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GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

THE ASSESSMENT OF ESSENTIAL DERIVATION IN GRAPEVINE

Document prepared by experts from Spain

The assessment of essential derivation in grapevine

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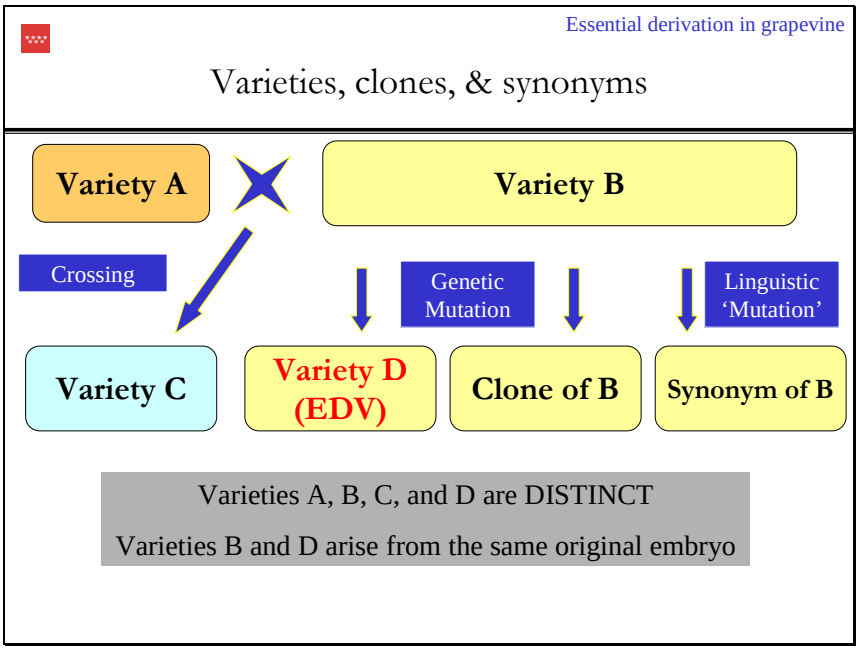
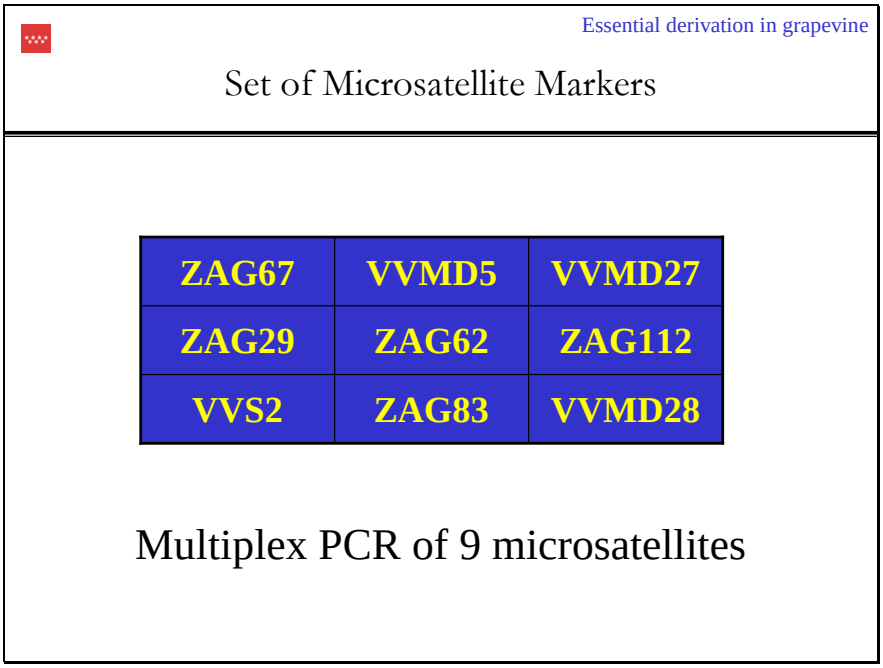
Comunidad de Madrid

BMT 11. Madrid, September 16 to 18, 2008

Vitis vinifera L.

