# Research into analysing apples with pear marker-pairs

## Motivation of Research

Confidence that two varieties, Cherry Pearmain and Skyrme’s Kernel, are indeed different was a major spur in developing this research study.

These varieties have subtly different morphology, but substantially more different blossom and leaf opening times, taste and tree habit. There is only one difference in the apple SSR fingerprints in the second allele of CH01f03b being 162 vs 136. Details have been described in the accreditation Registration Request Evidences for them.

Dr Danny Thorogood of IBERS was asked by Museum of Cider to test further the fingerprint difference by running samples with the standard set of pear marker-pairs, of which only GD147 is common with apples.

Several marker-pairs were found to have differences in excess of 2bp and are discussed in the section on Cherry Pearmain and Skyrme’s Kernel below.

Two benefits were seen. As above it could provide additional SSRs with which to assess whether two (similar) fingerprints were indeed the same or different. And in assessing a parentage and progeny relationship, the additional SSRs would increase its confidence.

## Sample checking

Samples for analysis pear marker-pairs were generally taken from the same tree sampled in earlier fingerprinting studies. For convenience and in some cases necessity, some of the samples were taken from trees at Paramor and Ty Glyn that had not been previously sampled. Twelve trees were resampled and analysed conventionally for avoiding inadvertent propagation or sampling errors. It provided a benefit to MAN for confirming no propagation errors have occurred. Results are listed in the table below.



Most MAN samples were found to match the NFC accessions or prior samples, including A4870, A4871, A4874, A4875, A4876, A4877, A4899, and A4900

A4878 matched A636 and fourteen other samples; they have a single allele differing in CH01h01 by 4 bp from the NFC ‘Keswick Codlin’.

A4883 was found to be Irish Peach, not as named at Berrington Hall ‘Nutmeg’.

A4901 from tree CU Dan y Coity 3 was found to match Corras, whereas the earlier fingerprint A4010 had two alleles each mismatching by 1 bp, and two with mismatches of 10 and 18 bp. It was partly to check this substantial mismatch that this tree was included in the research set.

A4872 is to be repeated by EMR.

That these analyses confirm prior ones lends confidence to all other samplings being most probably correct. Nutmeg and Ancient Brownlees Russet are now recognised as exceptions.

## Preliminary observations

All but one of 37 samples investigated with the SSR pear marker-pair set had previously been identified to be diploids from their SSR fingerprints with apple marker-pair sets.

Of the twelve original marker-pairs used for identification of pears, GD96 is no longer employed by EMR, CH03g07 was uniformly reported as nuls, and EMPc11 was found to be {138,0,0,0} for all samples.

All results are listed in Appendix A.

Of the 37 samples, there are 16 that are tri-allelic and 5 that are tetra-allelic in the CH05c06 marker-pair. This does appear unlikely an experimental glitch. By contrast among apples with apple marker-pairs only two of the 10000+ in Explorer v7.35, Rhead's Reinette and A580, have one maker-pair that is tri-allelic and another that is tetra, none are solely tetra-allelic in just on marker-pair and di-allelic in the other eleven.  For one sample TCTOP3 (= Lingens Lily), P1227, is also tri-allelic in CH04e03. I rather doubt that suddenly we've found hidden triploids and tetraploids. It seems another explanation is required....

It might be that marker-pair CH05c06 picks up two instances of the SSR rather than the usual one. I suggest that, as the pear marker-pairs weren't designed for Malus, there will have been no specific attempt or need to avoid such situations. It might also extend to CH04e03. Perhaps duplication arises somehow associated with the genome wide doubling in Malus x domestica

 <https://www.nature.com/articles/ng.654.>

As there is one marker-pair in common between the apple and pear marker-pair sets, GD147, a comparison of results for these provides an independent check on reproducibility. The table below lists these alleles for all 37 samples.

