Peanut (Arachis hypogaea L.) is an allotetraploid (2n = 4x = 40) crop species that arose from hybridization between two diploid (2n = 2x = 20) progenitors followed by chromosome doubling (Kochert et al., 1996; Stalker, 1997; Seijo et al., 2004, 2007; Moretzsohn et al., 2013; Bertioli et al., 2016). Genome duplication produced a new polyploid species that was isolated reproductively from its diploid progenitors and relatives, and thus limited genetic introgression from the diploid relatives has occurred, resulting in the relatively narrow genetic base of cultivated peanut (Halward et al., 1991, 1992). Therefore, additional sources of favorable alleles are needed to improve peanut for economically important traits.

ABSTRACT
Cultivated peanut (Arachis hypogaea L.) is an economically important crop grown around the world. Compared with the entire Arachis genus, cultivated peanut germplasm has low levels of genetic diversity for several economically important traits, resulting in the need for alternative sources of favorable alleles. Wild diploid species of Arachis are a source of such alleles to improve cultivated peanut for many economically important traits. An A. hypogaea × A. diogoi Hoehne introgression population was produced via the triploid–hexaploid method; the fourth generation after tetraploidy was used to initiate this study. The introgression lines were genotyped using a single nucleotide polymorphism (SNP) marker array to estimate the percentage of A. diogoi chromatin introgression. Morphologically, the introgression lines varied for an array of measured traits, with the majority being intermediate to the two parents. The average amount of A. diogoi genome introgressed was 8.12% across the tetraploid genome and ranged from 3.00 to 18.14% on individual chromosomes. The average A. diogoi introgression across all lines was 7.70% and ranged from 0.17 to 51.12%. Principal component analysis of morphological data and SNP markers revealed similarities and groupings of introgression lines. This introgression population demonstrates the potential of using wild diploid Arachis species for peanut improvement and has great potential for use in cultivated peanut breeding programs.

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Abbreviations: LD, linkage disequilibrium; NCDA&CS, North Carolina Department of Agriculture and Consumer Services; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

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genetic variation influencing economically important traits are needed for crop improvement.

The genus *Arachis* is composed of 77 naturally occurring diploid, three aneuploid, and three tetraploid species, many of which could serve as sources of valuable alleles for the improvement of economically important traits in cultivated peanut (Stalker and Moss, 1987; Valls and Simpson, 2005; Krapovickas and Gregory, 2007; Valls et al., 2013; Santana and Valls, 2015; Valls and Simpson, 2017; Stalker, 2017). Technical challenges exist in using wild *Arachis* germplasm for improving cultivated peanut, mainly due to sterility caused by ploidy and genomic differences, hybrid necrosis, and cross-incompatibilities (Stalker and Moss, 1987).

Several methods have been used to introgress favorable wild diploid species genes into cultivated peanut (Stalker et al., 1979; Stalker and Wynne, 1979; Stalker and Moss, 1987; Simpson, 1991, 2001). All strategies have involved the use of chromosome doubling agents to produce progenies with much greater levels of fertility.

One method, referred to as the “triploid–hexaploid introgression method,” involves hybridizing *A. hypogaea* with a diploid wild species and chromosome doubling the sterile triploid (2n = 3x = 30) with colchicine to restore fertility at the hexaploid (2n = 6x = 60) level, followed by self-pollination over many generations during which spontaneous chromosome loss occurs, resulting in 40–chromosome interspecific hybrid derivatives. The goal of self-pollination at a high ploidy level is to increase frequency of recombination between the wild and cultivated species. Little is known about how spontaneous chromosome loss occurs, but typically it is random and infrequent; therefore, the method requires many generations of self-pollination before tetraploids appear (Stalker et al., 1979; Stalker, 2017).

