomic regions hosting homoeologous recombination, we focused on polymorphic markers between \textit{Ipa} and \textit{Cor} which would have opposite genotype calls AA versus BB. Without taking into account recombination between subgenomes, we would naively expect \textit{IpaCor}^{4X} plants would form homoeologous genotyping clusters with AB genotype calls at these loci. In reality, we encountered unexpected genotype calls. All of the genotype calls in four categories AA, BB, AB, and NoCall were output as “call codes” using the Axiom analysis software. The data set was first filtered for polymorphic markers between \textit{Ipa} and \textit{Cor} using the “if” argument of Excel. Subsequently, the “countif” function was applied to curate the genotyping of \textit{IpaCor}^{4X} \textit{S}_2 and \textit{S}_1 plants deviating from expected homoeologous clusters. All of the markers with this type of sample distribution were visually inspected for clustering patterns. Markers with DNA samples clearly forming a separate cluster between homozygous and heterozygous clusters were assembled. The genotype calls of individuals falling in the separate cluster were adjusted to 75% of the closest homozygous calls. A stretch of at least three consecutive markers demonstrating the same recombination pattern was considered an event of homoeologous recombination.

Curation of new homoeologous recombination events in the F$_2$ populations of \textit{A. hypogaea} × \textit{IpaCor}^{4X} \textit{S}_0 crosses

Manually curating homoeologous recombination events among polymorphic markers of the \textit{F}_2 populations is an arduous task due to the large population size and the abundance of markers. However, monomorphic markers with \textit{F}_2 individuals grouped outside the majority of the population indicated the presence of new homoeologous recombination in these \textit{F}_2 outiers. To curate this type of new homoeologous recombination event, monomorphic markers with \textit{F}_2 individuals demonstrating genotype calls other than the expected AB calls were curated by Excel sorting and countif functions.

Figures of the clustering patterns were exported from the Axiom Analysis Suite software, which carried out clustering in two dimensions (Axiom Analysis Suite User Guide, Affymetrix.com). The X dimension is called “contrast” and the Y dimension is called “size.” They are log-linear combinations of the two allele signal intensities. To illustrate the allele exchange among the groups, sampler nucleotides G and C were used. The actual nucleotides at the targeted loci could be any one of the A, T, C, G. Superscripts next to the nucleotides denoted the sources of alleles. For instance, in the allele combinations such as G$^{AC}C^{G}$, C$^{CH}$, and G$^{AC}C^{CH}$, superscript indicated A genome from \textit{Ipa}, superscript denoted B genome from \textit{Ipa} and \textit{ab} denoted genome composition from \textit{A. hypogaea}.

To determine the frequency of homoeologous recombination across the genome, the number of lines hosting the recombination was counted for each marker. Genome positions of markers demonstrating homoeologous recombination were referenced to the \textit{Ipa} genome (Bertioli et al. 2016).

Results

Homoeologous recombination in the \textit{IpaCor}^{4X} neo-tetraploid

Out of 300 cuttings of \textit{IpaCor}^{2X} hybrids treated by colchicine, 21 \textit{S}_0 seeds were harvested. The new \textit{IpaCor}^{4X} allotetraploid was highly fertile. DNAs from six of the \textit{S}_0 seedlings were genotyped by the SNP array. The occurrence and segregation of chromosome regions demonstrating homoeologous recombination were curated in \textit{IpaCor}^{4X}_\textit{S}_0 and its seven progenies \textit{IpaCor}^{4X}_\textit{S}_0.2\textit{S}_1.1 to \textit{S}_1.7 (Table 1). During meiosis of \textit{IpaCor}^{4X}, genomic regions undergoing homoeologous pairing may result in homoeologous recombination. As one segment of the chromatid is replaced by the opposite subgenome, a set of contiguous homoeologous markers within the replaced chromosome segment should shift as a block from the expected “heterozygote” cluster (alternate SNPs in the two homoeologs are detected) toward the donor cluster. As illustrated at SNP marker AX-147221175 (Figure 1), diploid parents \textit{Cor} (red triangles, example base call as G$^{AC}G^{AC}$, ac denotes A subgenome from \textit{Cor}) and \textit{Ipa} (blue triangles, C$^{CH}$, bi denotes B subgenome from \textit{Ipa}) were grouped in the genotype clusters AA and BB respectively. Most of the \textit{IpaCor}^{4X}_\textit{S}_0 plants (S0.5, S0.6, S0.8, S0.10, S0.11, and S0.12) (G$^{AC}G^{AC}C^{CH}C^{CH}$, magenta circles) were grouped in the AB cluster as expected indicating their allele composition as G$^{AC}G^{AC}C^{CH}C^{CH}$ due to the merging of the \textit{Ipa} and \textit{Cor} genomes. However, \textit{IpaCor}^{4X}_\textit{S}_0.2 (two circled magenta triangles) was an outlier since it had a genotype call of AA. This was caused by the replacement of one chromosome segment from \textit{Ipa} by the homoeologous region from \textit{Cor}. Consequently, the allele composition became G$^{AC}G^{AC}G^{AC}C^{CH}$ and the hybridization signal was shifted toward the AA cluster where \textit{Cor} samples were located. Although the software assigned AA genotype calls for the two DNA samples from \textit{IpaCor}^{4X}_\textit{S}_0.2, the clustering pattern clearly indicated that \textit{IpaCor}^{4X}_\textit{S}_0.2 and four of her \textit{S}_1 progenies (S1.2, S1.3, S1.4, and S1.7) actually formed a fourth cluster (green oval) between the AB and AA clusters. Their genotype calls were adjusted to 75% of AA (re-coded as 75% of 0 in Table 1) implying one allele from the \textit{Ipa} subgenome was replaced by \textit{Cor}. The remaining three \textit{S}_1 progenies S1.1, S1.5, and S1.6 were grouped with \textit{Cor}. This implied that the allele composition of these individuals became G$^{AC}G^{AC}G^{AC}G^{AC}$ i.e., quadrilect for \textit{Cor}. A set of 19 adjacent markers at the end of chromosome \textit{A04}/\textit{B04} demonstrated the same pattern of homoeologous recombination and segregation among the \textit{S}_1 progenies of \textit{IpaCor}^{4X}_\textit{S}_0.2 (Table 1). The size of the chromosome segment was 6.7 Mbp with a marker density of 353 kb/marker. This was the only homoeologous recombination event identified in \textit{IpaCor}^{4X}_\textit{S}_0.2.

