Overview of my research works

Lab meeting at Chicago Botanic Garden

July 2016

Alice Ledent, first year of the PHD's degree in Biology
of Organisms and Ecology, University of Liège (ULg)

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   - Introduction and objectives
   - State of arts

1. Brief introduction

Biography

Alice Ledent

- Born in Liège (Belgium) in 1992

- Master degree obtained in June 2015

  - Historical biogeography of European temperate bryophytes flora

  - Supervisors : Alain Vanderpoorten (Ulg) and Jairo Patiño (UNIL)

- First year of the PHD's degree

  - Four years program

  - The epiphytic bryophytes flora of the Amazonian rainforest : habitat fragmentation and climate change

  - Supervisors : Alain Vanderpoorten (ULg) and Patrick Mardulyn (ULB)

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Work environment

- Alain Vanderpoorten
  - F.R.S-FNRS Senior Research Fellow
  - University of Liège (ULg)
  - Bryologist
  - Lab : ecological and historical biogeography of bryophytes

- Patrick Mardulyn
  - F.R.S-FNRS Research Director
  - Free University of Brussels (ULB)
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2. Master thesis

Master thesis title

Historical biogeography analysis of the last glacial maximum on European temperate bryophytes flora through Approximate Bayesian statistics

Master thesis title

Historical biogeography analysis of the last glacial maximum on European temperate bryophytes flora through Approximate Bayesian statistics

Introduction

- Impact of global warming on species distribution ?
  → Study of past climate changes

➡ Impact of global warming on species distribution ?

→ Study of past climate changes

➡ What explains the actual species distribution ?

→ Quaternary glacial periods

➡ Last Glacial Maximum (LGM : 26 000 - 19 000 years) = the most virulent

➡ Europe : stronger consequences than in North America or in the Southern hemisphere

➡ Why ? Harder climatic conditions and dispersal barriers W-E

➡ 2 biogeographical hypotheses

➥ Impact of global warming on species distribution ?

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➥ Why ? Harder climatic conditions and dispersal barriers W-E

➥ 2 biogeographical hypotheses

➡ Animals : South Refugia Hypothesis (SRH)

➡ Survival possible in 52 Mediterranean refugia identified through genetic diversity
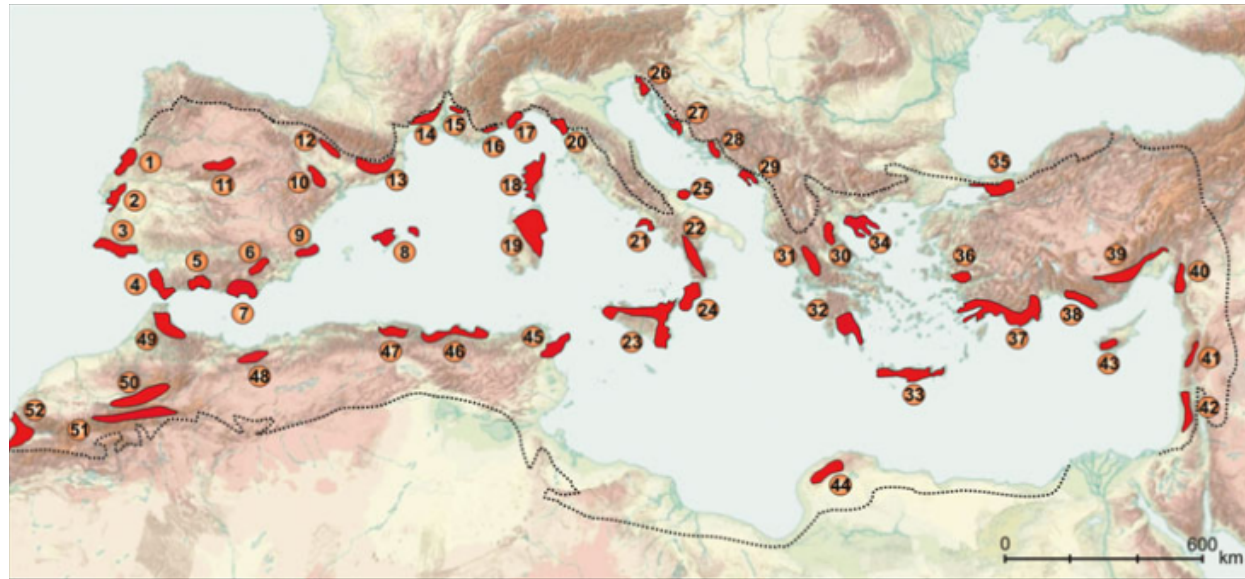
Refuges méditerranéens (Médail & Diadema, 2009)

➡ Slow recolonization of temperate regions from these refugia

➡ (Hewitt, 1996, 1999, 2000)

➤ Animals : South Refugia Hypothesis (SRH)

  ➤ Survival possible in 52 Mediterranean refugia identified through genetic diversity

  ➤ Slow recolonization of temperate regions from these refugia

    ➤ (Hewitt, 1996, 1999, 2000)

➤ Angiosperms : North micro-Refugia Hypothesis (NRH)

  ➤ Mediterranean region isolated in view of genetic divergence between Mediterranean and extra-Mediterranean regions

  ➤ Survival possible into Northern Europe micro-refugia

  ➤ Recolonization of temperate regions from these refugia

    ➤ (Petit *et al.*, 2003, 2005)

➡ Bryophytes particularities

➡ Poïkilohydry

▶ Bryophytes particularities

  ▶ Poïkilohydry

  ▶ Long distance dispersal capacity

→ **Model organisms for studying climate change**

- Bryophytes particularities
  - Poïkilohydry
  - Long distance dispersal capacity

- Dispersal capacity
  - Transoceanic migrations
  - Recruitment of extra-European individuals
    - From North America and Macaronesia
    - East-West structuration of Europe
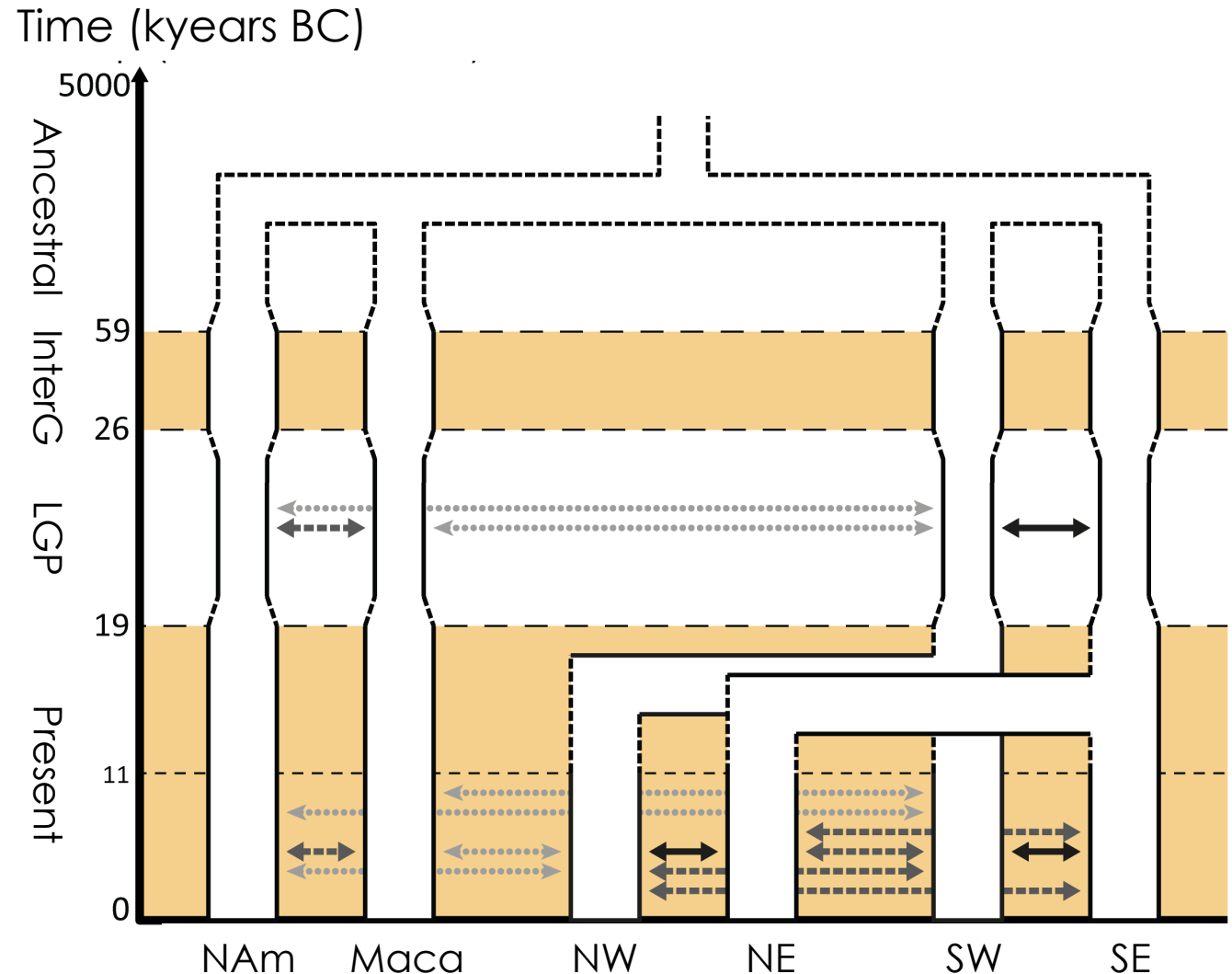   - → New biogeographical hypotheses

Objectives

➡ To asses, in Europe, the impact of the Last Glacial Maximum on bryophytes distribution through Approximate Bayesian statistics

➡ Especially,

  ➡ To determine if different scenarios of evolution lead up to the actual temperate distribution
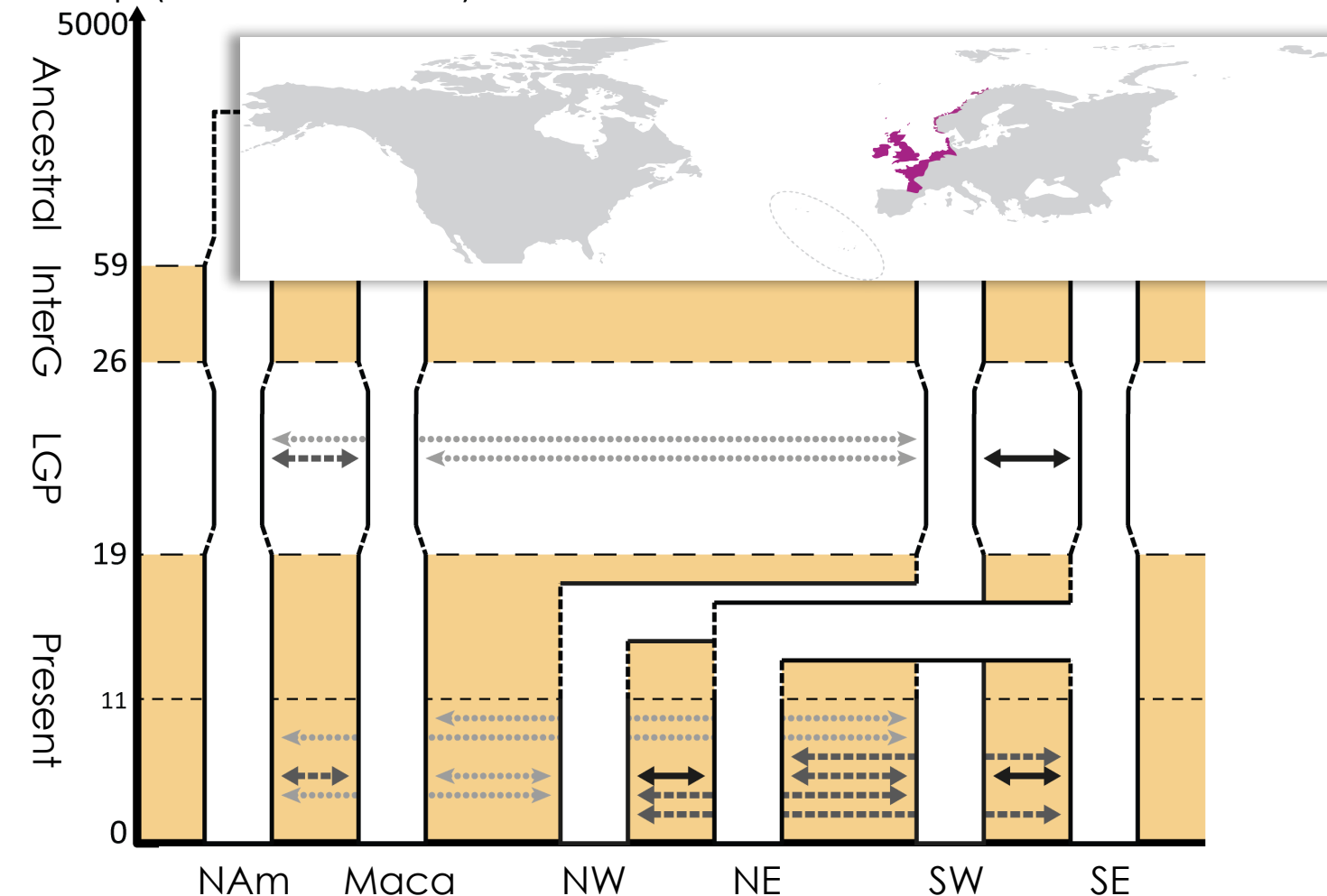
  ➡ To contrast the following biogeographical hypotheses :

South Refugia Hypothesis (scenario 1)

- North-South structuration

- No survival in extra-Mediterranean regions

- Survival in Mediterranean refugia

  - Recolonization of temperate regions from these refugia

- Low migration rate between West Europe and, North America and Macaronesia

Time (kyears BC)

5000

Ancestral InterG

59

26

LGP

19

Present

11

0

NAm   Maca   NW   NE   SW   SE

South Refugia Hypothesis (scenario 1)

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- Survival in Mediterranean refugia