Successful use of the triploid–hexaploid introgression method was demonstrated in crosses between the diploid species *A. cardenasii* Krapov. & W.C. Gregory and *A. hypogaea* (Smartt and Gregory, 1967; Stalker et al., 1979). Many *A. cardenasii*–derived materials possess high levels of disease and insect resistance (Stalker, 2017), and numerous germplasm lines and cultivars derived from *A. cardenasii × A. hypogaea* have been released (Stalker and Beute, 1993; Stalker et al., 2002a, 2002b; Stalker and Lynch, 2002; Isleib et al., 2006; Tallury et al., 2013; Stalker, 2017). Molecular marker analysis of *A. hypogaea × A. cardenasii*–derived interspecific hybrids have shown that introgression occurs into both *A. hypogaea* subgenomes (Garcia et al., 1995). In addition, it has been proposed that the mechanism of wild species chromatin introgression from *A. cardenasii* into the *A. hypogaea* genome is chromosome recombination rather than chromosome substitution (Garcia et al., 1995). These introgressed chromosome segments also appear to be in large blocks (Garcia et al., 1995; Nagy et al., 2010).

Much of the multiple disease resistance present in some breeding programs and peanut cultivars can be attributed to the abovementioned *A. cardenasii*–derived introgression lines (Stalker, 2017). However, wide use of the same disease resistance mechanisms may place great selection pressure on pathogens, resulting in a decrease in the effectiveness of the resistance or rendering it ineffective over time (McDonald and Linde, 2002). Thus, additional sources of multiple disease resistance in peanut are needed. One wild diploid species, *A. diogoi* Hoehne (syn. *A. chacoense* nom. nud.), specifically accession GKP 10602 (PI 276235), is highly resistant to many diseases affecting peanut production (Abdou et al., 1974; Company et al., 1982; Subrahmanyan et al., 1985; Lyerly et al., 2002; Stalker, 2017). The objectives of this research were to characterize an *A. hypogaea × A. diogoi* introgression population developed by the triploid–hexaploid method. Molecular markers were used to determine whether tetraploid introgression lines have *A. diogoi* chromosome substitutions or whether recombination occurred between the diploid and tetraploid parents. The percentage of *A. diogoi* chromatin that was introgressed into each line was determined, as well as the amount of introgression present across the *A. hypogaea* genome. Morphological characteristics of the introgression lines also were evaluated.

**MATERIALS AND METHODS**

**Introgression Population Development**

During the summer of 2000, the large-seeded Virginia-type peanut cultivar ‘Gregory’ (Isleib et al., 1999) was hybridized with *A. diogoi* accession GKP 10602 (PI 276235) to produce sterile triploid interspecific F1 hybrids (2n = 3x = 30). In 2001, cuttings from one sterile triploid were treated with a 0.2% colchicine solution for 8 h at room temperature to double the chromosome number and restore fertility at the hexaploid (2n = 6x = 60) chromosome level. Cuttings were treated with Rootone rooting hormone (GardenTech) and placed in sand in a mist chamber under shade for ~6 wk to develop roots. The plants were grown to maturity in the greenhouse and one seed was harvested, which was a hexaploid individual. The seed was planted in a 38-cm by 52-cm flat containing soil, and the plant was cytologically confirmed as being hexaploid (2n = 6x = 60). It was grown to maturity and seed were harvested by hand. The resulting progeny from this hexaploid individual were then spaced planted and self-pollinated with no artificial selection for 11 generations (2002–2012) at the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Sandhills Research Station near Jackson Springs in Moore County, North Carolina. Plots were grown according to common agricultural practices for peanut production in North Carolina (North Carolina Cooperative Extension, 2017) with the exception of planting and harvesting methodologies. For planting, seeds were sown in 5.5-cm by 5.5-cm peat pots (Jiffy-pots, Jiffy International) containing equal parts steamed sand, steamed topsoil, and Fafard 2P soil mix in the greenhouse and transplanted to the field in mid-May. Plants were hand dug and pods were manually removed from each plant in mid-October.

