# Parentage of some Malus triploid varieties

## Introduction

A number of ‘iconic’ varieties, such as Cox’s Orange Pippin, Oxbo, Ashmead’s Kernel, were reportedly bred from parents, one or both of which were triploids. Yet it has long been known that pollen and seed of triploids have low viability. It added a mystique, perhaps because it was also inconsistent with the observed low fertility of triploids.

Triploid varieties have three, rather than the usual two of diploids, copies of all 17 chromosomes. Considine et al. (2012) and others have shown this usually arises from one parent (often the mother) donating all its DNA and the usual half (haploid) set from the other parent. However as triploids have 51 chromosomes, the normal process of cell division into two by meiosis is messed up and results in both pollen and ovules with gametes likely having more than a haploid set. Hence they have low viability.

The advent of DNA has revealed that there are few if any examples of triploids as parents. Cox’s Orange Pippin, like other progeny of a purported triploid is actually from a pair of diploids, in this case Margil x Rosemary Russet. The mystique has evapourated.

MAN has shown that for barely half the documented parentages record by Smith (1971) does SSR support this.

Several groups have studied the parentage of apple varieties and begun to develop family trees with which pedigrees can be shown. Ordidge et al. (2018) used SSR and DArT to assess accessions among the 2138 varieties at the National Fruit Collection (NFC), of which 287 are triploids. They found the parentage of 15 triploids matched the documented parentage and at least one parent of a further 37 varieties was found.

Hélène Muranty et al. (2020) built upon this work and employed DNA SNP whole-genome data in a study of parentage and pedigree of over 1400 varieties held by European collections. Its prime focus was on diploids, however they also considered triploids. By applying various tests, they found 56 triploids had an inferred parent-progeny relationship where one of the parents contributed a diploid 2n-gamete, i.e. it contributed its entire chromosome bundle. However, there remained uncertainty, as it is possible that they are siblings. In a few cases there was confidence in identifying one or both as parents rather than siblings,

Howard et al. (2022) have used SNP data for studying parentage of 2498 diploids and 219 triploids. They found no examples of a diploid having a triploid as a parent, including some recorded in historic literature compiled by Smith (1971); it thus refutes all those prior documented cases investigated. For over 100 triploid progeny, a diploid parent was identified, and of these a second parent was found for about 40.

Other triploid varieties found as part of the fruitID SSR campaign, together with SSR data in fruitID Explorer-P2P v 7.42, have been analysed and added to this growing dataset.

These and other studies have begun to unlock what had been something of a mystery. Comparing morphology of progeny with parents, particularly with that of the diploid donating gamete (usually the mother) often reveals close similarities further building confidence in these relationships.

Results from these studies are compiled into a single file of parentages that may be readily searched. In it, wherever possible, parentages with the higher confidence level that comes from SNP analysis are quoted rather than SSR. In some cases these are augmented with additional findings. It is available under the Research pages.

While triploid varieties may have desirable properties, such as a greater disease/pest resistance, crop yield, and size, they appear to have little or no use for further breeding. Howard et al. (2022) have further argued that triploids are essentially an evolutionary dead-end.

Two examples are presented of (partial) family trees based upon these deduced parentages, That of Reinette Franch x Reinette des Carmes is covered in the article on Siblings. Dutch Mignonne has been found by Ramos-Cabrer et al. (2007), Ordidge et al. (2018), Muranty et al. (2020), and Howard et al. (2022) as a parent to a dozen varieties as illustrated below.

In this family tree the thicker lines indicate donation of a diploid gamete. These progeny are various degrees of siblings; not even Zabergaurenette and Tequenne are conventional full-siblings.

Some of the parent-progeny relationships are illustrated in a series of ‘parentage panels’ that give SSR and Dart results along with photographs of parent(s) and progeny. They are available under the Research pages.

The original report of 2022 describing SSR/DArT methodology, results and morphological comparisons is available on the fruitID.com help pages under ‘Genetics and Plant Breeding Science’.

## Genetic expectation

In a fascinating study Michael J. Considine et al. found that natural crosses between a range of Malus diploid varieties may lead to diploid (ca. 99% of crosses), triploid (ca. 0.25%), tetraploid (ca. 0.05%) and aneuploidy (ca. 0.7%) varieties. Triploid varieties usually result from a (maternal) diploid 2n-gamete being fertilised by (paternal) haploid pollen. As a consequence, the progeny inherit the entirety of the maternal DNA, but only half the paternal DNA. Considine et al. illustrated how these outcomes may arise during meiosis I and II in the elegant figure above.

