Molecular Phenology in Trees

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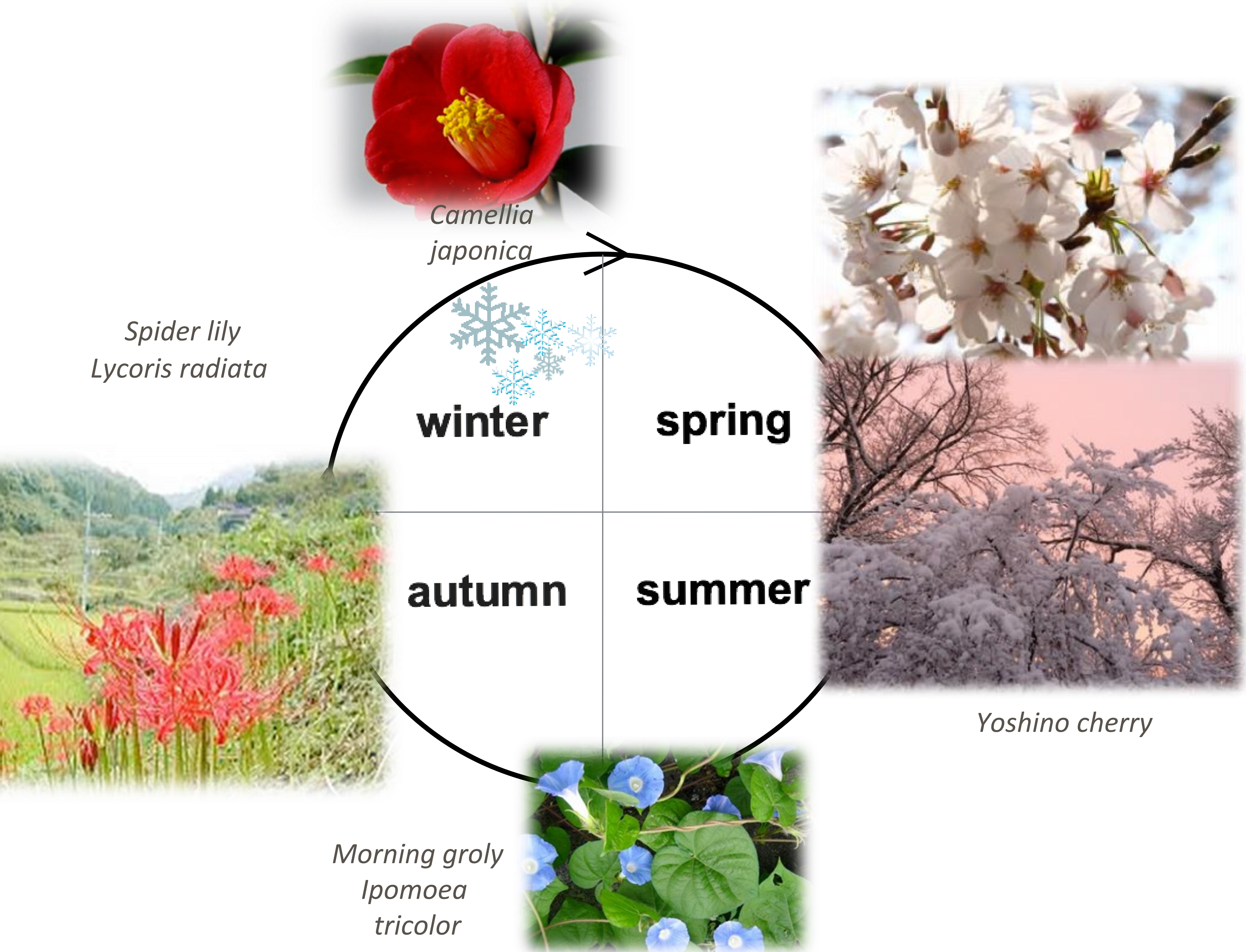
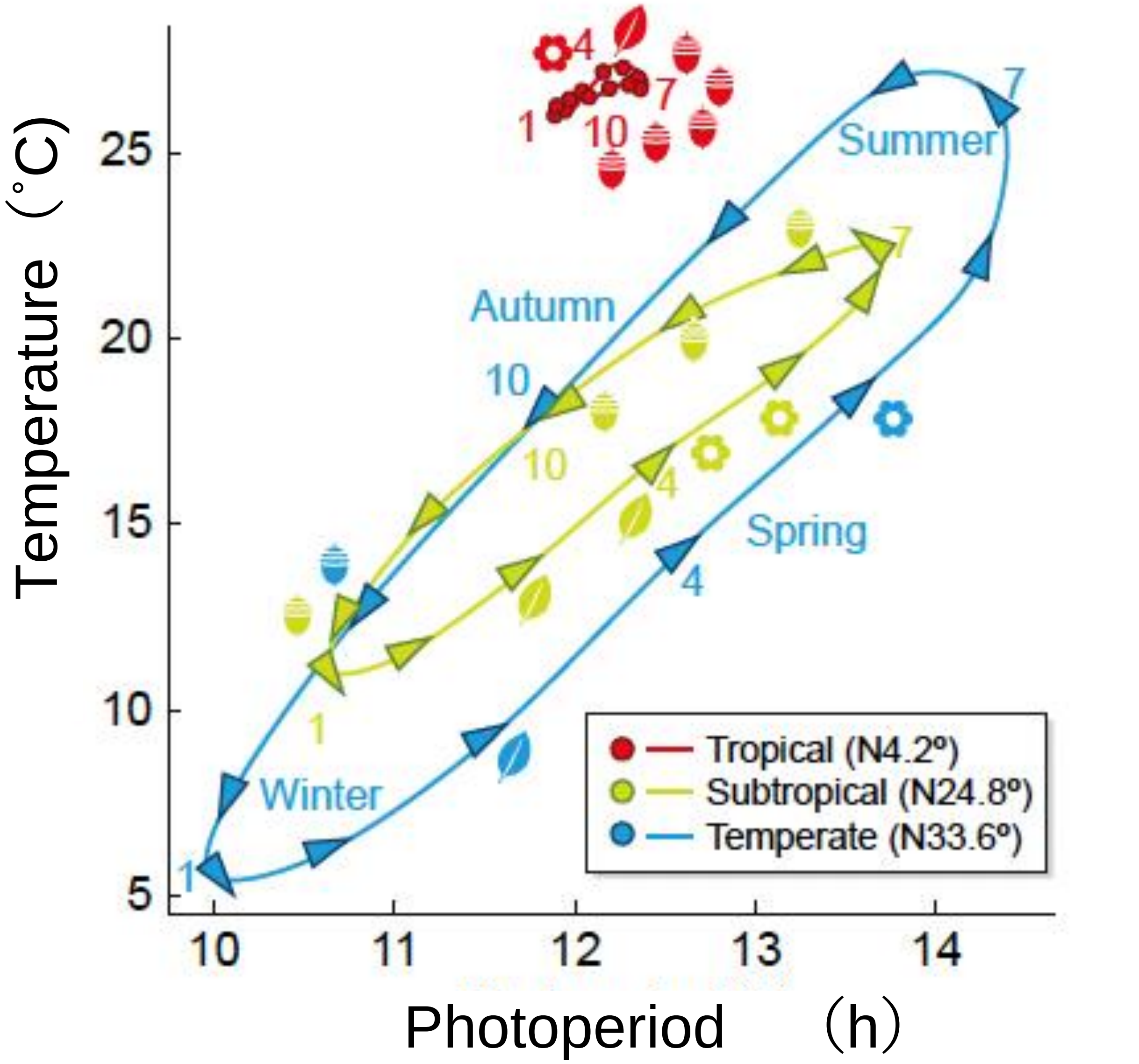
June 24, 2022


Camellia japonica
Spider lily
Lycoris radiata
winter
spring
autumn
summer
Yoshino cherry
Morning groly
Ipomoea tricolor

Seasonal variations in environmental factors

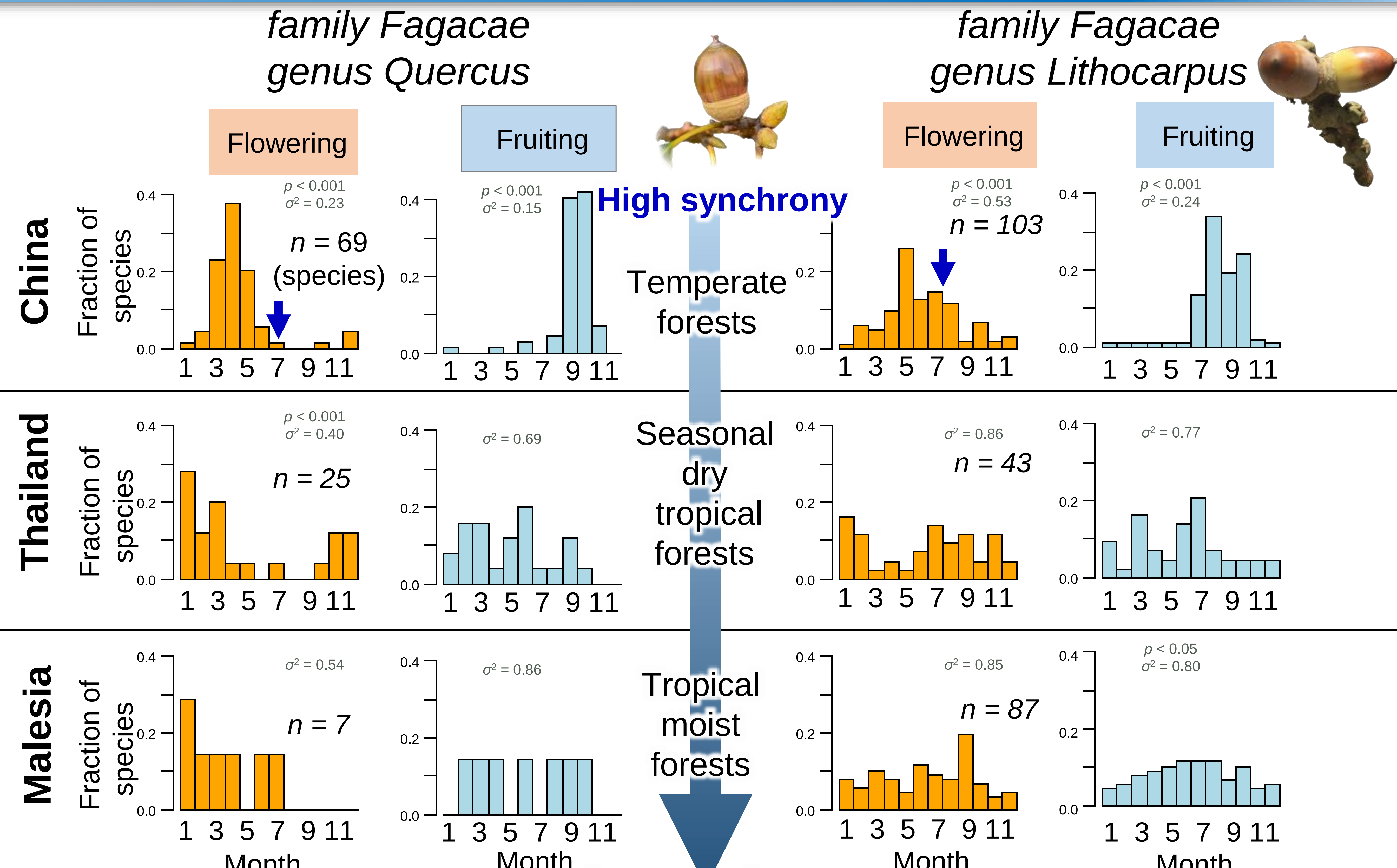
Satake et al. New Phytol 2022



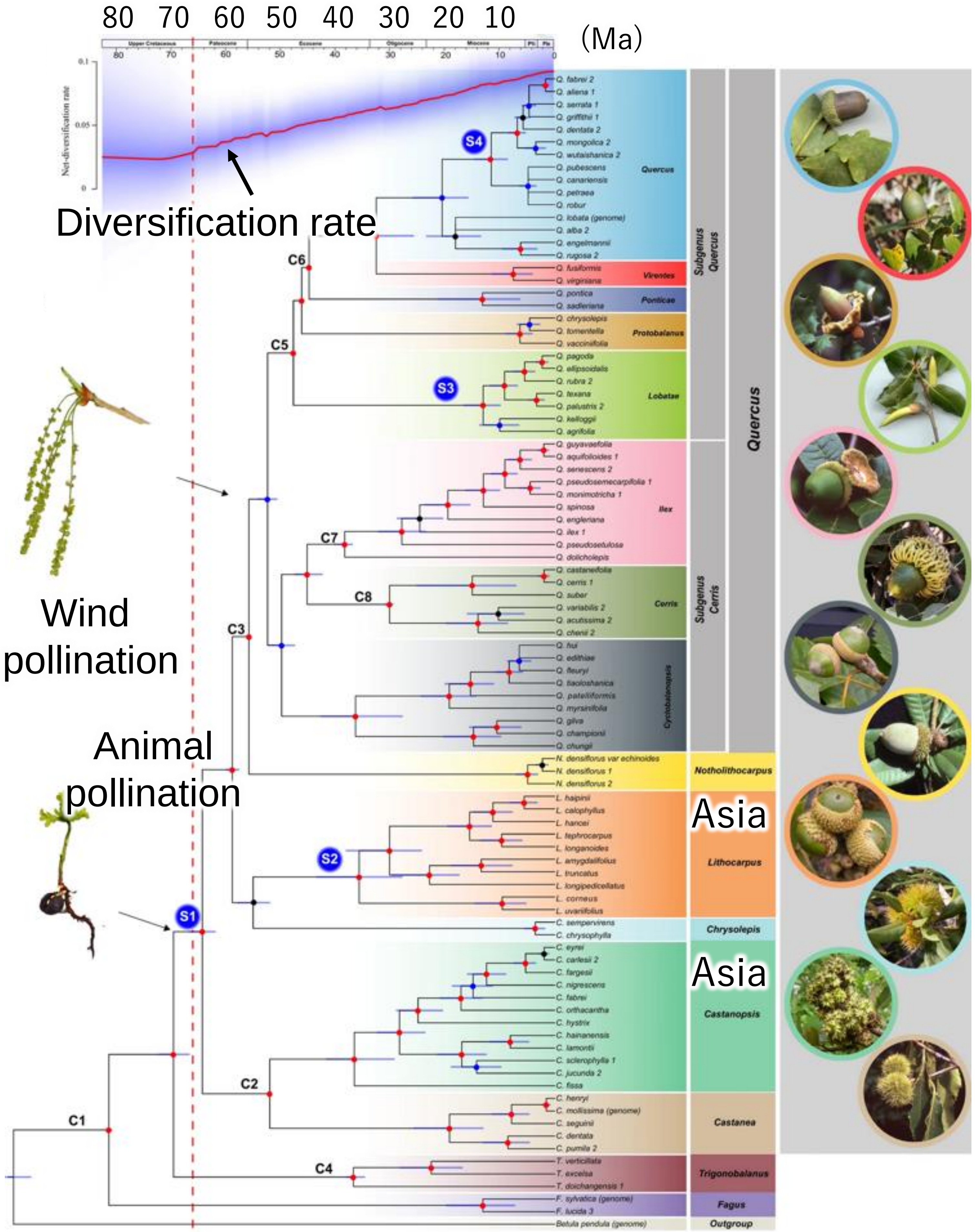
Japan (Kyushu) Taiwan Malaysia

Seasonality of photoperiod and temperature virtually disappear in tropical rain forests

Latitudinal gradient of reproductive phenology



Araye, Yahara, Satake (under review)



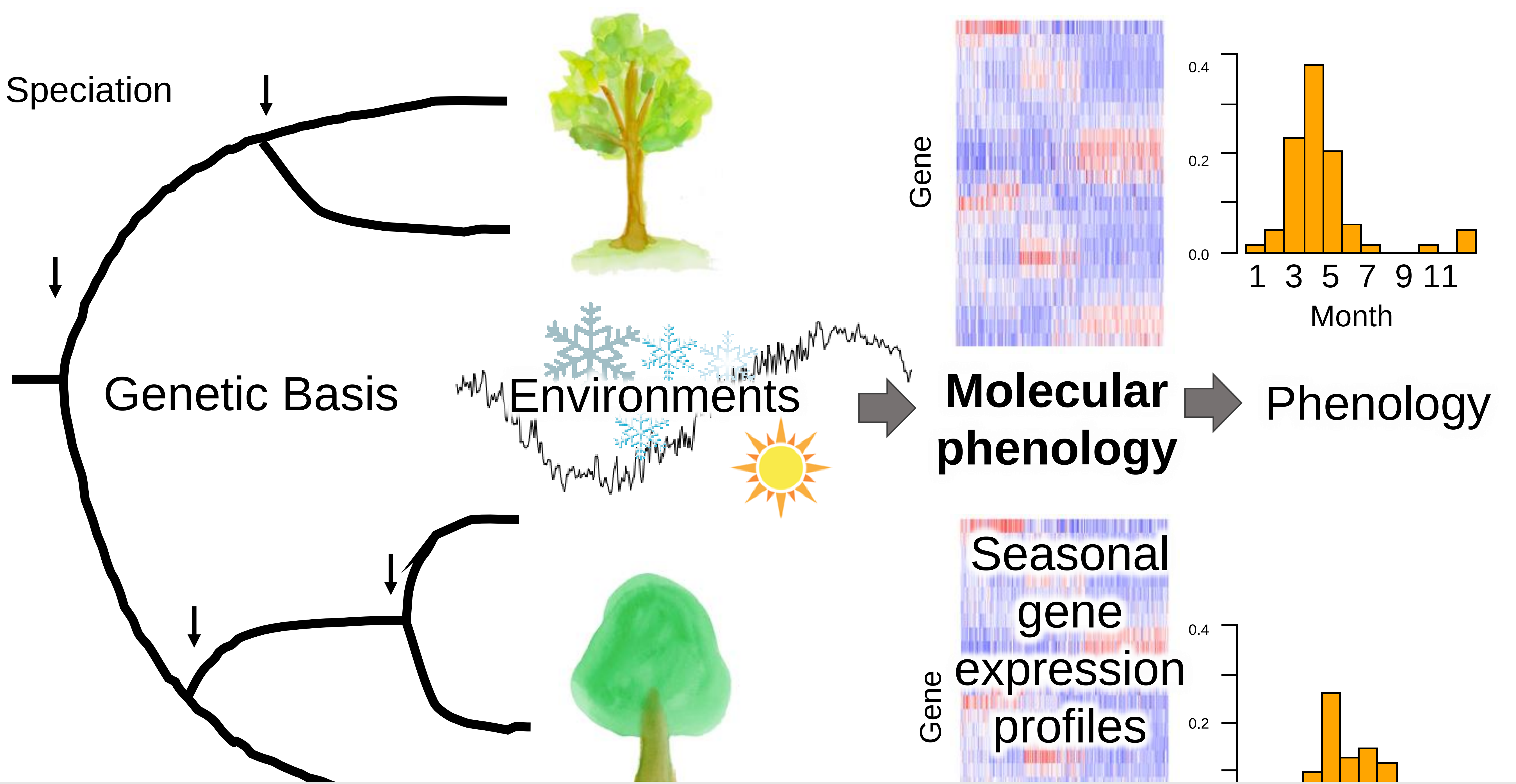
Genus *Quercus* and *Lithocarpus* diverged c.a. 55 million years ago.

Quercus
Wind pollination

Lithocarpus
Animal pollination

Zhou et al. 2022 Nat Commun

A cross-scale approach for phenology



We can understand the physiological mechanism and evolutionary history of phenology.

that have evolved from a
common ancestral gene

Molecular and genetic knowledge in model plants

Vernalization

FLC

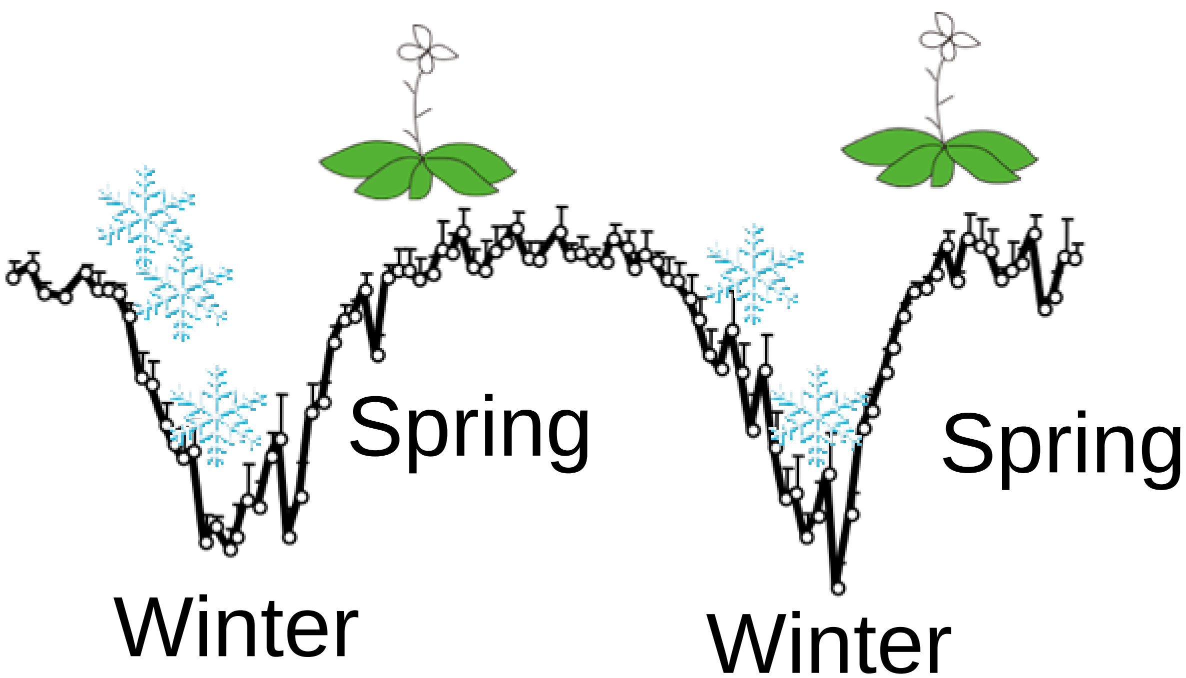
Flowering

Bud burst

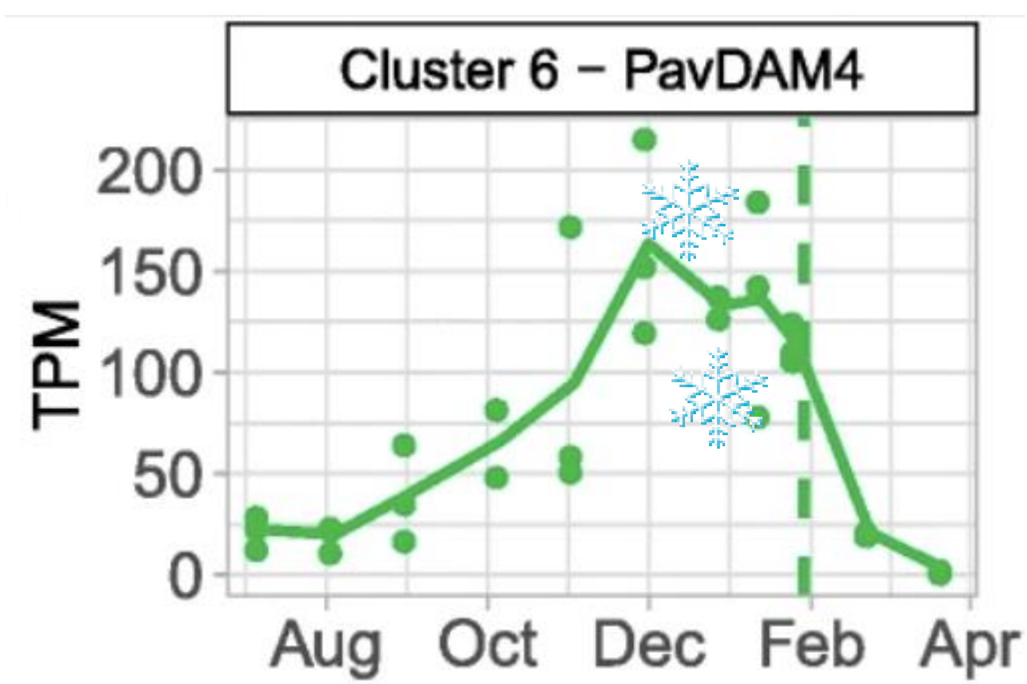
DAMs

ABA

But dormancy break



Aikawa et al. 2010 PNAS



Vimont et al. BMC Genomics 2019

Molecular phenology in non-model plants

Field monitoring of phenology
Field transcriptome
Target gene
expression analyses