During 2011, one of the field-grown hexaploid plants produced three progenies that had an *A. hypogaea* plant growth...
habit when grown in 2012. Flow cytometric analysis confirmed that these progenies were tetraploid (Tallury et al., 2014). In 2013, 12 individual tetraploid plants were selected on the basis of seed size (both large and small seeded lines), and in 2014, 87 introgression lines were derived from these selections. The 87 introgression lines were advanced using small plots of bulked seed for three generations. During the fall of 2016, one seed from each of the 87 introgression lines was planted in a separate pot in the greenhouse, grown to maturity, and seed harvested by hand. During the summer of 2017, seed of each single plant progeny from each of the 87 introgression lines were planted in a seed increase nursery at the NCDA&CS Peanut Belt Research Station near Lewiston–Woodville in Bertie County, North Carolina. For this nursery, the seeds of each introgression line were planted in two-row plots 3.7 m in length with 25–cm within-row seed spacing. Plots were grown according to common agricultural practices for peanut production in North Carolina (North Carolina Cooperative Extenson, 2017), and seeds from each introgression line were harvested in bulk.

**Phenotyping of Morphological Characteristics**

Introgression lines were evaluated for 13 morphological characteristics on plants in the seed increase nursery and on pods and seeds harvested from both the 2016 and 2017 seed increase nurseries at the Peanut Belt Research Station. The following morphological traits were evaluated: pod length (cm), pod width (cm), seed length (mm), seed width (mm), number of seeds per pod, seed weight (g), meat content (%), growth habit (spreading = 1, semibunch = 2, bunch = 3, segregating bunch = 4, prostrate = 5), presence of flowers on the main stem, flowering pattern on lateral stems (alternate pattern of reproductive nodes = 1, sequential pattern of reproductive nodes = 2), prominence of the main stem (not prominent = 1, semiprominent = 2, prominent = 3), plant height (cm), and canopy width (cm). Using these data, three other traits were calculated: pod length/width ratio, seed length/width ratio, and percentage row closure. Row closure was estimated as the ratio of canopy width to row spacing (91.4 cm). Pod and seed measurements were taken on 20 random pods from each line. Seed weight was evaluated on seed lots from one to two replications in 2016 and one replication in 2017. Meat content was estimated by the ratio of seed weight to pod weight of 50 randomly selected pods. Growth habit and visibility of the main stem were evaluated on whole plots. Four mature plants were sampled from each plot for branching pattern, plant height, and plant width. The morphological characteristics, percentage row closure, and presence of flowers on the main stem were not included in subsequent analysis resulting in 14 total traits. Principal component analysis (PROC PRINCOMP) of SAS version 9.4 (SAS Institute, 2013b) was performed on the averages for 14 morphological traits. The introgression lines were plotted on two dimensions using the first two principal components.

**Molecular Marker Genotyping and Introgression Analysis**

One folded leaf consisting of all four leaflets was collected from a single plant from each introgression line and the two parents during the winter of 2016–2017. DNA was isolated using a Qiagen DNAeasy Plant Mini Kit. DNA was quantified using PicoGreen (Thermo Fisher Scientific) and diluted to 30 ng μL^{-1} according to Affymetrix guidelines. Samples were genotyped using the Affymetrix Axiom Arachis2 48K single nucleotide polymorphism (SNP) array (Clevenger et al., 2017). Quality control and SNP genotype determination were performed using Axiom Analysis Suite version 3.0.1.4 (Thermo Fisher Scientific, 2016) using the default polyploidy threshold parameters. In addition to the 87 introgression lines and the two parents, nine other diploid Arachis species were included in the analysis to assist in genotype clustering by Axiom Analysis Suite software and for additional downstream introgression analysis. The nine diploid Arachis species included A. batizooi Krapov. & W.C. Gregory accession K 9484; A. cardenasii accession GKP 10017; A. correntina (Burkart) Krapov. & W.C. Gregory accession GKP 9548; A. duranensis accession SeSn 2848; A. goyepi C.E. Simpson, Krapov., & Valls accession V 6389; A. ipaenasis accession KGBSPSc 30076; A. magna Krapov., W.C. Gregory, & C.E. Simpson accession KG 30097; A. stenosperma Krapov. & W.C. Gregory accession V 10309; and A. villosa Benth. accession V 12812. All Axiom Analysis Suite array calls were visually checked for each potential SNP marker according to Affymetrix guidelines and manually corrected when the default clustering was not accurate.

