1st Meeting of the Iberian Ecological Society & XIV AEET Meeting
Barcelona, 4-7 February 2019

Phenotypic and genomic data reveal adaptive genetic variation in flowering phenology in a Mediterranean alpine plant

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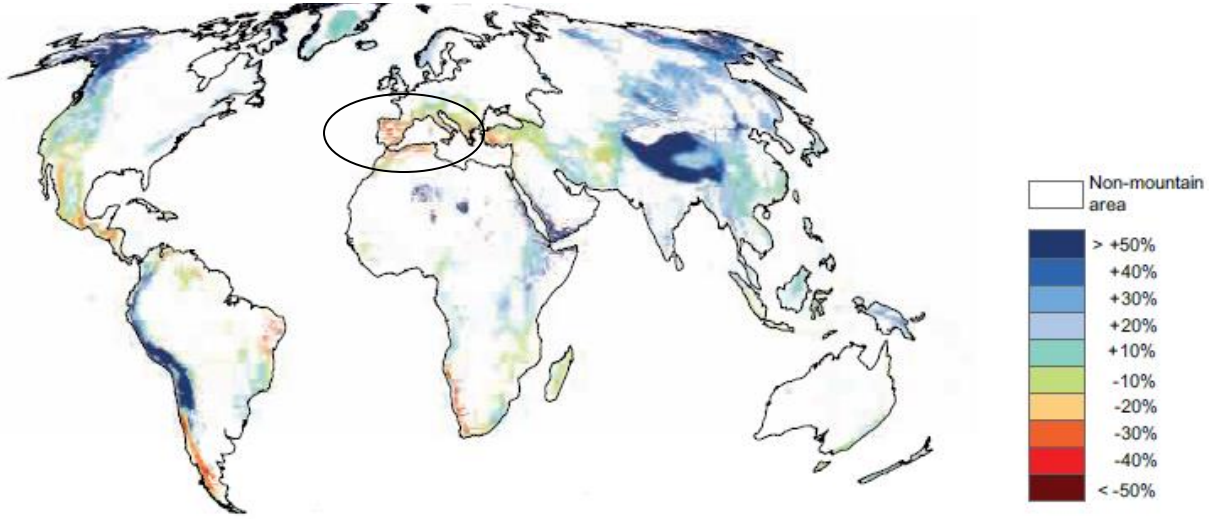
Mediterranean Alpine Environments

- Mediterranean alpine plants are among the organisms most vulnerable to climate change

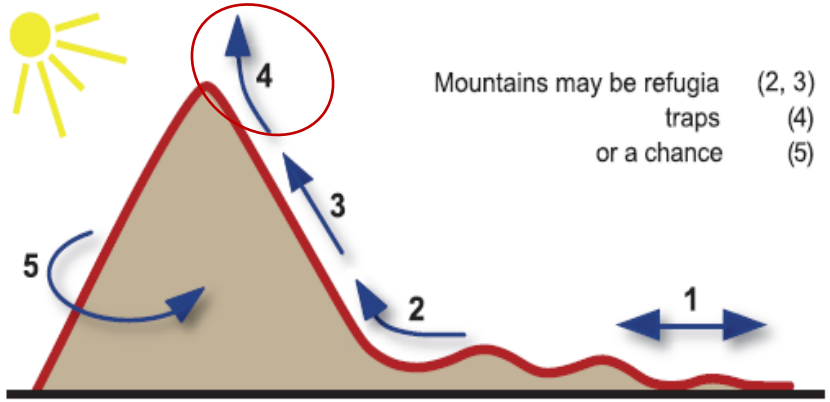


Precipitation change for the period 2081–2100 (RCP8.5)

IPCC Fifth Assessment Report

Mediterranean Alpine Environments

- Mediterranean alpine plants are among the organisms most vulnerable to climate change
- Migration is not feasible for many species → Mountain is a trap
- Persistence depends mainly on the genetic adaptation to the new conditions

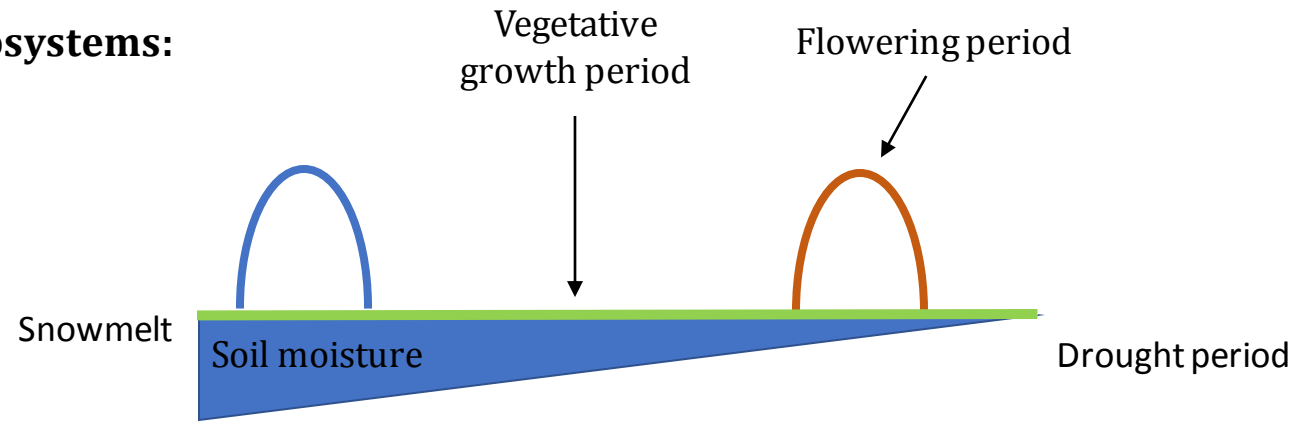


Comte et al 2014 *Nature communications* , Kohler, et al 2014. Mountains and Climate Change A Global Concern

Climate Change

Mediterranean alpine ecosystems:

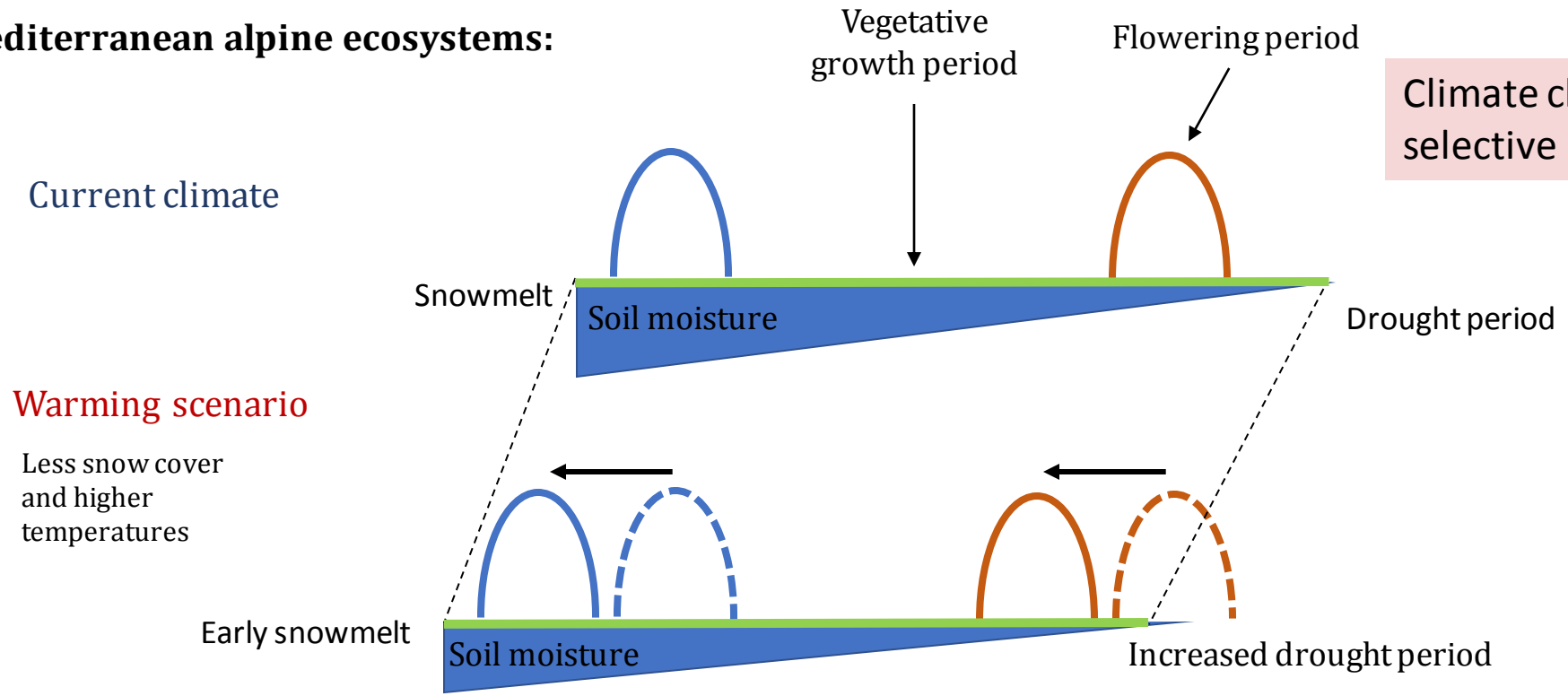
Current climate



Giménez-Benavides et al 2018 *Plant Biology*, Lambert et al. 2010 *AJB*

Climate Change

Mediterranean alpine ecosystems:



Climate change will probably generate selective pressures to flower earlier

Giménez-Benavides et al 2018 *Plant Biology*, Lambert et al. 2010 *AJB*

Climate Change

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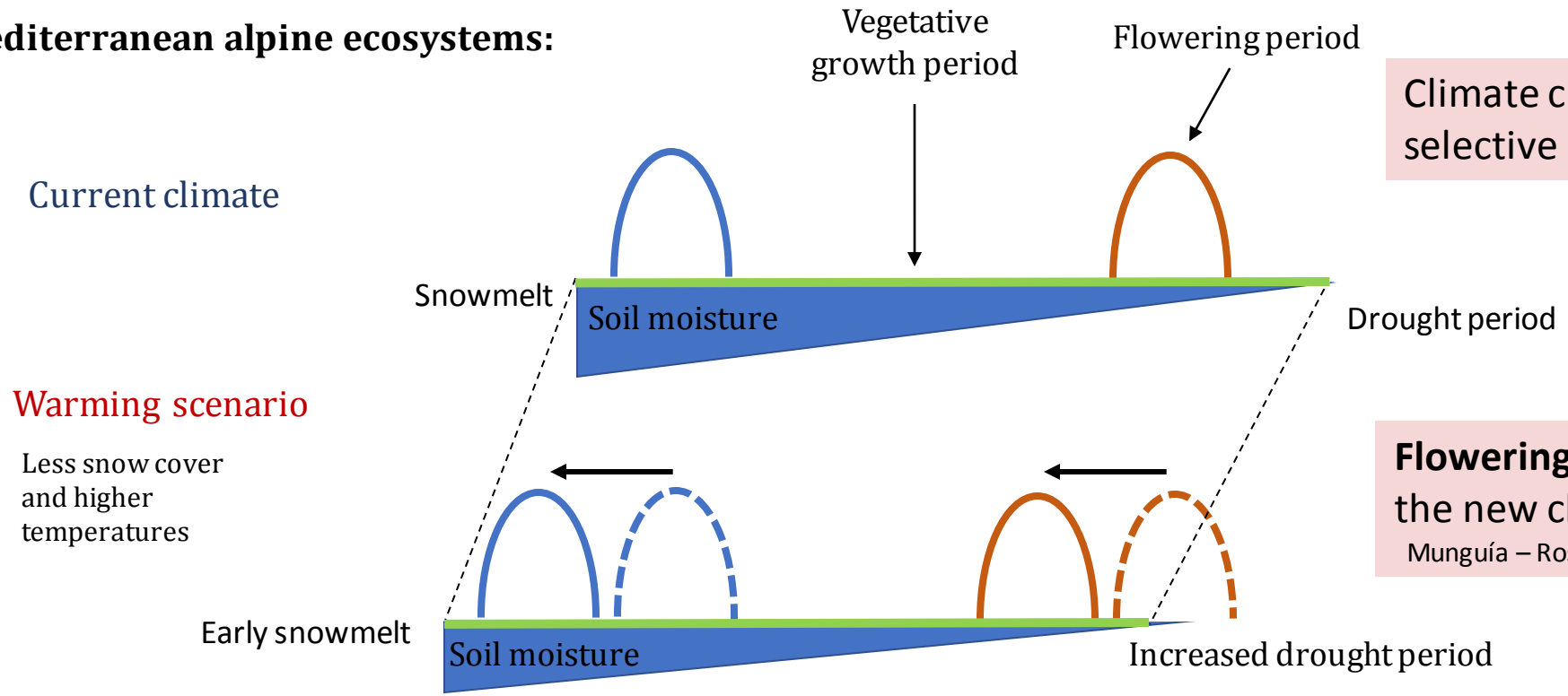