Twenty two samples had no differences. Fifteen did. Differences of 1 or 2 bp are seen in 40% of the sample set. Are these differences experimental glitches or something systematic? Typically allele lengths differ by 1-2 bp in a few %, and usually <1%, in routine apple or pear identification results. Of eight samples for which the second allele differs by +2 bp, four have an apple SSR of 154, and three have 143, and one 137. All instances with apple SSR of 141 or 152 differ from the apples as pear SSR. The five cases with a difference of -1bp in second allele are with apple SSR of 148 and apples as pear 147, a call that is one of the most frequently difficult call among apple SSR interpretations. These thirteen cases do suggest a systematic cause, perhaps in a slight difference of calibration standard set.

On this basis, when comparing alleles in the apples as pears set, differences of 1 bp are ignored, but 2 bp are retained. Arbitrary, yes, but ignoring differences of 2 bp would detract so much from the differences that the study would be undermined.



## Results with pear marker-pairs – identities and parentage

Selection of the 29 varieties was made in summer 2021 and a further 8 in 2022. Since then new Malus SSR information with apple marker-pairs and studies has clarified earlier uncertainties. Some of the selections now appear less interesting than previously thought.

### Nutmeg Pippin

MAN has an accession of Nutmeg Pippin from Scott’s; the DNA A1202 matches the NFC accession of the same name. MAN had a tree at National Trust Berrington Hall, named Nutmeg, fingerprinted as A1239 several years ago. There were 13 minor mismatches of 1-3 bp compared with A1202. NT permitted MAN to have scions for accessing Nutmeg into MAN’s collection; it was thought to be a good case to test SSR with the pear marker-pair set. Results from the conventional fingerprinting showed that a propagation error has likely occurred as A4883 was found to be Irish Peach. The pear marker pair result P1222 is thus also of Irish Peach.

### Leebotwood

The reason Leebotwood was selected has now disappeared following further close study of DNA records.

### Wyatt’s Endurance and Blanc Mollet

The reason for this probing these two varieties was their seemingly similar SSRs despite being having different morphology. The confusion arose from an erroneous SSR number in the accreditation Registration Request and WPCS Welsh Heritage Pomona. As the varieties are now seen to have both different morphology and SSR, there is no longer a concern or a confusion.

### Cherry Pearmain and Skyrme’s Kernel

In the standard SSR fingerprint, there is only one difference in the apple SSR fingerprints in the second allele of CH01f03b being 162 vs 136, a difference of 26 bp.

The initial study by Dr Thorogood of using the pear marker-pair set showed thee marker-pairs were found to have differences in excess of 2bp:

EMPc117 with {106, 106} vs. {100, 106},

CH02b10 with {132, 134} vs. {126, 138},

CH01f07a {193, 193} vs {175, 191}.

These differences supported the standard apple DNA fingerprint finding that these two varieties are genetically different and could be accredited as such.

Results were repeated at EMR as part of this wider study:

EMPc117 with {105, 105} vs. {99, 105},

CH02b10 with {130, 132} vs. {124, 138},

CH01f07a {192, 192} vs {173, 190}.

These small differences between the allele lengths recorded at Aberystwyth University IBERS and EMR are 1 or 2 bp and are likely of experimental origins, calibration, stutter, overlapping peaks etc. Most significantly both labs show a close consistency. It indicates that Cherry Pearmain and Skyrme’s Kernel do have substantially different SSR, and using the pear marker-pairs increases the confidence in discriminating between varieties with closely similar ‘apple marker-pair’ SSR.



### Tupstones and Hagloe Crab (Phelps),

An early example of an ambiguous identification was noted at the RLC accreditation meeting in August 2019. It was seen that Hagloe Crab (Phelps) A295 had the same fingerprint as Tupstones A1177 but for a mismatch in one allele of CH01f02 by 12bp. Both these fingerprints and two repeats (A4899, A4900), which confirm the earlier results, are shown in the panel to the right. Furthermore, both have similar morphology. It was thought that one may be a mutation of the other, or possibly they are full siblings.

It was one of the key examples that triggered this piece of research. Would analysing both varieties with the pear marker-pairs support or refute the similarity of fingerprints?