The SNP genotypes were converted to a proportional scoring scale, with “0” equal to the SNP marker identifying a homozygous genotype for Gregory, “1” equal to the SNP marker identifying a heterozygous genotype, and “2” equal to SNP marker identifying a homozygous genotype for A. diogoi to estimate the total amount of A. diogoi introgression. This was estimated using R (R Core Team, 2017) and R Studio version 1.0.143 (R Studio Team, 2017). The SNPs were assigned to subgenome A or B based on the assignments on the SNP chip used for this analysis as assigned by J. Clevenger (personal communication, 2017) and P. Ozias-Akins. Graphical representation of introgression across all chromosomes were prepared using the software GGT2 (van Berloo, 2008). The marker-derived genomic relationship matrix was used for principal component analysis in JMP Genomics 8 (SAS Institute, 2013a) to estimate the genetic relationships among introgression lines.

A preliminary marker–trait association analysis for the 14 morphological traits was conducted to gain insight on the phenotypic contributions of the various introgressed A. diogoi alleles in the Gregory genetic background. The introgression line IL 01 was dropped from the analysis as an outlier with respect to the amount of introgression present in that line because it had a very large number of SNPs not present in the other lines and its inclusion would have resulted in a skewed representation of the introgression in the population. Markers detecting no introgression within the population after removal of introgression line IL 01 were dropped from the analysis. Thus, a total of 4300 SNP markers were analyzed to estimate the amount of A. diogoi introgression. Markers were eliminated based on linkage disequilibrium (LD, r^2 > 0.8) using the LD tagSNP package in JMP Genomics 8 to reduce the total number of markers used in marker–trait association analysis. The LD tagSNP package eliminates redundant information in the marker data set and keeps SNP markers that provide the maximum information (Carlson et al., 2004). The marker–trait association analysis was...
conducted in JMP Genomics 8 using the single marker–trait association package. For the marker–trait association analysis, the critical significance level for declaring a significant marker–trait association was set at 0.0001 (logarithm of odds = 3) for all morphological traits.

RESULTS

Morphological Characteristics

Morphologically, the introgression lines varied for all traits including canopy characteristics, plant height, growth habit, and pod and seed traits (Supplemental Table S1). The majority of introgression lines were morphologically intermediate between the two parents. A smaller number of lines were found to be identical to either parent for certain traits. A range of variation among the introgression lines was observed for the measured pod and seed characters (Fig. 1, Supplemental Fig. S1). The overall average for individual seed weight of the 87 introgression lines (0.65 g) was greater than the mid-parent average (0.52 g), but less than the average of Gregory (0.999 g) (Fig. 2). Seed weights for the introgression lines ranged from 0.33 to 1.02 g. A few lines were identified as having seed size equal to or greater than Gregory. For meat content, Gregory (68%) was slightly lower than \( A. \text{diogoi} \) (73%), with the meat content of the introgression lines ranging from 48 to 77% with an average of 67% (Supplemental Table S1). On average, the majority of lines had two seeded pods typical of Virginia-type peanut cultivars, and a small number of lines had one-seeded pods similar to the wild species.

Main stem height and plant width varied across the introgression lines, with the majority of lines being intermediate to the two parents for plant height and slightly closer to \( A. \text{diogoi} \) for plant width. Row closure was estimated from plant width to provide an estimation of canopy closure, an important agronomic trait. At the time of measurement (31 Aug. 2017, 105 d after planting), row closure ranged from 48% for the bunch type lines to 100% for the spreading type lines. Two introgression lines had a bunch growth habit, even though this trait was not observed in either parent. The majority of introgression lines had the alternate branching pattern typical of \( A. \text{hypogaea subsp. hypogaea} \). Although the visual prominence of the main stem was somewhat related to growth habit, variation was observed for this characteristic regardless of growth habit.