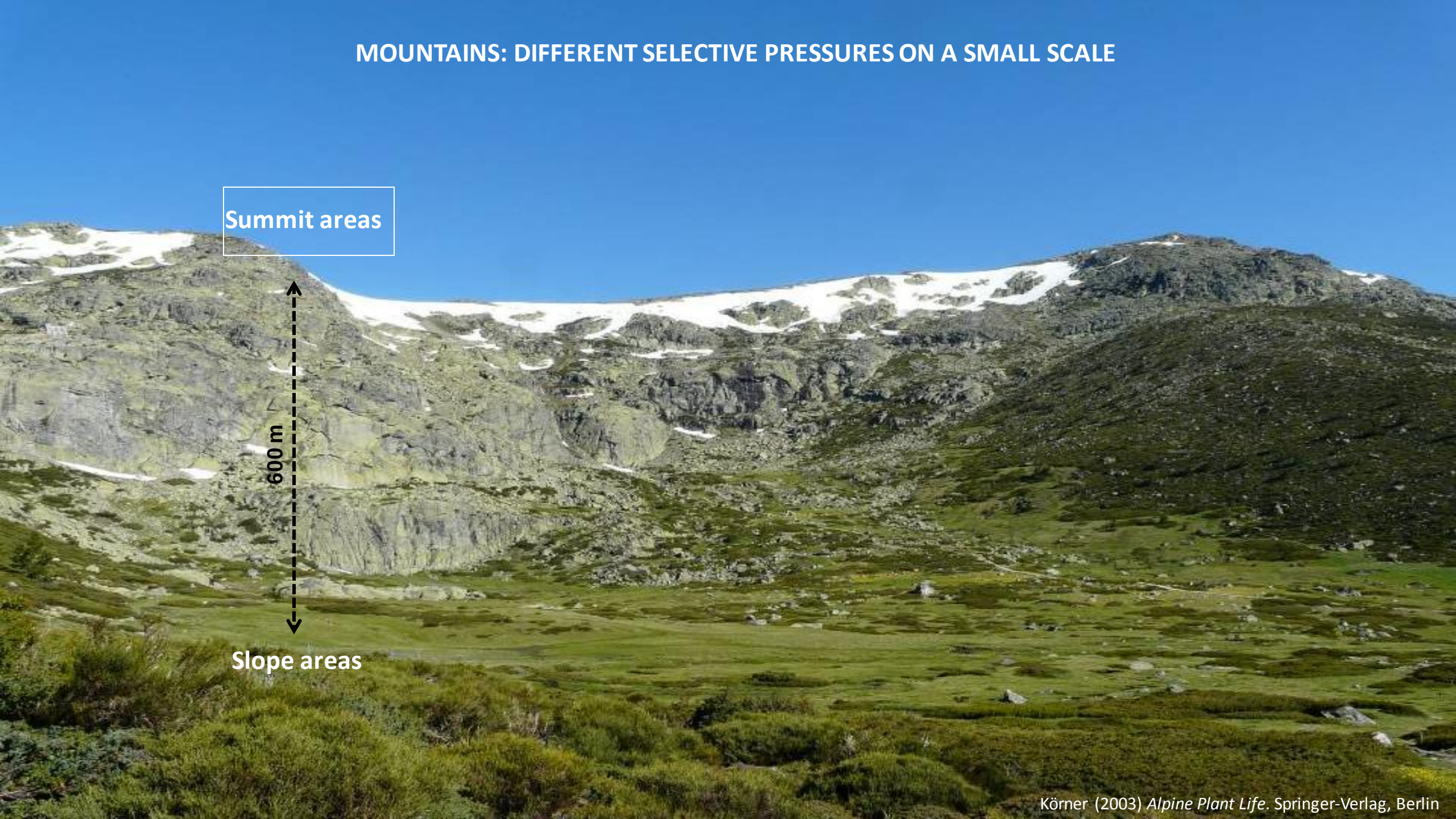
Climate change will probably generate selective pressures to flower earlier

