Pedigree reconstruction for triploid apple cultivars using single nucleotide polymorphism array data

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RESEARCH ARTICLE

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Pedigree reconstruction for triploid apple cultivars using single nucleotide polymorphism array data

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Societal Impact Statement

Many economically, culturally, and historically important apple cultivars are triploids, which have three copies of each chromosome instead of the more typical two copies in diploids. Despite their prevalence and importance, there have been conflicting reports regarding their origin and their ability to beget diploids. New genetic analysis methodologies outlined in this study have clarified the genetic origin of triploid apple cultivars and suggest that triploidy has been a dead end in historic apple pedigrees. The specific results of this study have resolved the pedigrees of many cultivars, including the famous English cultivar Cox's Orange Pippin and the oldest known US cultivar Roxbury Russet.

Summary

- In apple (*Malus* × *domestica*), most cultivars are diploid, though a sizeable number are triploids, which tend to be stronger growing, more robust, and bear larger fruit. However, triploidy is also associated with strongly reduced fertility. Some recorded pedigrees for historical apple cultivars include triploids as parents of diploids, despite this reputation of poor fertility. This information, coupled with some initiatives using triploids in breeding efforts, result in confusion about how possible or common it is for triploids to be parents of diploid offspring. To date, no studies have systematically evaluated and identified pedigrees of triploid apple cultivars to resolve these contradictions.
- Here, we describe a method to make triploid genotype calls using Illumina Infinium single nucleotide polymorphism (SNP) array data through a novel Python script: ploidyClassifier. SNP data for 219 unique triploids was compared alongside 2498 unique diploid apple accessions to conduct pedigree reconstruction.
- Unreduced gamete-donating parents were identified for over half of the triploid accessions. From those, reduced gamete-donating parents were identified for nearly half. Full or partial pedigrees for many classic triploids were uncovered, including that of the oldest known American cultivar, 'Roxbury Russet'. All tested pedigrees from literature that listed triploids as parents of diploids were deemed

false, including that of the well-known 'Cox's Orange Pippin', whose previously unreported second parent was also identified here as 'Rosemary Russet'.

- These results together suggest that historic triploids are mostly or solely the product of diploid parentage and that triploidy has been a dead end in historic apple pedigrees.

KEYWORDS
apple, genetics, *Malus*, pedigree reconstruction, polyploidy, single nucleotide polymorphism array, triploid

1 | INTRODUCTION

Polyploidy, or the presence of more than two homologous sets of chromosomes in an organism, is a wide-spread phenomenon studied for many reasons, including understanding speciation or for breeding purposes. While many of these studies have concerned even-numbered multiplications, which allow normal reproduction, uneven ploidies typically have been given less attention. Nevertheless, triploidy is a well-documented phenomenon occurring in many wild plants (Husband, 2004), crop plants (e.g., McClintock, 1929; Sattler et al., 2016; Siadjeu et al., 2018; Singh et al., 1967), and animal species (e.g., in fish; Piferrer et al., 2009; Tiwary et al., 2004). Triploids are generally believed to be less fertile and therefore have diminished fitness relative to their diploid and tetraploid counterparts. This is because triploids are less able to produce balanced gametes necessary for the formation of fertile offspring (Husband, 2004; Pelé et al., 2018). However, triploids may not be completely sterile (Husband, 2004; Ramsey & Schemske, 1998), and even reduced fertility may be enough to allow further contribution to the gene pool, in part through the formation of tetraploids (Bretagnolle & Thompson, 1995). Generally, two different pathways to generate triploidy are possible: fusion of a reduced gamete with an unreduced gamete from two diploid parents or the fusion of reduced gametes from one diploid and one tetraploid parent (Pelé et al., 2018). The former method is thought to be the most common way in which triploids originate in the wild, since this matches the genome dosage in the endosperm (Ramsey & Schemske, 1998), though many triploids in cultivated species are artificially produced via crossing tetraploids with diploids (Bergström, 1938; Sattler et al., 2016; Sedysheva & Gorbacheva, 2013; Wang et al., 2016). Unreduced gametes are most commonly the result of non-disjunction of either homologs during meiosis I, or sister chromatids during meiosis II, named first and second division restitution (FDR and SDR), respectively (Bretagnolle & Thompson, 1995; Pelé et al., 2018). The genetic difference between 2n gametes produced in FDR and SDR is that, if there is no recombination, the former retains the heterozygosity of the parent, whereas the latter leads to homozygous, diploid gametes (Bretagnolle & Thompson, 1995).

In plant breeding, crosses with triploids generally result in either no viable seeds being produced or, when seeds are produced, in aneuploid offspring (Sattler et al., 2016). For example, crosses between triploids and diploids resulted in aneuploidy in maize (*Zea mays*) (McClintock, 1929), in greatly reduced germination and aneuploidy in melon (*Cucumis melo* L.) (Ezura et al., 1994) and grape (*Vitis* spp.) (Park et al., 2001), and in no viable seeds in highbush blueberry (*Vaccinium corymbosum*) (Vorsa & Ballington, 1991) and banana (*Musa* spp.) (Perrier et al., 2011).

However, the fertility of triploids varies among studies of cultivated plants. For example, in cultivated rose (*Rosa* × *hybrida*), triploids have been recorded as being able to produce both haploid and diploid gametes in bidirectional crosses, albeit with lower efficiency than diploids or tetraploids, in one study (Van Huylenbroeck et al., 2005), but in another study no viable seeds were set using triploids as mothers (Abdolmohammadi et al., 2014). Despite the typically marked reduction in fertility of triploids, in *Hydrangea macrophylla*, a study conducting pedigree reconstruction through the use of SSR markers concluded that crosses involving triploids were used to create new diploid cultivars (Hempel et al., 2018). This sort of conflicting information regarding the nature of fertility in triploids is particularly prevalent in cultivated apple (*Malus* × *domestica*).

In apple, most cultivars are diploid, though a sizeable number are triploid. Incidences of triploid accessions in collections have been reported to be as high as 28% (Pereira-Lorenzo et al., 2007). Triploid cultivars include many that are culturally, historically, and/or economically important (e.g., 'Boskoop', 'Ribston Pippin', and 'Jonagold'). Triploid cultivars are generally thought to be produced via unreduced gametes in diploid × diploid crosses (Brownfield & Köhler, 2011; Considine et al., 2012), though they can also be produced via crosses between tetraploids and diploids (Bergström, 1938; Einset, 1945; Lespinasse et al., 1976; Sedov et al., 2014). Unreduced gametes have been reported to come from either the mother (Ordidge et al., 2018; Pikunova et al., 2018) or the father (Zhang et al., 1988) and as being produced either only in FDR (Considine et al., 2012) or in both FDR and SDR (Zhang et al., 1988).