The first five principal components accounted for 83% of the total variation, with the first and second principal components accounting for 37 and 20% of the variation, respectively (data not shown). Based on the loadings in the eigenvectors, the seed and pod morphological variables (pod length, pod width, seed length, seed width, and seed weight) had the greatest effects on the first principal component, whereas the plant architectural morphological variables (plant width, growth habit, branching pattern, and main stem prominence) had the greatest effect on the second principal component. Based on the principal components, most introgression lines were more morphologically similar to the cultivated parent than the diploid wild species, with the wild species having an effect on the introgression lines where the two groups are clustered between both parents.

Marker Polymorphism

A diverse set of unique SNP markers was also found to differentiate \( A. \text{hypogaea} \) from other diploid \( A. \text{arachis} \) species. The final SNP marker set consisted of a total of 7017 markers, all of which were polymorphic between the two parental lines \( A. \text{diogoi} \) and Gregory. The 7017 markers densely covered the genome of \( A. \text{hypogaea} \) with an average...
of 351 markers per chromosome. Of these 7017 markers, 6070 were polymorphic between Gregory and A. batizocoi, 6289 were polymorphic between Gregory and A. carde- nasis, 6212 were polymorphic between Gregory and A. corentina, 5771 were polymorphic between Gregory and A. duranensis, 6524 were polymorphic between Gregory and A. gregoryi, 5833 were polymorphic between Gregory and A. ipaënsis, 5508 were polymorphic between Gregory and A. magna, 6529 were polymorphic between Gregory and A. stenosperma, and 6162 were polymorphic between Gregory and A. villosa.

**Introgression Analysis**

Of the 7017 total markers polymorphic between Gregory and A. diogoi, 391 did not identify A. diogoi introgression in any of the introgression lines, whereas 6626 markers exhibited introgression of A. diogoi in one or more lines (Table 1). These markers showed two different A. diogoi chromatin introgression patterns: (i) substitution of A. diogoi chromatin into either subgenome of A. hypogaea, or (ii) small and large insertions of A. diogoi chromatin. Across all chromosomes, the average amount of A. diogoi introgression was 8.12% and ranged from 3.00 to 18.14%. There was a greater amount of introgression on chromosomes A.04/B.04, A.05/B.05, and A.06/B.06 and the least amount on chromosome A.07/B.07.

A subset (1205 SNP markers) of the total number of SNP markers was found to differentiate the two A. hypogaea subgenomes based on SNP marker polymorphisms between the progenitor species, and these markers were used to estimate the amount of A. diogoi chromatin introgression due to the substitution introgression pattern. These markers showed that A. diogoi chromatin was introgressed into both the A. duranensis (A) and the A. ipaënsis (B) subgenomes of A. hypogaea. However, percentages were not calculated because of the tentative assignments of SNPs in the two genomes, since there has not been genetic confirmation of assignments. In all cases, however, the A. diogoi allele replaced the progenitor allele. Tetrasomic recombination was identified on chromosomes A.04, A.06, B.04, and B.06 with 129 total markers showing tetrasomic recombination on the four chromosomes of A. hypogaea. The A. diogoi allele was present in zero to four copies for the 129 markers showing tetrasomic recombination.

Varying levels of introgression were observed across the 87 introgression lines. The average introgression for all lines was 7.70% and ranged from 0.17 to 51.12% (Supplemental Table S2). Two introgression lines were outliers with respect to the amount of introgression present, with line IL 36 having the least amount of introgression (0.17%) and line IL 01 having the most introgression present (51.12%), respectively. Excluding these two lines, the majority of individuals (85 of 87 lines) had an average introgression of 7.28% and ranged from 4.49 to 15.89%. No introgression line harbored all of the introgressed A. diogoi SNP markers analyzed. Anachis diogoi introgressions were present in large blocks across numerous neighboring markers and in other cases only in single markers (shown as graphical genotypes in Supplemental Fig. S2). Similar patterns of introgression were observed across several introgression lines. These similar introgressions across numerous introgression lines may have been a product of a single introgression event that occurred in earlier generations of population development in the hexaploid materials.

