

**LA GÉNÉTIQUE DES POPULATIONS COMME OUTIL
D'ÉLUCIDATION DES RÉSERVOIRS DE PATHOGÈNES :
LEÇONS TIRÉES DE *PSEUDOGYMNOASCUS DESTRUCTANS*,
L'AGENT RESPONSABLE DE LA MALADIE DU NEZ BLANC
CHEZ LES CHAUVES-SOURIS**



Nicola M. Fischer, Andrea Altewischer, Surendra Ranpal, Serena E. Dool, Gerald Kerth, & Sébastien J. Puechmaille

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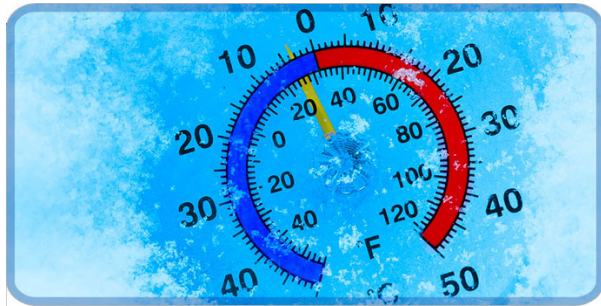


@SJPuechmaille

JOURNÉE PETITS MAMMIFÈRES
CBGP, MONTPELLIER, FRANCE

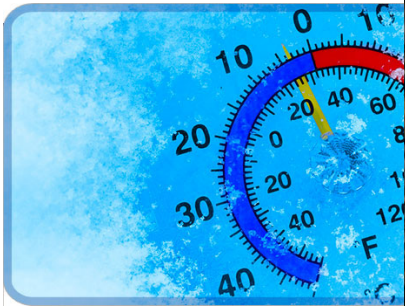
22/11/2021

WHITE NOSE DISEASE (WND)



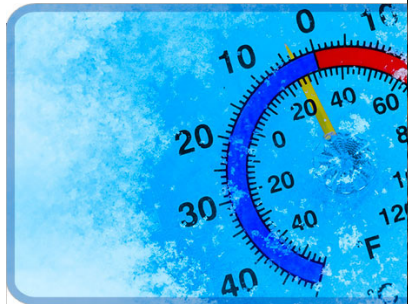
Puechmaile et al. 2011, Trends Ecol. Evo.

WHITE NOSE DISEASE (WND)



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WHITE NOSE DISEASE (WND)

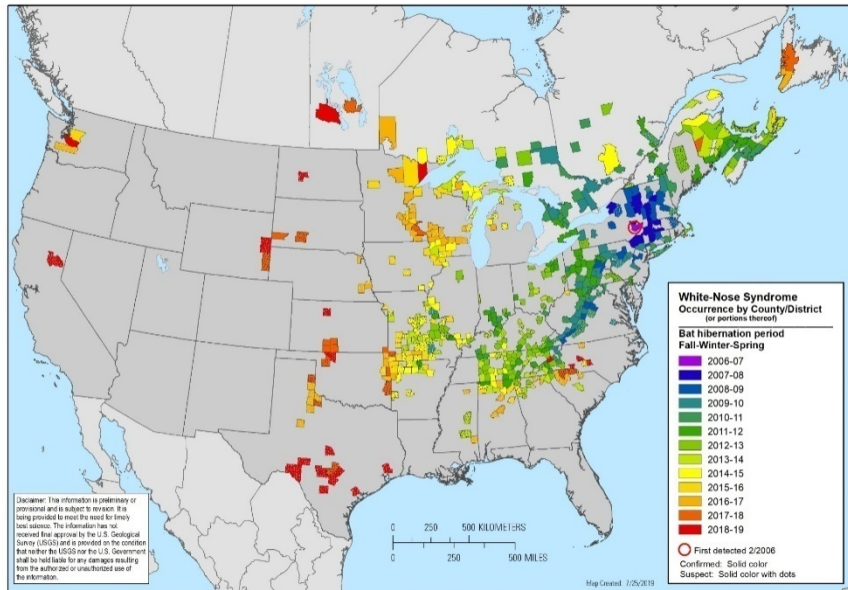
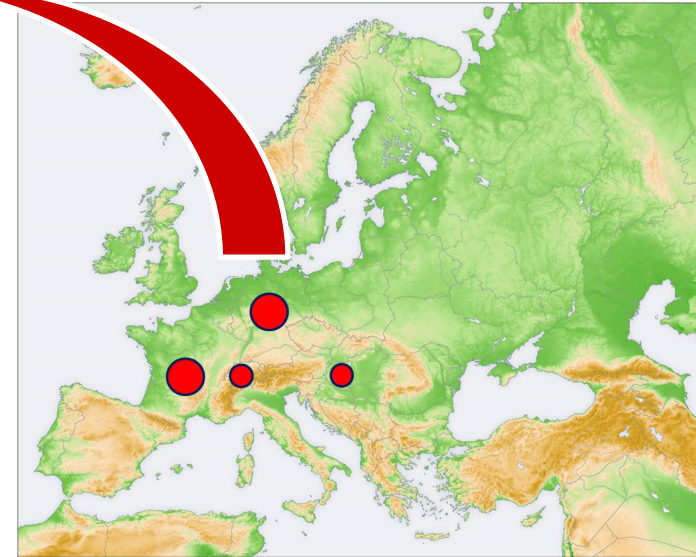
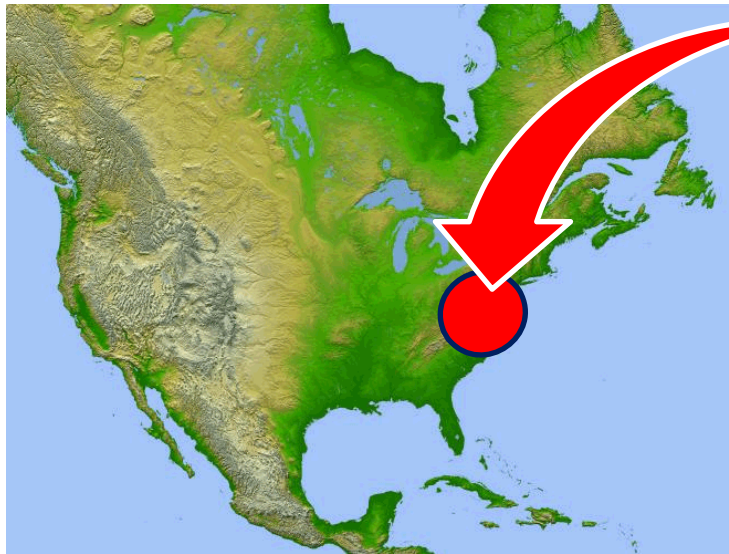


Pre-WNS count (Year)	Post-WNS count (Year)	Overall decline
2276 (2008)	1 (2010)	100.0%
1511 (2005)	1 (2008)	99.9%
9432 (2003)	24 (2010)	99.7%
1722 (2009)	5 (2010)	99.7%
1604 (2006)	8 (2010)	99.5%
720 (2004)	6 (2010)	99.2%
183 542 (2000)	2049 (2010)	98.9%
1102 (2004)	22 (2009)	98.0%
953 (1999)	22 (2010)	97.7%
1213 (2005)	37 (2010)	96.9%

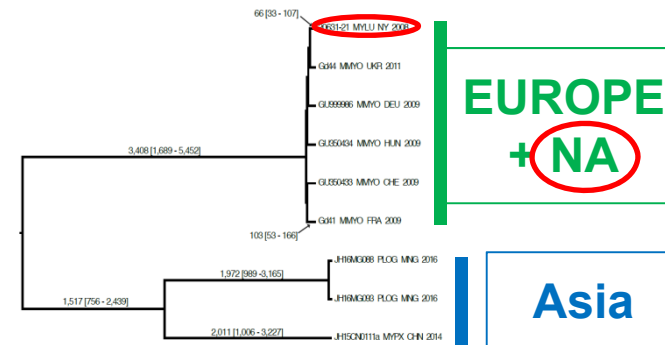


Puechmaille et al. 2011, Trends Ecol. Evo.; Fritze & Puechmaille 2018, Mamm. Rev.

A EUROPEAN ORIGIN OF THE FUNGUS



Citation: White-nose syndrome occurrence map - by year (2019). Data Last Updated: 7/25/2019. Available at: <https://www.whitenosesyndrome.org/static-page/wns-spread-maps>.

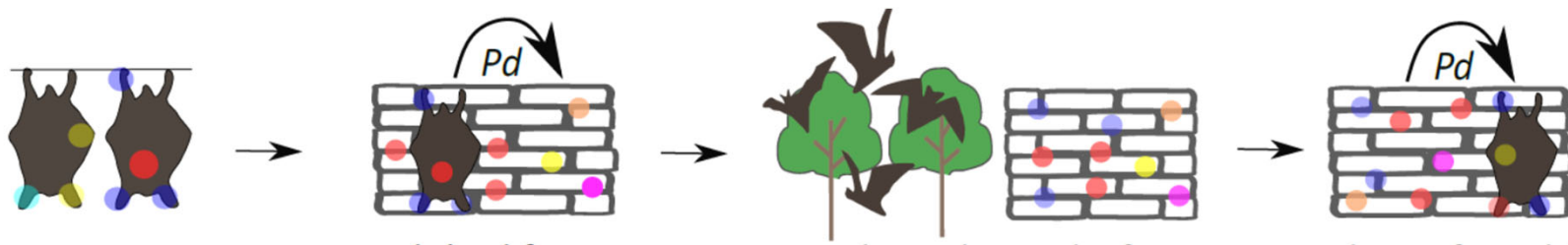


Puechmaille et al. 2010, *Emerg. Infec. Dis.*
 Leopardi et al. 2015, *Curr. Biol.*
 Drees et al. 2017, *mBio*

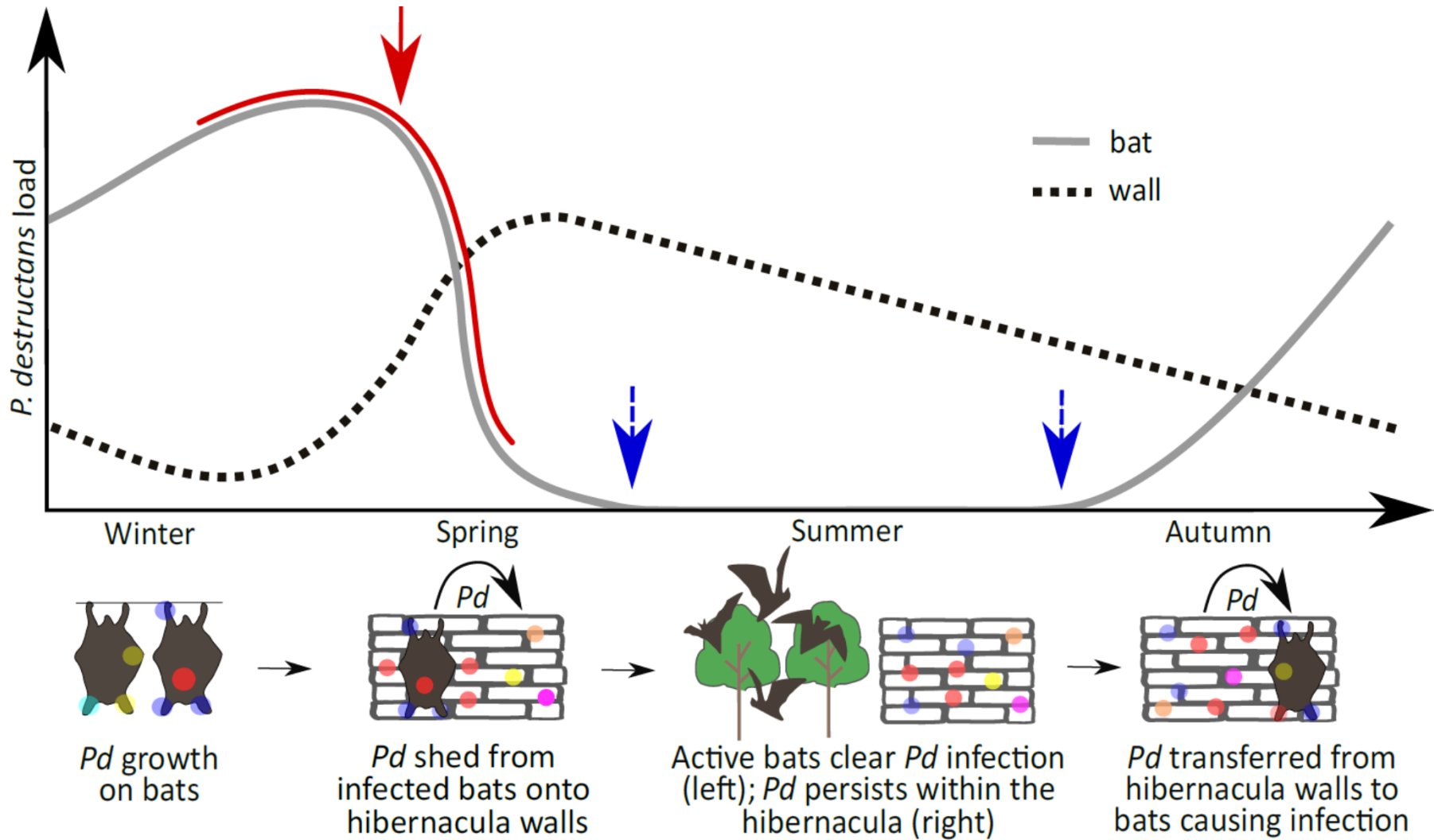
OBJECTIVES OF THE STUDY

Answer key questions regarding the infection of bats:

- where & when do bats get infected?
- what is the environmental reservoir from which bats get infected?
- what is the infection dose?