The results from previous literature on the fertility of triploid apples are conflicting. Many triploid cultivars have been noted for producing large fruit and for being particularly vigorous and robust (Sedysheva & Gorbacheva, 2013). These attributes have led some breeders to experiment with triploids in breeding programs (Einset, 1945; He et al., 2018; Magness, 1937; Sato et al., 2007), with variable levels of success noted, but only when triploids were used as the mother in crosses, as triploids are generally believed to be pollen sterile (Brown, 2012). Early work with controlled crosses that included

triploids universally noted low seed set and low germination, and when seeds did germinate, weak growth and aneuploidy were observed (Crane & Lawrence, 1930; Dermen, 1936; Einset, 1945; Magness, 1937). In contrast, several contemporary breeding efforts have reported some level of success with using triploids as parents (He et al., 2018; Pikunova et al., 2018; Sato et al., 2007; Sedov et al., 2014). These studies observed diploid offspring resulting from the use of triploid parents, despite an element of the reduced fertility and increased frequency of aneuploidy that was reported in earlier studies. In a 40 year-long study investigating heteroploid crosses, those between triploid mothers and diploid fathers resulted in 32.9% diploid offspring, whilst an equivalent number of seeds generated from crosses between diploid mothers and triploid fathers resulted in 96% diploid offspring (Sedov et al., 2014). Another study noted that 32.6% of seedlings grown from open-pollinated seeds of the triploid cultivar Jonagold were diploid (He et al., 2018), with the rest being either triploid, tetraploid, or aneuploid.

In addition to the conflicting literature regarding the fertility level of targeted crosses involving triploids, many historically recorded apple pedigrees cite triploids as parents of diploid cultivars. Whilst untested, the existence of these pedigrees is in conflict with the reduced fertility reported in some of the literature previously mentioned, and it is unclear how so many triploids could have donated balanced gametes to diploids. An important example of this is the pedigree of 'Cox's Orange Pippin', a common parent or ancestor of many modern cultivars, which was listed in the UK National Apple Register (Smith, 1971) as being a cross between the triploid 'Ribston Pippin' and possibly another triploid, 'Blenheim Orange'. This pedigree was recently proven to be at least partially incorrect, as 'Ribston Pippin' and 'Cox's Orange Pippin' have been demonstrated to instead share diploid 'Margil' as one parent (Muranty et al., 2020; Ordidge et al., 2018). However, the second parent has not previously been identified, nor has marker data for 'Blenheim Orange' been used to confirm or deny it as being a possible parent of 'Cox's Orange Pippin'. Removing the confusion for this and other pedigrees involving triploids would help to resolve outstanding questions about the fertility of triploid apple cultivars and the nature of their formation, which would be useful for future studies and breeding efforts involving them.

Genetic studies evaluating triploids offer a way to test these conflicting reports on the fertility and usefulness of triploids in apple breeding. Unreduced gamete-donating parents (UGDPs) that produced 2n gametes have been confirmed or identified in several studies through the use of SSR data (Evans et al., 2011; Larsen et al., 2017; Ramos-Cabrer et al., 2007; Storti et al., 2013), DArT markers (Ordidge et al., 2018), and SNP markers (Muranty et al., 2020; Vanderzande et al., 2017). However, none of these studies has systematically evaluated pedigrees in which triploids are the recorded parents of diploid cultivars. Additionally, probing large SNP datasets this way may shed some light into the origins and historic breeding use of triploid apple cultivars, as exemplified for 'Ribston Pippin' (Muranty et al., 2020).

In this study, we demonstrate a method for making accurate genotype calls for triploids using the Illumina Infinium apple 20K SNP array (Bianco et al., 2014) and a custom python script, named ploidyClassifier, and how to use this data for pedigree reconstruction for triploid apples. This new pedigree information was then used to address questions about the origins of triploidy in apple, to address whether triploid apples are dead ends in breeding, and to enable some new pedigree reconstruction results for diploid cultivars for use in breeding and to elucidate previously unknown origin information for historic cultivars.

2 | MATERIALS AND METHODS

2.1 | Plant material

A set of 3594 accessions (2715 unique) genotyped on the Illumina apple Infinium 20K array (Bianco et al., 2014) and/or the Affymetrix apple Axiom 480K array (Bianco et al., 2016) were evaluated in this study (Table S1). Germplasm included in the study was assigned MUNQ (Malus UNiQue genotype) codes (Denancé et al., 2020), as previously described (Muranty et al., 2020), based on SSR data, where available. For accessions that did not have SSR data available for MUNQ attribution, MUNQ codes were assigned if their SNP data was over 99.5% identical with samples that have been previously assigned MUNQ codes. No triploids genotyped on the 480K SNP array were included in this study, as the method described in this study for making triploid genotype calls was developed specifically for material genotyped on Illumina SNP arrays.

2.2 | SNP genotyping

A genetic map composed of 10,295 SNPs deemed to be of acceptable quality that was previously described (Howard, Troggio, et al., 2021) was used in this study, with 20 SNPs excluded that had null alleles in more than 50 diploid individuals. Genotype calls for diploid samples were made as previously described (Howard, Troggio, et al., 2021; Vanderzande et al., 2019). This process included visual inspection of B-allele frequency plots to assess ploidy as a check to the automated ploidy calling used in the Python script described below. Samples with poor or problematic allele call quality (as described in Vanderzande et al., 2019) and duplicates were excluded from all analyses. Duplicates were identified as having the same genotype calls for at least 99.9% of SNPs.

SNP genotyping for triploids was conducted using a custom Python script named ploidyClassifier. ploidyClassifier determines the ploidy of each individual by fitting of the smoothed histogram of the B-allele-frequency for heterozygous SNPs derived from the Final Report of GenomeStudio through three different models: (i) ModDiplo: composed by one Cauchy-Lorentz distribution with a prior center at 0.5 and with a sigma of 0.04 to represent the typical distribution of the B-allele-frequency histogram of a diploid accession, (ii) ModTriplo: composed by the sum of two Cauchy-Lorentz distributions with prior centers at 0.33 and 0.66 and with a prior sigma of

0.02 to represent the typical distribution of the B-allele-frequency histogram of a triploid accession, and (iii) ModTetra: composed by the sum of three Cauchy-Lorentz distribution with prior centers at 0.3, 0.5, and 0.7 (which were determined to be more accurate through empirical approximation from the theoretical values of 0.25, 0.5, and 0.75) and with a prior sigma of 0.02 to represent the typical distribution of the B-allele-frequency histogram of a tetraploid accession. All these models were built using the lmfit (Non-Linear Least-Squares Minimization and Curve-Fitting) module (Newville et al., 2014) for Python. The best fitting model was chosen using the Bayes Information Content (BIC). The script then reclassifies SNP calls for triploids to consider allele dosage. Homozygous SNP calls are then re-coded as *AAA* and *BBB*, and heterozygous SNP calls are divided into *AAB* and *ABB* depending on the value of Theta. Triploid SNP calls are designated *AAB* when the value of Theta is lower than the median of the values of Theta for heterozygous diploids for the considered marker and the genotype is set as *ABB* when the value of Theta is higher than the median of the values of Theta for heterozygous diploids for the considered marker. SNP calling for tetraploids and classification of ploidy levels greater than four was not programmed into ploidyClassifier. More details on the algorithm and usage are available in the documentation of ploidyClassifier that is available at bitbucket (https://bitbucket.org/michelettd/ploidyclassifier).