- Woody, asexually multiplied
- Very old culture
- Widely spread culture
- Very high number of varieties
- Synonyms and homonyms


Essential derivation in grapevine
Varieties, clones, & synonyms
Variety A
Variety B
Crossing
Genetic Mutation
Linguistic 'Mutation'
Variety C
Variety D (EDV)
Clone of B
Synonym of B
Varieties A, B, C, and D are DISTINCT
Varieties B and D arise from the same original embryo


Essential derivation in grapevine
Set of Microsatellite Markers
ZAG67
VVMD5
VVMD27
ZAG29
ZAG62
ZAG112
VVS2
ZAG83
VVMD28
Multiplex PCR of 9 microsatellites

Characterization of grapevine varieties with 9 microsatellites

- Study of 991 accessions (2 plants / accession)
- 490,545 pair wise comparisons
- 3,170 pairs fully matched (18 alleles):
  - 639 accessions
  - 138 genotypes

Essential derivation in grapevine

Characterization of grapevine varieties with 9 microsatellites

- Full-matching accessions could include:
  - Same variety (clone, synonym)
  - Distinct variety:
    - EDV (same original embryo)
    - Non-EDV (different original embryos)

Full-matching accessions

Literature / morphology data

- 594 full-matching accessions classified as:
  - Same variety (clone, synonym)
  - Distinct variety:
    - EDV (same original embryo)

Essential derivation in grapevine

Full-matching accessions

Doubts remaining: 45 accessions

- 20 microsatellites:
  - 19 linkage groups
  - 4 of the first set of 9 (full-matching expected)
  - 16 different

Full-matching accessions

Doubts remaining: 45 accessions

- Full-matching in 25 microsatellites
- Conclusions:
  - Same variety (clone, synonym) OR
  - Distinct variety: EDV (same original embryo)

Characterization of grapevine varieties with 9 microsatellites

- Study of 490 non-redundant genotypes
- 119,805 pair wise comparisons
- 1 pair matched 17 of 18 alleles:
- 2 pairs matched 16 of 18 alleles:
- Literature: considered distinct varieties:
  - EDV (same original embryo)
  - Non-EDV (different original embryos)

Characterization of grapevine varieties with 9 microsatellites

- 1 pair matched 17 of 18 alleles:

'Chasselas Blanc' and 'Chasselas Gros Coulard'

- Full-matching in 25 microsatellites (but 1 allele)
- Conclusion:
  - EDV (same original embryo)

Essential derivation in grapevine

Characterization of grapevine varieties with 9 microsatellites

- 2 pairs matched 16 of 18 alleles:

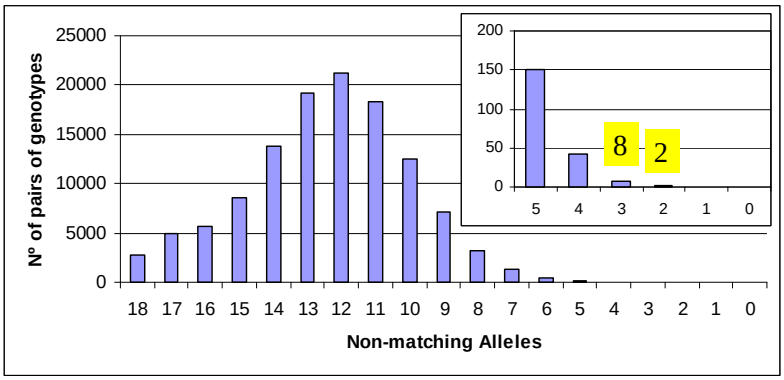
  Alphonse Lavallée with Princeps

  Pizzutello Moscato Biondo with Galletta Rosa

- 25 microsatellite analysis:
  - 10 different alleles (8 loci) in both cases
- Conclusion:
  - Non-EDV (different original embryos)

Essential derivation in grapevine

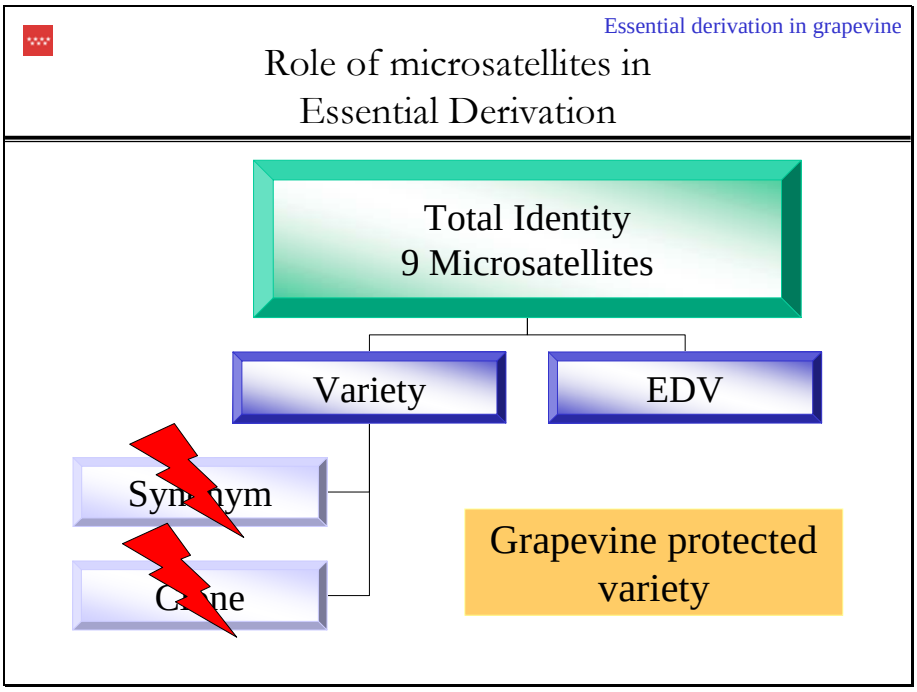
Pair wise comparison between non-redundant genotypes

