



Monitoring and protection of local genetic resources of sweet chestnut (*Castanea sativa* Mill.) by integrated analysis of cultivated and wild populations: case study of Umbria and Lombardy regions (Italy)

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- PSR Region Lombardy 2014-2020 «**CASTADIVA**»
- PSR Region Umbria 2014-2020 «**MULTICAST**»



Objectives

- Characterization of sweet chestnut genetic resources
- Genetic identification of chestnut products
- Improving traceability and protection of local products



Strategy

Integrated genetic analysis of cultivated orchards, fruits, and wild stands

The approach is derived from previous studies of gene flow between natural populations of chestnut in hybrid zones of Turkey (Villani et al. 1999. J. Evol. Biol. 12: 233-244)

Genetic structure of natural populations of *Castanea sativa* in Turkey: evidence of a hybrid zone

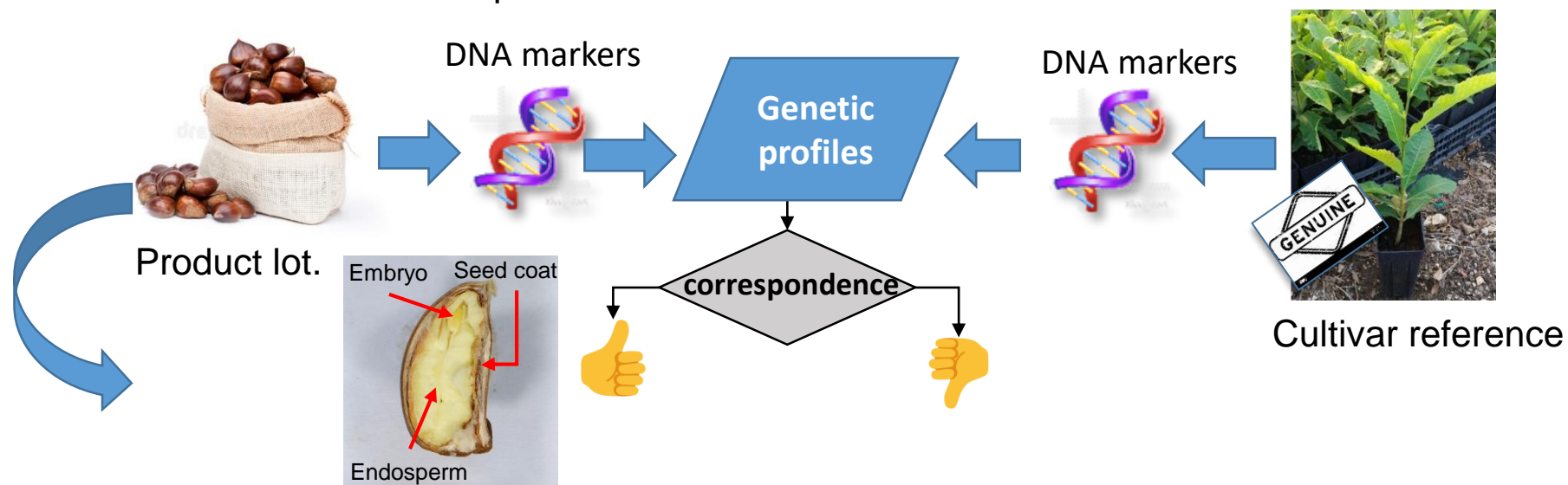
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J. EVOL. BIOL. 12 (1999) 233-244 © 1999 BLACKWELL SCIENCE LTD

<https://www.mapchart.net/italy.html>

Conventional approach based on genetic reference of cultivars

Comparison of genetic profile between investigated samples and the genetic reference of cultivars or orchard plants



Genetic analysis of chestnut fruit tissues

- **Seed coat**, maternal tissue (correspondence with cultivar)
- **Embryo** (Pollen contribution)
- **Endosperm** and transformed products (Pollen contribution)

(Torello *et al.* 2022)*

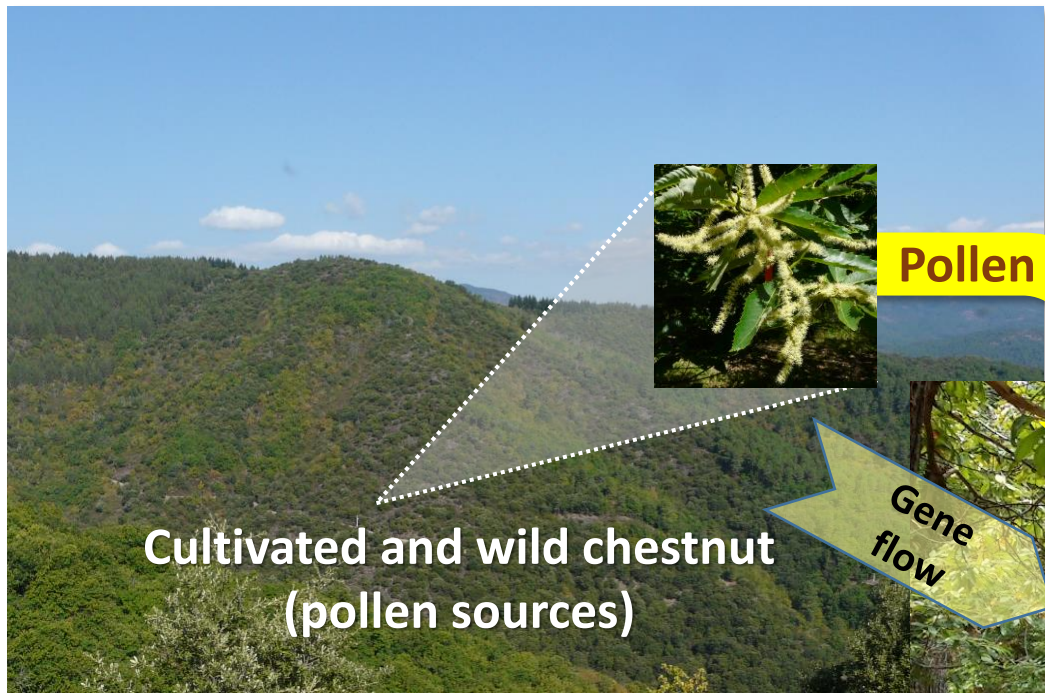
* Torello *et al.* 2022. Ottimizzazione di un protocollo di tracciabilità genetica per la salvaguardia delle produzioni di castagne. VIII Convegno nazionale del castagno 14-16 Settembre 2022, Portici (NA).