2.3 | Triploid pedigree reconstruction

Triploid pedigree reconstruction was initially conducted under the assumption that triploids were produced through the union of an unreduced gamete from one diploid (the UGDP) and a regular gamete from another diploid (the reduced gamete donating parent, or RGDP). In all pedigree reconstruction tests, SNP calls in diploids that included one or two null alleles were treated as missing data. Genotypic profiles for triploid accessions were each compared with that of all diploid accessions to identify diploid UGDPs of triploids. Two types of UGDPs were considered (Figure 1). An UGDP1 was defined as any parent that had donated a 2n gamete where parental heterozygosity was maintained in full (i.e., formed without recombination as demonstrated in Figure 1). An UGDP1 would be expected to share both alleles with their triploid offspring at every SNP, except in rare cases involving aneuploidy or in cases of problematic clustering previously described (Howard, Troggio, et al., 2021). Thus, an UGDP1 was considered identified when it shared both alleles with a triploid for at least 99.5% of SNPs. This threshold exceeded a previously established threshold of 99% in a study that used the apple 8K SNP array (Vanderzande et al., 2019). This higher stringency was chosen because there are more heterozygous SNPs in triploids and homozygous SNPs are needed for most of the cases of pedigree reconstruction described below. The identification of UGDP1s (and as described later, UGDP2s, and RGDPs) was first conducted through HapShared and later for triploids that did not have both parents identified an excel tool was used (similar to that used in Vanderzande et al., 2019) to enable pedigree reconstruction using the Axiom data.

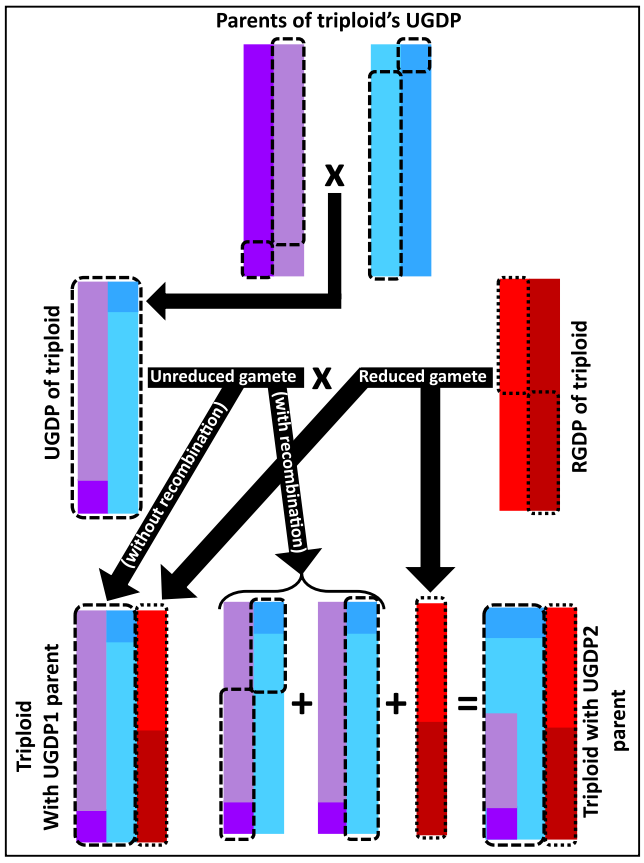

FIGURE 1 Depiction of a triploid pedigree and inheritance with an unreduced gamete-donating parent (UGDP), where the unreduced gamete was formed through first division restitution (FDR) without recombination (UGDP1) and with recombination (UGDP2). The colored fragments represent homologs for a single chromosome. Areas encircled in long dashes represent the transmission of gametes from the parents (top) of the triploid's UGDP to the triploid's UGDP (middle left) and of the unreduced gamete from the UGDP to the triploid (bottom). Haplotypes encircled in shorter dashes represent the transmission of a regular gamete from the triploid's reduced gamete donating parent (RGDP) (middle right) to the triploid (bottom).

An UGDP2 was defined as any parent that had donated a 2n gamete where parental heterozygosity was not completely maintained (i.e., formed with recombination as demonstrated in Figure 1). This presumably would have happened via recombination during 2n gamete formation at either FDR or SDR, resulting in a loss of heterozygosity (relative to a 2n gamete produced at FDR without recombination), or via a lack of recombination during 2n gamete formation at SDR, which would have resulted in a completely homozygous 2n gamete. Thus, in contrast to the case with UGDP1, some SNPs that are heterozygous in an UGDP2 could be homozygous in the 2n gamete donated to their triploid offspring. However, like with UGDP1s, no SNPs that are homozygous AA or BB in an UGDP2 could be ABB or AAB, respectively, in their triploid offspring since homozygosity would not be altered by recombination. This difference between UGDP1 and UGDP2 was not sufficient to confirm an UGDP2, as the triploid

could have also inherited two thirds of its 3n chromosome complement from an individual diploid if that diploid was both its RGDP and one grandparent of its UGDP1. Thus, an individual was considered a possible UGDP2 if it shared at every SNP either both its alleles or one of its alleles at least twice for at least 99.5% of homozygous SNPs with a triploid. Each triploid with an identified or confirmed UGDP1 was further evaluated to identify its RGDP. To identify these RGDPs, the SNP alleles that must have originated from this RGDP were deduced by subtracting the SNP alleles attributable to the UGDP1 from the triploid offspring. For example, if the triploid's SNP alleles were "*AAB*," and the identified UGDP1 had SNP alleles "*AA*," then the SNP allele from the RGDP must have been "*B*." These deduced RGDP contributions were then compared with the diploid dataset to identify the RGDPs. Any diploid that could account for at least 99.5% of these deduced SNP alleles was assigned as the RGDP. A list of acceptable SNP allele inheritances for triploids that are the product of an UGDP and a RGDP is found in Table 1.

Triploids lacking identified UGDPs were then evaluated for other types of pedigree reconstruction. First, pairs or groups of those triploids were identified that shared at least two alleles at each SNP for over 99.5% of SNPs. Those that did were noted and assumed as either sharing an unidentified, putative UGDP1, or as having the same parents, but with reciprocal crossings where each triploid had an UGDP1 and not an UGDP2. The genotypic profiles for triploids lacking identified UGDP1s were then compared with that of other triploids without identified UGDP1s to determine if they shared at least one, but not two, alleles for at least 99.5% of SNPs (or in other words, not more than 0.5% of SNPs where one triploid was "*AAA*" and another triploid was "*BBB*"). Sharing at least one, but not two, alleles at every locus was considered as being that the UGDP1 in one is either the RGDP or the grandparent of the UGDP1 of the other triploid, or the offspring of the UGDP1 of the other triploid. Next, genotypic profiles for triploids lacking identified UGDP1s were compared with the deduced contribution coming from unidentified RGDPs of triploids with identified UGDP1s to determine whether any unidentified, but deduced, UGDP1s of triploids could be the RGDP of other triploids. If the alleles in a triploid's haplotype deduced to come from an unknown RGDP were present in the homozygous SNP calls of a second triploid for at least 99.5% of SNPs, the putative UGDP1 of the second triploid was considered the RGDP of the first.

TABLE 1 Possible single nucleotide polymorphism (SNP) genotype calls resulting in no Mendelian inconsistent errors between a triploid and its unreduced gamete donating parent (UGDP) and reduced gamete donating parent (RGDP)

Triploid SNP call	UGDP SNP call[a]	RGDP SNP call
AAA	*AA* (*AB*)	*AA* or *AB*
AAB	*AA* (*AB*)	*AB* or *BB*
AAB	*AB*	*AA* or *AB*
ABB	*AB*	*AB* or *BB*
ABB	*BB* (*AB*)	*AA* or *AB*
BBB	*BB* (*AB*)	*AB* or *BB*

[a]Additional genotype calls in parentheses are only possible in UGDP2s. UGDPs are parents that donated to their triploid offspring a 2n gamete in which parental heterozygosity was not completely maintained to triploid offspring.