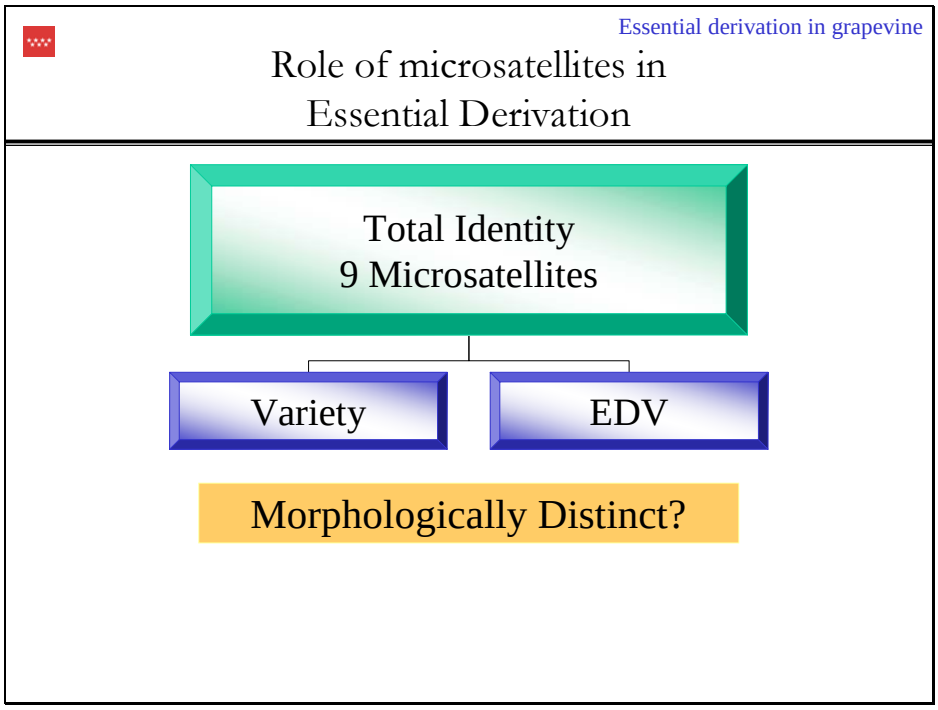


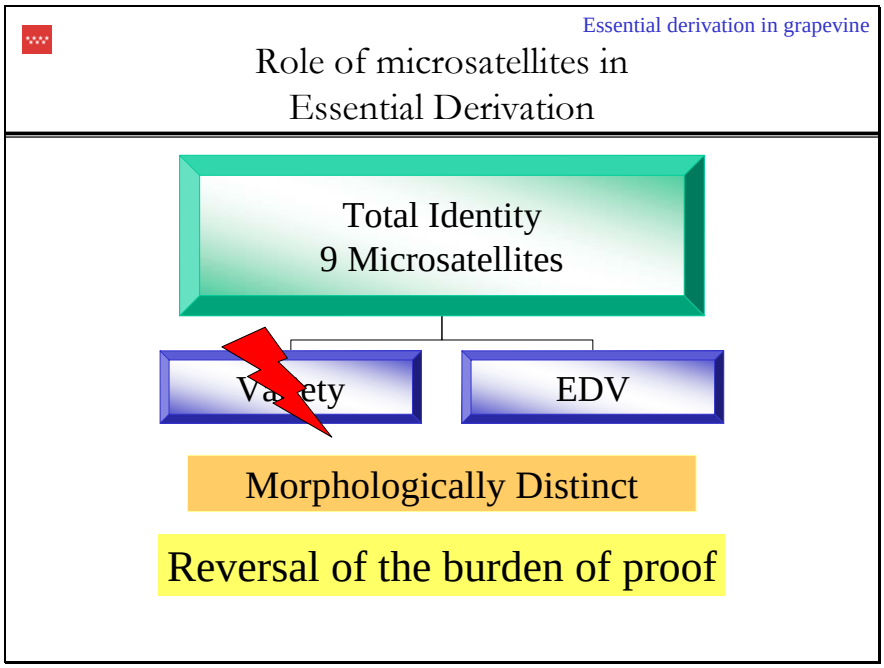
Essential derivation in grapevine

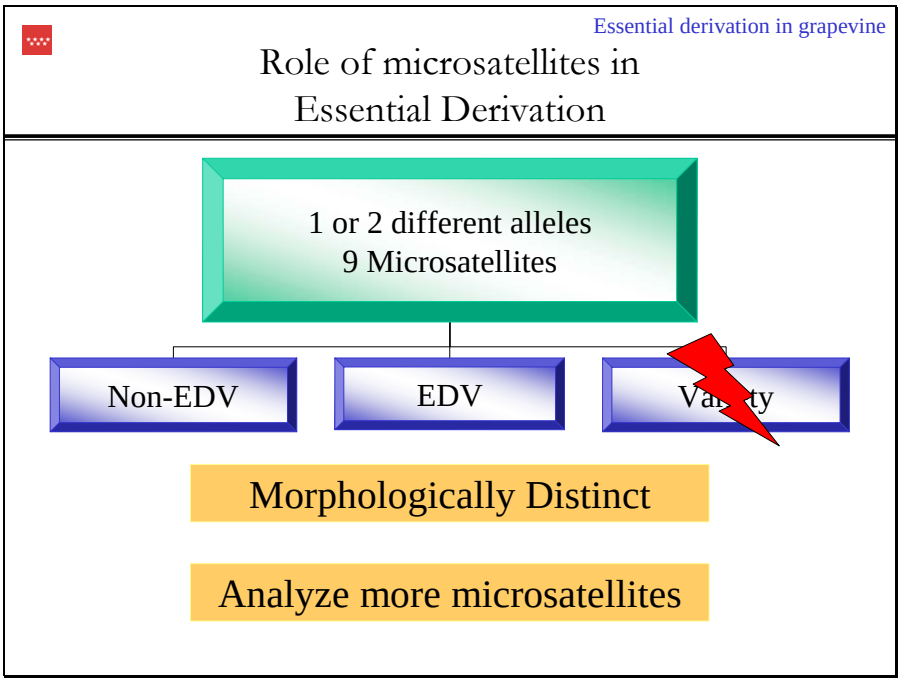
Characterization of grapevine varieties with 9 microsatellites

- Conclusions:
  - All varieties arising from different embryos (=Non-EDVs) were distinguished by 2 or more alleles
  - All varieties arising from the same embryo, including EDVs, matched in the 18 alleles (except one case, one different allele)


Essential derivation in grapevine
Role of microsatellites in Essential Derivation
Total Identity
9 Microsatellites
Variety
EDV
Grapevine protected variety