Flowering onset: key trait for fitness in the new climate change scenarios

Munguía – Rosas et al. (2011) *Ecology Letters* 14: 511-521

Giménez-Benavides et al 2018 *Plant Biology*, Lambert et al. 2010 *AJB*

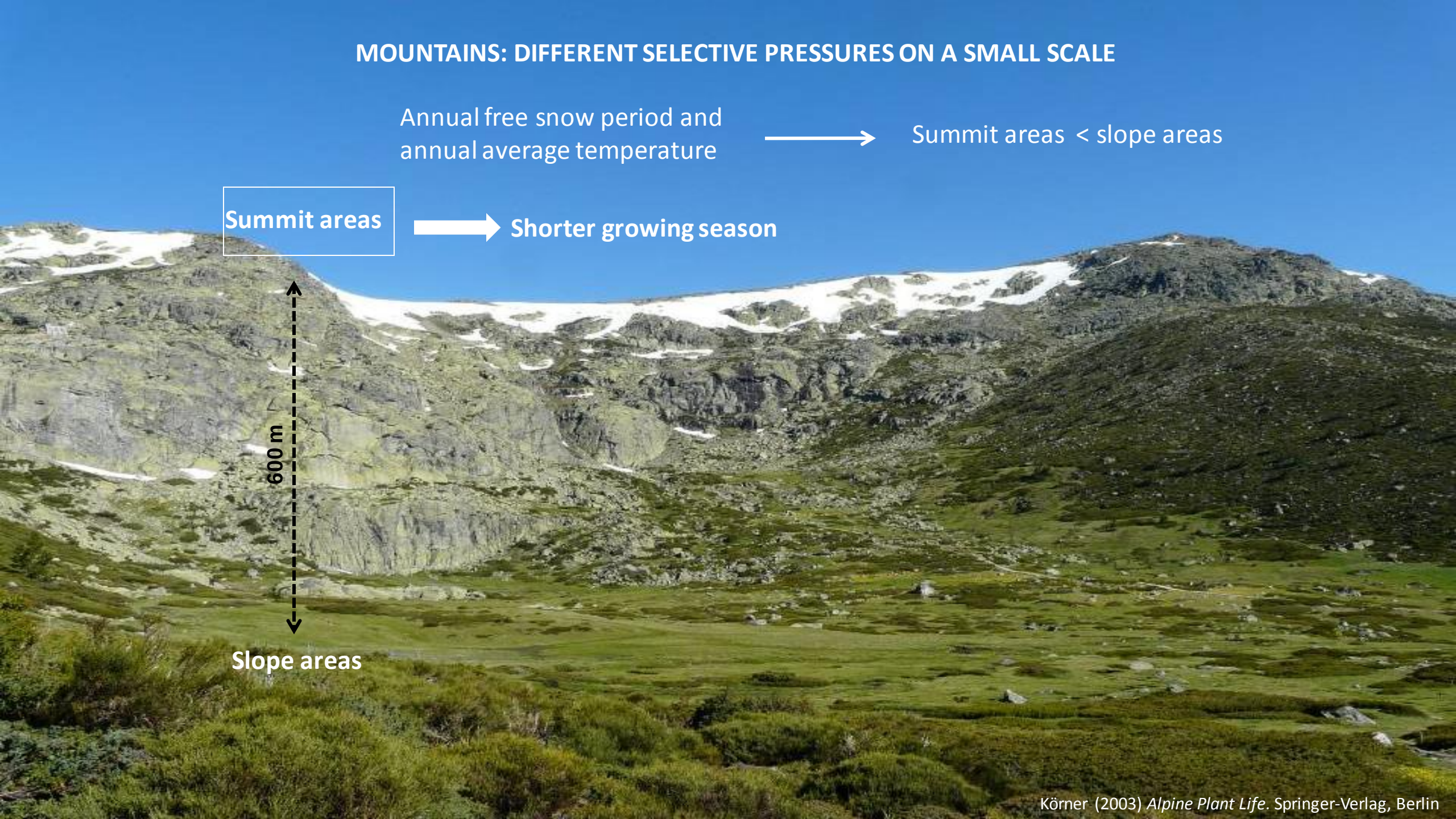
MOUNTAINS: DIFFERENT SELECTIVE PRESSURES ON A SMALL SCALE



Körner (2003) *Alpine Plant Life*. Springer-Verlag, Berlin

MOUNTAINS: DIFFERENT SELECTIVE PRESSURES ON A SMALL SCALE

Annual free snow period and annual average temperature → Summit areas < slope areas



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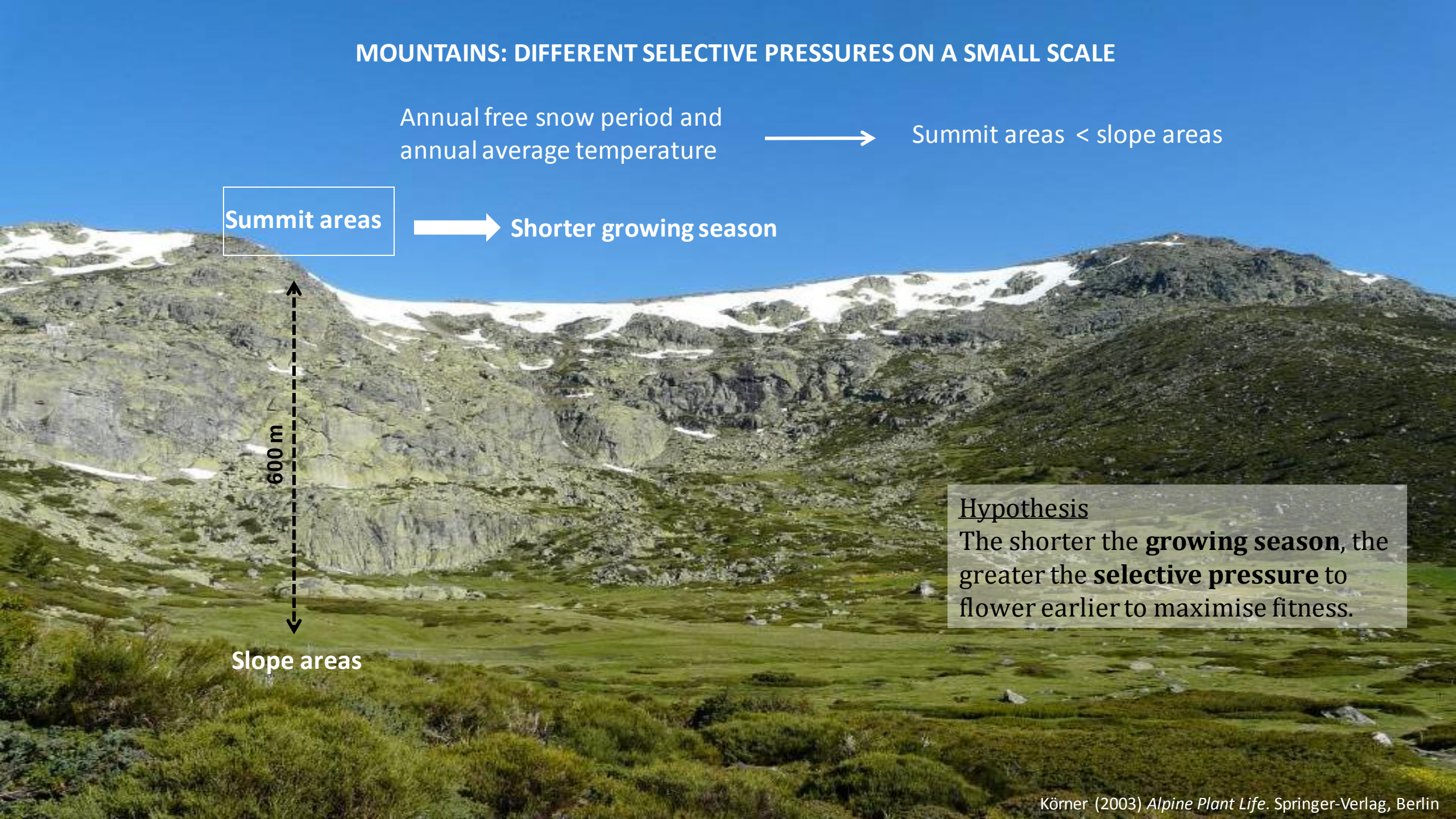