Four new homoeologous recombination events were found in three \textit{S}_1 progenies, \textit{IpaCor}^{4X}_\textit{S}_0.2 S1.1, S1.5, S1.7 (Table 2, Supplementary Table S2). At the top of chromosome \textit{A03}/\textit{B03}, a 5 Mbp chromatin segment had \textit{Cor} replacing \textit{Ipa} in \textit{IpaCor}^{4X}_\textit{S}_0.2_\textit{S}_1.1. Most of the chromosome \textit{B04} (~120 Mbp) had homoeologous recombination in both \textit{IpaCor}^{4X}_\textit{S}_0.2_\textit{S}_1.1 and \textit{S}_1.7 yet in opposite directions, i.e., the \textit{Cor} allele replaced the \textit{Ipa} allele in \textit{S}_1.1 and vice versa in \textit{S}_1.7. At the top of chromosome \textit{B05}, a 14 Mbp segment had one copy of alleles from \textit{Cor} replaced by \textit{Ipa} in \textit{IpaCor}^{4X}_\textit{S}_0.2_\textit{S}_1.5. These new events among the \textit{S}_1 progenies indicated that active homoeologous recombination continues to occur during generation advancement of the neo-tetraploid.

In addition to the \textit{IpaCor}^{4X}_\textit{S}_0.2 family, homoeologous recombination was identified in the four other \textit{IpaCor}^{4X}_\textit{S}_0 plants on chromosomes \textit{A04}/\textit{B04}, \textit{A05}/\textit{B05}, and \textit{A07}/\textit{B07} (Table 2). The two events on chromosome \textit{A04}/\textit{B04} were opposite in direction of
Table 1

Homoeologous recombination between A04 and B04 in IpaCor_4x_S0.2 and its segregation among S1 progenies

| Probeset_id | Chromosome | SNP Position (bp) | Genotype call | Genotype calls from the SNP array were re-coded as follows, genotype call AA ¼ 0; BB ¼ 2; AB ¼ 1 to avoid confusion with the description of subgenomes. Seventy-five percent of a genotype call indicates the dosage of a subgenome allele was increased by 25% as a result of subgenome recombination. | Chromosome and SNP positions were given relative to genome (peanutbase.org). The recombination events occurred at these loci were actually between the homoeologous chromosomes. Arachis ipaensis dark purple triangles) fell in this new group. The remaining three S1 circles) were clustered in the expected AB group. Figure 1 A SNP marker demonstrating a Cor allele replacing an Ipa allele in IpaCor_4x_S0.2 and S1 progenies. Cor, red triangles with G^C^C^C as an example of base call, and Ipa (C^C^C, blue triangles) were grouped in genotype calls of AA and BB respectively. ¼ superscript indicates A genome from Cor, ¼ superscript denotes B genome from Ipa. IpaCor_4x (C^C^C^C, green circles), and IpaCor_4x_S0.5, 6, 8, 10, 11, 12 (G^C^G^C^C^C^C^C, magenta circles) were clustered in the expected AB group. IpaCor_4x_S0.2 (C^G^G^G^C^C^C^C^C, two circled magenta triangles) and some other samples formed a separate group between cluster AB and AA which was enclosed by a green oval. Four of the S1 progenies from IpaCor_4x_S0.2 (G^G^G^C^C^C^O^O, dark purple triangles) fell in this new group. The remaining three S1 progenies (G^G^G^C^C^C^C^O^O, dark purple triangles) grouped with Cor forming a quadriplex locus. | homoelogous recombination and occurred in two separate S0 plants. The event in IpaCor_4x_S0.11 spanned a small segment of 0.5 Mbp whereas the event in IpaCor_4x_S0.12 almost covered the whole chromosome A04/B04 (124 Mbp). The event captured on chromosome A05/B05 also encompassed nearly the whole chromosome (148 Mbp) in IpaCor_4x_S0.6. The event identified on the top of chromosome A07/B07 was 1.4 Mbp in IpaCor_4x_S0.12. Among the nine events found in IpaCor_4x_S0 and S1 plants, five of them had Ipa replacing the Cor subgenome and four of them had Cor replacing the Ipa subgenome. Segregation of genomic regions subjected to recombination among F1 hybrids Three of the IpaCor_4x_S0 plants S0.2, S0.5, and S0.6 were used as males to cross with two elite A. hypogaea breeding lines 13-2113 and 13-1014 and produced 37 F1 progenies. Hybridity of all 37 F1 progenies was confirmed by the expected heterozygous calls from 1024 polymorphic markers between the A. hypogaea and IpaCor_4x_S0 parents (Supplementary Table S3). Each of these three IpaCor_4x_S0 male parents had a genomic region with chromosomal recombination on chromosomes A04/B04, A07/B07, and A05/B05, respectively (Tables 1 and 2). Segregation of these genomic regions harboring homoelogous recombination among F1 hybrids was observed (Supplementary Table S4; red font |
genotype calls). Two examples demonstrating segregation of the inherited homoeologous recombination from the IpaCor\(^{4x}\) \(_{S_0}\) parents among F\(_1\) hybrids are illustrated in Supplementary Figures S1 and S2. In both cases, the IpaCor\(^{4x}\) \(_{S_0}\) parent had a genomic region with pre-existing homoeologous recombination and the A. hypogaea female parent presented in either the heterozygote (Supplementary Figure S2) or homozygote (Supplementary Figure S3) cluster.