Pollen gene flow and diversity of Pollen Sources

Cross pollinization is necessary in male-sterile plants (i.e. Marron)
The effect of pollen gene flow is related to the genetic diversity of local pollen sources

- Gene flow by pollen makes cultivar attribution difficult
- *May pollen gene flow be useful for genetic characterization and traceability of local products?*

Gene flow between cultivated and wild chestnut

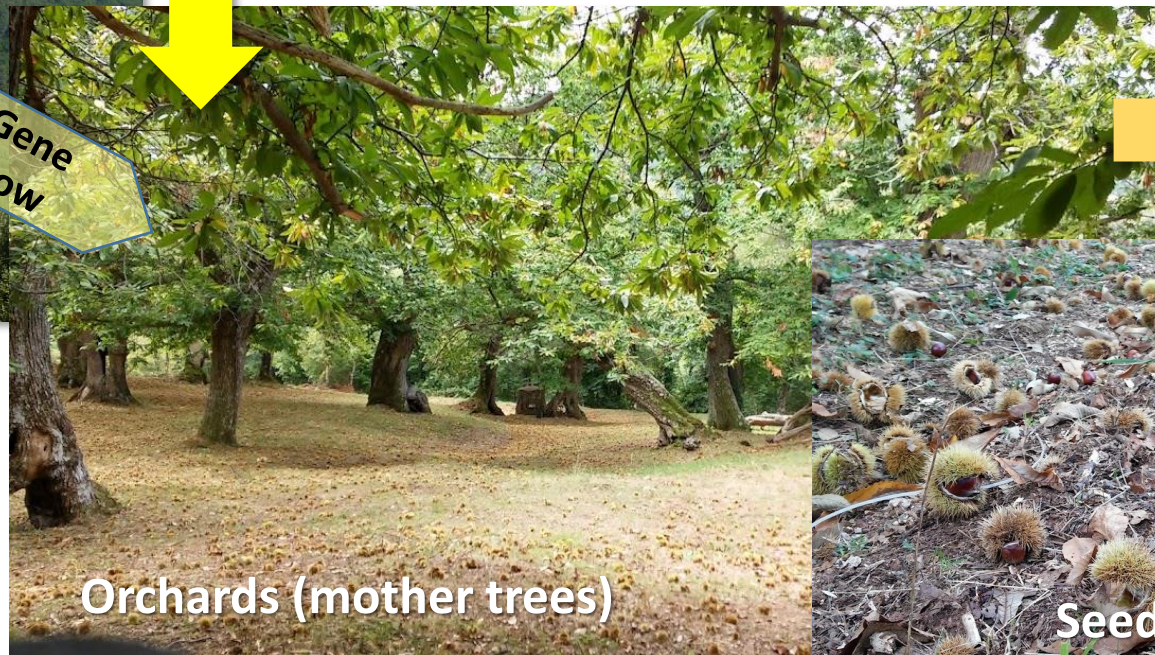
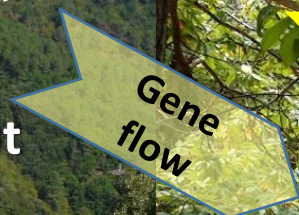


Pollen

Features of sweet chestnut

Coexistence of heterogeneous genetic resources

- *Natural and naturalized populations*
- *Managed coppice*
- *Orchards with grafted cultivars*
- *Male-sterility of Marron cultivars*



Seeds

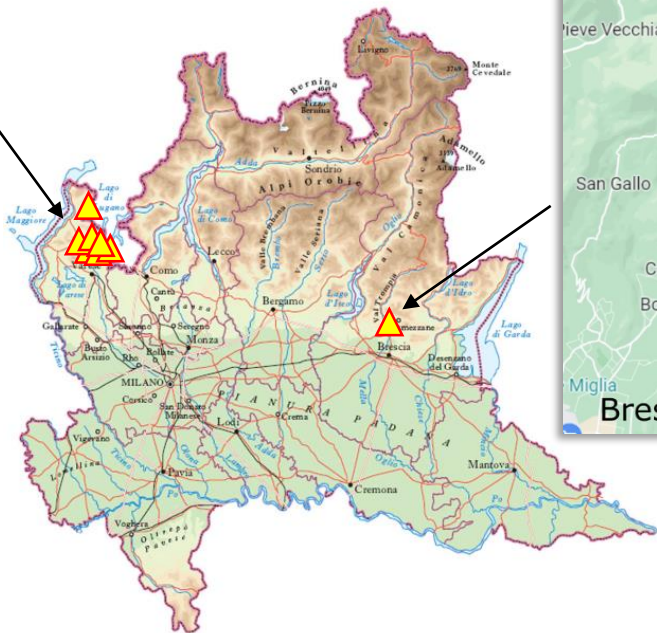


Lombardy region: sampling and experimental design

Province Varese



Map of study sites



Province Brescia



| Site | Fruits |
|----------------------------------|--------|
| Province Varese | |
| Curiglia | ✓ |
| Castelveccana | ✓ |
| Aga (Castelzuigno) | ✓ |
| Brinzio | ✓ |
| Orino | ✓ |
| Velate | ✓ |
| Arcisate | ✓ |
| <i>Castello Cabiaglio (Wild)</i> | |
| <i>Curiglia (Wild)</i> | |
| Province Brescia | |
| Serle | ✓ |
| Meder | ✓ |
| <i>Madonnina (Wild)</i> | |

- 20 orchard trees per site
- 15-20 fruits (embryos) per site
- 20 trees from some wild stands