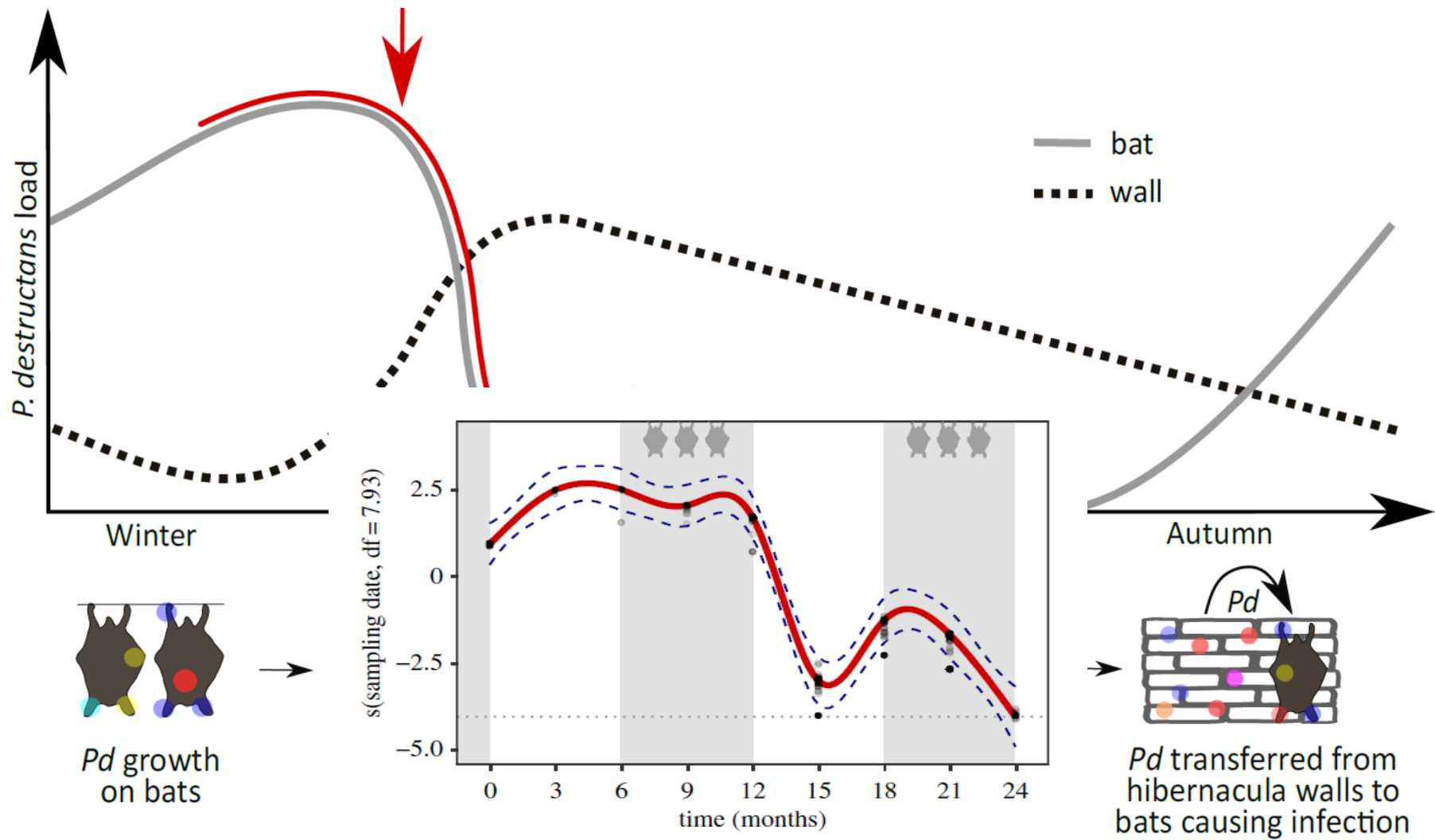


THE CONTEXT



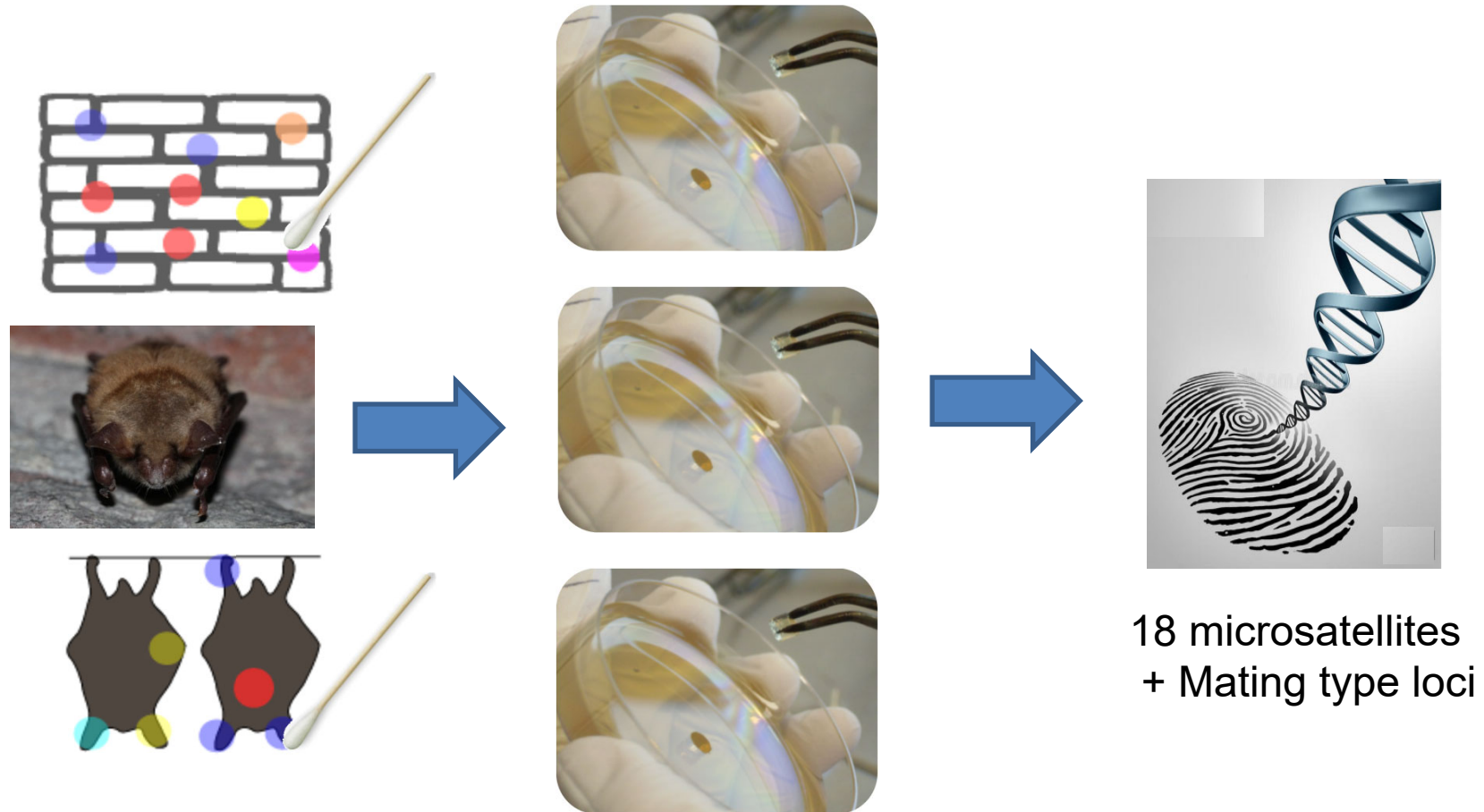
Fischer et al. 2021, Mol. Ecol.

THE CONTEXT



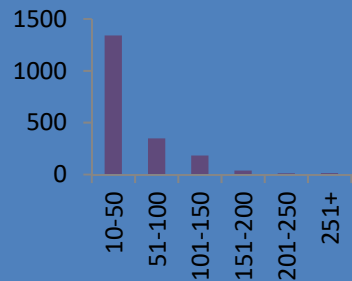
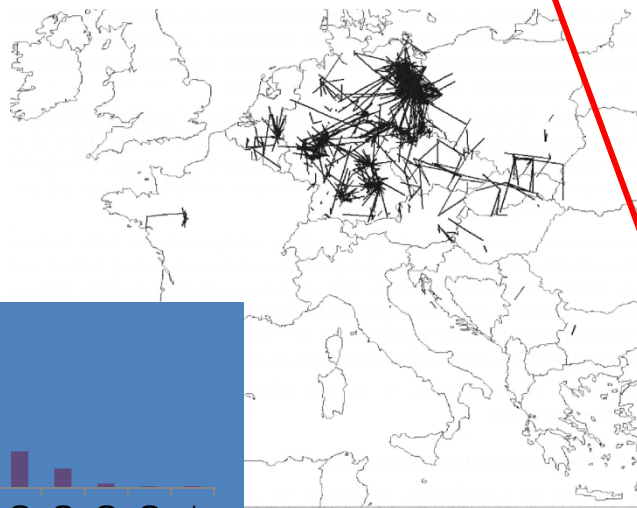
Fischer et al. 2020, Biol. Lett; Fischer et al. 2021, Mol. Ecol.

METHODS

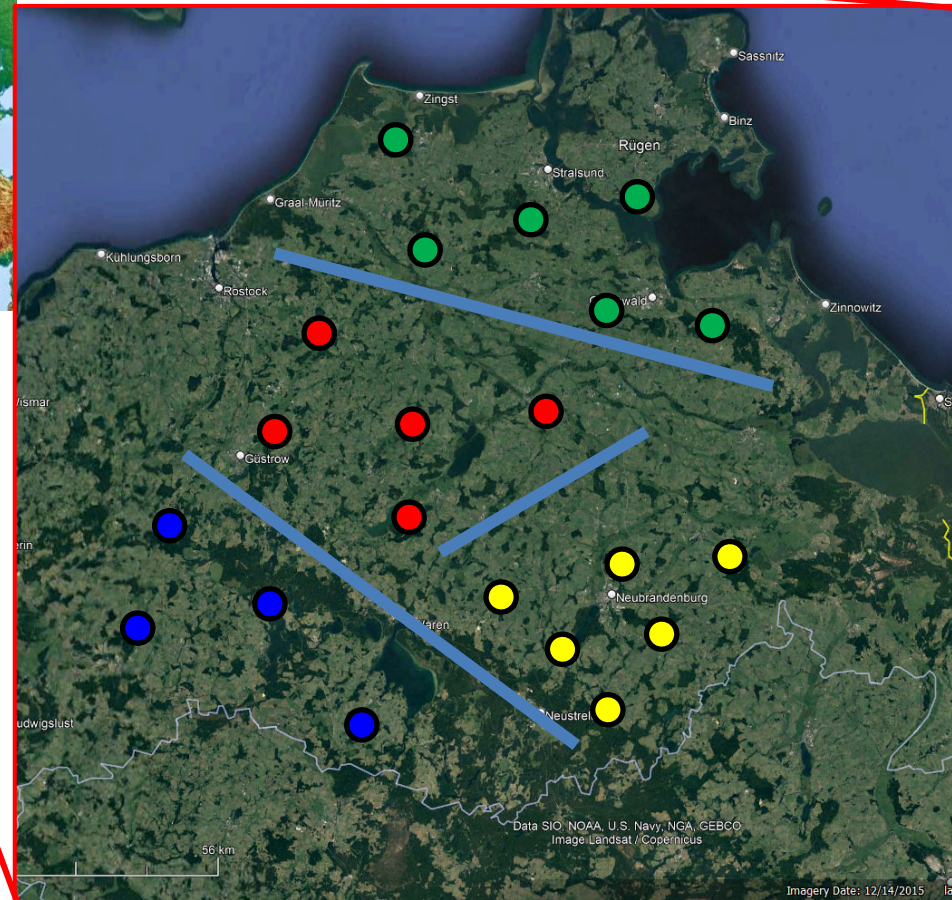


Drees et al. 2017, J. Dis. Wild; Dool et al. 2020, Cons. Genet Res.