For all alleles, they match exactly. It further supports their genetic similarity, giving some added confidence that one maybe a mutation of the other rather than them having a parent-progeny or sibling relationship.

### Corras

A sample from a tree known locally as Ancient Brownlees Russet A4010 (source unknown?) was found to be similar to Corras A500, accessed from Trevithel near Three Cocks, but with two mismatches in CH01h01 of 1 bp, in CH01f03b of 18 bp and in GD147 of 10bp. Morphologically they appeared very similar.

From experience with Tupstones and Hagloe Crab (Phelps), it was considered that both Corras and Ancient Brownlees Russet should be repeated and run with pear marker-pairs too in case the conventional markers failed to distinguish a difference.

Results of the two original and two new fingerprints (A4874, A4901) are shown opposite. Now it was reported A4901 matches that of Corras, and A4010 is established to be wrong.

What of the fingerprint obtained with pear marker-pairs?

P1212 and P1226 match one another exactly, just as do conventional fingerprints A4874 and A4901.

The results also support the view that A4010 is erroneous.

### Martin Nonpareil

Among many successes, the DNA SSR campaign has enabled parentage of some varieties to be proposed with some confidence. Many cases give support to those reported in the literature, especially those by breeders at government research stations or major nurseries. However, more than half the reported parentages have been found inconsistent with SSR fingerprints, and thus are implausible.

Martin Nonpareil was accredited at the RLC6 in 2021. There is strong morphological and circumstantial evidence that supported the case. Yet the parentage study suggested the only plausible parents were Keswick Codlin x Chatley Kernel. This is problematic as provenance of the former parent is contemporary and of the latter about 100 years after the progeny. Yet provenance records are not infallible.

As an example of the uncertainty on provenance data, Pearson’s Plate A2119, a triploid, probably has as maternal parent Gipsy King A398. The entire diploid SSR is incorporated into the progeny’s triploid SSR. Morphologically this looks plausible. National Apple Register gives Pearson’s Plate as described in 1831 whereas Gipsy King as catalogued in 1872. Despite the seeming contrary provenance, it is worth noting that SSR indicates plausible parents of the latter to be Reinette Franche and Reinette des Carmes, old French varieties from medieval times and which are also parents to varieties such as Golden Reinette and Hubbard’s Pearmain that have an earlier provenance than Pearson’s Plate. Gipsy King might well have been bred before 1800.

It was wondered whether analysing the trio Keswick Codlin, Chatley Kernel and Martin Nonpareil with pear marker-pairs might alternatively show that the parentage was unlikely, and therefore not call into question the provenance dates.

Results of P1218, P1221 and P1216 are shown to the right. All but one allele are consistent with the possible parentage, and the exception has a mismatch in CH01f07a of 1 bp. It is more likely an experimental glitch than indicative of an error in assigning parentage.

The puzzle of parentage and provenance remains.

### Wrought of Beet and Sops in Wine

Anna Baldwin has noted that there is much confusion surrounding red fleshed apples. A well-known variety is the ornamental Polish Niedzwetzkyana. Its colouring is intense and extends into the cambium of branches, veins of leaves and tissue of young leaves. A heritage variety, Sops in Wine, is described by Hogg (1884) and Vintage Fruit (1886).

There are trees called Sops in Wine variously:

NFC 1992-133 is thought to be typical example, whereas a matching accession under the name Devon Crimson Queen were questioned.

NT A1449 described as false matches A1023 from Endsleigh Gardens Nursery and others do not match the NFC accession.

SAR (MAN) A5482 was sourced from NT as Sops in Wine (and is a fair match to A1449) is an exact match with the accession ISSA\_118NIEDZWETZKYANA\_TYPE\_LIMERICK\_05

MAN A2416 was accredited with a (temporary) name Wrought of Beet. It is a match to an unnamed trees A4552; these are very similar to the morphological description by Hogg of Sops in Wine, though perhaps of a better dessert quality.