Molecular analyses

- SSR markers, 12 loci
- Data analysis, GenAlex software



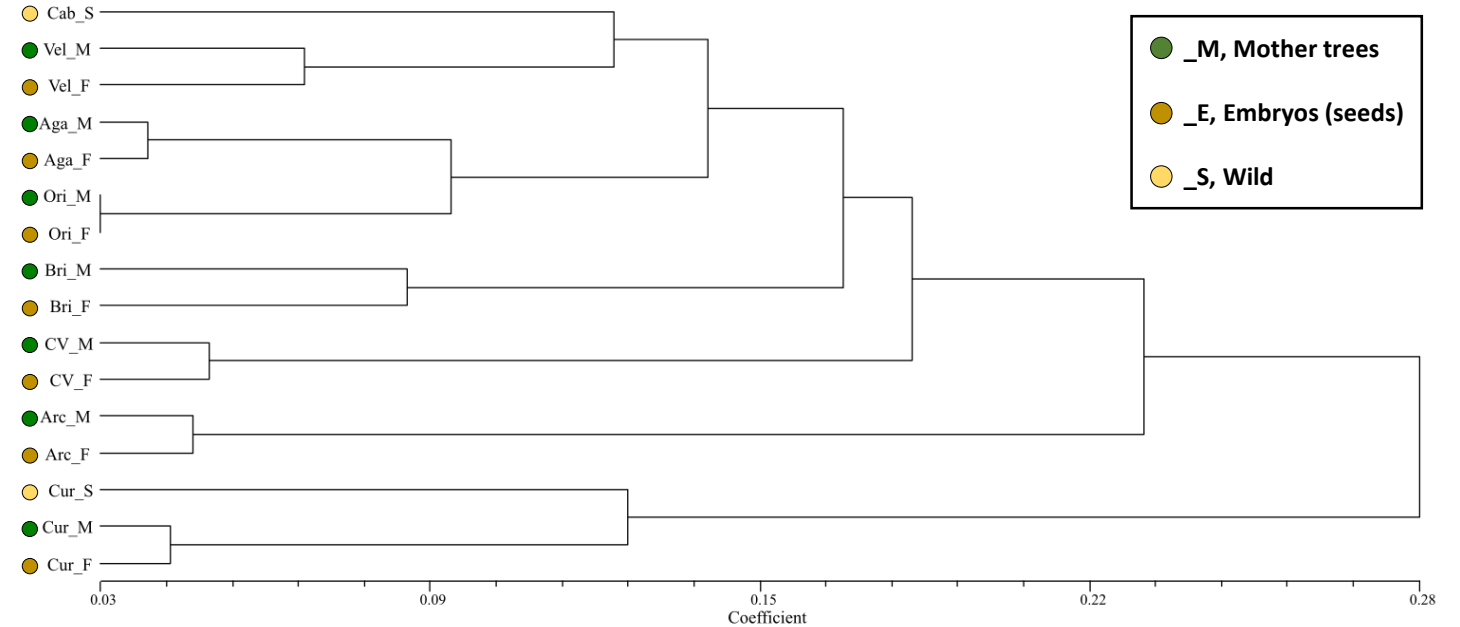


Clonality

| Genotype CV | Ramets | Site |
|--------------|--------|----------|
| Gen. 1 unkn. | 2 | Castelv. |
| Gen. 2 unkn. | 2 | Curiglia |
| Gen. 3 unkn. | 3 | Brinzio |
| Gen. 4 unkn. | 2 | Orino |

| Site | Label |
|---------------------------|-------|
| Curiglia | Cur |
| Castelveccana | CV |
| Aga (Casalzuigno) | Aga |
| Brinzio | Bri |
| Orino | Ori |
| Velate | Vel |
| Arcisate | Arc |
| Castello Cabiaglio (Wild) | Cab |
| Curiglia (Wild) | Cur |

Genetic distance: orchards, seeds, and wild stands



UPGMA dendrogram, from matrix of genetic distance (Nei)

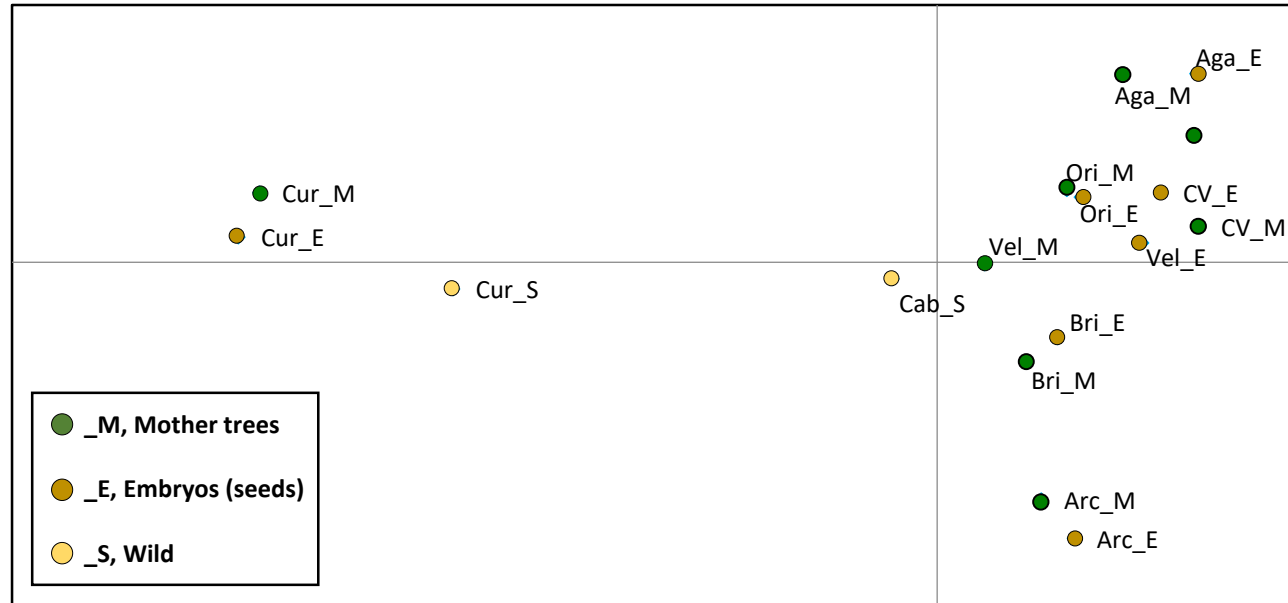
Management and structure

- Most of orchard trees have **unique genotypes**
- **Few clonal replicates** by grafting were found, of undefined cultivar
- Absence of Marron trees

Genetic diversity

- The pattern of genetic differentiation is consistent with the geographic distance
- Orchards are genetically similar to local wild stands
- Fruit offspring of some sites are genetically similar to orchard trees

Principal Coordinates (PCoA)



- The pattern of genetic distance reflects the geographic distribution. *Small effect of selection and breeding*
- Orchards are genetically similar to local wild chestnut. *Cultivated chestnut likely derives from local genetic resources*
- Fruit offsprings are genetically similar to orchard trees. *Pollen gene flow is within the orchards*



