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UNIVERSITÀ DEGLI STUDI DI MILANO

DIPARTIMENTO DI SCIENZE AGRARIE E AMBIENTALI - PRODUZIONE, TERRITORIO, AGROENERGIA

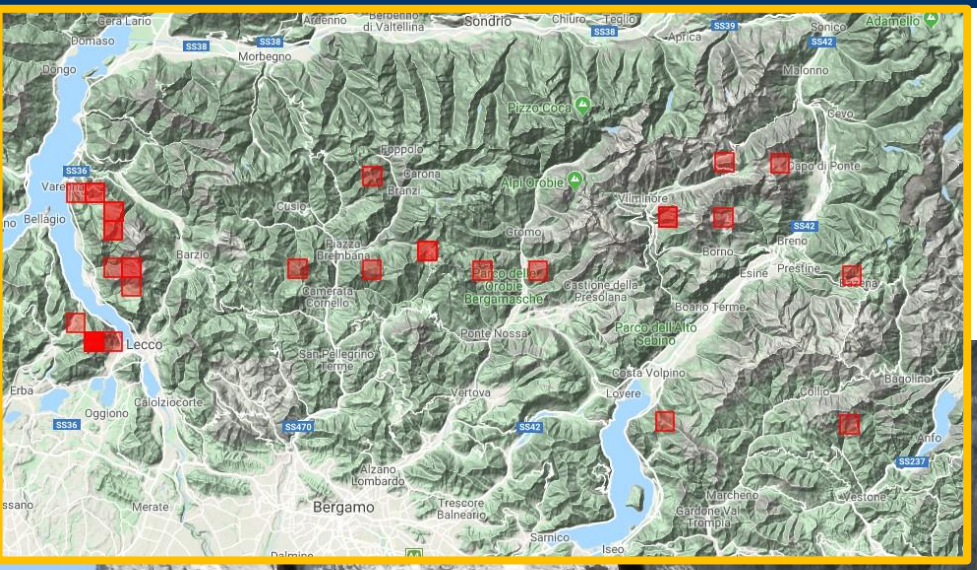
Inter-population variability in seed germination, reproductive ecology and genetic structure of a rare chasmophyte (*Campanula raineri*, Campanulaceae): insights for conservation purposes

SEED ECOLOGY VII

2022 • SEPTEMBER 6-9 • ASTURIAS

Tutors:
prof. Simon Pierce;
Matteo Montagna;
Cristiano Vernesi

Case study



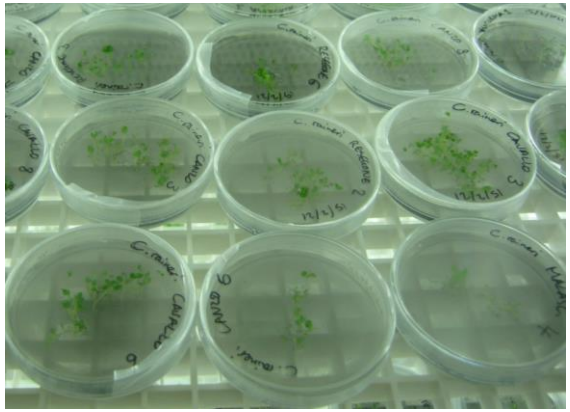
Endemic chasmophyte.

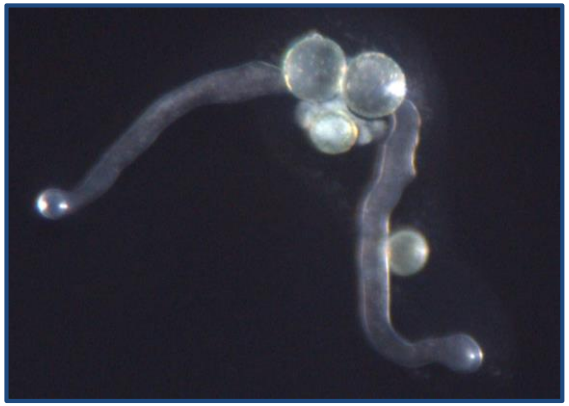
1000-2000 m a.s.l. on calcareous-dolomitic cliffs.

Aim

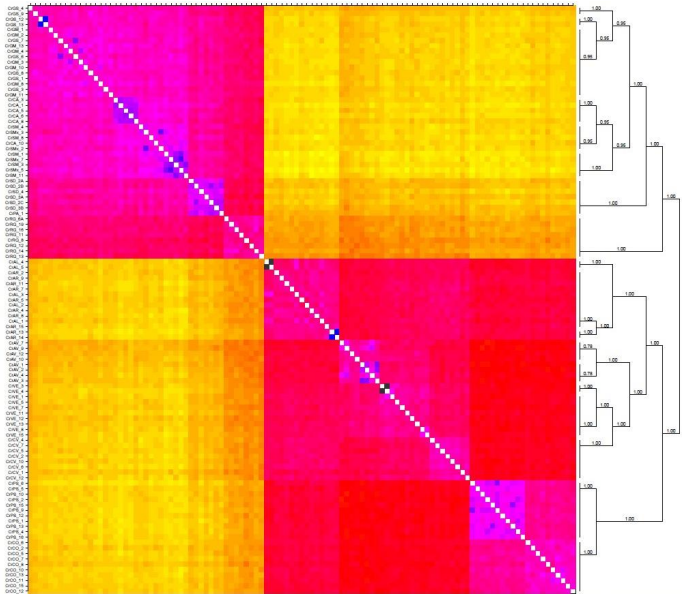
Are conservation actions necessary for this species?

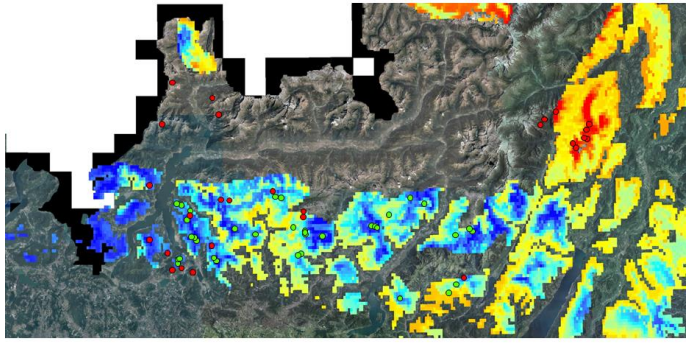
Reproductive fitness assessment





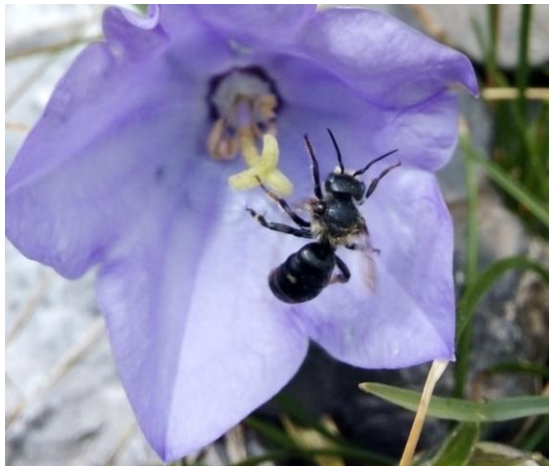
Population genomics

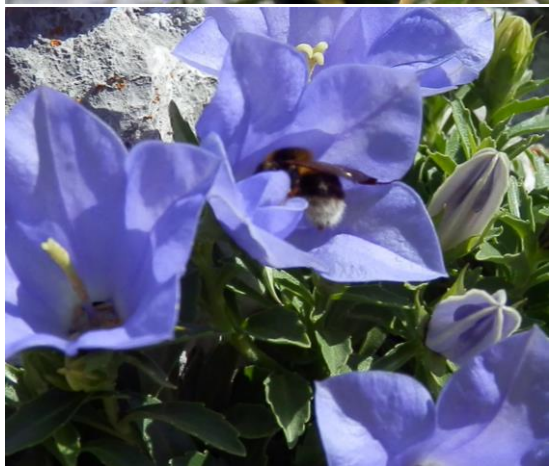




Species Distribution Models (SDMs)

Pollinator assessment

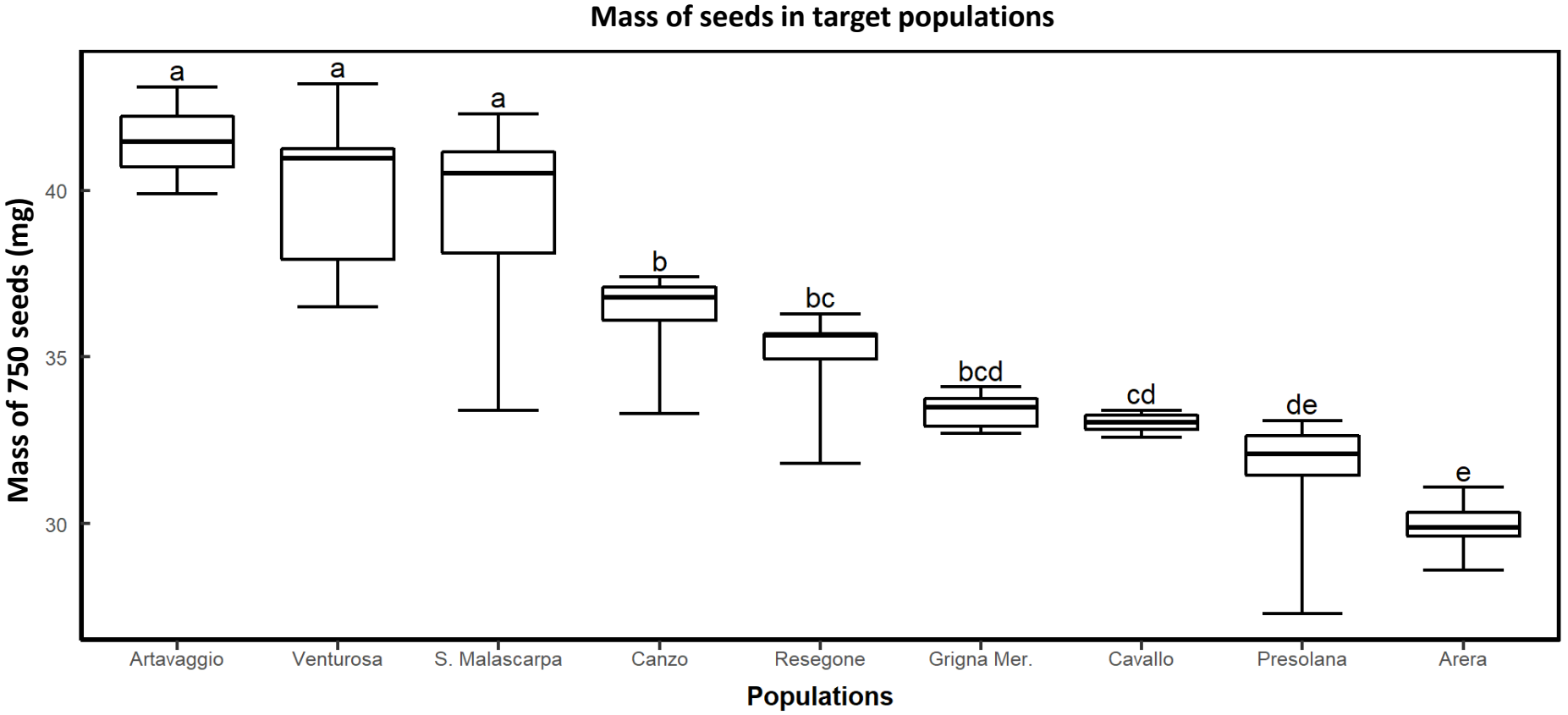




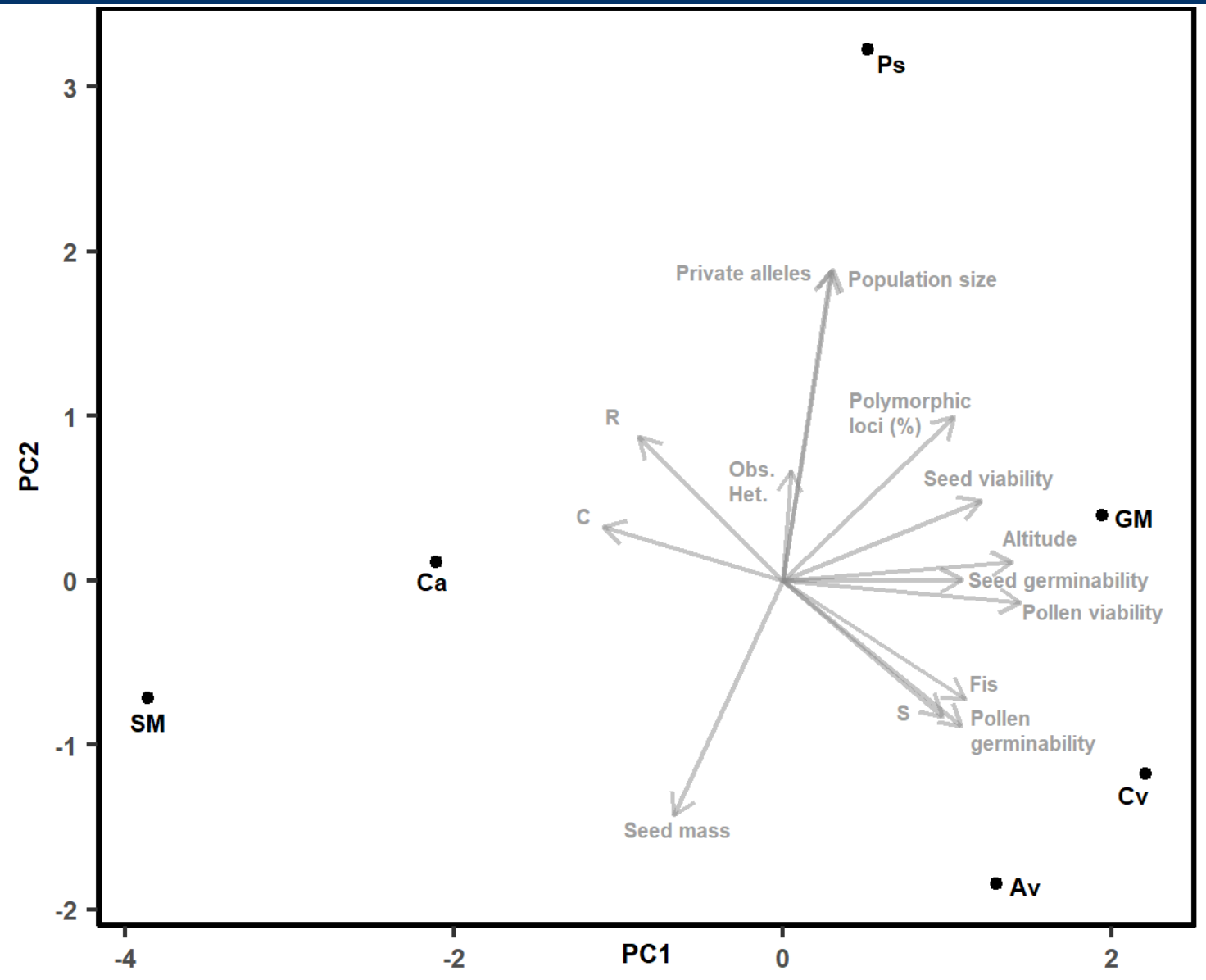
Results: seed mass across target populations