2.4 | Testing SNP call accuracy in triploids

SNPs were considered problematic if they demonstrated at least four Mendelian inconsistent inheritance errors (Sobel et al., 2002) across triploids that had either UGDPs or both UGDPs and RGDPs identified. Cluster plots of these SNPs were evaluated to determine the nature of the SNP call inconsistency. Data for SNPs deemed to be inaccurately called were set to missing and the UGDP and RGDP identification process was repeated using the subset of SNPs that remained to identify any relationships that had been previously rejected.

Further genotype call accuracy evaluations and a demonstration of triploid phasing were made by comparing genotype calls for triploid 'Jonagold' to its reported UGDP 'Golden Delicious' and RGDP 'Jonathan' (Gianfranceschi et al., 1998). SNP data for Jonathan was phased using FlexQTL™ software (v0.99) and various cultivar offspring of Jonathan, 20 seedlings from a 'Jonathan' × 'Prima' cross (Di Pierro et al., 2016), and using the known parent of 'Jonathan', 'Esopus Spitzenburg'. Genotype calls for 'Golden Delicious' were compared with 'Jonagold' to demonstrate that both share at least two alleles at every locus and to confirm 'Golden Delicious' as an UGDP1. Genotype calls from 'Golden Delicious' were then subtracted from the triploid genotype calls of 'Jonagold' and the remaining alleles were compared with the phased data from 'Jonathan' to demonstrate that the data represented an accurate phased haplotype. All genotype calls were evaluated for both Mendelian inconsistent errors and Mendelian consistent errors (Sobel et al., 2002). Cluster positions of SNPs with such errors were evaluated in GenomeStudio to determine the nature of the errors.

2.5 | Use of triploid data for diploid pedigree reconstruction

Genotypic profiles for unidentified, putative UGDP1s were deduced and used for diploid pedigree reconstruction (Figure 2a). This SNP allele deduction was accomplished using the homozygous calls of these triploids. In other words, when the triploid's SNP calls were "*AAA*" or "*BBB*," the UGDP1's SNP alleles must be "*AA*" or "*BB*" for those SNPs, respectively. Genotypic profiles deduced this way would have many instances of missing data, but there were still sufficient genotype calls available for pedigree reconstruction. Deduced genotypic profiles for putative UGDP1s were compared with the diploid dataset to identify parent-offspring relationships, as described in Vanderzande et al. (2019). Any individual from the diploid dataset that had a parent-offspring relationship with a putative UGDP1 was assumed to be either an offspring or a parent of a putative UGDP1 or the RGDP of the original triploid offspring. The latter case is because as the

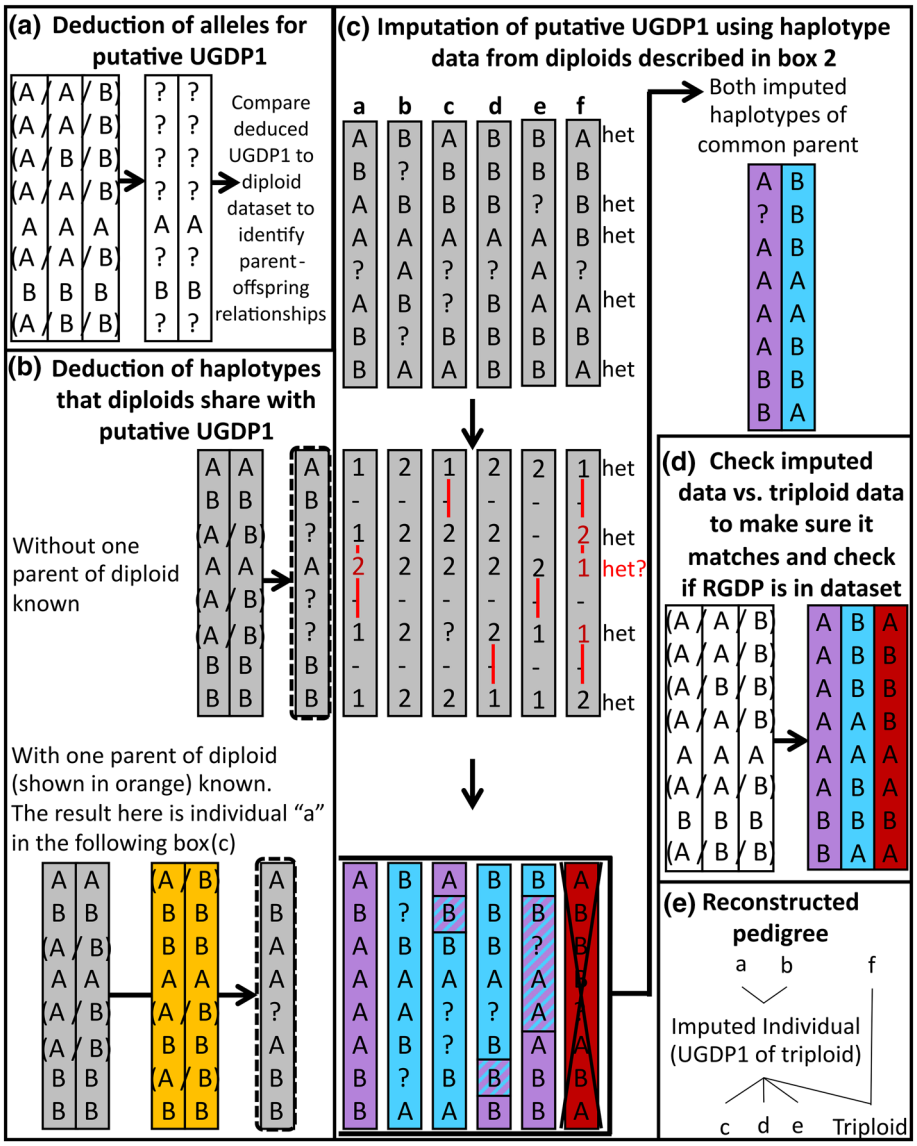

FIGURE 2 Imputation of a putative unreduced gamete donating parent (UGDP1) of a triploid. Figure 2a shows the allele deduction for putative UGDP1s. Deduced single nucleotide polymorphism (SNP) data for putative UGDP1s were used to identify diploids that had parent-offspring relationships with the putative UGDP1s. Figure 2b shows the deduction of haplotypes that such diploids have in common with the triploid's putative UGDP1. Imputation was conducted using the homozygous genotype calls in the diploids (depicted in grey) if no other parent was known (top of Figure 2b) but, if one parent of the diploid was known, homozygous calls in the known parent were also used to deduce the allele in the haplotype where the diploid relative was heterozygous (bottom of Figure 2b). Figure 2c shows how the genotype calls for UGDP1s were imputed. SNPs were first filtered to show only those that would be heterozygous (shown as "het") in the putative UGDP1. The alleles were then assigned as being from either one of the two possible haplotypes (1 and 2 in the middle of Figure 2c) of the putative UGDP1. These assignments were made on the basis of minimizing the level of recombination (shown as red lines in Figure 2c), which would otherwise be required to explain the switching between haplotypes 1 and 2. Diploid f shows haplotype switching that is inconsistent with the other relatives. When five or more individuals were identified that had realistic SNP segregation patterns across all 17 chromosome pairs, genotype calls for the individual being imputed were determined for each SNP over regions that had clear representation from both possible haplotypes. In the example in Figure 2c, this was possible for all but the second SNP for the first haplotype, as individual a is missing an allele and individuals c and e have recombinations over this interval, rendering their genotype calls ambiguous as to their haplotype assignments. Then, the newly imputed putative UGDP1 was checked to confirm that it was in agreement as an UGDP1 of the triploid (Figure 2d). Finally, if there was a diploid that had a parent-offspring relationship with the putative UGDP1 but did not have a parent-offspring relationship with the imputed UGDP1, it was checked to see if it could be the reduced gamete donating parent (RGDP) of the original triploid (individual f, depicted in red). The result is a reconstructed pedigree involving the imputed individual (Figure 2e—transmission of unreduced gamete depicted in red).

