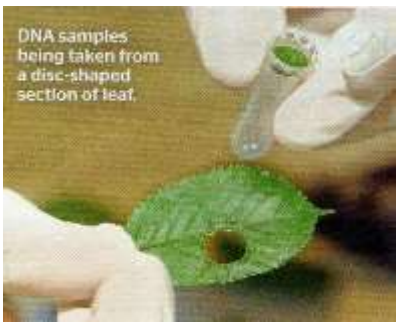
Progress in Apple & Pear Cultivar Identification and Validation by DNA Analysis

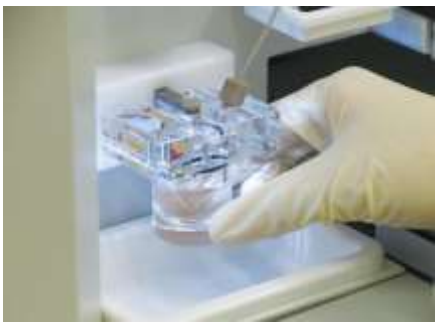
by Andy Gilchrist, Chairman, South Lakeland Orchard Group (SLOG)

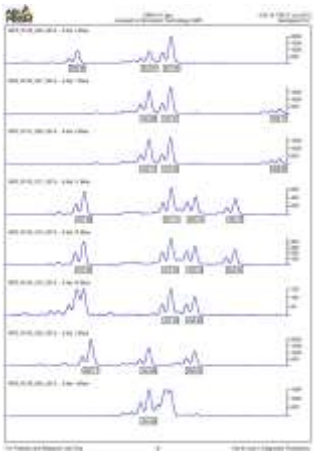
Gardeners who purchase an apple tree from a garden centre or nursery assume its labelled identity to be accurate. We all prefer certainty to uncertainty and expect things like cultivar identity to be black and white. Historically, it was difficult and time-consuming to prove otherwise. Consequently most mistaken identities went unnoticed and if the tree was subsequently used for propagation, the mistake endured, for decades or even centuries. Such mislabelling may occur during propagation either by carelessness or negligence. Labels over time become brittle and break off. In years gone by, an identity may have been forgotten, so a new name was given, leading to one or more synonyms for the same cultivar.

Fortunately in August 2010 the complete genome of *Malus domestica* was published, showing a structure of 17 pairs of chromosomes bearing 57,386 genes. East Malling Research then undertook a DNA analysis study of the entire UK National Collection of over 2,000 apple cultivars and over 500 pear cultivars. This project demonstrated that around 20% of accessions to the National Collection were in fact synonyms (duplicates), meaning that literally hundreds of assumed unique cultivars, carefully propagated, grown and maintained at Brogdale were imposters which had been a waste of time and scarce resources. East Malling then announced that their DNA service was commercially available at £300 per sample: cost-effective perhaps for a few plant breeders but out of reach for societies and individuals. However, in 2015 an organisation called FruitID negotiated a bulk package deal such that if the various regional orchard groups like SLOG around the British Isles together submitted hundreds of samples on a co-ordinated basis, the cost per sample would fall to within an economical £30-£35 range.

Up to this point, from 2012, SLOG had established a comprehensive collection of all known Northern English apple cultivars in the SLOG orchard in Kendal, many of which were not in the National Collection. We suspected that some of these were duplicates under different names and were attempting to resolve these questions by planting the suspect cultivars simultaneously side-by-side on the same rootstock, then waiting for them to fruit for the ultimate comparison. The DNA analysis service offered us the opportunity to accelerate this process, so we have participated annually from 2016. We are required to submit leaves only, so we don't have to wait for the tree to bear fruit. The leaves are frozen and then towards the end of the year, the DNA is extracted by a robotic phenol based method, then amplified by polymerase chain reaction. The genetic fingerprint of each sample is established by printing out 12 microsatellite markers using a semi-automated genetic analyser which then compares it against the database of over 2,000 cultivars and, using an algorithm based software, declares the sample to be either a match against a known cultivar or else a unique unknown cultivar.