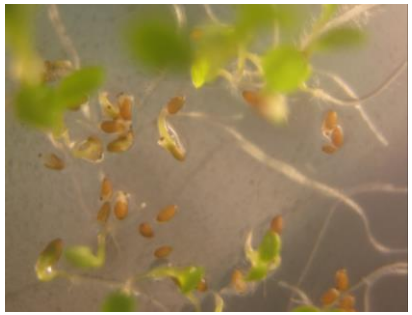
Analysis on seed mass

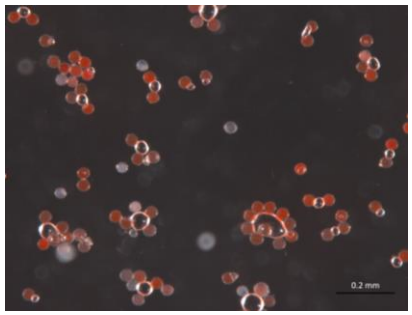
Different letters denote significant differences between means (n = 10) at P ≤ 0.01 (ANOVA, df = 7, F = 74.63, P = <2e-16; followed by Tukey multiple comparison procedure)

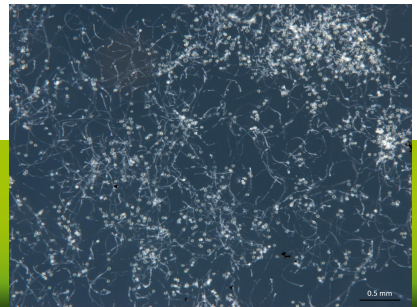


Results: relationships between reproductive traits, elevation and genetics









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E AMBIENTALI - PRODUZIONE,
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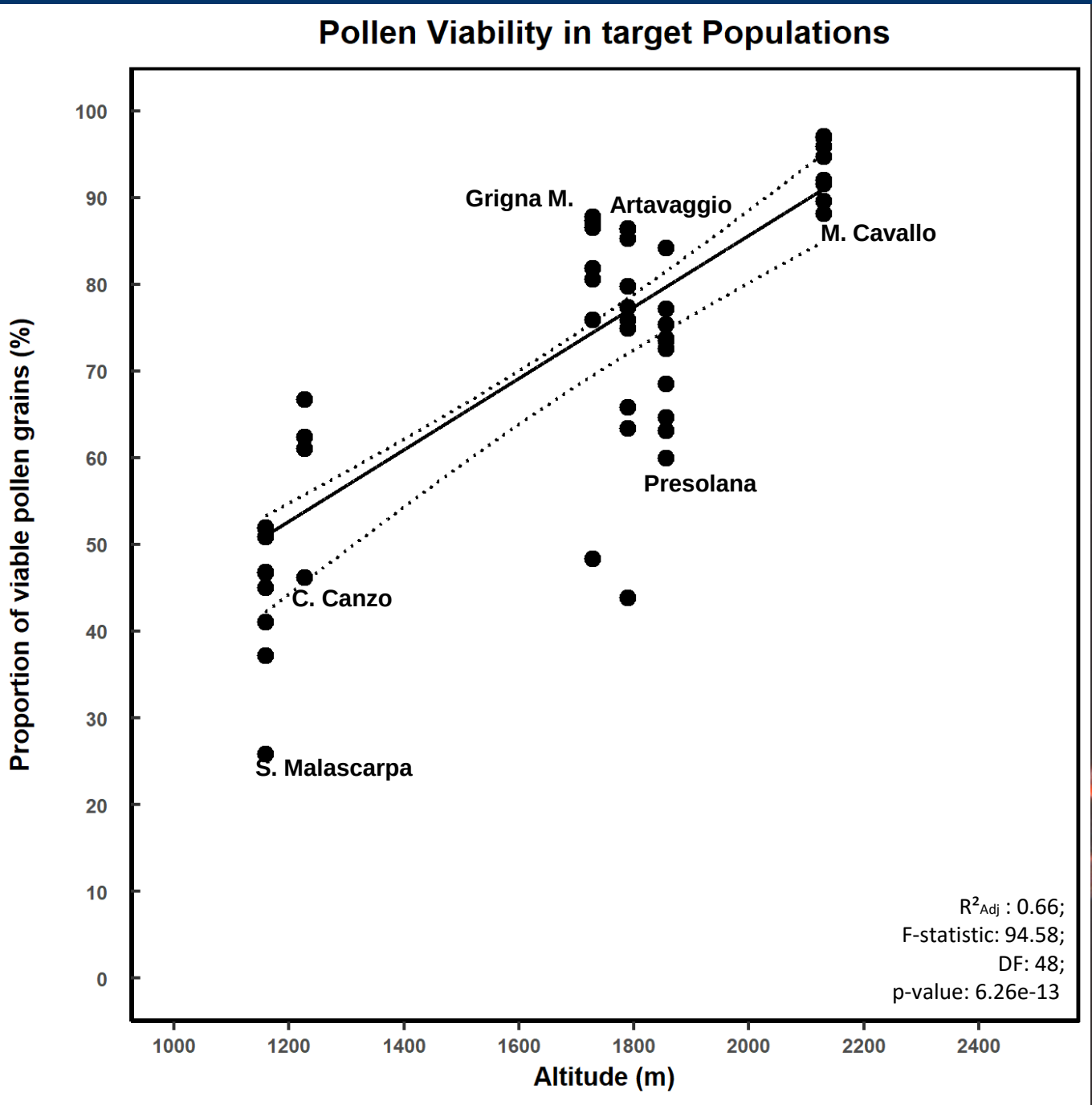
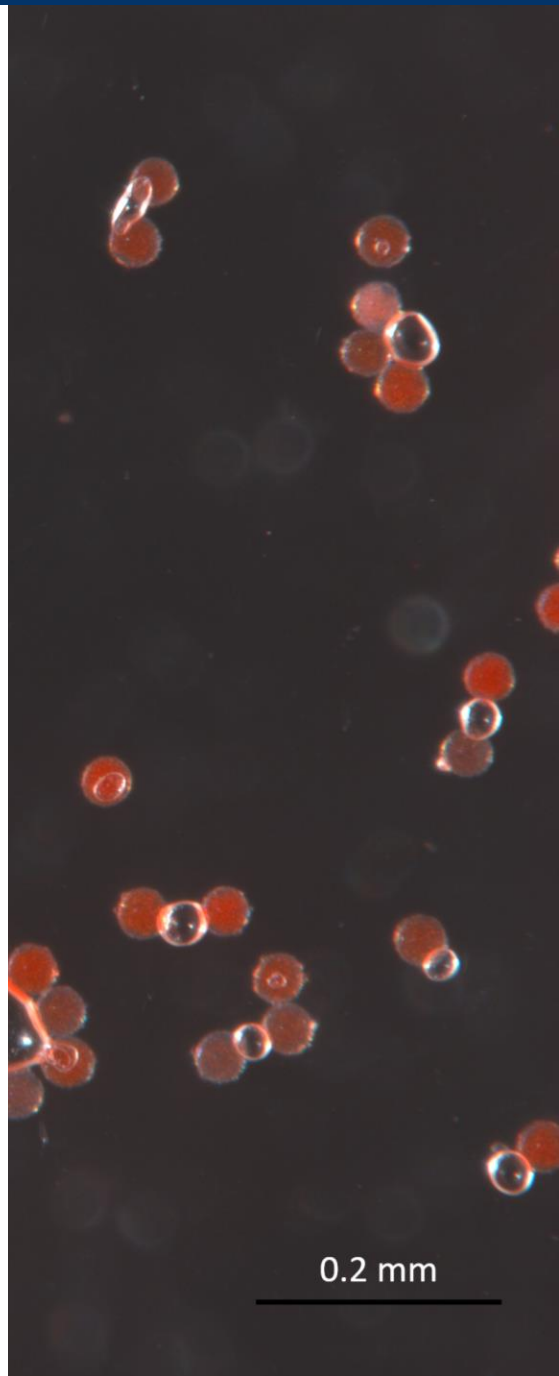
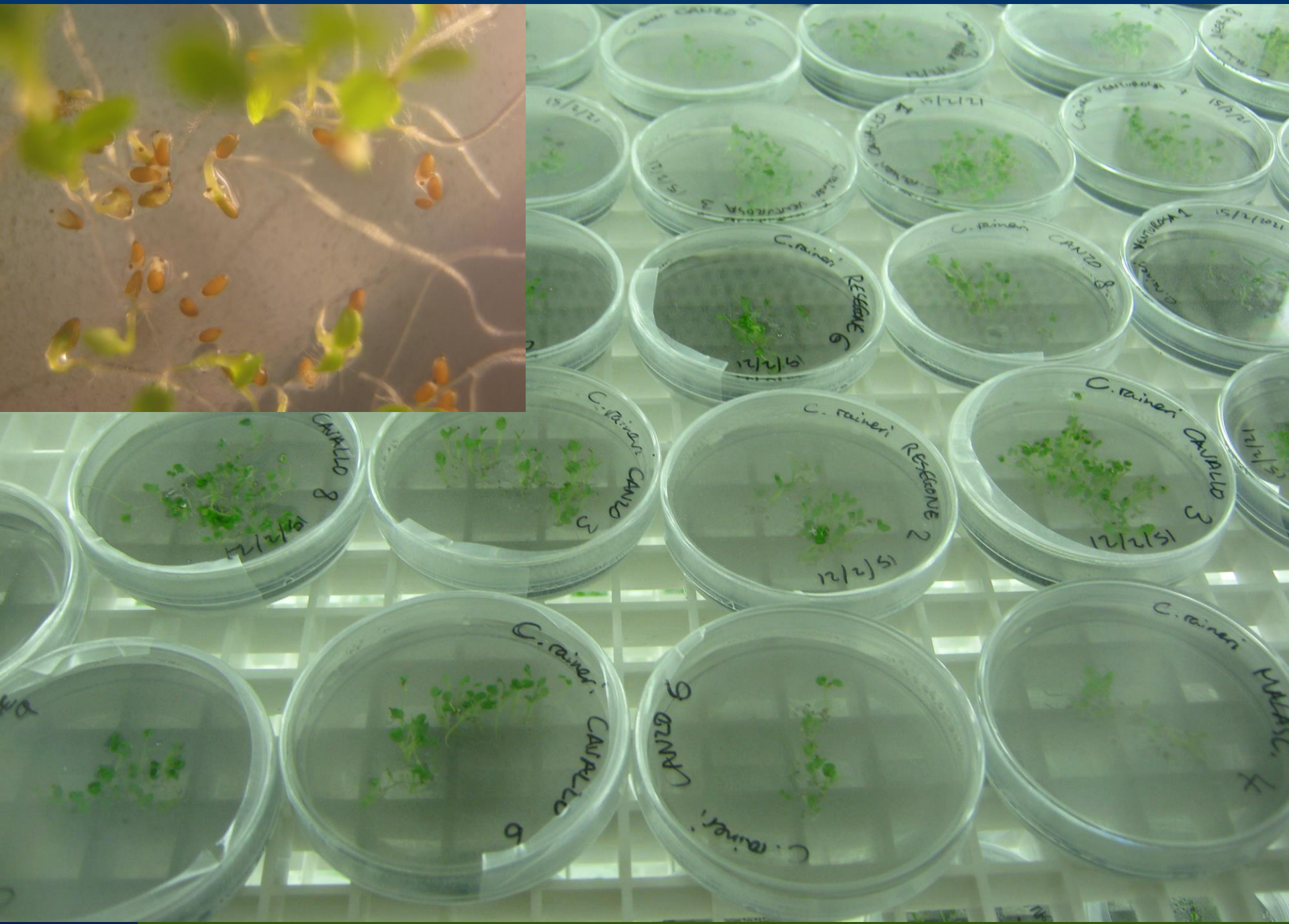
Results: pollen viability vs elevation