deduced genotypic profiles of the putative UGDP1s were made using only the homozygous SNP calls in the triploids, any true RGDPs would also have at least one of its alleles in common with each SNP of the deduced genotypic profiles. In cases where at least five parent-offspring relationships between putative UGDP1s and diploids were identified, haplotypes from diploids deduced to be shared with a triploids' putative UGDP1 (Figure 2a) were used to impute the genotypes of the putative UGDP1. Five parent-offspring relationships were chosen as a threshold for imputation because fewer relationships would result in a very high level of missing SNP data and ambiguity in conducting imputation (Howard et al., 2022). Imputation was accomplished as described in Figure 2c. This method was previously used to impute "Unknown Founder 1" (Howard, Peace, et al., 2021) and is described in detail there. There were three differences between the methods used to impute "Unknown Founder 1" and the method used in this study. First, the groups of relatives were identified via putative UGDP1s instead of via the use of summed potential lengths of shared haplotypes. Second, the imputation process in this study was also used to identify RGDPs of the triploid whose UGDP1 was being imputed. In such cases the RGDP would be identified via the presence of inconsistent segregation by comparison to the majority of the diploid relatives (as shown for individual f in Figure 2). After imputation, the imputed individuals were confirmed as UGDP1s (Figure 2d). Following imputation of a UGDP1, a RGDP could also be identified or confirmed via the pedigree reconstruction methods previously described. Third, after imputation and confirmation that the imputed individual was a credible UGDP1 of a triploid, further SNP calls could be imputed using SNP calls from the triploid offspring. In the example in Figure 2c, the second SNP was able to be fully imputed during this final step.

In cases where imputation was not able to clarify the relationships between diploids and putative UGDP1s, POR (parent-offspring order resolution) tests (Howard et al., 2022) were used to elucidate the relationships between them. These tests can be used to determine which individual in a parent-offspring duo relationship is the parent and which is the offspring. This is made possible via phasing (in the POR-1 test) or deduced phasing (POR-2 test) when phasing data is not sufficiently available for the individual (s) in question.

The POR-1 test was used to clarify relationships between diploids and a putative UGDP1 when there were at least 10 offspring available for diploids to use for phasing. Phased SNP data were generated using FlexQTL (Bink et al., 2014). Through this test, a putative UGDP1 or the triploid itself would be considered the parent of a diploid if there were no evidence of the putative UGDP1 being composed of recombinant haplotypes of the diploid. If the putative UGDP1 was instead found to be composed of recombinant haplotypes of the diploid, the diploid was deduced to be either the RGDP, one parent of the UGDP1, or possibly as having the same pedigree as the triploid. The first was a possibility because the deduced homozygous SNP calls of the putative UGDP1 also represented the haplotype from the RGDP of the triploid offspring of the UGDP1. In other words, the deduced SNP calls in the UGDP1 represented SNPs that are both homozygous in the UGDP1 and that had the same allele in the gamete the RGDP donated to the triploid.

The POR-2 test was used to clarify relationships between diploids and putative UGDP1s when there were between five and nine offspring of a diploid available for the test. The interpretation of the POR-2 test is conceptually similar to the POR-1 test, but it identifies evidence of an individual being composed of recombinant haplotypes of another individual differently. In the POR-2 test, one individual in an unordered parent-offspring duo relationship is considered a candidate grandparent of the confirmed offspring of the second individual (termed the "Parent" in the test) used in the test. This candidacy status is validated or invalidated on the basis of recombination patterns among the offspring. Problematic recombination patterns are identified via "common areas of apparent recombination" (CAAR) among the offspring in the test. CAAR are areas where all offspring in the test would only have had unlikely coinciding recombinations if the candidate grandparent were a true grandparent. In this study, the putative UGDP1s were always used as the candidate grandparent in the POR tests because all instances in which five confirmed offspring of any putative UGDP1 were available would have been addressed via the imputation steps described above. To be conservative and in accordance with the rubric defined in Howard et al. (2022), a candidate grandparent was deemed false if there were more than two CAAR identified. If the candidate grandparent (i.e., the putative UGPD1 of a triploid in this study) were deemed false, then it would be a sibling of the offspring used in the test (i.e., the other individual in the parent-offspring duo relationship being tested). The interpretation for this study would be that the Parent individual would either be the RGDP, one parent of UGDP1, or would have the same pedigree as the triploid. Alternatively, if the candidate grandparent was deemed true, then the putative UGDP1 or the triploid itself would be concluded as a parent of the other individual.

In a final step, literature was scanned to identify historical pedigrees where triploids were listed as the parents of diploids or of other triploids. These pedigrees were evaluated to determine if any were valid through the identification of Mendelian inconsistent errors (Sobel et al., 2002). During this step a small number of additional pedigrees involving diploid individuals were identified using methods described in Vanderzande et al. (2019).

3 | RESULTS

3.1 | Triploid allele calling

Analysis of SNP array data confirmed polyploidy of 218 unique triploids and one tetraploid in this germplasm (Table S1). Ploidy levels for all individuals matched the expected levels from inspection of B-allele frequency plots in GenomeStudio. Typical clustering and associated allele calling for triploids using ploidyClassifier can be found in A of Figure S1. There were 33 duplicate pairs, 12 groups of three duplicates, four groups of four duplicates, three groups of three duplicates, and one group of six duplicates. Across the unique triploids, the median number of missing SNP calls prior to any level of data curation was 858 (8.4%), with a maximum of 1570 (15.3%) and a minimum of

524 (5.1%). Following confirmation or identification of UGDPs and RGDPs described below, the median numbers of Mendelian inconsistent errors were nine and 11 (maximums of 36 and 47) among triploids with identified UGDPs and with both UGDPs and RGDPs, respectively. There were 19 SNPs that had more than four identified Mendelian inconsistent inheritance errors involving triploids with either UGDPs or both UGDPs and RGDPs identified. These SNPs were typically problematic due to the presence of more than one *AB* cluster for diploids (B in Figure S1), shifted clusters that were very close together (C in Figure S1), or, occasionally, wide *AB* distributions (D in Figure S1). The first issue was the most common and would sometimes result in the wrong heterozygous allele call being made, which in turn resulted in the presence of some false Mendelian errors during pedigree reconstruction.