Körner (2003) *Alpine Plant Life*. Springer-Verlag, Berlin

Study species

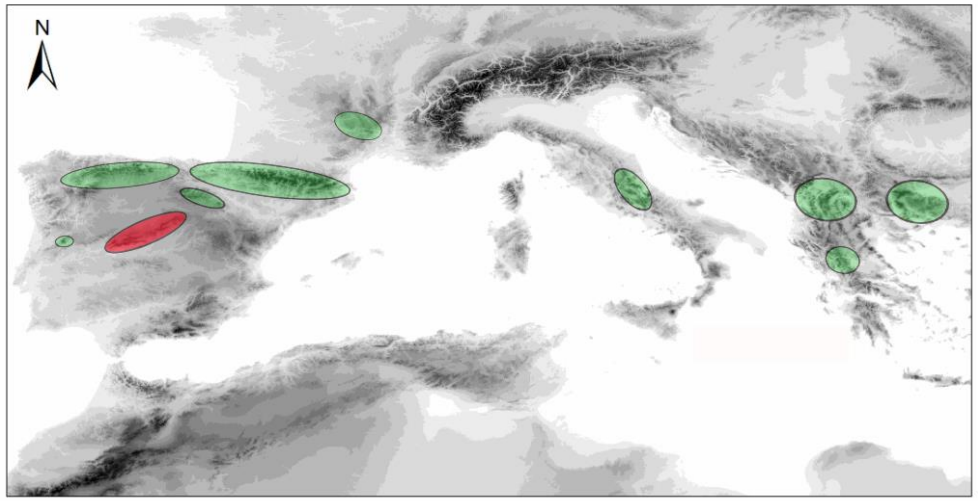
Silene ciliata Pourr. (Caryophyllaceae)

Long-lived perennial plant (Cushion plant)

Study site

The species occurs in areas above the tree line in the Mediterranean mountain ranges of Southern Europe

Central Spain populations are isolated from more northern populations



Kyrkou et al 2015, *PeerJ* 3; Morente-López et al 2018, *Frontiers in Plant Science 9*

Study site

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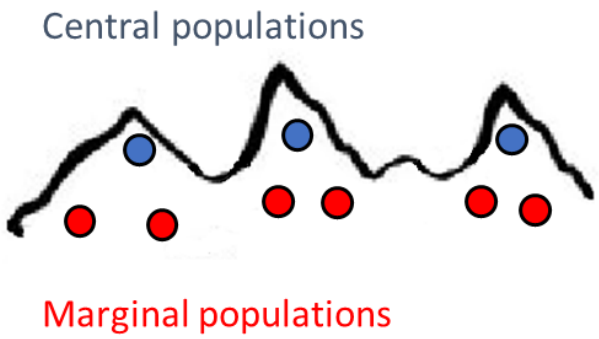
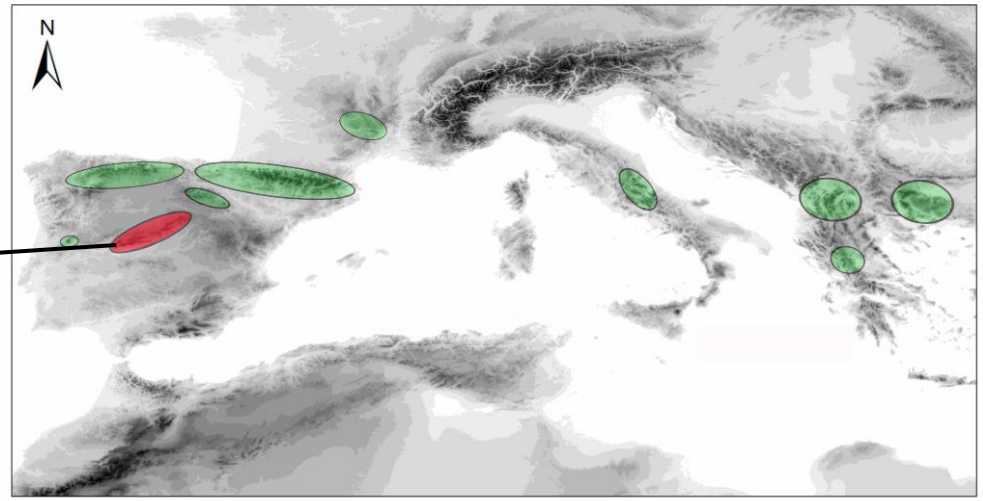
Central Spain populations are isolated from more northern populations

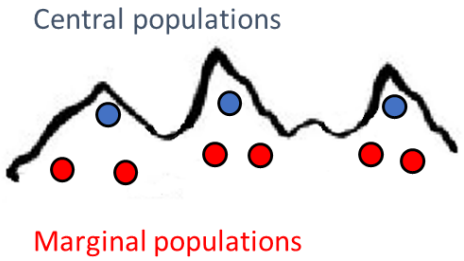
Elevational gradient experiment: 9 populations (3 central & 6 marginal)





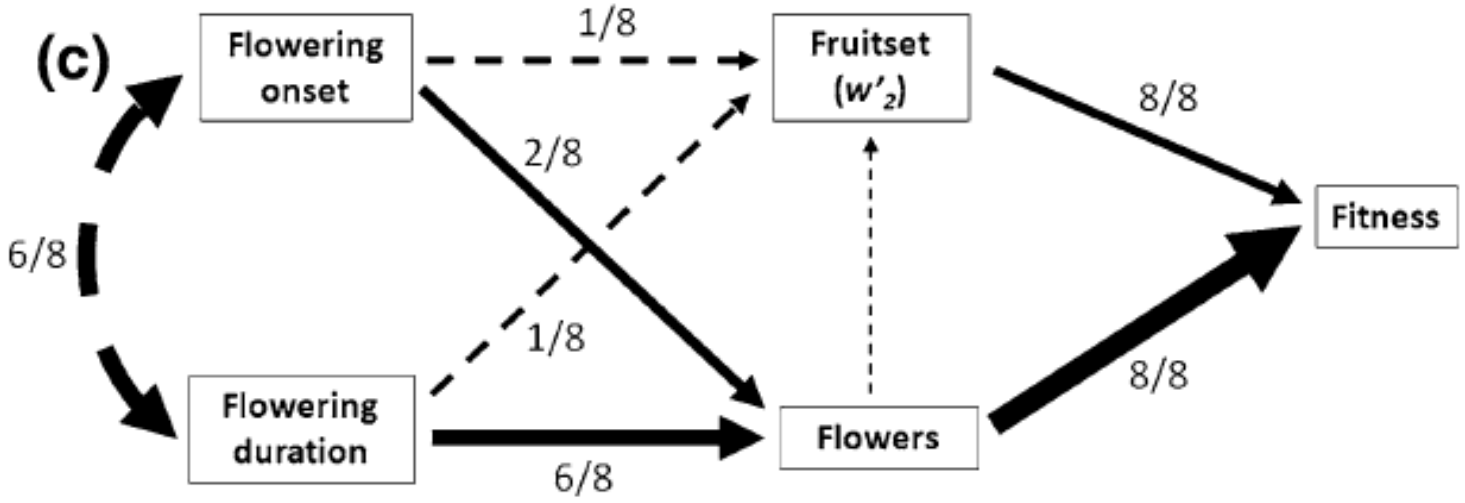
Kyrkou et al 2015, *PeerJ* 3; Morente-López et al 2018, *Frontiers in Plant Science 9*