Fagus crenata
(Japanese beech)

Fagaceae

Quercus glauca

Lithocurpus edulis

Perennial herb
Arabidopsis halleri

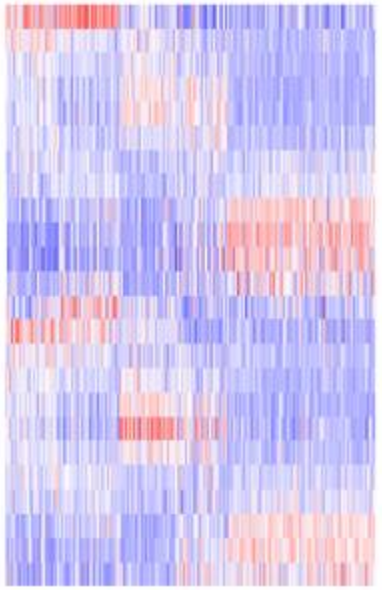
Shorea curtisii
Shorea leprosul

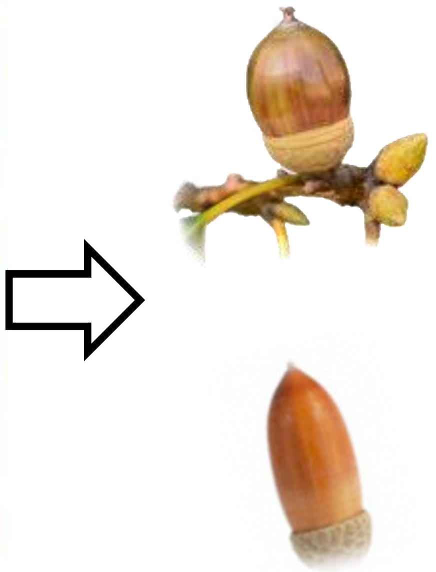
Dipterocarpaceae

Today's topics

1. Tropical phenology and impacts of climate change

2. Comparative molecular phenology in Fagaceae



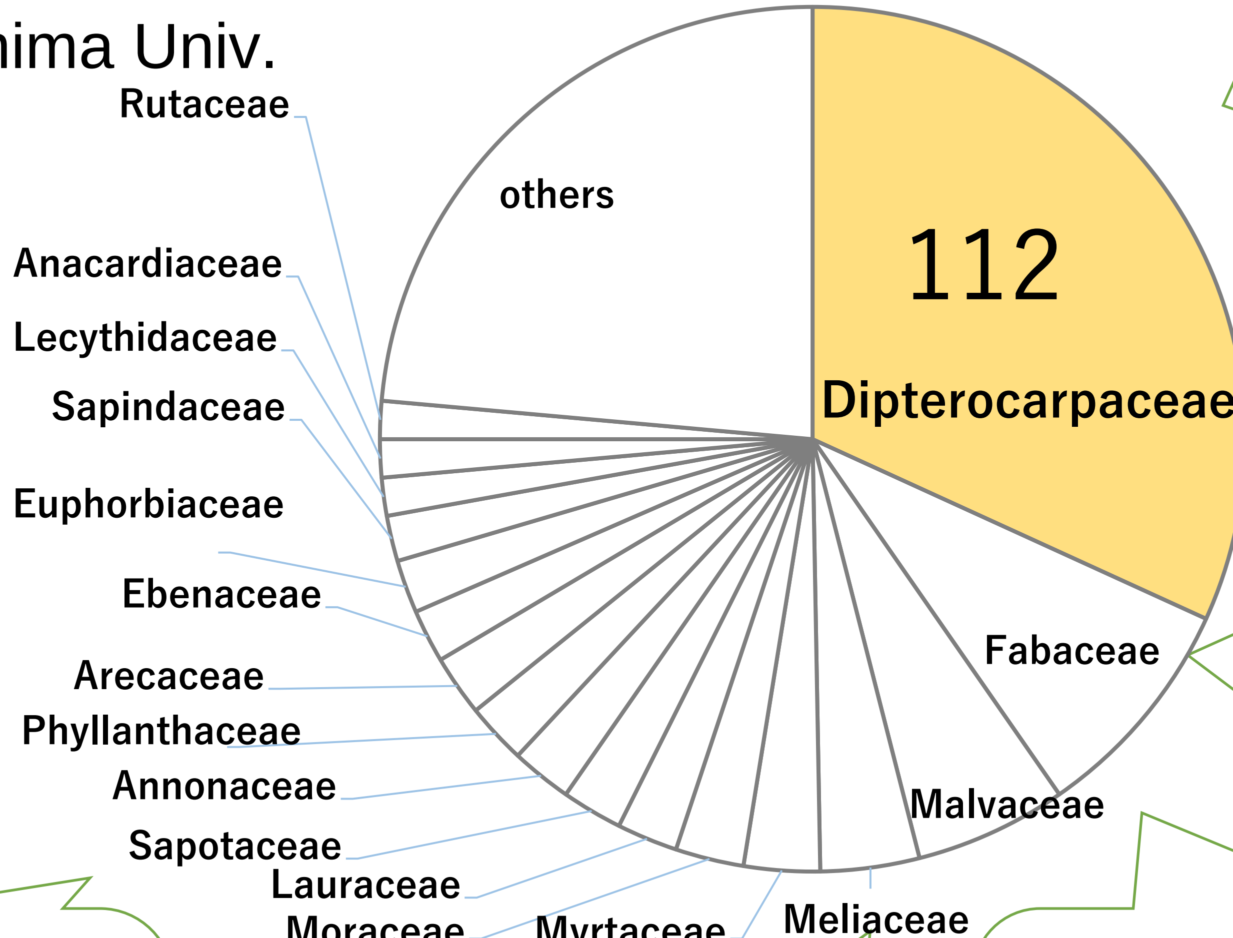


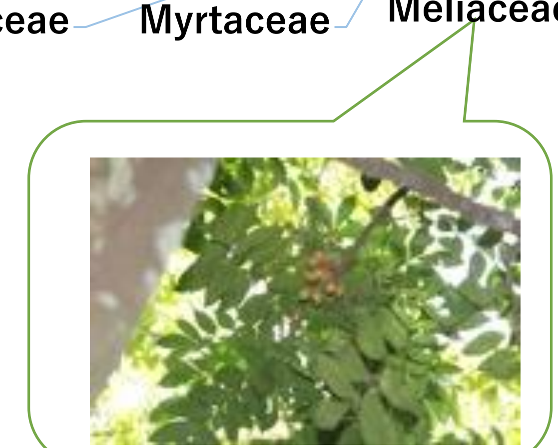
Long-term flowering and fruiting data

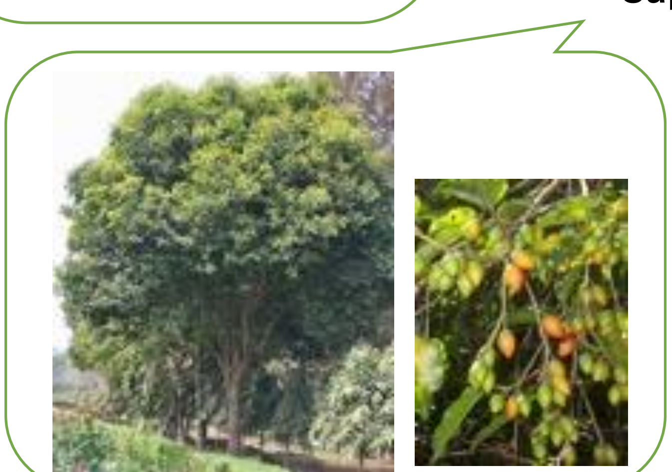
Monthly record of present or absence of flowers and fruits
in **210 tree species from 41 families in arboretums in Malaysia.**

In collaboration with
Dr. Numata@Tokyo Met and
Dr. Hosaka@Hiroshima Univ.

1976~2010





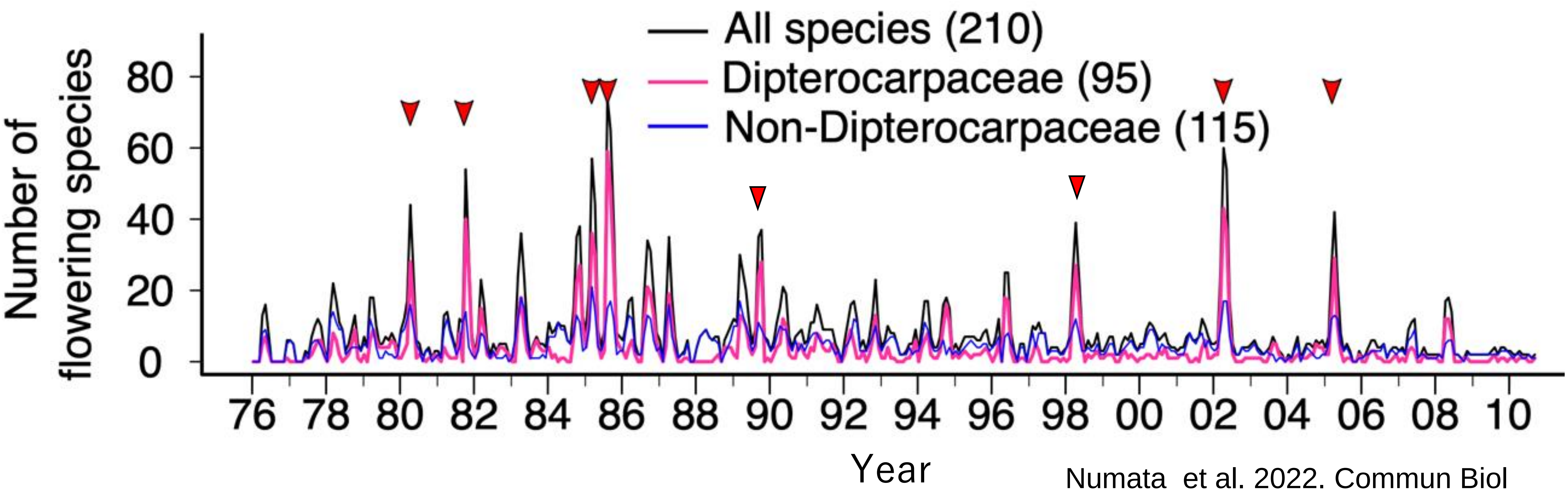


Useful Tropical Plants ,http://tropical.theferns.info,2019/04/20

Tropical phenology in Southeast Asia

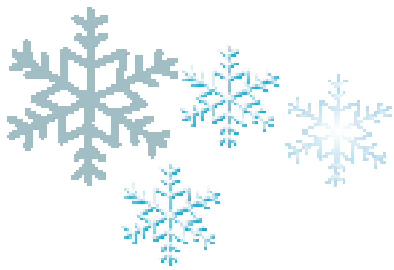
- Number of flowering species is low in late 1970s.
- More than 70 species flowered in 1985.
- Synchronized flowering, in which more than 20% of species participate (▼), occurred once in 2–8 yrs.

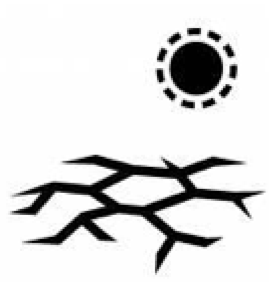
➡ **General flowering**



Numata et al. 2022. Commun Biol

What is a trigger of general flowering?





Low temperature

Several nights of low temperature
Ashton et al. 1988

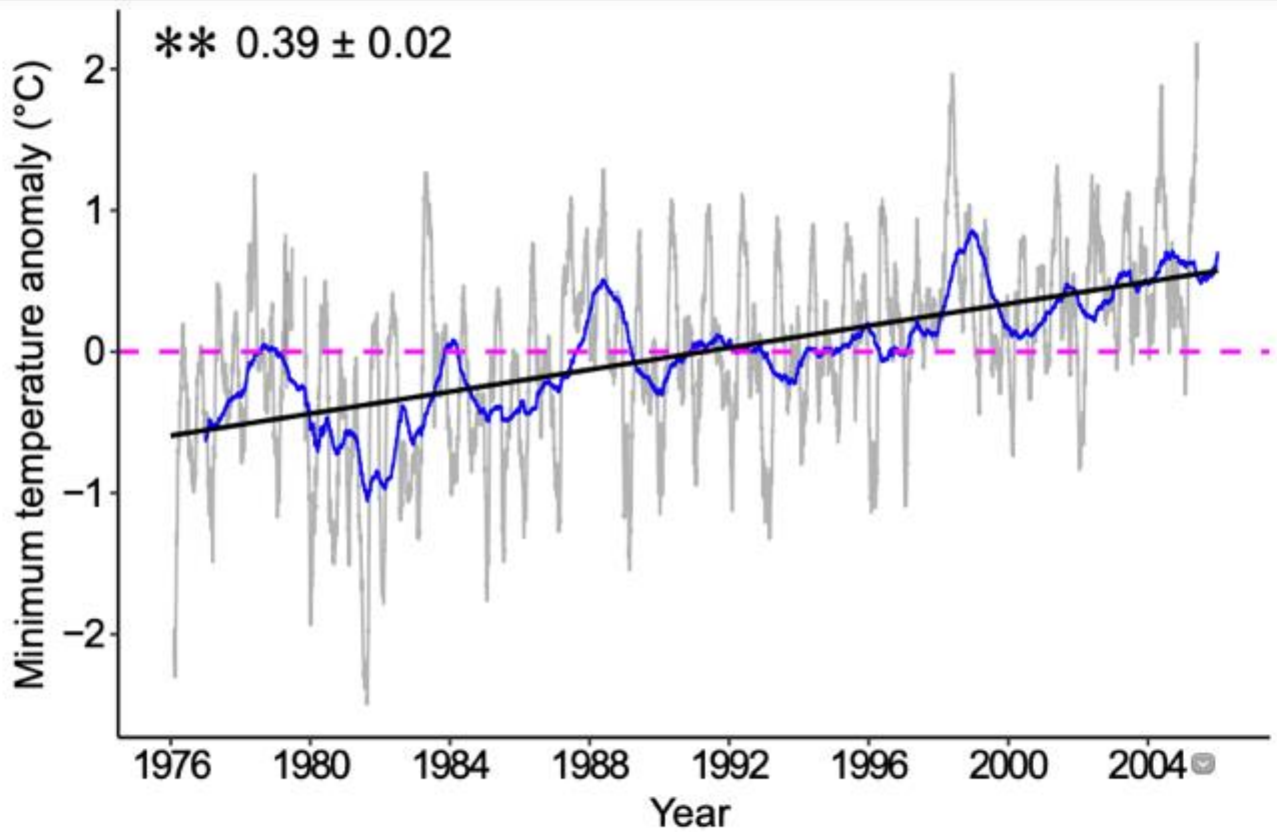
Drought

30 days total rainfall
that is less than 40mm
Sakai et al. 2006

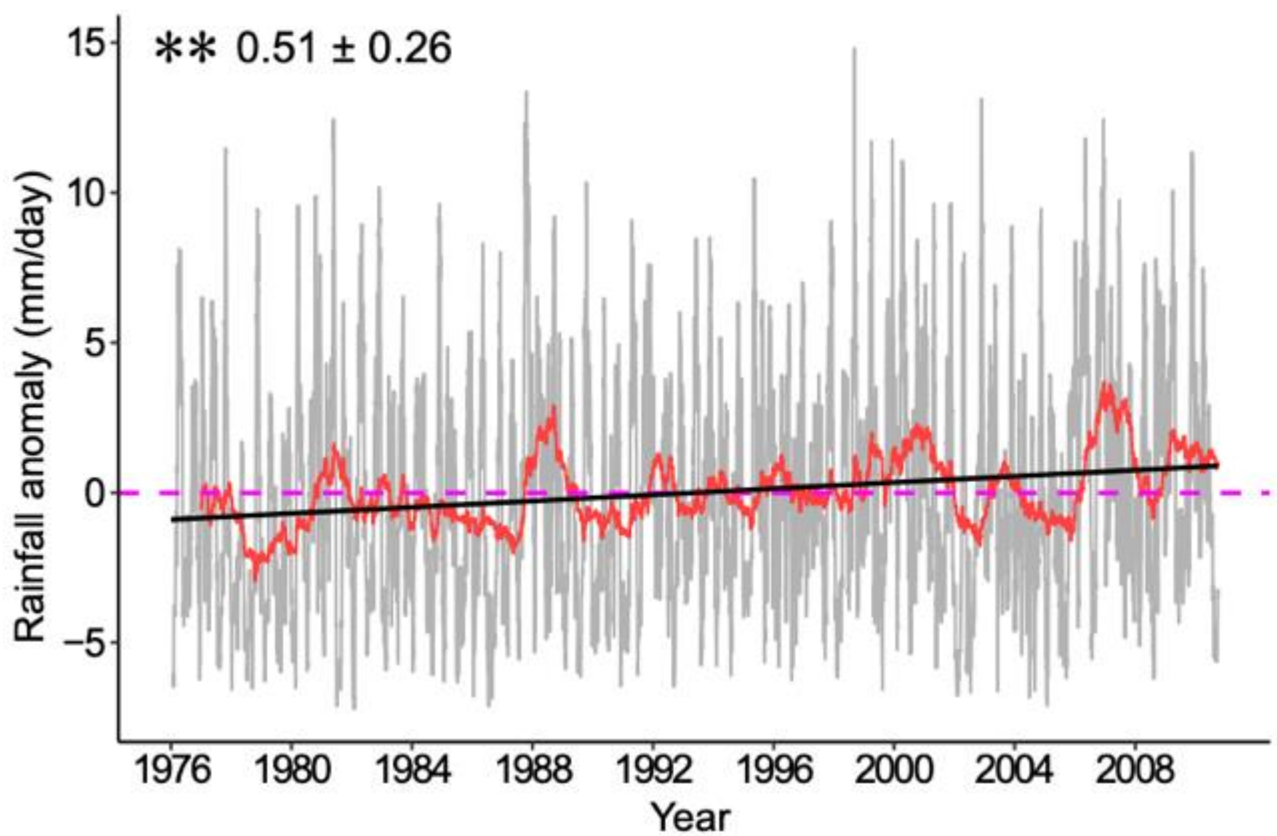
Low temperature × Drought

Synergistic effect
Chen et al. 2018

Observed climate change over 30 yrs



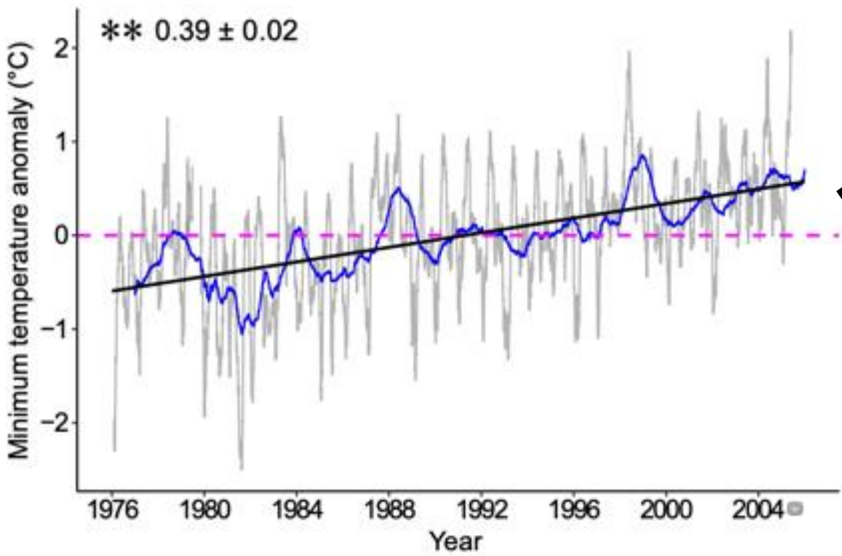
Temperature increased.
(0.39 ± 0.02 ˚C /decade)

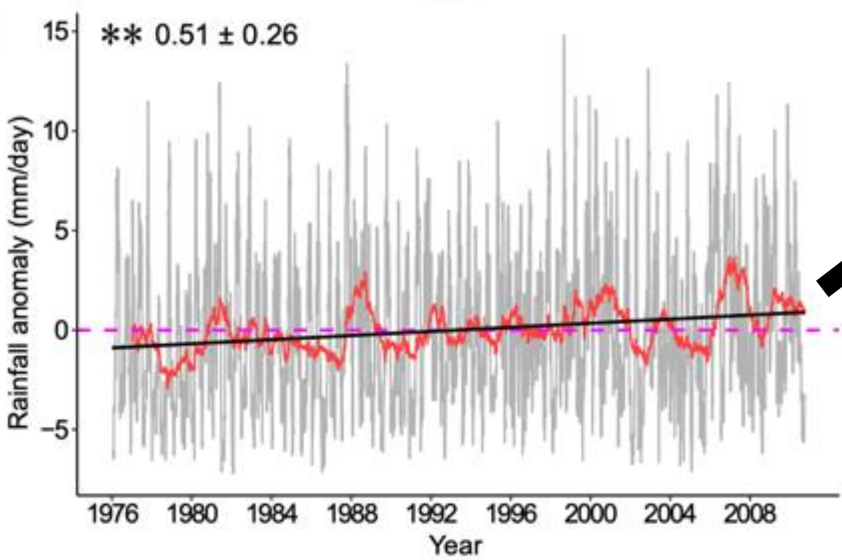


Precipitation
slightly increased.
(0.51 ± 0.26 mm/day/decade)

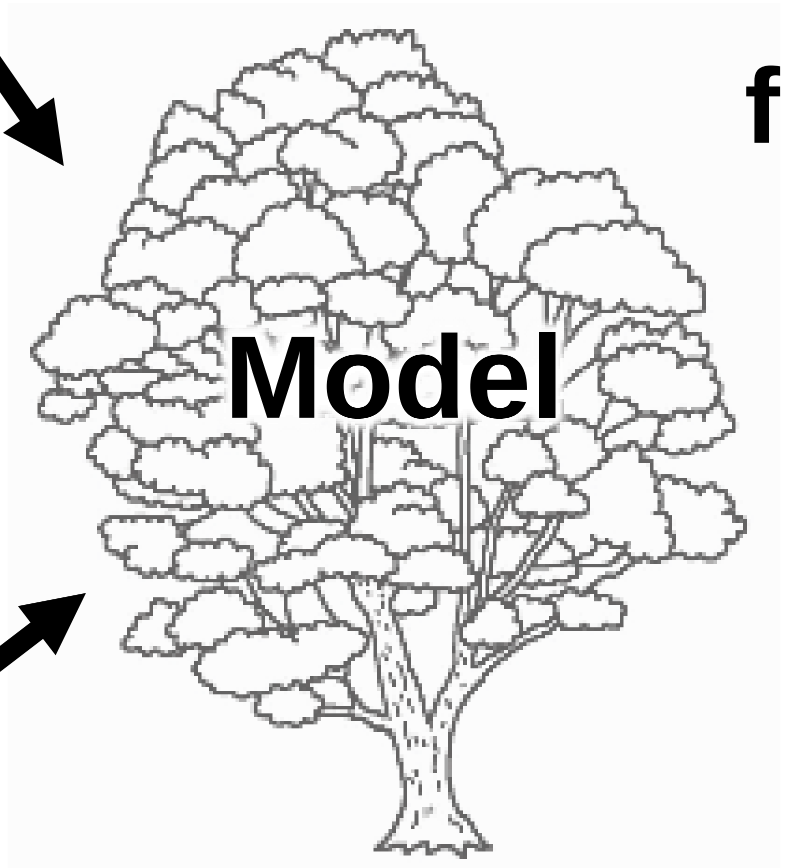
How climate change impacts on tropical phenology?