'Jonagold' and its parents exhibited mismatches at 11 loci. These were all due to the previously mentioned issues. The haplotype in 'Jonagold' coming from its RGDP, 'Jonathan', was nearly perfectly composed of a gamete sample from 'Jonathan' (Table S2), with recombination of 'Jonathan' haplotypes evident on chromosomes 5, 6, 7, 8, 9, 10, 12, 14, 15, and 16. Nine instances of Mendelian consistent errors, or double recombination single points, were identified in the 'Jonathan' haplotype of 'Jonagold' (columns highlighted in yellow in Table S2). Seven of these were due to the presence of multiple *AB* clusters whereas the other two were due to clusters being too close together.

3.2 | Pedigree reconstruction

UGDPs that donated a 2n gamete without recombination, referred to in this study as UGDP1s, were identified for 126 of the 218 triploids. Ten cases involved imputed individuals that were not genotyped nor identified in this study. RGDPs were identified for 45 of the triploids with identified UGDP1s (Table S3). Additionally, the putative UGDPs of 'Arkansas' and 'Lutticher Ananaskalvill' were deduced to also be the RGDPs of 'Clozette' and 'Beauvais 4 Côtes', respectively, resulting in complete parentage for 'Clozette' and 'Beauvais 4 Côtes' (included in Table S3). Twenty-one diploids were UGDP1s of more than one triploid, with 'Brabant Bellefleur', 'Glane', and 'Reinette Franche' each being UGDP1s of six triploids, the latter being also the RGDP of 8 additional triploids. There were 47 diploids that were UGDP1s of only one triploid.

Three pairs of triploids and one trio of triploids were identified as either having common unknown UGDP1s, or as having the same parents with their UGDP1s and RGPDs switched. 'Graue Herbstrenette' and 'Reinette de Macon' composed the first pair, 'Geflammter Kardinal' and 'Dredge's Fame' the second pair, 'Smokehouse' and 'Vandevere' the third pair, and 'Piattona', 'Suzanne', and 'Double Rose' composed the trio. Seven pairs of triploids and two trios of triploids, all without identified UGDP1s, were identified as having at least one, but not two, alleles in common at every SNP (Table S4). Three triploid cultivars were identified as possibly having parents who donated a 2n gamete that underwent recombination (such parents were termed UGDP2 here) in this study: 'Reinette de France' (potential UGDP2 'Court Pendu Plat'), "'Baron Wood' ('Orange Goff'), and 'Sächsischer Königsapfel' ('Danziger Kantapfel') (Table S5). One triploid breeding selection, 2004_015a_099, intentionally included in the study because it was the only example of a confirmed UGDP2, was identified as such as it matched its recorded pedigree and pedigree reconstruction methods confirmed that its recorded maternal parent was an UGDP2.

Diploid accessions having at least one allele in common with triploids for every SNP were identified for 50 of 102 triploids without identified UGDP1s (Table S5). This left 52 triploids with no identified direct relationships with diploids. Fifty diploids were all deemed offspring of the imputed common UGDP1 of 'Piattona', 'Suzanne', and 'Double Rose' (Table S5). The high number of offspring of this individual, dubbed "Unknown Italian Founder," enabled genotype imputation for all but 39 SNPs (Table S6). Genotype calls for 7626 (74%) SNPs were imputed for the unknown UGDP1 of 'Early Strawberry' (Table S7) using SNP data from five diploids that had at least one allele in common with it at every locus. Through this imputation, "UGDP Early Strawberry" was determined to be an offspring of 'Reinette Franche' and one parent of 'Ralls Janet', 'Milam', 'Buckingham', and 'Tender Skin' (Tables S5 and S7). Unknown, putative UGDP1s of triploids matched as being the second parent of diploids in 11 instances (Table S5). Eight of these were offspring of "Unknown Italian Founder" and one was an offspring of "UGDP Early Strawberry." In the other two instances, both involving the unknown, putative UGDP1 of 'Stay Close', imputation was not possible due to a lack of offspring of its unknown, putative UGDP1. In these cases, the possibility that the triploid was the parent of these two diploids could not be excluded.

Triploids were excluded as parents of diploids in all but 38 cases (Table S5). Of these cases, 35 could not be further evaluated using POR tests because there were insufficient offspring of the diploids in question available to use for performing the tests. The remaining three cases were unresolved.

3.3 | Comparison of historical pedigree records to SNP data

Nineteen pedigrees were identified in literature that list one or more triploids as parents of diploids (Table S8). In 18 of these pedigrees, these relationships were all deemed false based on the existence of high numbers of Mendelian inconsistent errors that exceeded the threshold for parent-offspring relationships. Additionally, in 14 of these relationships, diploid parents and/or more distant relatives were identified that fully accounted for both parents.

'Cox's Orange Pippin' was a special case, as it had only one Mendelian inconsistent error with recorded triploid parent 'Ribston Pippin'. However, phased SNP data demonstrated that one chromosome complement in 'Cox's Orange Pippin' was completely composed of haplotypes from 'Margil', which had been previously identified as both the UGDP1 of 'Ribston Pippin' and one parent of 'Cox's Orange Pippin' (Muranty et al., 2020; Ordidge et al., 2018). Additionally, to further

demonstrate that 'Cox's Orange Pippin' was the product of two diploid cultivars, the previously unknown second parent of 'Cox's Orange Pippin' was identified in this study, and UGDP1s of both triploids that were incorrectly recorded as the parents of 'Cox's Orange Pippin' were also identified (Figure 3). The second parent of 'Cox's Orange Pippin' was identified as 'Rosemary Russet'. 'Rosemary Russet' was also identified as an offspring of 'Nonpareil' in the present study.

4 | DISCUSSION

This study successfully demonstrated that SNP array data could be used to make highly accurate SNP genotype calls that could be used for large-scale pedigree reconstruction of triploid apples. The resulting pedigree reconstruction results helped clarify the typical origin of triploidy in apples (via UGDP1s rather than UGDP2s) and suggest that triploidy is a dead end in pedigrees. The results also revealed previously unknown pedigrees of many culturally important triploids and helped elucidate pedigrees of diploid cultivars as well.

4.1 | Triploid allele calling

Our results demonstrated that a high number of SNPs on the Illumina Infinium 20K SNP array could be accurately called for triploids using ploidyClassifier. Previously, Chagné et al. (2015) demonstrated the ability to differentiate triploids (and aneuploids) from diploids using Illumina Infinium SNP array data, but that study did not attempt to make genotype calls for triploids. Methods for allele calling in triploids have been previously demonstrated for the Thermo Fisher GeneTitan platform for Atlantic salmon (*Salmo salar*) (Grashei et al., 2020), but our study is the first to do so on the Illumina Infinium platform.

Following the filtering of SNPs with problematic triploid clustering (see Figure S1), the resulting data were of high quality, with very few Mendelian inconsistent errors across identified or confirmed triploid pedigrees (Table S3). We were able to further demonstrate the accuracy of the triploid allele calling by demonstrating that when an UGDP1 was identified for a triploid, the SNP data for that triploid could be fully phased (Table S2).