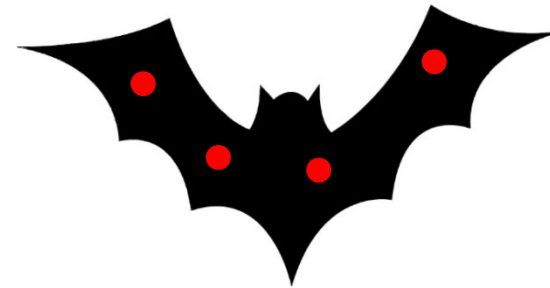
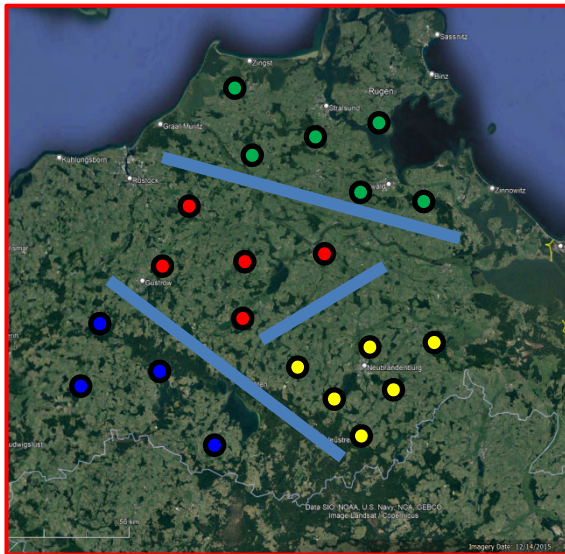
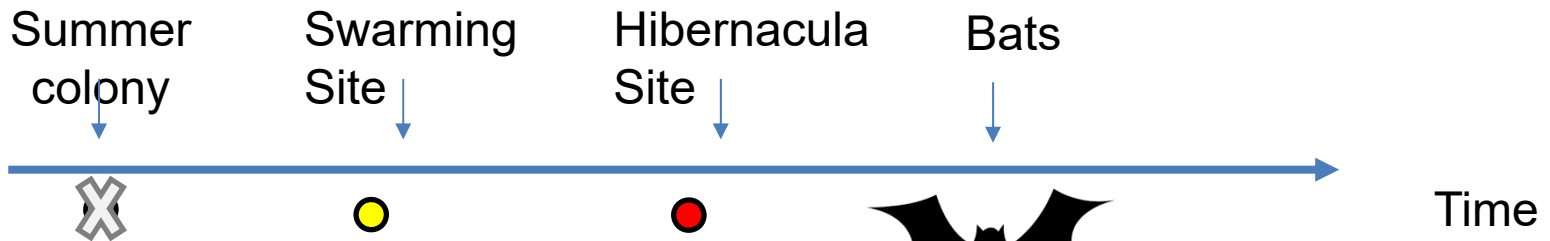
WHERE & WHEN DO BATS GET INFECTED?



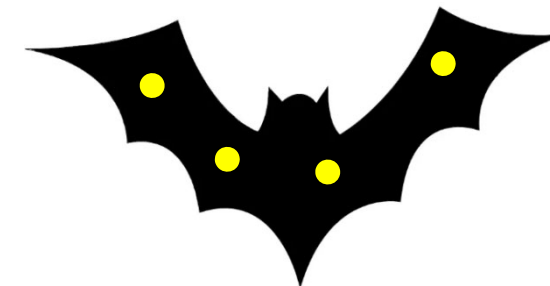
Distance movements of *Myotis myotis* in Europe (n=3273).



WHERE & WHEN DO BATS GET INFECTED?

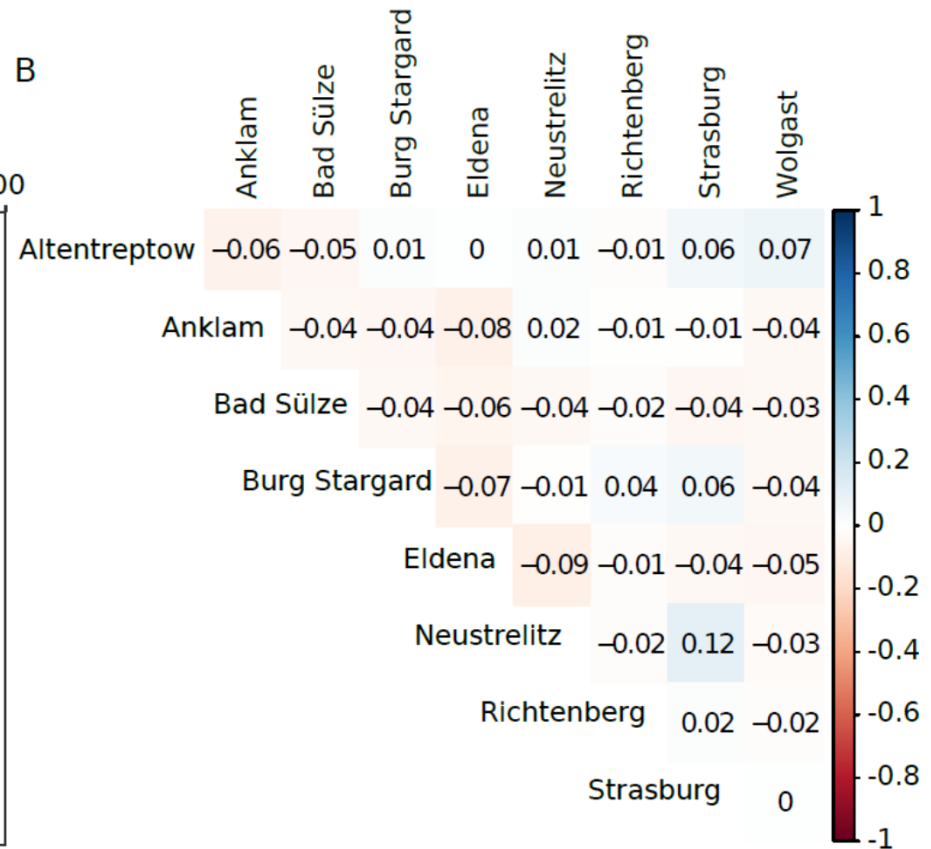
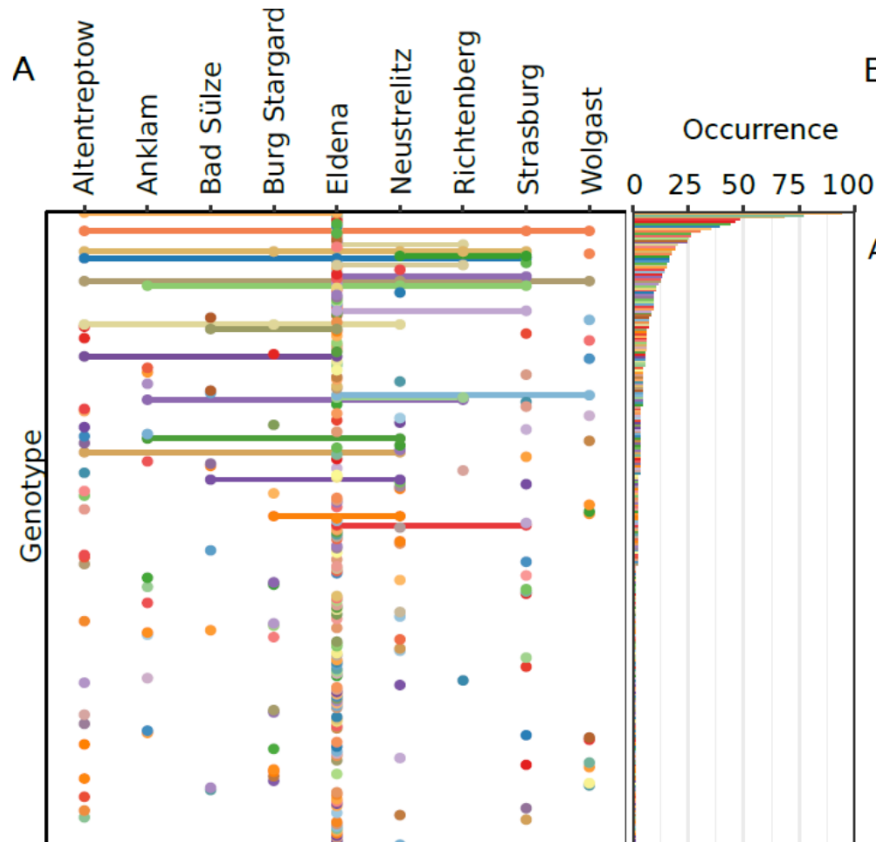
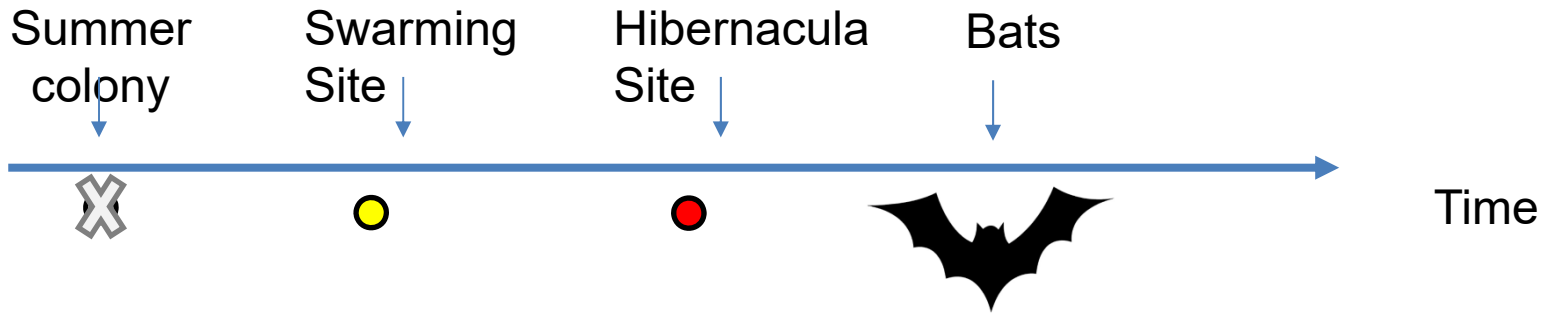


Infection during hibernation

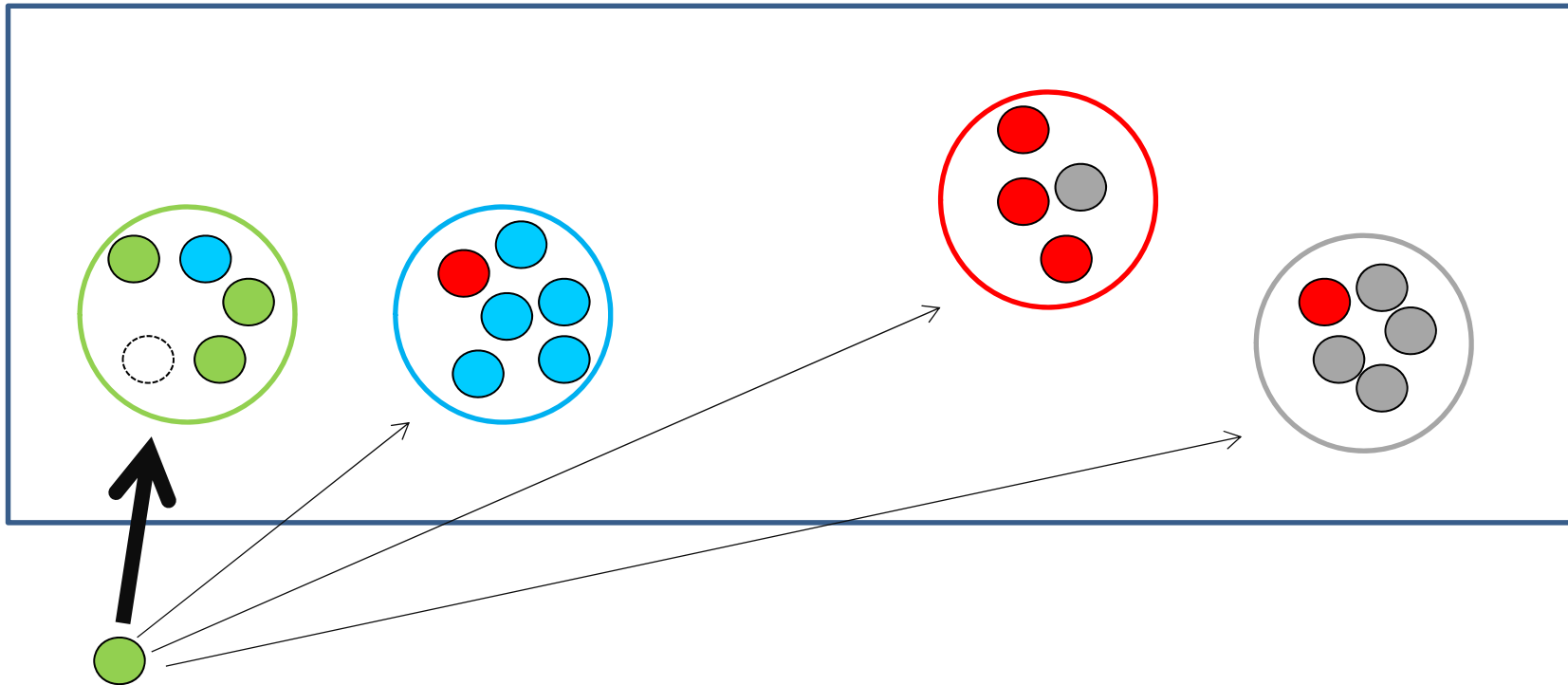


Infection during Swarming

WHERE & WHEN DO BATS GET INFECTED?