  - Recolonization of temperate regions from these refugia

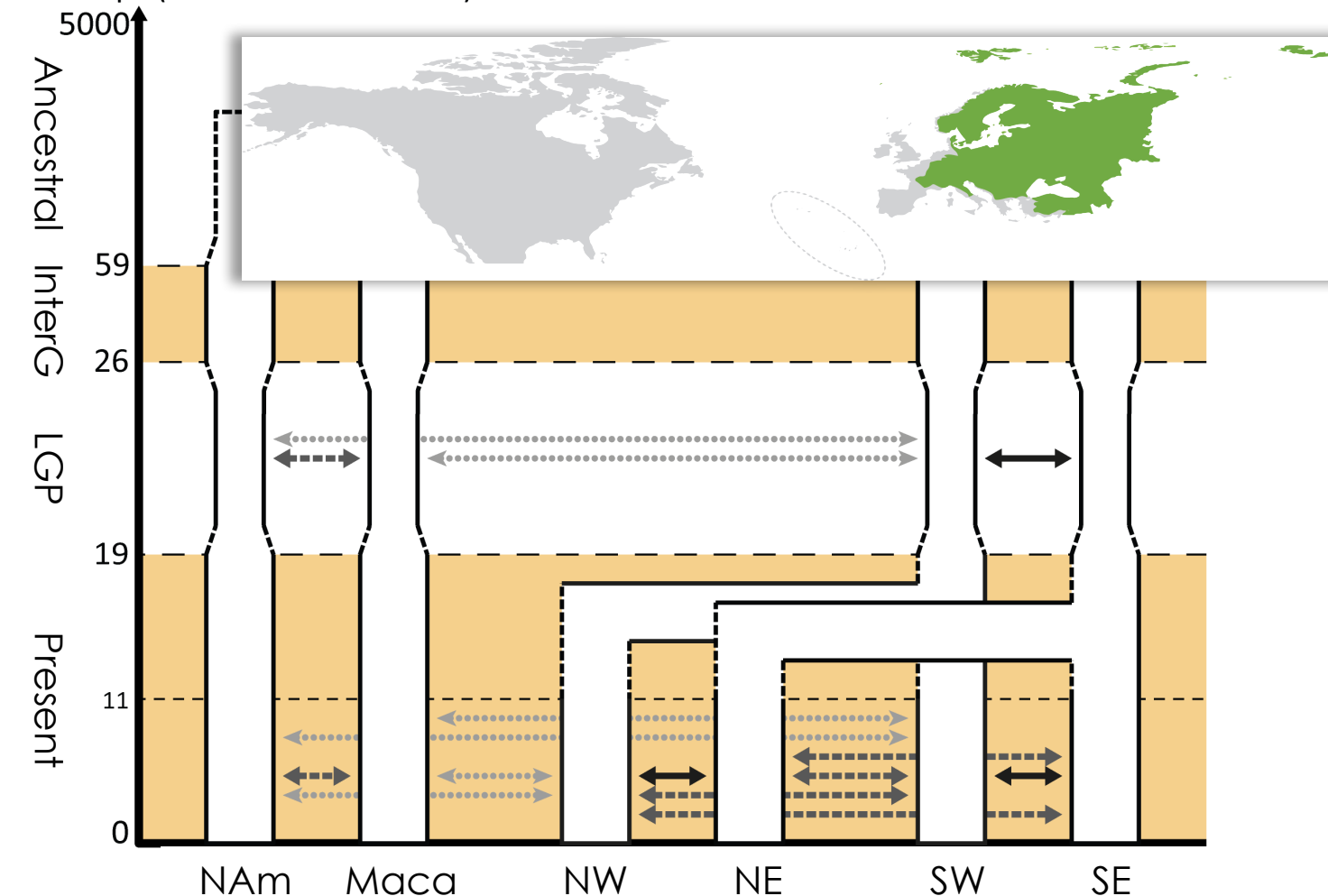- Low migration rate between West Europe and, North America and Macaronesia

Time (kyears BC)

Alice Ledent

08-12-16

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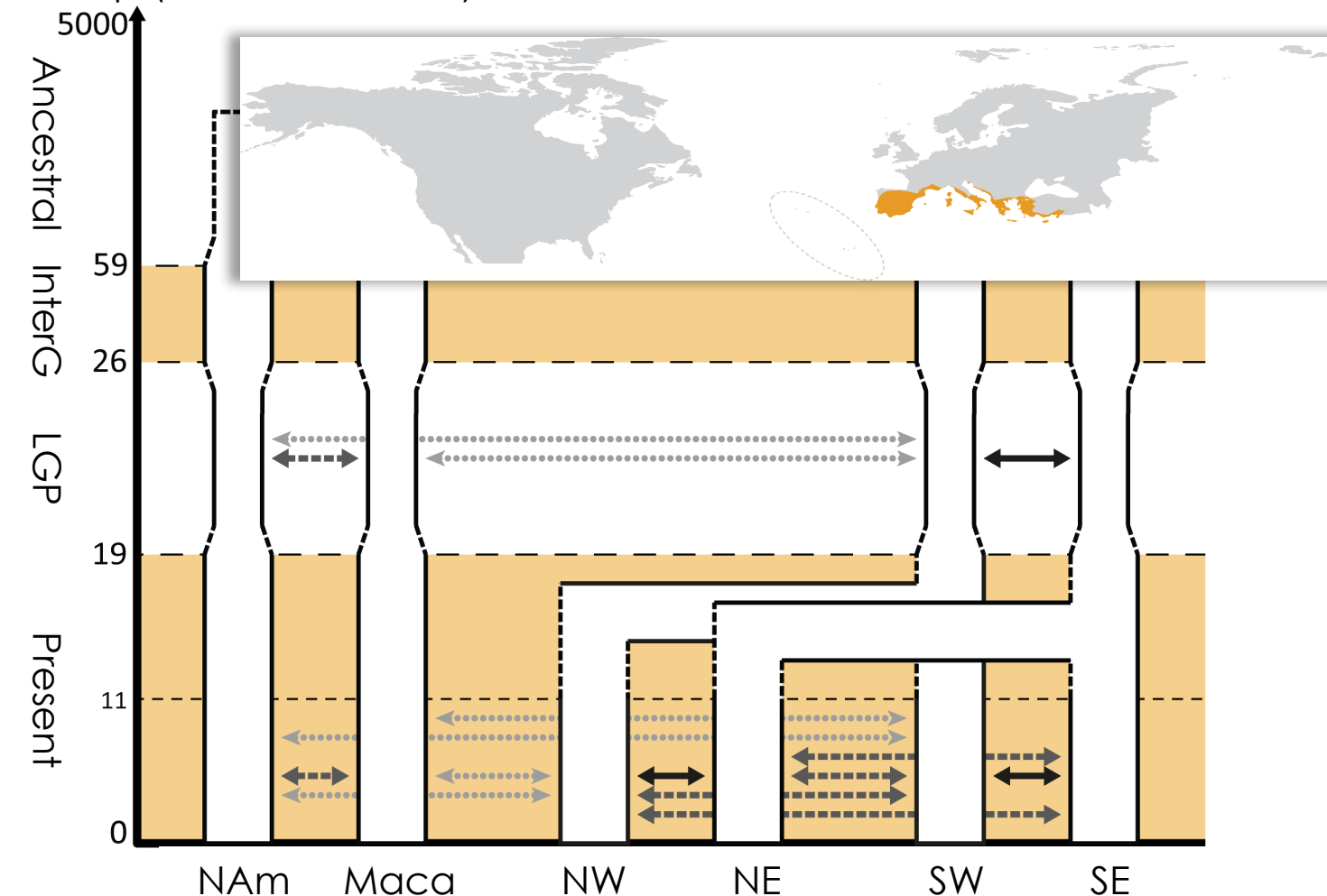
Time (kyears BC)

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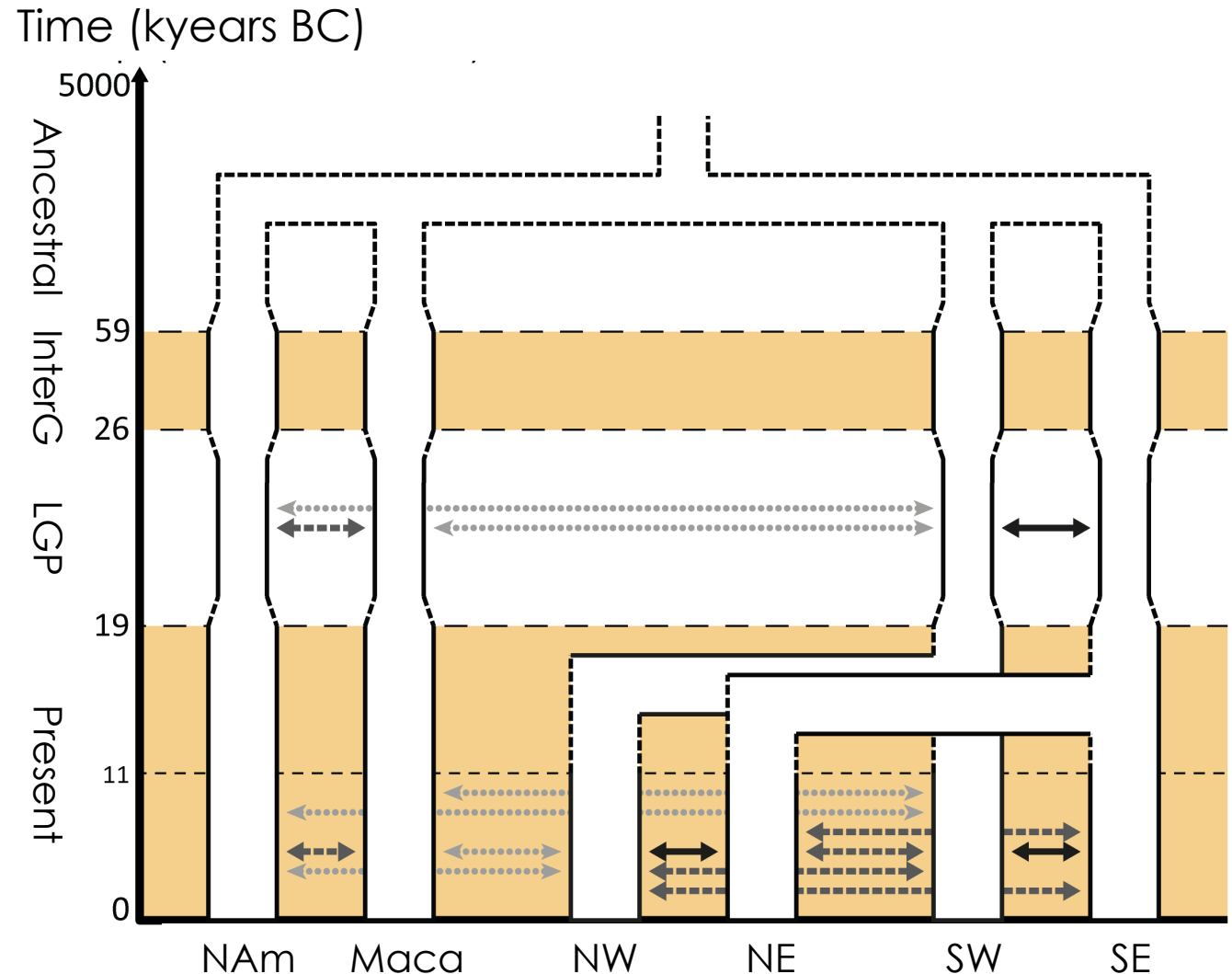

Time (kyears BC)

5000

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- No survival in extra-Mediterranean regions

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Time (kyears BC)

- Survival in extra-Mediterranean regions

- Recolonization of temperate regions from Northern micro-refugia

- No migrations between these two regions during the Last Glacial Period
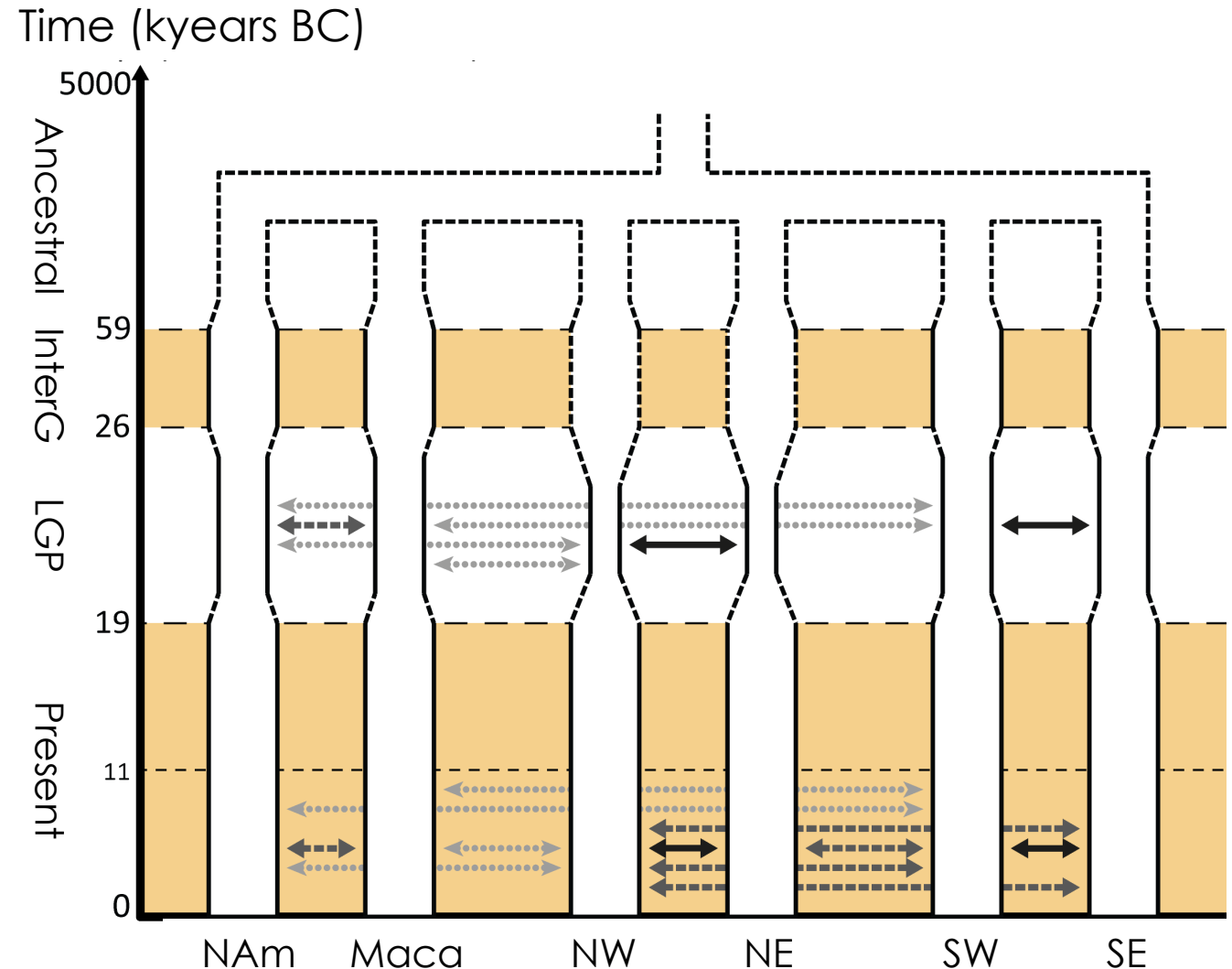
Est-West structuration Hypothesis (scenario 3)