Temperature



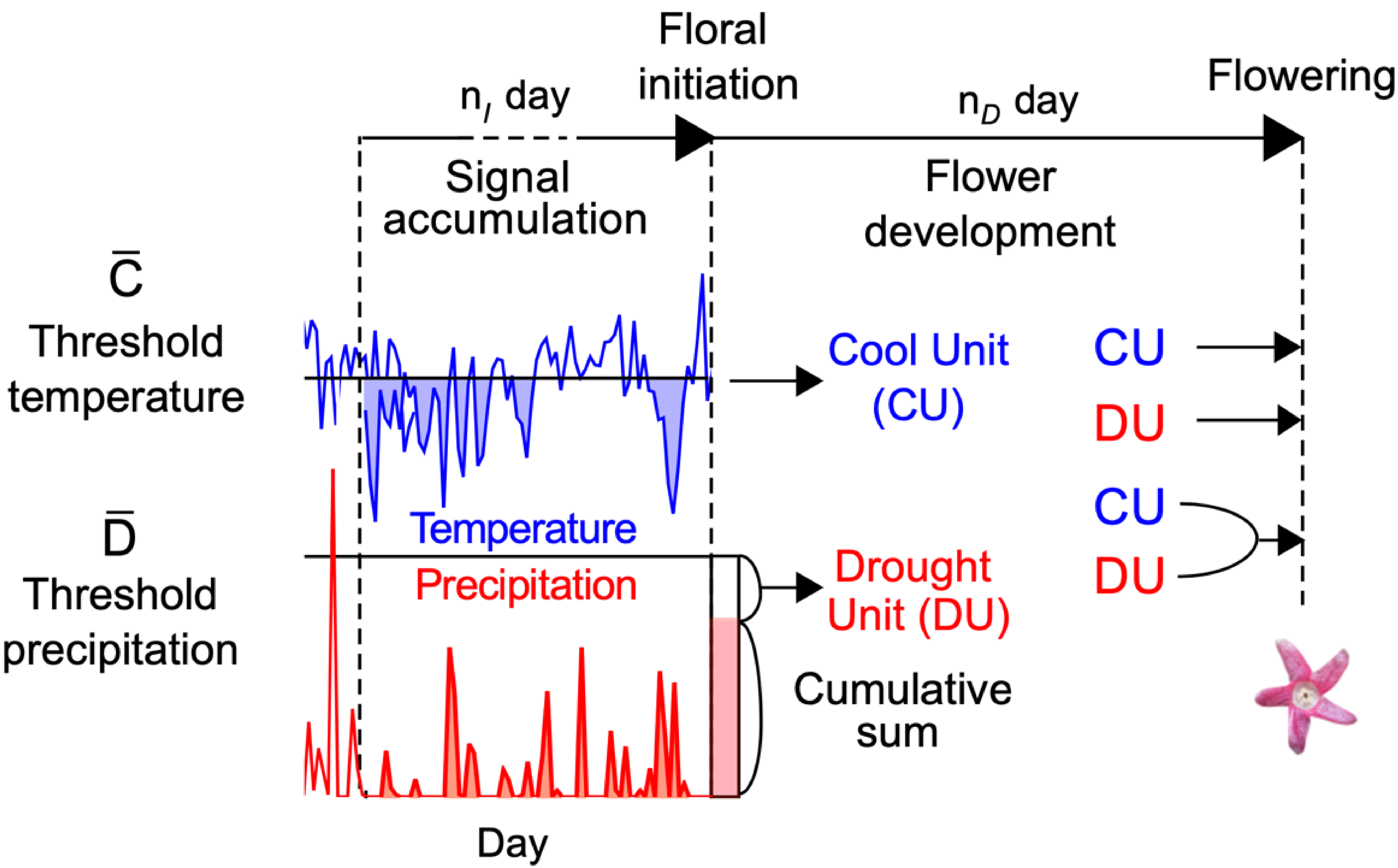


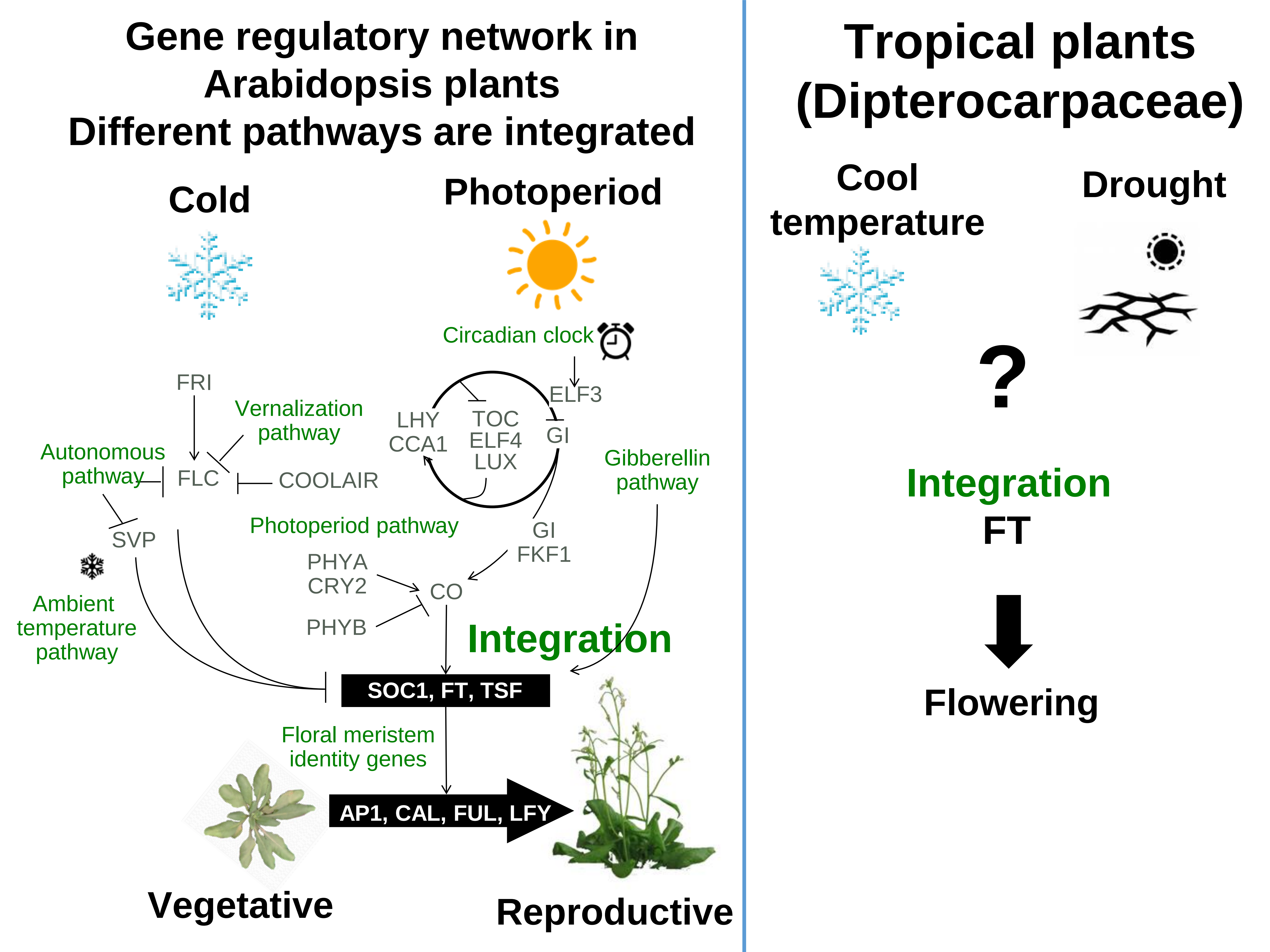
Precipitation



Prediction of future phenology

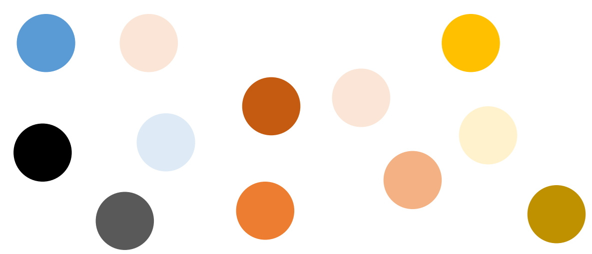
Modelling signal accumulation and integration



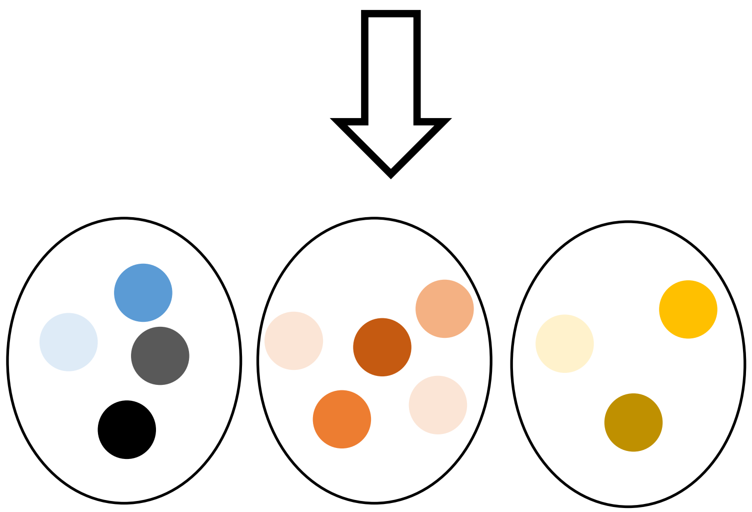


Gene regulatory network in Arabidopsis plants
Different pathways are integrated
Cold
Photoperiod
Circadian clock
FRI
Vernalization pathway
LHY
CCA1
TOC
ELF4
LUX
ELF3
GI
Autonomous pathway
FLC
COOLAIR
Gibberellin pathway
SVP
Photoperiod pathway
GI
FKF1
PHYA
CRY2
CO
PHYB
Ambient temperature pathway
Integration
SOC1, FT, TSF
Floral meristem identity genes
AP1, CAL, FUL, LFY
Vegetative
Reproductive
Tropical plants (Dipterocarpaceae)
Cool temperature
Drought
?
Integration
FT
Flowering

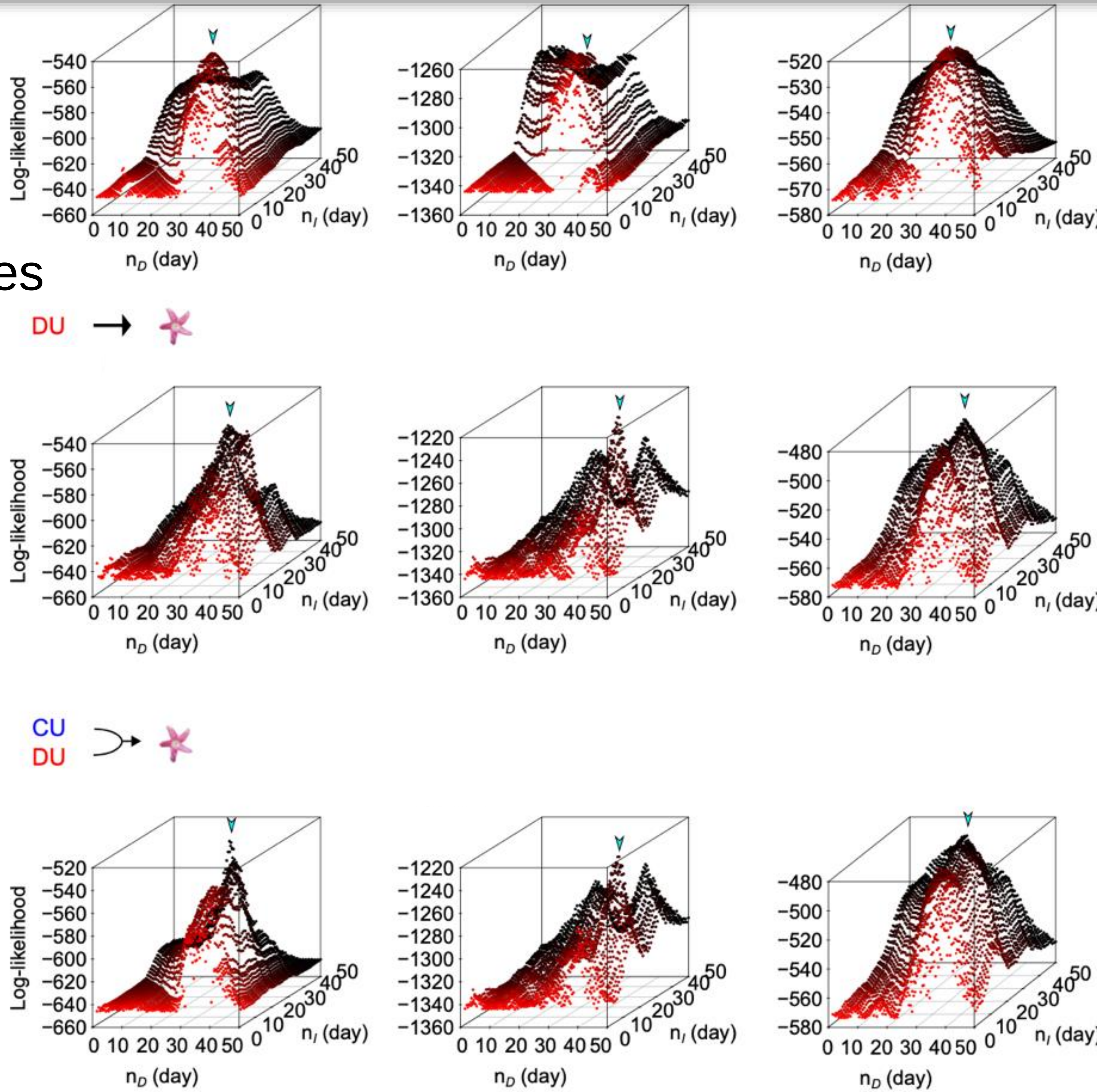
Parameterization and model selection



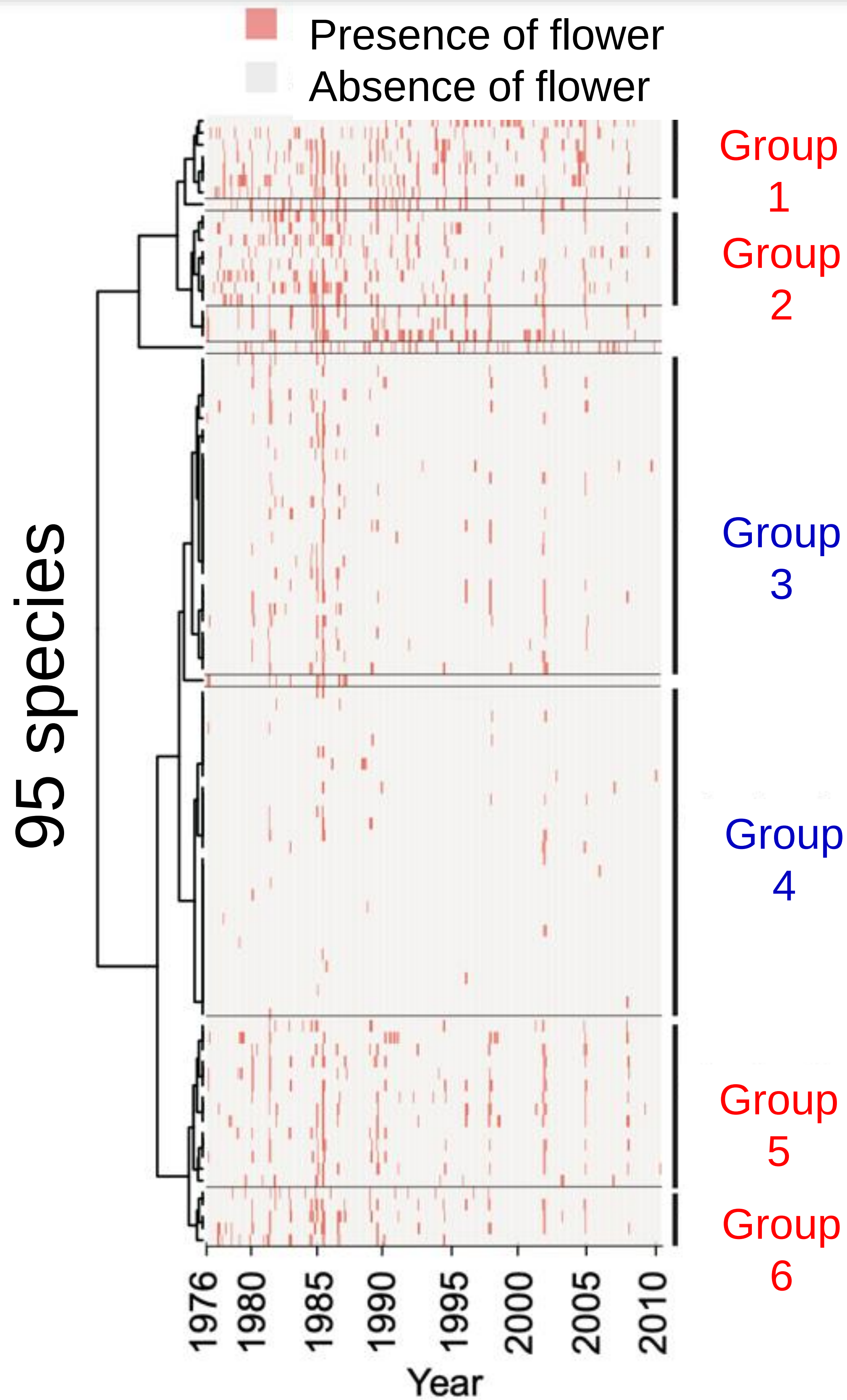
95 Dipterocarp species



Clustering into optimal number of phenological groups



We identified 6 phenology groups



Different pheno groups showt different environmental responses under same environment

Group **1, 2, 5, 6**
response to drought

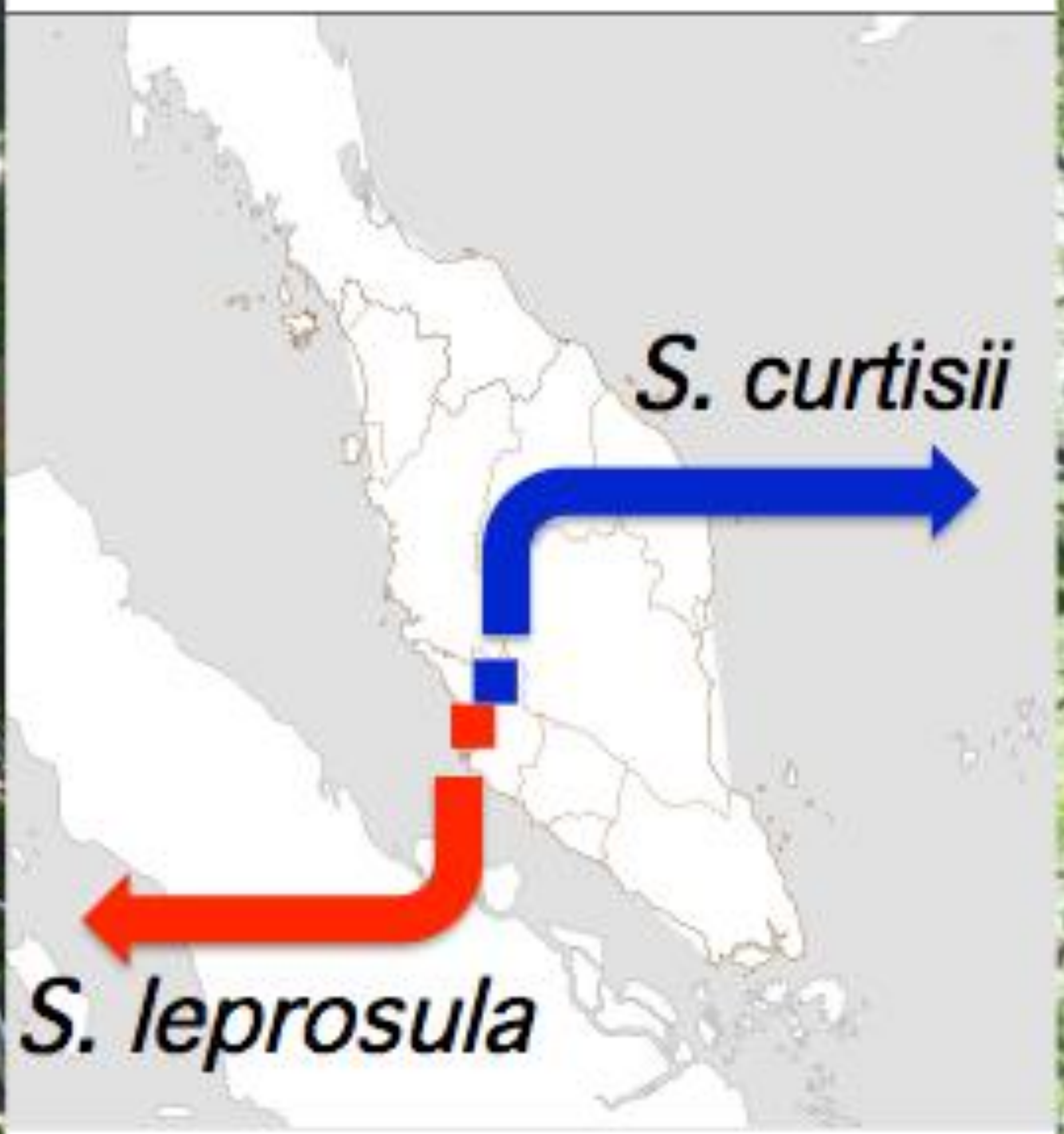
Group **3, 4**
(57% of total number of species)
response to low temperature
and drought

The differential sensitivity to environmental signals will have a profound effect on fitness.

How about molecular phenology in tropics?