Elevational gradient experiment



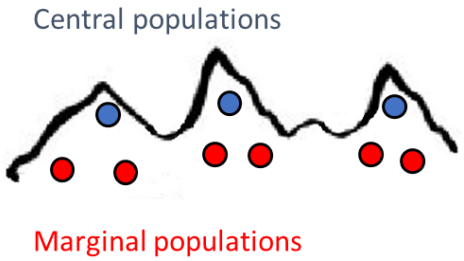
Field conditions: **Flowering onset in this species has been found to be under selection**

Plants that bloomed earlier flowered for longer periods and produced more fruits

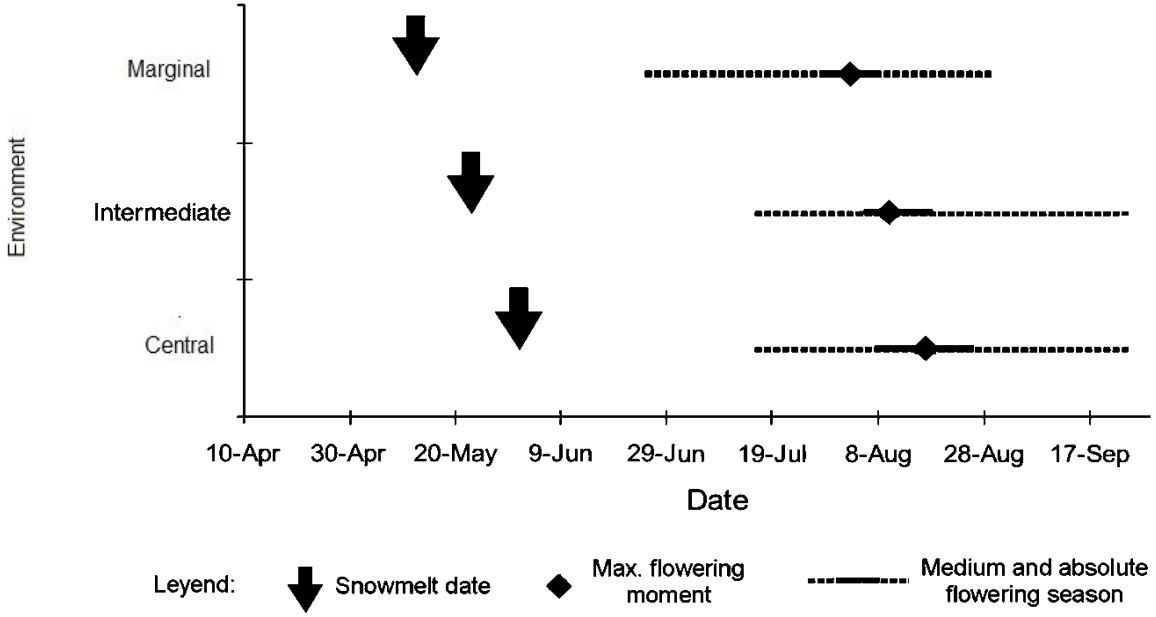


Gimenez-Benavides et al 2007 *Evolutionary Biology*

Elevational gradient experiment



Field conditions: marginal populations (more thermic) bloomed earlier



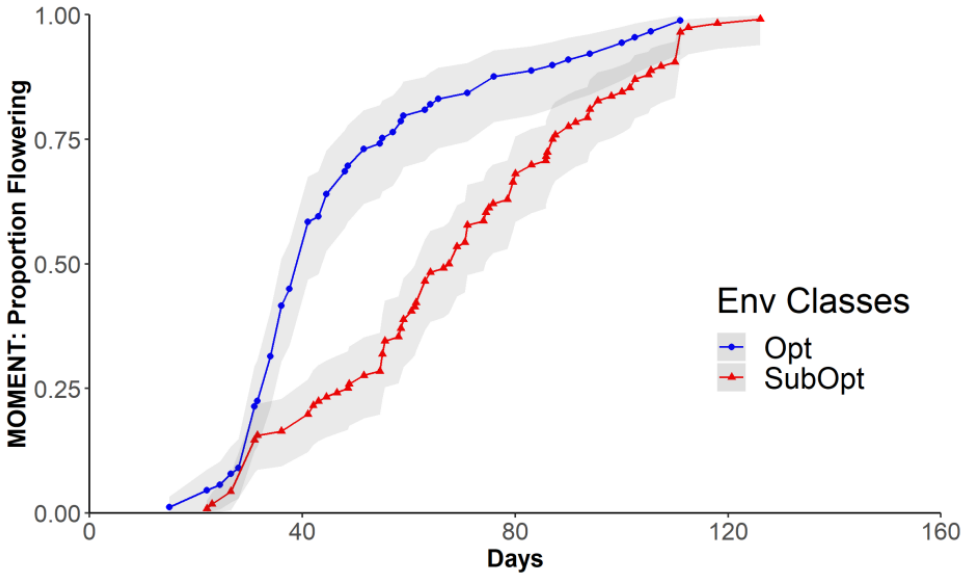
Gimenez-Benavides et al 2006 *New Phytologist*

Elevational gradient experiment

Field conditions: marginal populations (more thermic) bloomed earlier

Common garden experiment: central populations bloomed earlier → Genetic variation in flowering phenology