- Est-West structuration

- Survival in both Est- and West-European regions

- No migrations between these two regions during the Last Glacial Period

- Medium migrations between Western Europe and, North America and Macaronesia
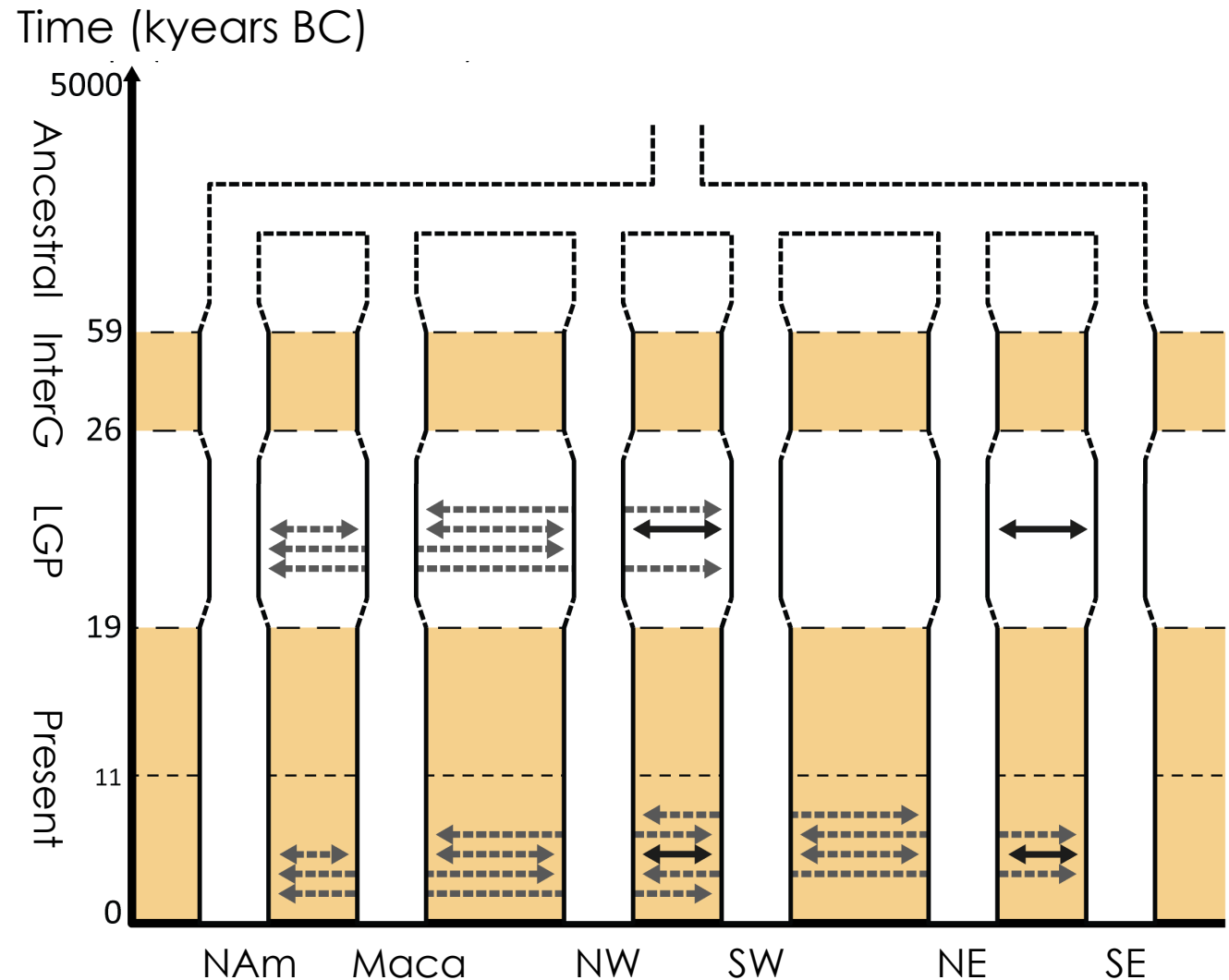
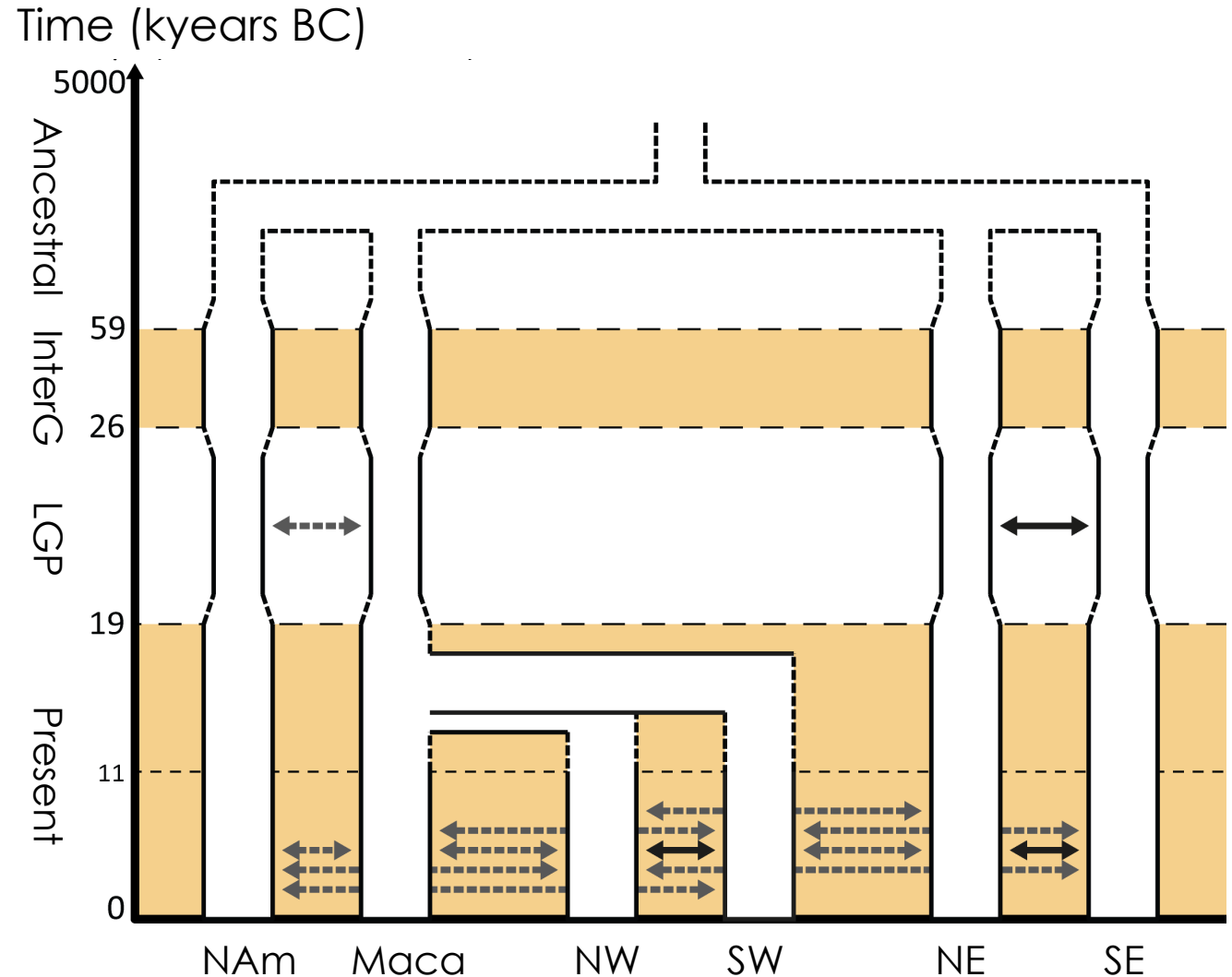# Extra-European recolonization Hypothesis : North-American origin (scenario 4a)

- No survival in the Western Europe region
  - Recolonization of this region from North America

Time (kyears BC)
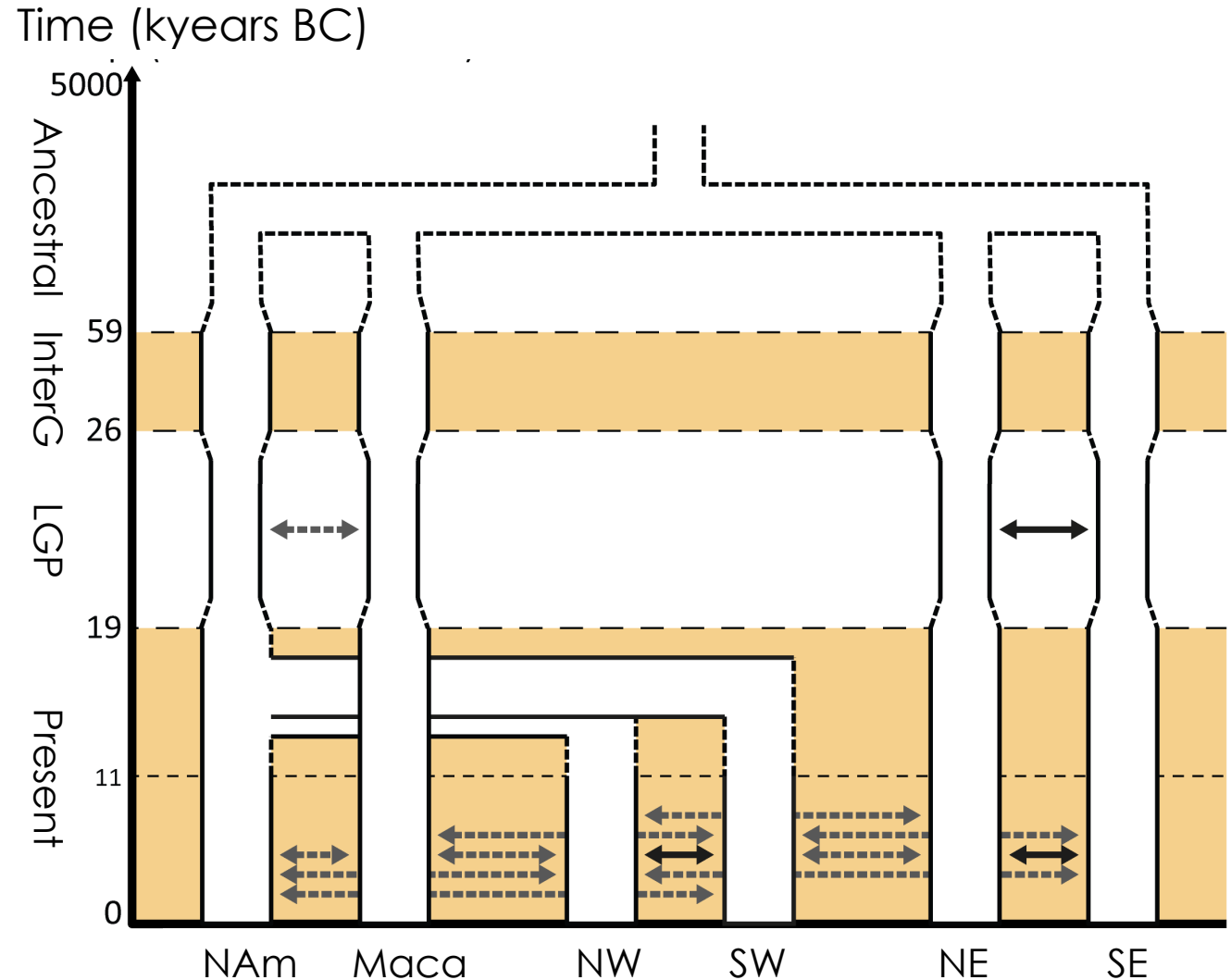
Extra-European recolonization Hypothesis : Macaronesian origin (scenario 4b)
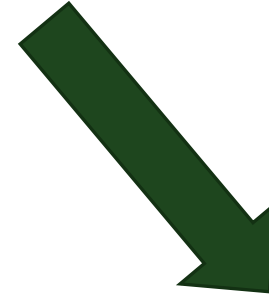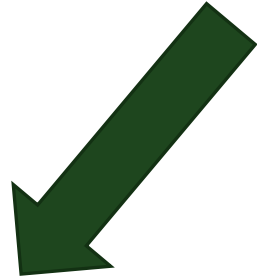
➡ No survival in the Western Europe region

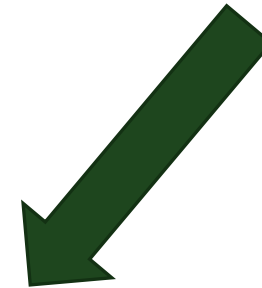➡ Recolonization of this region from Macaronesia

Material and methods

I
Taxonomic and populational sampling

II
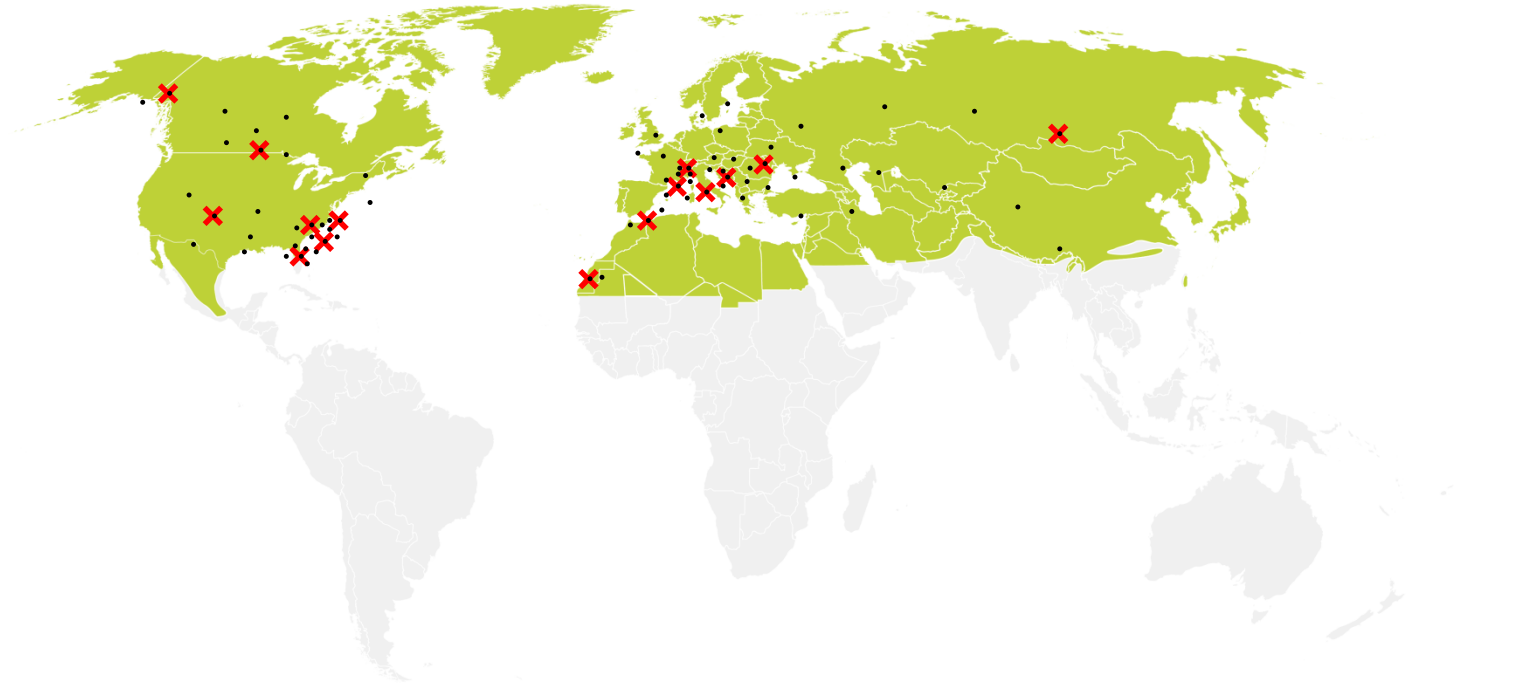## Extraction, amplification et sequencing of DNA

II'
Species Distribution models (SDMs)

III
Approximate Bayesian Computation analyze (ABC)

I

Taxonomic and populational sampling

- Presence point (occurrence)
- ✖ Sampled point (39-217)

✖ 13 species

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I
Taxonomic and populational sampling

II
Extraction,
amplification et
sequencing of DNA

X_1 = ACGATCCCAA ... TAGTGGCATG
X_2 = CGGATGCATG ... ACGTTAGCTC

X_n = CGAATTACGG ... CATGCAATAT

locus 1 ... locus z

Matrix of observed sequences

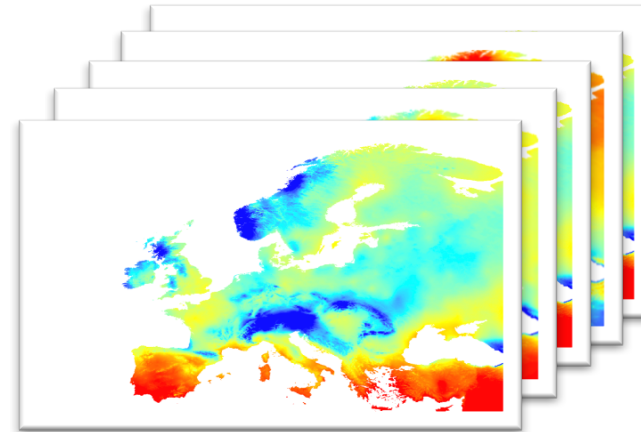X 13 species

X = Sampled point (39-217)

