Microsatellite DNA fingerprinting has revealed the identities of both parents of the hybrid grape cultivar, Isabella - a 200 year-old mystery finally solved.

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Version 3 (31 December 2023)
DOI: 10.13140/RG.2.2.15746.86720/5

Summary

Microsatellite DNA-marker analysis shows that the Isabella hybrid grape cultivar originated from a crossing between a *Vitis vinifera* grape cultivar, Meslier Petit and a *Vitis labrusca*-containing cultivar called Ives (also known as Bordô in Brazil) and historically known as Ives' Madeira Seedling.

Introduction

The Isabella cultivar has long been suspected to be a hybrid cultivar between the American genotype *Vitis labrusca* (or 'fox' grape) and the European *Vitis vinifera* (D'Onofrio et al., 2016). In fact, the Isabella hybrid cultivar is sometimes identified with other similar labrusca-containing hybrids which have been referred to as belonging to the group, *Vitis labruscana* - a loosely designated grouping, displaying various degrees of vinifera characteristics. Only recently, through microsatellite DNA analysis has it been revealed that the vinifera parent of Isabella is the very rare white-berried French cultivar, Meslier Petit, but the "wild" *Vitis labrusca* parent has remained unknown, until now.
According to Wikipedia, the skin of Isabella grapes, when ripe, is dark purple, almost black, with a tender green-yellow flesh. It has large, well-formed fruit clusters with a thick bloom. It is called a "slip-skin" variety, meaning that the skin separates easily from the pulp. The grapes may be used to make wine, most notably Uhudler (Austria) and Fragolino (Italy) -- the latter literally meaning "strawberry". Isabella is one of the most widely planted grape cultivars in the southern states of Brazil and is used there for processing into both juice and jam (Gonçalves, 1986).

The Isabella is also grown on the Madeira Islands as well as in the Azores. But because it is an American hybrid grape it may only be used for personal home wine production since it is on the banned list of American hybrid cultivars as an export wine variety according to European Union (EU) region regulations (Fig. 1).

Figure 1. Typical Isabella hybrid grapevine cultivar.

We scanned the scientific literature for any available microsatellite DNA fingerprinting data on the Isabella and Ives hybrid cultivars and discovered that there exists a number of mutant clones according to the available literature. The *V*IVC database reports more than 100 synonyms for the Isabella hybrid cultivar (Maul and Röckel, 2015).

Materials and Methods

We have used the simple sequence repeat (SSR) data of the Isabella and Ives varieties which became recently available (de Oliveira et al., 2020). Furthermore, the *V*IVC database was also consulted for the Meslier Petit microsatellite DNA markers (Maul and Röckel, 2015).

Results

Table 1 provides a subset of 9 SSR DNA markers which show that the Isabella hybrid cultivar is very likely the result of a random crossing between the Ives' Seedling hybrid cultivar and the European vinifera, Meslier Petit.

Table 1. Parent-offspring relationship between Meslier Petit, Ives' Madeira Seedling and Isabella.
A subset of 9 SSR markers (loci) shows that Meslier Petit and Ives' Seedling could be the parents of Isabella. Fragment lengths in base pairs (bp) are given for the two alleles of each cultivar. The corresponding consistent fragment lengths (± 2 bp) for Meslier Petit and Ives' Seedling are shown in red and blue colours respectively.

Microsatellite Markers* (SSRs)	Meslier Petit (Petit Meslier) (bp)	Isabella (a.k.a. Isabel) (bp)	Ives' Seedling (a.k.a. Bordô) (bp)
VVS2	133:151	123:151	123:135
VVMD5	236:240	240:240	238:238
VVMD7	249:257	235:249	235:249
VVMD25	239:249	241:249	241:241
VVMD27	180:190	180:184	184:186
VVMD28	236:246	226:236	226:230
VVMD32	272:272	248:272	248:248
VrZAG62	194:204	202:204	202:204
VrZAG79	237:251	237:247	247:247

SSRs = Simple Sequence Repeats; bp = base pairs.
*These markers are normalized to *V*IVC standards.

Discussion

If we look carefully at the microsatellite DNA data presented in Table 1 it's apparent that one of Ives' VVMD5 alleles of 238 bp differs somewhat from Isabella's VVMD5 of 240 bp. This is not surprising because as we mentioned earlier in the introduction, there are a number of Isabella clones which display small differences in some of their microsatellite DNA markers. These differences are likely the result of somatic mutations which contribute to some clonal variations.
It is well-known that hybrid American cultivars in the southeastern regions of the US have undergone intense natural selection amid the extreme biotic (disease-related) and abiotic (climatic conditions-related) pressures that exist in that environment. The final effect of all these external pressures is the survival and propagation of hybrid cultivars that resemble their ancestors both genetically and often, phenotypically.

An alternative and plausible explanation for the apparent difference found at the VVMD5 locus would be to postulate that Ives carries a null allele at that locus. Since SSR alleles display typical Mendelian segregation, it is necessary to postulate that Isabella inherited a null allele from Ives' Seedling at the VVMD5 locus.
This latter explanation, however, would need to be verified through actual genome sequencing – but it is an interesting thought, as the Microsatellite DNA data in Table 1 would then present a perfect fit.