At the first locus (Supplementary Figure S2A), Cor (G\(^{c_c}\)G\(^{c_c}\)) and Ipa (C\(^{c_c}\)C\(^{c_c}\)) had genotype calls of AA and BB, respectively. The two DNA samples from IpaCor\(^{4x}\) \(_{S_0}\) that showed homoeologous exchange (G\(^{c_c}\)G\(^{c_c}\)G\(^{c_c}\)G\(^{c_c}\)) grouped in a separate cluster between the AB and AA clusters indicating that one Ipa allele was replaced by Cor. The two A. hypogaea female parents had the genotype call of AB indicating the presence of polymorphism between its own subgenomes and fell in the BB genotype cluster with 75% BB indicating their allele composition of C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\).

The two LB strains were C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\) and F\(_{1}\) hybrids had genotype calls of AB (G\(^{c_c}\)G\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)) and seven F\(_1\) hybrids had genotype calls of 75% AA (G\(^{c_c}\)G\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)). At the second locus (Supplementary Figure S2A), Cor (G\(^{c_c}\)G\(^{c_c}\)) and Ipa (C\(^{c_c}\)C\(^{c_c}\)) had genotype calls of AA and BB, respectively. The IpaCor\(^{4x}\) \(_{S_0}\) formed a separate group between AB and AA indicating that the Ipa allele was replaced by Cor (C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)). The A. hypogaea female parent (C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)) was monomorphic between its subgenomes and fell in the BB genotype cluster with Ipa and a few other samples. The expected allele compositions of F\(_1\) hybrids were C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\) and C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\) (Supplementary Figure S2B) and realized; three F\(_1\) hybrids had genotype calls of AB (C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)) and seven F\(_1\) hybrids had genotype calls of 75% AA (C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)C\(^{c_c}\)). Among the 16 F\(_1\) hybrids from 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) crosses, 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) segregated for most of the 60 markers within the 6.7 Mbp chromosome 4 region. The expected segregation patterns among the F\(_1\) hybrids of the homoeologous recombination events from neo-tetraploid parents confirmed the presence and inheritance of these events.

### New homoeologous recombination events captured in F\(_1\) hybrids

In addition to the inherited recombination events, 27 new recombination events on chromosomes A02/B02, A03/B03, A04/B04, A05/B05, A06/B06, and A07/B07 were captured among the F\(_1\) hybrids (Table 3, Supplementary Table S4). There were four events on chromosome 2, where the segment sizes ranged from 2 to 106 Mbp. One event was identified on chromosome 3 with a segment size of 5 Mbp. There were seven events on chromosome 4, where the segment sizes ranged from 0.5 to 130 Mbp. The 0.5 Mbp recombination event was identified in two F\(_1\) hybrids, i.e., 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) and 13-2113 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\). The inheritance of this 0.5 Mbp region in the F\(_2\) population descending from 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) was demonstrated (Table 3). Three events were found on A05/B05 with the segment sizes ranging from 3 to 17 Mbp. Seven events were identified on chromosome 6 where the segment sizes ranging from 0.3 to 124 Mbp. For the direction of genome recombination, there were 16 events where Cor alleles replaced the Ipa alleles and 11 events that demonstrated the opposite direction of recombination. Most of the recombination break points occurred closer to the ends of the chromosome arms. Most of the events were in different regions except for two consecutive recombination events in opposite directions on A06/B06 in 13-2113 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) (Supplementary Table S4).