I
Taxonomic and populational sampling

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Extraction,
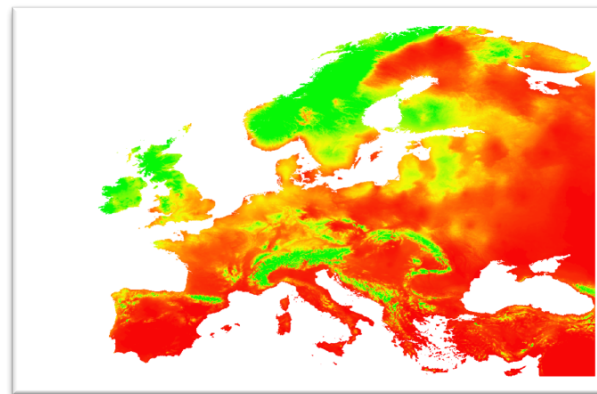amplification et
sequencing of
DNA

Presence points of a
species

+

Climatic variables

=

Species Distribution
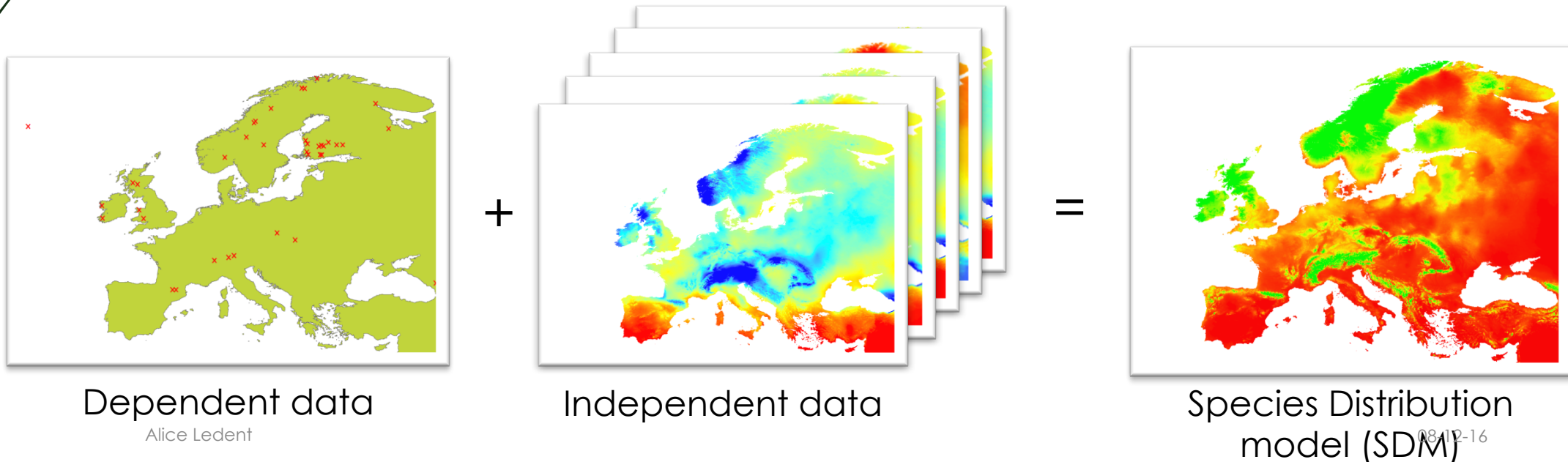model (SDM)

II'
Species
Distribution
models (SDMs)

X 13 species

Species Distribution Model (SDM)

➡ Models → simplification

➡ SDMs → prediction of suitable area

➡ Mathematical association between dependent (data on distribution of species) and independent variables (environmental factors)
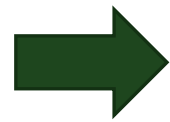

Dependent data

+

Independent data

=

Species Distribution model (SDM)

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Species Distribution Model (SDM)
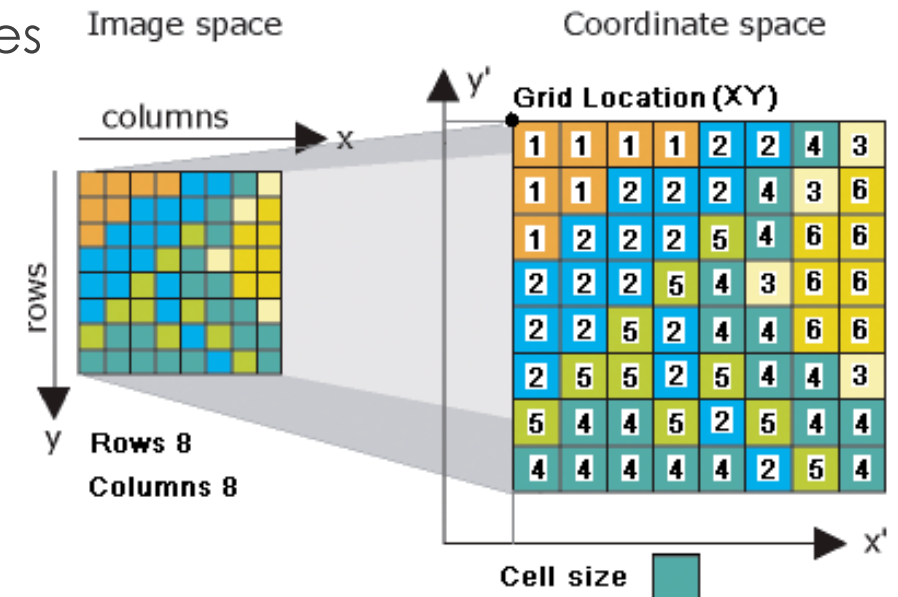
- Independent data
  - Type
    - Climatic variables: Worldclim 1.4, present, past and future
    - Soil, lithology and geology
    - Elevation and derived variables
    - …

    Different scales

  - Raster
    - Each pixel = a value of the climatic variable

Image space — columns, x, rows, y, Rows 8, Columns 8

Coordinate space — Grid Location (XY)

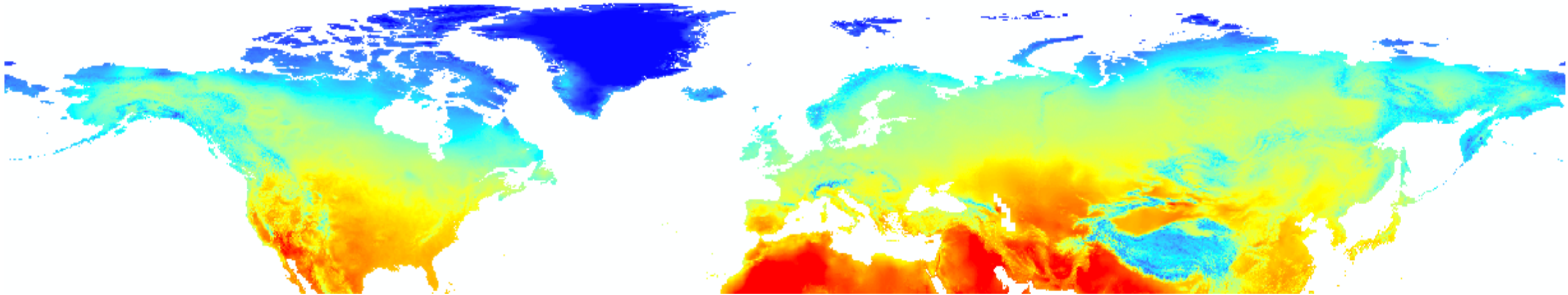| 1 | 1 | 1 | 1 | 2 | 2 | 4 | 3 |
1	1	2	2	2	4	3	6
1	2	2	2	5	4	6	6
2	2	2	5	4	3	6	6
2	2	5	2	4	4	6	6
2	5	5	2	5	4	4	3
5	4	4	5	2	5	4	4
4	4	4	4	4	2	5	4

Cell size

List of cell values

[1111224311222436122254662225436622524466255254435445252544444254]

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Species Distribution Model (SDM)

➡ Independent data

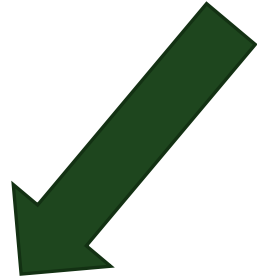　　➡ e.g. climatic variable

Species Distribution Model (SDM)

- ➡ Dependent data
  - ➡ Shapefile point
  - ➡ *e.g. Amphidium mougeotii*

I
Taxonomic and populational sampling

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Extraction, amplification et
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II'
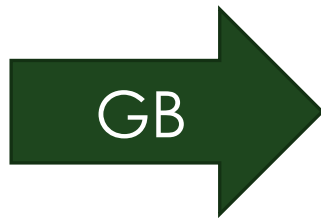Species Distribution models (SDMs)

4 steps

Species Distribution Model (SDM)

➡ SDM in four steps

   ➡ I Define models

      ➡ Define rules that explain the presence or absence of the species

      ➡ On the Geographic Background (GB)

      ➡ Machine learning

      ➡ Use 80% of the independent data

GB

Species Distribution Model (SDM)

➡ SDM in four steps

➡ I Define models

➡ II Test models

➡ Test the robustness of the models (*e.g.* TSS, AUC)

➡ Use 20% of the independent data

		Real data	
		Presence	Absence
Model	Presence	40%	3%
	Absence	7%	50%

Species Distribution Model (SDM)

- SDM in four steps
  - I Define models
  - II Test models
  - III Choose one model
    - Choose the best model
    - Combine some models in one
      - Ensemble modelling
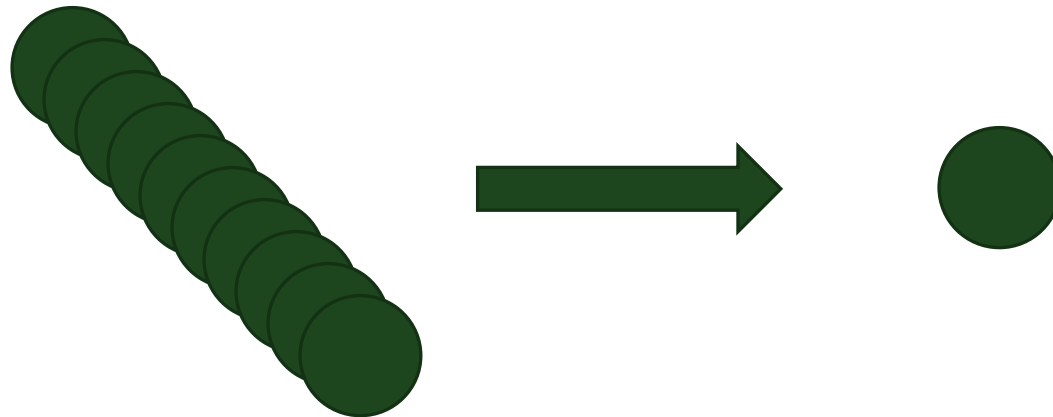
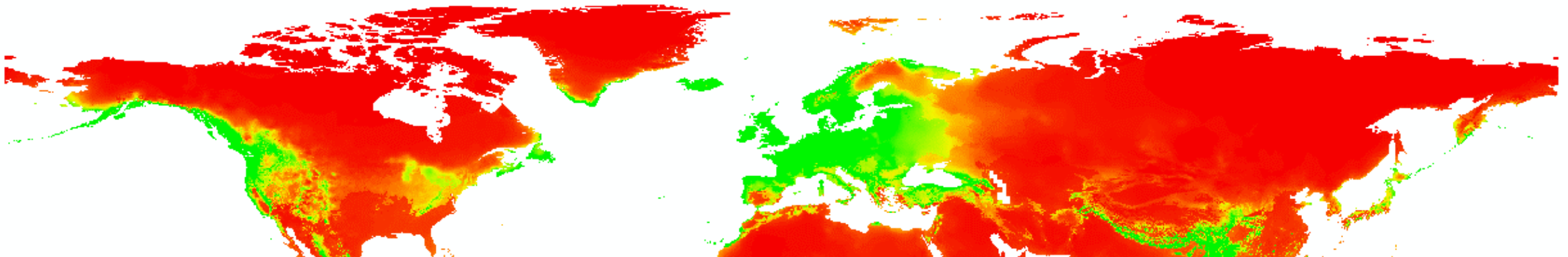# Species Distribution Model (SDM)

Index of suitability

Highly suitable

Not suitable

➡ SDM in four steps

- ➡ I Define models

- ➡ II Test models

- ➡ III Choose one model

- ➡ IV Project the model into the whole studied area

  - ➡ Where environmental data are available

  - ➡ Could be in the present, the past or the future!

  - ➡ Obtain the suitability of the species in each pixel, *i.e.* the SDM

I
Taxonomic and populational sampling

X 13 species

II
Extraction, amplification et
sequencing of DNA

X 13 species

II'
Species Distribution models (SDMs)

X 13 species

III
Approximate Bayesian Computation analyze (ABC)

X Amphidium mougeotii

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Taxonomic and populational sampling

X 13 species

II
Extraction, amplification et sequencing of DNA

X 13 species

II'
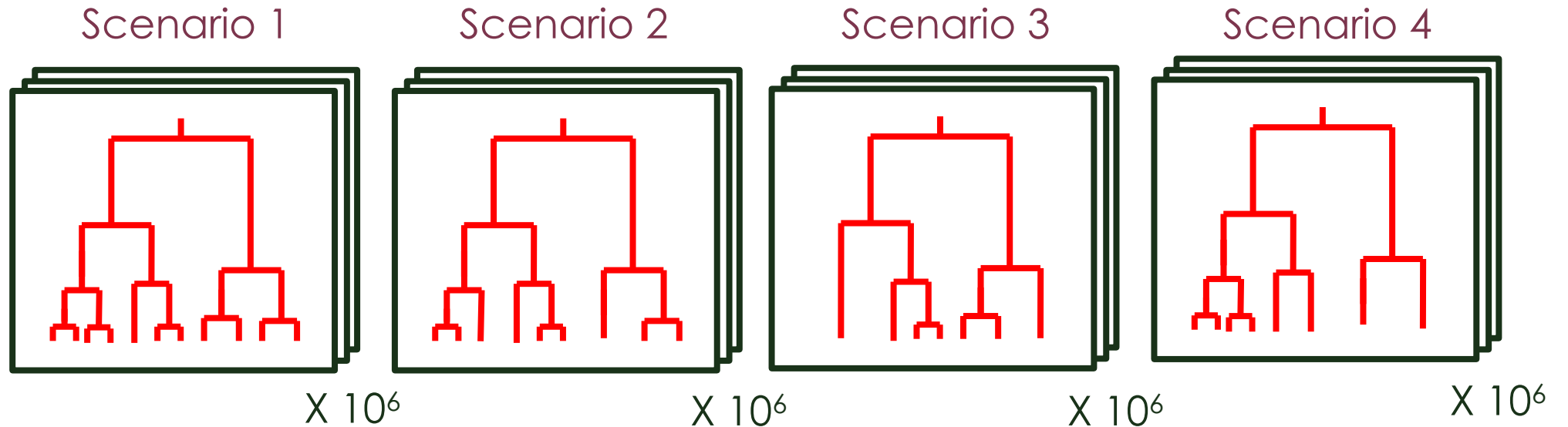Species Distribution models (SDMs)

X 13 species

III
Approximate Bayesian Computation analyze (ABC)

X Amphidium mougeotii

3 steps

1. Simulation of individuals genealogies

- Coalescence technique
- Under the constraint of different biogeographical scenarios