This suggests A5482 and A1449 and their (closely) matching samples A1023, A2843, A4090, A5323 and A056 match to ISSA\_118NIEDZWETZKYANA\_TYPE\_LIMERICK\_05 2022

These are different from, yet have some similarities to, Wrought of Beet and to NFC Sops in Wine, with ca. 10-12 ‘apple’ and 7 ‘pear’ alleles in common. At most these three genotypes might be half-siblings.

There are a number of other accessions of red-fleshed apples including groups with genotypes A1021, A3256, A059, A057, A2026. These are under separate study but appear to be related to the Polish NIEDZWETZKYANA

### Roxbury Russet

In a study of parentage of triploid varieties by Stephen Ainsleigh Rice that you can find at

<https://www.marcherapple.net/wp-content/uploads/Malus-triploid-variety-parentage-10Apr22.pdf>

it was proposed that Roxbury Russet has Herceg Bathyanyi alma as it’s maternal parent. This was based upon the implication of having the entire apple SSR of Herceg Bathyanyi alma 1948-375 contained within Roxbury Russet 1952-111.

It causes an issue with provenance, though. National Apple Register has first date for parent as 1872 and progeny as early 1600s! Also while Ordidge et al. (2018) had noted the SSR inclusion they also recognised that a low score of 0.53 in the Diverse Array Technology (DArT) ran contrary to a parentage relationship.

Dr Ordidge has subsequently pointed out that Herceg Bathyanyi alma is a synonym of Reinette Franche, the provenance of which is to medieval times. Also there is a suspicion that one or other of the samples used in DArT analysis had been mis-sampled. Given the DArT scores between Reeinette Franche and two of its progeny, Golden Reinette and Golden Harvey are also quite low, 0.64 and 0.62 respectively, the suspicion remains that this (rather than Roxbury Russet) was mis-sampled.

Using pear marker-pairs provides an additional check on the parentage. Of the ten marker-pairs included, five have exactly the entire putative parent SSR included in progeny, and a further four do so with alleles that differ by 1 or 2 bp, plausibly experimental glitches. Only one is a puzzle, CH05c06. On unpicking homozygosity, there are two matching alleles of 99. However, it appears that for apples this marker-pair finds two instances of the SSR, as this is often tri-allellic and sometimes tetra-allelic. That neither 114 nor 123 were included in Roxbury Russet might indicate a further limitation or complexity in using ‘apples as pears’ SSR.



### Golden Harvey

Reinette Franche has a synonym Hherceg Bathyanyi alma used at the NFC. Nick Howard (2022) has advised that SNP shows this to be a parent of Golden Harvey.

The conventional SSR also shows that such a parentage is plausible.

Finally the SSR with ‘apples as pears’ is fully consistent with this parentage.

The other parent is as yet unknown



### Bringewood Pippin, Downton Pippin, Grange, Yellow Ingestrie

 These four varieties were bred by Thomas Andrew Knight between 1792 and 1806. He stated that all were from Golden Pippin, with Bringewood Pippin a cross with Golden Harvey and the others a cross with Orange Pippin. They are so similar morphologically and SSR that it is hard to believe they are not full siblings. Nick Howard (2022) states that SNP shows Golden Harvey is a known parent common to all four. Furthermore the other unknown parent is itself progeny of Reinette des Carmes, which is given as a synonym Karmelitská reneta in Ecxplorer-P2P Malus v7.41

This situation was presented to and approved at the RLC7 meeting at Reading University of 15Nov22.

From these relationships, conventional and ‘apples as pears’ SSRs of the unknown parent can be reconstructed. The conventional SSR has just five alleles uncertain, two having either of the values of Reinette des Carmes (CH01h01 & CH02c11), and the other three being undetermined (CH01h10, CH04e05 & CH02d08). SSR with ‘apple as pears’ has five alleles undetermined, of which two (EMPc117 & EMPc11) are often homozygous, two for which all four siblings have the allele values (CH03d12 & CH01d09) and that of CH01d08 is unconstrained again because of similarity of the parents allele values.