Malaysia research institute (FRIM)

Semangkok



Depterocarpaceae

Shorea curtisii

S. leprosula

From 2012

From 2011

Monitoring molecular phenology of tropical trees

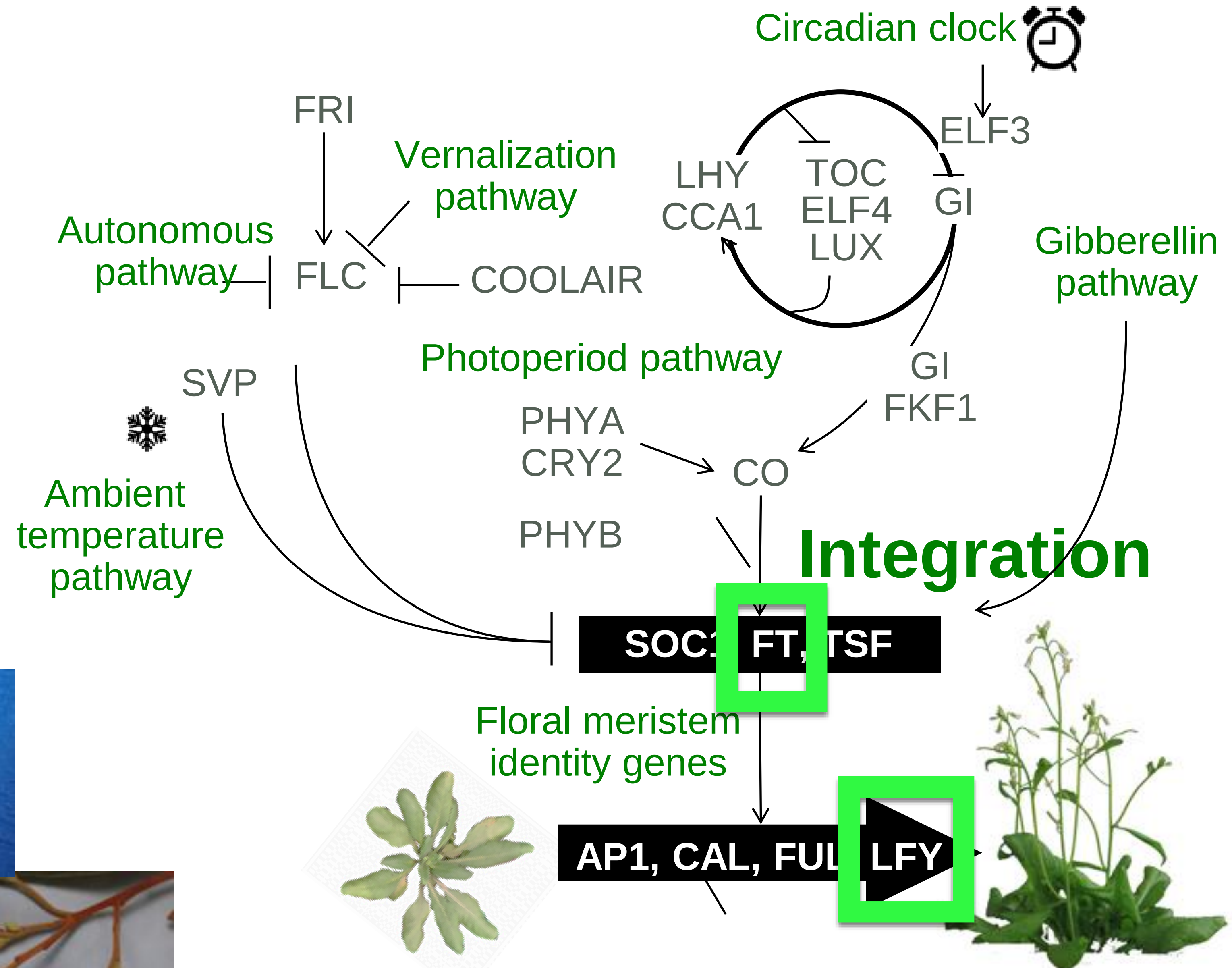




Sample collection

RNA extraction

Measurement of gene expression level by qPCR



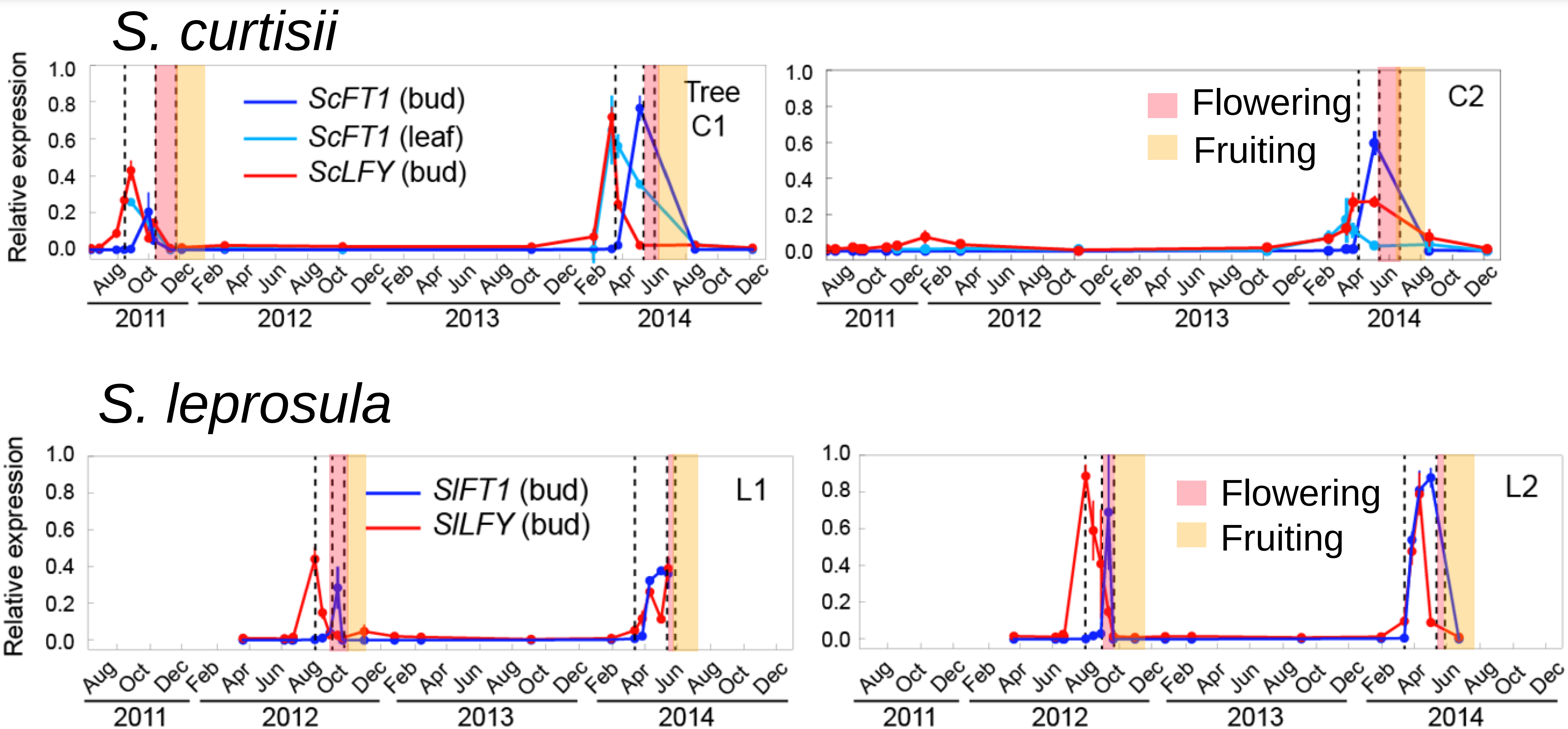
Target genes

Most important genes for floral transition

FT
(*FLOWERING LOCUS T*)

LFY
(*LEAFY*)

Molecular phenology of tropical trees

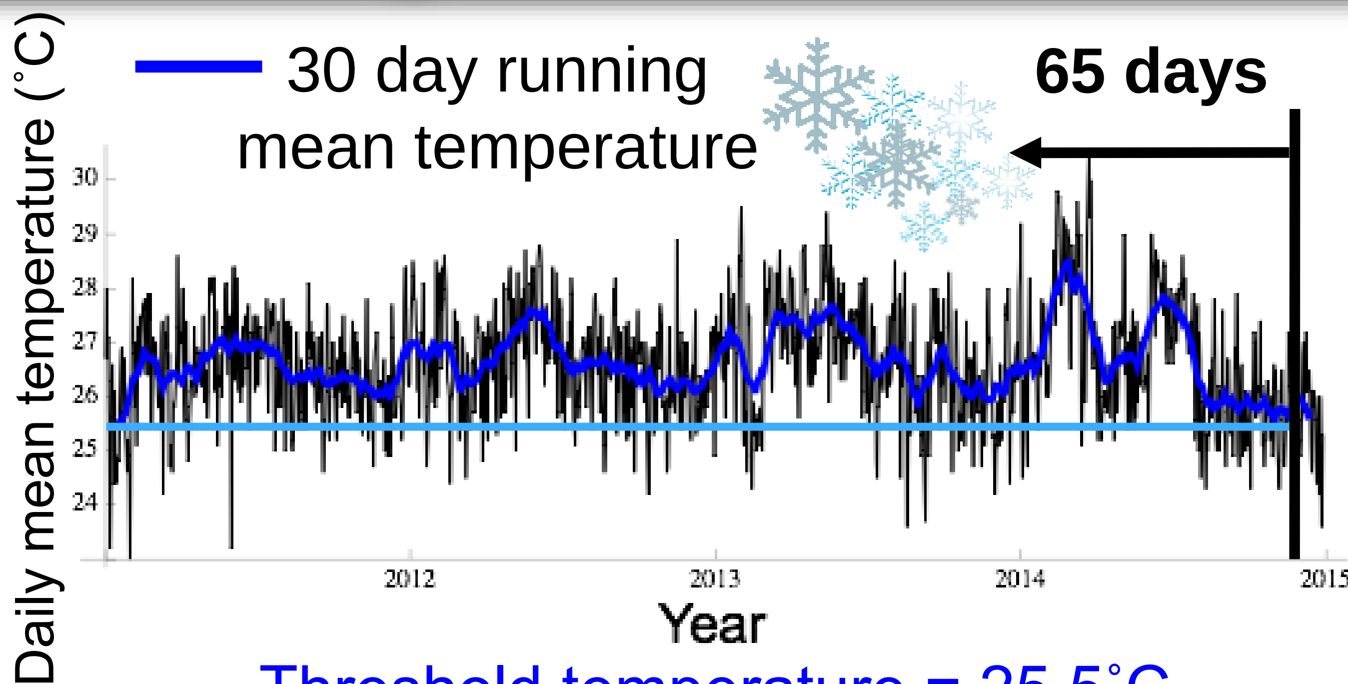


Yeoh et al. 2017. Mol Ecol

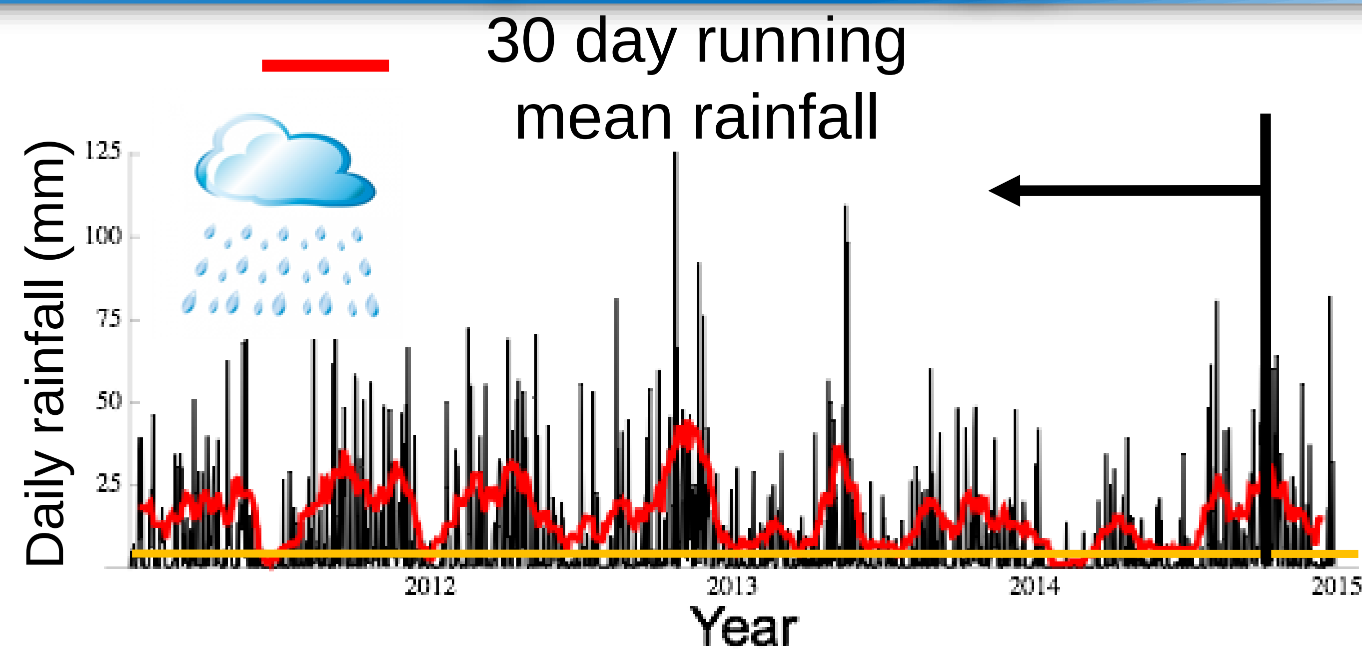
Activation of *FT* and *LFY* induces floral induction.

Molecular phenology is effective to monitor physiological changes that cannot be seen with eyes.

Integrated drought and low temperature signals explain observed molecular phenology

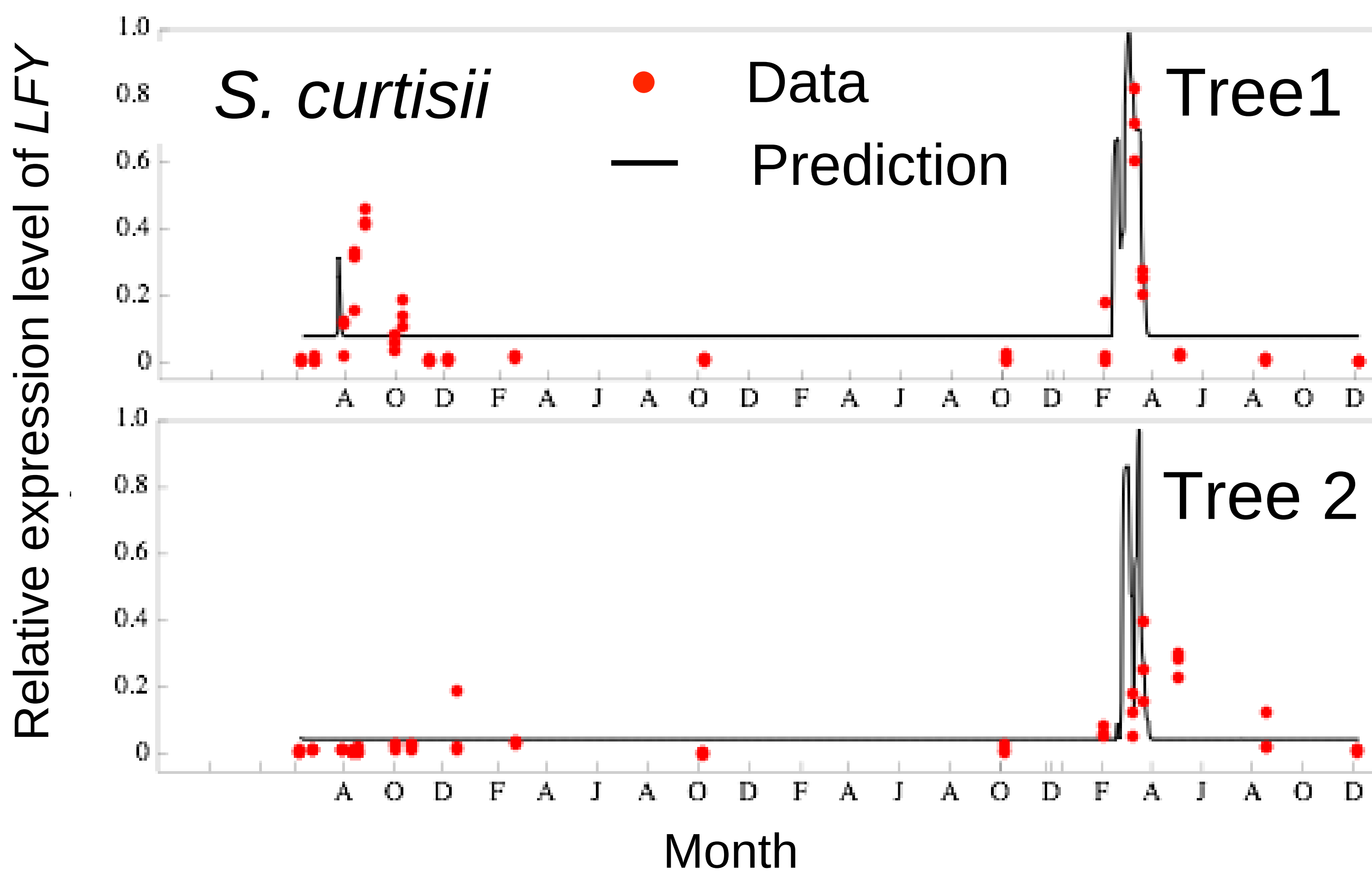


Threshold temperature = 25.5˚C



Threshold rainfall = 2.4mm/day

Yeoh et al. 2017

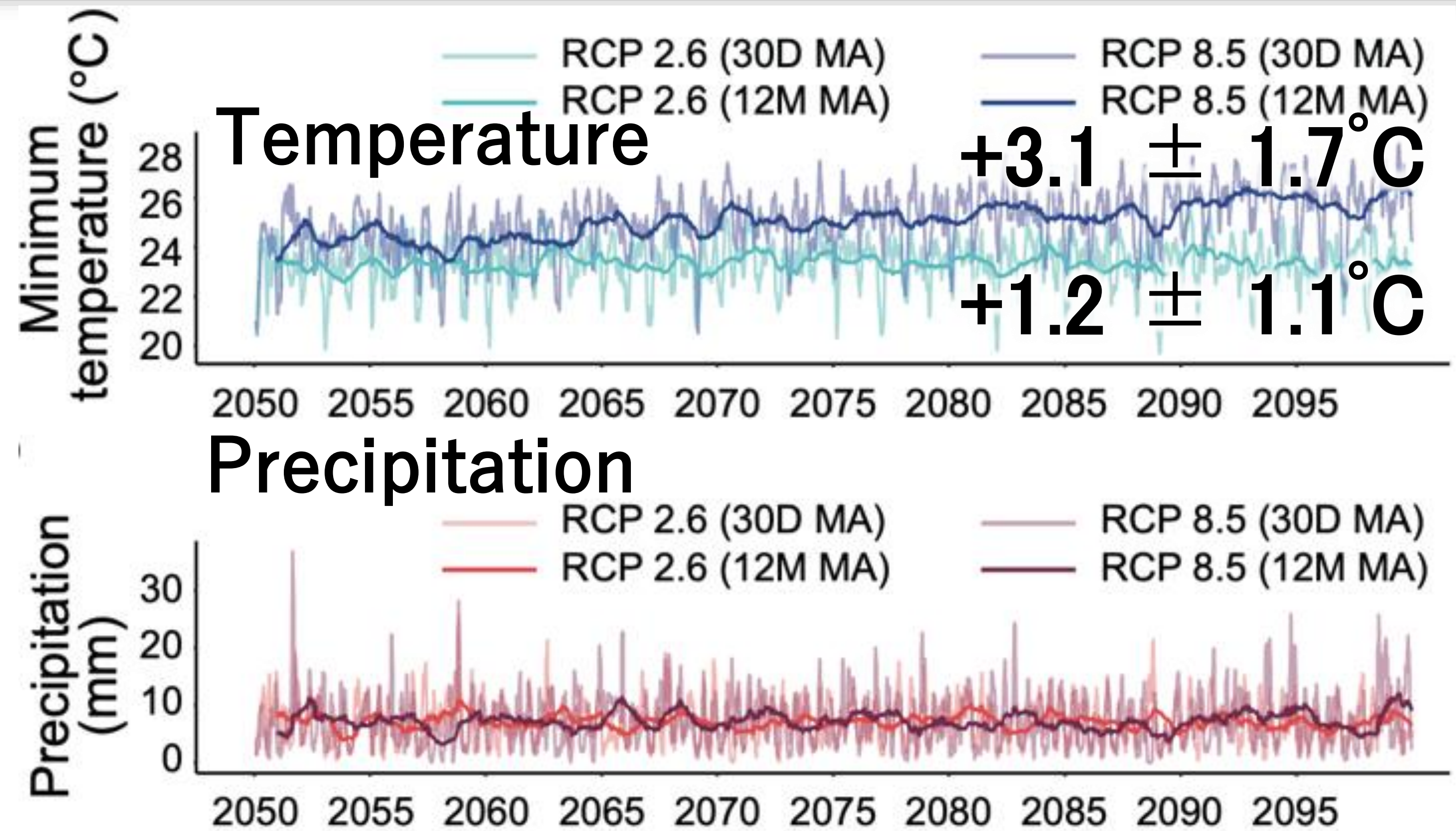


Impacts of climate change on tropical phenology

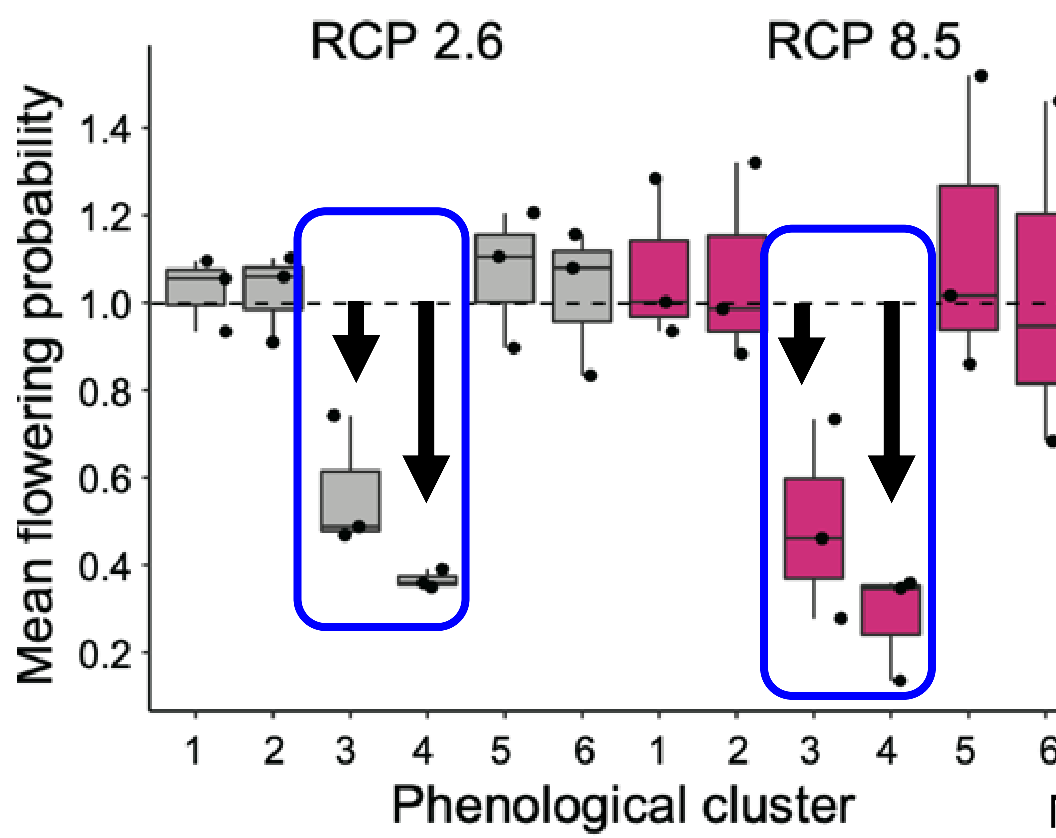
High CO_2 emission scenario (RCP8.5)