Finding cases where a triploid contains all alleles of a diploid variety is evidence that the former maybe progeny of a diploid variety; it is generally thought more likely to be maternal than paternal. There are complications when the diploid variety has sports or mutations, as the DNA SSR is likely the same for all these, and thus it is unclear which diploid to assign as parent. Statistically, it is unlikely that a triploid variety will appear to be a full-sibling of a diploid progeny of the same maternal parent, even if crossed with a paternal parent with very similar DNA.

When using DNA SSR fingerprints for studying parentage, another complication can arise if the variety thought to be a triploid is actually an aneuploid with a few less than 51 chromosomes. This, it appears, may arise from a cross between a (maternal) haploid gamete being fertilised by pollen from an aneuploid paternal gamete. Such aneuploids may be interpreted to be triploids on the basis that because several of the marker-pairs are tri-allelic, the other (partially) homozygous marker-pairs are also to be assumed as tri-allelic with some allele counts duplicated (or triplicated).

Interestingly, Considine et al. found that none of the 27542 F1 crosses they studied had 2n+2 = 36 chromosomes. Either one extra or at least three seems favoured. This may have guided the approached employed by Urrestarazu et al. of treating varieties with three or more tri-allelic marker-pairs as triploids (their study used 16 marker-pairs rather than 12 as in EMR studies).

Considine et al. also noted that all aneuploids in their study, which here may be interpreted as triploids as a result of (partial) SSR homozygousity in some of the marker-pairs, arise from fertilisation of a (maternal) haploid gamete and an aneuploid (paternal) gamete.

De Storme and Mason presented a review of meiotic cell division and how diploids, triploids and aneuploids arise in a wider range of plant genera. There remains a possible third mechanism for formation of a (apparent) triploid by crossing of two aneuploids which, it is thought, are often to be viable.

It raises a simple question. Do triploids result from a cross of diploid and haploid gametes, and if so is it the dominant route? A way to begin answering this is seeking examples where maternal parents or siblings can be identified with some confidence. If a significant fraction of extant triploids have an identifiable parent or sibling, then natural (unforced) breeding is more likely a larger contributor. This study is an attempt to contribute towards answering the question. It builds upon the signal work of Ordidge et al. (2018) and Muranty et al. (2020), though has now been partly superceded by Howard et al. (2022).

Whether varieties that are assumed to be triploid are actually aneuploid and thus have a different way for genetic inheritance requires a separate assessment of ploidy, from flow cytometry or whatever. It is a subject for subsequent investigation.

Provenance of the varieties were taken from the NAR (Smith, 1971) or from any accreditation reports available on fruitID.

## Analysis for finding the maternal parent

DNA SSR fingerprinting samples about one base pair in 500,000. Despite this sparseness, the elegant design of the SSR-12 methodology results in a beautiful method for identification, and increasingly to parentage assessment, based upon matching with a reference collection. There is little ambiguity. For understanding of SNP analysis and interpretation, you are cordially invited to read papers by Muranty et al. (20110) and Howard et al. (2022). Parentage results from SSR are open to some interpretation, whereas SNPs are unambiguous.

SSR methodology does however reveal many of the principles behind SNP approach and so is worth introducing. There is a large pan-European SSR dataset now available, and this warrants exploiting to the full. First diploids are found whose SSR fingerprint was contained in entirety, or nearly so, by a triploid variety. These are generally considered more likely the maternal than paternal parent. Then the mother-progeny pair was checked to see if a diploid variety could contribute the missing alleles of the triploid progeny. Wherever choice has to be made in expanding homozygous marker-pairs or the matching between parents and progeny the most favourable case was selected for avoiding missing possible connections. Finally, checks were made for those with available DArT score, provenance and morphology. Here a few details are given to amplify the method.

The dataset used encompasses genotypes taken from Explorer-P2P v7.42 which comprises 5287 accessions, of which 4537 are diploid (2n, 2n+1), 734 are triploid (3n and 3n+1), and 16 are tetraploid. Some of these are indeed aneuploids.

For testing whether the entire (or most of) diploid fingerprint was included in the triploid, fingerprints of diploids and triploids were first expanded to remove the homozygosity in each marker-pair, rather in the manner of Hélène Muranty et al. As an example of this, take three different hetero- or homozygous marker-pairs of the variety Baker’s Delicious.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker-pair | fingerprint | | | | expansion | | | |
| CH04c07 | 106 | 114 | 129 | 0 | 106 | 114 | 129 | 0 |
| CH01h10 | 96 | 107 | 0 | 0 | 96 | 96 | 107 | 107 |
| CH01f02 | 205 | 0 | 0 | 0 | 205 | 205 | 205 | 0 |

The first and third are certain within the limits of assumed triploidy. The second expansion covers against the two possibilities {96,96,107,0} and {96,107,107,0}. For diploids the expansion of a homozygous marker-pair is certain e.g. for Colwall Quoining CH01h10 {96,0,0,0} becomes {96,96,0,0}.