The *V*IVC database (Maul and Röckel, 2015) lists the putative parentage of Ives (accession no. 5592). They have stated that the Hartford hybrid cultivar is a parent of Ives (a.k.a. Bordô), but provide no microsatellite DNA for Hartford – although we found a 2020 publication which lists 17 microsatellite DNA markers attributed to a putative Hartford hybrid cultivar they claim originated in the USA (de Oliveira et al., 2020). If they are assuming that Rochester is a synonym for the Hartford hybrid, Rochester cannot be a parent of Ives simply because there are no matching VrZAG79 alleles. Rochester's VrZAG79 alleles are 237:265, while Ives' corresponding VrZAG79 alleles are 247:247. These markers are widely different and unlikely to be simple mutations (Leão et al., 2009).
Another discrepancy is apparent which involves both alleles of marker VVMD5. Rochester's VVMD5 alleles are 240:240, whereas the corresponding alleles in Ives are 238:238.
Accepted differences (possibly due to stutter and/or slippage) are usually short repetitions of 2 - 4 base pairs. However, in the case of the 240:240 VVMD5 alleles, there is a chance that this discrepancy is the result of null alleles being involved, as we previously discussed in the case of Ives and Isabella.
A similar argument would apply to the very closely-related Perkins hybrid cultivar (misidentified as Hartford) according to *V*IVC (Maul and Röckel, 2015).

We have consulted a number of historical accounts describing the possible origins of the Isabella and the Ives' Seedling hybrid cultivars and discovered that some of the reported dates attributed to the earliest appearance of some of these hybrid cultivars just don't add up (Munson, 1905).
For example it has been reported that the Isabella hybrid was introduced as early as 1816.
However, according to Hedrick, Ives was grown by Henry Ives from a seed planted in 1840 in his garden in Cincinnati, Ohio. Ives had claimed that his seedling was derived from a seed of Madeira grapes which had been sent to him from abroad. This would explain the designation, "Ives' Madeira Seedling".
There has been general agreement that Ives' Madeira probably became accidentally mixed with a different chance seedling. Some of the Ives' characters and perceived labrusca properties were noticeably reminiscent of other labruscana varieties such as Isabella, Alexander, Hartford and others (Hedrick, 1908).
We maintain a similar position on this matter by postulating that the seedling which became known as Ives likely originated accidentally from a seed mix-up containing wild labruscana species. The original seed-bearing cultivar was probably already well established elsewhere in Cincinnati, Ohio. So the actual date of 1840 in this case is meaningless to say the least.

What we find most interesting is the fact that the microsatellite DNA data has revealed that the now very rare white-berried French *Vitis vinifera* cultivar, Meslier Petit, is the second parent of Isabella. It is very likely that Meslier Petit acted as the pollen donor in the crossing since we also know Meslier Petit's parentage and chlorotypes (Cretazzo et al., 2022). Since the chlorotypes of Heunisch Weiss (a.k.a. Gouais Blanc) and Savagnin (a.k.a. Traminer) are of the "C" and "D" types respectively, and that the chlorotype of Isabella (offspring) is of the "B" type, one can conclude that Meslier Petit must be the pollen donor.

Although we don't yet have data on the chlorotype of the Ives hybrid cultivar, it would be safe to say that if Ives was the other parent of Isabella, then it would have been the seed parent. This would imply that the chlorotype of the Ives hybrid cultivar would also be of the "B" type.

I present the pedigree of the Isabella hybrid grape cultivar in a simplified infographic (Fig. 2).