Clonality

| Genotype CV | Ramets | Site |
|-------------|--------|-------|
| Marrone | 20 | Serle |
| Rossera | 5 | Serle |

| Site | Label |
|-------------------|-------|
| Meder (seedlings) | Med |
| Serle | Ser |
| Madonnina (wild) | Mad |

Management and structure

- **Meder**, unusual orchard composed of **seedlings**
- **Serle**, grafted CVs of **Marrone** and **Rossera**

Genetic diversity

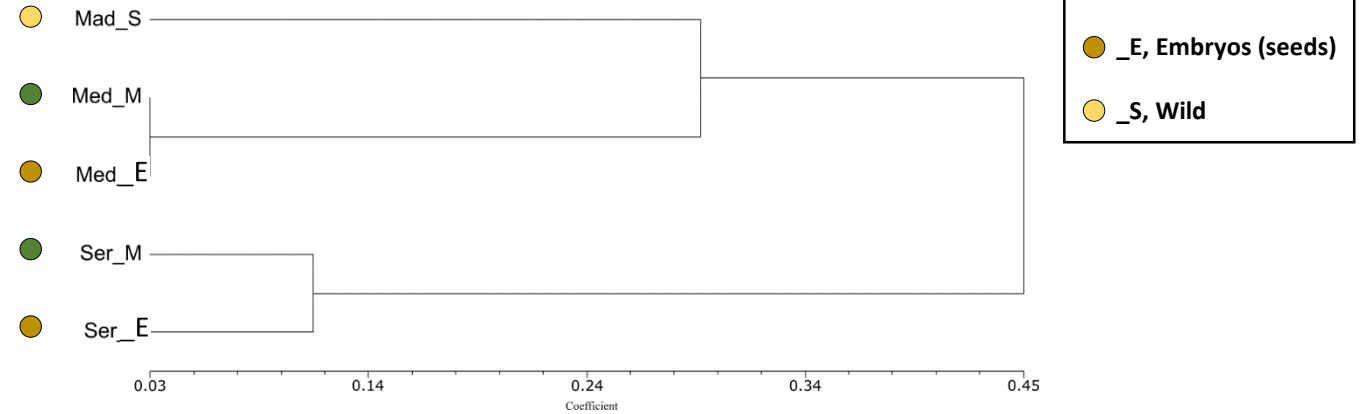
- Relatively high differentiation consistent with different propagation modes
- **Meder**. Fruit offspring from seedling trees is very close to orchards

Pollen gene flow is within the orchards

- **Serle**. Fruit offspring from grafted trees display the genetic effect of local wild pollen sources

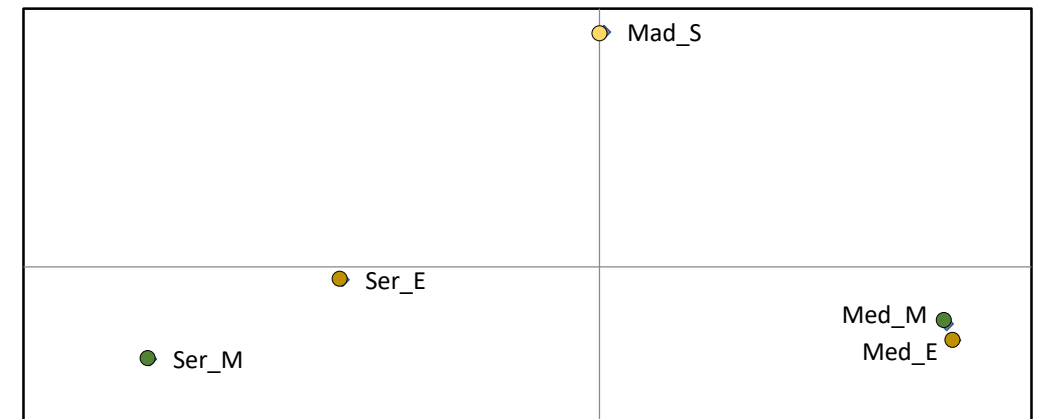
Male-sterile Marron trees receive pollen from wild pollen sources

Genetic distance: orchards, seeds, and wild stands



UPGMA dendrogram, from matrix of genetic distance (Nei)

Principal Coordinates (PCoA)



Varese province

- Genetic differentiation among sites at a small geographic scale. *Possibility to discriminate provenances.*
- Unexpected genetic diversity within orchards. *Resources for conservation and breeding*
- Unconventional orchard management with scarce diffusion of grafted cultivars
- Local landraces and cultivars are poorly characterized. *Need for varietal standards*

Brescia province

- Mixed orchard management system in a small area: Seedling orchards, grafted cultivars, Marron
- High genetic diversity within orchards. *Resources for conservation and breeding*
- Fruit samples display the gene flow from local wild pollen sources. *Possibility to discriminate local Marron fruits*



Umbria: sampling and experimental design

Map of study sites



Marradi (FI)

1. Com. Marradi
2. Loc. Serravalle

Città di Castello (PG)

1. Loc. Lippiano Pescaloto
2. Loc. Lippiano Ransola

Orvieto (TR)

Loc. Sugano

Baschi (TR)

Loc. Morre

Montecchio (TR)

Loc. Melezzole



Spoleto (PG)

1. Loc. Montebibico
2. Loc. Montebibico
3. Loc. Montebibico
4. Loc. Borgiano
5. Loc. Acqualacastagna
6. Loc. Battiferro
7. Loc. Montebibico

Stroncone (TR)

Loc. Cimitelle

Study sites in Umbria

- **Spoleto** (PG): 7 zones
- Città di Castello (PG): 2 localities
- Stroncone (TR)
- Baschi (TR)
- Montecchio (TR)
- Orvieto (TR)

External reference of Marron cultivar

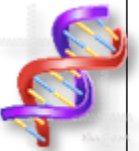
- **Marradi (FI), 2 localities**

Experimental design

- 7-29 orchard trees per site
- Subset of selected sites*
- 7-16 fruits per site
- 20 trees from surrounding wild stands