GCM Prediction

Low CO_2 emission scenario (RCP2.6)



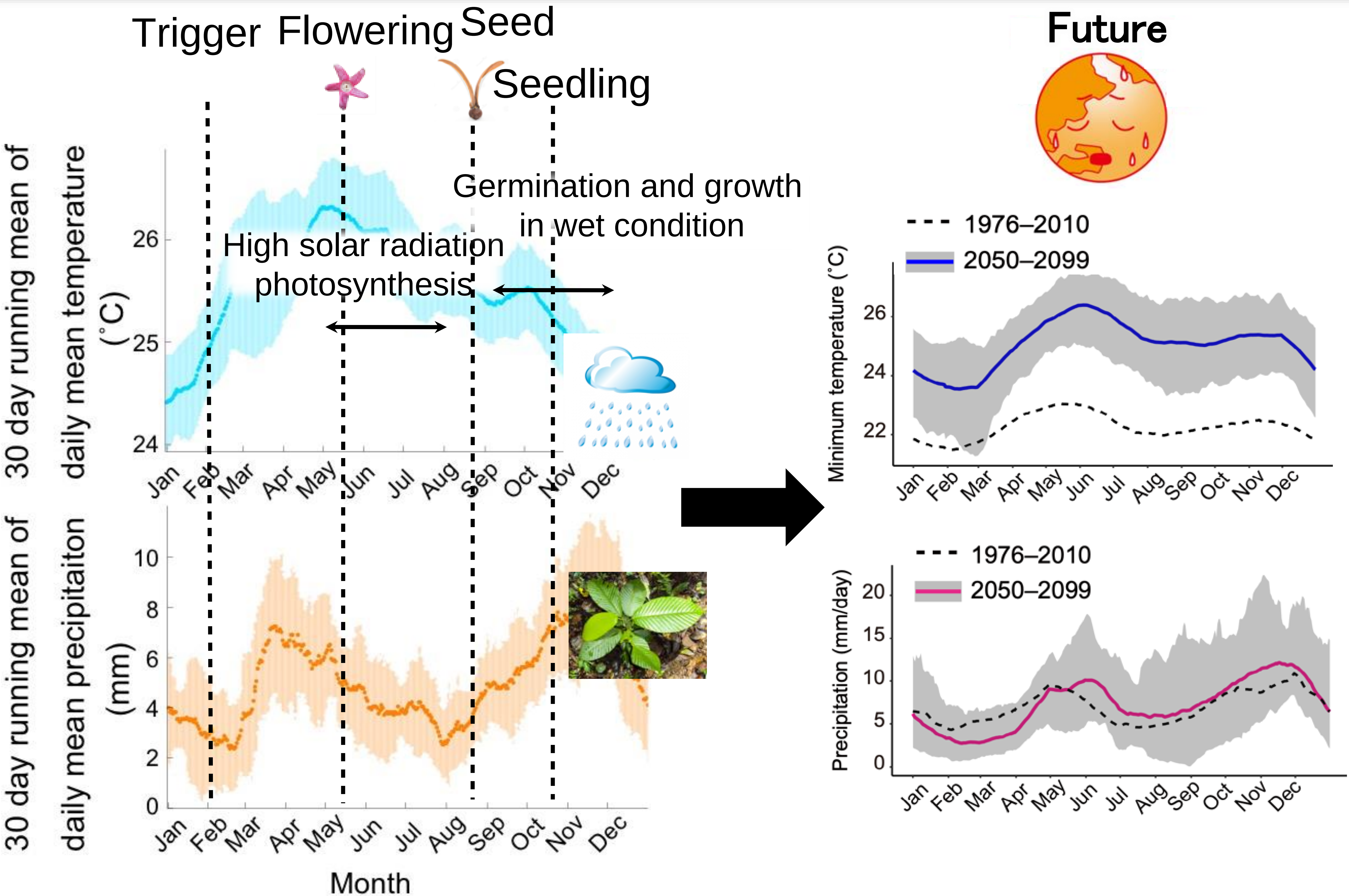
Prediction



1.2˚C increase of temperature results in the loss of flowering in 57% of Dipterocarp species.

Numata et al. 2022. Commun Biol

Cool temperature response may be adaptive to the past but not to the future climate



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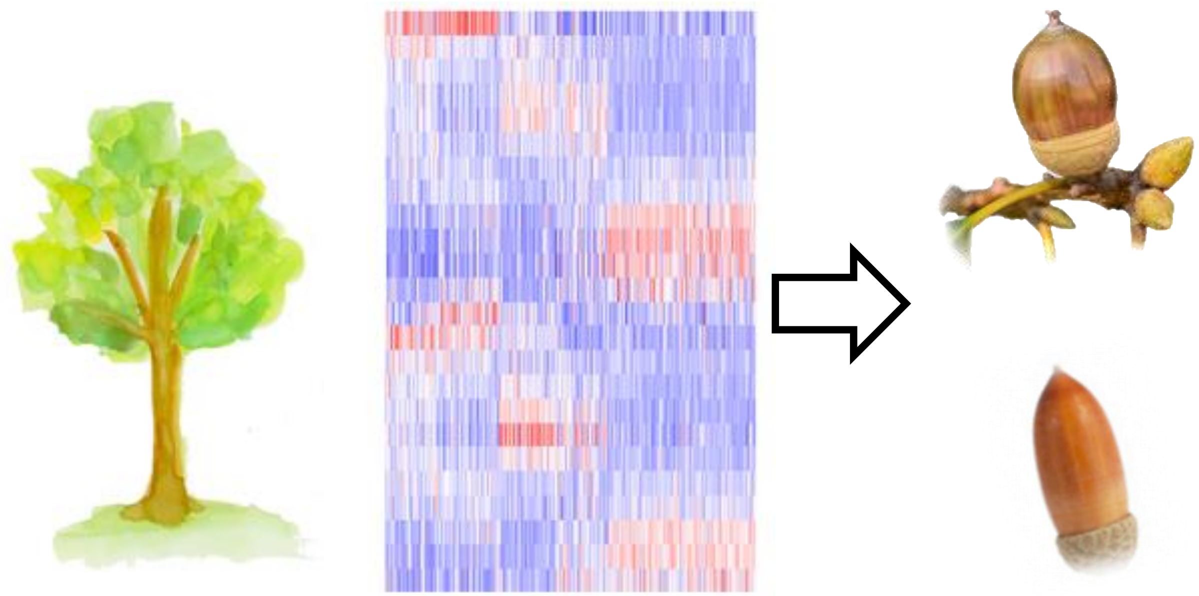
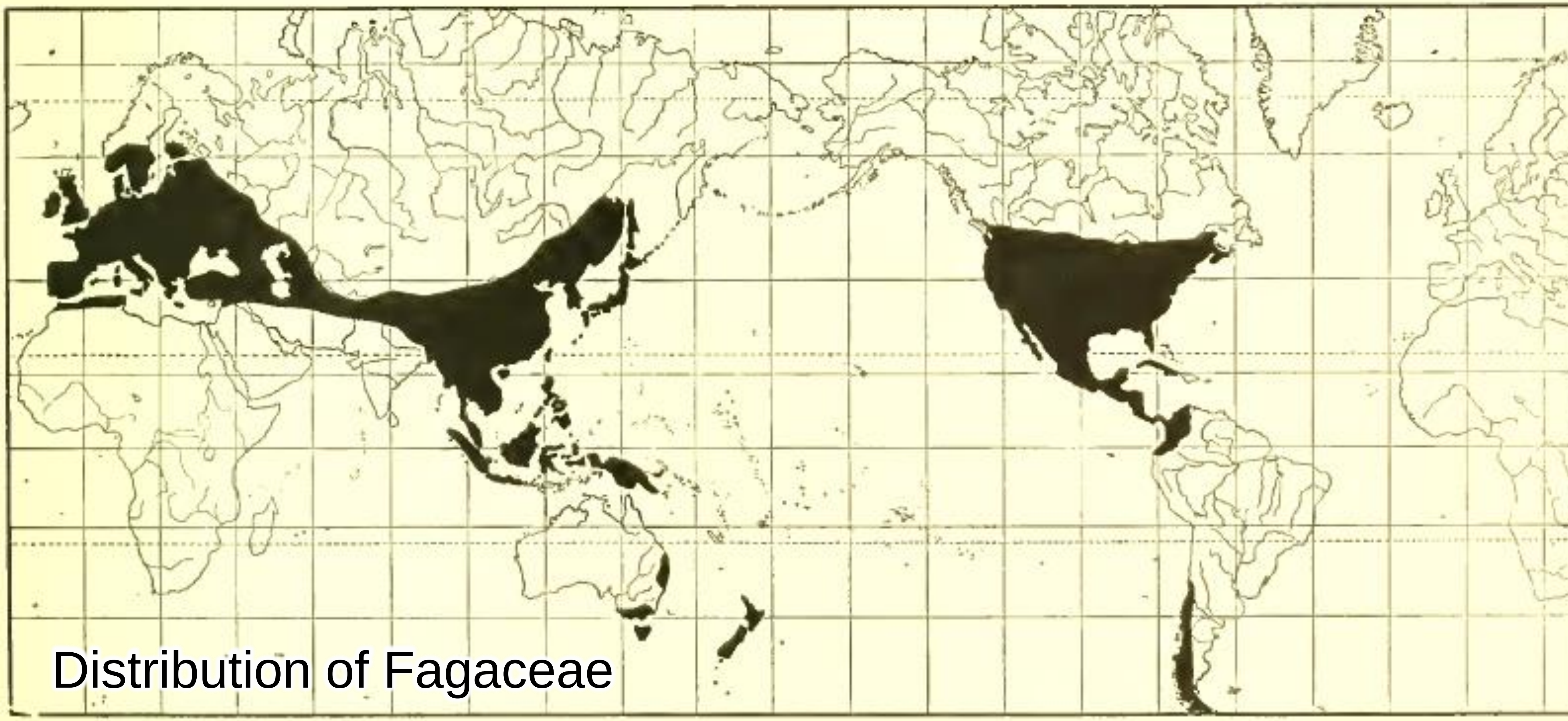
2. Comparative molecular phenology in Fagaceae





Soepadmo 1972

Fig. 1. Present distribution of *Fagaceae*. Add: New Caledonia.

Genus *Lithocarpus*

Rich species diversity in Southeast Asia

Quercus glauca

Lithocarpus edulis

From target gene analysis to genome-wide transcriptomics

RNA seq
DNA microarray
for two years

Qg
21,168
Independent genes

Quercus glauca
Evergreen
Wind pollinated

Le
21,336
Independent genes

Lithocurpus edulis
Evergreen
Animal pollinated

- Transdecoder
- Blast
- Pfalm database

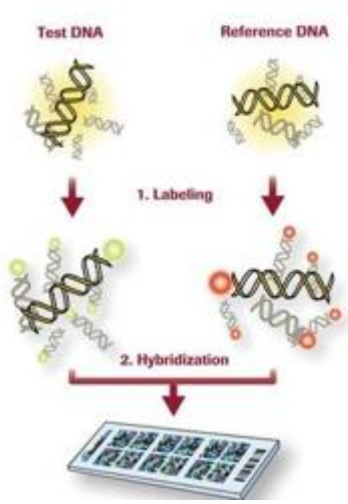
ORF estimate

- Ortholog finder

Orthologous gene

7,707 orthologous genes

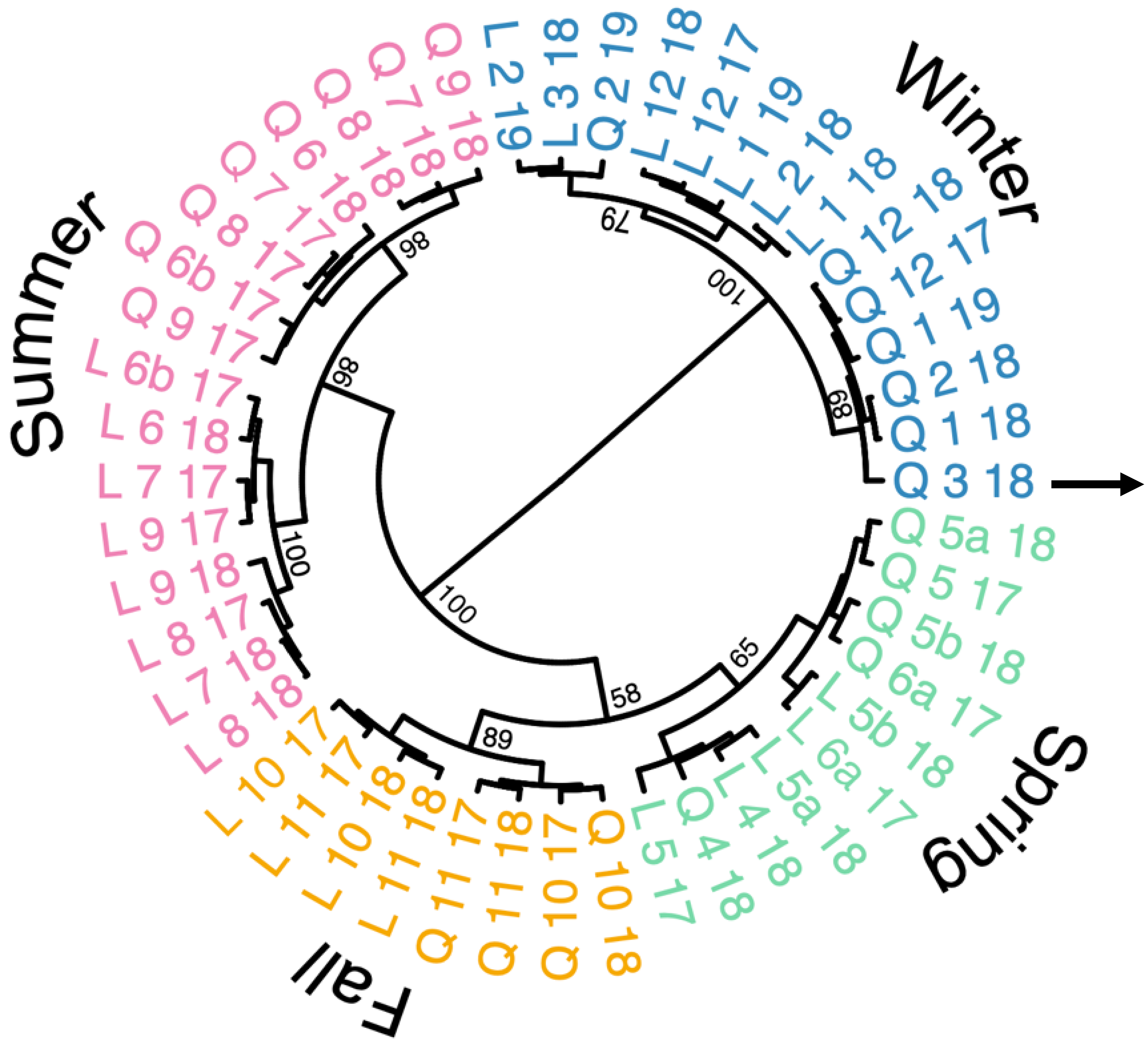
Duplicated OG groups were removed.
High variation genes across individuals were removed



Leaf and bud tissues
n = 3 per species

We identified four seasons in genome-wide expression profiles

Hierarchical clustering of monthly expression profiles



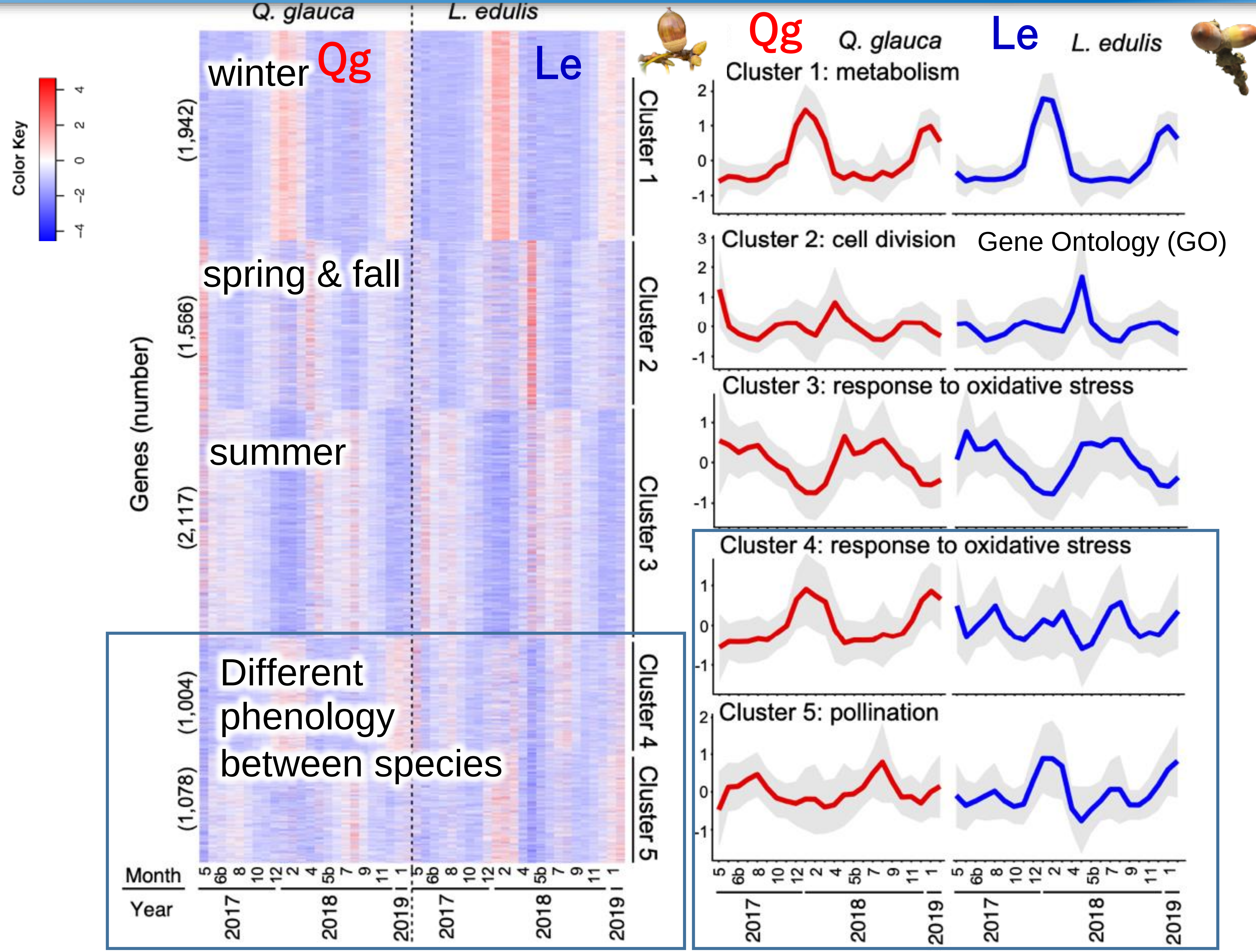
Q

Q. glauca

L

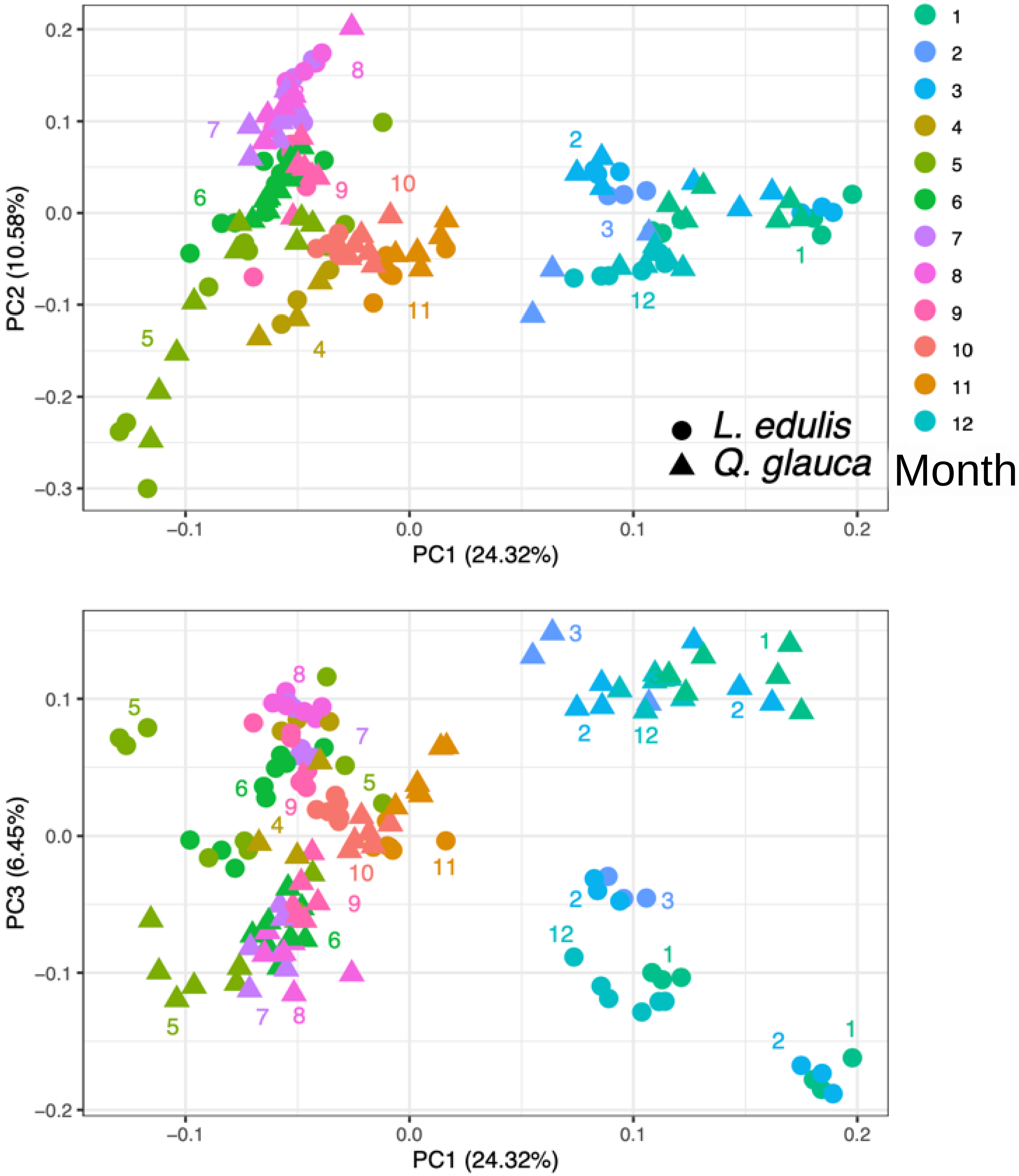
L. edulis

27 % of genes revealed different phenology

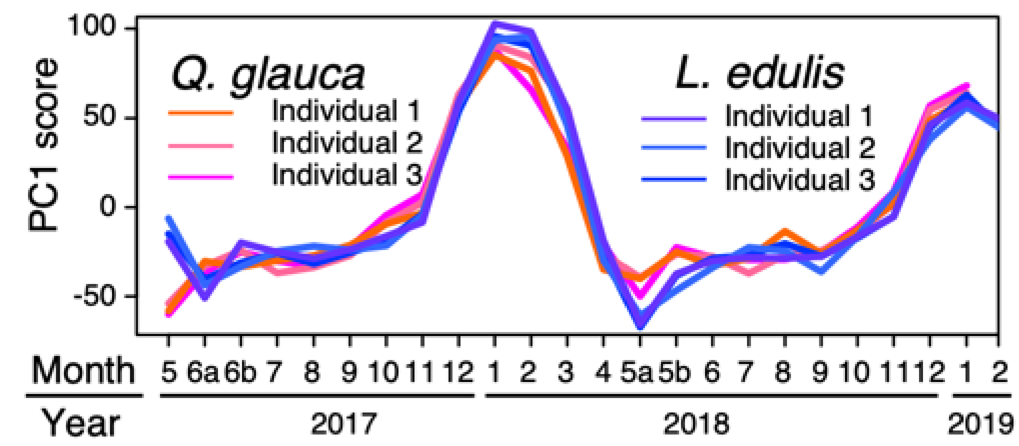


PC3 characterizes phenological difference between species

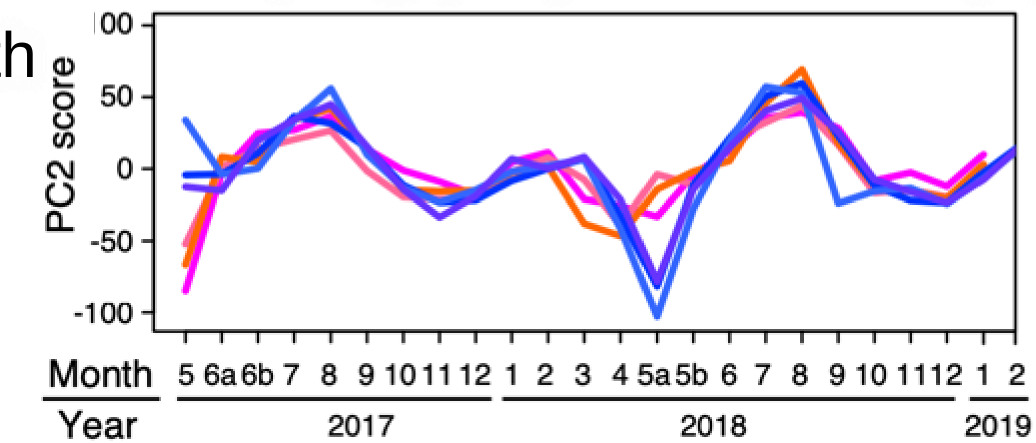
Results of Principal Component Analysis (PCA)