In 2016, SLOG submitted 55 apple & pear samples out of a national total of 820 samples. The results told us that 27 showed database matches and 28 were unique. We had suspected that two Lyth Valley apple cultivars “Churn Lid” and “Royal” were identical, and the DNA results confirmed this. Furthermore we suspected that both names were local synonyms for the Scottish cultivar “Cambusnethan Pippin”, a dual purpose apple dating from 1750 or possibly earlier in Stirlingshire.

Churn Lid syn. Cambusnethan Pippin

Wheaten Loaves

Fallbarrow Favourite

The computer generated result denied this so we challenged the project scientist who, after studying the 12 microsatellite marker printouts, agreed that whilst there were minor differences, these were within the range of experimental error. This incidentally is a grossly simplified version of a rather convoluted debate but I’ll spare you the details. However it does demonstrate that the most sophisticated technology cannot be relied upon to give “black and white” results (or maybe it can be too “black and white”) and that human interpretation is still vital to avoid further errors. The value of this result to SLOG is that we now don’t need to waste our limited resources preserving these two presumed Westmorland cultivars. We still have two more unresolved Westmorland identity cases. We believe that “Fallbarrow Favourite” and “Wheaten Loaves” are (slightly) different. Both are early season culinary apples and the first record of “Fallbarrow Favourite” is 1936 though both are probably much older. The DNA analysis result validated our “Fallbarrow Favourite” but showed that our presumed “Wheaten Loaves” was also a match for “Fallbarrow Favourite”. We now suspect that the presumed “Wheaten Loaves” in the SLOG orchard was grafted from the wrong tree in a Lyth Valley orchard, so we have now taken samples for analysis from other trees in the hope of identifying the correct “Wheaten Loaves” – which incidentally would give a “unique” DNA result because it does not exist in the National Collection. The second case involves another Lyth Valley cultivar, “Taylor’s Favourite”, a mid-season culinary apple dating from the 19thC. The sample we submitted was a match for the Nottinghamshire cultivar “Maltster”, also a mid-season culinary apple dating from the 19thC. Again, we suspect that the presumed “Taylor’s Favourite” in the SLOG orchard was grafted from the wrong tree in a Lyth Valley orchard, so in 2018 we submitted samples from other trees and found the correct “Taylor’s Favourite”. Resolving these two cases is important because people have already grafted scion wood from our two mislabelled trees and therefore have perpetuated these mistakes. Obviously we don’t want this confusion to continue, but it does illustrate how these mistakes occur with heritage cultivars.

On a more positive note, analyses have validated the presumed identity of the following Cumbrian apple cultivars in the SLOG orchard: “Autumn Harvest”, “Bradley’s Beauty”, “Carlisle Codlin”, “John Huggett”, “Lancashire Pippin 1” (which had been a lost variety but was found in Tasmania of all places), “Lancashire Pippin 2”, “Nelson’s Favourite”, & “Tiffen”. We still have a further five presumed Cumbrian cultivars to validate, but these are cases where we have a high level of confidence about their authenticity based on the very close match between fruit grown and literature descriptions. Completion of this group will enable us to offer scion wood for propagation of seventeen listed Cumbrian cultivars with a guarantee of their authenticity, something that had previously been impossible. Although a few of the better known of these cultivars such as “Keswick Codlin” and


A typical old Westmorland pear tree

"Duke of Devonshire" are offered by commercial suppliers, many are not, so SLOG can ensure the survival of the authentic version of these lesser known local heritage cultivars.

In addition we have established the genetic fingerprints for ten locally found "unique" apple cultivars: "Alan's Apple", "Nancy Crow" "Mower's Quench", "Burgh Beauty", "Eddie Potts", "Brantwood Golden Codlin", "Hazelslack Striped", "Crosthwaite Fragrance", "Dobson's Green" & "Dobson's Pink". Again, more will follow which, when fully profiled, will be proposed for entry into the national "Register of Local Cultivars" to ensure that they too are properly recorded for posterity.