Molecular analyses

- SSR markers, 8 loci
- Data analysis: GenAlex software



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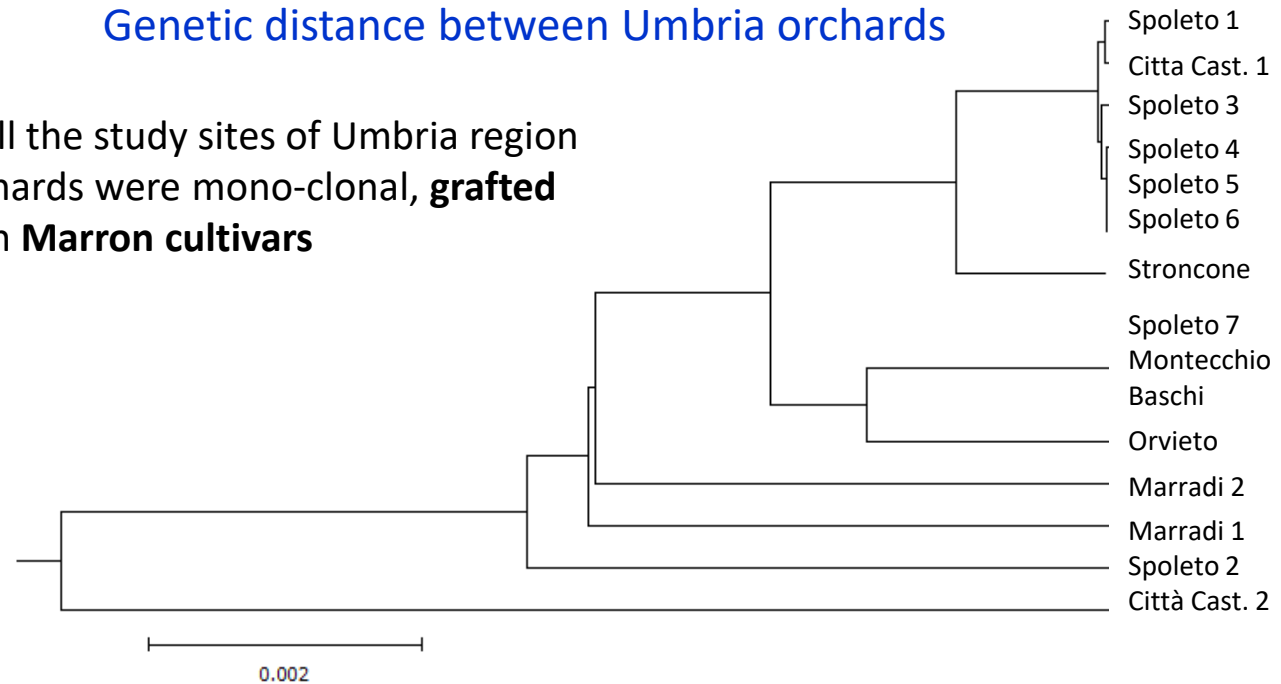
Genetic analysis of orchards



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Genetic distance between Umbria orchards

In all the study sites of Umbria region orchards were mono-clonal, **grafted** with **Marron cultivars**



UPGMA dendrogram based on matrix of genetic distance (Nei).

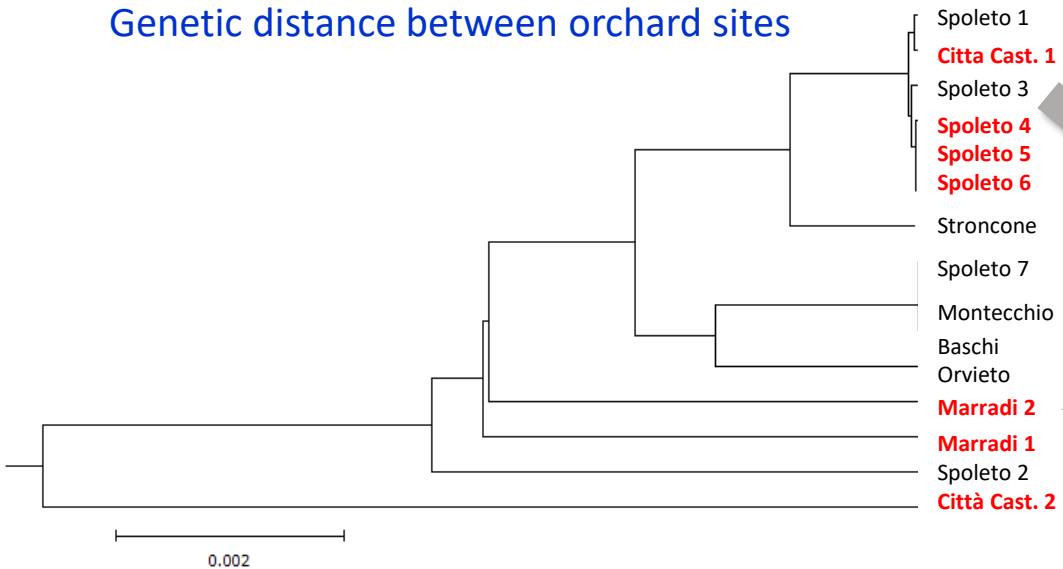
- Very **low overall genetic differentiation** among Umbria orchards. *Selected Marron germplasm*
- Genetic clustering not fully consistent with the geographic provenance of orchards. *Effect of selection and cultivar deployment*
- Spoleto sites display multiple Marron genotypes, with a **major genotype spread in several sites**. *Suitable model to test geographical discrimination within one cultivar.*

Results Umbria – Genetic diversity of offspring

Genetic analysis of orchard offsprings

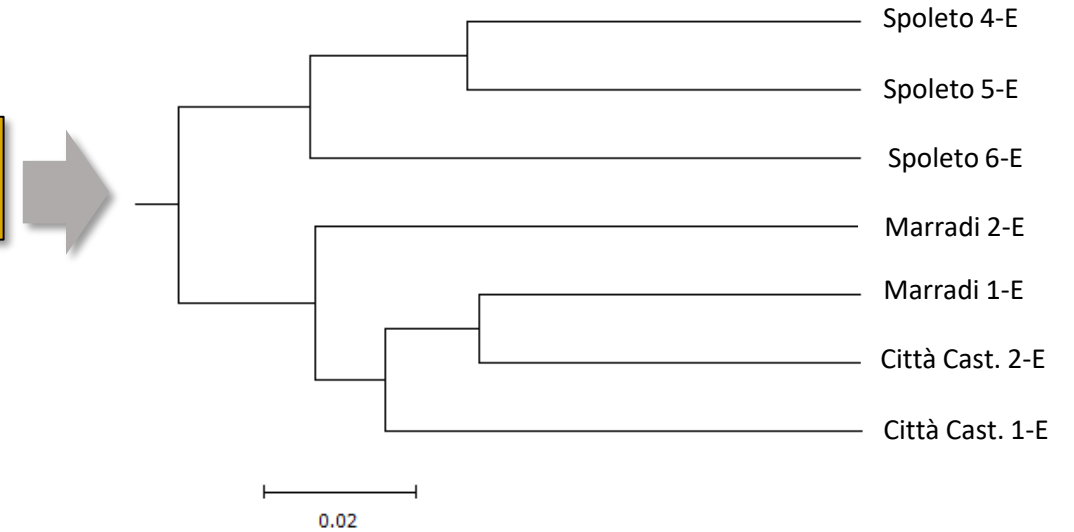
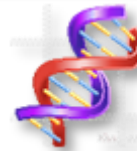