### Segregation of the F\(_2\) population descending from 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) at the bottom of chromosome 4

Of the two F\(_2\) populations descending from 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) and 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) that were genotyped by the SNP array, there was only one pre-existing homoeologous genomic region at the bottom of A04/B04 of 13-1014 \(_{IpaCor}\) \(_{S_0}\)/C\(_2\) \(_{F_1.4}\) identified in this study (Supplementary Table S4). In the genotyping profile of the parental lines and F\(_1\) hybrids

### Table 2 Homoeologous recombination events in IpaCor\(^{4x}\) \(_{S_0}\) and S\(_1\) plants other than those listed in Table 1

| Genotype          | Direction of subgenome recombination | Chromosome* | Left border (bp) | Right border (bp) | Segment size (bp) | No. of markers | bp/marker |
|-------------------|--------------------------------------|-------------|-----------------|------------------|-------------------|----------------|-----------|
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2}\) \(_{S_1}\) | Cor replaced 50% of Ipa              | Araip.B03   | 2,065,843       | 7,115,308        | 5,049,465         | 19             | 265,761   |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2}\) \(_{S_1}\) | Cor replaced 50% of Ipa              | Araip.B04   | 4,420,103       | 124,301,830      | 119,881,727       | 96             | 1,248,768 |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2}\) \(_{S_1}\) | Ipa replaced 50% of Cor              | Araip.B04   | 613,773         | 124,301,830      | 123,688,057       | 109            | 1,134,753 |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2}\) \(_{S_1}\) | Ipa replaced 50% of Cor              | Araip.B05   | 1,426,280       | 15,568,257       | 14,141,977        | 23             | 614,869   |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2,1}\) | Ipa replaced 50% of Cor              | Araip.B04   | 123,807,791     | 124,301,830      | 124,494,039       | 7              | 70,577    |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2,1}\) | Ipa replaced 50% of Ipa              | Araip.B04   | 613,773         | 124,298,527      | 123,684,754       | 108            | 1,145,229 |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2,1}\) | Ipa replaced 50% of Ipa              | Araip.B05   | 1,426,280       | 148,997,085      | 147,570,805       | 91             | 1,621,657 |
| IpaCor\(^{4x}\) \(_{S_0}\) \(_{S_2,1}\) | Ipa replaced 50% of Cor              | Araip.B07   | 686,538         | 2,077,018        | 1,390,480         | 13             | 106,960   |

* Chromosome and SNP positions were given relative to A. ipaensis genome (peanutbase.org). The recombination events occurred at these loci were actually between the homoeologous chromosomes.
Cor and Ipa had genotype calls of BB (G^{4c}G^{4c}) and AA (G^{4c}C^{4c}) respectively, at marker AX-147221124 (one of the markers at the end of B04). The A. hypogaea female parent was polymorphic between its subgenomes (G^{4c}C^{4c}+G^{4c}C^{4c}+G^{4c}C^{4c}) Ipacor_{4xS0.2} and six F1 hybrids from A. hypogaea × Ipacor_{4xS0.2} had one allele from Cor replacing that of Ipa (G^{4c}C^{4c}+G^{4c}G^{4c}) forming a cluster between genotypes AB and BB. Four other F1 hybrids fell in the AB cluster as expected. However, Ipacor_{4xS0.2F1.4} and a few other samples formed a cluster between AA and AB (G^{4c}C^{4c}+G^{4c}C^{4c}) indicating that a new round of homoeologous recombination during hybridization led to the opposite direction of exchange in this individual, i.e., Ipa replaced Cor.

Segregation of the F2 population descended from 13-1014 × Ipacor_{4xS0.2F1.4} confirmed the genome composition of this hybrid. The F2 population consisted of 456 individuals (Figure 2B). The majority of the population segregated into three clusters following a ratio of 109:219:121 among three genotypes GGGG: CCCG: CCCGGG as expected from the genotype composition of G^{4c}C^{4c}C^{4c}C^{4c} of the F1 hybrid (Figure 2C). This ratio was close to 1:2:1 (Chi-square = 0.91, P = 0.63) indicating that disomic segregation was predominant. In addition, there were three F2 individuals that clustered with Ipacor_{4xS0.2} (CGGG) indicating a new homoeologous recombination event in these three individuals resulting in 75% allele composition from Cor and 25% from Ipa. A stretch of 19 adjacent markers including 15 markers within the region inherited from Ipacor_{4xS0.2} and four neighboring markers came from a new homoeologous recombination event in the F1 hybrid (Table 4).