Examples of the required matching between a triploid, here given on the first line, and its two parents, on second and third lines, are illustrated here.

For Horneburger Pfannkuchen marker-pair Hi02c07 the three alleles of triploid are different to each other {114,116,118,0}. Two of these can come from the diploid gamete Boiken and one only is needed from Prinzen Apfel, the 114; 110 is not inherited.

In some cases both parents have are homozygous and have the same value of allele, there are actually four (parental) alleles, in the case of Mutsu CH04e05 it is 173, available for inheritance. DNA SSR doesn’t differentiate between these.



Oranje de Sonnaville Hi02c07 shows that both parents have the same fingerprint {116,150,0,0}. In this case the triploid (unexpanded) fingerprint will inevitably match its parents.

For Horneburger Pfannkuchen marker-pair CH01h10 it is homozygous with three instances {96,96,96,0}. It can acquire one each from the given two parents Boiken and Prinzen Apfel, but not a third. In this case the allele 98 may be a mis-interpretation or glitch; the corresponding allele in Horskreiger is indeed 96. That Ordidge et al, found Boiken the diploid parent suggests that a glitch did occur.

Diploid-triploid pairs with at least ten marker-pairs containing two alleles in common were evaluated in Excel. These are possible diploid parent-progeny pairs. They were manually screened and only the only instances accepted were with a complete match of all 24 alleles or with a single mismatch of no more than 2 bp. About 70 triploid varieties and possible diploid gamete parents were identified, and of these 52 are ones previous identified by Ordidge et al.

Haploid parents were found with Explorer-P2P using the mother-progeny relationship as a basis.

A fascinating group of siblings discussed on our website under Siblings, is with the old French varieties parents Reinette Franche x Reinette des Carmes. The siblings Reinette d’Anjou and Braddick Nonpareil likely have Reinette des Carmes as diploid gamete donating parent, whereas Claygate Pearmain has Reinette Franche. They are full siblings, well, not quite; there are mismatches of 6 and 10 bp between them. Perhaps they should be called ‘two-thirds plus siblings’.

It may be noted that both parts of Explorer-P2P, “master” and “grid”, were developed originally for seeking parentage of diploid varieties. Neither were fully developed for seeking parentage of triploids that inherit diploid from one and haploid gametes from the other. The shortcoming in “Master” is indeed miniscule and rarely arises; that in “Grid” is more significant. When looking for parents of a triploid, double counting of allele matching may occur, something not evident with diploid parent matching. In the case of Horneburger Pfannkuchen the Boiken and Prinzen Apfel CH01h10 alleles of 96 is treated as if it is they could be duplicates so that the three Horneburger Pfannkuchen alleles effectively derive one each from these two parents and a third by the implicit double counting.

Users of Explorer-P2P are advised to make manual checks on any triploid parental match to see whether a full diploid gamete of parent is common to the triploid.

This additional constraint was implemented into the algorithms and workflow used in this study. However, the experience of doing so suggested inclusion into Explorer-P2P would likely make it an unwieldy addition.

## Analysis for finding siblings

It may happen that the maternal parent is no longer extant, or hasn’t yet been fingerprinted. In this case finding two or more varieties that contain the same diploid set of alleles suggests strongly they had a common parent, and are thus siblings. If there is no diploid parent that can be identified, they are likely orphaned siblings.

### Established siblings

This investigation was used as a test of the methodology before applying it to find orphaned triploids. Triploids were compared against each other to find families that had at least 24 alleles in common with at least two per marker-pair.

From the DNA SSR-12 study, four sets of half (or full) siblings were identified and investigated having a (probable) diploid gamete in common. These were tested to check that DArT score between parent and progeny supported them. In all case the DArT score is >0.79, this is encouraging, especially as scores between progeny of different parents is <0.70.