A principal component analysis on the molecular marker data was performed to provide an estimate of

### Table 1. Genome-wide estimation of percentage A. diogoi introgression per chromosome and totals for markers for the 87 A. hypogaea × A. diogoi introgression lines.

<table>
<thead>
<tr>
<th>Chr.†</th>
<th>Total no. of SNP‡ markers per chromosome</th>
<th>No. of SNP markers detecting A. diogoi introgression§</th>
<th>Avg. A. diogoi introgression¶</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.01/B.01</td>
<td>690</td>
<td>654</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td>A.02/B.02</td>
<td>759</td>
<td>726</td>
<td>5.16</td>
<td></td>
</tr>
<tr>
<td>A.03/B.03</td>
<td>760</td>
<td>724</td>
<td>8.11</td>
<td></td>
</tr>
<tr>
<td>A.04/B.04</td>
<td>591</td>
<td>552</td>
<td>12.75</td>
<td></td>
</tr>
<tr>
<td>A.05/B.05</td>
<td>630</td>
<td>599</td>
<td>16.98</td>
<td></td>
</tr>
<tr>
<td>A.06/B.06</td>
<td>659</td>
<td>634</td>
<td>13.96</td>
<td></td>
</tr>
<tr>
<td>A.07/B.07</td>
<td>971</td>
<td>937</td>
<td>3.05</td>
<td></td>
</tr>
<tr>
<td>A.08/B.08</td>
<td>567</td>
<td>521</td>
<td>5.99</td>
<td></td>
</tr>
<tr>
<td>A.09/B.09</td>
<td>836</td>
<td>771</td>
<td>4.32</td>
<td></td>
</tr>
<tr>
<td>A.10/B.10</td>
<td>564</td>
<td>518</td>
<td>5.69</td>
<td></td>
</tr>
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<td>Total</td>
<td>7017</td>
<td>6626</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>665</td>
<td>–</td>
<td>8.12</td>
<td></td>
</tr>
</tbody>
</table>

† Chr., chromosome. Chromosome information supplied by Affymetrix.
‡ SNP, single nucleotide polymorphism.
§ SNP markers identifying introgression of A. diogoi in one or more lines.
¶ Average A. diogoi introgression (sum of the number of lines harboring an A. diogoi genotype at each SNP marker across all markers of the corresponding chromosome divided by total number of marker calls of the corresponding chromosome).
genetic relatedness among the introgression lines. The first two principal components accounted for 77.4% of the total variation, with the first and second principal component accounting for 69.5 and 7.9% of the variation, respectively (Fig. 3). The two-dimensional separation of the lines in a scatterplot of the first two principal components shows that many lines are highly similar genetically. Four distinct groups can be identified from this separation with the majority of individuals clustering around both parents. Thirty-four introgression lines clustered tightly together, and the genetic relationship among these 34 introgression lines, estimated as the correlation between SNP markers, ranged from $r^2 = 0.70$ to $r^2 = 0.99$. Two smaller groupings of four and seven introgression lines were observed. The clusters did not correspond with the three original tetraploid revertants, as progeny from each of these three were present in each of the clusters. Thus, it does not appear as though introgression occurred during the first hexaploid generation, but more likely occurred over many of the 12 generations of selfing during subsequent years.

The preliminary marker–trait association analysis revealed 1082 total marker–trait associations with the 14 morphological traits (Supplemental Table S3). Chromosomes A.08 (23) and B.06 (112) harbored the lowest and highest number of significant ($P < 0.0001$) markers, respectively. Individual SNP markers were associated with one to nine morphological traits resulting in 286 unique SNP markers with significant ($P < 0.0001$) marker–trait associations. However, the population was highly variable and many of the traits are very complex, and the traits with large numbers of marker associations do not present usable data for selection. No marker–trait associations were identified for seed width. Two, three, and five significant ($P < 0.0001$) marker–trait associations were identified for branching pattern, pod ratio, and number of seeds per pod, respectively.