The last four markers at the end of chromosome A04/B04 (Table 4) shared a different segregation pattern due to the homozygous genotype call of the female parent (Figure 3). At marker AX-147221375, Cor and Ipa had genotype calls of BB (G^{4c}G^{4c}) and AA (G^{4c}C^{4c}), respectively. The female parent was monomorphic between its two subgenomes (Table 3) and positioned in the genotype CB cluster along with Cor. The male parent Ipacor_{4xS0.2} had Cor alleles replacing Ipa (G^{4c}G^{4c}G^{4c}C^{4c}) due to homoeologous recombination. Two types of F1 hybrids were expected to be produced with genotype compositions of G^{4c}G^{4c}C^{4c}G^{4c} and G^{4c}G^{4c}C^{4c}C^{4c} (Figure 3C). Indeed, six of the F1 hybrids fell in the 75% BB (G^{4c}G^{4c}G^{4c}C^{4c}) genotype group; four of the F1 hybrids fell in the BB (G^{4c}G^{4c}G^{4c}G^{4c}) genotype group 13-1014 × Ipacor_{4xS0.2F1.4} shifted right from the other F1 hybrids and grouped in the AB genotype due to a new round of homoeologous recombination. As expected, the F2 population from this hybrid segregated into five clusters, i.e., GGGG, GGGG, GGGC, CCCG, CCCGGG (Figure 3C) at this locus in a ratio of 30:107:181:102::38 or close to the expectation for a disomic segregation ratio of 1:4:6:1 (Chi-square = 6.78; P = 0.14) (Table 3). The remaining three markers at the bottom of B04 shared similar segregation ratios. Therefore, disomic segregation of the F2 population was observed for all of the markers at the bottom of A04/B04 where the subgenome exchange from the male parent was inherited.

### Table 3 New homoeologous recombination detected among F1 progenies of A. hypogaea × Ipacor_{4xS0} crosses

| Genotype | Direction of subgenome recombination | Chromosome* | Left border (bp) | Right border (bp) | Segment size (bp) | No. of markers | bp/marker |
|----------|--------------------------------------|-------------|-----------------|------------------|-----------------|----------------|-----------|
| 13-2113 × Ipacor_{4xS0.6F1.1} | Cor replace 50% of Ipa | Araip.B02 | 102,655,016 | 104,599,332 | 1,944,316 | 9 | 216,03 |
| 13-2113 × Ipacor_{4xS0.5F1.1} | Cor replace 50% of Ipa | Araip.B02 | 61,718,684 | 106,235,603 | 44,516,919 | 25 | 1,780,677 |
| 13-2113 × Ipacor_{4xS0.5F1.7} | Cor replace 50% of Ipa | Araip.B02 | 295,929 | 95,321,834 | 95,025,905 | 73 | 1,301,725 |
| 13-2113 × Ipacor_{4xS0.5F1.7} | Cor replace 50% of Ipa | Araip.B02 | 295,929 | 106,235,603 | 105,977,776 | 103 | 1,028,910 |
| 13-2113 × Ipacor_{4xS0.6F1.1} | Cor replace 50% of Ipa | Araip.B02 | 2,029,212 | 6,739,025 | 4,709,827 | 25 | 188,39 |
| 13-2113 × Ipacor_{4xS0.6F1.1} | Cor replace 50% of Ipa | Araip.B02 | 123,807,791 | 124,298,527 | 490,736 | 4 | 122,684 |
| 13-2113 × Ipacor_{4xS0.6F1.6} | Ipa replace 50% of Cor | Araip.B04 | 123,807,791 | 124,298,527 | 490,736 | 4 | 122,684 |
| 13-2113 × Ipacor_{4xS0.5F1.3} | Ipa replace 50% of Cor | Araip.B04 | 129,385,417 | 130,961,045 | 1,575,628 | 8 | 196,954 |
| 13-2113 × Ipacor_{4xS0.5F1.7} | Cor replace 50% of Ipa | Araip.B04 | 3,490,627 | 6,128,842 | 2,638,215 | 15 | 175,881 |
| 13-2113 × Ipacor_{4xS0.5F1.6} | Cor replace 50% of Ipa | Araip.B04 | 204,623 | 6,097,596 | 5,892,973 | 44 | 133,931 |
| 13-2113 × Ipacor_{4xS0.5F1.6} | Ipa replace 50% of Cor | Araip.B04 | 140,971 | 23,049,636 | 22,908,665 | 70 | 327,267 |
| 13-2113 × Ipacor_{4xS0.4F1.2} | Ipa replace 50% of Cor | Araip.B04 | 647,417 | 46,100,243 | 45,452,826 | 63 | 721,47 |
| 13-2113 × Ipacor_{4xS0.4F1.4} | Ipa replace 50% of Cor | Araip.B04 | 605,143 | 130,961,045 | 130,352,902 | 132 | 987,545 |