Scenario 1   Scenario 2   Scenario 3   Scenario 4

X 10^6 X 10^6 X 10^6 X 10^6

1. Simulation of alleles genealogies

Coalescence

- Goal : make a tree
  - grouping the alleles of a gene
  - backward in the time until the last recent common ancestor

- Coalescent event : two alleles of a population shared the same ancestral allele at the previous generation

- Probability of coalescence : probability that two alleles taken randomly into sample of a population coalesce

1. Simulation of alleles genealogies

Coalescence

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1. Simulation of alleles genealogies

Coalescence

➡ $prob\_coal(n, Ne) \approx n.(n-1)/2Ne$

   ➡ Depends on :

      ➡ The sample size

      ➡ The effective population size

      ➡ Probability increase when Ne decrease

➡ The time of coalescence is the time between two coalescent events

      ➡ Time decrease when probability increase

      ➡ Time decrease when Ne decrease

1. Simulation of alleles genealogies

Coalescence

➡ $prob\_coal(n, Ne) \approx n.(n-1)/2Ne$

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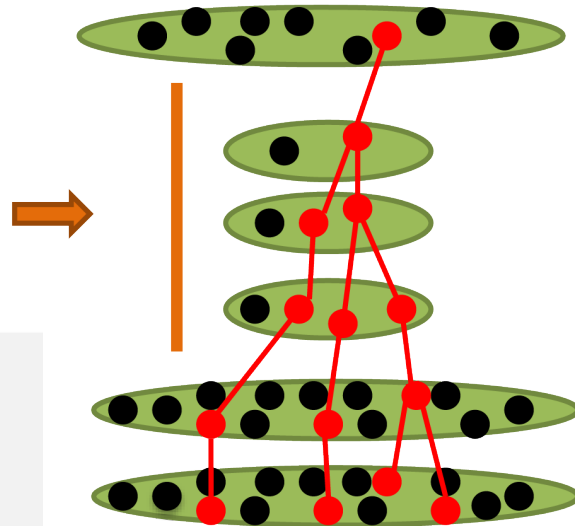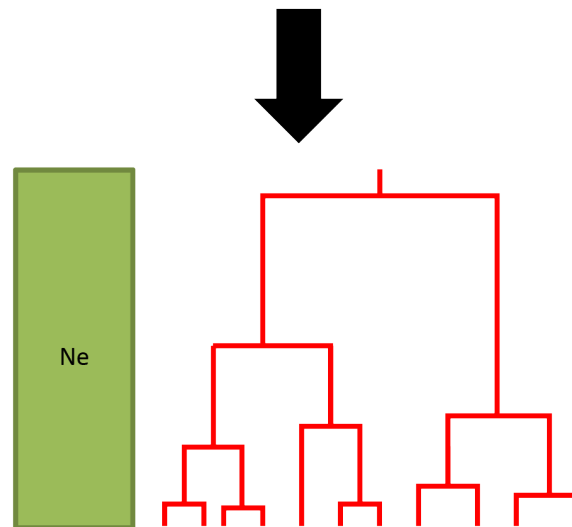
With "bottleneck effect"

Without "bottleneck effect"

Present time

Non-sampled individual

Sampled individual

"Bottleneck effect"

Ne

Ne

1. Simulation of individuals genealogies

- ► Coalescence technique
- ► Under the constraint of different biogeographical scenarios

Scenario 1 $\times 10^6$ Scenario 2 $\times 10^6$ Scenario 3 $\times 10^6$ Scenario 4 $\times 10^6$

1. Simulation of alleles genealogies

Coalescence with ABC

- For every scenario, define *prior* distribution of demographic parameters
  - Effective population size
    - Of each population
    - At different times
  - Sampling size of each population
  - Migration rates between populations
- The distributions are sampled 10^6 times
- For each sampling a tree is built
- 10^6 trees for every scenario

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1. Simulation of alleles genealogies

Coalescence with ABC

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Scenario 1

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X 10^6
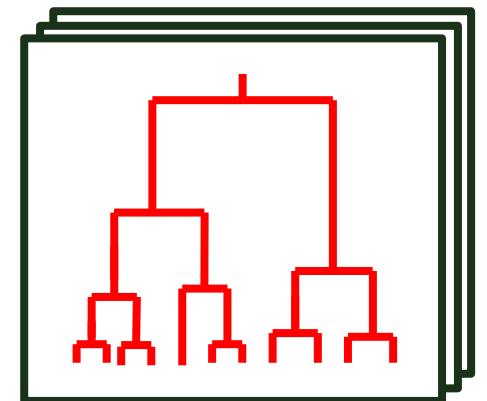
1. Simulation of alleles genealogies

Coalescence with ABC

➡ Other parameters need to be provided

  ➡ Generation time

  ➡ Number of populations

  ➡ Time period
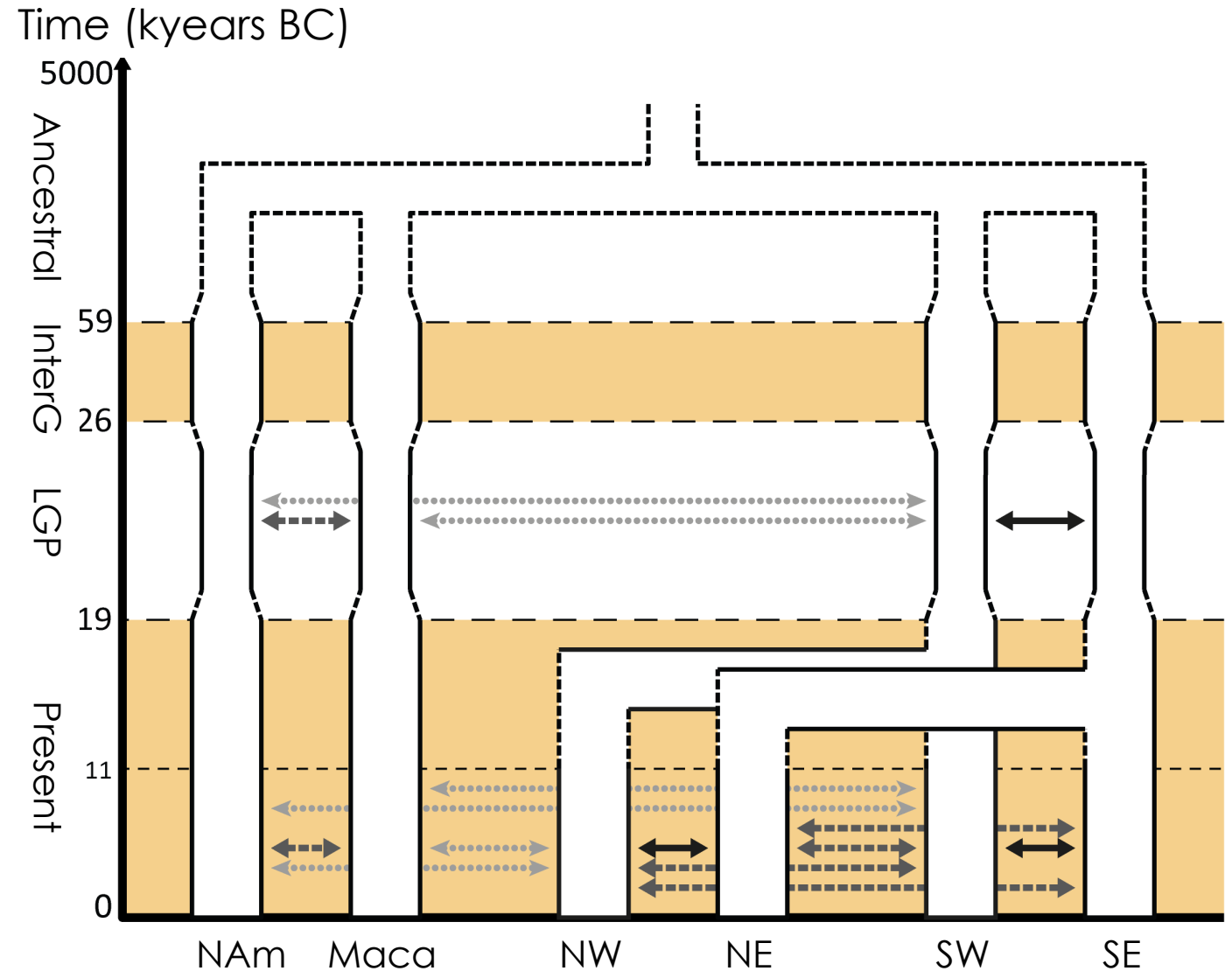
1. Simulation of alleles genealogies

➡ How to choose the *prior* distribution of demographic parameters for every scenario ?

  ➡ Range of values compatible with every scenario

  ➡ Ex. : effective population size

1. Simulation of alleles genealogies

➡ How to choose the *prior* distribution of demographic parameters for every scenario ?

➡ Range of values compatible with every scenario

➡ Ex. : effective population size

$$\sum \blacksquare \times 1 = Ne_{min}$$

$$\sum \blacksquare \times 50 = Ne_{max}$$
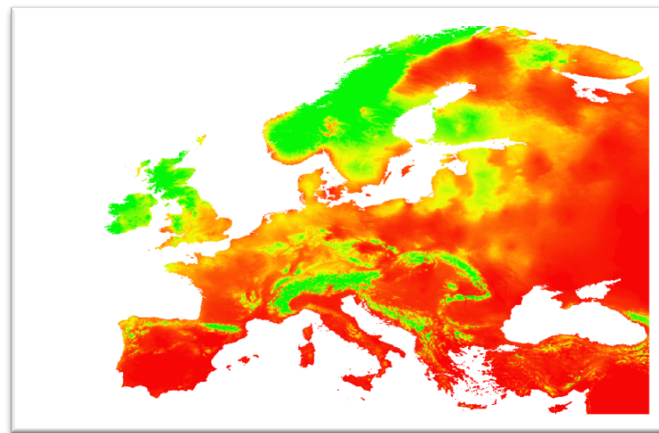
SDM
with index of suitability **Binarization with Threshold**
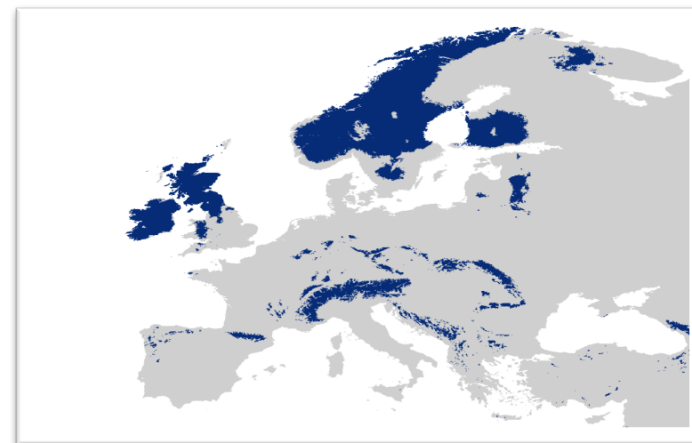
■ Area suitable for the species (1)
■ Area unsuitable for the species (0)
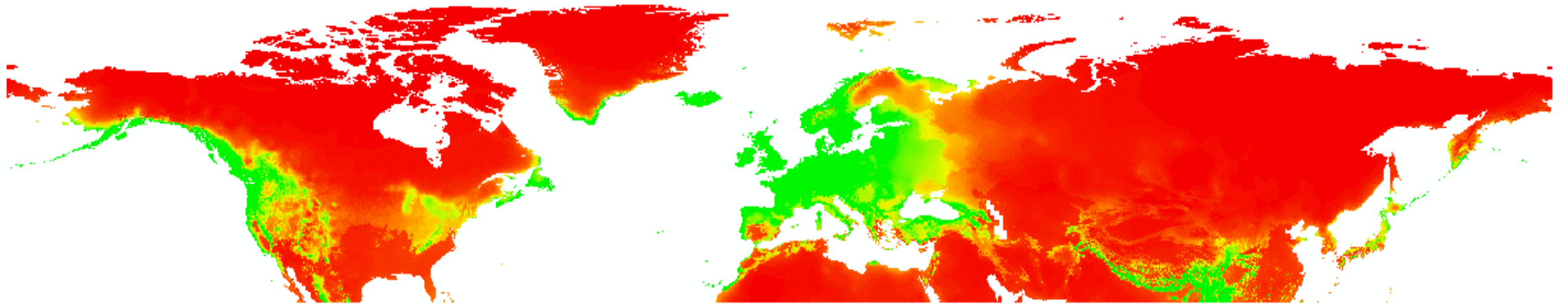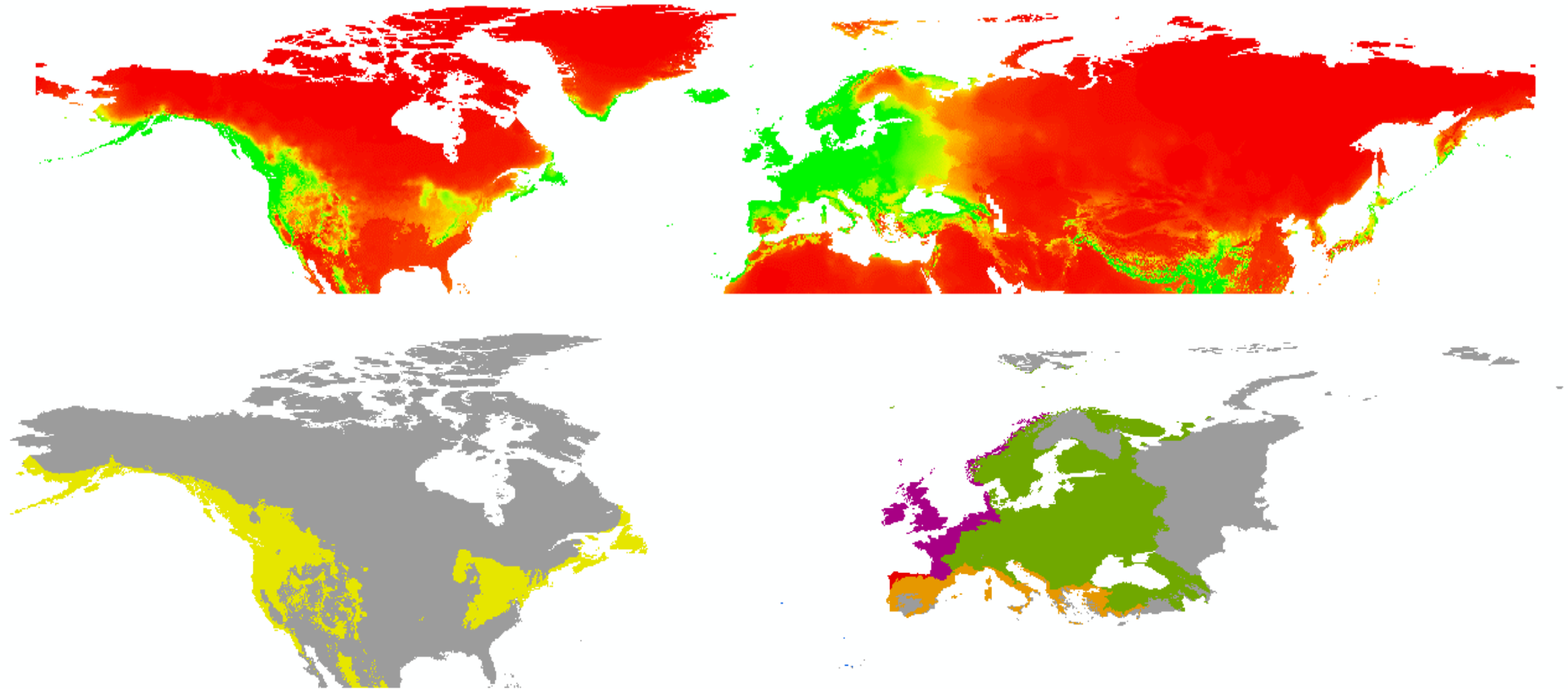
2. Matrices of sequences simulation