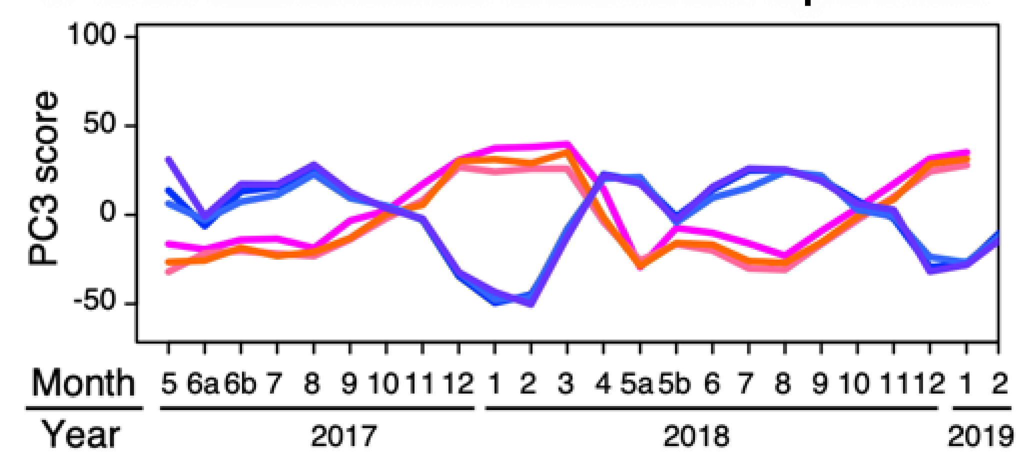
PC1: winter



PC2: spring (low) and summer (high)



PC3: difference between species



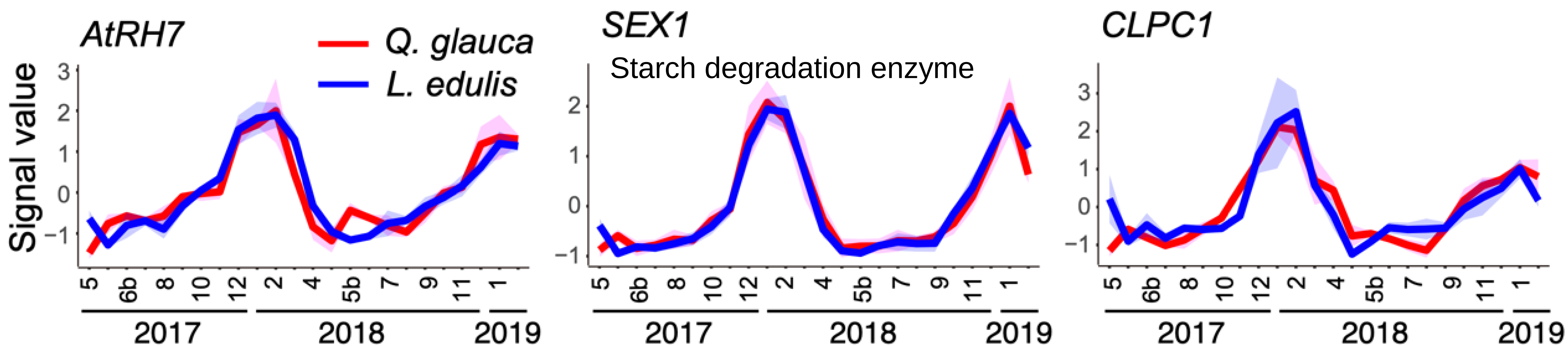
PC1 is characterized by genes associated with stress response

Top 2.5% genes (*n*= 175) with high loading values for PC1

Acclimation to low temperature

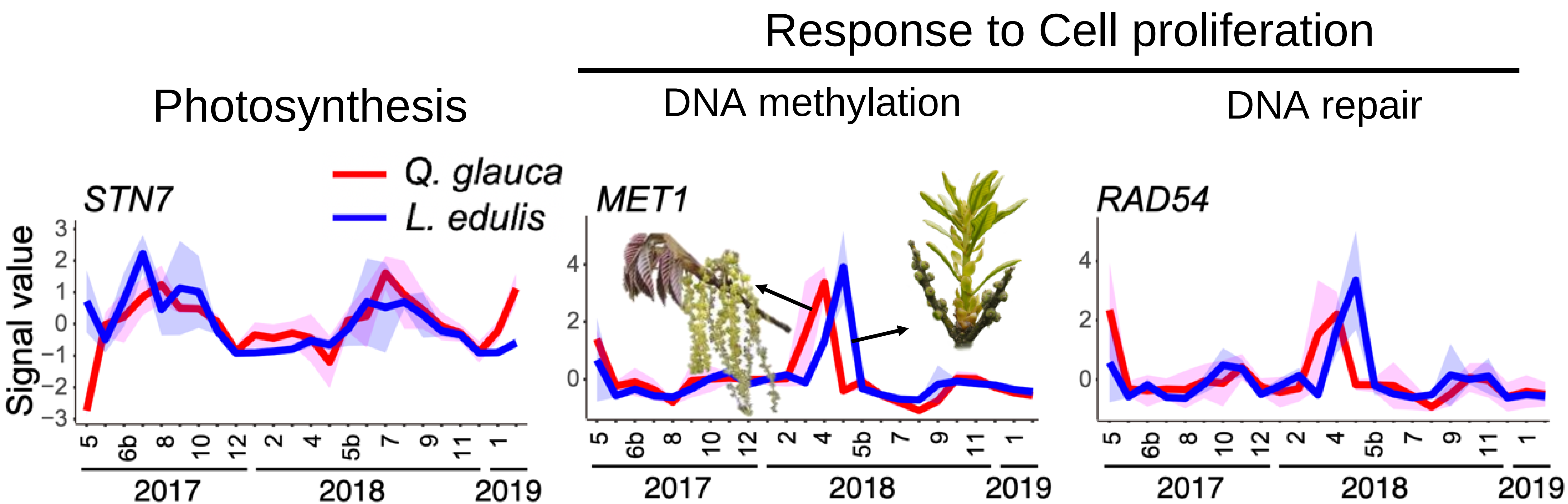
Tolerance to freezing

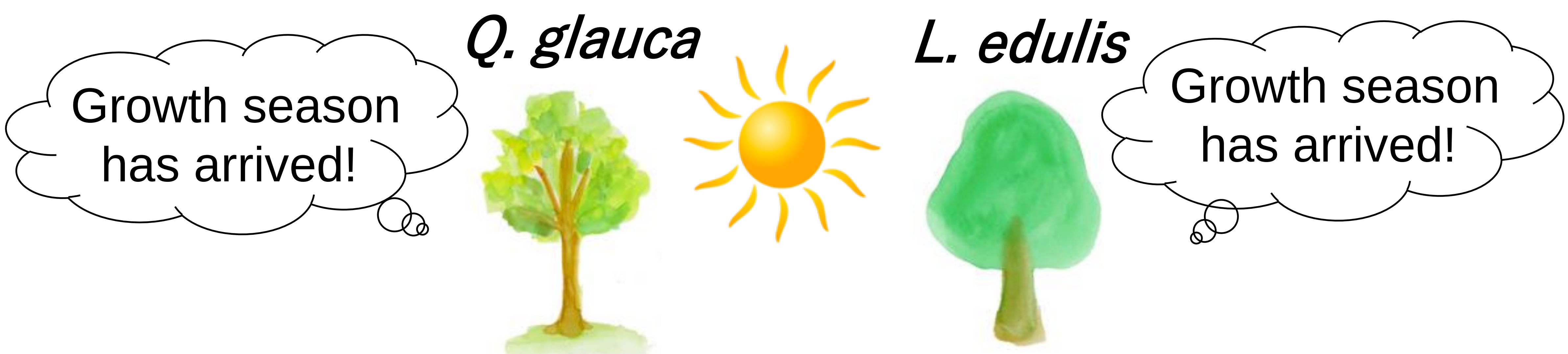
Proteome and RNA homeostasis



PC2 is characterized by genes associated with energy acquisition and growth

Top 2.5% genes (*n*= 175) with high loading values for PC2



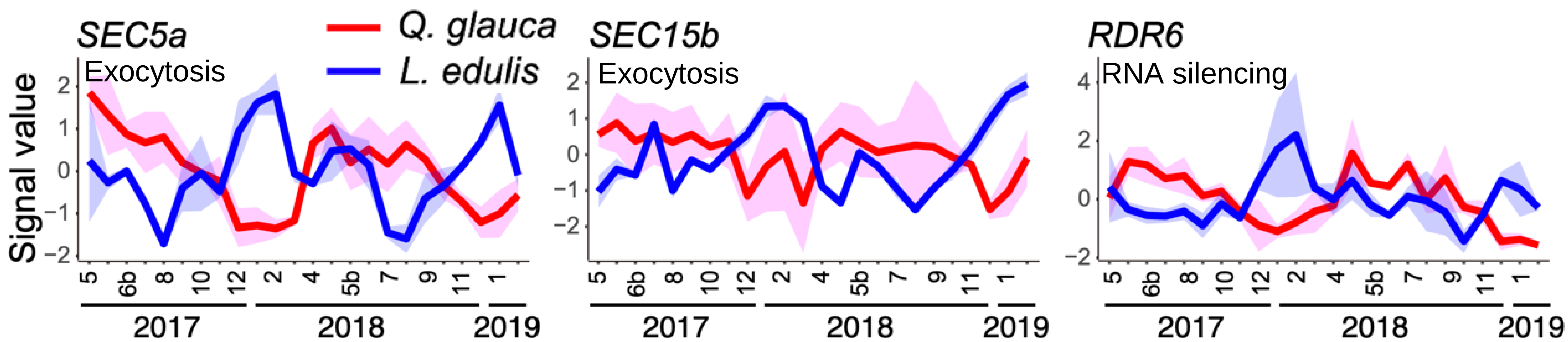


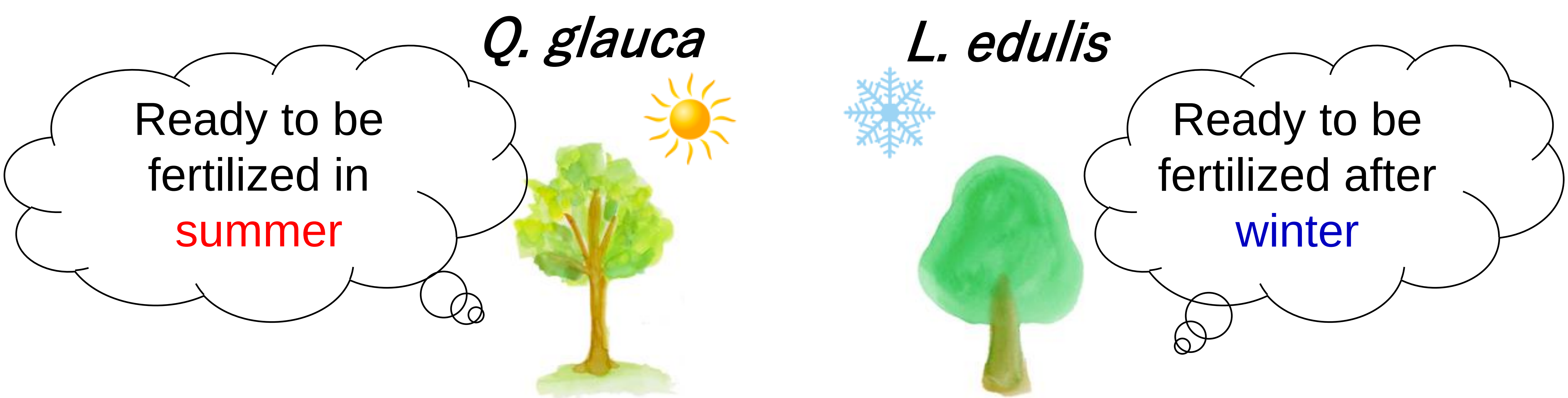
PC3 is characterized by genes associated with pollination

Top 2.5% genes (*n*= 175) with high loading values for PC3

Pollen acceptance

Acquisition of female gametophyte identity

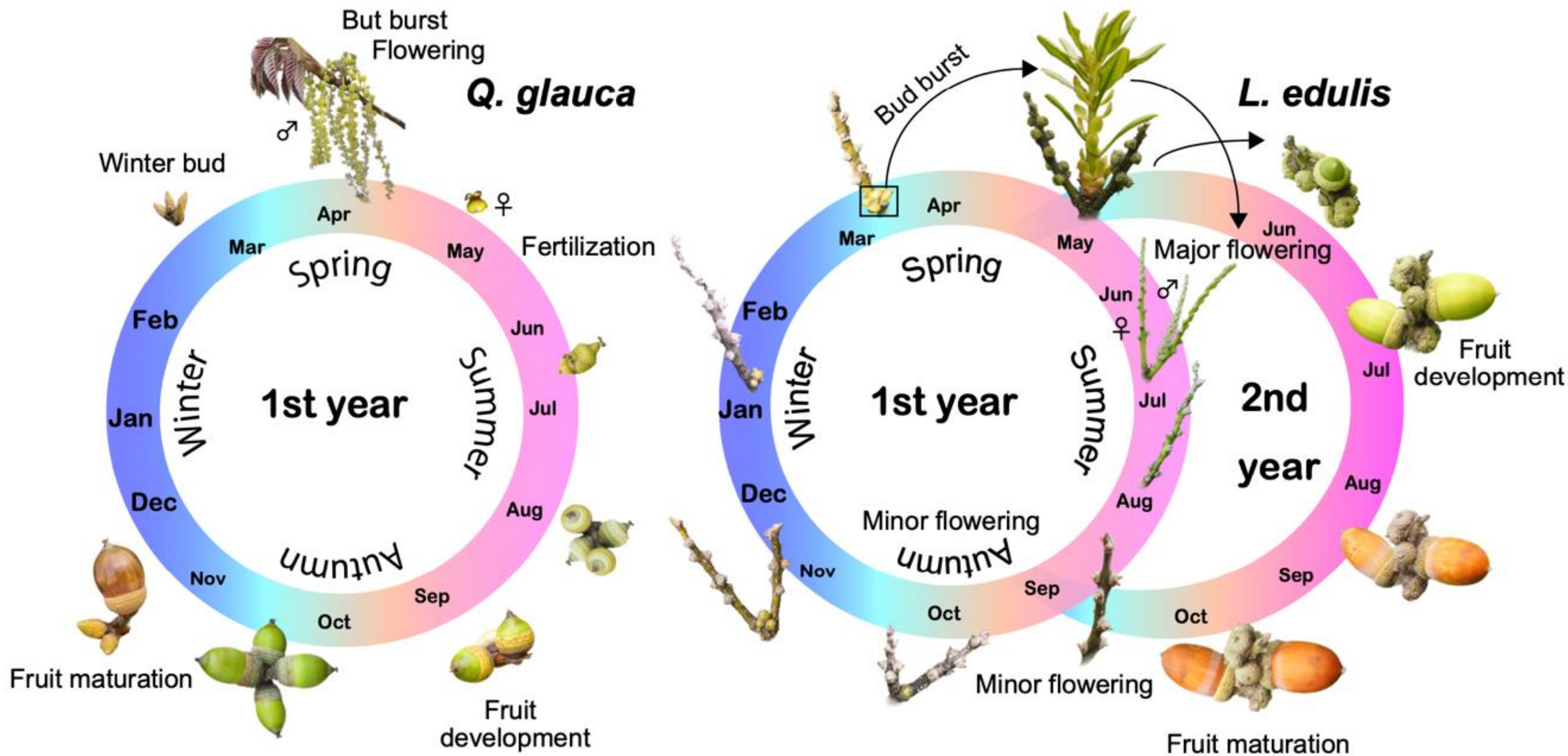




Different molecular phenology in PC3 genes would be the basis for different fruiting habits

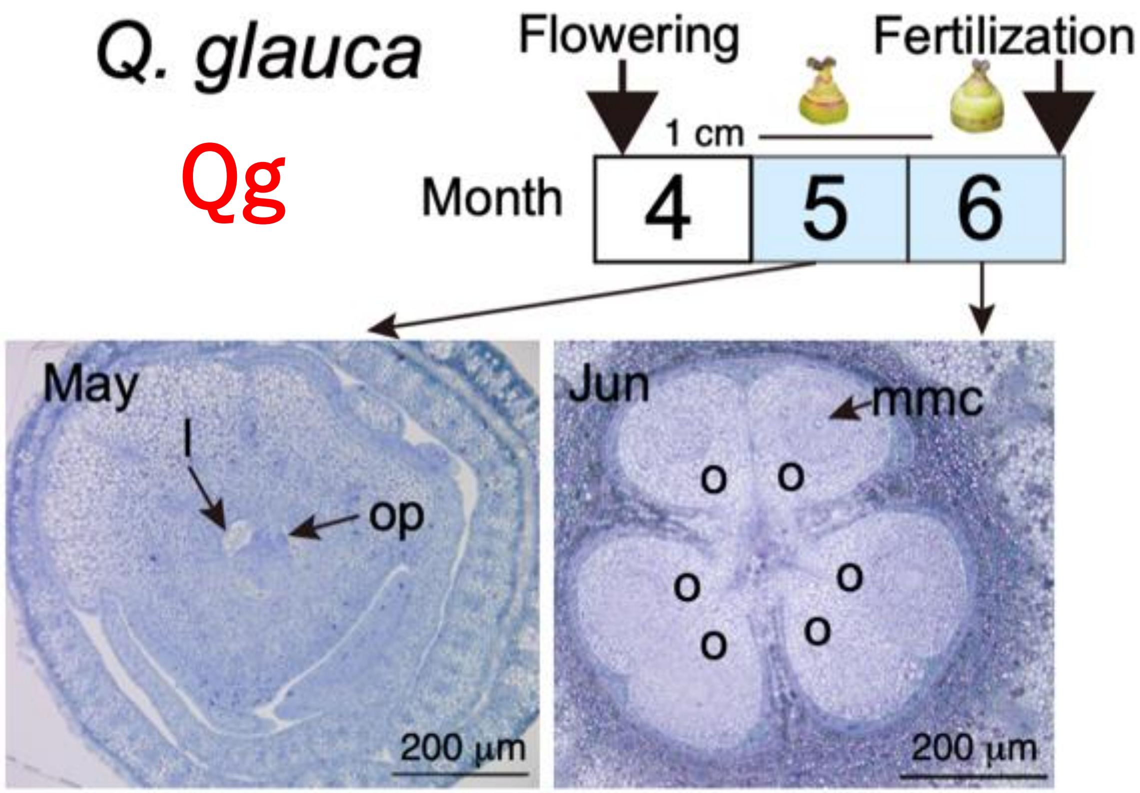
Qg
1-year fruiting species

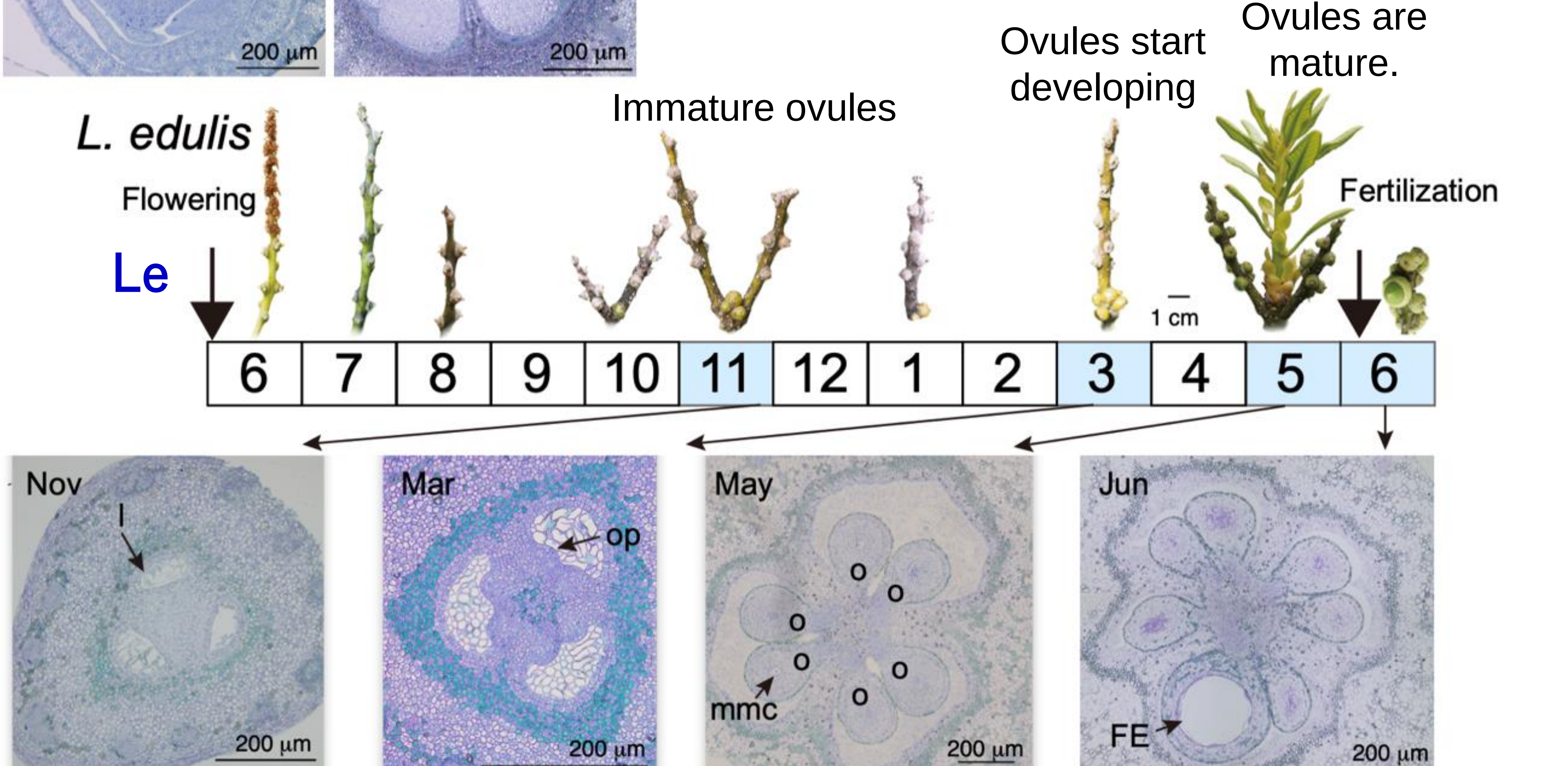
Le
2-year fruiting species



Delayed fertilization

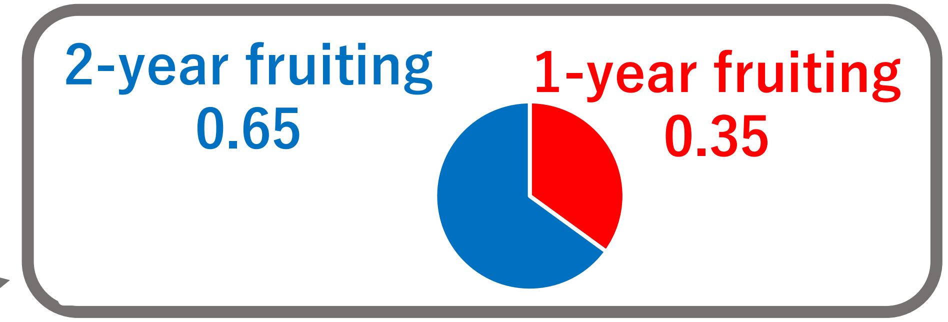
Ovules develop quickly in Qg.
Ovules are immature until next spring in Le.
Delayed activation of PC3 genes may regulate delayed fertilization.