* Chromosome and SNP positions were given relative to A. pannosa genome (peanutbase.org). The recombination events occurred at these loci were actually between the homoeologous chromosomes.
Figure 2 Segregation of the $F_2$ population derived from $13\text{-}1014 \times \text{IpaCor}^{4x}_{S0.2} \text{F}_1$ when the female parent was genotyped as AB at this locus. (A) Genotyping profile from the SNP array of the parental lines and $F_1$ hybrids. Homoeologs at this locus were detected in the $A. \text{hypogaea}$ parent ($G_{ah}G_{ah}C_{bh}C_{bh}$; golden circles), and the $\text{IpaCor}^{4x}_{S0.2}$ parent ($C_{bi}G_{ac}G_{ac}G_{ac}$; two circled magenta circles) had one allele from $\text{Ipa}$ replaced by $\text{Cor}$. It was expected to produce two types of $F_1$ hybrids (panel C, black font), i.e., $G_{ah}G_{ac}C_{bh}G_{ac}$ (genotype call 75% BB) and $G_{ah}C_{bi}C_{bh}G_{ac}$ (genotype call AB). Indeed, six of the $F_1$ hybrids (circled white circles in the green oval) were in the 75% BB group and four $F_1$ hybrids (circled white triangles in the yellow oval) were in the AB group. The 11th $F_1$ hybrid, $13\text{-}1014 \times \text{IpaCor}^{4x}_{S0.2} \text{F}_1$ (green font), experienced a new round of homoeologous recombination with the $\text{Ipa}$ allele replacing $\text{Cor}$ resulting in $G_{ah}C_{bi}C_{bi}C_{bh}$ (genotype call 75% AA). (B) The $F_2$ population derived from this hybrid segregated disomically in three clusters following a ratio close to the expected 1:2:1. Although most $F_2$ individuals fall in the expected clusters, there were three $F_2$s that clustered in the 75% BB group indicating that new homoeologous recombination occurred in these three individuals during selfing. The $^{ah}$ superscript indicates $A$ subgenome from $A. \text{correntina}$ (red filled samples), $^{bi}$ superscript denotes $B$ subgenome from $A. \text{ipaensis}$ (blue filled samples). The ancestral origin of the $A. \text{hypogaea}$ parents is unclear at this locus, therefore, $\text{ah}$ is used to denote their genome composition.
five percent of a genotype call indicates the dosage of a subgenome allele was increased by 25% as a result of subgenome recombination.

In an effort to increase the genetic diversity of cultivated peanut, crosses were made between two peanut diploid relatives Ipa and Cor. The resulting IpaCor F2 hybrids were highly sterile, consistent with the well-documented sterility of diploid hybrids from A and B genome species (Krapovickas et al. 2007). Formation of seeds from the colchicine-treated hybrids indicated the success of chromosome doubling. In newly formed allotetraploids, the two distinct genomes are expected to function in one cytoplasm and to be inherited through regular meiotic divisions (Lukens et al. 2006). However, it is known that while the majority of the genome of many allotetraploids does pair bivalently within each subgenome during meiosis, meiotic recombination between the subgenomes can occur when multivalent associations form. In addition, homoeologous chromosomes may be used as templates to repair double-stranded breaks. Both of these mechanisms, homoeologous meiotic recombination and homoeologous repair, can result in the duplication or elimination of corresponding homoeologous chromosomes (Figure 5). The frequency of Cor replacing Ipa was higher than the frequency of Ipa replacing Cor on chromosome A03/B03 and lower on chromosomes A04/B04, A05/B05, and A06/B06. The incidence of homoeologous recombination events was higher at distal regions of chromosomes and lower near the centromeres. Among the 907 F1 lines, 416 (46%) hosted at least one event of homoeologous recombination. Tetrasomic recombination was identified in four F2 individuals on chromosome A03/B03 (Supplementary Table S5 dark blue and dark pink highlighted events).

**Discussion**

### Table 4 Segregation of the F2 population from 13-1014 × IpaCor F2.4_S0.2_F1.4 at the end of chromosome B04

| Event history | probeset_id | Start position (bp)a | Ipa | Cor | IpaCor F2.4_S0.2 | IpaCor F2.4_S0.2_F1.4 | Genotype call 0 | Genotype call 75% of 0 | Genotype call 1 | Genotype call 75% of 2 | Genotype call 2 | No Call |
|---------------|-------------|----------------------|-----|-----|------------------|----------------------|----------------|------------------------|----------------|------------------------|------------|--------|
| New event in F1 hybrid | AX-147248422 | 123,807,791 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 103 | 228 | 120 | 2 | 0 | 3 |
| Inherited from IpaCor F2.4_S0.2 | AX-147220907 | 124,824,690 | 2 | 0 | 75% of 0 | 1 | 75% of 2 | 0 | 2 | 127 | 226 | 103 | 0 |
| AX-147244848 | 124,804,923 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 105 | 224 | 121 | 2 | 0 | 4 |
| AX-147248475 | 125,438,618 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 102 | 226 | 120 | 2 | 0 | 6 |
| AX-147248476 | 125,440,304 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 104 | 225 | 125 | 2 | 0 | 0 |
| AX-147248477 | 125,440,304 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 104 | 225 | 125 | 2 | 0 | 0 |
| AX-147220849 | 124,269,424 | 2 | 0 | 75% of 0 | 1 | 75% of 2 | 0 | 3 | 123 | 222 | 107 | 4 |
| AX-147248617 | 124,469,894 | 2 | 0 | 75% of 0 | 1 | 75% of 2 | 0 | 2 | 116 | 220 | 108 | 10 |
| AX-147248627 | 124,518,680 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 105 | 219 | 121 | 2 | 0 | 9 |
| AX-147221124 | 129,198,098 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 109 | 219 | 119 | 3 | 0 | 7 |
| AX-147221160 | 129,385,417 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 110 | 219 | 119 | 2 | 0 | 6 |
| AX-147221161 | 129,385,417 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 110 | 218 | 117 | 2 | 0 | 9 |
| AX-147248696 | 124,600,869 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 110 | 218 | 117 | 2 | 0 | 9 |
| AX-147221357 | 129,385,417 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 110 | 218 | 117 | 2 | 0 | 9 |
| AX-147221364 | 131,423,712 | 0 | 2 | 75% of 2 | 1 | 75% of 0 | 110 | 218 | 117 | 2 | 0 | 9 |

**Notes:**

- Genotype calls from the SNP array were re-coded as follows: genotype call AA = 0; BB = 2; AB = 1 to avoid confusion with the description of subgenomes. Seventy-five percent of a genotype call indicates the dosage of a subgenome allele was increased by 25% as a result of subgenome recombination.
- Chromosome and SNP positions were given relative to A. ipaensis genome (peanutbase.org). The recombination events occurred at these loci were actually between the homoeologous chromosomes.