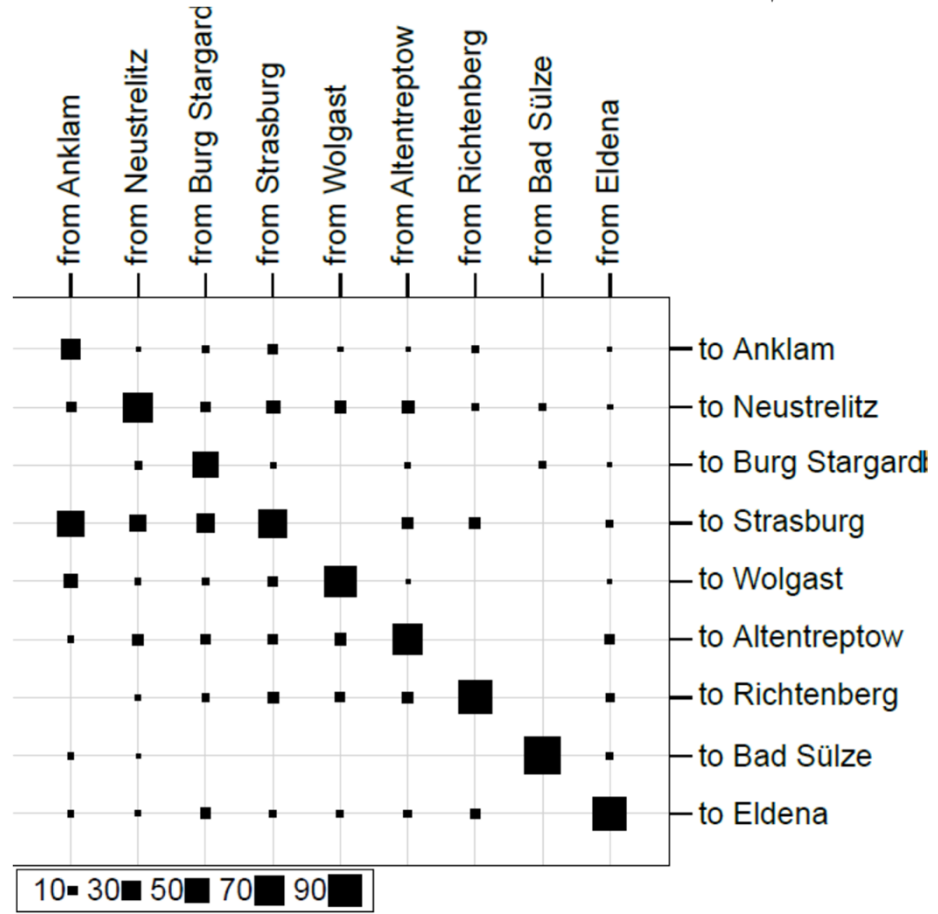
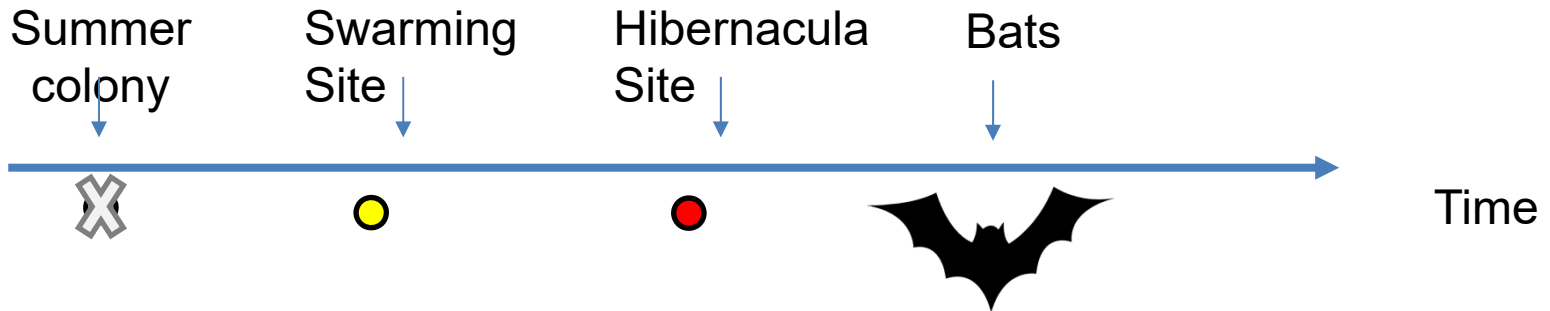
GENETIC ASSIGNMENT



Reassignment to the correct site

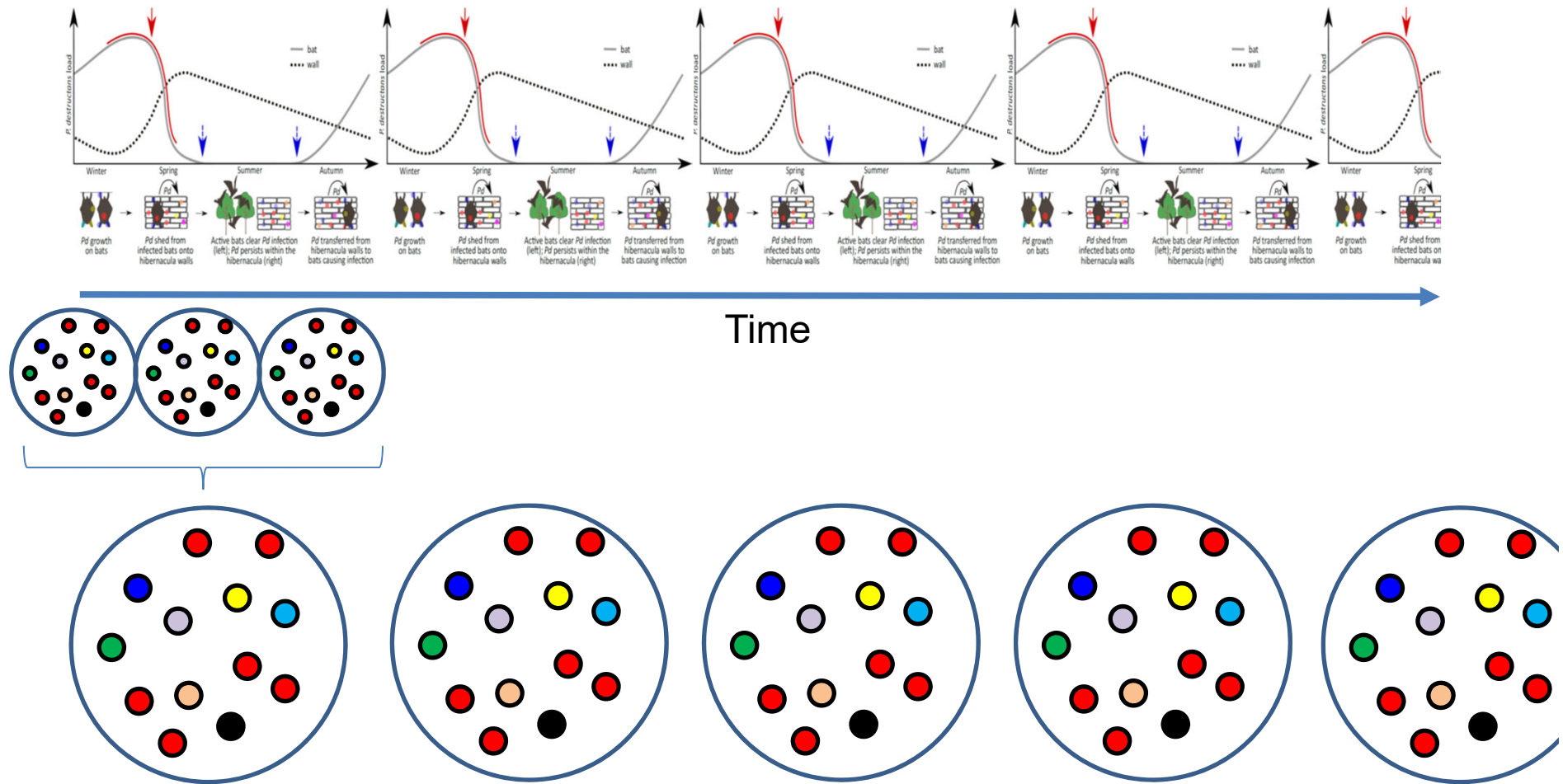
e.g. DAPC, Discriminant Analysis of Principal Components

WHERE & WHEN DO BATS GET INFECTED?

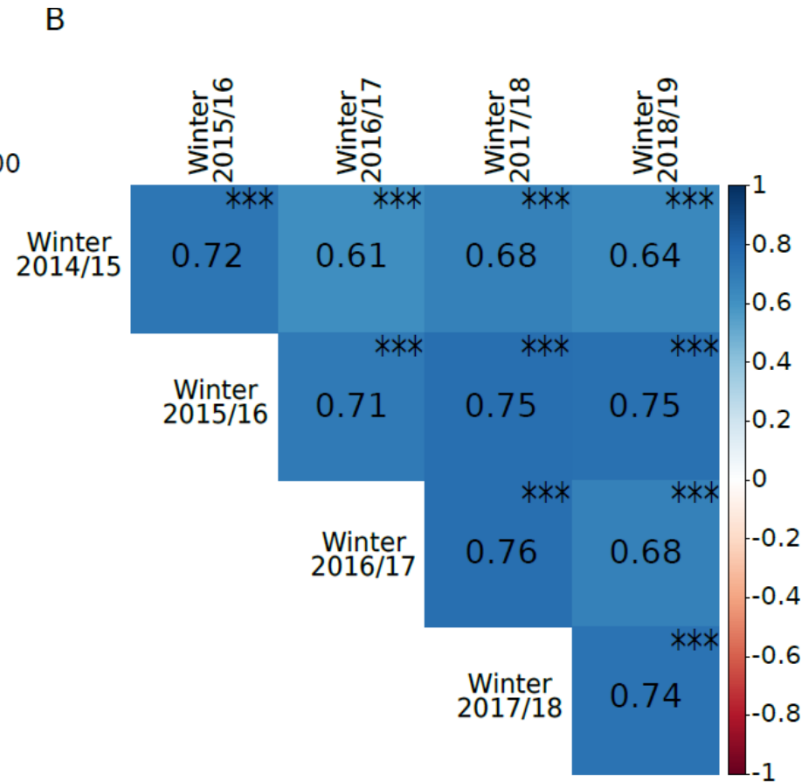
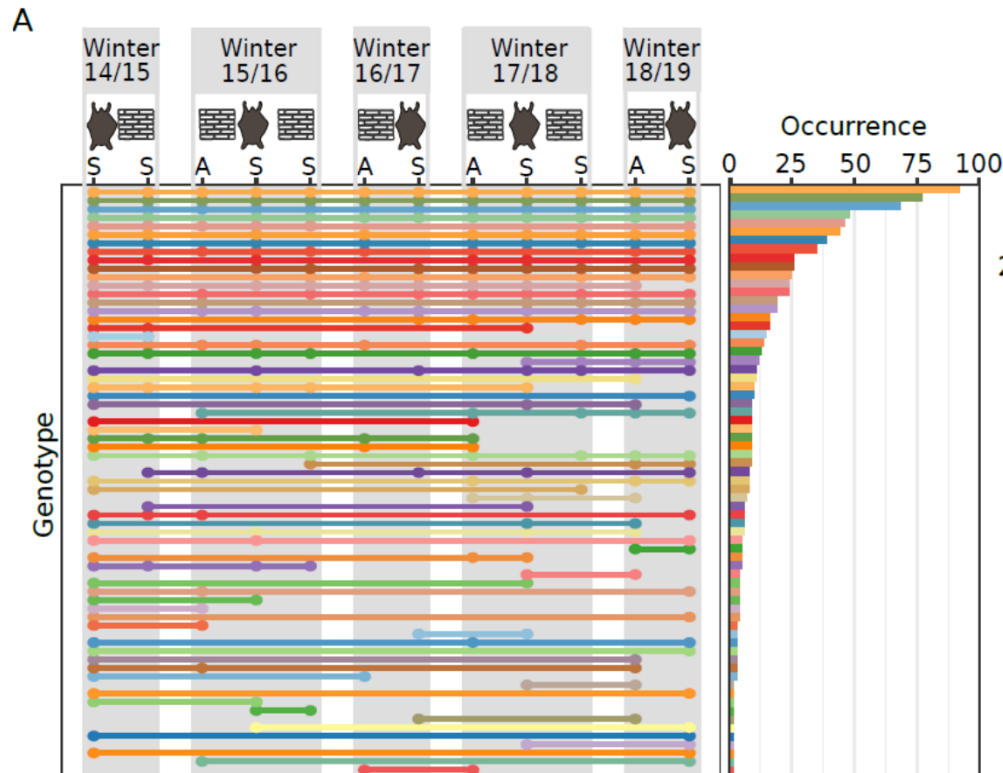
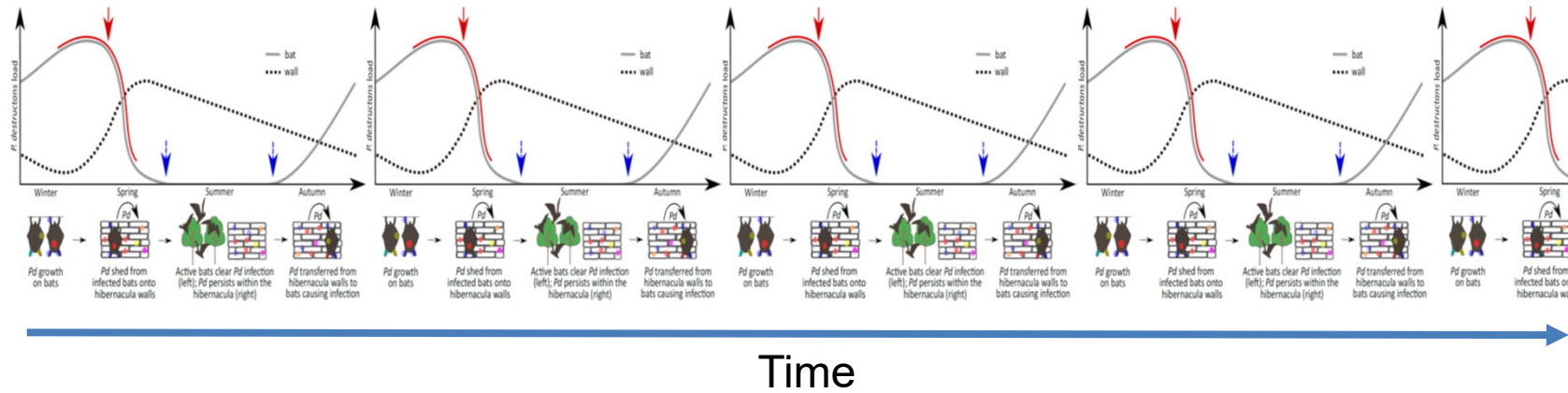