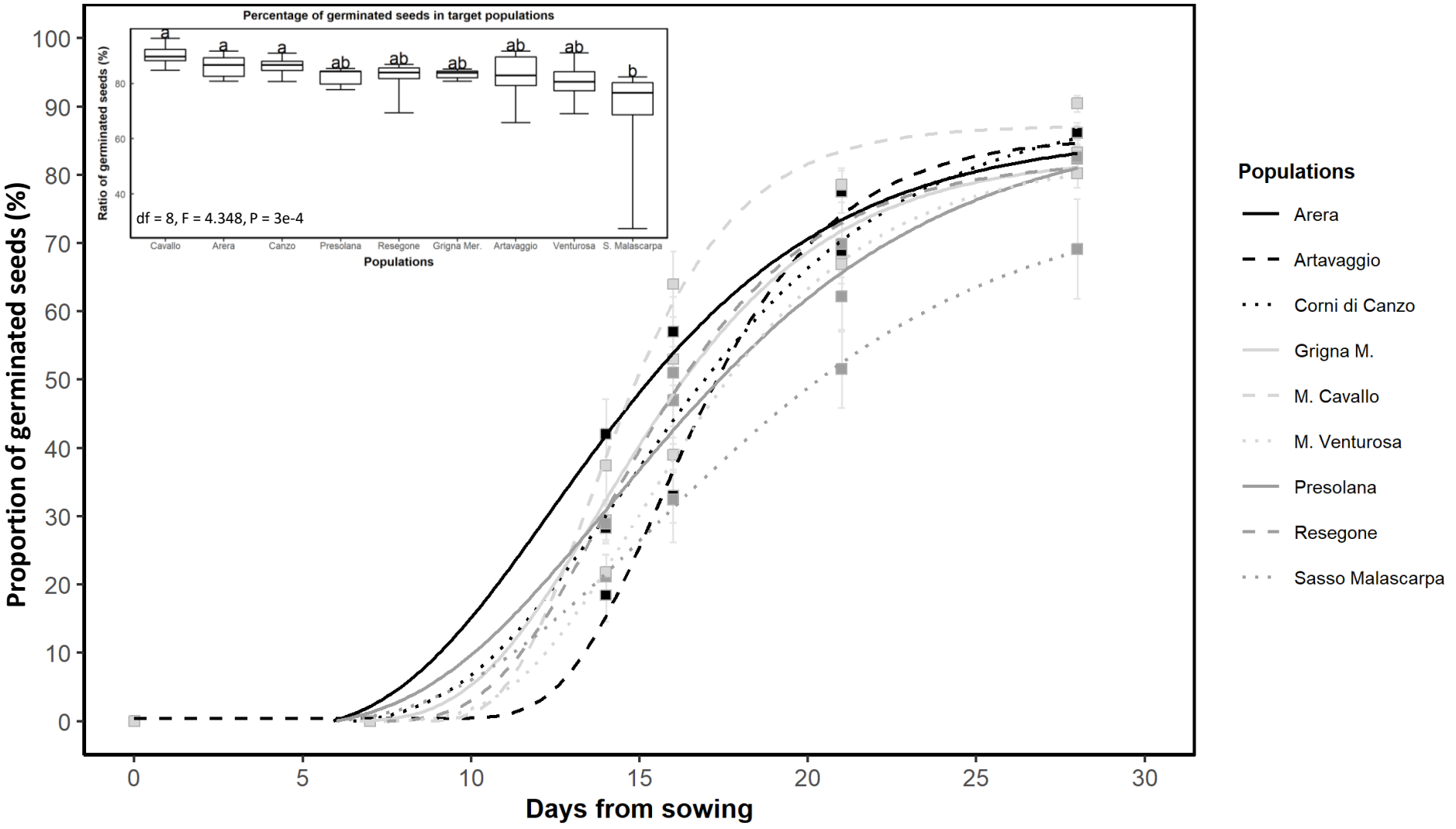


Results: seed germination test

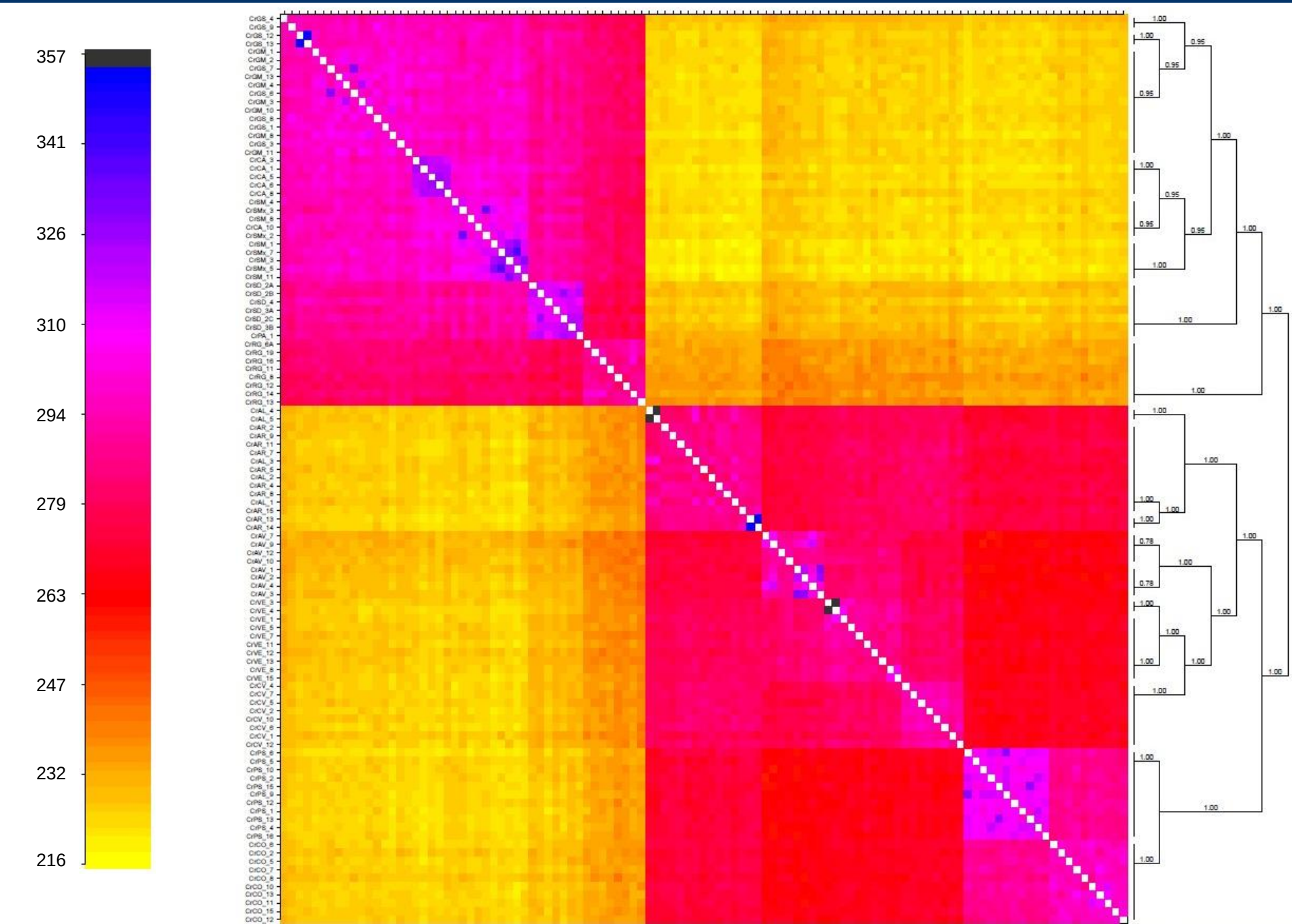


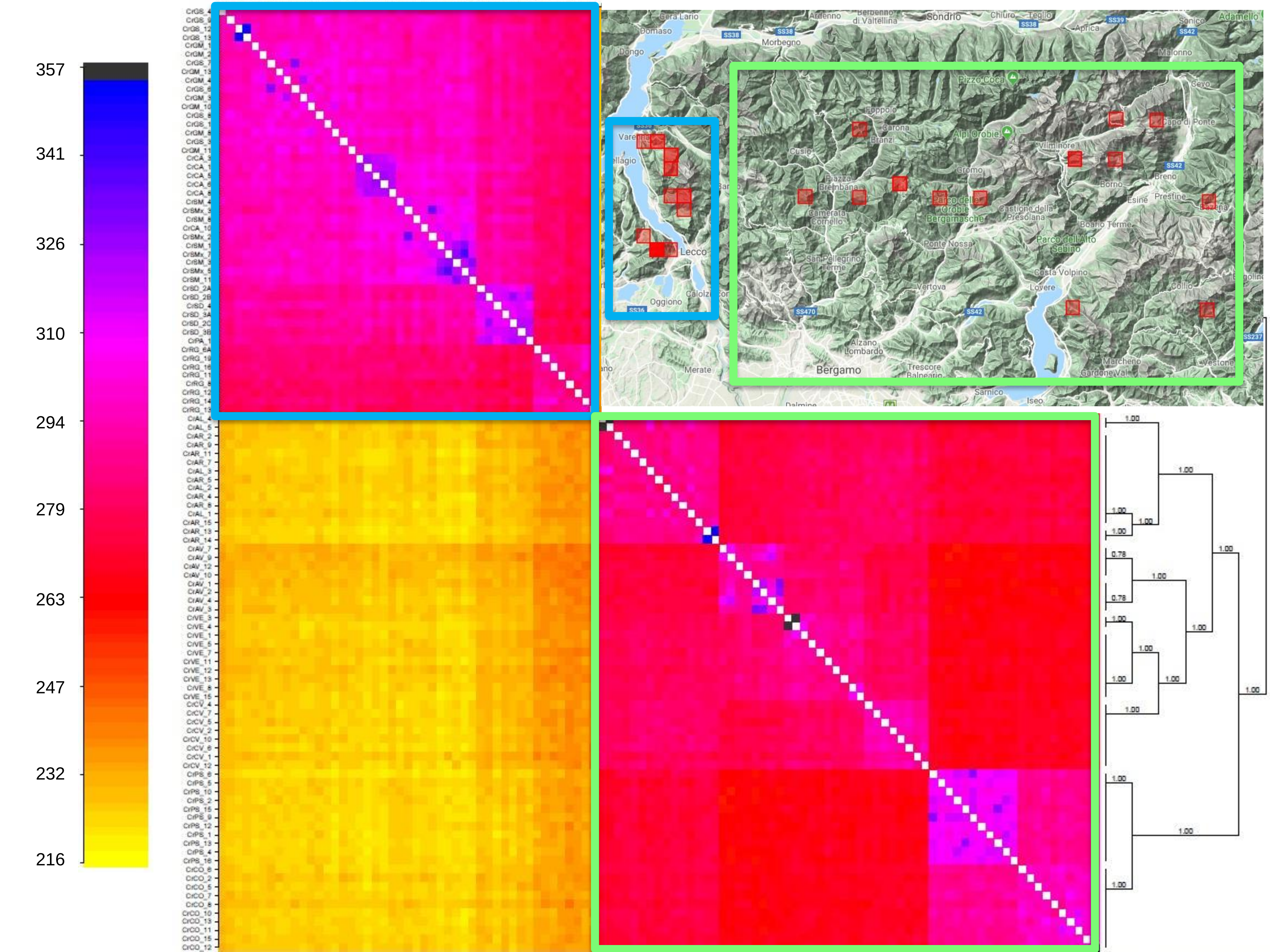
Results: seed germination test

Germination rate in target populations



Results: genomic analysis of populations

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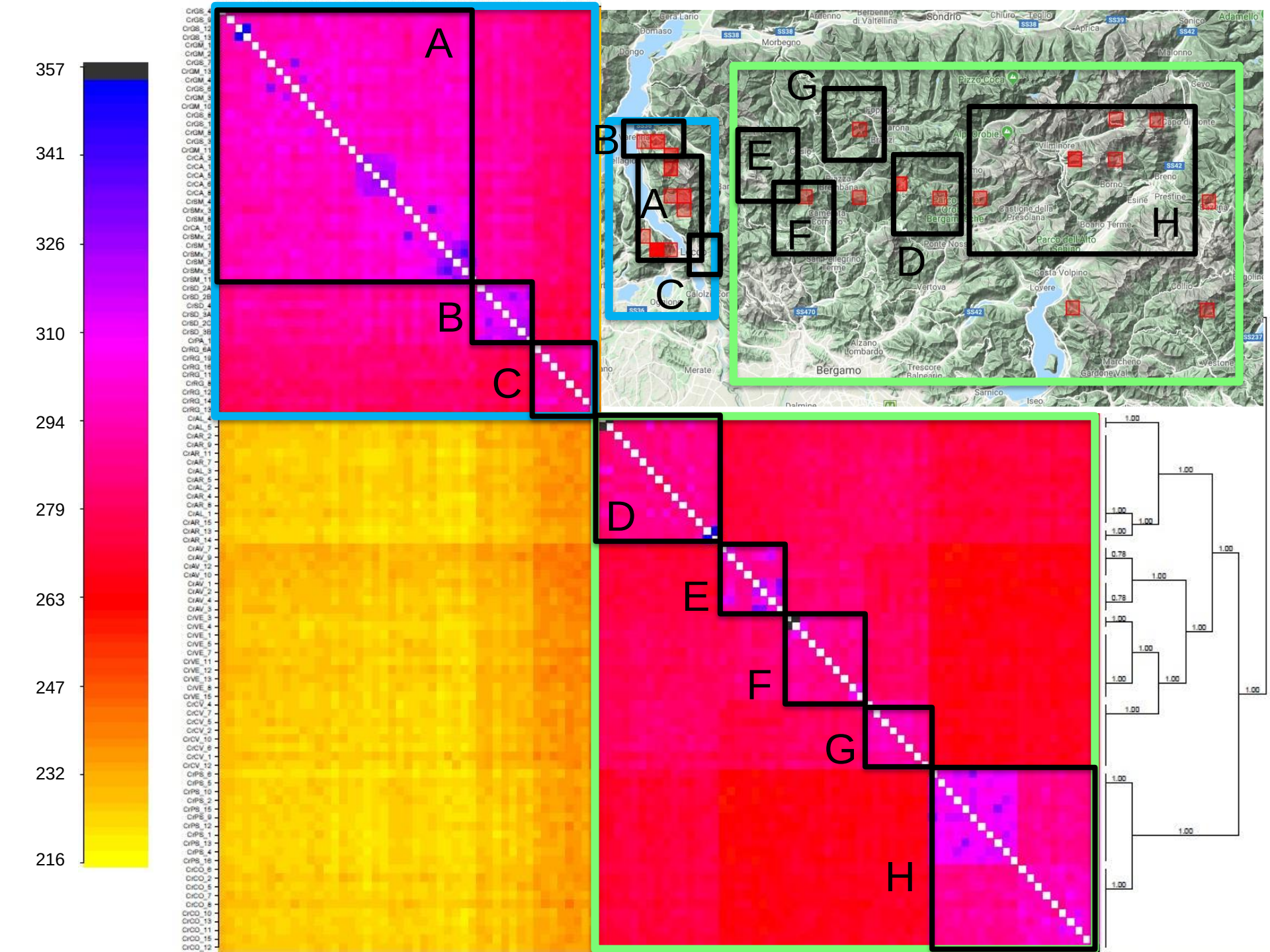
Morbegno
Domaso
Dongo
Pizzo Coca
Alpi Orobie
Carona
Branzi
Foppolo
Cusio
Gromo
Vilminore
Capo di Ponte
Breno
Borno
Esine
Prestine
Piazza Brembana
Camerata Cornello
Parco delle Orobie Bergamasche
Castione della Presolana
Boario Terme
Parco dell'Alto Sebino
Ponte Nossa