Ipa (C^{(2)}b^{(2)}) had a genotype call of AA. The male parent IpaCor F2.4_S0.2 (G^{(2)c^{2}}C^{(2)c^{2}}b^{(2)b^{(2)}}) had an AB genotype call indicating there was no pre-existing homoeologous recombination in the male parent at this locus. The female parent 13-1014 (G^{(1)c^{1}}C^{(1)c^{1}}b^{(1)b^{(1)}}), 13-1014IpaCor F2.4_S0.2_F1.4 (G^{(1)c^{1}}C^{(1)c^{1}}b^{(1)b^{(1)}}), and most of the F2 individuals were grouped in the AB cluster as well. However, there were 18 F2 individuals that shifted right from the AB cluster and formed a separate cluster (GCCC) between the AB and AA genotype calls. This suggested that an Ipa allele replaced a Cor allele in these individuals as a result of homoeologous recombination. There were another 29 F2 individuals that shifted left from the AB cluster and formed a separate cluster (GGGC) between AB and BB genotype calls. These F2 individuals were evidence of homoeologous recombination with a Cor allele replacing an Ipa allele at this locus. Two F2 individuals (GGGG) had become quadriplex at this locus with all alleles from Cor. Therefore, there were 49 F2 progeny that deviated from the expected monomorphic AB calls and demonstrated homoeologous recombination in both directions at this locus. New homoeologous recombination events captured in the two populations were listed (Supplementary Table S5) and presented as a zoomed-out image for global view (Supplementary Figure S4). The two populations shared a similar distribution of recombination events across the chromosomes (Supplementary Figure S4). A03/B03, A04/B04, A05/B05, and A06/B06 were densely populated with homoeologous recombination events and accounted for 95% of the total number of events. The ratio of markers showing Ipa replacing the Cor subgenome versus those with Cor replacing the Ipa subgenome was 0.99% across both populations. However, there was a preference for one direction of homoeologous recombination over the other on individual chromosomes (Figure 5). The frequency of Cor replacing Ipa was higher than the frequency of Ipa replacing Cor on chromosome A03/B03 and lower on chromosomes A04/B04, A05/B05, and A06/B06. The incidence of homoeologous recombination events was higher at distal regions of chromosomes and lower near the centromeres. Among the 907 F1 lines, 416 (46%) hosted at least one event of homoeologous recombination. Tetrasomic recombination was identified in four F2 individuals on chromosome A03/B03 (Supplementary Table S5 dark blue and dark pink highlighted events).
Figure 3 Segregation of the F2 population derived from 13-1014 × IpaCor<sup>−</sup>-S<sub>6</sub>,<sub>2</sub>−F<sub>1</sub> when the female parent was genotyped as BB at this locus. (A) Genotyping profile from the SNP array for the parents and F1 hybrids. The *A. hypogaea* parents (GahGahGbhGbh; golden triangles) were monomorphic at this locus and IpaCor<sup>4</sup>-S<sub>0</sub>,<sub>2</sub> parent (GacGacGacCbi; two circled magenta circles) had one allele from *Ipa* replaced by *Cor*. It was expected to produce two types of F1 hybrids (panel C black font), i.e., GahGacGahGac (genotype call BB) and GahGacGbhCbi (genotype call 75% BB). Indeed, six of the F1 hybrids (circled white circles) were in the 75% BB group and four (circled white triangles) were in the BB group. The 11<sup>th</sup> F1 hybrid, 13-1014 × IpaCor<sup>−</sup>-S<sub>6</sub>,<sub>2</sub>−F<sub>1</sub> (green font), experienced a new round of homoeologous recombination in which the *Ipa* allele replaced *Cor* resulting in the genome composition of GahCbiGbCbi (genotype call AB). (B) The F2 population from this hybrid was expected to segregate into five clusters. (C) The distribution of the F2 population followed a ratio close to the disomic segregation, i.e., 1:4:6:4:1.
regions and alter gene dosage (Szadkowski et al. 2010; Youds and Boulton 2011; Leal-Bertioli et al. 2015). Our genotyping data from IpaCorS0 plants revealed five independent homoeologous recombination events in each of the five S0 plants. All had one block of alleles on a chromosome segment replaced by its homoeologous alleles. This observation suggests that during meiosis of the tetraploid cells, multivalent association of homoeologous chromosomes and homoeologous recombination occurred as early as the formation of S0 neo-tetraploids. Our finding is consistent with previous cytological studies with Arachis interspecific F1 hybrids (Stalker 1991) and F1 hybrids derived from A. hypogaea × allotetraploids (Gardner and Stalker 1983). Both studies presented evidence of multivalent formation in meiotic cells of the hybrids although at low frequencies.