► Evolution of nucleotidic sequences along each genealogy

X 10^6

Evolution of nucleotidic sequences

X 10^6

I_1 = | CAGATCCCAA | ... | TATGAGCCAT |
I_2 = | ACGACGAAAG | ... | CATGAGACAG |
.
.
.
I_n = | CCAAACGATC | ... | ATGTGCGTGC |

locus 1 ... *locus* z

Matrices of simulated sequences

X 4 scenarios
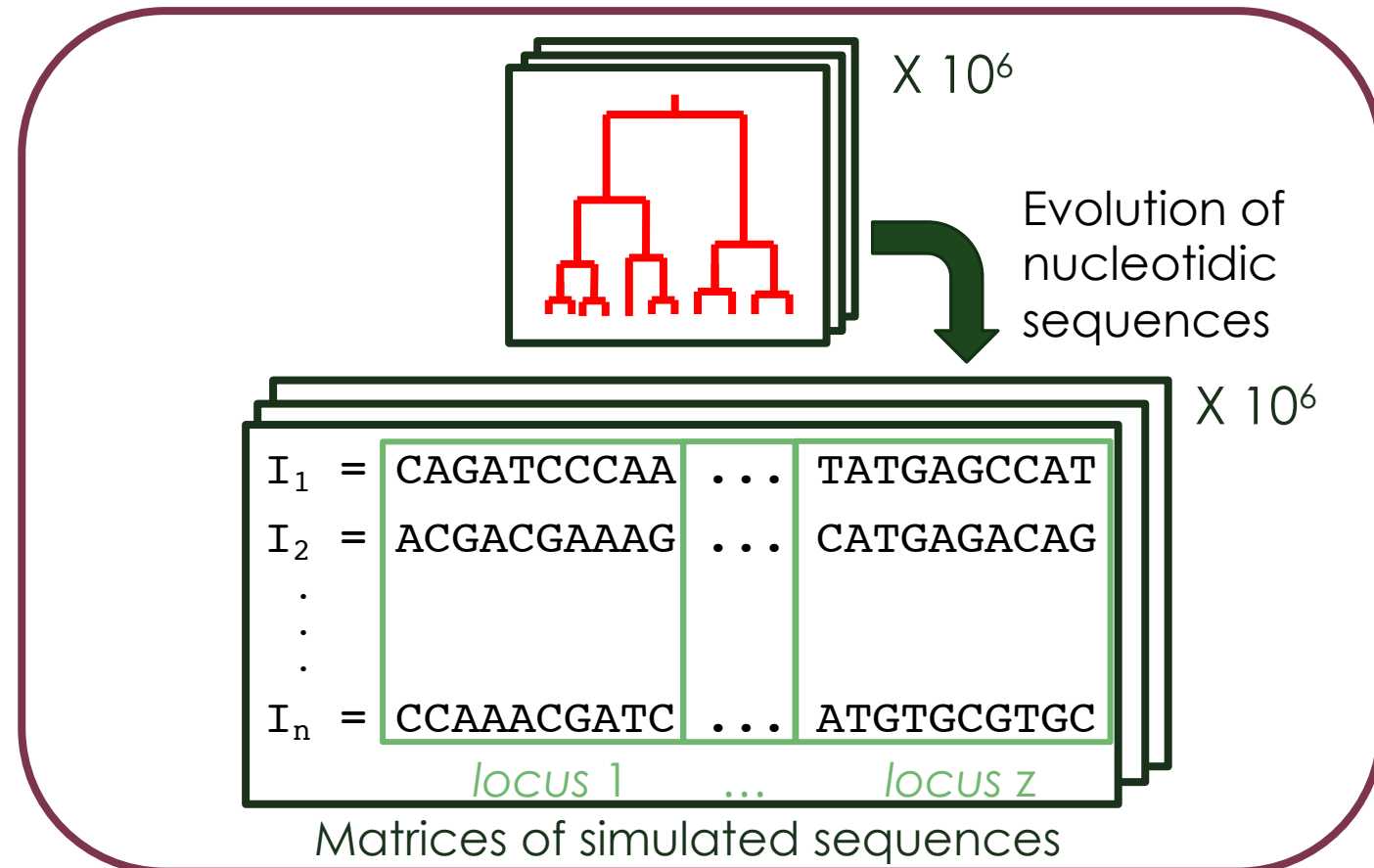
2. Matrices of sequences simulation

- Evolution of nucleotidic sequences along each genealogy

  - Choose a model of sequences evolution

  - *e.g.* : Kimura 2-parameter

    - Substitution rate

    - Ratio T/T

  - Apply this model for each tree

  - The tree topology will modify the resulted sequences

Tree topology influence sequences

With "bottleneck effect"

Without "bottleneck effect"

12 substitutions
5 different alleles

12 substitutions
9 different alleles
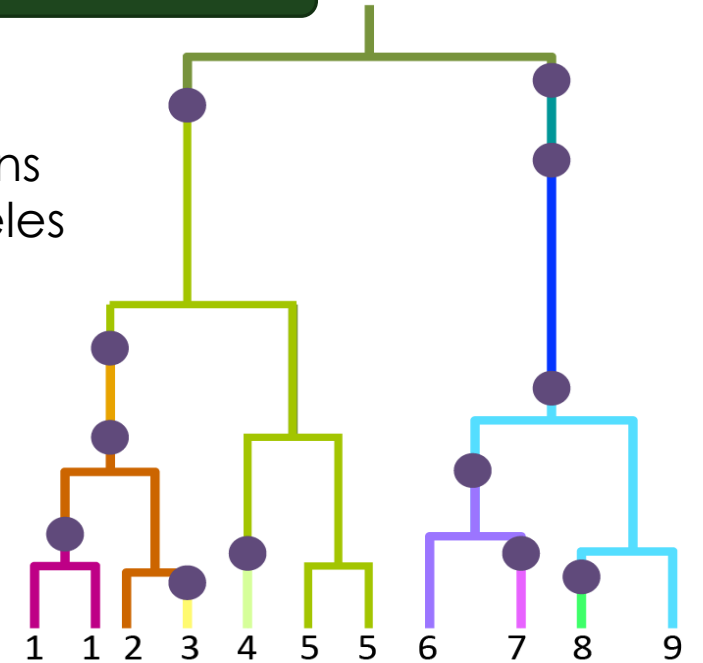
Substitution in one site
"Bottleneck effect"
1,2,...9 Different alleles

2. Matrices of sequences simulation

▶ Evolution of nucleotidic sequences along each genealogy

X 10^6

Evolution of nucleotidic sequences

X 10^6

$$I_1 = \text{CAGATCCCAA} \quad \ldots \quad \text{TATGAGCCAT}$$
$$I_2 = \text{ACGACGAAAG} \quad \ldots \quad \text{CATGAGACAG}$$
$$.$$
$$.$$
$$.$$
$$I_n = \text{CCAAACGATC} \quad \ldots \quad \text{ATGTGCGTGC}$$

locus 1 ... *locus* z

X 4 scenarios

Matrices of simulated sequences

Alice Ledent

08-02-16

3. Selection of the Best Scenario

➡ Compute the distance
between observed data and
every simulated dataset

X NbSc.10^6

$\mathbf{X}_1 = $ ACGATCCCAA ... TAGTGGCATG
$\mathbf{X}_2 = $ CGGATGCATG ... ACGTTAGCTC

.
.
.

$\mathbf{X}_n = $ CGAATTACGG ... CATGCAATAT

locus 1 ... *locus z*

Observed dataset

Distance

$I_1 = $ CAGATCCCAA ... TATGAGCCAT
$I_2 = $ ACGACGAAAG ... CATGAGACAG

.
.
.

$I_n = $ CCAAACGATC ... ATGTGCGTGC

locus 1 ... *locus z*
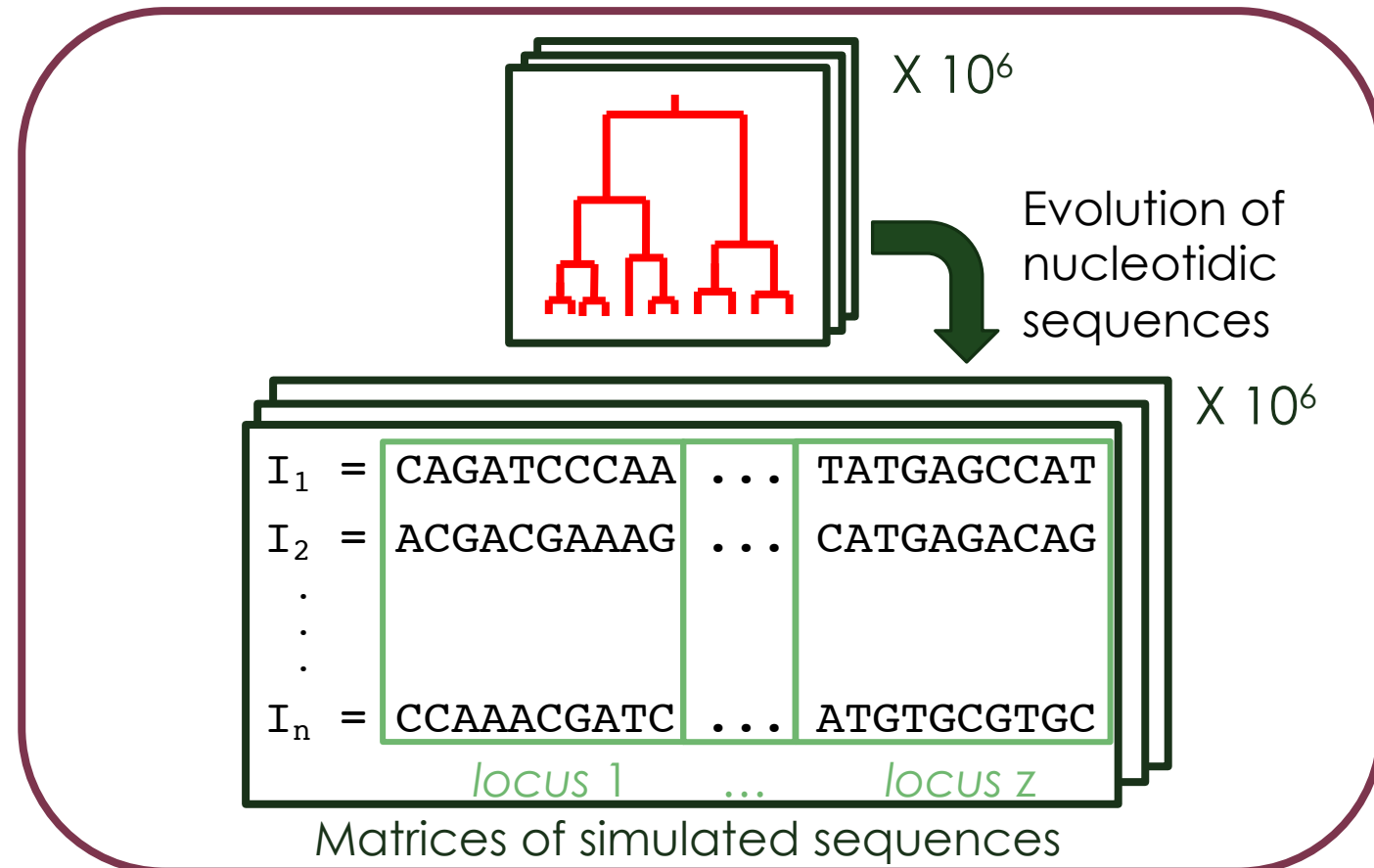
Simulated dataset

❌ = Sampled point (39-217)

08-12-16

3. Selection of the Best Scenario

Distance : **δ**

- Compute the distance between observed data and every simulated dataset

- Sort ascending Euclidian distances

- Select the 1000 shortest distances

- Select the best scenario *i.e.* with the highest proportion of simulations within the 1000 best simulations

δ — 0

sim. x **sc. 1**

sim. y **sc. 2**

sim. z **sc. 1**

sim. w **sc. 4**

…
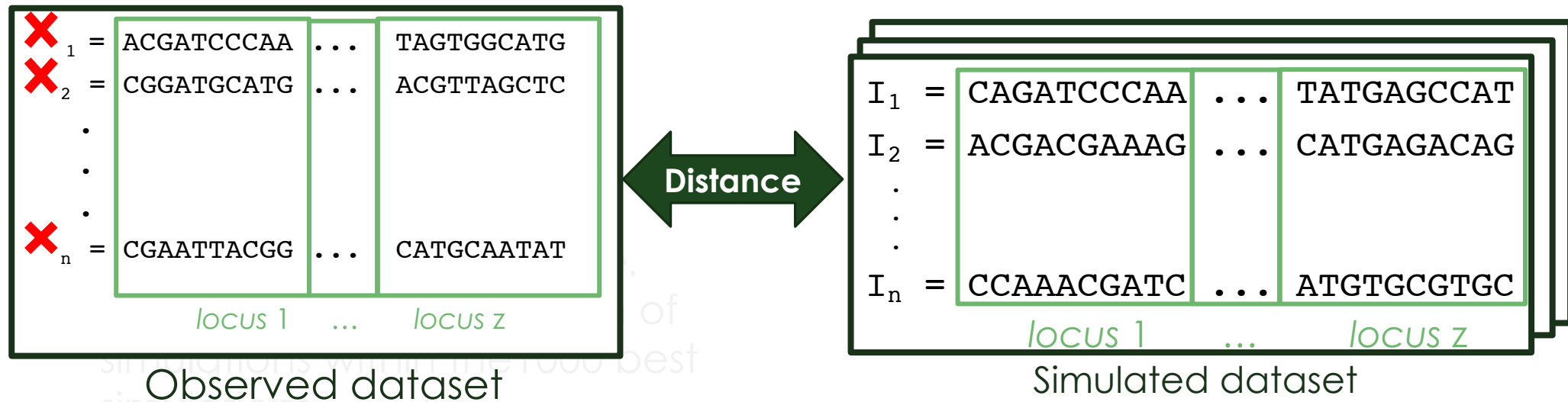
1 000 first simulations

∞

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........sim. x **sc. 1**
........sim. y **sc. 2**
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.... …