Re-constructing the SSR of Golden Pippin (or whatever it actually was), there are no inconsistencies between SSR of Golden Harvey. the four siblings nor with the conventional one for Reinette des Carmes. A caveat remains that, as with many other ‘apple as pears’ SSR, there are ‘apparent’ mismatches of 1-2 bp in three marker-pairs (CH05c06, CH01f07a & CH04e03).

### Red Ingestrie (Clumber Park)

NFC 1947-381 was obtained from a Plantation House in Gildersome, Leeds. Though originally named ’Orange Pippin’ it has been de-named. It matches the MAN DNA sample A1206. Two other accessions that MAN has were from Bernwode and R V Rogers Nurseries; these are marketted as Red Ingestrie, and samples are A1198 and A2498.

MAN considers them a questionable morphological match to Red Ingestrie of TAK, Ronalds, Lindley, Hogg and others, and is here described as Red Ingestrie (Clumber Park). DNA evidence against is compelling especially the SSR with ‘apples as pears’.

Red Ingestrie was bred by TAK from another seed of the same fruit from which Yellow Ingestrie was also grown. Given how different the Ingestries are described to be, they may have been half-siblings, sharing the same maternal parent.

Nick Howard states that SNP shows Golden Harvey is a parent of Yellow Ingestrie. At the RLC7 MAN presented the case that TAK may have mistakenly had an Orange Pippin that was a sport of Golden Harvey and this was the maternal parent.

SSR of parent and progeny should have at least one allele in common for each of the marker-pairs.

Golden Harvey cannot contribute conventional SSR alleles for the marker-pairs CH01h01 and CH02c11 as these each mismatch by at least 4 bp. For ‘apples and pears’ mismatching alleles are in CH01d09 of 15 bp, and CH02b10 of 8bp.

It is less likely that the possible parent in common between this and Yellow Ingestrie was Golden Pippin as above (or whatever it actually was, and is substantially reconstructed above). Conventional SSR allele that could not be contributed is CH01f03b by 2bp, and of ‘apples as pears’ SSR CH01f07a by 7bp.

Additionally Nick Howard noted that NFC 1947-381 has a conventional SSR match with a Danish accession of the Swedish variety Hayoske Guldreinette which has been assigned code MUNQ3036 and this was found to have parents of Reinette Franche and Reinette des Carmes. SNP also found these two old French varieties were two of the grandparents of Yellow Ingestrie and Downton Pippin.

### Golden Pippin (Miller)

Nick Howard (2022) has confirmed that DNA SNP shows that Golden Pippin (Mlller) is progeny of Yellow Ingestrie. The other parent is as yet unknown.

It is fully consistent with DNA SSR data with both conventional and ‘apples as pears’ marker-pairs. Also the DArT score between Yellow Ingestrie and Golden Pippin (Miller) is 0.67.

At first this might seem a little odd, how can it be its grandparent? The caveat ‘(Miller)’ makes clear it is not the lost Golden Pippin.

### Glebe Gold

Its parentage was given as Yellow Ingestrie x Cellini by the breeder, James Walker in 1945.

DArT and conventional SSR confirm that this is so. SSR has three instances of ‘apples as pears’ marker-pairs having mismatches of 2bp. CH05c06 does appear to have experimental glitches or calibration issues with several other varieties. Neither EMPc117 nor EMPc11 have mismatches observed with other varieties.

This warns a little against acceptance that a few mismatches of 1 or 2 bp among the alleles of marker-pairs ‘apples as pears’ are significant. Among conventional SSR, while one mismatching allele of 1 or 2bp may not be significant, two or more of that magnitude are probably so.

### Dol-afallen

Dol-afallen is early to mid-season cooker found near the Elan valley reservoirs. I’s conventional and ‘apples and pears’ SSR strongly suggested it a cross of Yellow Ingestrie x Keswick Codlin. Both varieties were likely available early in C19 neat this location.