4.2 | Triploid pedigree reconstruction

Over half of the triploids evaluated were found to be the product of extant diploid UGDP1s (Table S3). Additionally, the RGDP was also identified or confirmed for 41% of the triploids with identified or confirmed UGDP1s. The present study is the largest apple triploid pedigree reconstruction study to date, with many of the pedigrees identified being previously unknown. False negatives (failing to identify true pedigree relationships) could have resulted, in rare instances that involved individuals with high numbers of null alleles, if null alleles could have remained unaccounted for during the SNP data curation steps due to a lack of pedigree relationships (see Vanderzande et al., 2019). Individuals with higher numbers of null alleles have tended to be those that are more distantly related to *M. domestica*, such as *Malus floribunda* 821 (see Howard, Troggio, et al., 2021). However, false negatives were considered unlikely in the present study due to the high degree of SNP data curation and because there was no indication from literature that the triploids included in this study were related to other *Malus* species, with the notable exception of the smaller fruited triploid 'Virginia Crab', which successfully saw its UGDP1 identified in this study as 'Manchurian', which is a small fruited crab classified in the USDA-ARS apple collection as *M.baccata* (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1006696).

The results of this study revealed many previously unknown pedigree links for culturally important cultivars:

'Reinette Franche', a French cultivar from the early 16th century, was identified as one of the most common UGDP1s. This cultivar has also previously been identified as a very common pedigree parent of

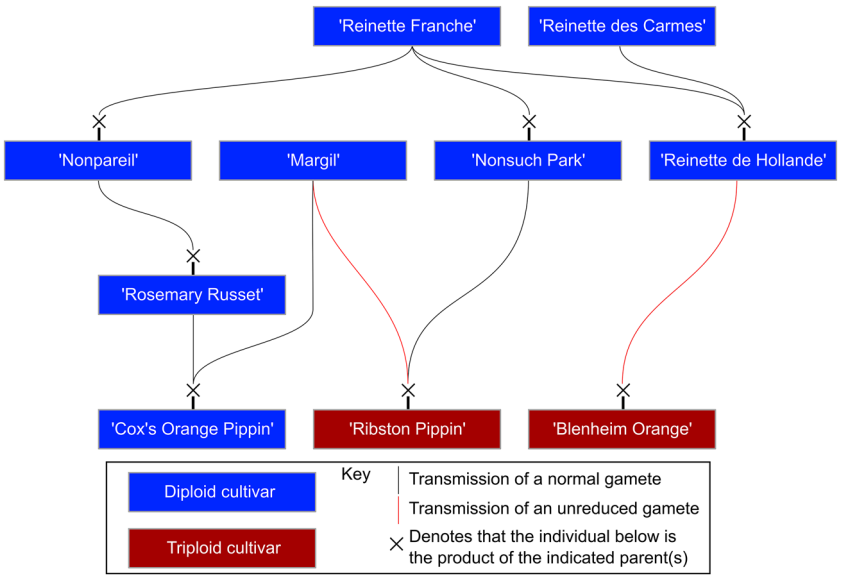

FIGURE 3 The true pedigrees of apple cultivars Cox's Orange Pippin, Ribston Pippin, and Blenheim Orange

diploid cultivars, including many historically important cultivars (Muranty et al., 2020). Thus, its high frequency as an UGDP1 in this study is consistent with its high frequency as a parent of diploids. 'Reinette Franche' was identified as the UGDP1 of 'Roxbury Russet,' which has been commonly cited as being the oldest US cultivar (Bussey & Whealy, 2016), so either 'Reinette Franche' or its seeds may have been imported to North America by European colonists in the 17th or 18th centuries. 'Reinette Franche' was also identified as a possible grandparent through an UGDP1 or as the RGDP of 'Baldwin', which was at one point an important commercial cultivar in the United States (Dolan, 2009).

'Glane' was the UGDP1 of five "Locard" apples. 'Glane' is known as a French cider apple probably originating in Normandy (Boré & Fleckinger, 2007). The 'Glane' sampled was maintained only as a single copy in a private collection and thus is very rare, in contrast with its numerous descendants (see also Muranty et al., 2020). The French "Locard" apple series is a rather large one where most of the members are triploids (Lassois et al., 2016) and a number are morphologically similar. The cultivar Gros-Locard was described by A. Leroy (Leroy, 1873) as originating in the Sarthe region near Normandy in the early 19th century. Fruits are generally big, spherical or slightly flat and very juicy. A more in-depth analysis would be necessary to associate specific pomological traits to the Locard series, but it is interesting to identify that a number of apples associated by name are also associated by unreduced gamete donating parentage and this new information provides previously unknown provenance for a regionally important group of apples.

Interestingly, the qualifier "Gros" (i.e., "big" in French) is sometimes attributed to the triploid accession names in addition to the initial names of the UGDP: e.g., 'Rouget de Dol Gros' (3n) with its UGDP 'Rouget de Dol' and 'Grosse Piquette' (3n), with its UGDP 'Petite Piquette' ("Petite" meaning small in French). Such a qualifier is consistent with the generally larger fruit size of the triploids.

4.3 | The source of triploidy in apple

Our results strongly suggest that triploid apples have historically been produced primarily or completely through the union of one unreduced gamete and one reduced gamete both originating from diploid parents, with the unreduced gamete being formed without recombination. For many of the triploids without identified UGDP1s, we could not confirm nor deny alternate origin hypotheses. However, we also identified three instances where likely or confirmed unknown UGDP1s were shared between pairs of triploids and one instance involving a trio of triploids. In the three pairs of triploids that possibly share UGDP1s, insufficient data existed to confirm the exact nature of the relationship between the triploids but, in the case of the trio, imputation of the genotypic profile of the UGDP1 from diploid offspring of the unknown UGDP1 confirmed that all three were indeed the product of the imputed UGDP1 (Table S4). It is likely that additional genotypic data for more cultivars would enable the confirmation of an UGDP1 origin for many of the other triploids that currently lack identified UGDP1s in the dataset.

Triploid production through UGDP2s (i.e., with heterozygosity reduced through recombination) is far rarer, as there were only three instances of possible UGDP2s in this study and only a single confirmed UGDP2 (Table S4). The only confirmed UGDP2 in this study was a breeding selection and was included intentionally in the study because it represented the only confirmed case of an UGDP2 across the data available for this study. The three instances of possible UGDP2s could not be confirmed because, unlike the breeding selection, no pedigree records were available for them and two alternative hypotheses for the observation of possible UGDP2s were possible. The first alternative hypothesis is that the possible UGPD2s were instead both the RGDP and a grandparent through an UGDP1. The second is that the assumed UGDP of the possible instances of UGDP2 was actually a tetraploid version donating a diploid gamete, resulting in triploid offspring. Tetraploid versions of diploid cultivars are possible, as some exist in germplasm collections such as that of the USDA collection in Geneva, NY (https://www.ars-grin.gov/). Our finding that UGDP2s are exceedingly rare is in agreement with the observation by Ordidge et al. (2018) that all but one of the triploid offspring identified in their study contained a full diploid complement (albeit, based on a small number of SSRs in the latter). Both studies were based on collected and/or named cultivars and it is possible that there has been an element of artificial selection, although the finding is also in line with that of Considine et al. (2012) which was based on seedlings prior to selection.

The low frequency of possible UGDP2s and high frequency of UGDP1s suggests triploid formation through FDR, since FDR without recombination is the only process that would retain full heterozygosity (Pelé et al., 2018). This result is in contrast to Zhang et al. (1988), which found SDR as being the more common means by which unreduced male gametes formed in a particular diploid individual. However, the atypical genetic status of this individual ("R1–23" was a selfed offspring, that is, I1, from the cultivar Lowry) may have favored a particular abnormality pattern during microsporogenesis.