Kaplan-Meier survival curves comparisons $p < 0.01$



Morente-López et al 2019, *Annals of Botany*, under review

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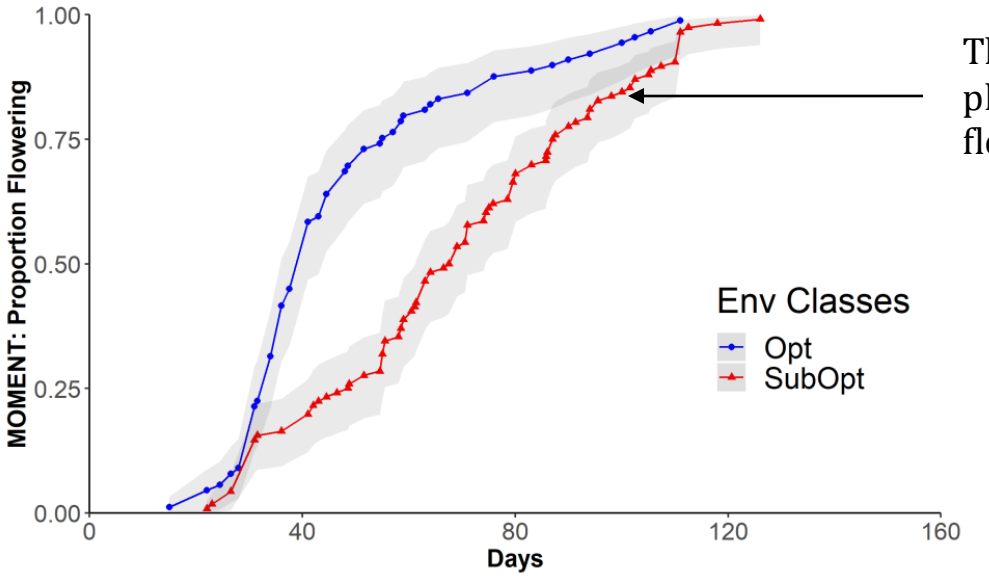
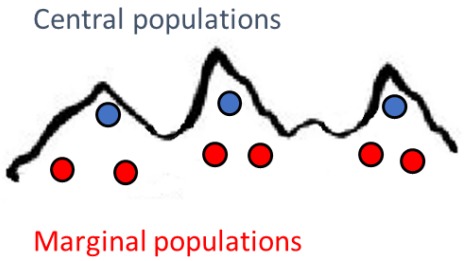


The curves represent the proportion of plants that reach their peak of flowering activity at a particular day.

Morente-López et al 2019, *Annals of Botany*, under review

Elevational gradient experiment



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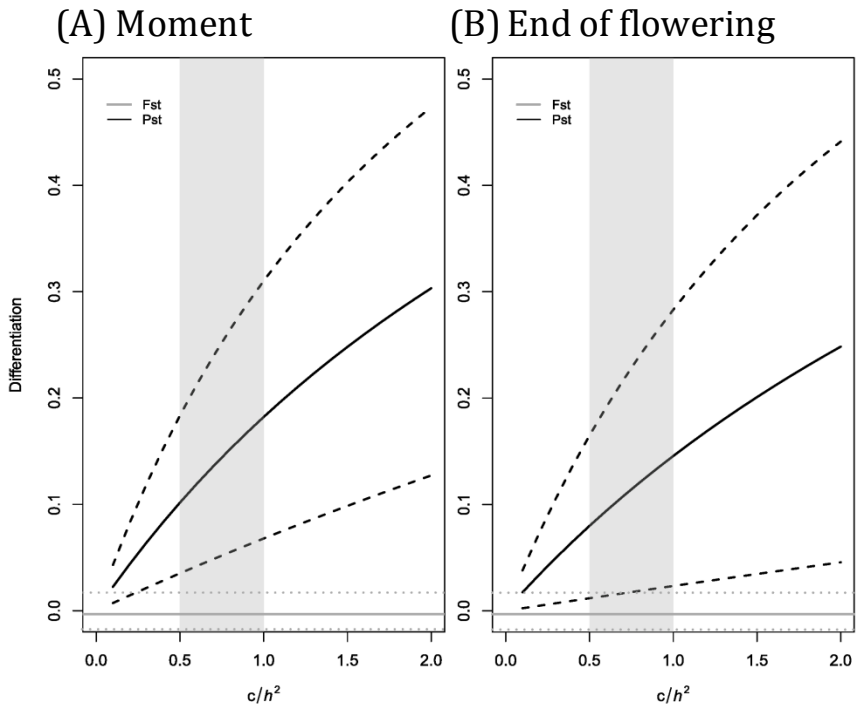
Quantitative genetic approach (Pst >> Fst) → Past diversifying selection for moment and end of flowering.

Neutral genetic differentiation (F_{ST})
Quantitative trait differentiation (P_{ST})



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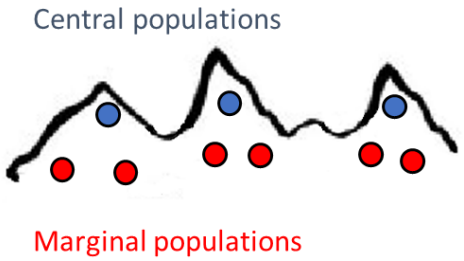
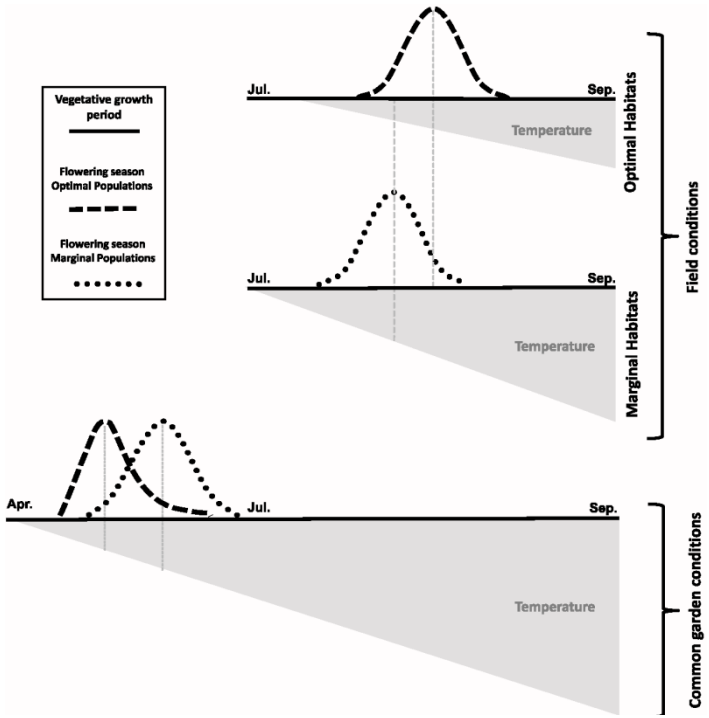


Field conditions: marginal populations (more thermic) bloomed earlier

Common garden experiment: central populations bloomed earlier → Genetic variation in flowering phenology

Quantitative genetic approach (Pst >> Fst) → Past diversifying selection for moment and end of flowering.