There was only one mismatched allele of 2bp in the entire SSR; in marker-pair CH01f07a one that similarly had mismatches for several other varieties.

At the RLC7, Panel approved it being so named. Its morphology is quite similar to Onibury Pippin (see below). That this is so is not surprising as they are half-siblings with common parent Keswick Codlin; their other parents are themselves full–siblings, Yellow Ingestrie and Grange.



### Onibury Pippin

Historic records suggest that this variety arose from a TAK nursery near his home at Downton Castle.

DNA DArT and SSR show this to be progeny of Keswick Codlin, and SSR further shows the other parent is Grange and not Yellow Ingestrie, Downton Pippin nor Bringewood Pippin.

As mentioned about Dol-afallen, they are siblings somewhere between half and full.



### Gulley Green

Only Grange could be identified as a parent from both DNA SSRs. The other parent remains unknown.



### Lingens Lily

Lingens Farm is located just west of Worcester. In the old cider and perry orchard, once in the ownership of the Bishops of Worcester, a late cider apple was noted as morphologically quite likely a seedling of Blanc Mollet, a variety present there. SSR confirms this to be likely.

SSR indicates the other parent is Grange. While Grange is not currently present, a connection between Lingens Farm and TAK is likely given that there is another old variety, accredited as Knight Bishop at RLC6, found here and at Elton Hall.

There is a query though about an allele of the ‘apples as pears’ marker-pair. In CH05c06, Blanc Mollet is {99,0,0,0} and can only contribute 99, whereas Lingens Lily is {99,104,114,119}. It might be expected to have had 114 and 119 contributed from Grange {103,114,119,0}. It suggests a mismatch of 5 bp, and this might be the result of a mutation. It should be noted that the Blanc Mollet tree from which sample leaves were taken for DNA was from near Ross-on-Wye.



### Freetown Yellow (etc.)

Court of Wick likely is progeny of Golden Harvey x Golden Reinette, based upon the Dart scores of 0.7 and the fact that all alleles of Court of Wick (apple) SSR can be contributed from these two parents. It is also true when considering the apples as pears SSR dataset, if the small mismatches in CH01f07a and CH04e03.

Court of Wick is a full sibling of Martin Becker and a half sibling Doree de Tournai, Pitmaston Pine Apple, Yellow Ingestrie etc….

Another variety of interest to MAN and the Museum of Cider is one recently recognised at Freetown near Tarrington as likely the Eggleton Styre described by Hogg and presented in the Vintage Fruit (1886).

A number of samples matching the apple SSR of Freetown Yellow was reported in the Malus Compilation of 2022:

* A103 (OLRM Thornhayes Nursery, Collumpton (TN) - White Close Pippin)
* A1525 (NT PCC 21173 - White Close Pippin)
* A1767 (WPCS Tretower Tag 0222)
* A2551 (MAN Cui Parc Tennis Ct 54 Tir Chanter 2nd top W - unknown)
* A2552 (MAN Cui Parc Tennis Ct 55 Tir Chanter Russet - unknown)
* A2824 (TVO Ash Barn Garden 144+ Mr Andrews' Pitcher)
* A5245 (A5245 MOC Norman Orchard, Freetown Farm, HR1 4JB Row 13, 7th from S

So far morphological checks support these matches. Conventional SSR suggests only a few of the known diploids could be a plausible parent:

* Beauty of Stoke (1889)
* Delincée (not in Ass’n du Domaine de Merval)
* Calville Rouge d'Hiver (big and red)
* Mele Ubriache (Italy?)
* Chodské (Czech?)
* Golden Reinette
* Zoete Bloemee (Netherlans, C19)
* Court of Wick

Golden Reinette and Court of Wick stand out as most likely. SSR with ‘apples as pears’ further supports either. Is it suggestive that Vintage Fruit (1886) notes the eye of Eggleton Styre to be “open with reflex segments like Court of Wick”?

## Further Work – additional samples

?

## Appendix A – Table of 37 apple varieties with DNA SSR **pear** marker-pairs