San Pellegrino Terme
Costa Volpino
Lovere
Vertova
Collio
Lecco
Varenna
Bellagio
Oggiono
Merate
Alzano Lombardo
Bergamo
Trescore Balneario
Sarnico
Iseo
Marcheno
Gardone Val
Vestone
Malonno
Aprica
Teglio
Chiuro
Adamello
Sonico
Cevo
Dalmine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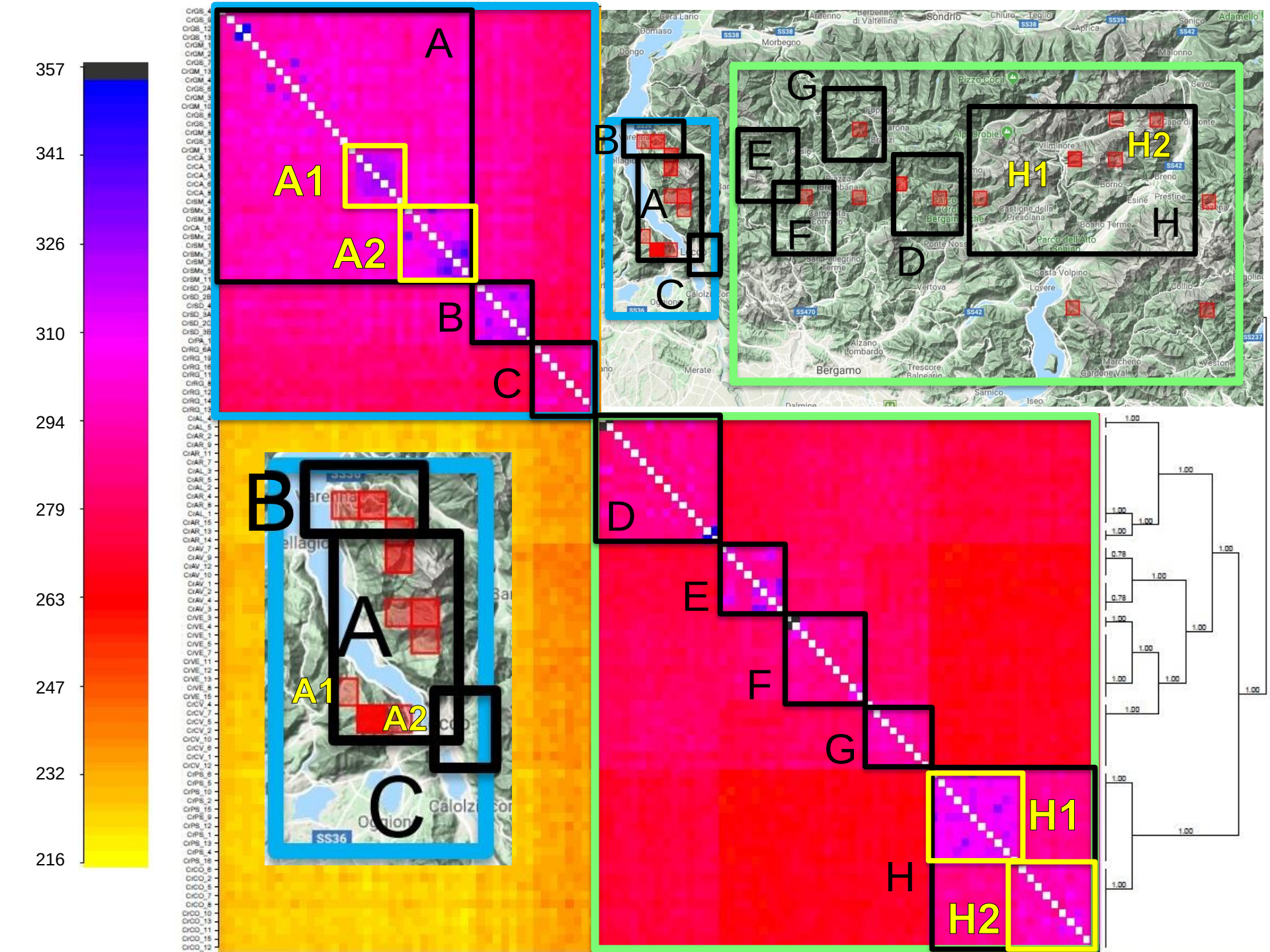
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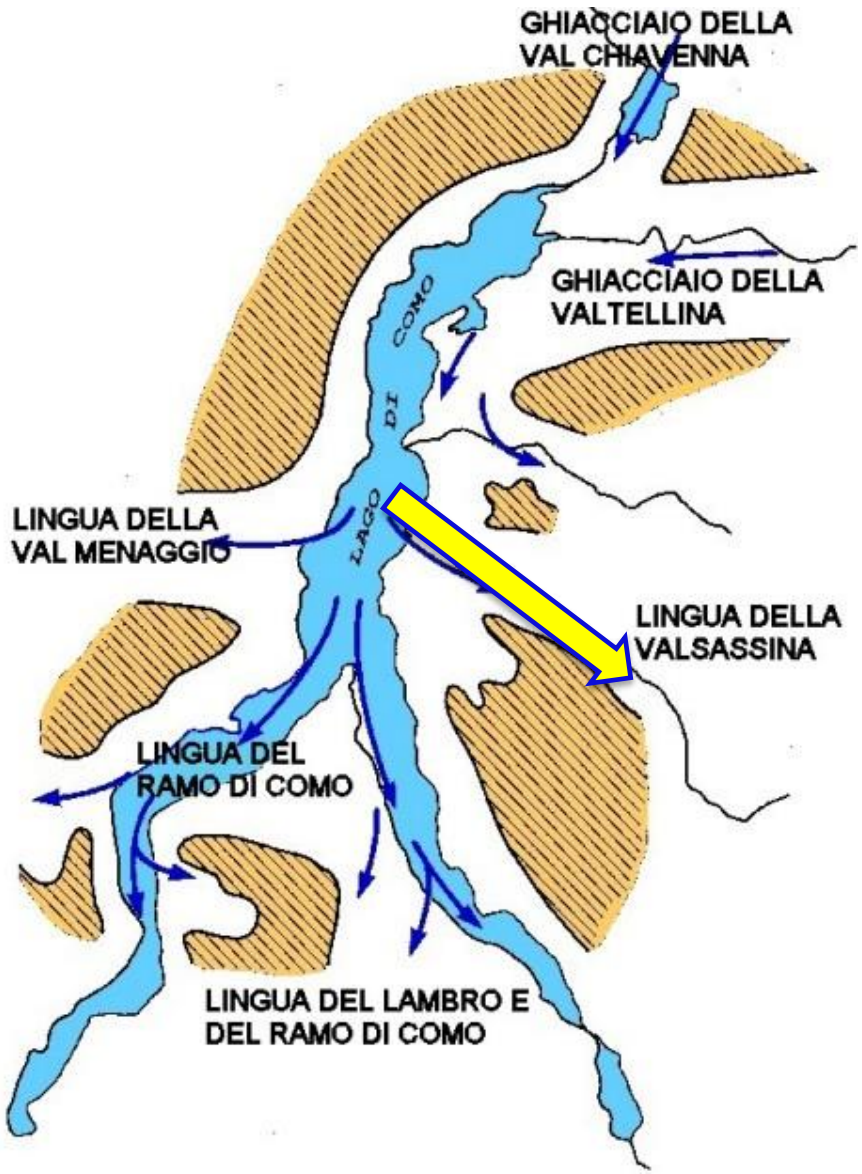
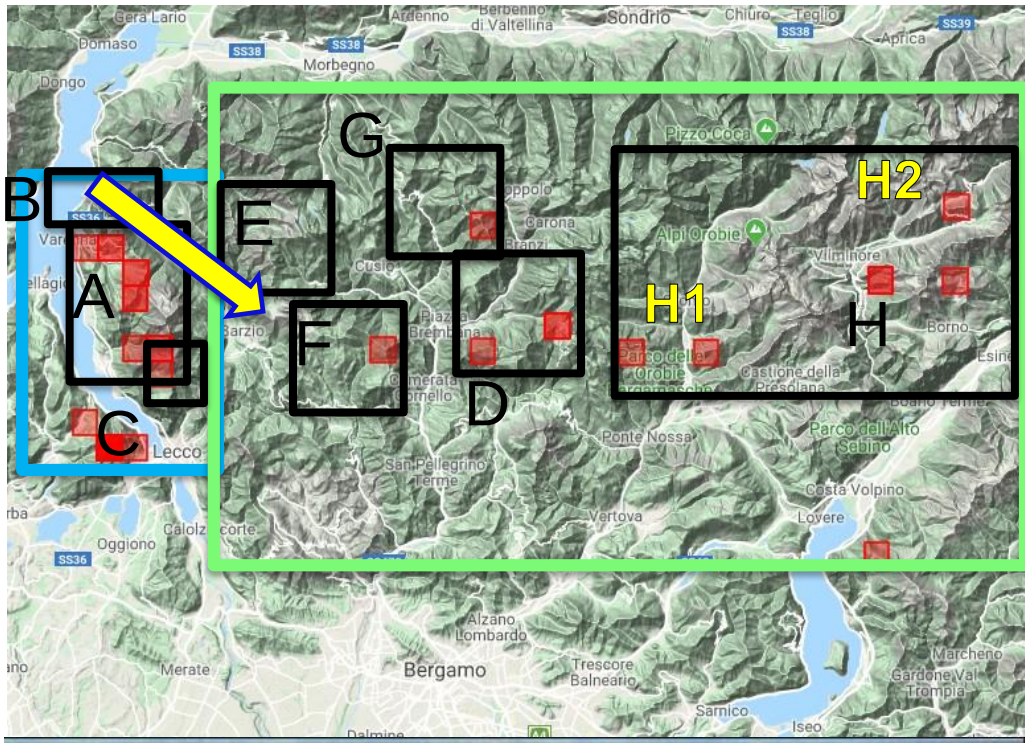
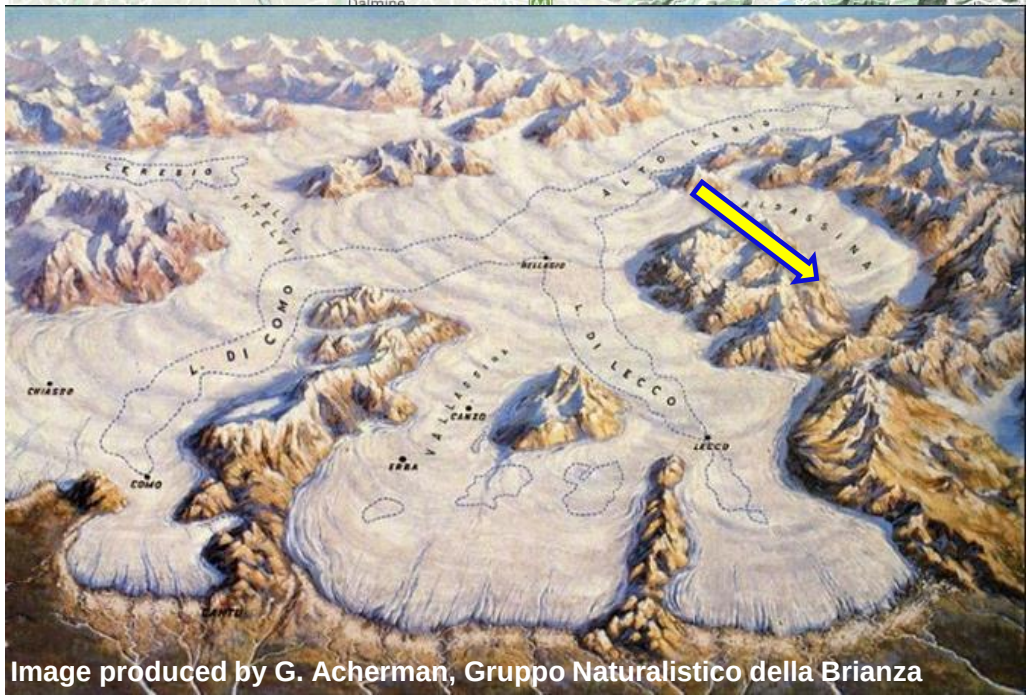


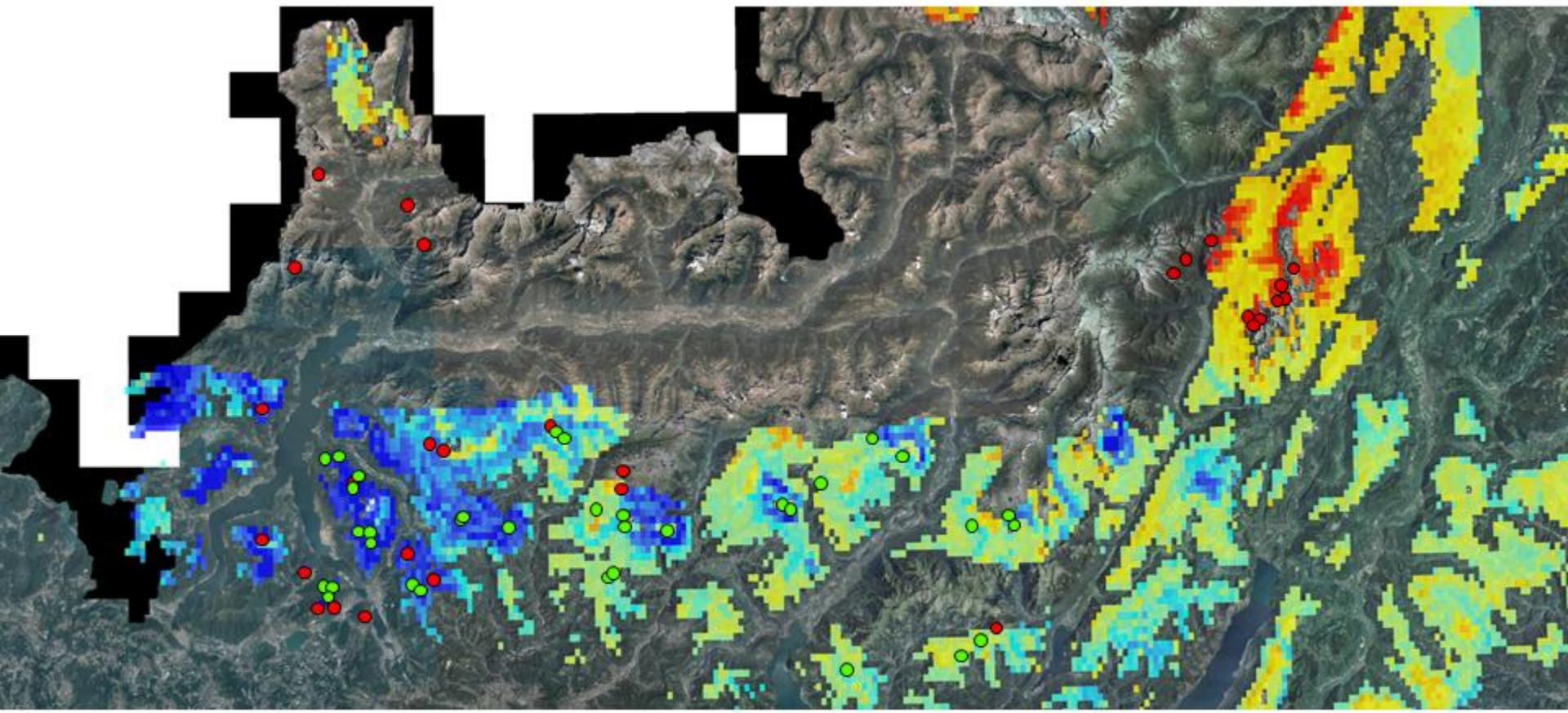
Expansion dynamics of the Adda glacier during LGM (~ 20,000 years bp). Bini, 1990.