THE ENVIRONMENTAL RESERVOIR

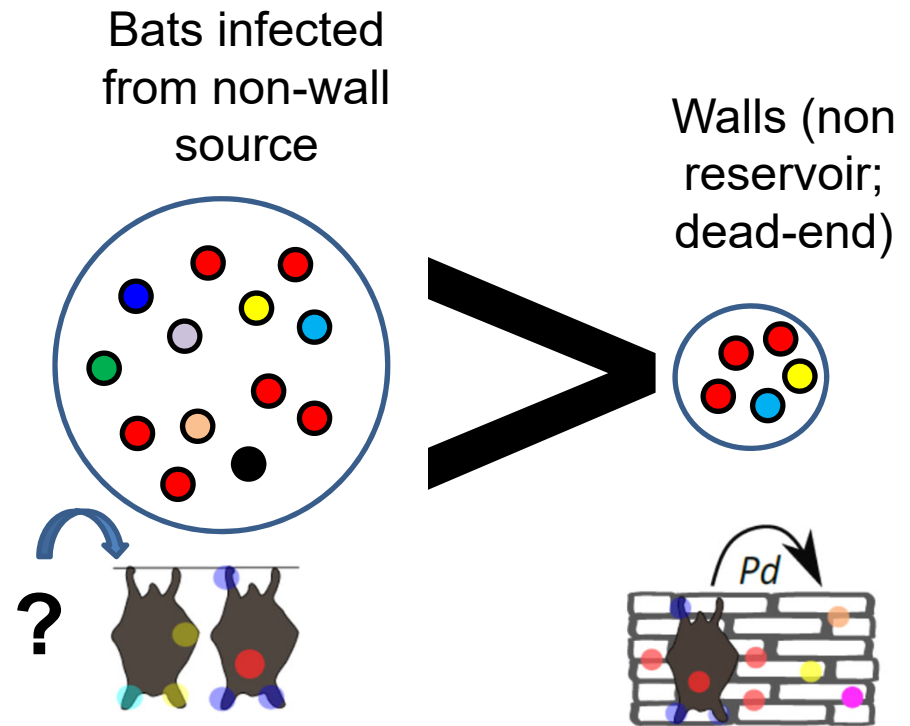
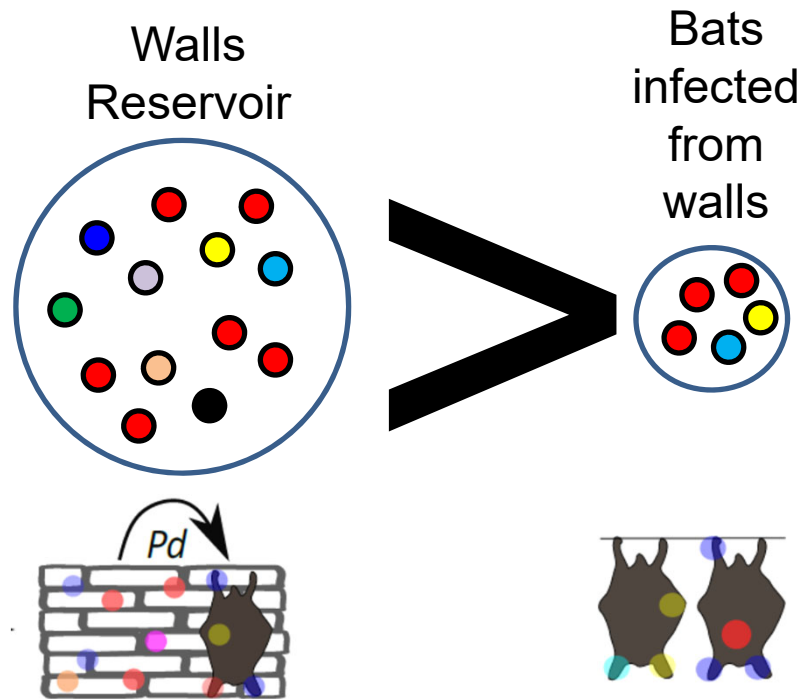
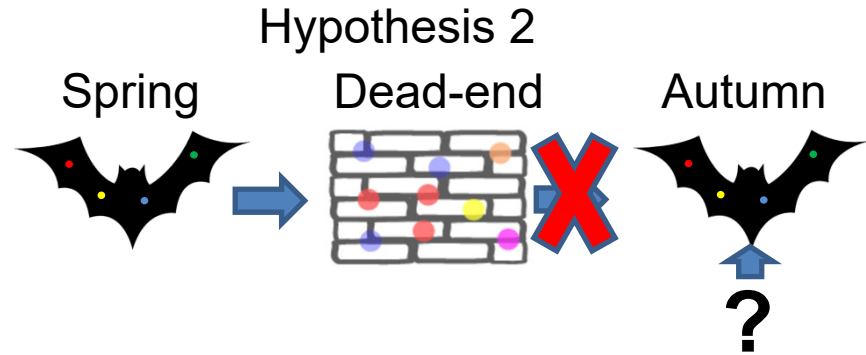
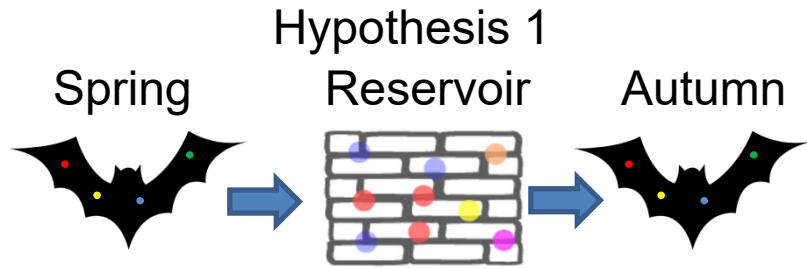
ELDENA; 2015-2019; 1062 isolates



THE ENVIRONMENTAL RESERVOIR

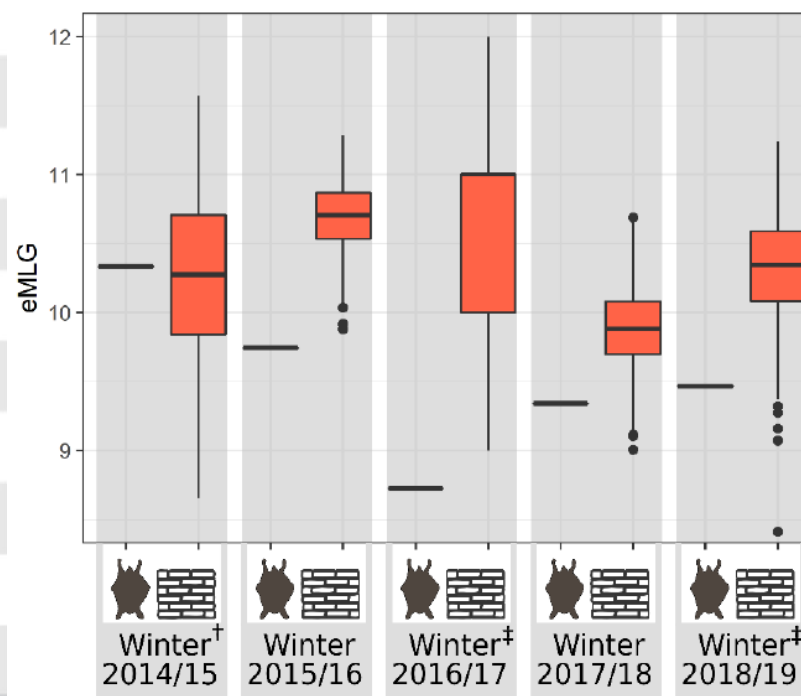


THE ENVIRONMENTAL RESERVOIR



THE ENVIRONMENTAL RESERVOIR

Winter and substrate	Swabs	Isolates
2014/15 bats	139	391
2014/15 walls ^a	8	34
2015/16 bats	31	80
2015/16 walls	25	77
2016/17 bats	10	26
2016/17 walls ^b	6	22
2017/18 bats	53	149
2017/18 walls	24	89
2018/19 bats	53	142
2018/19 walls ^b	15	52

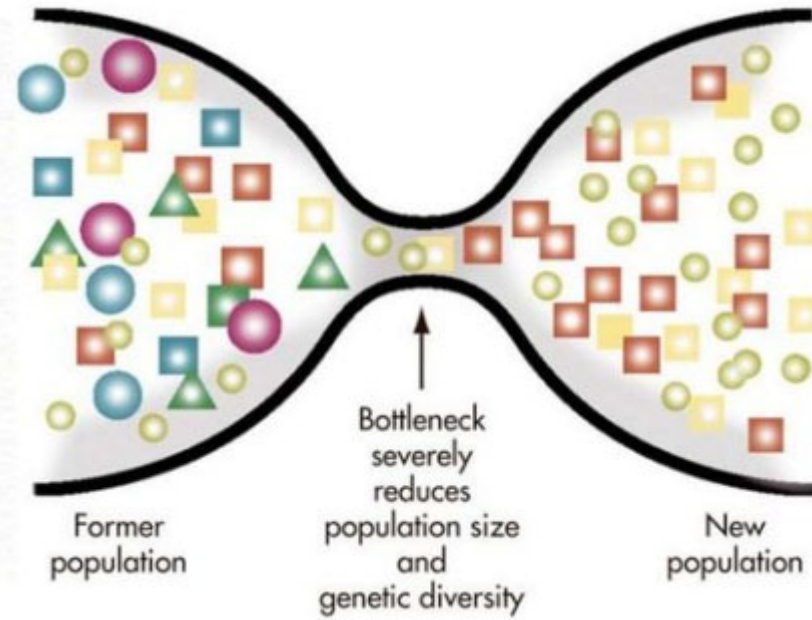
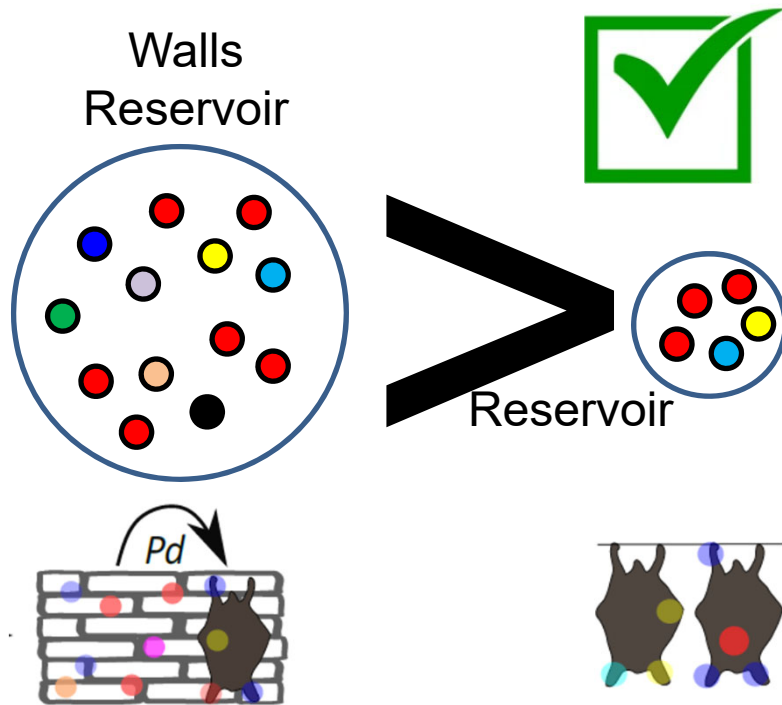


^aContains isolates from wall sampling in April only.