∞

1 000 first simulations

40 sim. for the **sc. 1** = 4%
25 sim. for the **sc. 2** = 2,5%
900 sim. for the **sc. 3** = 90%
35 sim. for the **sc. 4** = 3,5%
_____
Sc. 3 = 90%
➔Best scenario
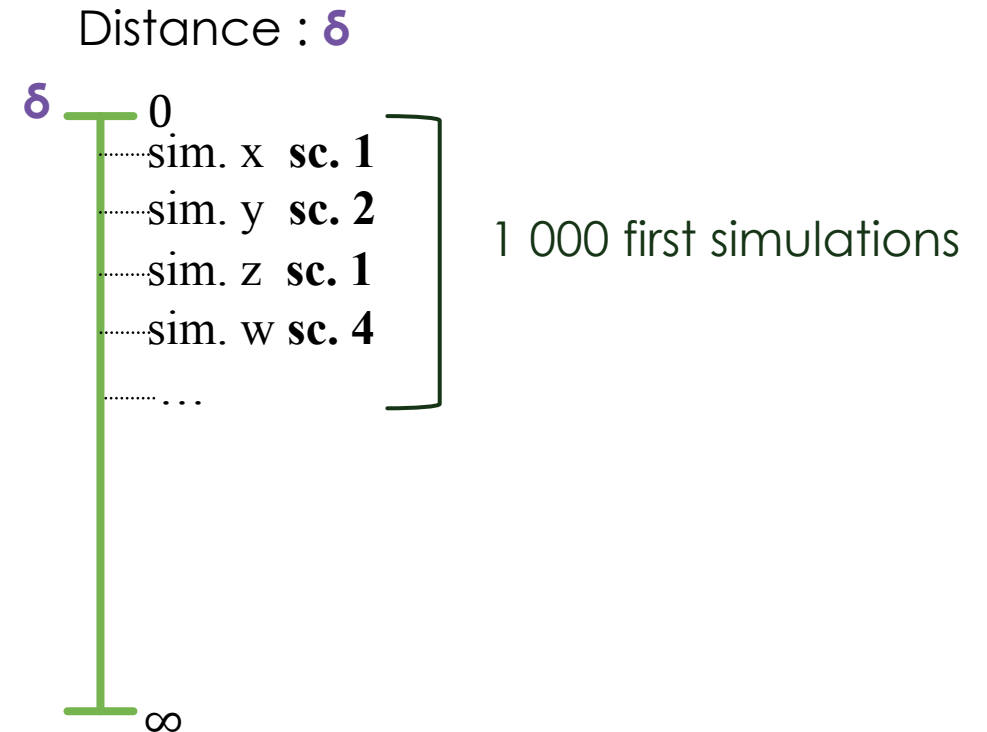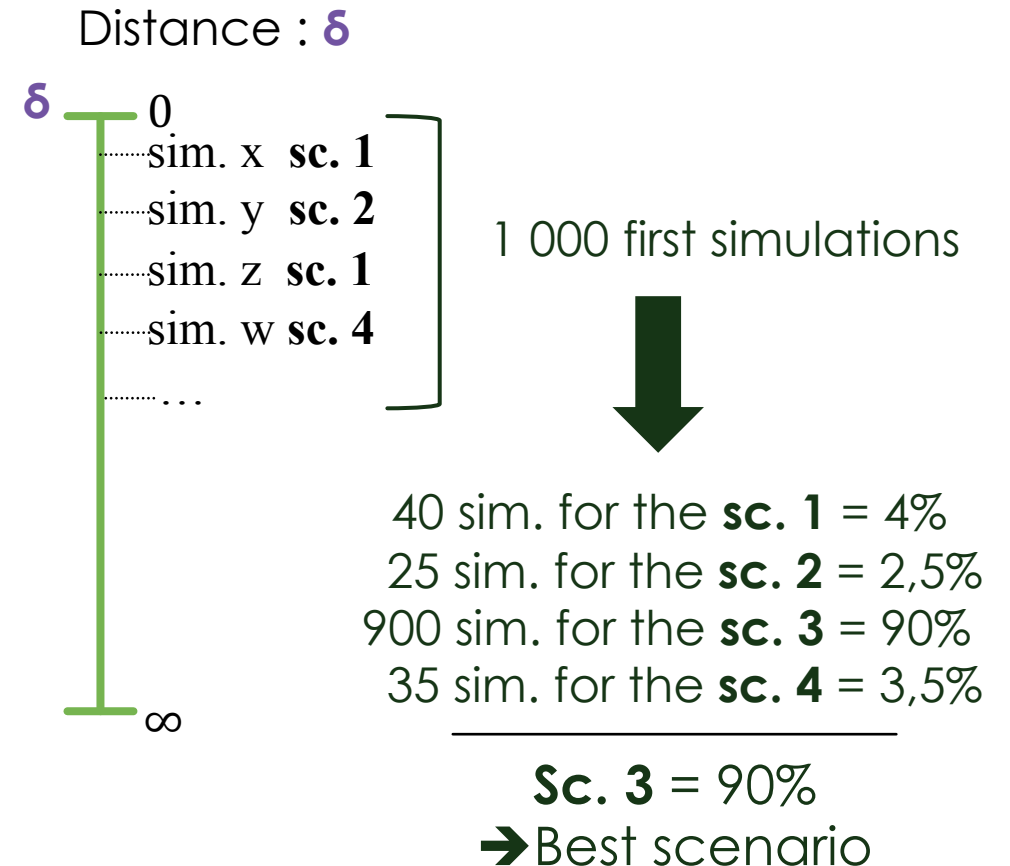
Results and discussion

Proportion of each scenario within the 1000 best simulations

Legend:
- South (scenario 1)
- North (scenario 2)
- East/West (scenario 3)
- Asource (scenario 4a)
- Msource (scenario 4b)

238
19
0
370
373
743
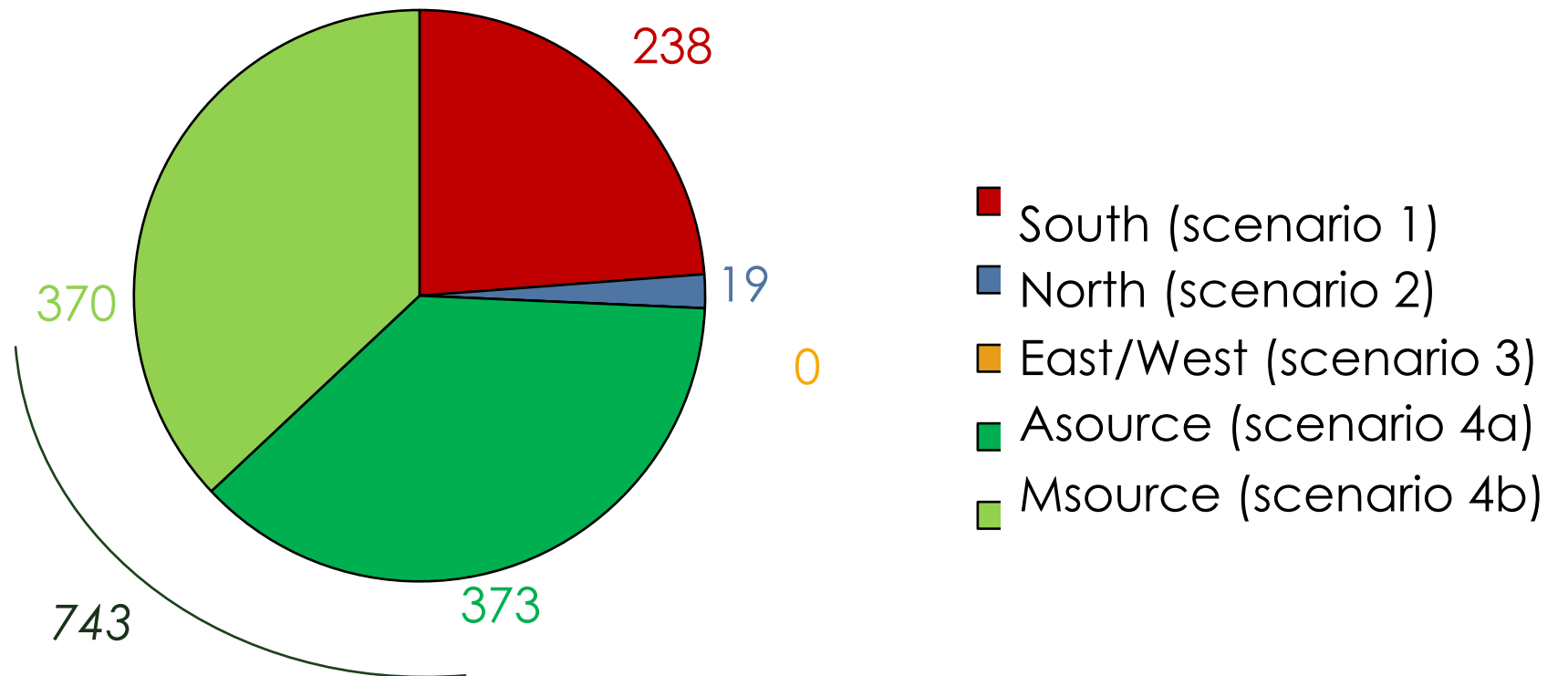
Amphidium mougeotii

Two scenarios are highlighted by the study (4 and 1)

- Scenarios 4 et 1 : common points
  - Extinction in temperate regions
    - Scenario 4 : Atlantic region
    - Scenario 1 : extra-Mediterranean regions
  - Recolonization from extra-temperate regions
    - Scenario 4a : North America
    - Scenario 4b : Macaronesia
    - Scenario 1 : Mediterranean region

  → Climatic conditions of temperate regions unfavorable compared to extra-temperate regions

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Climatic differences

- Temperate regions drier than extra-temperate regions
  - Temperate regions : cover with a tundra or a steppe
  - Extra-temperate regions :
    - Mediterranean region : the only European region with forests
    - North America : tundra less extensive; forest more numerous
    - Macaronesia : Preponderance of laurel forests (hygrophile) on xerophile vegetation

  - Bryophytes are sensitive to drought
    → Sensitivity to drought = important characteristic to explain their biogeographical histories

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Recolonization from extra-temperate regions

- Dispersions transoceanic (scenario 4)
- Dispersions beyond the usual dispersal barrier (European seas and mountains; scenario 1)

→ Long dispersal capacities = important characteristic to explain their biogeographical histories

→ Multiply the possible origins of migrants

→ Explain why several origins of migrants were observed for bryophytes while unique origins of migrants was observed for animals and angiosperms

- Animals : origin of migrants = Mediterranean regions
- Angiosperms : origin of migrants = Northern micro-refugia

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Conclusion & perspectives

Conclusion

➤ ABC → contrast divers biogeographical scenarios

  ➤ Coexistence of several scenarios for only one species

  ➤ Heterogeneity in the origins of migrants

  → Contrast compared to unique origins observed for other group of living organisms

  ➤ Heterogeneity explained by long dispersal capacities

➤ Methodology has been developed as part of a meta-study

  ➤ Results from *Amphidium mougeotii* will be combine to the results of the 12 other temperate species

  → Homogeneity of answers between species ?

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State of arts

- ➡ Scenarios simplified and better contrasted

- ➡ Addition of a ghost population

- → Routine study for the 13 species soon

- ➡ New study: An Amazonian panmictic world **?** Epiphytic bryophytes testify
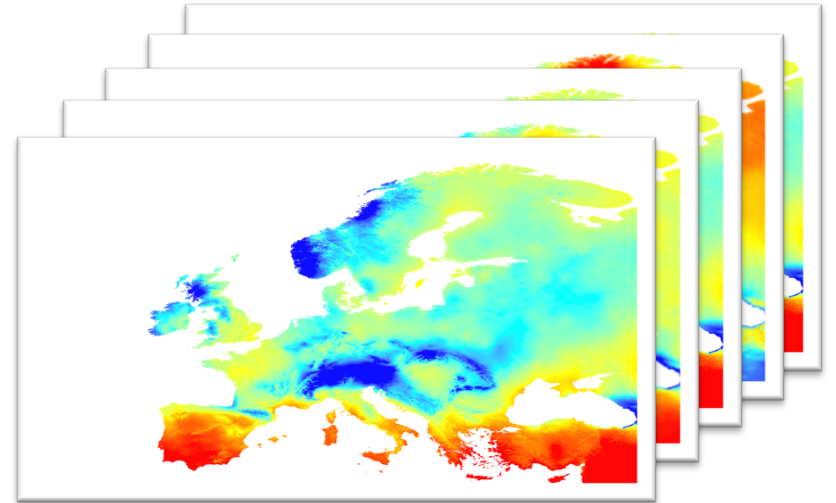
3. PHD degree

Return on SDMs

Presence points of a species   Climatic variables
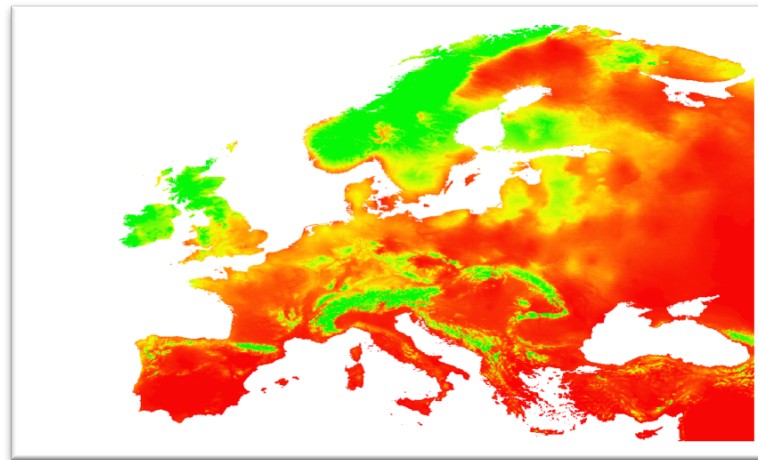
Species Distribution model
(SDM)

Applications

- ▶ Biogeography
  - ▶ Study the distribution of a species in the past
    - ▶ e.g. using Worldclim variables
  - ▶ Study the effects of global change
    - ▶ e.g. using Giec RCP scenarios

- ▶ Biodiversity
  - ▶ Search for new populations of endangered organisms
  - ▶ Selecting areas for reintroduction
  - ▶ Biodiversity patterns and hot spots
  - ▶ …

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Limitations

- **Pseudo-equilibrium hypothesis**

  - Assume that the population is in balance or pseudo-equilibrium with environmental conditions

- No space component

  - Biogeographical barriers

  - Limitations in the dispersal capacity of the species

    Bryophytes are good model because of their high dispersal capacities

    Dynamic model

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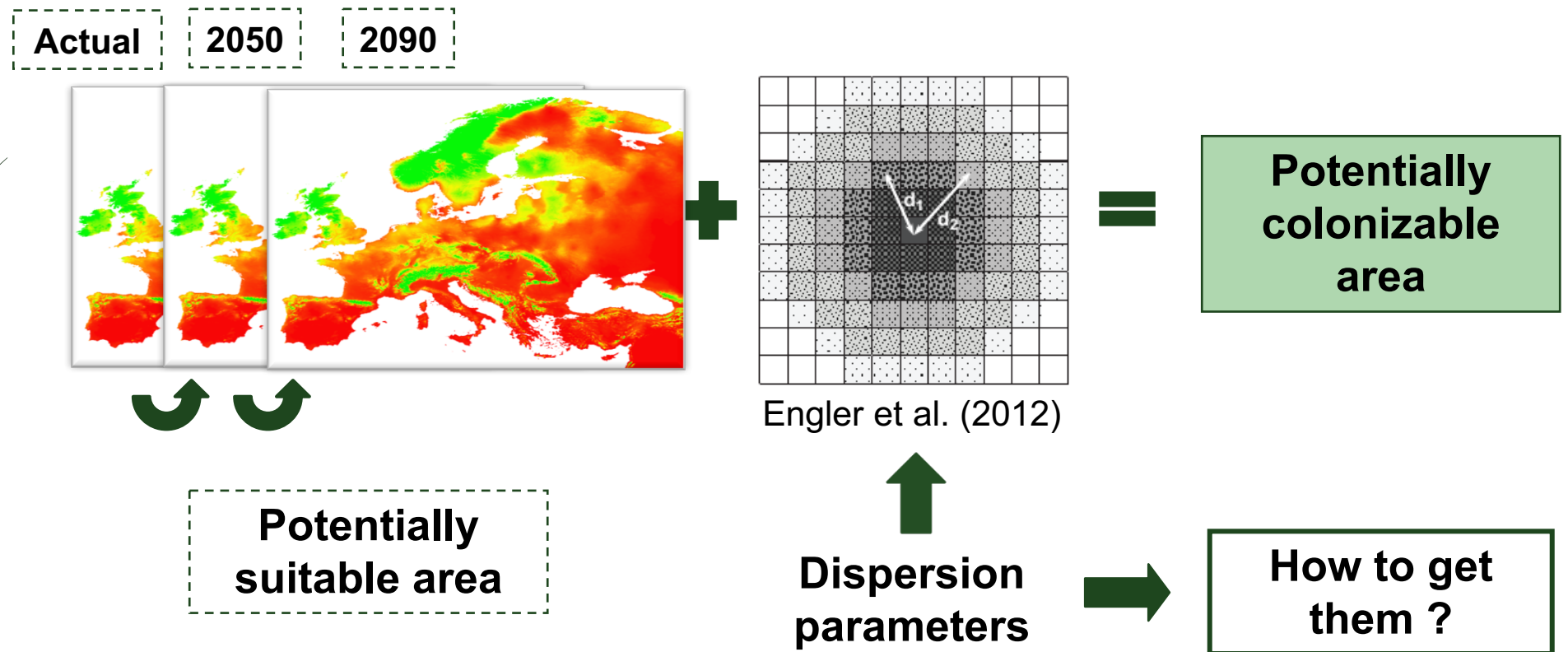 - Limitations in the dispersal capacity of the species

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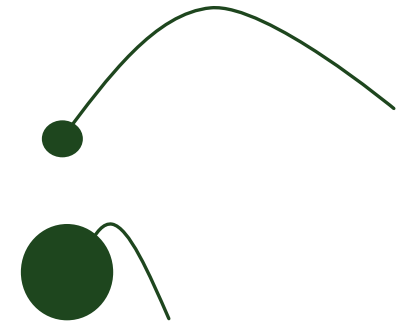
    ➡ Dynamic model

Dynamic models

Actual   **2050**   **2090**

Engler et al. (2012)

Potentially suitable area

Dispersion parameters ➡ **How to get them ?**

+ **=** **Potentially colonizable area**

Dynamic models

Dispersion parameters ?