Genetic distance between orchard sites



Genetic distance between orchard offsprings

Samples of embryos



UPGMA dendrogram based on matrix of genetic distance (Nei).

- Orchard offsprings are much more differentiated than the respective mother trees
- The genetic clustering of offsprings is consistent with their geographic origin

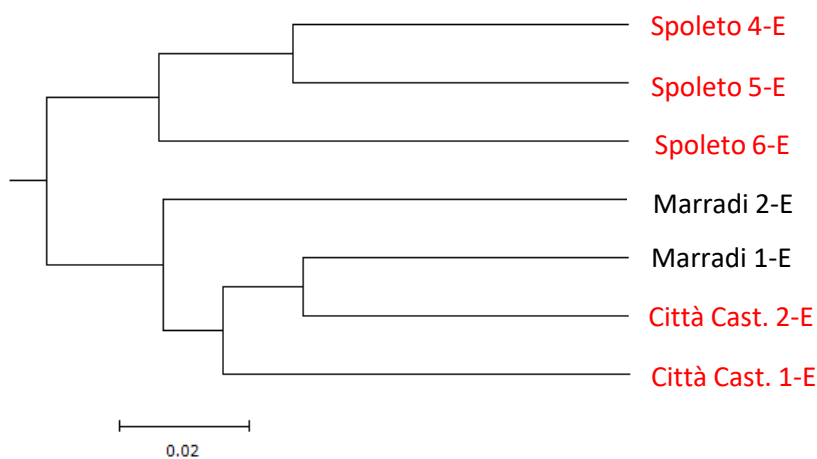
Results Umbria – Gene flow from local pollen sources