The homoeologous event in the neoallotetraploid was heritable, as evidenced by the segregation of the recombined region among the S1 progenies. In our study, three S1 from IpaCorS0 became quadruple and the other four shared the genotype of the mother plant. This suggests normal segregation of the established homoeologous recombination event during generation advancement. In addition to the inherited recombinant subgenome, four new homoeologous recombination events were found in three of the S1 progenies indicating that recombination between subgenomes continued throughout generation advancement. Therefore, it is apparent that homoeologous recombination occurred at the nascent and early generations of the neo-tetraploids similar to reports on synthetic polyploids of Brassica (Song et al. 1995; Lukens et al. 2006; Szadkowski et al. 2010).

Crossing the neo-tetraploids with cultivated breeding lines initiates introgression of chromosomal segments from wild genomes. The inheritance of the established subgenome exchange from IpaCorS0 S0 parents was observed in the F1 hybrids from crosses between A. hypogaea and IpaCorS0. In the meantime, new events of homoeologous exchange were identified in the F1 hybrids suggesting active homoeologous recombinants were captured in the first zygotes of crossing. The established homoeologous recombination event on chromosome 04 captured in the IpaCorS0 male parent was found to segregate diosomically in most of the F2 population. The impact of homoeologous recombination events on allele dosage is potentially large given their accumulation within the F2 population with nearly half of the population possessing at least one event. Interestingly, the new events were preferentially distributed on chromosomes A03/B03, A04/B04, A05/B05, and A06/B06. These chromosomes hosted over 95% of the homoeologous recombination events. The A. hypogaea genome contains historical quadruplex loci on these chromosomes derived from the homoeologous recombination between the ancestral A. duranensis and Ipa (A. ipaensis) subgenomes (Leal-Bertioli et al. 2015; Bertioli et al. 2016, 2019). It is possible that the existing tetrasomic regions in cultivated peanut increase the frequency of homoeologous recombination among progeny derived from crosses between A. hypogaea and interspecific materials. Most recently, hot spots of homoeologous recombination were found pre-dominantly located within genic regions in wheat and other polyploids including peanut (Zhang et al. 2020). Consequently, novel gene transcripts and proteins produced would contribute to neo- and sub-functionalization of genes and phenotypic changes. For instance, spontaneous flower color change from yellow to orange was reported in the new synthetic allotetraploid A. ipaensis × A. duranensis4x, which was ascribed to homoeologous recombination (Bertioli et al. 2019). Yield increase due to intersubgeomic heterosis was reported in B. napus upon introgression from Brassica rapa (Qian et al. 2005).

The frequent homoeologous recombination in these genetic materials derived from the neo-tetraploid poses a challenge in genetic mapping. Most often, genetic markers linked to the homoeologous recombination were excluded from genetic map construction since they appear to be present/absent or cause severe segregation distortion from the disomic inheritance model. However, QTL analysis with genetic markers associated with presence and absence (PAV) variations was able to identify major effect QTL for seed quality and flowering pattern in B. napus (Stein et al. 2017). Disease resistance QTL against Sclerotinia stem rot and blackleg disease for oilseed rape were identified in the PAV regions (Gabur et al. 2018). Homoeologous recombination
was found to be the underlying cause of these trait variations. More recently, mixed inheritance following both disomic and tetrasomic patterns of inheritance among the population from a cross between *A. hypogaea* and (*A. duranensis*/*C2* *A. batizocoi*)4x supported the occurrence of homoeologous recombination in these progenies (Nguepjop et al. 2016). Therefore, genetic analysis of peanut populations with wild introgression needs to consider the markers associated with homoeologous recombination.

The high frequency of genome instability of the neo-tetraploid and its derivative lines may offer both possibilities and perils from the breeding perspective. On the one hand, the instability offers an unprecedented opportunity to introduce new phenotypes and variations. Conversely, the desired traits may be unstable and can be lost as generations advance. Backcross and generation advancement may stabilize the genome and trait expression. To this end, we are advancing a BC1F1 population by single seed descent with *A. hypogaea* as the recurrent parent. The population, being on average 75% of the domesticated genome should predominantly express cultivated phenotypes. The other 25% of wild genome composition may be stabilized through generations of selfing. This type of genetic material will provide the peanut breeding community with valuable genetic diversity to improve disease resistance and other agronomic traits in cultivated peanuts.

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Data availability

Five supplemental Tables were included in file Supplemental_Table.xlsx. Four supplemental figures were included in the Supplemental_Figures.pdf file. Supplemental Material available at figshare: https://doi.org/10.25387/g3.14043620.

Author contributions

P.O.-A., H.T.S., and Y.C. conceived the experiments; H.T.S., Y.C., and C.C.H. and C.M.L. executed the experiments; Y.C. and D.B. drafted the manuscript; P.O.-A. secured funding for this research; all authors approved the manuscript.

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