^bContains isolates from wall sampling in October only.

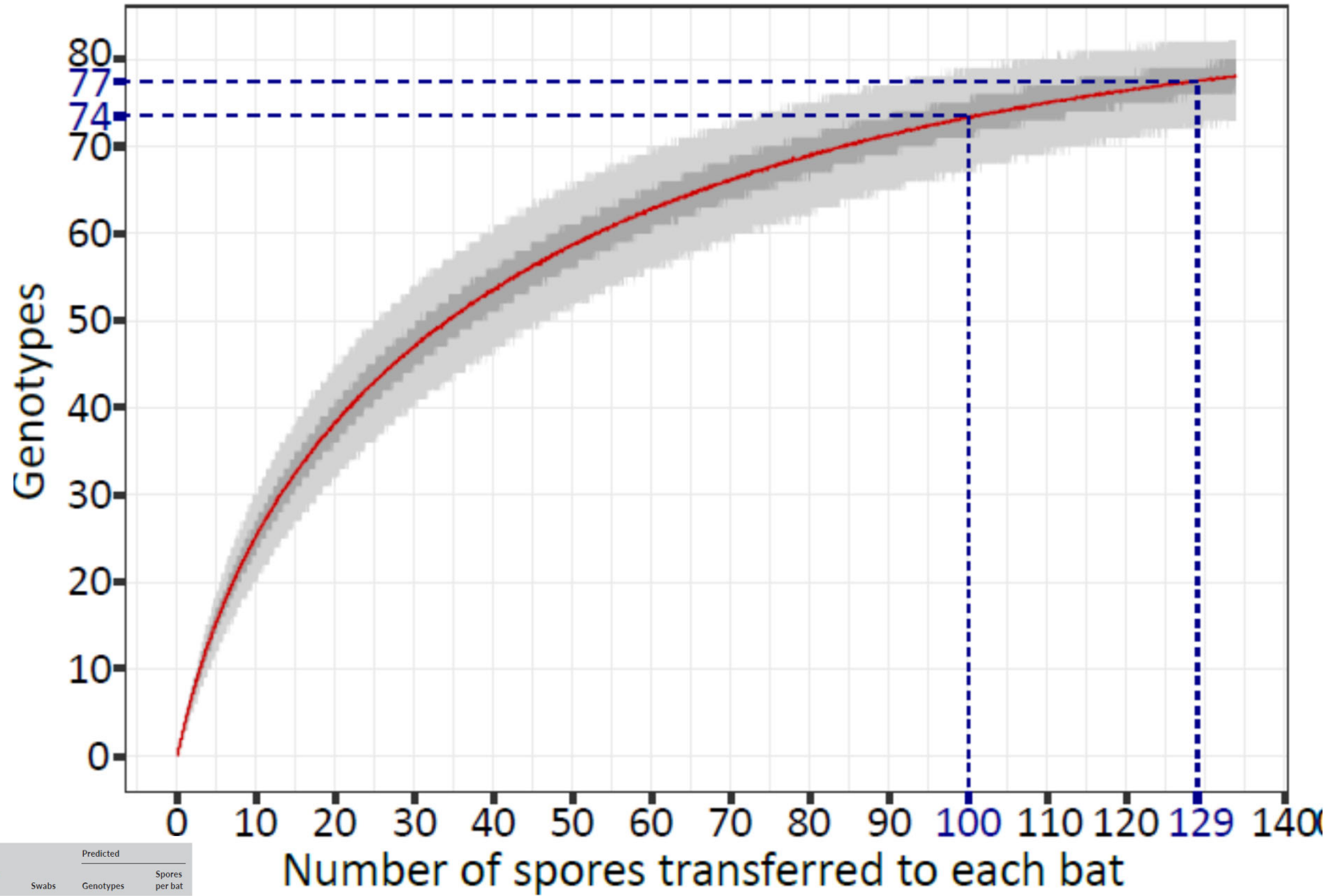
To test for the difference in genotypic richness (eMLG) between bats and walls of each year a one-sided, paired t test was calculated ($t = -2.83$, $df = 4$, $p = .038$)

THE INFECTION DOSE



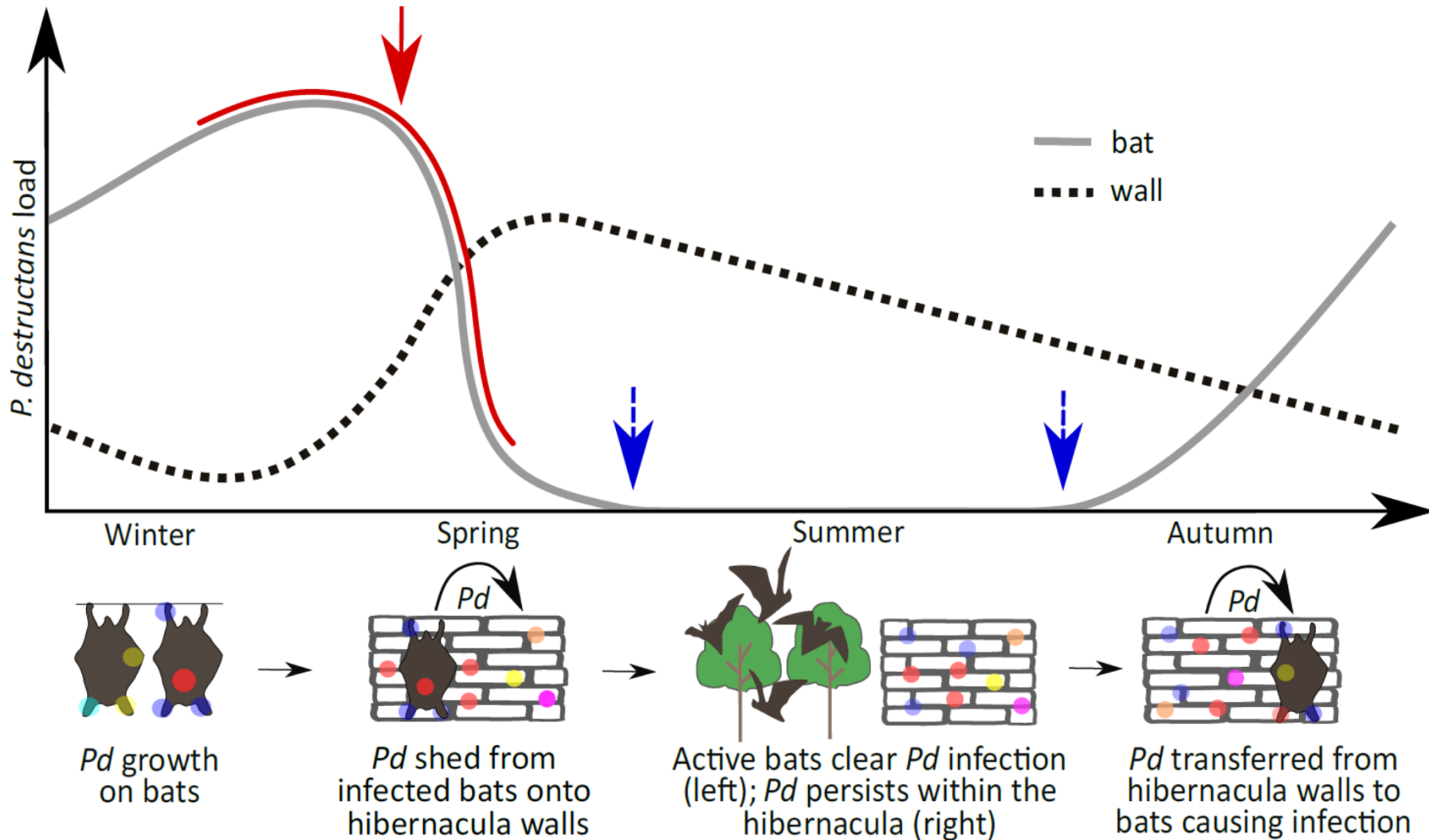
Infected host population

THE OBJECTIVES



Winter season (bat samples only)	Swabs	Predicted	
		Genotypes	Spores per bat
Winter 14/15	139	74-77	100-129
Winter 15/16	31	35-48	74-143
Winter 16/17	10	19-52	88-530
Winter 17/18	53	37-41	48-61
Winter 18/19	53	47-56	77-121

CONCLUSION



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Received: 8 February 2021 | Revised: 19 October 2021 | Accepted: 21 October 2021

DOI: 10.1111/mec.16249

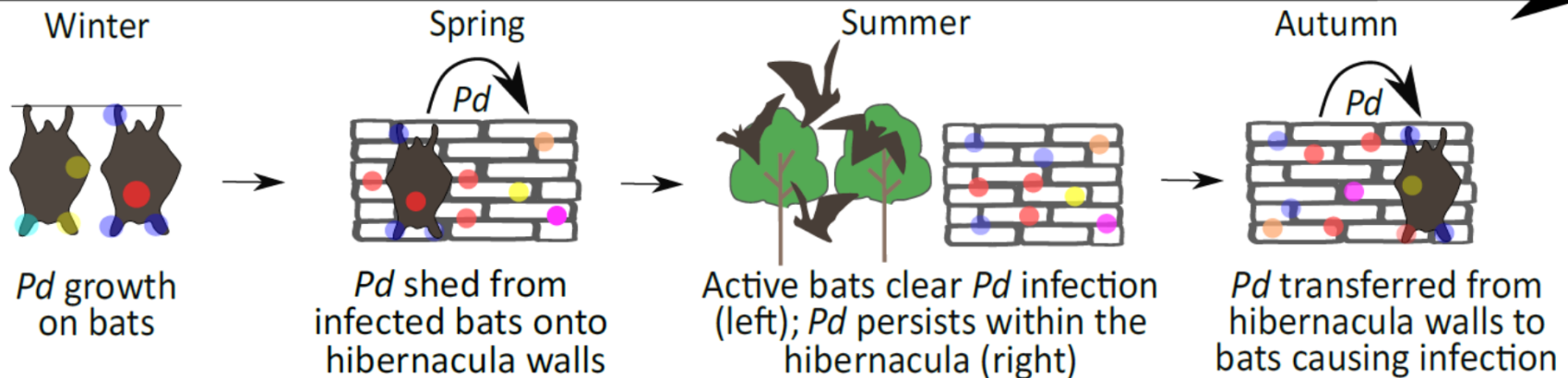
ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Population genetics as a tool to elucidate pathogen reservoirs: Lessons from *Pseudogymnoascus destructans*, the causative agent of White-Nose disease in bats

Nicola M. Fischer^{1,2} | Andrea Altewischer¹ | Surendra Ranpal¹ | Serena Dool^{1,3} |
Gerald Kerth¹ | Sebastien J. Puechmaille^{1,2,4}

P. destructans load



Acknowledgements

Jens Berg, Anne Petzold, Holger Schütt, Dirk Karoske, Thorsten Blohm and Axel Griesau of NABU Mecklenburg Vorpommern bat conservation group for facilitating access to the sampling sites and Dagmar Brockmann for sharing information on bat movements between the studied hibernacula and Frauke Meier for helpful advice on swarming literature.

Ina Römer, Silke Fregin, Ruth-Marie Stecker and Marcus Fritze for their help relating to labwork and sample collection.