Genetic analysis of wild chestnut stands

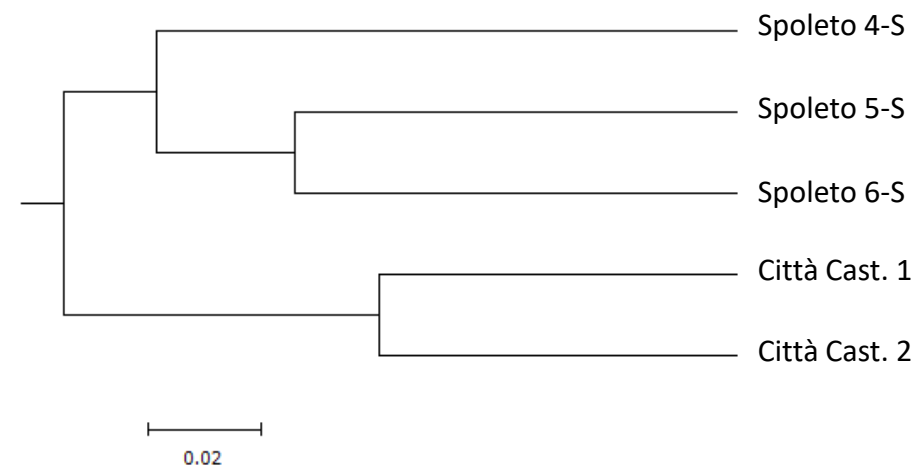


Genetic distance between orchard offsprings



Genetic distance between wild populations close to orchards

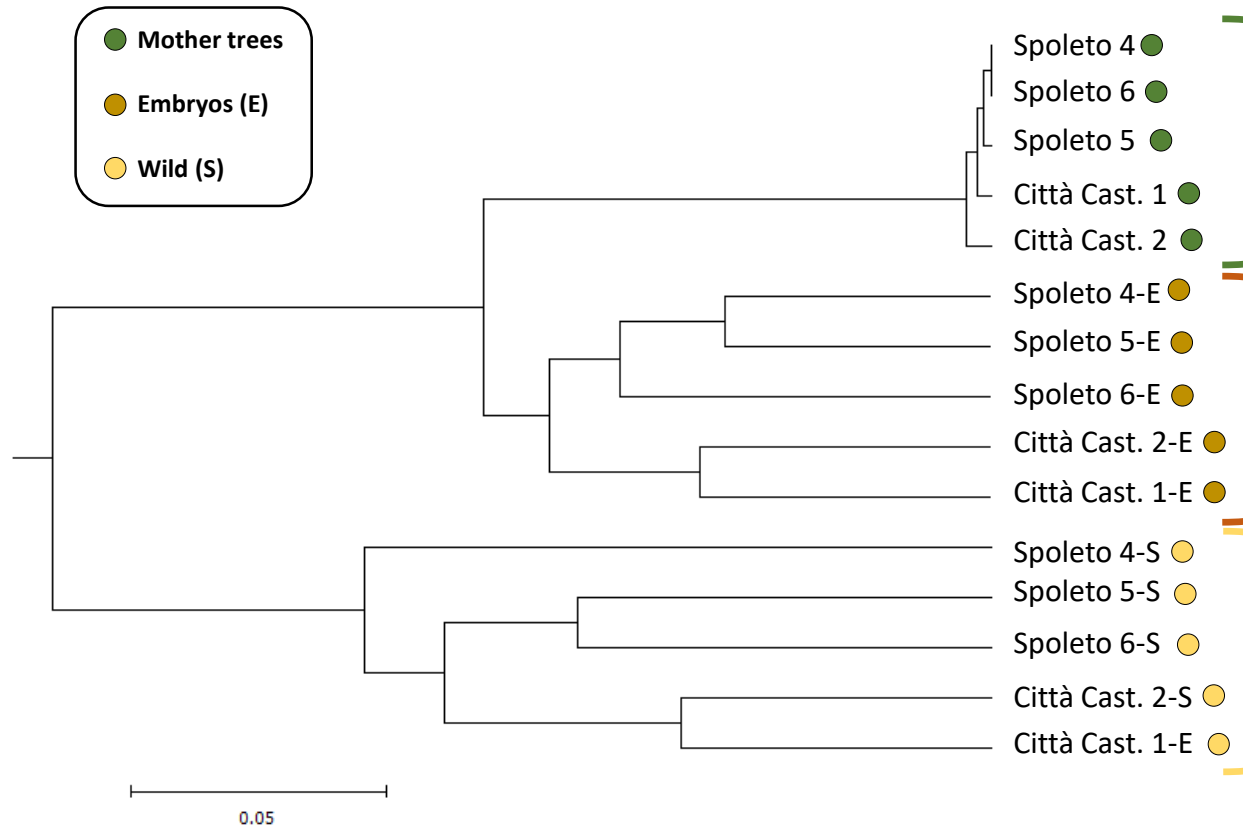
Similar clustering pattern




UPGMA dendrogram based on matrix of genetic distance (Nei).

- The genetic clustering of orchard offsprings is consistent with clustering of wild populations
- The orchard offsprings display the gene flow from local wild pollen sources

Genetic distances: orchards, seeds, and local wild populations

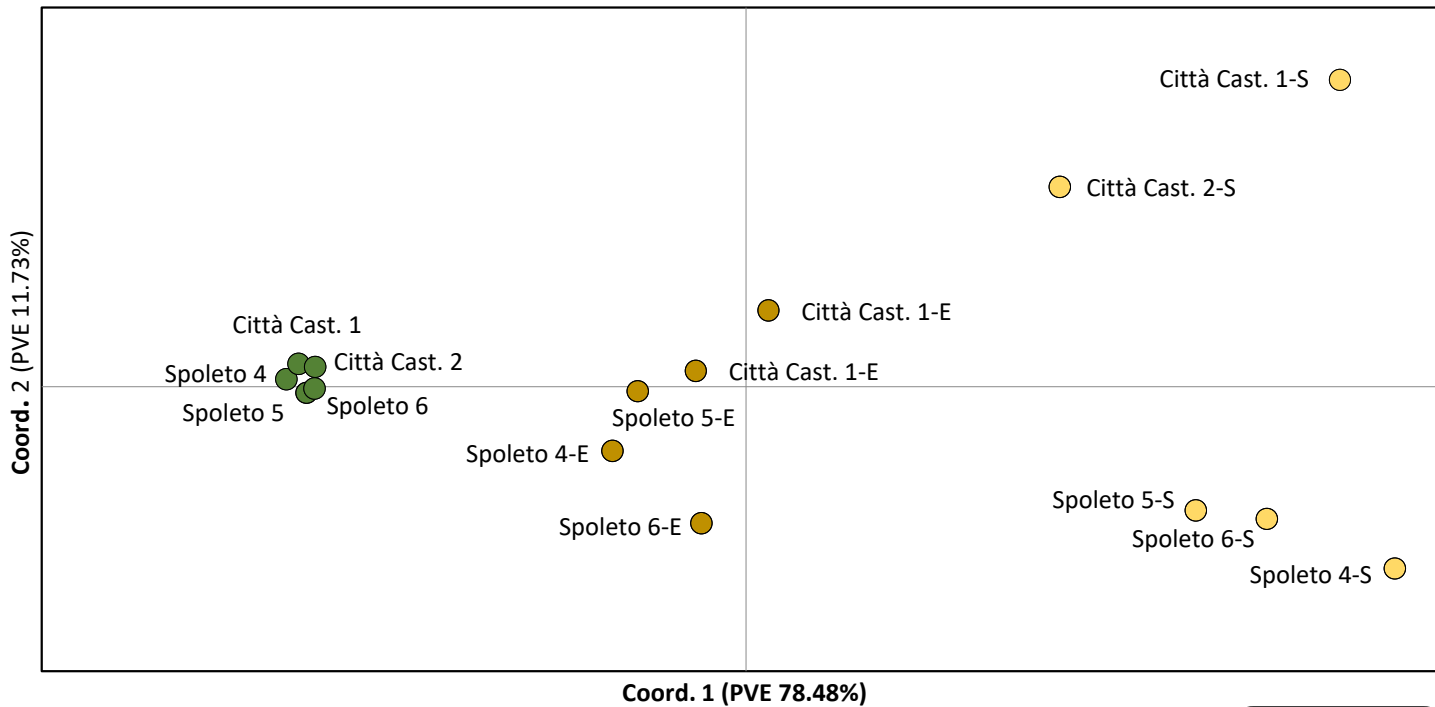


- The genetic differentiation between orchard seeds (offspring) is much stronger than between orchard trees
- The genetic structure of offsprings fits the genetic structure of local wild populations
- The forced pollen gene flow from wild chestnut to male-sterile Marron trees confers a unique genetic footprint to local chestnut fruit productions

UPGMA dendrogram based on matrix of genetic distance (Nei).

Genetic relationships between orchards, seeds, and local wild pollen sources

Principal Coordinates (PCoA)



- Offsprings from Marron orchards display a genetic profile intermediate between cultivars and local wild chestnut
- This mixed genetic profiles represents a genetic and geographic identity of Marron products
- Fruits from a unique cultivare genotype can be genetically differentiated by origin with a fine geographic resolution

Principal Coordinate Analysis based on matrix of genetic distance (Nei)

- Mother trees
- Embryos (E)
- Wild (S)