**DISCUSSION**

A wide range of values for the morphological characteristics collected on introgression lines was observed, presumably due to alien *A. diogoi* chromatin introgressed into the Gregory genetic background. Pod and seed size are extremely important for incorporation of exotic germplasm in Virginia market-type breeding programs due to the stringent requirements for Virginia-type cultivars. Thus, selection on the basis of large seed size was conducted among the progeny of the tetraploid plants derived from self-pollinating the hexaploid individual. A number of introgression lines were identified that had acceptable seed size for a Virginia market-type cultivar. The majority of lines possessed seed size acceptable for use in a runner market-type breeding program. Although the seed size was acceptable for a number of lines, no data were recorded on market grades or yield in the present study.

Previous studies indicated that 40-chromosome derivatives derived from the triploid–hexaploid interspecific hybridization between *A. cardenasii* and *A. hypogaea* had large blocks of introgression, were intermediate to both parents morphologically, and possessed high levels of resistance to numerous plant pathogens that were derived from *A. cardenasii* (Stalker et al., 1979; Stalker, 1984; Garcia et al., 1995, 1996). Likewise, large blocks of *A. diogoi* introgressed chromosome segments were observed in addition to smaller regions of single markers that detected introgression. The findings in this study were similar to those of Garcia et al. (1995).

A reduction in recombination has been found in other introgression populations (Garcia et al., 1996; Nagy et al., 2010) and may be expected in the *A. diogoi* lines. Nagy et al. (2010) found recombination to be suppressed around the root-knot nematode resistance gene (*Rma*) harbored on an alien chromosome segment that comprised one-third to one-half of chromosome A.09. The suppression of recombination around introgressed regions is also found...
in tomato (*Lycopersicon esculentum* Miller) introgression populations (Chetlet et al., 2000; Canady et al., 2006).

Similar to Garcia et al. (1995, 1996) for *A. cardenasi* introgression lines, our results indicate that the mechanism of *A. diogoi* chromatin introgressions is not due to chromosome substitution. Rather, it appears that introgression is due to crossing-over and reciprocal recombination involving both subgenomes. This is evident because in no case was an entire *A. diogoi* chromosome or chromosome arm found in an introgression line. Bivalent chromosome paring in *A. hypogaea* is most common, although multivalent chromosome paring has been reported (Husted, 1936; Stalker and Wynne, 1979; Stalker, 1985; Leal-Bertioli et al., 2015). Cytological analysis of triploid individuals derived from the interspecific hybridization of *A. hypogaea* with diploid *Arachis* species shows that 10 bivalents and 10 univalents are typically observed, but trivalents have also been reported (Company et al., 1982; Stalker, 1985). The presence of intergenomic pairing (Singh and Moss, 1984) and the presence of trivalent formation within triploid hybrids (Company et al., 1982), although somewhat rare, are likely the basis of the genetic exchange and recombination between chromosomes of the wild species and *A. hypogaea*, which are manifested over the numerous cycles of self-pollination at a high ploidy level that is necessary to develop such individuals.

The mechanism of chromosome elimination in the triploid–hexaploid method of producing tetraploid introgression lines in *Arachis* is not fully understood. The unique genomic composition of two different parental genomes within one nucleus can lead to intergenomic conflicts such as chromosome elimination. Chromosome elimination has been reported in interspecific hybrids within other crop species genera such as *Hordeum* (Subrahmanyan and Kasha, 1973) and *Nicotiana* (Hancock et al., 2015). Hypotheses to explain the mechanisms of chromosome elimination after interspecific hybridization in various crop species include differences in timing of the mitotic cycle between the two species, histone changes resulting in the failure of centromeres to attach to the spindle microtubules, host–specific nucleases capable of degrading alien chromatin, or the formation of micronuclei followed by selective removal (Kasha and Kao, 1970; Subrahmanyan and Kasha, 1973; Finch, 1983; Gernand et al., 2005; Ravi and Chan, 2010, 2013). In these previous studies, chromosome elimination typically occurs over one generation, resulting in haploid individuals. It is unclear if any of these chromosome elimination mechanisms are at play in the triploid–hexaploid method of *Arachis* hybridization, primarily due to the fact that hexaploid individuals are maintained at that ploidy level for a number of generations, during which time the selective elimination of chromosomes occurs randomly but presumably in a few generations. Although requiring a large time and labor expense, cytological investigations during the numerous generations of inbreeding of hexaploid individuals would be needed to confirm the mechanism and rate of chromosome elimination.