Some example families are listed below.

|  |  |  |  |
| --- | --- | --- | --- |
| **Diploid gamete donating parent** | **Triploid Progeny** | **DArT score of triploid with diploid gamete** | **Haploid parent** |
| Brabant Bellefleur | Belle-Fleur de France | 0.85 |  |
| Brabant Bellefleur | Belle-Fleur Large Mouche | 0.81 |  |
| Brabant Bellefleur | Dubbele Belle Fleur (VT) | 0.85 |  |
| Brabant Bellefleur | Gros Croquet | 0.84 |  |
| Brabant Bellefleur | Marie Doudou | 0.86 |  |
| Brabant Bellefleur | Marroi Rouge | 0.79 |  |
| Cox's Orange Pippin | 3022 | 0.83 |  |
| Cox's Orange Pippin | Carswell's Orange | 0.82 | Worcester Pearmain |
| Cox's Orange Pippin | Holstein (LA 69A) | 0.83 | Ananas Reinette |
| Cox's Orange Pippin | Honey Pippin | 0.81 | Worcester Pearmain |
| Cox's Orange Pippin | Jupiter | 0.80 | Starking |
| Cox's Orange Pippin | Oranje de Sonnaville | 0.83 | Jonathan |
| Cox's Orange Pippin | Polly Prosser | 0.90 | Duke of Devonshire |
| Cox's Orange Pippin | Suntan | 0.82 |  |
| Dutch Mignonne | Belle de Boskoop | 0.79 |  |
| Dutch Mignonne | Bielaar | 0.82 |  |
| Dutch Mignonne | Reinette Coulon | 0.80 |  |
| Golden Delicious | Charden | 0.85 | Reinette Clochard |
| Golden Delicious | Jonagold | 0.87 | Jonathan |
| Golden Delicious | Mutsu | 0.87 | Indo |
| Golden Delicious | Mutsu spur type | 0.86 |  |

### Orphaned siblings methodology

Now on applying this methodology to varieties where either the maternal parent has been lost or it has not yet been fingerprinted, should find groups of orphaned progeny. Over 100,000 pairs were checked with the ‘most advantageous’ expansion of homozygosity applied. Progeny of known diploid parents were excluded. Only those with at least ten marker-pairs each having two alleles in common and, for those with marker-pairs having mismatching alleles, they were excluded if a mismatch exceeded 2 bp. It reduced the number of pairs of possible orphan pairs to less than 100, allowing manual inspection of them all.

|  |  |
| --- | --- |
| Cockpit | Improved Cockpit |
| Huntingdon Codlin | TVm168 |
| Jersey Beauty | A1337 |
| Lady Henniker | Vicar of Beighton |
| Muscadet de Dieppe | Omont |
| Peter Lock | A1996 |
| Graue Herbstrenette | Reinette de Macon |

Further inspection showed there were some duplicates, sports and mutations. This reduced the number of pairs to about 40.

### Siblings

#### Pairs of triploid siblings

Seven pairs of possible siblings lacking a known maternal parent are suggested from this analysis.

#### Multiple triploid siblings

There are another seven more families of varieties, 24 in total, which may be siblings of each other, while lacking a known maternal parent. For two varieties, A484 and Dredge’s Fame the DNA match with other possible siblings is unconvincing.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Osnabrucker Reinette | Wheeler's Russet | Pinner Seedling |  | | |  |
| Gaillarde | Carrara Brusca | Rose de Bouchetiere |  | | |  |
| Gros Locard | Bassard | Pomme de Choux a Nez Creux | Pomme de Glace |  |
| Cornish Pine | A510 | A514 | Charlotte Daunt | A484 \* |
| Philadelphia | TVm122 | TVm234 |  | | |  |
| Ponsford | Profit | A060 |  | | |  |
| Dredge's Fame \* | Vajki Alma | A1333 |  | | |  |

\* these are much less likely to be siblings

## Family occurrence in collections

In their study Considine et al. found that the occurrence of triploids was 0.26% of ca. 19000 intra-specific Malus Domestica seedlings that they raised. The occurrence of aneuploids was 0.66%. These were suggested as indicative of the natural occurrence of such polyploidy. For these both parents and progeny are known.

Are triploids families more frequently found in the NFC or other collections than this natural occurrence? Yes, definitely.

From Explorer-P2P v7.42 the NFC has 247 triploid (3n or 3n+1) SSR-genotypes, though some may be aneuploids.[[1]](#footnote-1) Fernadez reported that cytometry showed 225 NFC genotype varieties to be triploids rather than aneuploids. Of another 20 varieties without cytometric analysis, all might be triploids, 16 of which have SSR with a ploidy of at least 2n+5, meaning that at most seven marker-pairs would have to be (partially) homozygous for them to be triploids (Bramley’s Seedling has only five heterozygous marker-pairs). There are a few without clear ploidy. It suggests a predominance towards triploidy rather than aneuploidy.