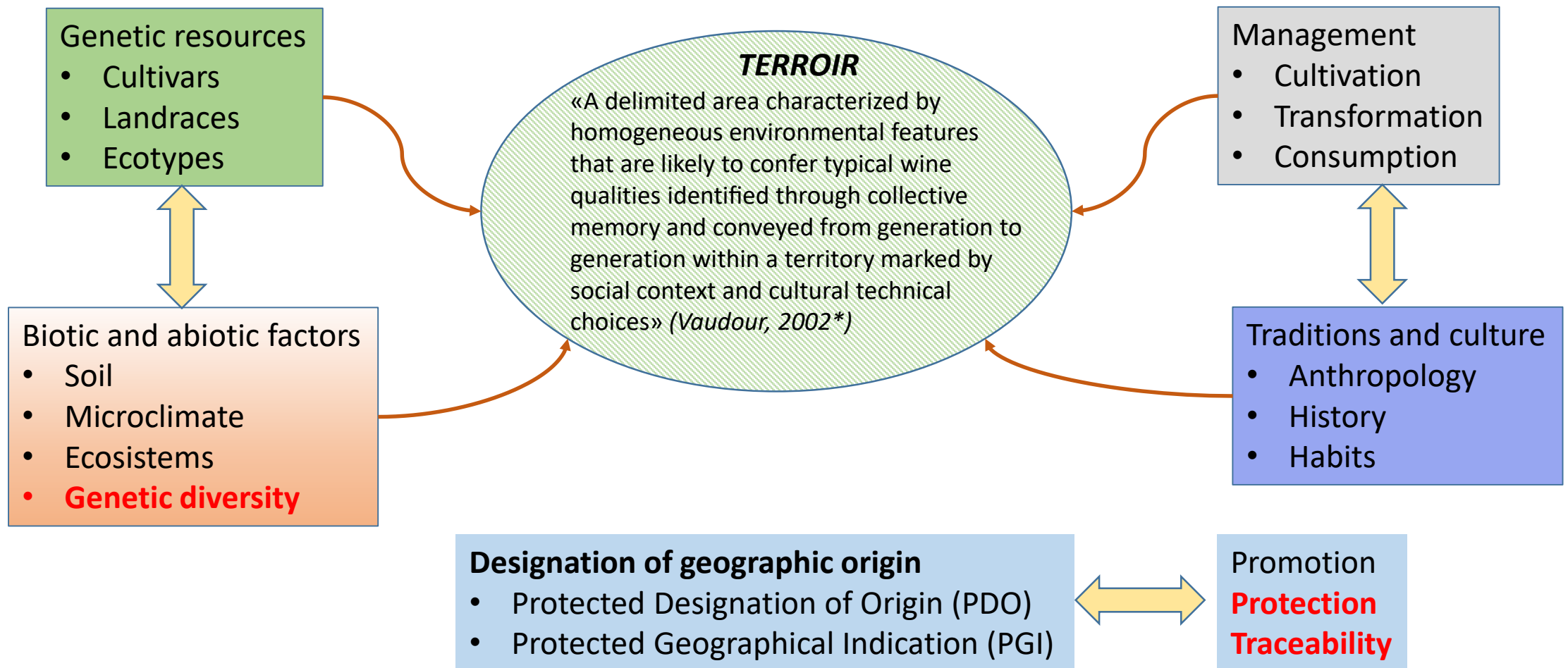
Genetic diversity of chestnut orchards in Umbria

- Conventional orchard management with mono-clonal stands of Marron graftings
- Relative richness of Marron genotypes at a small geographic scale.
- Low overall genetic differentiation of orchards and wide propagation of selected Marron germplasm
- The fruits from wide-spread Marron trees display the genetic footprint of local wild pollen sources



- Genetic analyses provide a clear characterization of genetic resources and management systems of traditional chestnut orchards
- Diverse management systems and germplasm of traditional chestnut orchards represent a reservoir of genetic and biocultural diversity
- An unexpectedly high genetic diversity is conserved in traditional orchards managed with poorly selected germplasm
- This integrated genetic analysis of orchards, fruits and wild chestnut
 - -> provides an informative characterization of chestnut resources,
 - -> opens perspectives for a fine-scale traceability of Marron fruit products
 - -> represents an implement of the *terroir* concept

Implementation of *terroir* concept with local genetic diversity



Vaudour, E. 2002. The quality of grapes and wine in relation to geography: notions of terroir at various scales, J. Wine Res., 13, 117–141, 2002.

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- Francesca Sparvoli

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- Giovanni De Simoni
- Muriel Gaudet
- Paola Pollegioni
- Liliana Ranzno
- Dania Valli



| Locus | Primer sequence | Repeat motif | Fragment size |
|---------|---|---|---------------|
| CsCAT1 | 5'-GAGAATGCCCACTTTTGCA-3' 5'-GCTCCCTTATGGTCTCG-3' | (TG) ₅ TA(TG) ₂₄ | 192-221 |
| CsCAT2 | 5'-GTAACCTGAAGCAGTGTGAAC-3' 5'-CGCATCATAGTGAGTGACAG-3' | (AG) ₁₆ | 195-232 |
| CsCAT3 | 5'-CACTATTTTATCATGGACGG-3' 5'-CGAATTGAGAGTTCATACTC-3' | (AG) ₂₀ | 195-261 |
| CsCAT6 | 5'-AGTGCTCGTGGTCAGTGAG-3' 5'-CAACTCTGCATGATAAC-3' | (AC) ₂₄ AT(AC) ₄ | 160-196 |
| CsCAT14 | 5'-CGAGGTTGTTGTTTCATCATTAC-3' 5'-GATCTCAAGTCAAAGGTGTC-3' | (CA) ₂₂ | 131-160 |
| CsCAT16 | 5'-CTCCTTGACTTTGAAGTTGC-3' 5'-CTGATCGAGAGTAATAAAG-3' | (TC) ₂₀ | 124-140 |
| CsCAT17 | 5'-TTGGCTATACTTGTCTGCAAG-3' 5'-GCCCATGTTTTCTCCATGG-3' | (CA) ₁₉ A(CA) ₂ AA(CA) ₃ | 133-164 |
| CsCAT34 | 5'-TGAGCAAGGATGGATGATGAG-3' 5'-GGTGGTCATCATGACTGCATC-3' | (GT) ₂₃ | 145-183 |
| CsCAT41 | 5'-AAGTCAGCAACACCACCATATGC-3' 5'-CCCCTGTTTCATGAGTTTCT-3' | (AG) ₂₀ | 201-243 |
| EMCs25 | 5'-ATGGGAAAATGGGTAAAGCAGTAA-3' 5'-AACCGGAGATAGGATTGAACAGAA-3' | (GA) ₁₂ | 140-160 |
| EMCs32 | 5'-TTCCACACTTATCTCTTAACCCAAAAA-3' 5'-CTCCGGTACGGTATTGACTTCCTT-3' | (AG) ₁₈ | 92-100 |
| EMCs38 | 5'-TTCCCTATTTCTAGTTTGTGATG-3' 5'-ATGGCGCTTTGGATGAAC-3' | (AG) ₃₁ | 230-270 |