Triploid production did not appear to be cultivar or cultivar group specific. We would speculate that the presence and frequency of cultivars as UGDP1s in this study is likely more a reflection of the cultural or breeding value of the cultivars rather than of their relative ability to produce unreduced gametes. Evidence of this is that some diploids that were frequently UGDP1s, like 'Brabant Bellefleur', 'Cox's Orange Pippin', 'Glane', and 'Reinette Franche' were also common parents of diploid cultivars (Muranty et al., 2020). The exact pedigree relationships among all identified UGDP1s and among the most common UGDP1s are mostly unknown, but these individuals are of diverse origin. This result is in contrast to Zhang et al. (1988) that stated some particular individuals were able to produce larger number of unreduced gametes relative to other individuals.

A possible compounding effect on unreduced gamete production is the influence of environment. However, it is impossible to understand from the present study whether certain environmental stresses caused an increase in unreduced gamete formation in UGDPs or whether unreduced gamete formation was simple chance. Cultivar-specific rates of unreduced gamete formation are not known for

apple, in contrast to *Brassica*, where individual-specific variation and cold temperatures have been shown to increase the rate of unreduced gamete formation (Mason et al., 2011). Nevertheless, Kanlić et al. (2016) suggested that triploids are more common in Southern Europe than in Northern Europe, which could point to heat stress or drought stress as inducers of higher rates of unreduced gamete formation in apple or to differential fitness for environmental or human selection at the seedling/adult stage according to the latitude or the cultural or agronomical preferences. Studies comparing various cultivars under different growing conditions could be helpful to understand mechanisms leading to unreduced gamete formation in apple.

4.4 | Diploid pedigree reconstruction using triploid data

Triploid data resulted in the reconstruction of pedigree data for many diploids through the identification of groups of half-sibs whose shared parents were the unidentified UGDP1s of triploids. A common parent of 50 diploid cultivars, dubbed "Unknown Italian Founder," was identified and subsequently 99.6% of its SNP data was imputed (Tables S5 and S6). This individual was given that name because its offspring included a set of 50 diploid offspring that are mostly very old and of Italian origin. In particular, one offspring is 'Decio', which has been widely regarded as being a cultivar that possibly originated during Roman times (Juniper et al., 1998; Smith, 1971). If this anecdote is correct and if the Unknown Italian Founder still exists in the landscape, it may be the oldest extant apple cultivar in Europe.

The genotypic profile of one unidentified parent has now been imputed for the diploid 'Ralls Janet', which is itself a parent of the important market cultivar Fuji. This parent, dubbed "UGDP Early Strawberry" because it is the unknown UGDP1 of triploid 'Early Strawberry', was also the parent of several other cultivars and the offspring of 'Reinette Franche'. 'Reinette Franche' has also now been identified as a great-grandparent of 'Cox's Orange Pippin' through this present study (Figure 3). These new results provide previously unknown links between 'Fuji', 'Cox's Orange Pippin', and many other diploid descendants of 'Reinette Franche' reported by Muranty et al. (2020).

The results of this study support the hypothesis that triploids are not, or at the very least are not commonly, the parents of diploids. There were 81 instances of triploids that share at least one allele, but not two, at every locus with diploids that had no identified parents and where the triploid's UGDP1 had neither been identified or imputed. While our results could not exclude the possibility that the diploid could be the offspring of the triploid in 39 of these cases (Table S5), alternate hypotheses that the diploid is either the RGDP of the triploid, a parent of the triploid's UGDP1, or an offspring of the triploid's UGDP1 could also not be excluded. This uncertainty was nearly entirely due to an insufficient number of relevant parent-offspring relationships available to conduct the POR-1 or POR-2 tests. Possibly additional genotyped accessions could determine whether these are diploid offspring of triploids. However, overall, there were only three instances where it was not possible to distinguish whether a diploid was either an offspring of a triploid's UGDP1 or an offspring of the triploid itself and in most other diploid-triploid relationships, the possibility of the diploid being an offspring of the triploid was denied using the POR tests.

4.5 | Comparison of historical pedigree records to SNP data

All the pedigree records considered from literature that list triploids as one or both parents of diploids were identified as false (Table S8). The only such pedigree that had less than 0.5% of SNPs with Mendelian inconsistent errors was between triploid 'Ribston Pippin' and its recorded diploid offspring 'Cox's Orange Pippin'. However, the UGDP1 of 'Ribston Pippin', 'Margil', was already previously identified as one parent of 'Cox's Orange Pippin' (Muranty et al., 2020). We further completed the pedigree of 'Cox's Orange Pippin' by identifying 'Rosemary Russet' as its second parent (Figure 3). The cultivar Rosemary Russet was first recorded in the literature in 1831 (Smith, 1971). These sorts of errors in the literature regarding pedigrees could be due to historic misidentification of cultivars. Likely in some cases triploids bear some resemblance to their UGDPs, which was likely the case between 'Ribston Pippin' and 'Margil'. The refutation of these pedigrees supports the hypothesis that triploids are not, or are not commonly, parents of diploids. Coupled with the results from pedigree reconstruction, our study suggests that triploidy is likely a dead end in pedigrees, at least concerning historical germplasm, which composed most of the triploids in this study. We speculate that gametes from triploid apple cultivars that undergo meiotic division only or mostly lead to aneuploidy, as has been previously noted in multiple studies (Crane & Lawrence, 1930; Dermen, 1936; Einset, 1945; He et al., 2018; Magness, 1937; Sedov et al., 2014), and that triploid apple cultivars do not produce diploid offspring. However, two previous studies have reported the possibility of diploid offspring of triploids (He et al., 2018; Sedov et al., 2014). In the future, SNP array data should be generated for putative diploid offspring of triploids to evaluate the SNP data phasing for these individuals using methods outlined in this study to better clarify how possible are diploid offspring of triploids. Additionally, cytogenetic analysis of gametes from triploid cultivars could be conducted to understand chromosomal inheritance from triploids.

4.6 | Conclusion

Our study demonstrates that highly accurate genotype calls can be made for triploid apple cultivars using Illumina Infinium SNP array data and ploidyClassifier, a new Python Script. The triploid allele call data generated in this study allowed us to conduct an unprecedented level of pedigree reconstruction for triploid cultivars. This pedigree reconstruction elucidated previously unknown historical information for many culturally important cultivars. The identification of UGDPs for

more than half of the triploids evaluated suggests that UGDPs are a common origin of triploid apple cultivars, with the unreduced gamete most typically being produced without recombination. Though we could not completely deny the possibility that triploids could be parents of diploids in some cases, the refutation of all tested pedigrees from literature that list triploids as one or both parents of diploids, the imputation of the two UGDPs, dubbed Unknown Italian Founder and UGDP Early Strawberry, and the denial of many triploids as parents of diploids through the use of the POR tests together suggest that triploids are either not, or are not commonly, parents of historical diploid cultivars and that triploidy is a dead end in apple pedigrees.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

NPH, DCA, and JJL conceived the study. NPH led the project and conducted the pedigree reconstruction analyses. NPH, DCA, and JJL wrote the first draft of the manuscript. DM wrote the Python script ploidyClassifier. CD, CED, and HM provided curated accession level meta-data for dataset organization. NPH, DCA, JJL, DM, CD, CED, HM, and MO provided critical feedback on the methods, results, and interpretations in the study. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Genome Database for Rosaceae at https://www.rosaceae.org/publication_datasets, reference number tfGDR1061.

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SUPPORTING INFORMATION

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