⬇

Estimations

Short distance

P_{Disp}

Distance

Long distance

$$\frac{\text{Spores size}}{\text{Dispersion distance}}$$
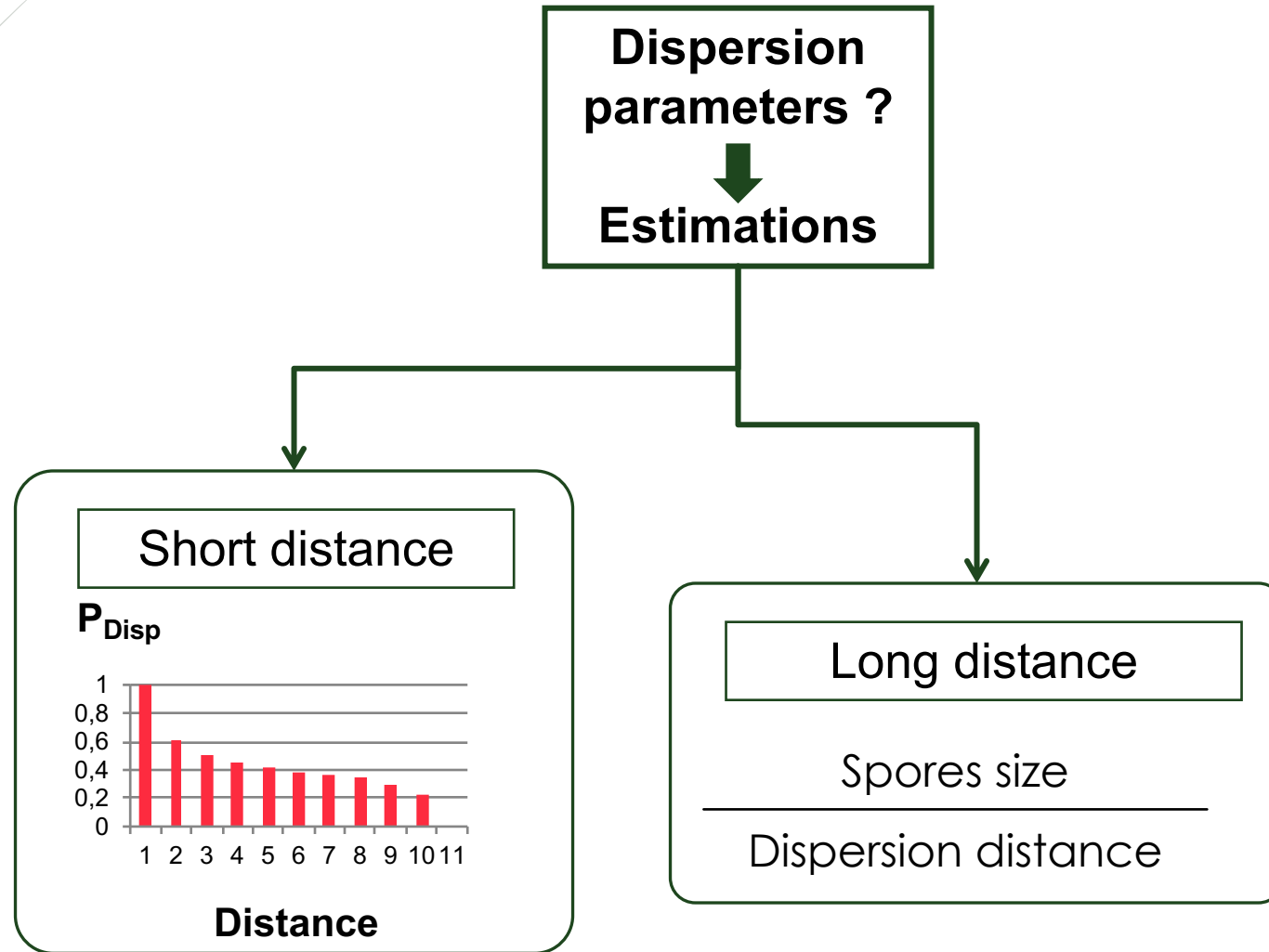
Dynamic models

➥ Green-red gradient =
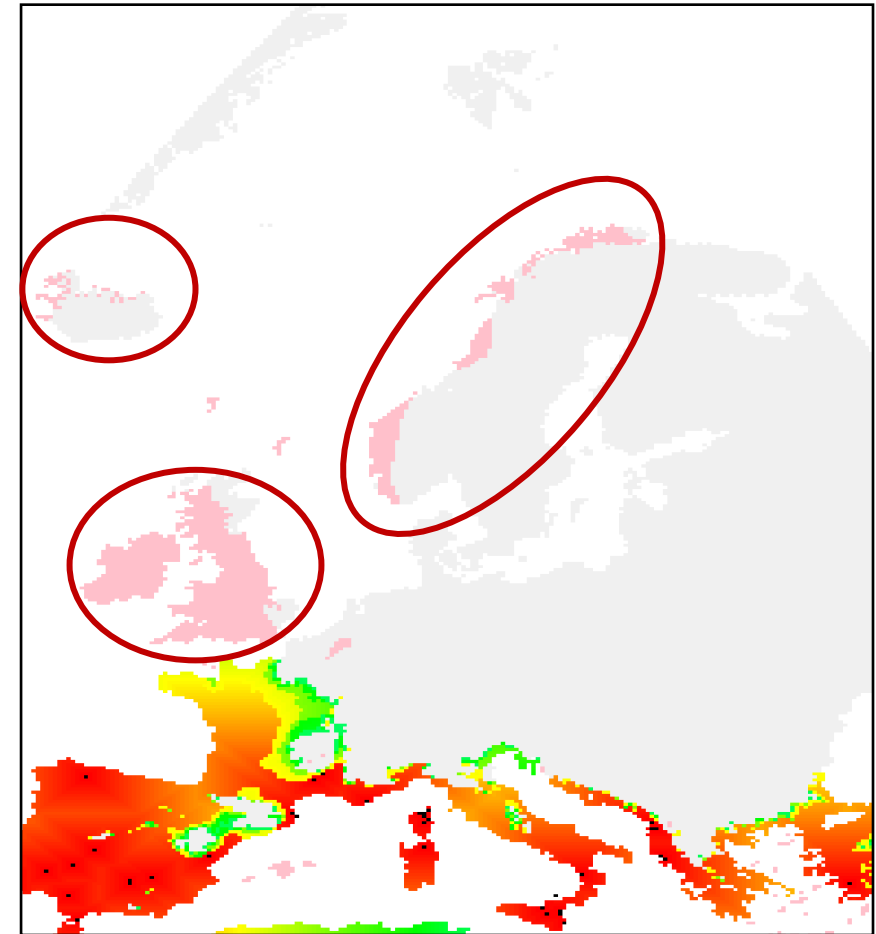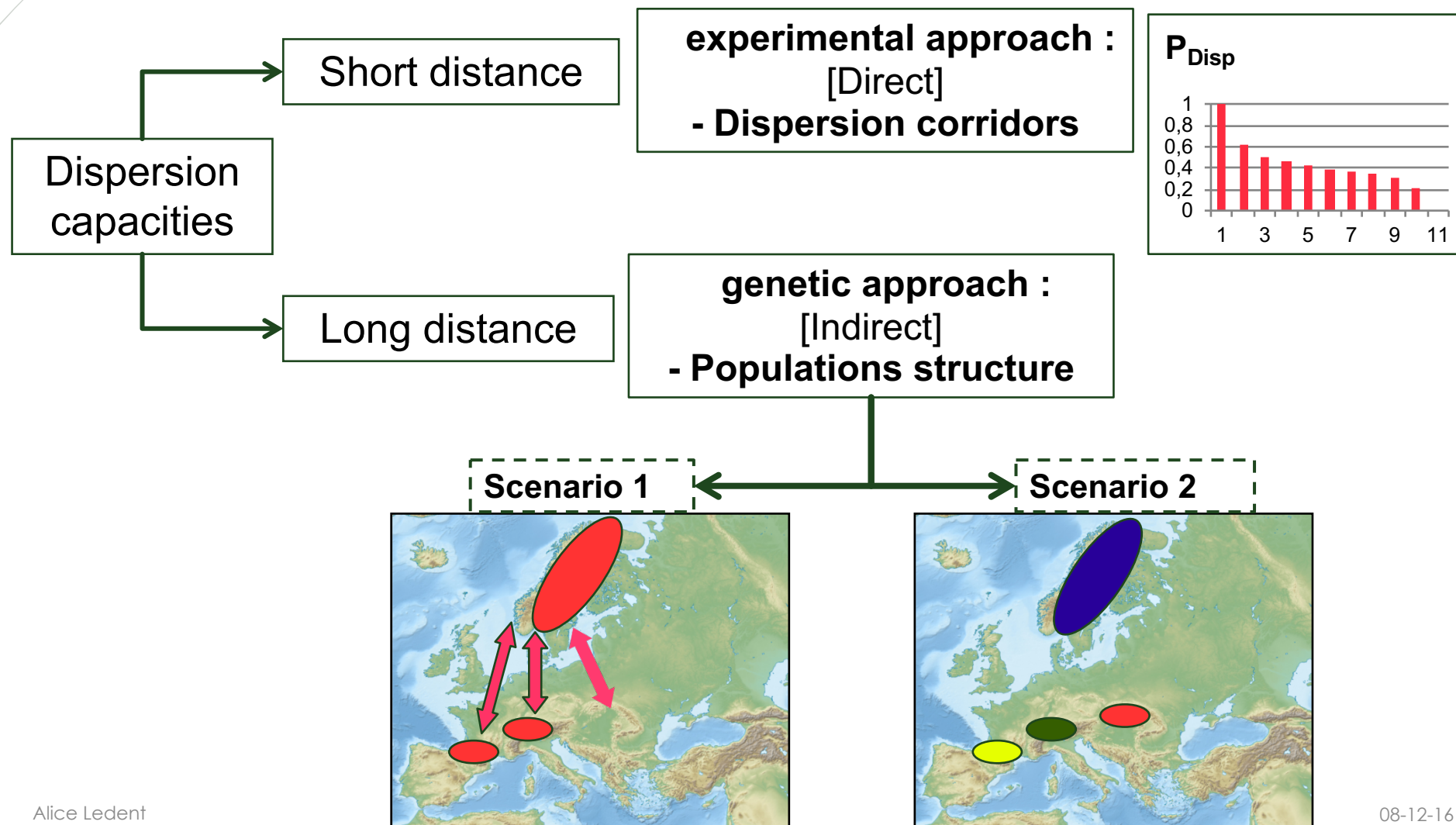gradient of suitability

➥ Pink = suitable but not
colonizable area

Corsinia coriandrina

Improved dynamic models

Short distance

experimental approach :
[Direct]
- Dispersion corridors

P_{Disp}

Dispersion capacities

Long distance

genetic approach :
[Indirect]
- Populations structure

Scenario 1

Scenario 2

Introduction and objectives

Introduction

- Understanding population connectivity is fundamental for biodiversity conservation and management
  - Different spatial scale
  - Different temporal scale

- Amazonian rainforest
  - One of the biggest biodiversity hotspot
  - 6 million square kilometers
  - High α–diversity but low β–diversity
  - Species broadly distributed

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- Bryophytes epiphytes
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  - Factors influencing distribution are variable
    - Niche segregation
    - Dispersal limitation
    - Historical factors
  - Mota & ter Steege (2015) : One Panmictic metapopulation
    - Null model analyze
    - Individuals randomly distributed across the Amazonian forest
    - No ecological mechanism driving their distribution
    - No modification because of global change and deforestation

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Objectives

- 1. Amazonian epiphytic bryophytes = one panmictic population ?
  - If not, isolation by distance pattern ?

- 2. Dispersal capacities sufficient to maintain genetic connectivity ?
  - To bridge their actual and future distribution of suitable areas ?
  - Context of human disturbance
  - Context of climate change

- 3. Historical biogeography
  - How the populations were affected by
    - Pleistocene climate oscillations ?
    - Recent habitat fragmentation ?

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      ➡ Population genetics

- 2. Dispersal capacities sufficient to bridge their actual and future distribution of suitable areas ?

      ➡ SDMs : dynamic models

- 3. Historical biogeography

      ➡ ABC

State of arts

Where are we ?

- 1. Amazonian epiphytic bryophytes = one panmictic population ?

  - Do Amazonian epiphytes exhibit population structure at regional (< 500 km) scales ?

    - Traditional population genetics : descriptive statistics (Fst, Nst,…)

  - At which spatial scale does genetic structuring occur ?

    - Molecular phylogeography : spatially explicit coalescence model (PhyoGeoSim 2.0)

  - Is niche conservatism rather than dispersal limitation the main driver of distribution across Amazonia ?

    - Partial Mantel test

What have we done ?

- Sampling
  - In the Amazonian forest
  - 14 species sampled
  - 301 individuals
  - Bryophytes(11) and liverworts (3)

- Extraction
  - Qiagen columns

- GBS sequencing
  - In progress

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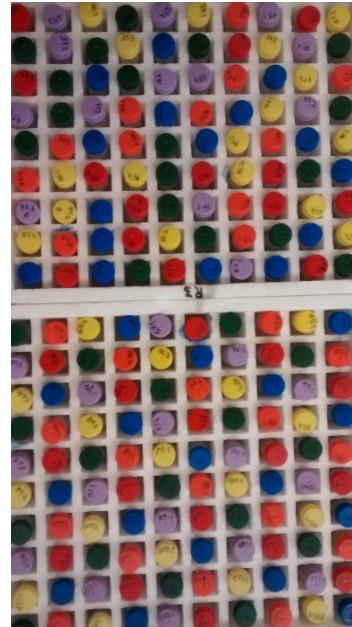
Acknowledgements

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- Dr. Jairo Patiño, Université de Lausanne

Thank you for your attention!

➡️ Questions ?