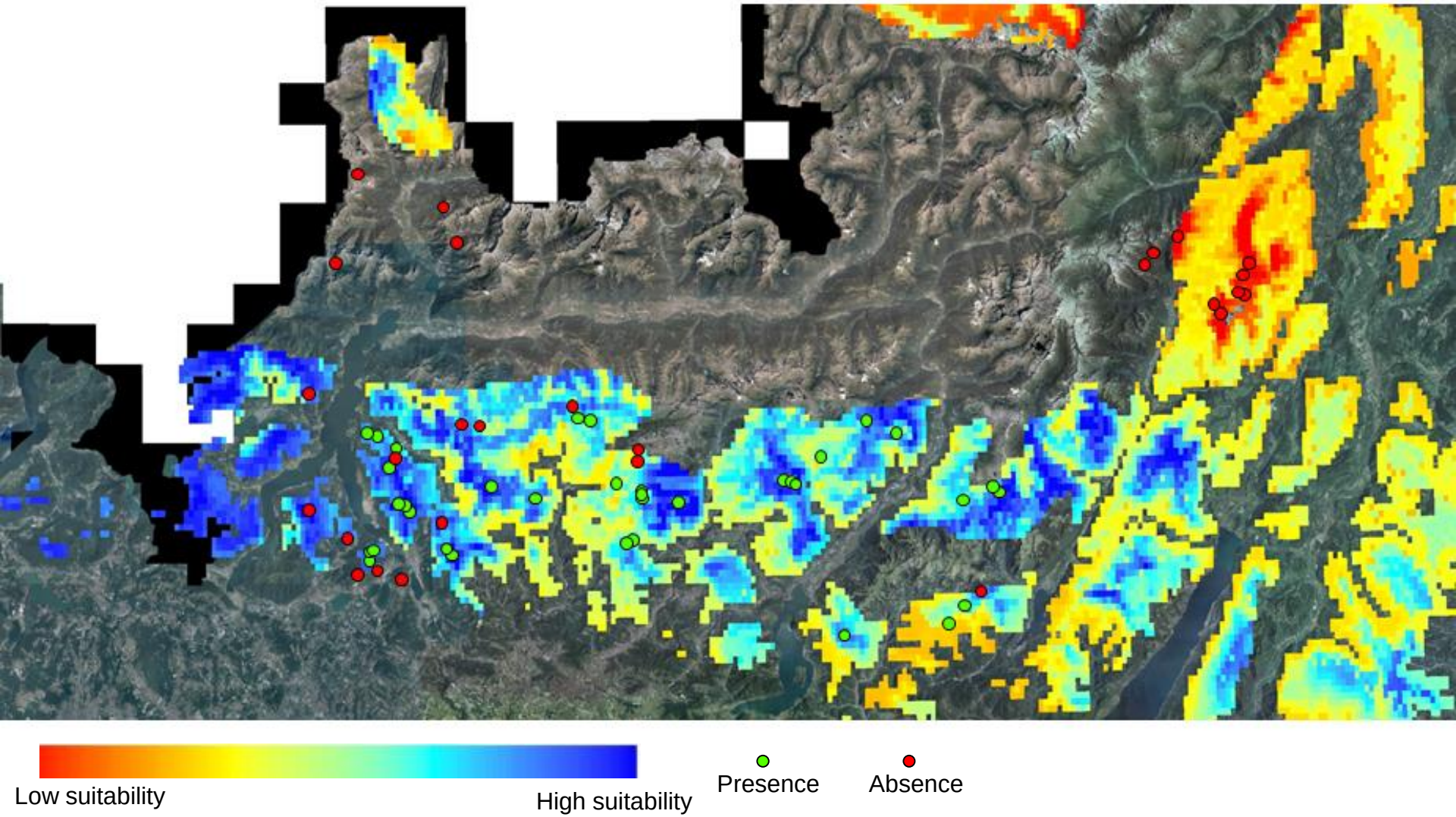
Results: SDMs

- Current, based on bioclimatic variables 1981-2010



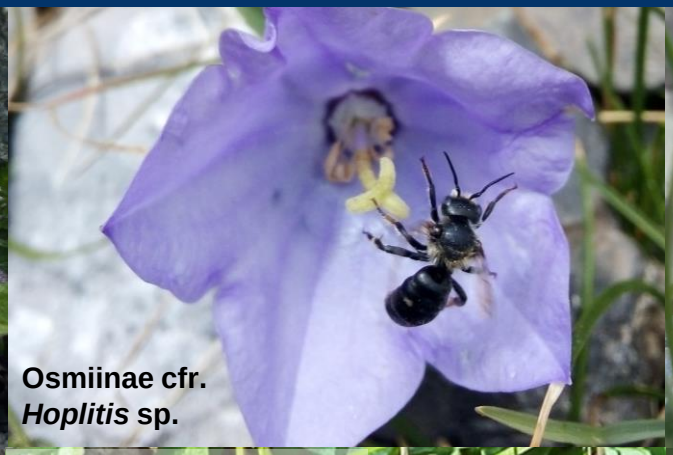
Results: SDMs

- Longer-term prediction, based on bioclimatic variables 2071-2100, rcp: 8.5



Results: pollinator assessment


Bombus cfr. *lapidarius*


Osmiinae cfr.
Hoplitis sp.


Apis mellifera


Eupeodes
cfr.
luniger


Xylocopa cfr. *violacea*

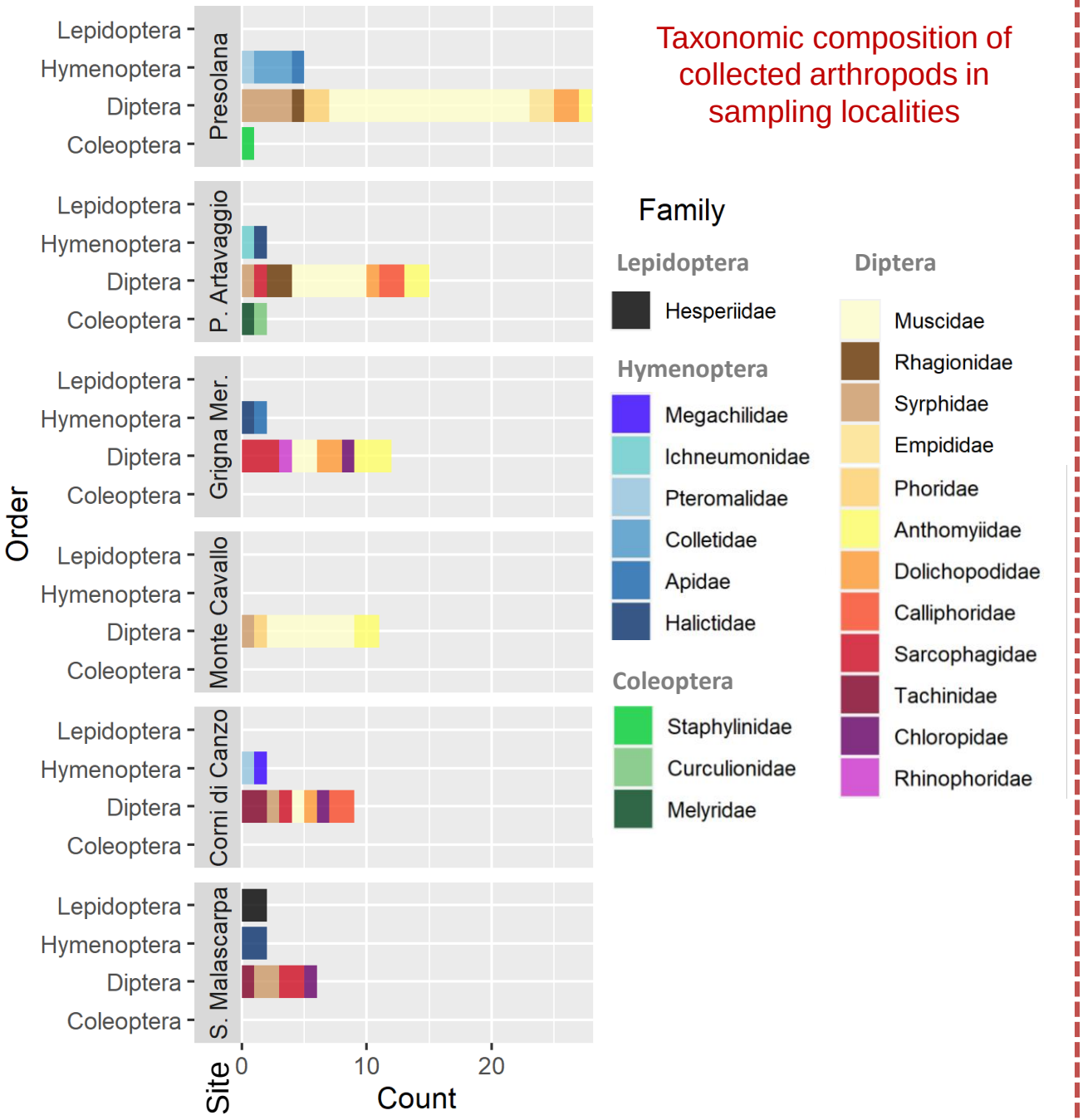

Andrena sp.

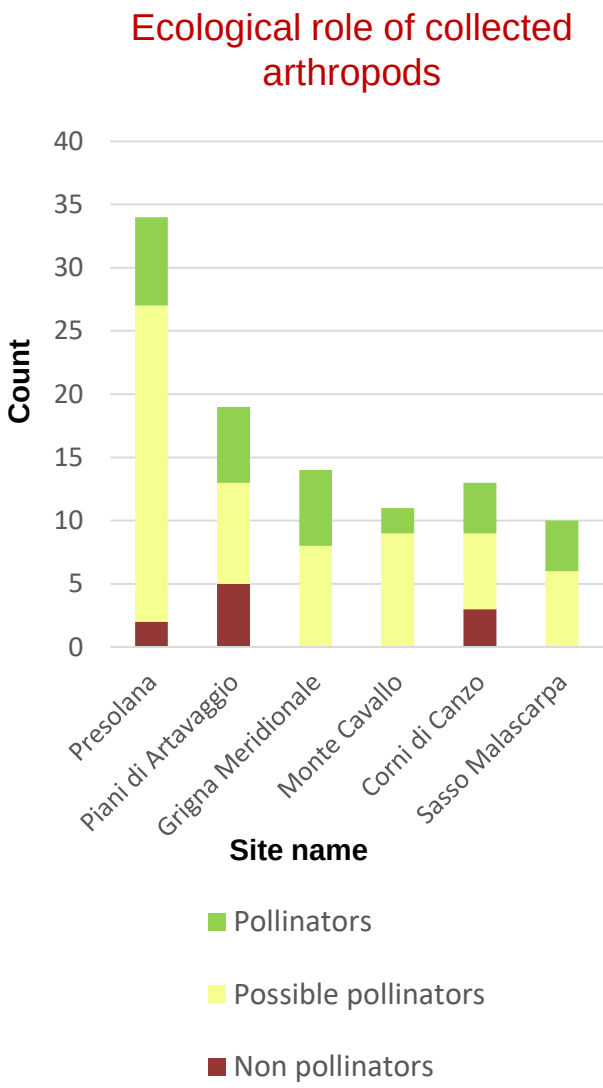

Bombus hortorum


Lasioglossum cfr. *nitidulum*

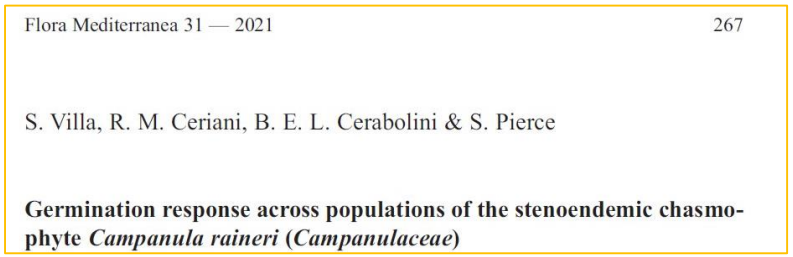

Satyrium spini

Results: pollinator assessment





Conclusions

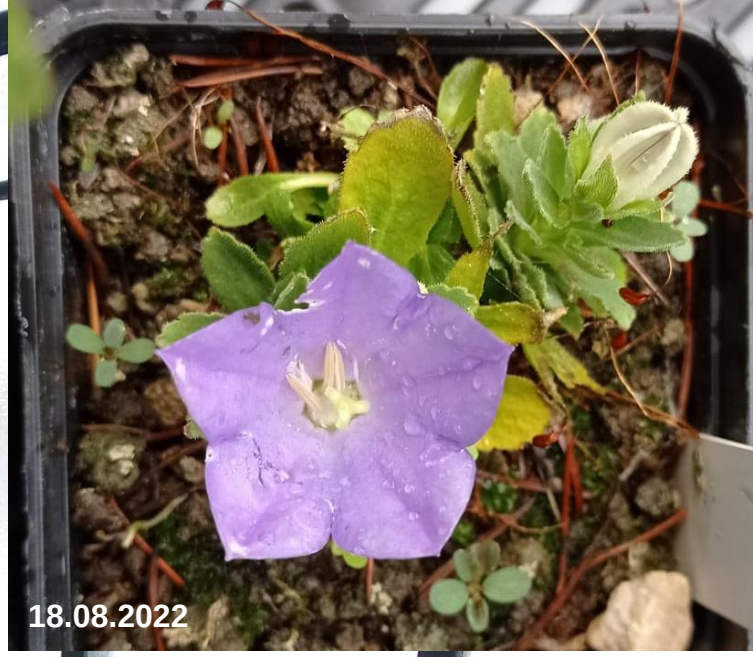

Flora Mediterranea 31 — 2021 267

S. Villa, R. M. Ceriani, B. E. L. Cerabolini & S. Pierce

Germination response across populations of the stenoendemic chasmophyte *Campanula raineri* (*Campanulaceae*)

- The **germination protocol** proved to be **effective** and resulted in the production of hundreds of plants from each population, albeit with different rates.
- The positive relationship between seed and pollen quality and elevation suggests a **pollen limitation at the lowest altitude**.
- The positive relationships between the percentage of polymorphic loci, the observed heterozygosity and the number of private alleles with population size reveal that **large populations** tend to have a **higher genetic variability** but associated with **specific local genetic adaptation**.
- Population genomics show a **three-level structuring**, with the distinction of two main groups (eastern and western sites) and further differentiation at the level of single localities, showing a degree of **genetic isolation among populations**, but within each variability and private alleles are a sign of **local adaptation**. In each population there is evidence of **diversifying selection**, and the maintenance of good levels of intra-population variability ensures that all populations have a **good chance of survival**.
- Preliminary projections of future habitat suitability suggest a clearer distinction between **low-altitude areas**, which will become climatically **less suitable** for the species, and areas around 2000 m above sea level, where *C. raineri* can more easily survive. Moreover, **new suitable areas** could emerge to the west of the current distribution area.
- **Propagation** of the species is a real possibility, and the results of modelling and genetic analysis can show us where interventions will be most urgent and effective, and which populations are the most appropriate sources.