A greater amount of *A. diogoi* introgression into the A genome of cultivated peanut is expected, since *A. diogoi* is classified as an A-genome species. Chromosomal homeolog assignment of the SNPs in this study is tentative in the absence of genetic data given the demonstration of homeologous chromosome pairing and recombination. However, evidence suggests that introgression into both subgenomes has occurred.

Garcia et al. (1995) conducted a restriction fragment length polymorphism (RFLP) evaluation of an *A. hypogaea* × *A. cardenasi* population derived via the triploid–hexaploid introgression method and observed most introgression into the A genome, but also introgression into the B genome. Thus, the results in this study were not unexpected. Because there is a high degree of chromosome synteny reported between *A. duranensis* and *A. ipaensis* and the corresponding A and B subgenomes of *A. hypogaea*, in addition to a high degree of synteny (~99%) between the two subgenomes within *A. hypogaea* (Seijo et al. 2004, 2007; Fončeka et al., 2009; Guo et al., 2012; Bertioli et al., 2009, 2016), there is likely a considerable amount of chromosome pairing between the two genomes. This sequence synteny likely results in pairing between chromosomes of either genome and the *A. diogoi* chromosomes within the hexaploid lines, followed by the potential for recombination and introgression of *A. diogoi* chromatin into either *A. hypogaea* subgenome. The *A. diogoi* introgression into the B genome could be biased upward due to ambiguities associated with preliminary chromosome assignments of the Affymetrix Axiom_Arachis2 SNP array caused by the high degree of similarity between subgenomes. Although there may be a bias of having assignments to the B genome, the critical point of the study is to illustrate *A. diogoi* introgression to *A. hypogaea* and its potential for cultivar improvement.

Marker–trait association analysis of morphological traits revealed a large number of markers significantly associated with certain traits in addition to a large number (226) of markers significantly associated with two or more traits. Future studies are needed to reduce the number and verify the associated markers with these traits of interest. Much of the *A. diogoi* introgression harbors unfavorable genetic variation influencing important morphological traits, such as smaller seed size, and molecular markers linked to traits of interest can be used in marker-assisted selection schemes. Nevertheless, with the array of *A. diogoi* introgression present in the population and its effect on plant morphological traits, all introgression lines are of the Virginia type with regards to plant structural classification.

Here we report the successful development of an interspecific hybrid derived population through the use
of the triploid–hexaploid introgression method with two Arachis species. This population will be phenotyped for an array of disease reactions to facilitate the determination of marker–trait associations that could be used in marker-assisted selection schemes. Additional crosses will be needed to move favorable introgressions into acceptable genetic backgrounds, and attempts to break any linkages between undesirable and desirable genetic variation should be made more efficient through the application of marker-assisted selection. Additionally, this research demonstrates the value of SNP marker arrays to peanut research and the genetic improvement of cultivated peanut.

This A. diogoi interspecific derived introgression population is highly variable even after selection for A. hypogaea morphological traits, and all progenies are subsp. hypogaea, var. hypogaea, an important consideration for use in most US peanut breeding programs. An array of A. diogoi introgression was observed on all chromosomes, with the majority of introgression on chromosomes A.04/B.04, A.05/B.05, and A.06/B.06 of both genomes. A significant amount of introgression into both the A and B genomes was observed even though A. diogoi is an A genome species.

Conflict of Interest
The authors declare that there is no conflict of interest.

Supplemental Material Available
Supplemental materials for this article are available online.

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