Because Central populations have a shorter growing season than Marginal populations, they are probably under greater selective pressure to bloom earlier due to the positive relationship between flowering duration and fitness



Lara-Romero et al 2014, *Bot J Linean Soc*; Morente-López et al 2019, *Annals of Botany*, under review

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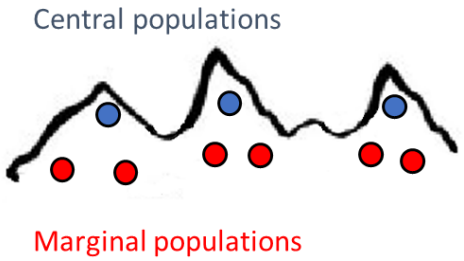

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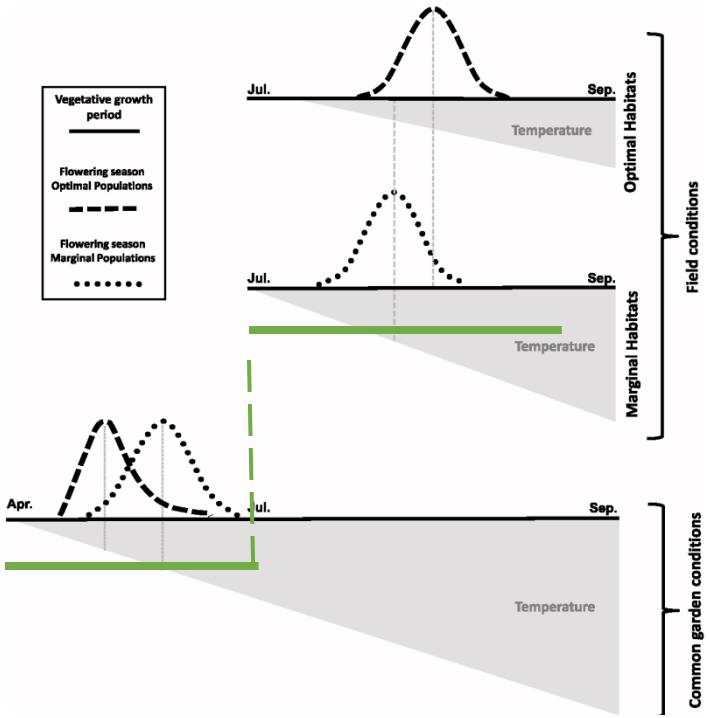
Common garden experiment: central populations bloomed earlier → Genetic variation in flowering phenology

Quantitative genetic approach (Pst >> Fst) → Past diversifying selection for moment and end of flowering.

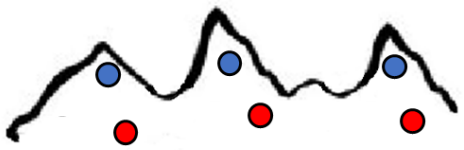
Plants in the common garden flowered three months earlier (mid of April) than populations in the field

- Flowering phenology in this species is strongly affected by temperature but not by photoperiod
- Adaptive plastic response that may enhance persistence under ongoing global warming

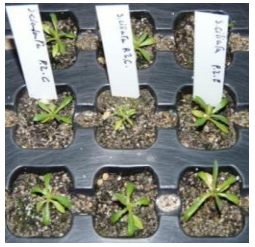


Lara-Romero et al 2014, *Bot J Linean Soc*; Morente-López et al 2019, *Annals of Botany*, under review

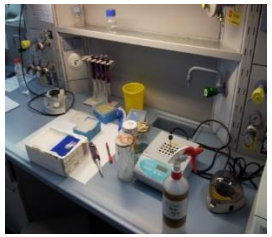
Exome capture experiment

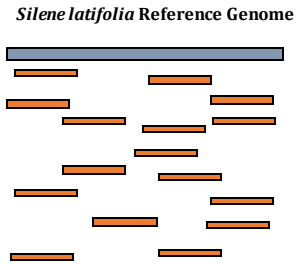


Seed collection & Greenhouse sowing



RNA extraction & Illumina sequencing



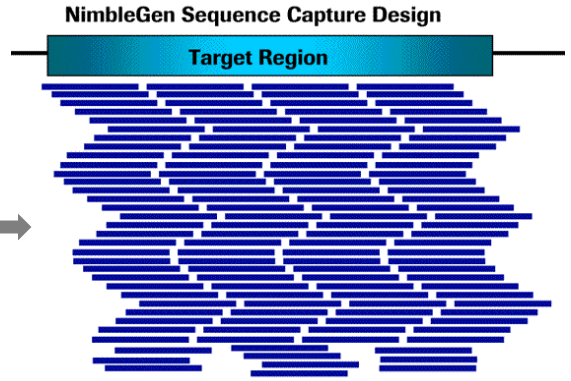


Functional annotation

Step 1: RNA-seq
(1 plant x 6 populations, n= 6)

Reference-based transcriptome assembly BWA

Step 2: Exon capture experiment
(16 plants x 6 populations, n= 96)



71,000 SNPs

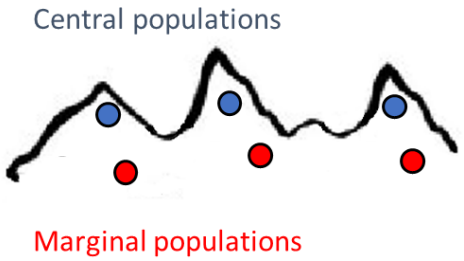
2414 genes selected for exome capture experiment considering annotation :

- 1925 abiotic stress-responsive genes
- 489 genes that promote flowering

Work flow. Genomic data

Sacristán et al 2019, *Plant Genetic Resources*, under review

Exome capture experiment



Detection of SNPs outlier: SNPs influenced by selection among marginal and central populations

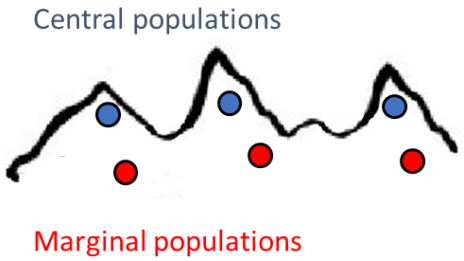
1. F_{ST} outlier analysis implemented in ARLEQUIN to control for strong genetic structure
2. Significant fixation index among environments within mountains (F_{SC})
3. Consistent AFDs in all pairwise comparisons among environments within mountains

} SNP outlier

19 genes that promote flowering had at least one SNP (loci) outlier → potential adaptive value
2 ± 1,22 SNPs per gene
F_{ST} = 0.3 ± 0.06

Lara-Romero, Iriondo & González-Martínez, unpublished results

Exome capture experiment



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F_{ST} = 0.3 ± 0.06

The combination of both genomic and phenotypic data suggested the existence of adaptive potential for an adjustment to environmental change.

Lara-Romero, Iriondo & González-Martínez, unpublished results

Acknowledgments

Collaborators

Santiago González-Martinez (INRA, BIOGECO, Bordeaux)
Alex Widmer (ETH-Zurich)
Luis Giménez-Benavides (URJC-Madrid)

Financial support

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Thank you

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