Thank you for your attention



Centro Flora Autoctona of Parco Monte Barro
(Galbiate, LC)

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You J *et a*l. 2018. Sci Rep 8(5879), DOI 10.1038/s41598-018-24360-9

Hypotheses

- **Climate oscillations are associated with changes in the distributional range of *Campanula raineri***. Species Distribution Models (SDMs) are consistent with the genetic structure of populations (genomic analysis) and with migration models elaborated by bioinformatic tools;

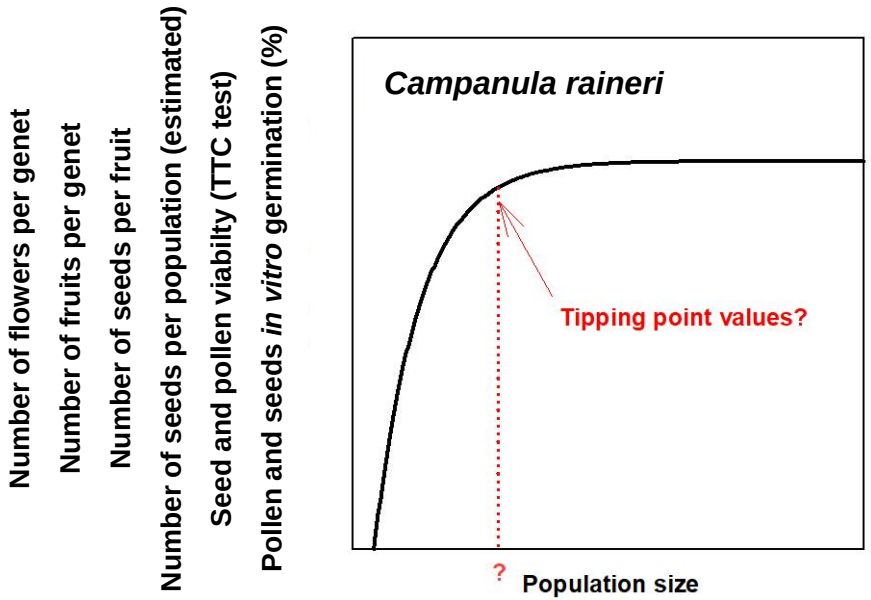
- **The current distribution area of the target species will no longer be ecologically suitable by 2100**, but potentially suitable areas will emerge in future;

- **Genetic variability and altitude affect the reproductive ecology of *C. raineri*, in terms of vegetative and reproductive traits** (flowers and seeds produced and pollen and seeds quality). Altitude also affects **the pollination network**;

- **Endemism in recently diverged angiosperms is associated with chromosome set duplication**

Estimating dangerous tipping points for the reproduction of alpine rupicolous endemics

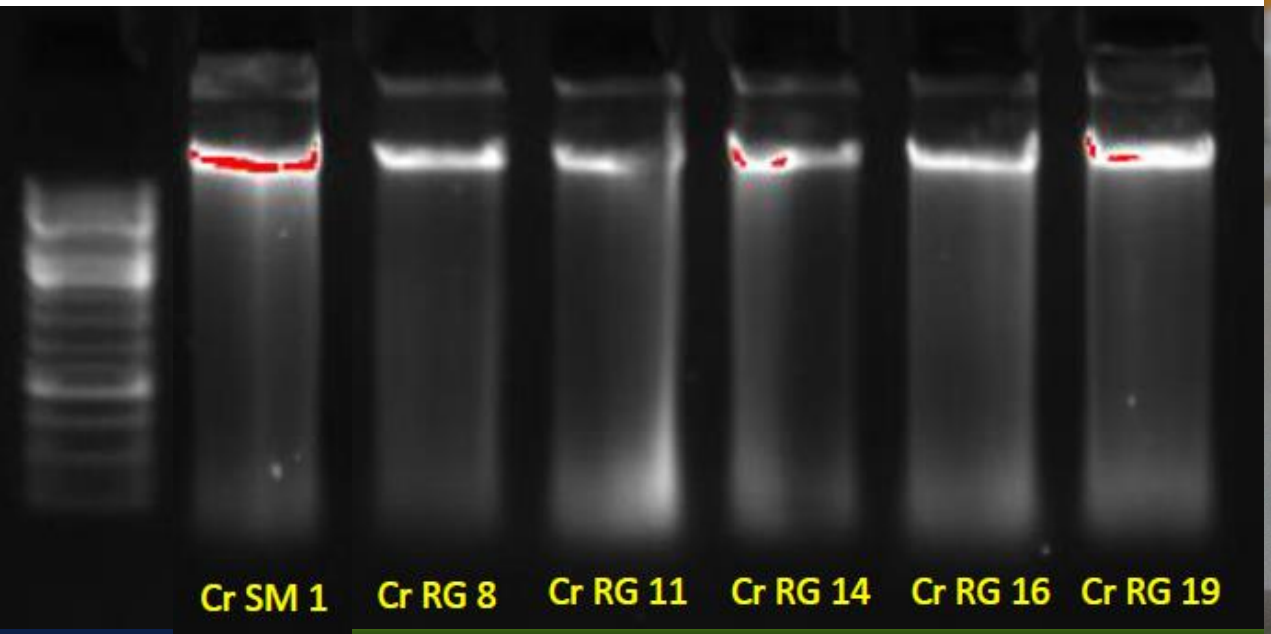
Visual hypothesis

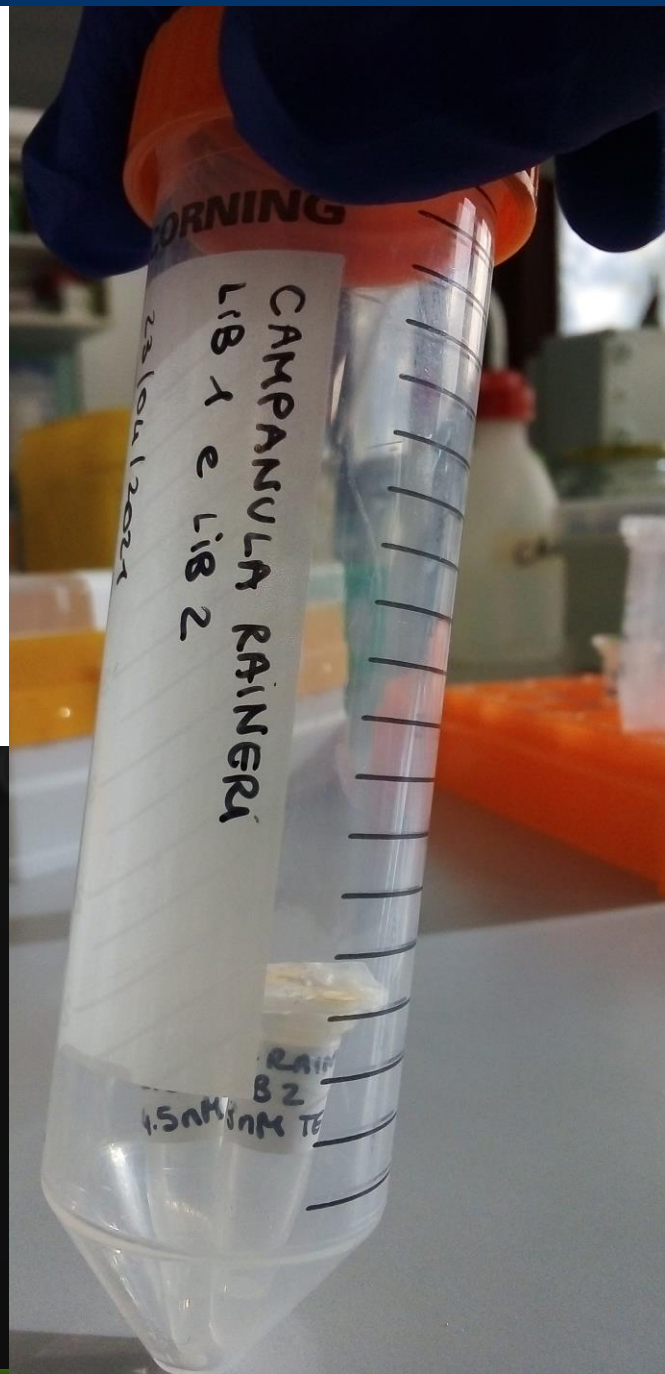


Hypothesis: reproductive traits and genetic variability exhibit exponential rise-to-maximum relationships with population size, from which minimum viable population size can be estimated

Phylogeography and SDM for the conservation of *Campanula raineri*.

- DNA extraction (110 samples) and preparation of 2b-RAD libraries for genome-wide genotyping (University of Trieste)
- Samples sequencing and sequences quality check
- Removal of sequence contamination (DeconSeq)
- Loci building, SNPs identification and calculation of population genomic summary statistics (Stacks)
- Calculation of the coancestry matrix (fineRADstructure)
- In progress: Integration to SDMs for phylogeographic analysis