Essential derivation in grapevine
Role of microsatellites in Essential Derivation
Total Identity
9 Microsatellites
Variety
EDV
Morphologically Distinct?


Essential derivation in grapevine
Role of microsatellites in Essential Derivation
Total Identity
9 Microsatellites
Variety
EDV
Morphologically Distinct
Reversal of the burden of proof


Essential derivation in grapevine
Role of microsatellites in Essential Derivation
1 or 2 different alleles
9 Microsatellites
Non-EDV
EDV
Variety
Morphologically Distinct
Analyze more microsatellites

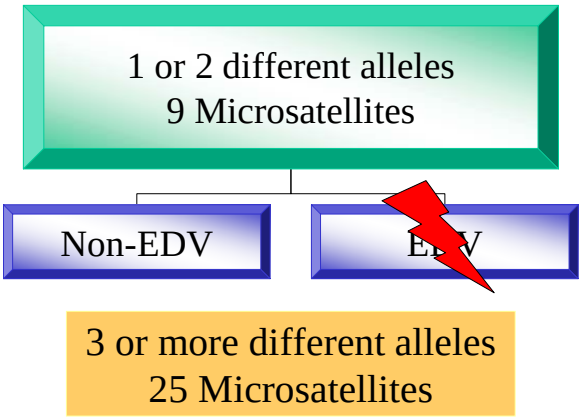
Role of microsatellites in Essential Derivation

Considerations:

- Study of 72 varieties involved in pedigrees:
  - Closest varieties differed in 7 alleles for the 16 additional microsatellites
- Probability of finding two different mutations in the microsatellites of one variety is 6.8 10^{-9} (the mutation rate in grapevine per microsatellite is 8.2 10^{-5} after Crespan, 2004)

Essential derivation in grapevine

Role of microsatellites in Essential Derivation



Role of microsatellites in Essential Derivation

1 or 2 different alleles
9 Microsatellites

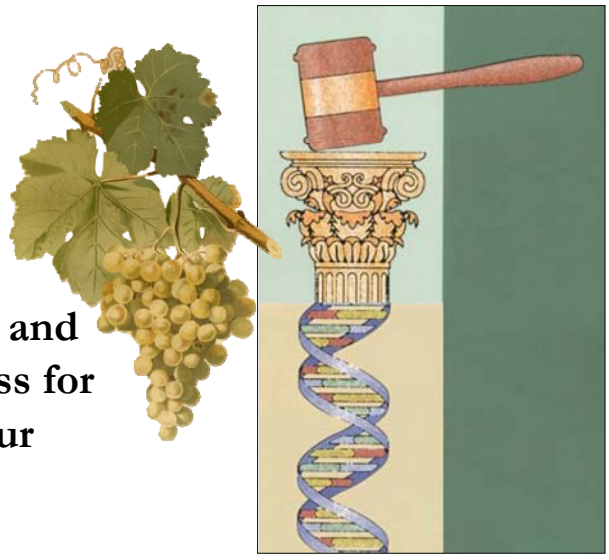
Non EDV

EDV

2 or less different alleles
25 Microsatellites

Reversal of the burden of proof

A case of essential derivation and distinctness for berry colour



(Modified from Evett & Weir, 1998)

A case of essential derivation and distinctness for berry colour

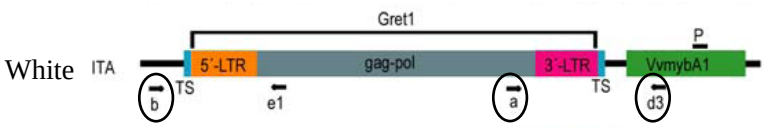
- Three grapevine varieties:
  - A: white colour, original variety
  - B: rose colour
  - C: rose colour
- Full-matched for the 9 microsatellites

Essential derivation in grapevine

A case of essential derivation and distinctness for berry colour

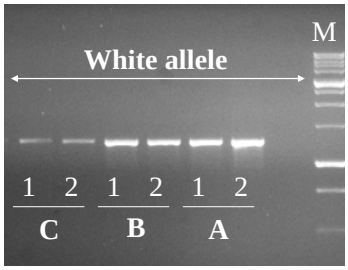
- Initial conclusion:
  - B and C are EDVs from A
- Possibilities regarding B and C:
  - Same variety
  - Distinct varieties

Molecular genetics of berry colour variation



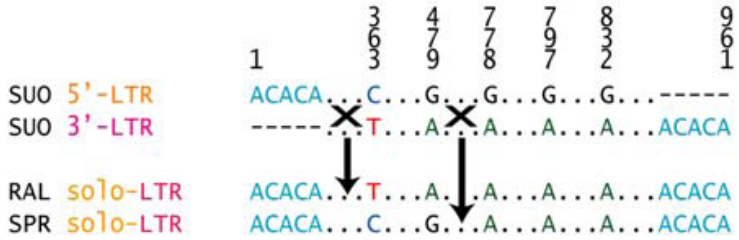
Lijavetzky et al. Mol Gen Genomics (2006) 276:427–435

A case of essential derivation and distinctness for berry colour



Two independent excision events gave place to B and C

Intra-LTR recombination events



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A case of essential derivation and distinctness for berry colour

- B and C are different, but are they distinct?
- Option 1 for distinctness?

A case of essential derivation and distinctness for berry colour

A B C

A case of essential derivation and distinctness for berry colour

- B and C are different, but are they distinct?
  - Differ in berry colour
  - Differ in the uniformity of berry colour
- Option 1 for distinctness?
  - Study for a correlation between marker and berry colour
  - Probably, different excision events may give place to a non-distinct berry colour

Conclusions

- Total identity for the set of 9 microsatellites between two distinct varieties are enough evidence as to reverse the burden of proof
- Differences of 1-2 alleles should be studied with more microsatellites
- Molecular analysis of berry colour is not ready for an option 1 approach yet

The assessment of essential derivation in grapevine

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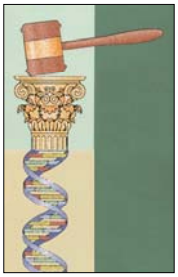

Thank you for your attention

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