Similarly, we have also submitted samples of Lancashire apple cultivars with similarly mixed success. We thought we had rediscovered the lost cultivar "Scarlet Tiffing", a colourful mid-season cooker dating from 1851 in the Lancaster area, but it turned out to be "Tiffen" and perhaps the former is simply a synonym of the latter. Another lost cultivar is "Trumpeter", a distinctly shaped green cooker dating from 1851 and according to Hogg (1884) "much esteemed in the orchards around Lancaster". This sample returned a "unique" fingerprint but discussion continues with the aforementioned project scientist and his panel about its authenticity.

Initially, apples were our primary interest, mainly because they were better known and to a certain extent could claim to be our "National Fruit". However, pears also have a long history in Westmorland, with some specimens in the Lyth Valley believed to be around 300yrs old. DNA analyses of local pear trees have yielded interestingly mixed results. Some proved to be recognised cultivars, albeit of Belgian ("Beurré Rance", "Beurré Capiaumont"), Scottish ("Golden Knapp") or Welsh (a perry pear) origin, whilst most returned "unique" fingerprints despite being grafted trees and therefore not seedlings. Most of these "unique" Westmorland cultivars are good flavoured juicy dessert pears which have stood the test of time and are worthy of preservation. We plan to characterise them and list them in the "Register of Local Cultivars" to ensure that they too are properly recorded for posterity. Furthermore, we have supplied newly grafted specimens to Sizergh Castle (National Trust) who have planted them as a row of cordons against the south facing wall of their orchard, to be known as "The Brian Fereday Westmorland Pear Collection" in memory of one our founder members who worked for many years as a Ranger there. A dozen were planted in early 2018 and there will be at least twice that number eventually.

The SLOG orchard contains over 160 different apple & pear cultivars, including all known Cumbrian, Lancashire & Yorkshire cultivars, each county collection being in a single row of twenty or so cordon trees. It is open to the public and has an information board with explanatory guidance to the collection and all trees are individually labelled. The orchard is on the Underley Road Allottments in Kendal, situated between Underley Road & Hallgarth Circle just east of Windermere Road. The easiest parking is on Hallgarth Close (southern edge of Hallgarth Circle) then walk through lane due south past sheds, up path on right into allottments and along to plot numbers 4, 7 & 10. The south entrance is off Underley Road from where a narrow green lane leads to a gate on the left which opens directly into the orchard.

The SLOG Orchard in Kendal with information board

In conclusion, DNA analysis is a valuable tool which speeds up the process of validating cultivar identity with greater accuracy than previously possible. Computer generated data is cost-effective, but human interpretation is still required in some instances. The current process involving only 12 microsatellite markers has limitations in that it cannot differentiate between sports, most of which are single gene mutations (example below), but these can usually be resolved visually.

Norfolk Royal

Norfolk Royal Russet

The match to the database of >2,000 apple & >500 pear cultivars offers presumed certainty for over 2,500 cultivars. This appears impressive, but depends on the accuracy of the database. The responsible scientists take it as an article of faith, but regional groups such as SLOG have doubts about the accuracy of at least 1% of the database – again, things are never quite as "black and white" as we would like! Nevertheless, keeping these minor issues in context, there is no doubt that DNA analysis is a wonderful tool which, by eliminating mis-identities and validating correct identities, allows orchard groups like SLOG to preserve our local heritage apple and pear cultivars with a level of confidence never before possible. Thanks are due to East Malling Research for making this service available, and to FruitID for making it economically feasible.

References

East Malling Research: Fingerprinting the National Apple and Pear Collections, 2010

FruitID: Introduction to DNA fingerprinting, 2017

FruitID: Register of Local Cultivars, 2018

Hogg: The Fruit Manual, 1884

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