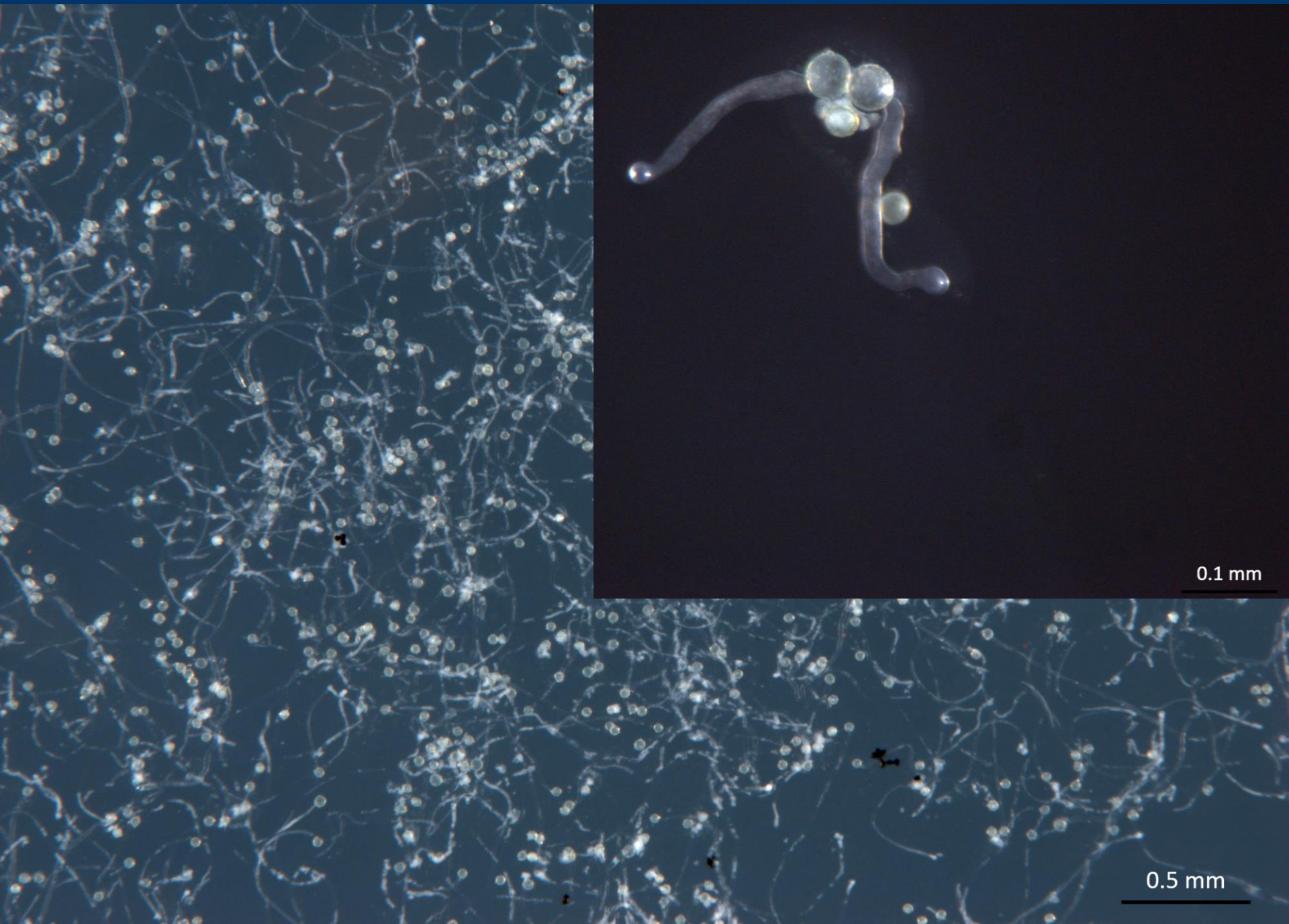


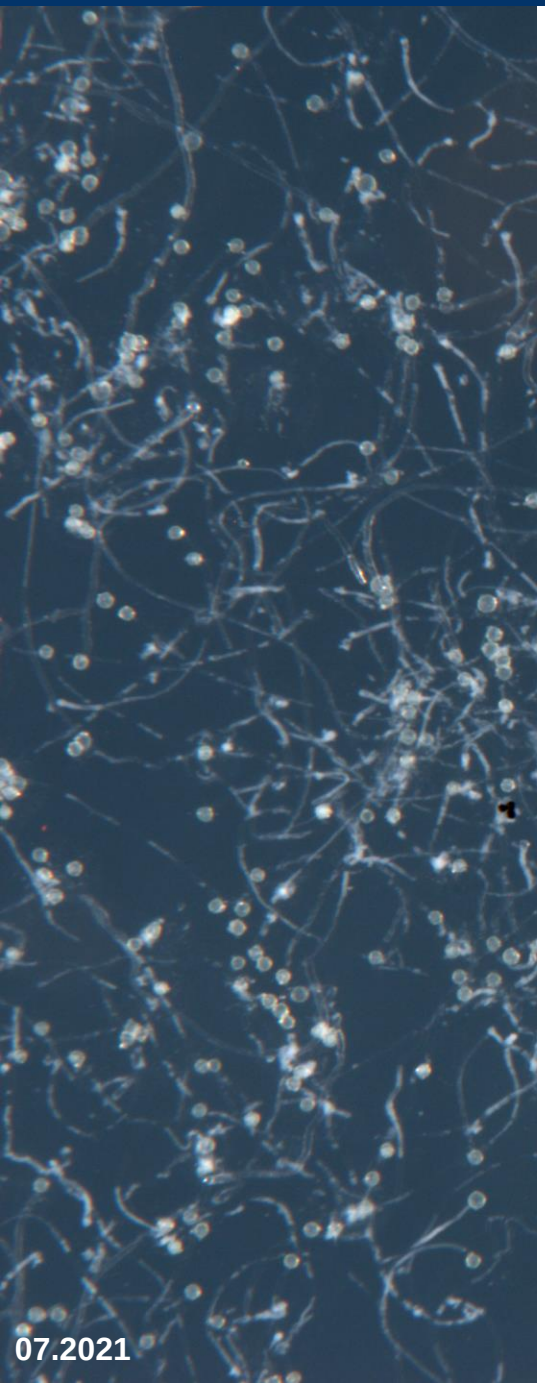


Methods: Pollen viability test (*Triphenyl tetrazolium chloride*)

0.2 mm

Methods: Pollen germination





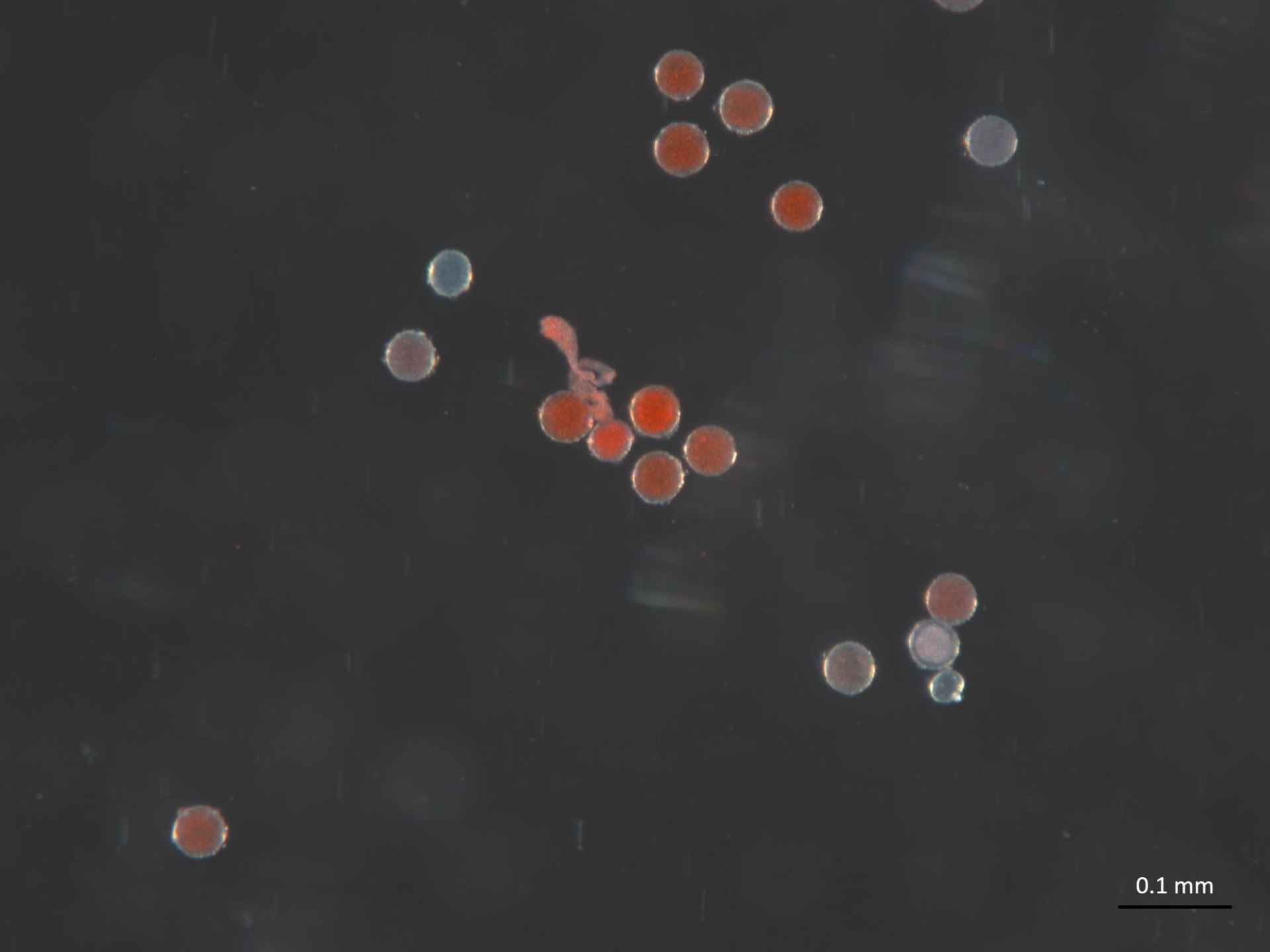
Pollen germination in target Populations

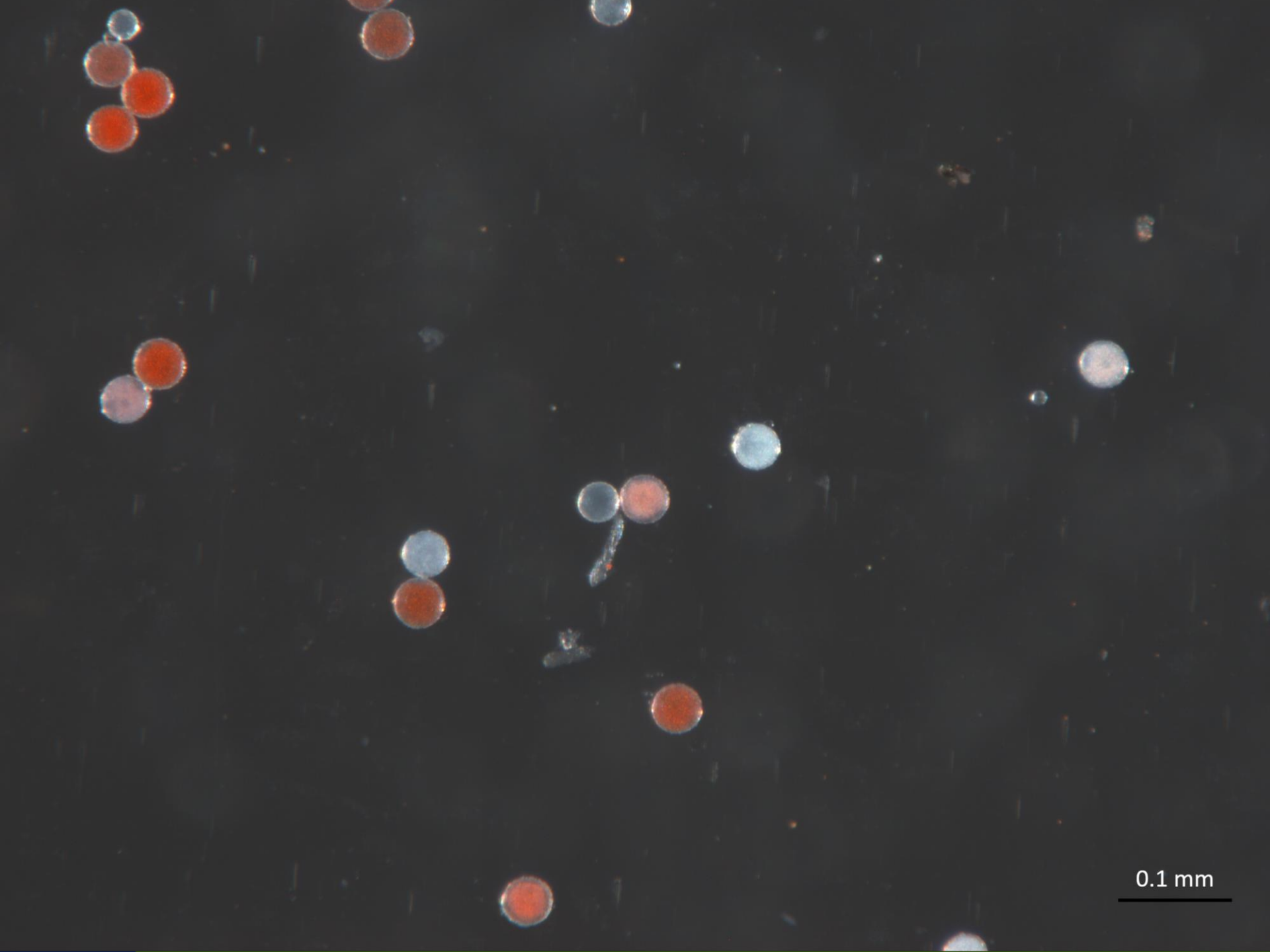
Proportion of germinated pollen grains (%)

Artavaggio
Presolana
M. Cavallo
Grigna M.
C. Canzo
S. Malascarpa

R^2_{Adj} : 0.28;
F-statistic: 23.92;
DF: 58;
p-value: 8.34e-06

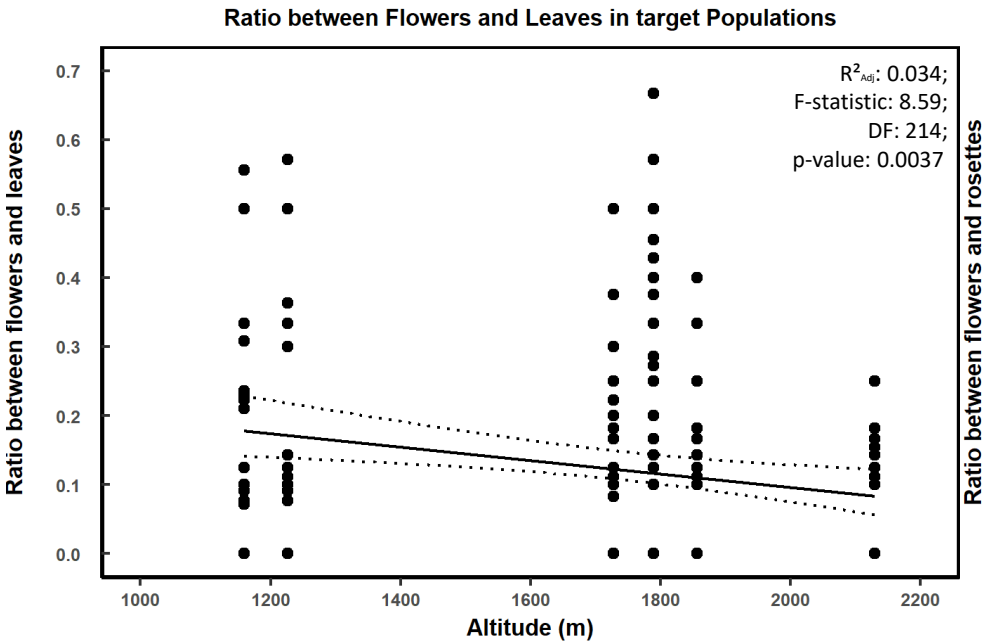
Altitude (m)