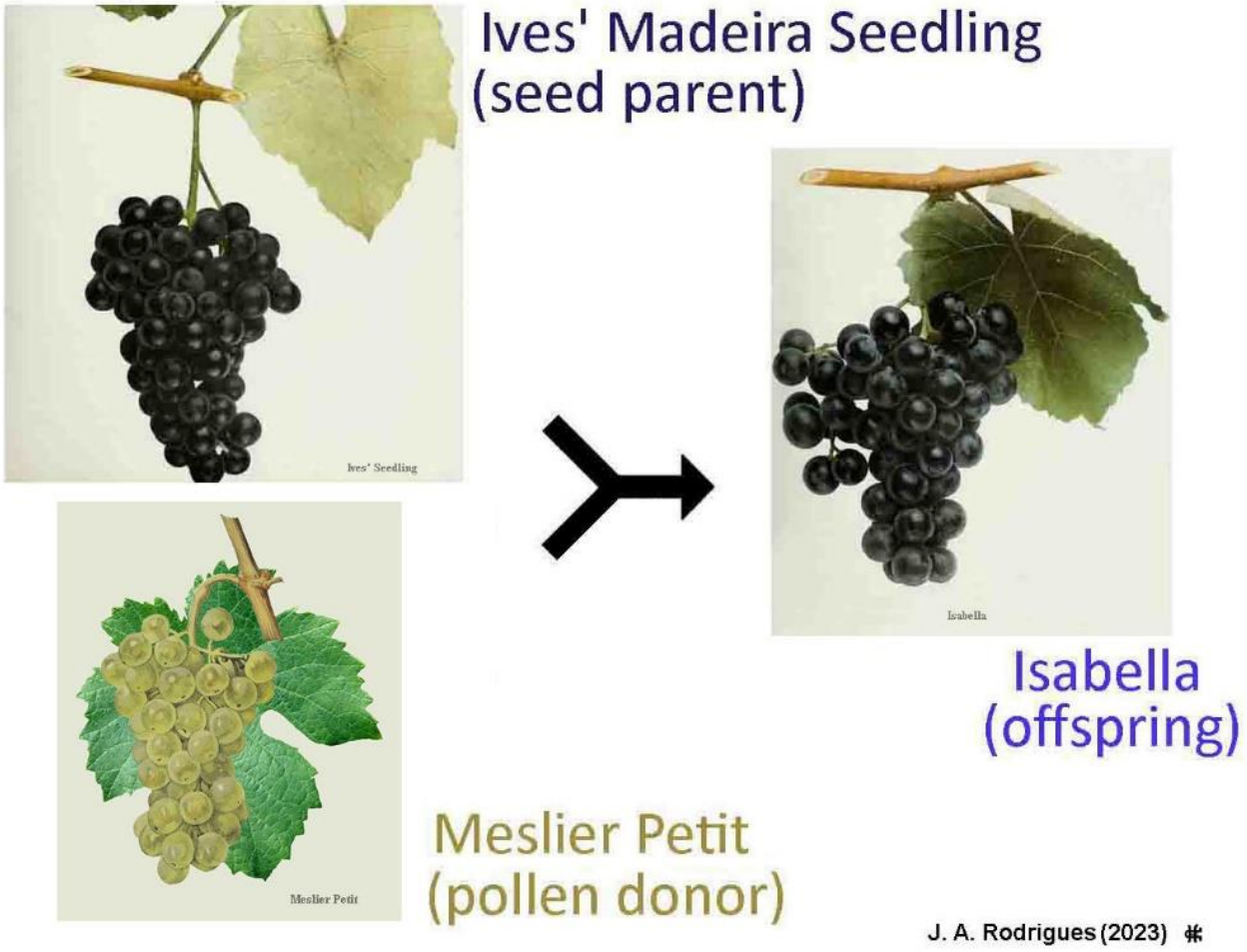

Figure 2. Pedigree of the Isabella hybrid cultivar.

Conclusion

The SSR data seems to confirm that the origin of the Isabella hybrid grape cultivar was likely a chance hybridization that took place between Ives' Seedling hybrid cultivar and that of Meslier Petit. That chance hybridization event must have occurred at the beginning of the 19th century, somewhere in the southeastern region of the US.

We can be fairly confident that through microsatellite DNA fingerprinting technology, both parents of the popular Isabella hybrid grape cultivar have been revealed after more than 200 years of uncertainty.

They are the relatively rare Meslier Petit (likely the pollen donor) and the so-called Ives hybrid labruscana cultivar (the likely seed parent).

Acknowledgements

This work has been funded solely by the author (JAR) and no other funder played any role either in the interpretation of the results or in the preparation of this manuscript.

References

1. Cretazzo E., Sanz, P.M., Lorenzi, S., Benítez, M.L., Velasco, L. and Emanuelli, F. (2022).
Genetic Characterization by SSR Markers of a Comprehensive Wine Grape Collection Conserved at Rancho de la Merced (Andalusia, Spain).
Plants 11, 1088-1107.
https://doi.org/10.3390/plants11081088

2. de Oliveira, G.L., de Souza, A.P., de Oliveira, F.A., Zucchi, M.I., de Souza, L.M. and Moura, M.F. (2020). Genetic structure and molecular diversity of Brazilian grapevine germplasm: Management and use inbreeding programs.
PLoS One 15(10): e0240665.
https://doi.org/10.1371/journal.pone.0240665

3. D'Onofrio, C., Fausto, C., Matarese, F., Materazzi, A., Scalabrelli, G., Fiorani, F., and Poli, I. (2016). Genotyping of grapevine varieties from Garfagnana (Northern Tuscany): Evidence of a Historical Center of Diversity.
Am. J. Enol. Vitic. 67, 120–126.
https://doi.org/10.5344/ajev.2015.15046

4. Gonçalves, B. Identificação ampelográfica de videiras americanas e híbridas cultivadas na MRH 311. Circular Téchnica, No. 12 da EMBRAPA. Agosto, 1986.

5. Hedrick, U. P. (1908). The Grapes of New York. J. B. Lyon Company, State Printers, Albany.

6. Leão, P.C.S., Riaz, S., Graziani, P., Dangl, G.S., Motoike, S. Y. and Walker, M.A. (2009). Characterization of a Brazilian Grape Germplasm Collection Using Microsatellite Markers.
American Journal of Enology and Viticulture 60(4).

7. Maul, E. and Röckel, F. (2015). "Isabella" Vitis International Variety Catalogue.
http://www.vivc.de/

8. Munson, T.V. In: Viala, P. and Vermorel, V. Ampélographie, Paris, Masson**,** 1905. v.6.