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batcon.org
BAT CONSERVATION
INTERNATIONAL

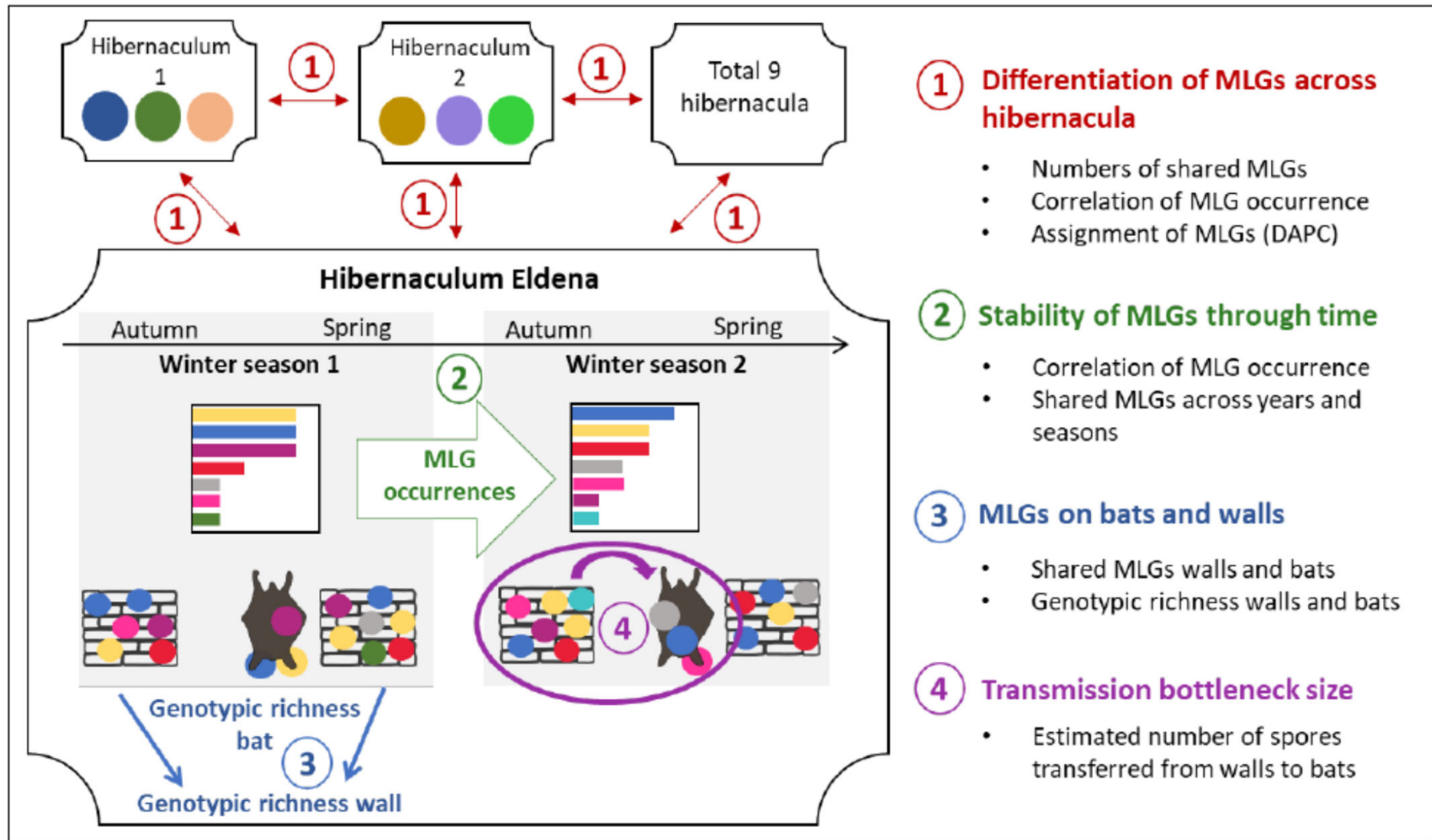


Deutsche
Forschungsgemeinschaft

DFG

DFG

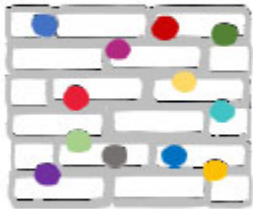
THE CONTEXT



Questions:

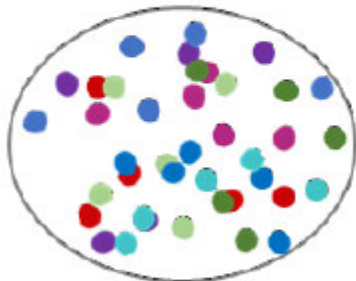
SUBSAMPLING INFECTION DOSE

All genotyped spores
from walls (**pool 0**).



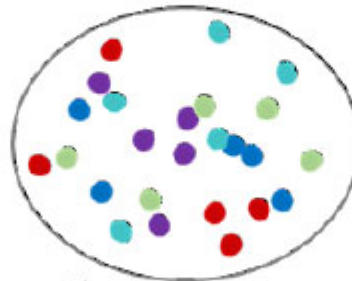
Bottleneck
Subsampling of N spores
with replacement from
pool 0. N varied
between 2 and 3100.

Pool 1



Germination
Subsampling of 17.5% of
the spores from pool 1
without replacement.

Pool 2



Quantification
Number of unique
MLGs from pool 2
(calculated separately
for every value of N).



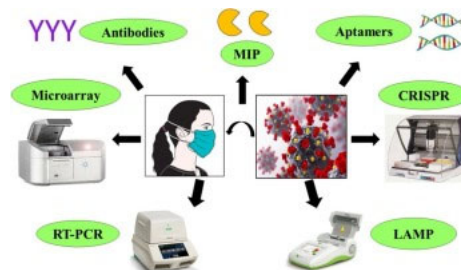
1000 times per N
(bottleneck size).

MONITORING DISEASES

Diseases are generally known to:

- Regulate populations (Anderson & May 1979, Nature)
- Drive populations to extinction (De Castro & Bolker 2004, Ecol. Lett.)

Monitoring disease outbreaks and dynamics is important



HYBRID INVASIVE SPECIES, THE CHYTRID

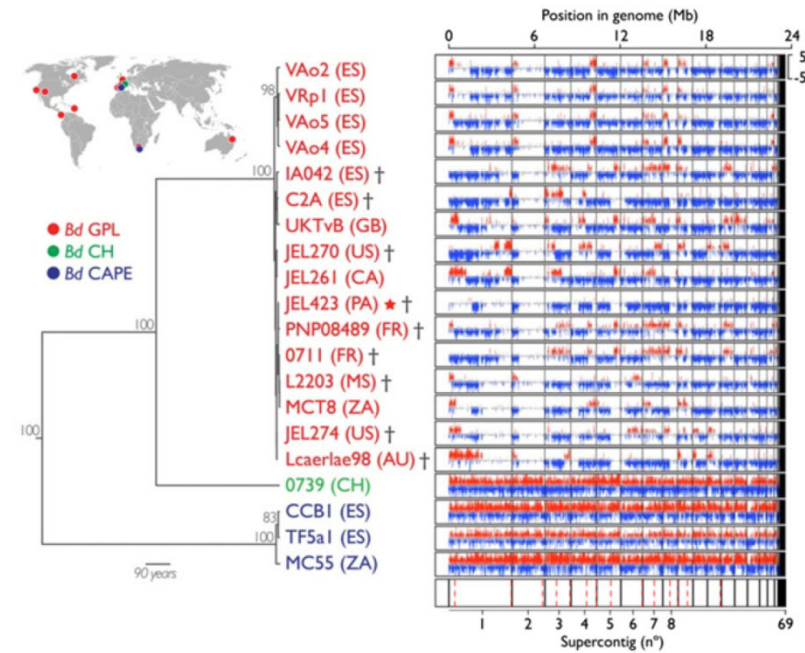


Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage

Rhys A. Farrer^{a,b,1}, Lucy A. Weinert^a, Jon Bielby^b, Trenton W. J. Garner^b, Francois Balloux^a, Frances Clare^{a,b}, Jaime Bosch^c, Andrew A. Cunningham^b, Che Weldon^d, Louis H. du Preez^d, Lucy Anderson^b, Sergei L. Kosakovsky Pond^e, Revital Shahar-Golan^a, Daniel A. Henk^a, and Matthew C. Fisher^a