0.1 mm

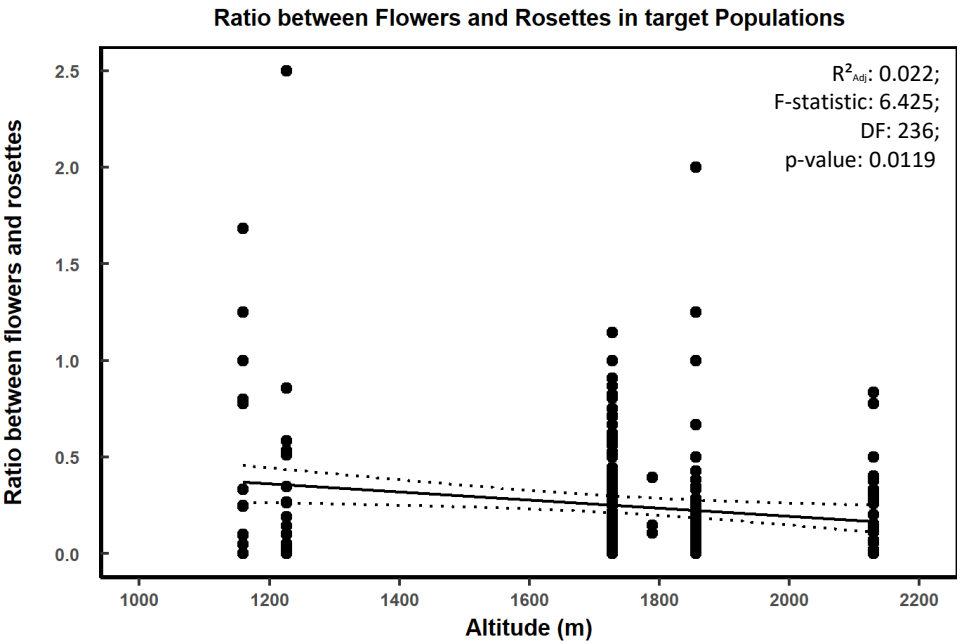

0.1 mm

Fitness analysis: effort in investments

reproductive vs vegetative effort



sexual vs vegetative reproduction

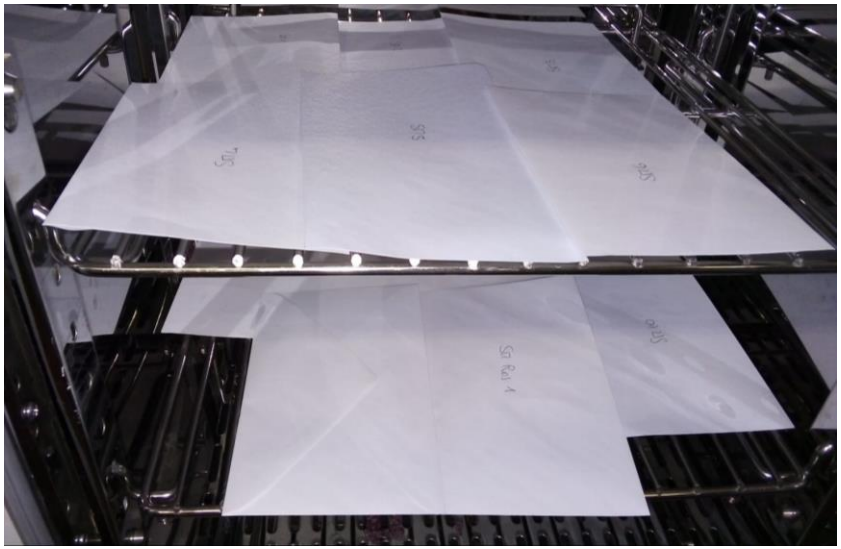


Adaptive specialization: CSR strategy (Competitive, Stress-tolerant or Ruderal)

Leaf Fresh Weight

+

Leaf Dry Weight



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Leaf Area

Pierce *et al*., 2017

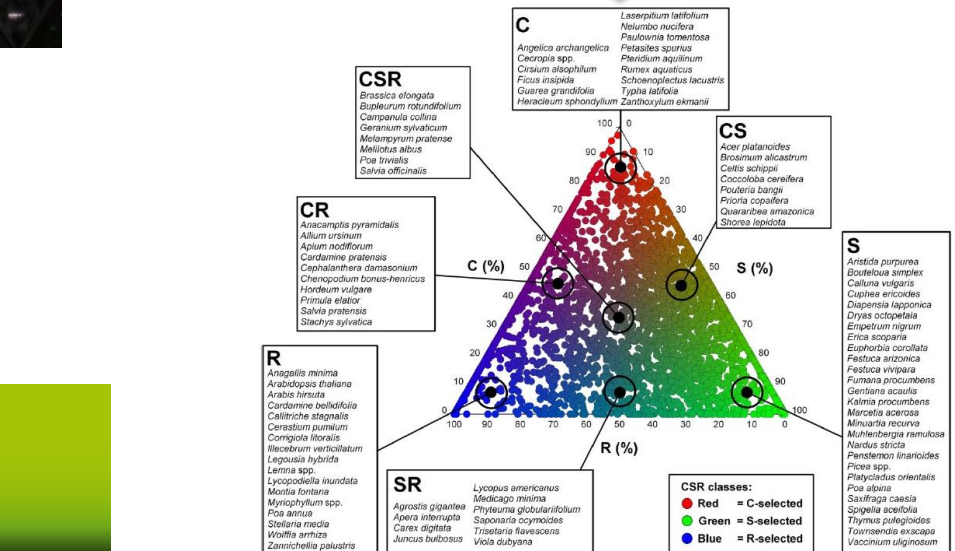
Specific Leaf Area,

Leaf Dry Matter Content,

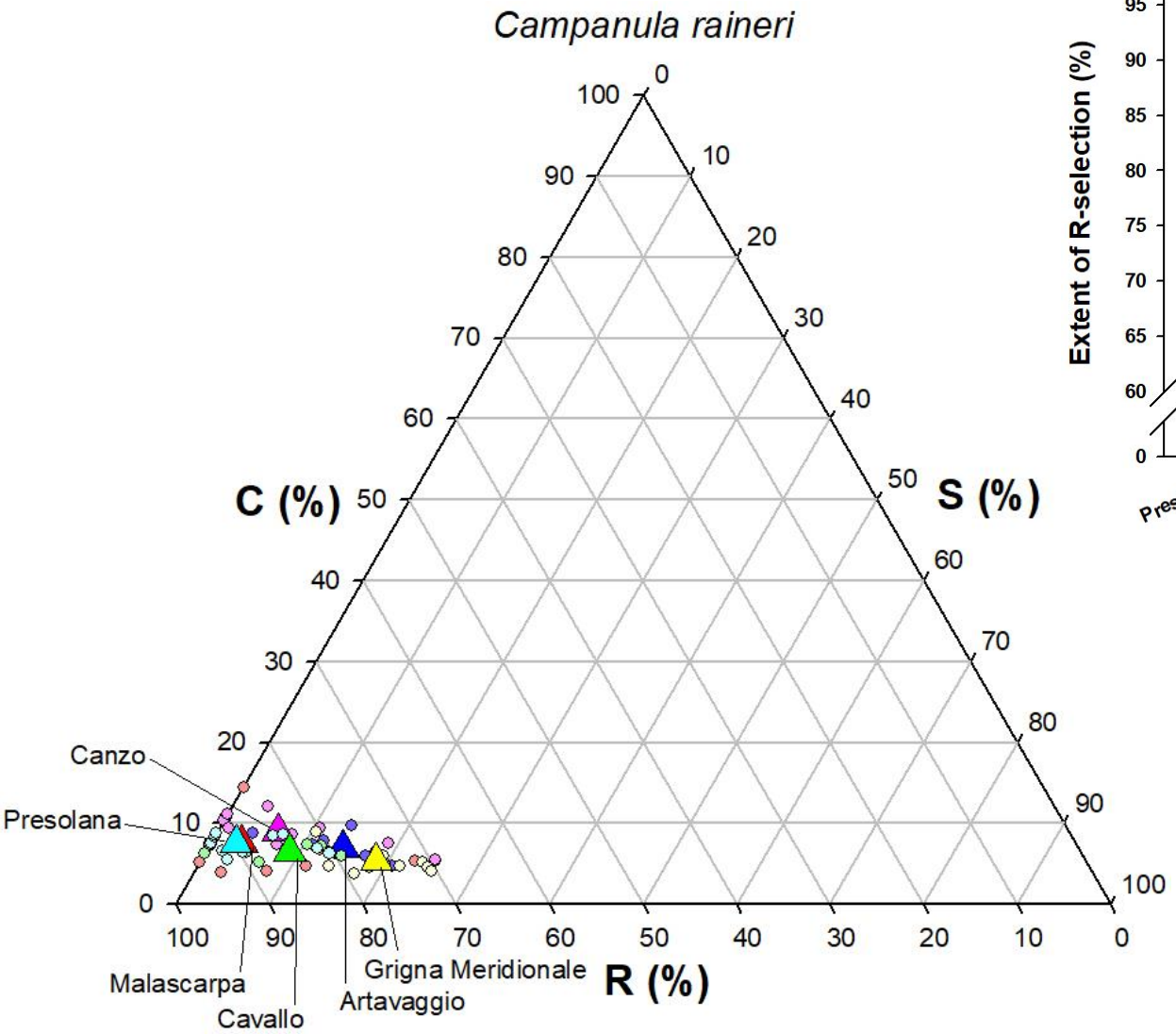
Leaf Mass per Area,

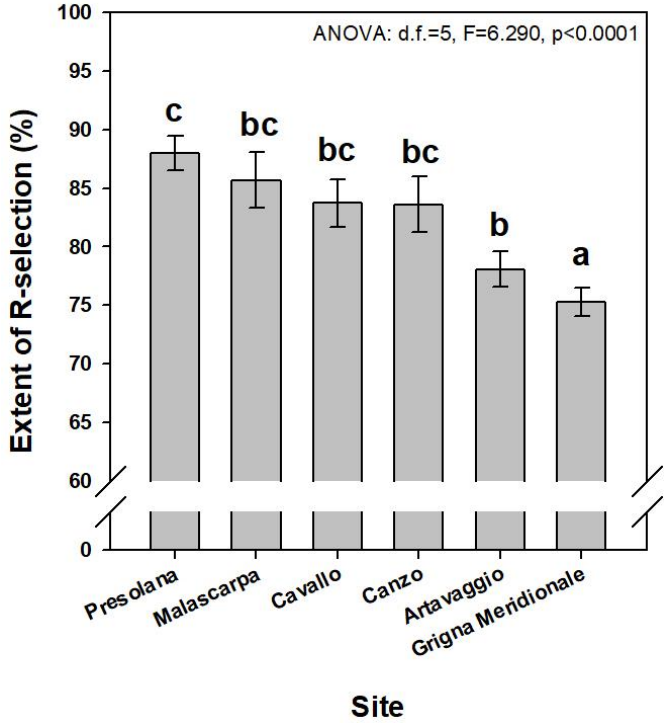
Leaf Water Content

CSR classification

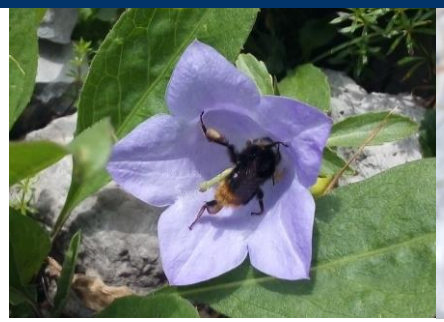


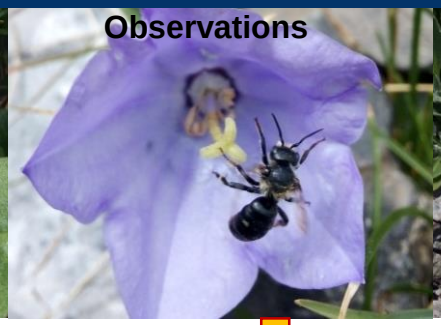
CSR strategy (Competitive, Stress-tolerant or Ruderal)





Methods: Assessment of pollination network





DNA extraction from samples

Metabarcoding on eDNA from flowers

Comparison

Identification of arthropods visiting flowers and assessment of potential pollinators.

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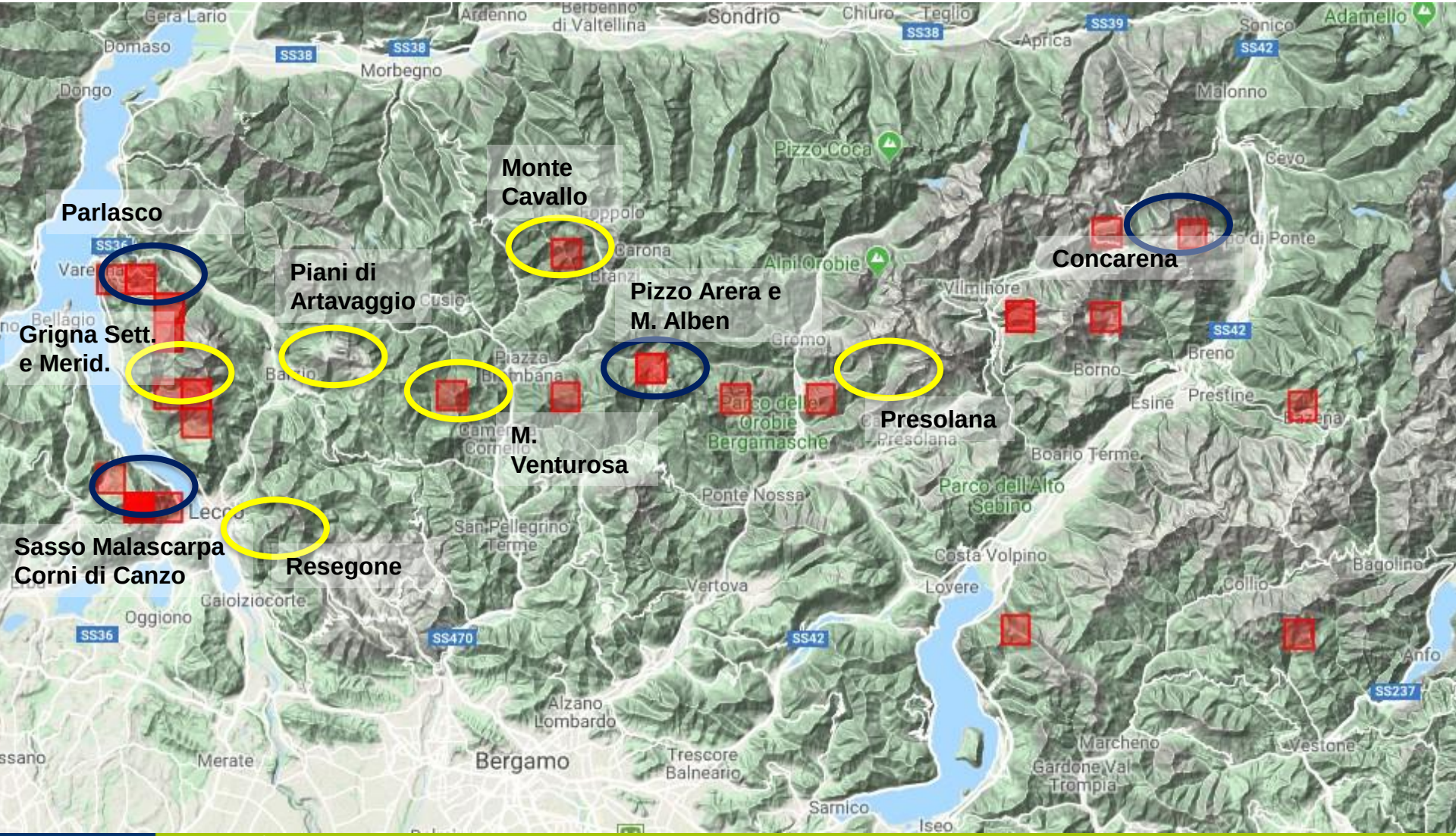
2019-2020 sampling

2019 sampling

2020 sampling

recorded occurrences

records from Osservatorio Regionale della Biodiversità – Regione Lombardia



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