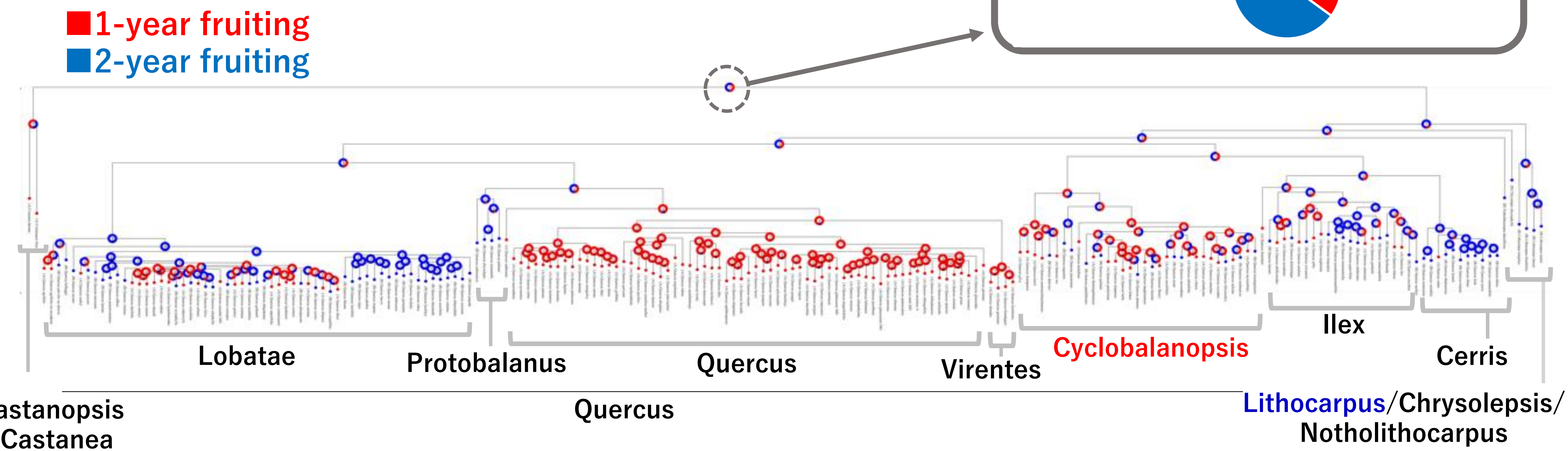




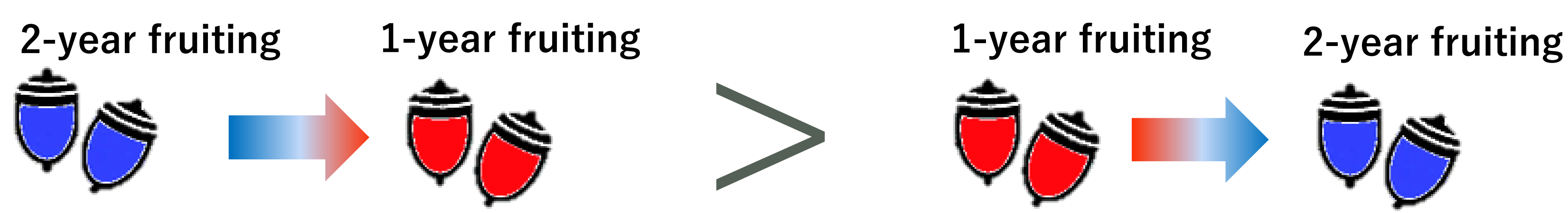
Ancestral trait estimation suggests firm genetic basis for delayed fertilization

The ancestral trait is
more likely to be 2-year fruiting





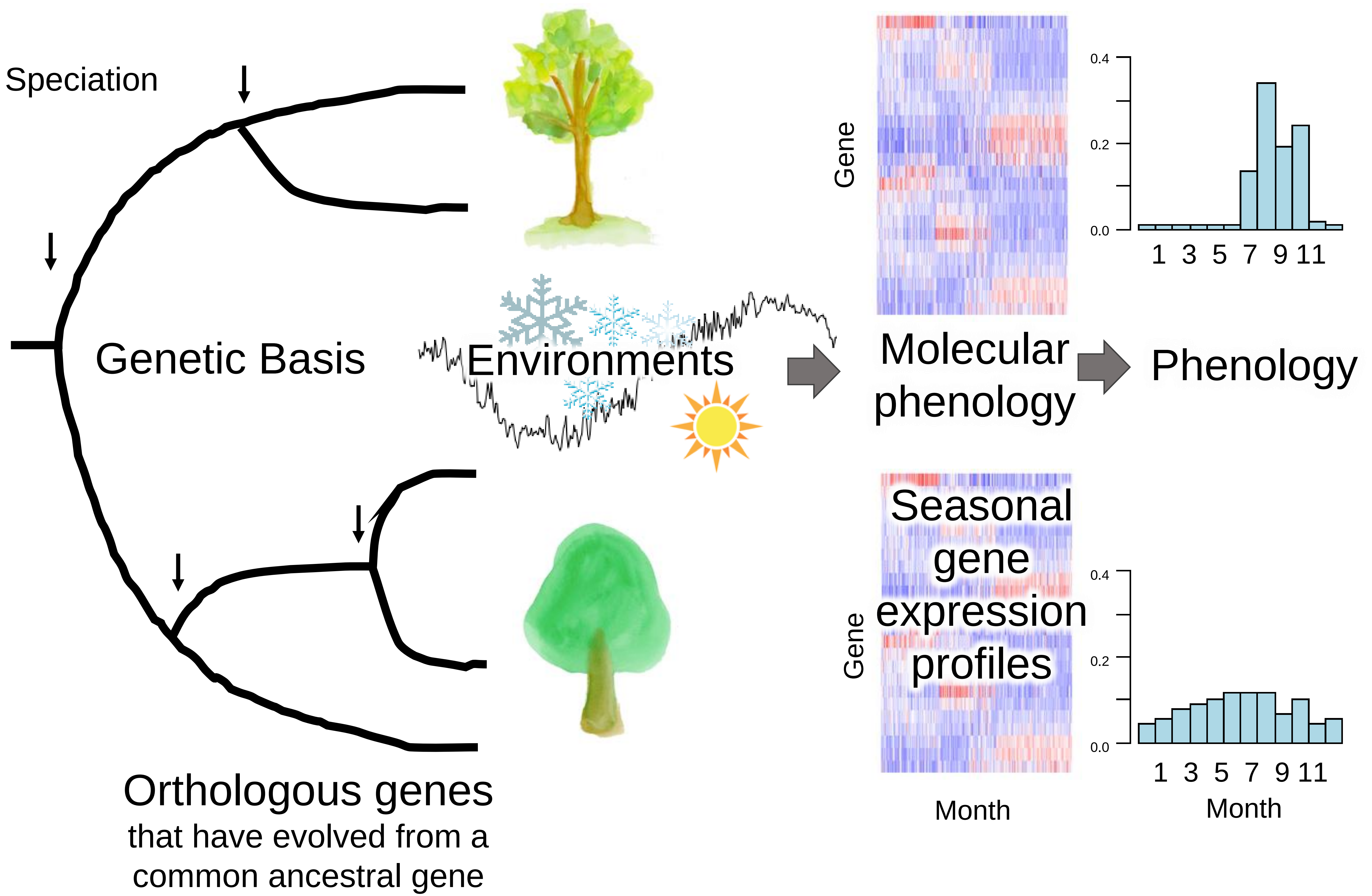
q_{11}	q_{12}
30.7	69.3
q_{22}	q_{21}
7.2	92.8



Evolutionary transition rate from 2-year to 1-year fruiting type is higher than the opposite.

PC3 genes can be the candidates for the genetic basis of delayed fertilization.

Conclusion



Speciation
Genetic Basis
Environments
Molecular phenology
Phenology
Seasonal gene expression profiles
Gene
Gene
0.4
0.2
0.0
1 3 5 7 9 11
0.4
0.2
0.0
1 3 5 7 9 11
Month
Month
Orthologous genes
that have evolved from a common ancestral gene

Future perspectives

Comparative molecular phenology
+
Genome resources
+
Development of predictive models

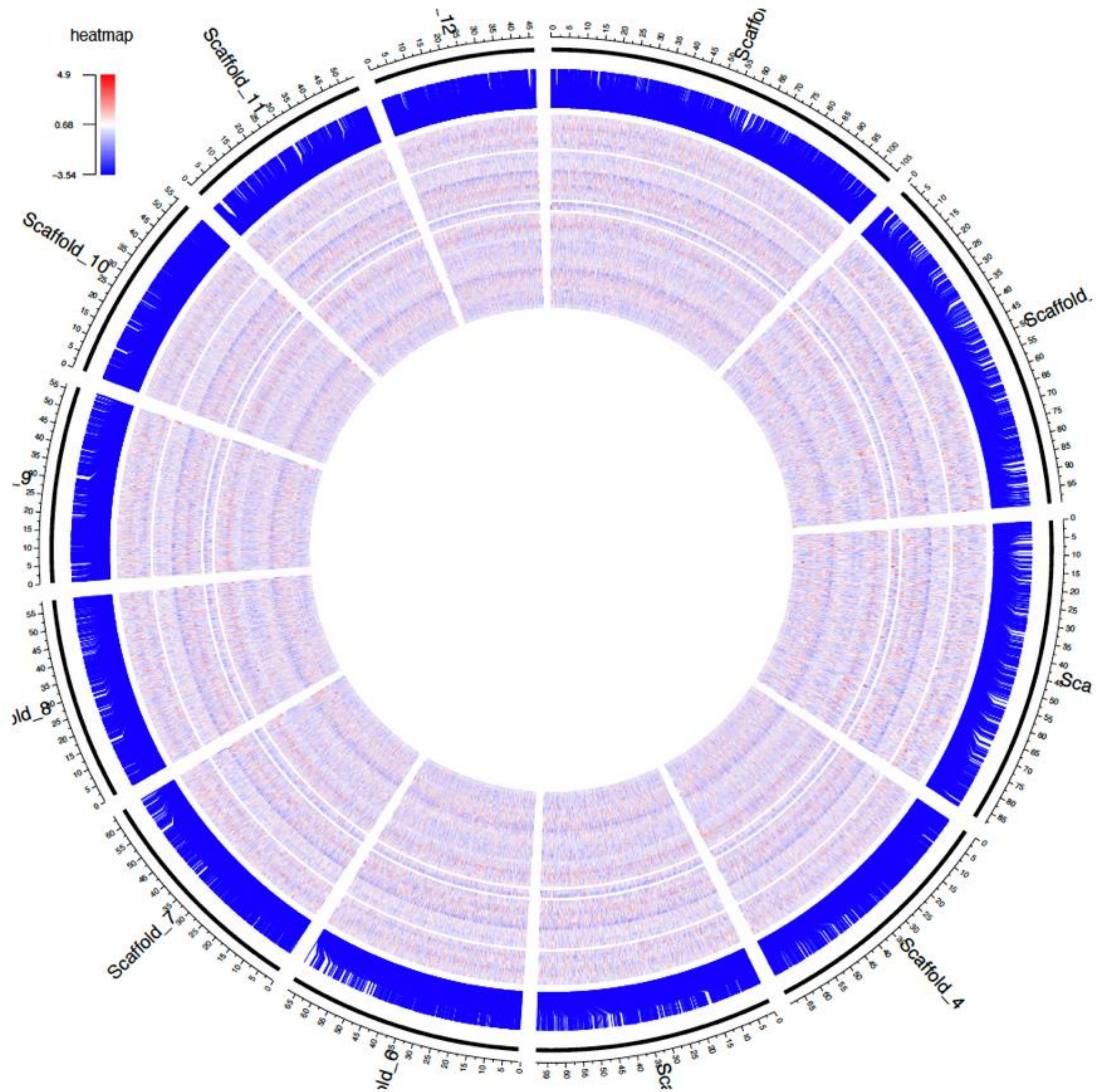
- **Evolution of phenotypic diversity**
- **Forecasting future flowering phenology under changing environments**

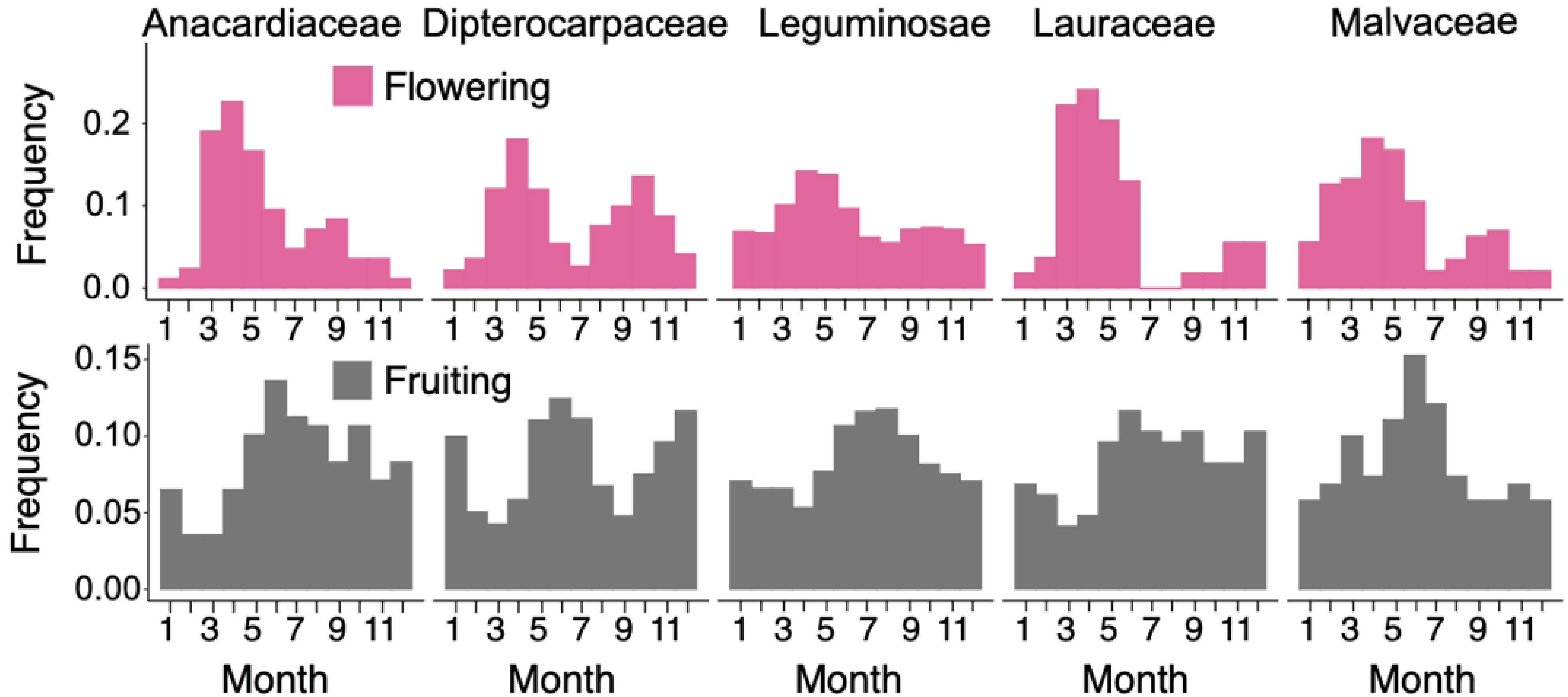
Acknowledgements

Shinya Numata (Tokyo Metropolitan University)
Koharu Yamaguchi (Kyushu University)
Masaaki Shimizu (Kyushu University)
Gen Sakurai (NARO)
Ayaka Morimoto (Tokyo Metropolitan University)
Noraliza Alias (FRIM)
Nashatul Zaimah Noor Azman (FRIM)
Tetsuro Hosaka (Hiroshima University)
Ai Nagahama (Kyushu University)
Tetsukazu Yahara (Kyushu University)
Myotoishi Chihiro (Kyushu University)
Junko Kusumi (Kyushu University)
Kayoko Ohta (Kyushu University)
Kiminori Toyooka (RIKEN)
Noriko Takeda (RIKEN)