Of the NFC genotype triploids, 65 have a diploid gamete parent (probably) identified, or 25%. During the eight years of the fruitID campaigns a total 1543 genotypes are listed in Explorer-P2P v7.42 of which 267 are triploids. That is about 17%.

The NFC has accumulated families with a parent and triploid progeny at an occurrence approximately one-hundred times greater than the frequency reported in breeding trials by Consideine et al. For the fruitID set something similar is seen. Some selection of varieties must have arisen from breeder and owners.

## Glossary

Allele Normally taken to be one of two or more versions of a gene. Here it is an oligonucleotide fragment extracted during the SSR anaylysis, that is one of the short DNA sequences of ca. 80-260 base pairs copied from that part of the genome which does not code for proteins. It is essentially synonymous with SSR.

Aneuploid A variety that has a non-integral complement of chromosomes. For Malus domestica having one extra chromosome appears not uncommon, that is having 35. They can arise spontaneously. The ability to reproduce, and tendency to gain or lose one or more chromosomes, is unclear. While SSR analysis can give an indication of the number of chromosomes, homozygosity may mask the total number of chromosomes.

Diploid A variety that has two sets of chromosomes, one from mother and one from father. For Malus domestica (and most Malus species) the chromosome number is 17.

Diverse Array Technology <https://www.diversityarrays.com/> A technology to reduce complexity of genome by assessing the occurrences of one or more given nucleotide sequences in two related varieties.

fruitID <https://www.fruitid.com/#main>

Gamete Reproductive cells, with male (pollen) and female (ovum). Usually they are haploid, that is they have half the number of chromosomes of the variety from which it originated (i.e one or other of each of the pairs of chromosomes). In this study diploid gametes are also considered in which the ovum (possibly pollen) contain all the chromosomes from that parent.

Genotype (SSR) Refers to entirety of an individual’s genome. Here it is used to indicate all individual Malus that have a particular SSR-12 fingerprint in which the quantity chksm\_stdev is the same where chksum is the sum of all 48 alleles and stdev is its standard deviation.

Heterozygous When the (non-zero) alleles for a given marker-pair are different to one another, e.g. for a diploid {88,96.0,0}, or triploid {88,96,104,0}, the marker-pair is referred to as being heterozygous.

Homozygous When the (non-zero) alleles for a given marker-pair are all the same, e.g. for a diploid {88,0.0,0}, the marker-pair is referred to as being homozygous., though for a triploid with three non-zero alleles {88,96,0,0} is partially homozygous.

Marker-pair Two short oligonucleotide fragments (each typically about 15 nucleotides long) that are the (DNA) complements of the end members bounding a SSR. In the case of the NFC and fruitID studies a set of 12 marker-pairs are used aligned with the ECPGR recommendation. Details on the NFC website and for marker-pairs (more commonly just called markers) are <https://www.rosaceae.org/search/markers>

National Fruit Collection <http://www.nationalfruitcollection.org.uk/>

Simple Sequence Repeat (SSR) A simple and repeated sequence of nucleotides (e.g. ATATAT or GCGCGC) contained between two end-members and which is relatively close to a gene but which does not code for proteins. A SSR is not subject to (normal) evolutionary pressures, yet these regions have (fortuitously) variable lengths in different varieties.

Single Nucleotide Polymorphism (SNP) This arises when a single nucleotide (A,T,G, or C) in the genome (or other shared sequence) differs between members of a species or paired chromosomes in an individual. Here it refers to such a difference being found in an extracted length of the genome (chromosome of whatever) between two or more different varieties of Malus. Often SNP is pronounced”snip”.

Triploid A variety that has three sets of chromosomes, which for Malus is 51. They arise as a result of Meosis I or II failures either spontaneously or induced chemically (etc.) which produce a diploid gamete, It may then be fertilised by a haploid gamete. Whether there are other mechanisms by which triploids form is unclear.

Tetraploid A variety that has four sets of chromosomes, which for Malus is 68. Similar to triploids, they arise from fertilisation of a diploid gamete with another such, or between a triploid gamete and a haploid. Whether there are other mechanisms by which triploids form is unclear.

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## Acknowledgements

Many fascinating discussions with Mike Porter, Dr Matthew Ordidge, Dr Danny Thorogood, Peter Laws, Bob Lever, Liz Copas and John Teiser have raised my awareness of genetics and subtleties, I am most grateful to them and many others for imparting their understandings and of their patience.

1. Considine et al. found no occurrences of 2n+2 nor 2n+4 ploidy. Of the 247 triploids 7 are 3n+1. [↑](#footnote-ref-1)