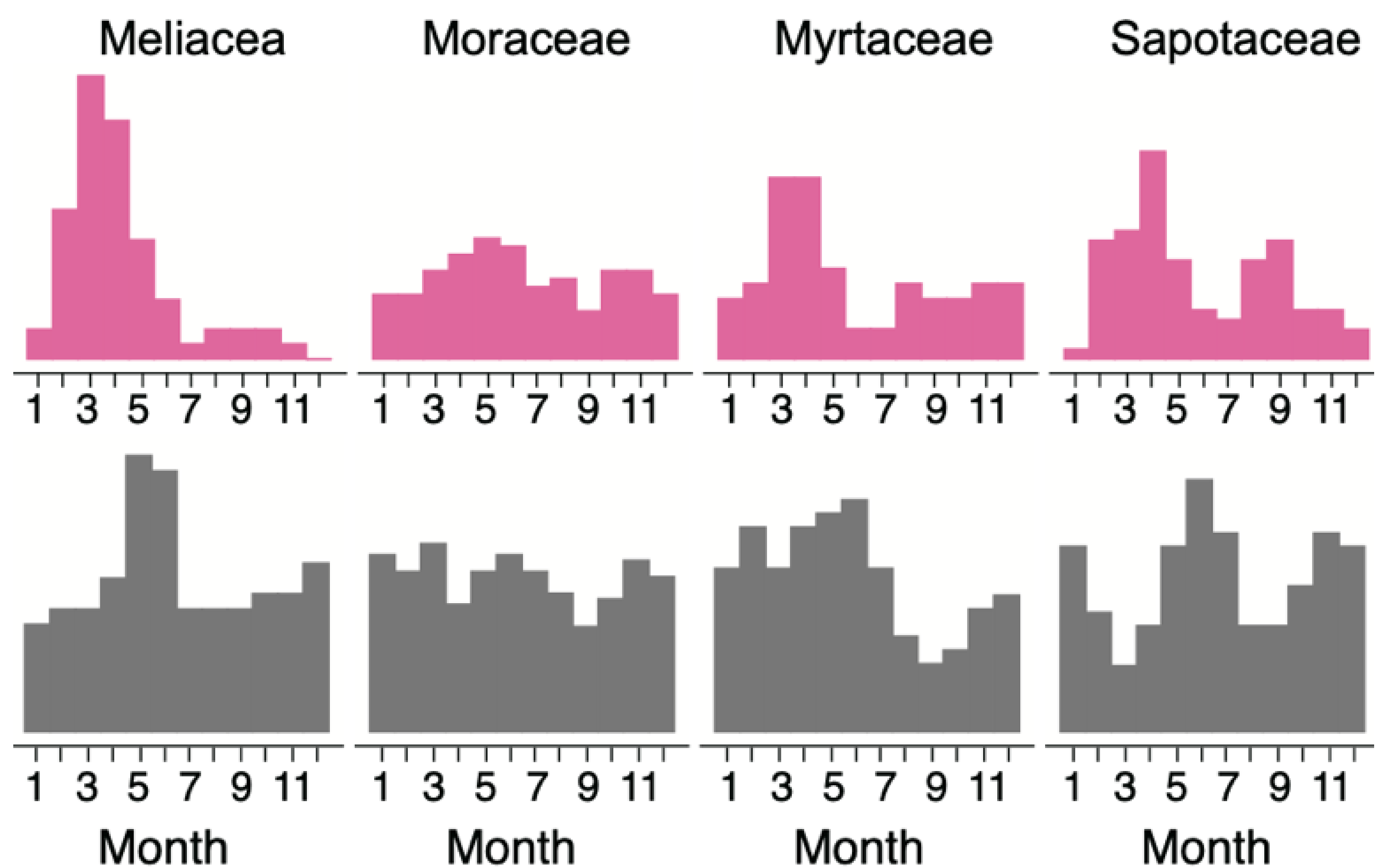
科研費 KAKENHI

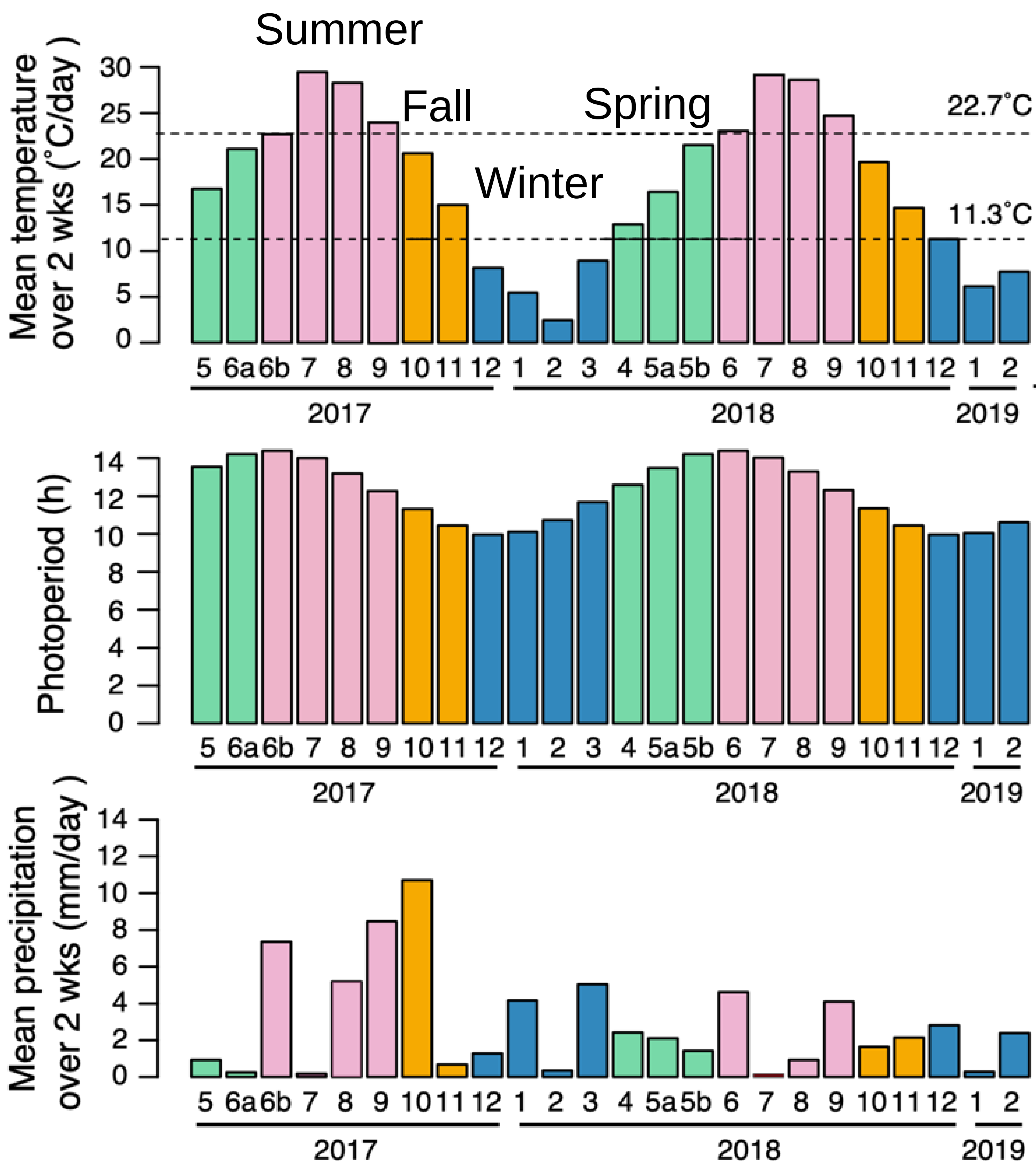
Bud Z-score


heatmap
4.9
0.68
-3.54
Scaffold_11
Scaffold_10
Scaffold_7
Scaffold_4
Scaffold



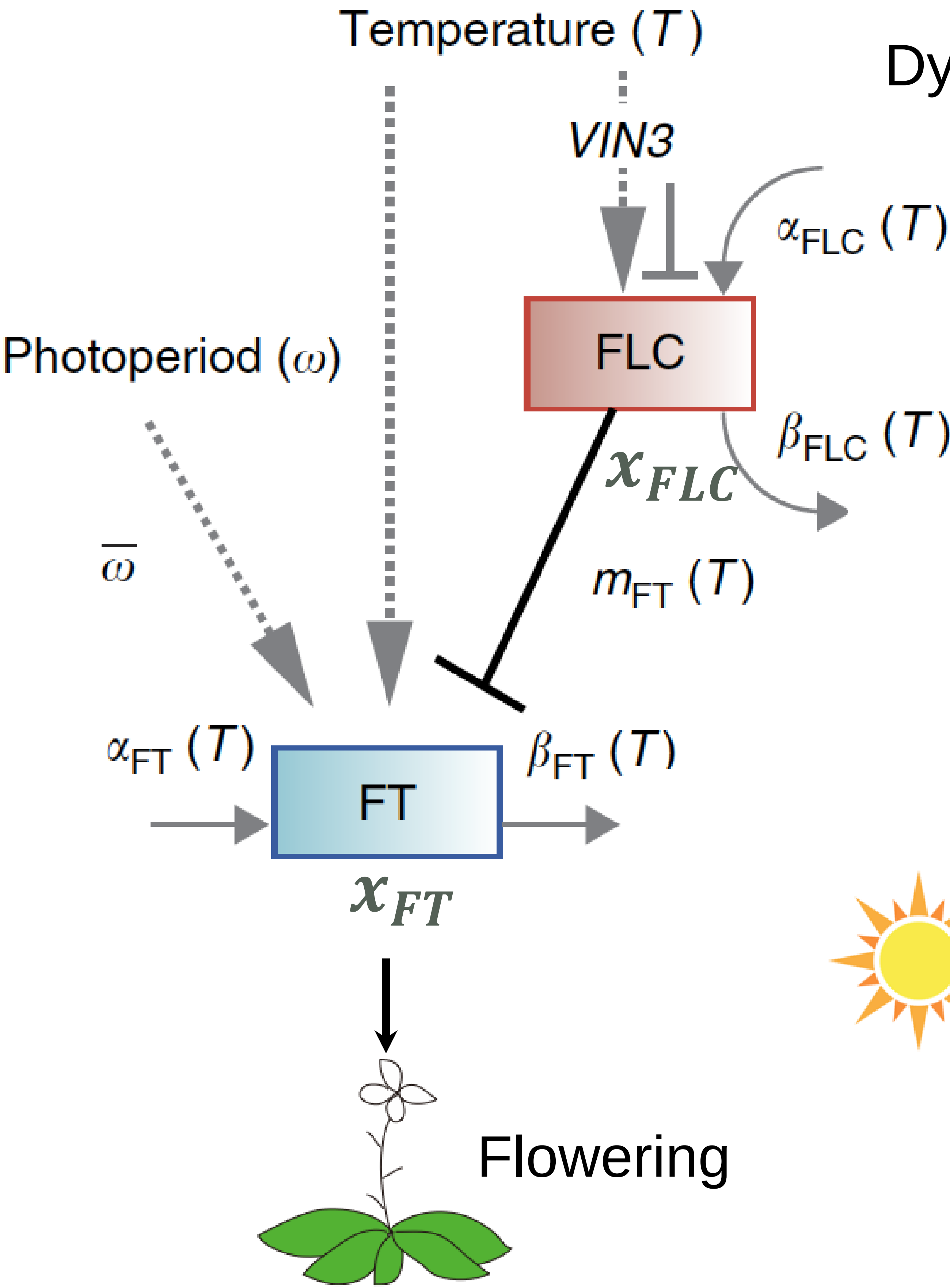
There are two flowering peaks: April and October.





Summer
Fall
Winter
Spring
22.7˚C
11.3˚C
Mean temperature over 2 wks (˚C/day)
30
25
20
15
10
5
0
5 6a 6b 7 8 9 10 11 12 1 2 3 4 5a 5b 6 7 8 9 10 11 12 1 2
2017
2018
2019
Photoperiod (h)
14
12
10
8
6
4
2
0
5 6a 6b 7 8 9 10 11 12 1 2 3 4 5a 5b 6 7 8 9 10 11 12 1 2
2017
2018
2019
Mean precipitation over 2 wks (mm/day)
14
12
10
8
6
4
2
0
5 6a 6b 7 8 9 10 11 12 1 2 3 4 5a 5b 6 7 8 9 10 11 12 1 2
2017
2018
2019

Modelling gene regulatory dynamics



Dynamics for *FLC* and *FT* transcript levels

$$\frac{dx_{FLC}}{dt} = \alpha_{FLC}(T) f(x_{FLC}) - \beta_{FLC}(T) x_{FLC}$$

$$\frac{dx_{FT}}{dt} = \alpha_{FT}(T) g(x_{FLC}) h(\omega) - \beta_{FT}(T) x_{FT}$$

temperature (T) dependent

Vernalization pathway

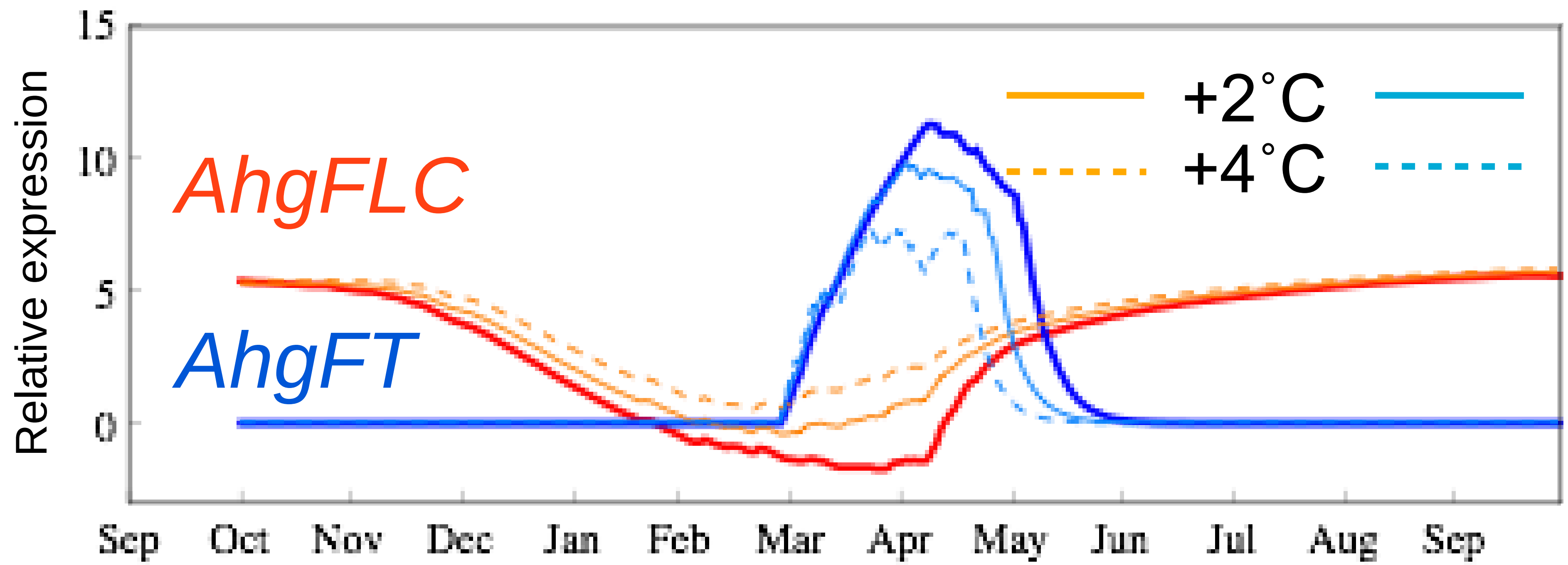
$$g(x_{FLC}) = \frac{1}{1 + \frac{x_{FLC}}{m_{FT}(T)}^{H}}$$

Photoperiod pathway

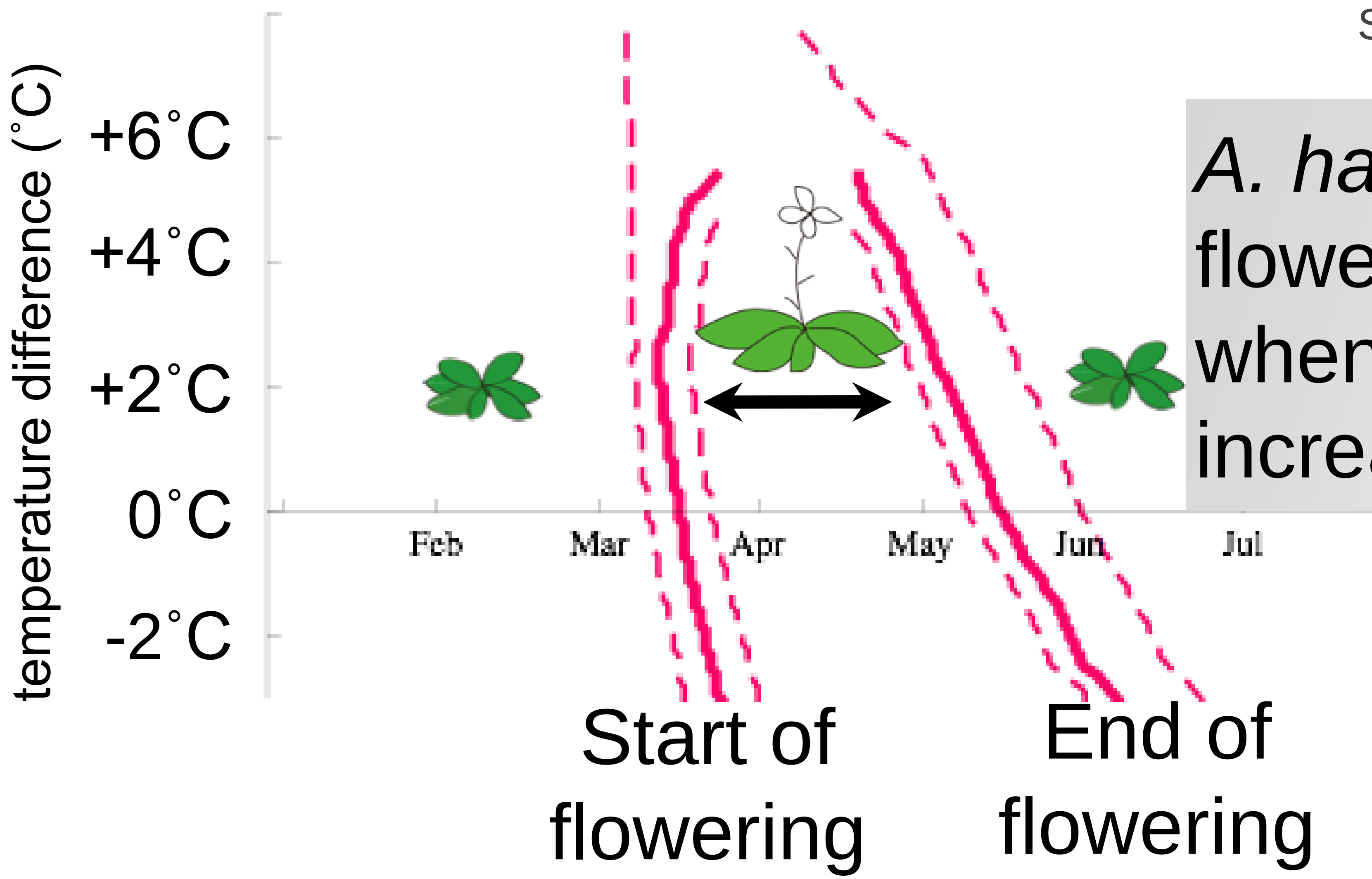
$$h(\omega) = \begin{cases} 1 \text{ if } \omega \geq \overline{\omega} \\ 0 \text{ if } \omega < \overline{\omega} \end{cases}$$

$\overline{\omega}$:critical photoperiod

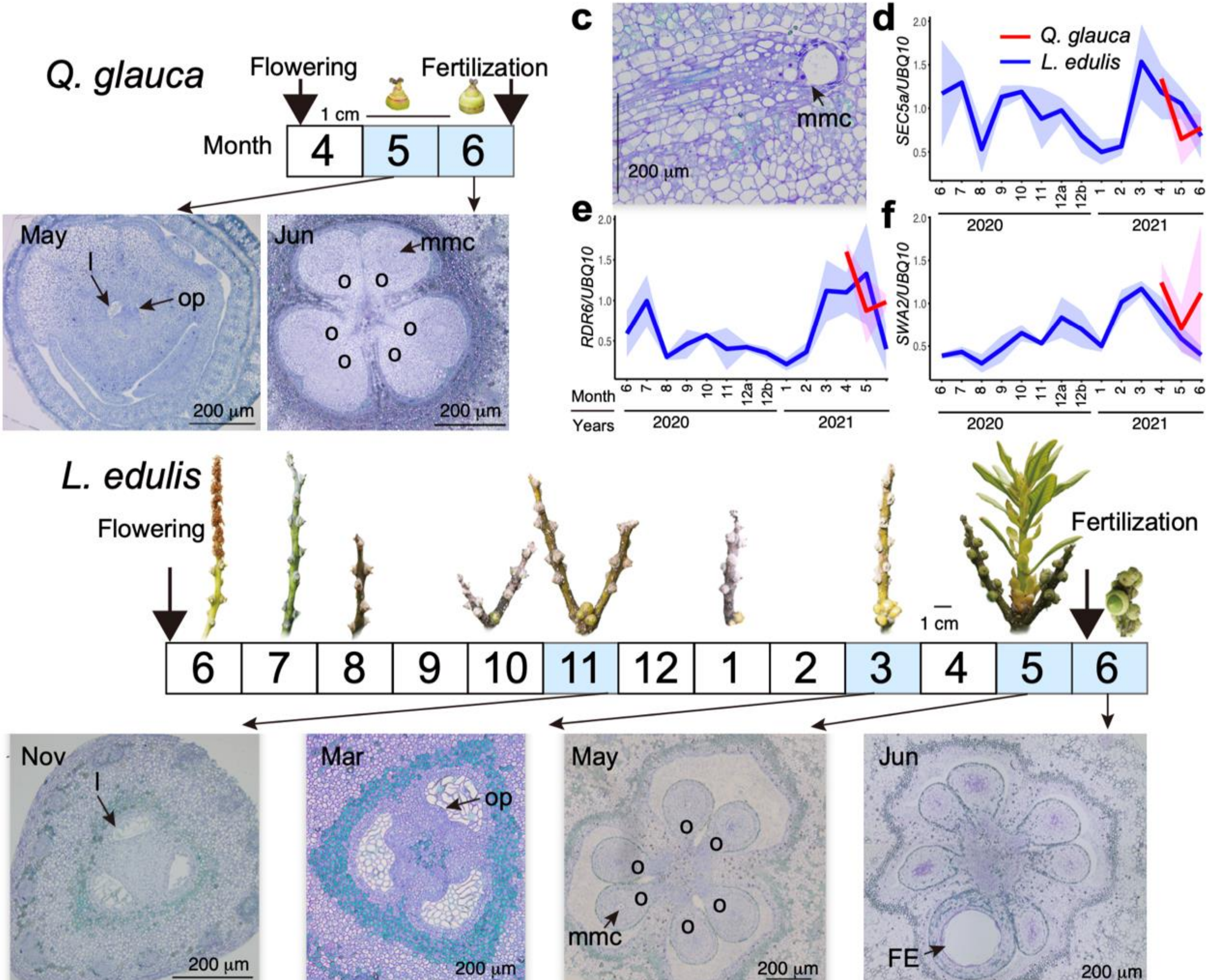
What happens in a warmer world?



Satake et al. 2013



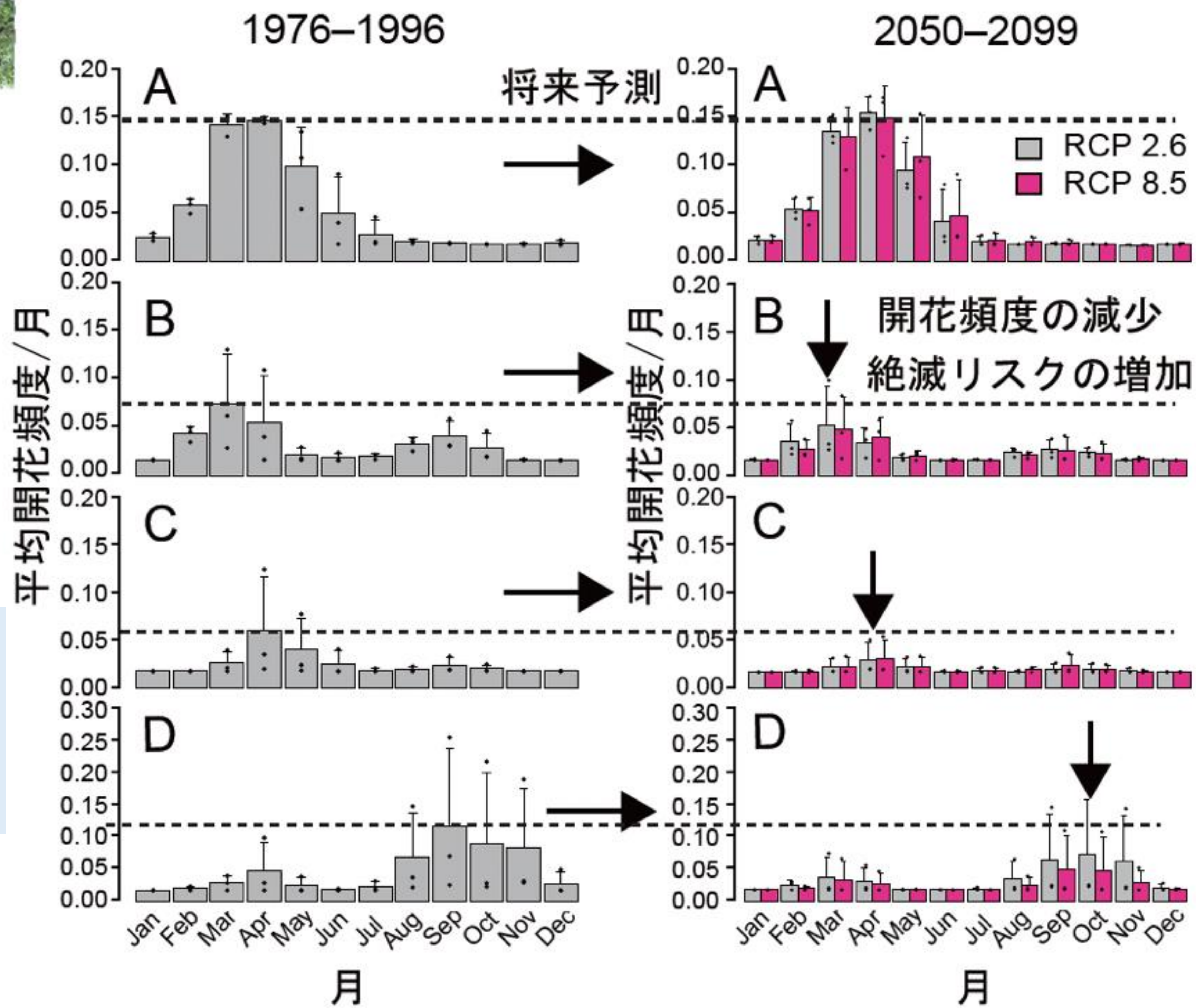
A. halleri may lose flowering opportunity when temperature increases by 5˚C.



Q. glauca
Flowering
Fertilization
1 cm
Month
4
5
6
May
l
op
Jun
mmc
o
200 μm
c
mmc
200 μm
d
Q. glauca
L. edulis
SEC5a/UBQ10
e
RDR6/UBQ10
f
SWA2/UBQ10
Month
Years
2020
2021
L. edulis
Flowering
Fertilization
1 cm
6
7
8
9
10
11
12
1
2
3
4
5
6
Nov
l
Mar
op
May
o
mmc
Jun
FE
200 μm

広域スケールのフェノロジー予測

Numata et al. 2022 Commun Biol



開花は次世代を残す重要なイベント

開花機会の減少は絶滅リスクの増大にもつながる可能性

- 3 pairs of leaf and bud were sampled every two weeks from 3 individuals.
- RNA sequence (Illumina Hiseq2500) using samples from May to Dec.
- Probe design for DNA microarray.

Quercus glauca
Qg (*n*=3)

Lithocurpus edulis
Le (*n*=3)