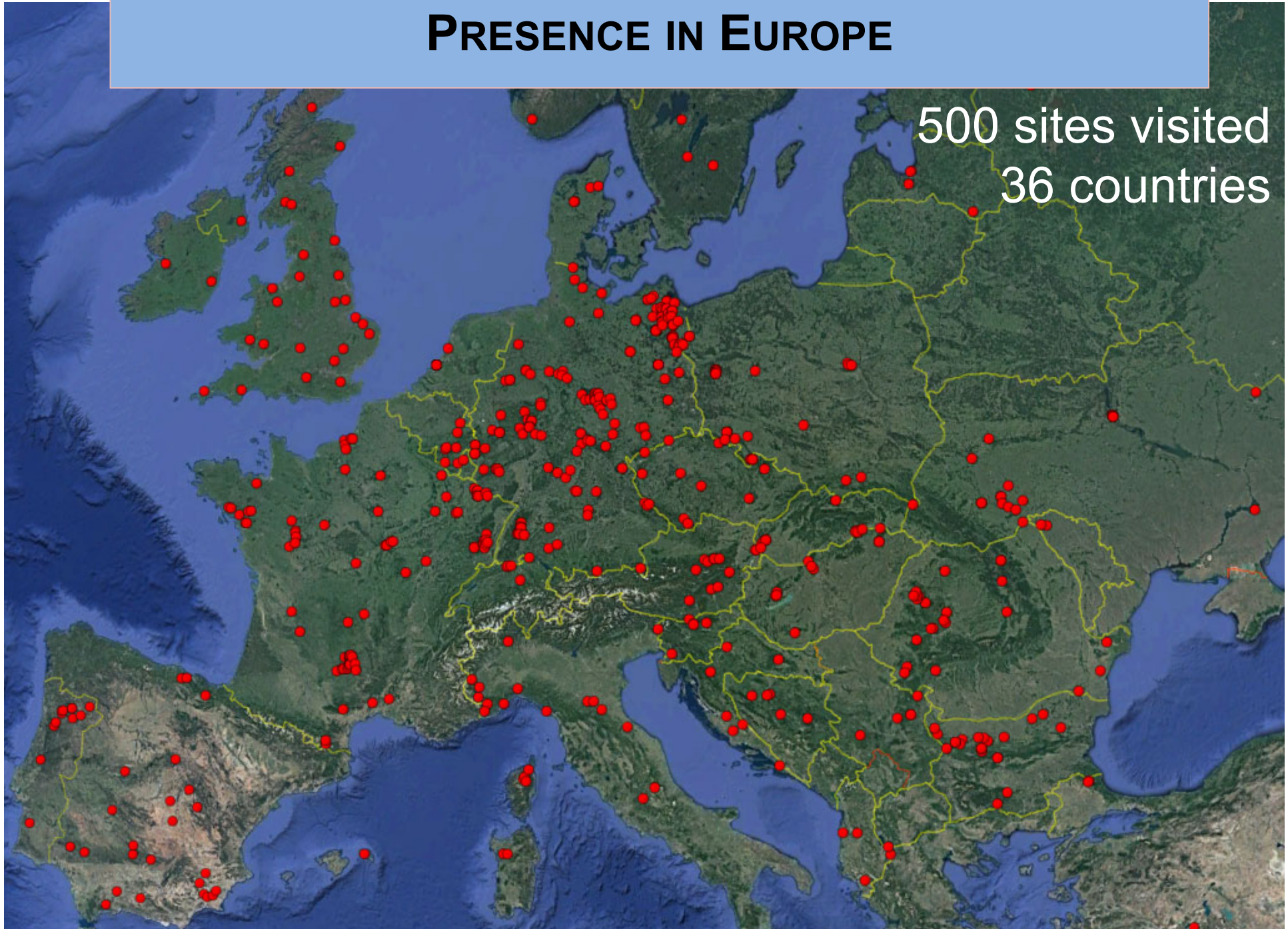
Batrachochytrium dendrobatidis



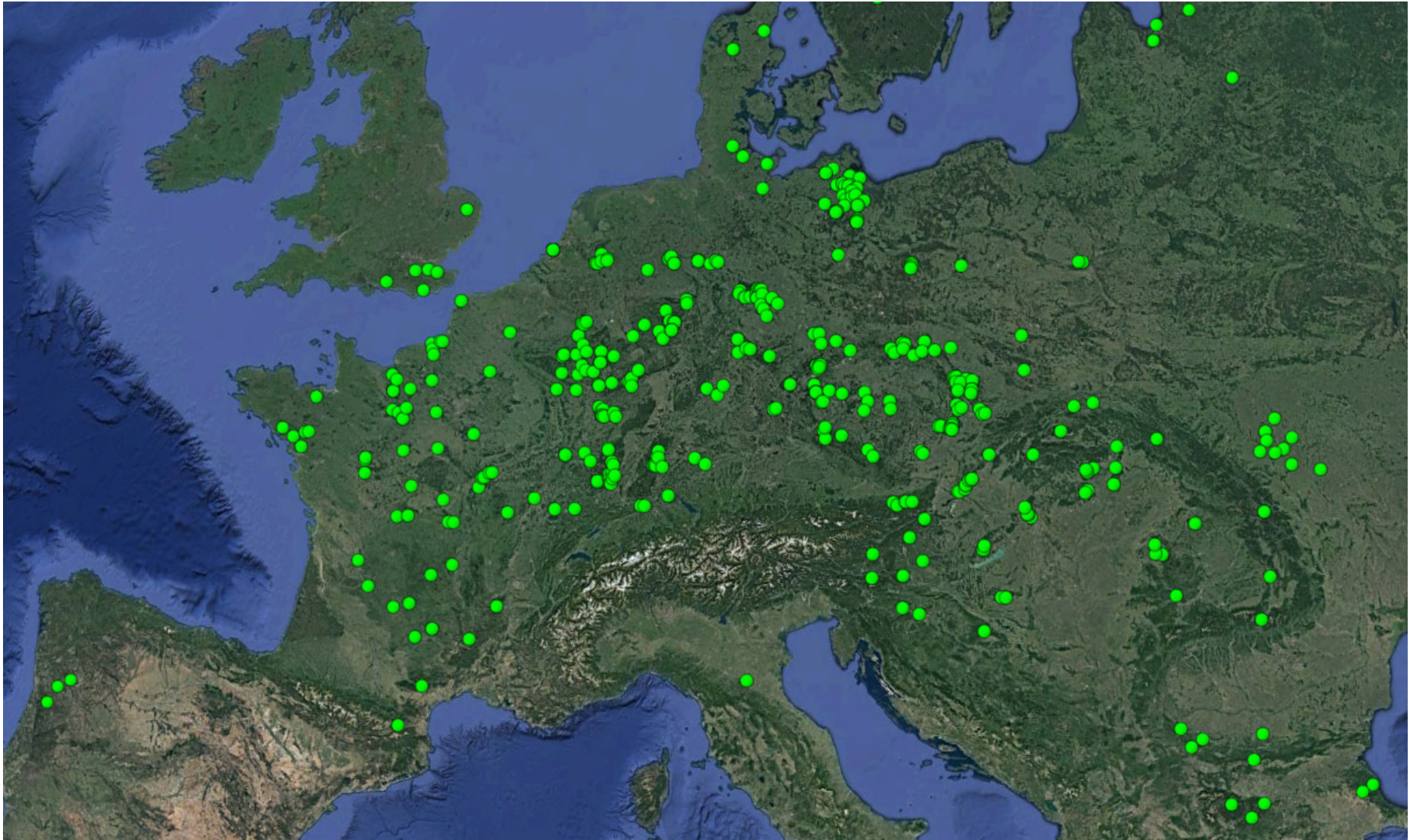
GPL:
Global Panzootic Lineage

PRESENCE IN EUROPE

500 sites visited
36 countries



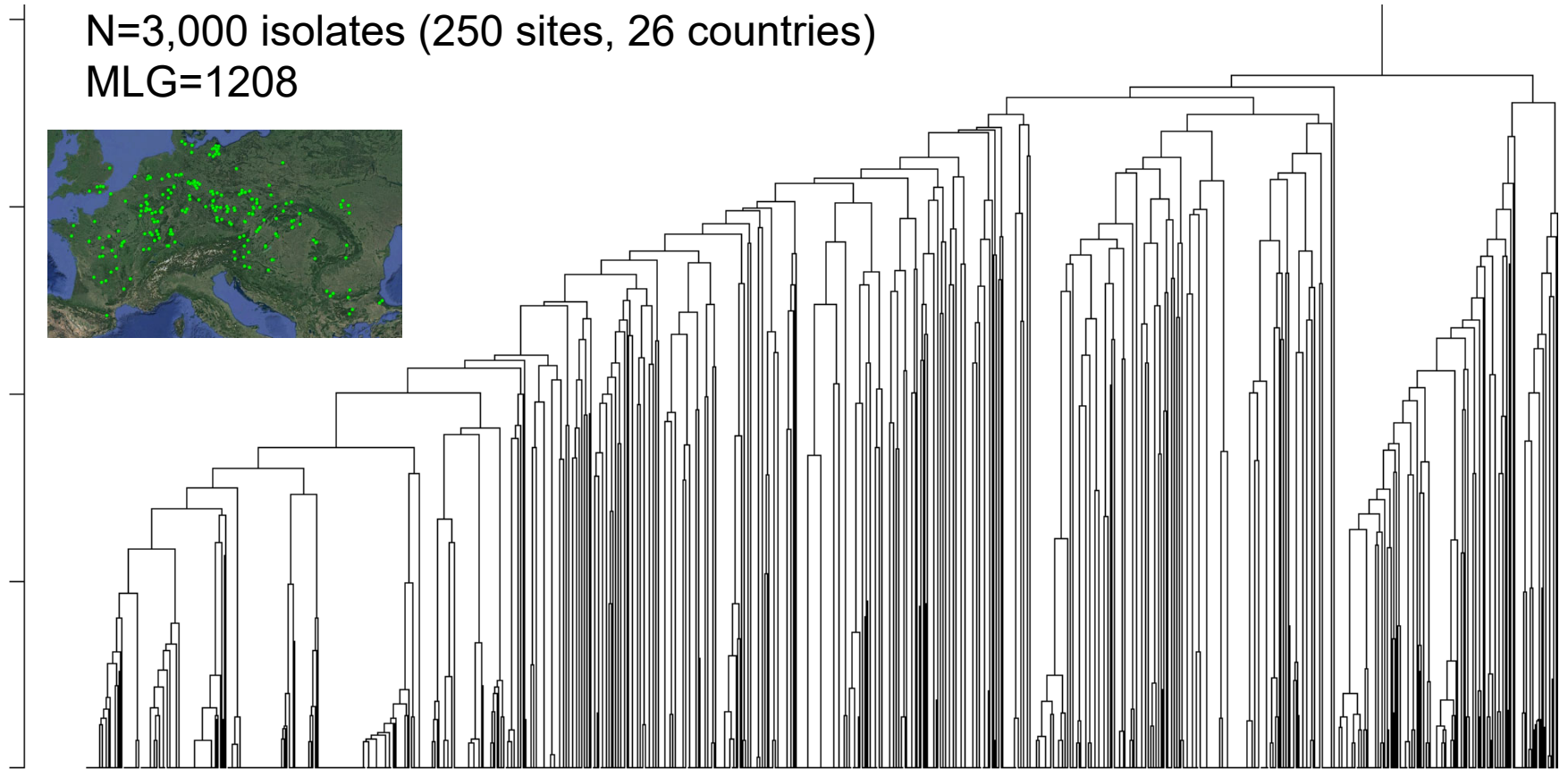
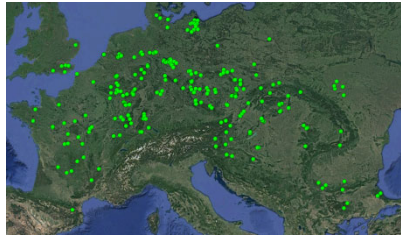
PAN EUROPEAN SAMPLING



Area: 2,000 x 2,500 km = 5 million km² (454 sites with *Pd*)

EUROPEAN POPULATION STRUCTURE

N=3,000 isolates (250 sites, 26 countries)
MLG=1208

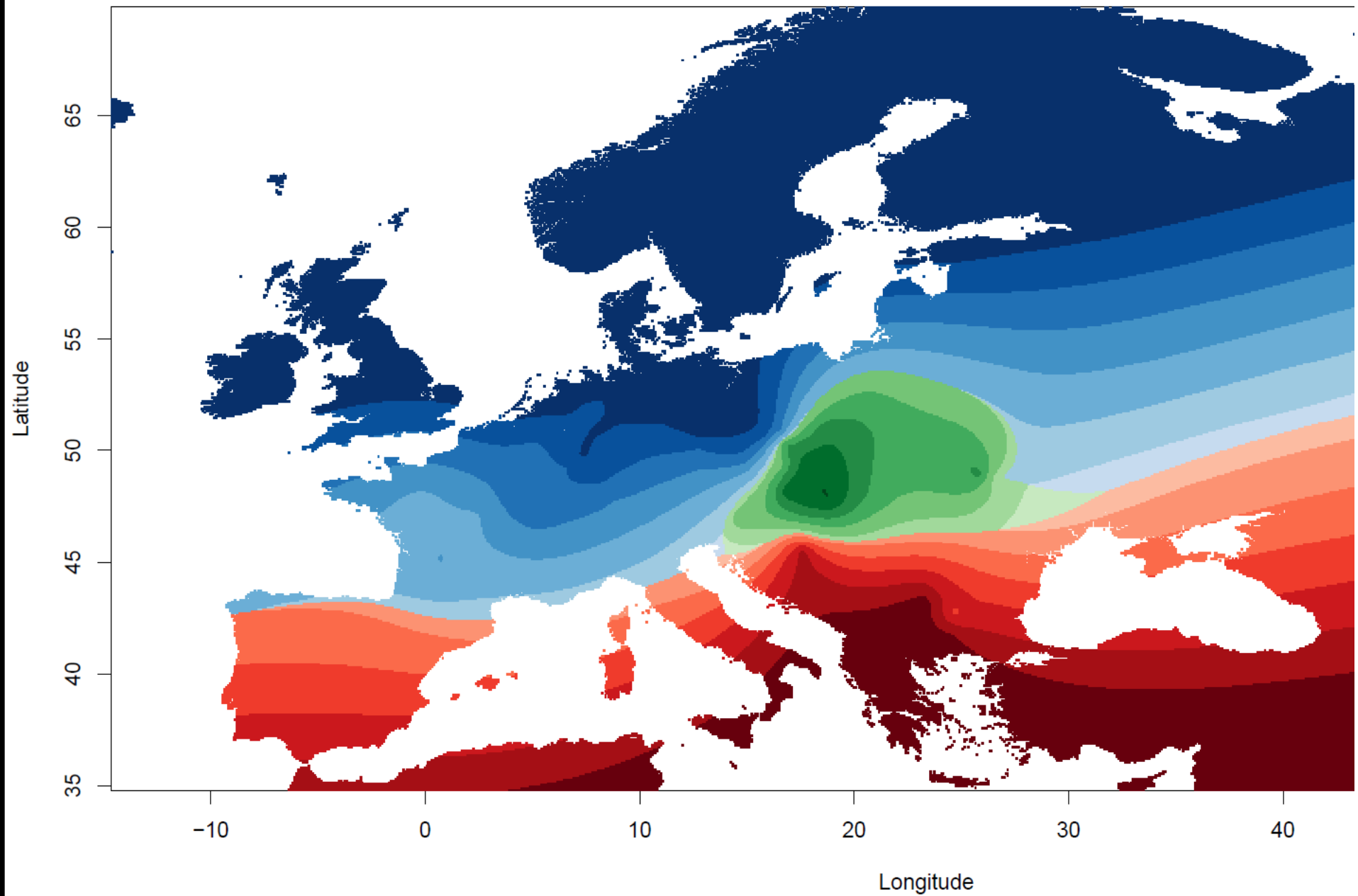


Western Europe
+ Scandinavia

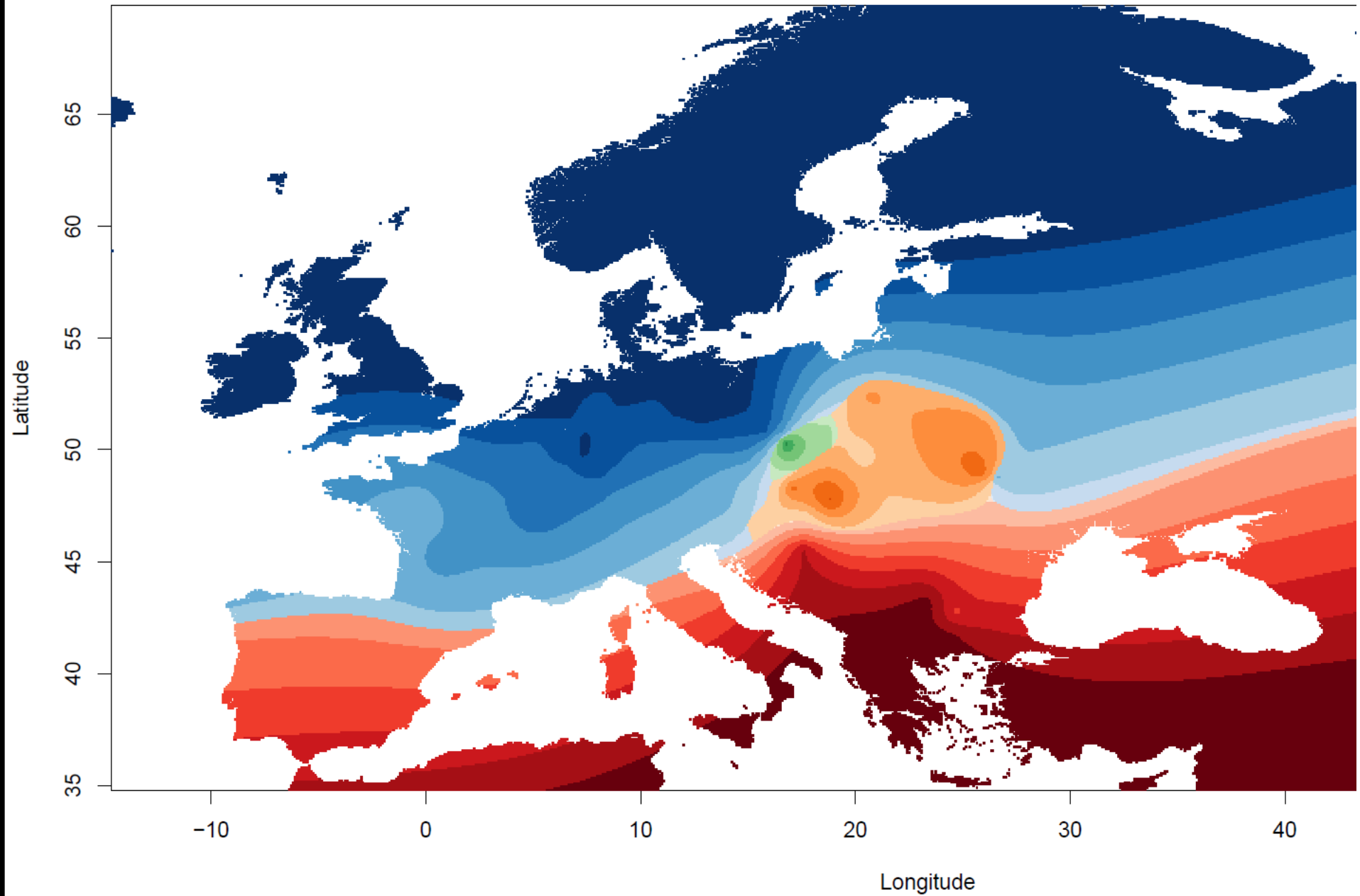
Central /
Northeastern

Southeastern

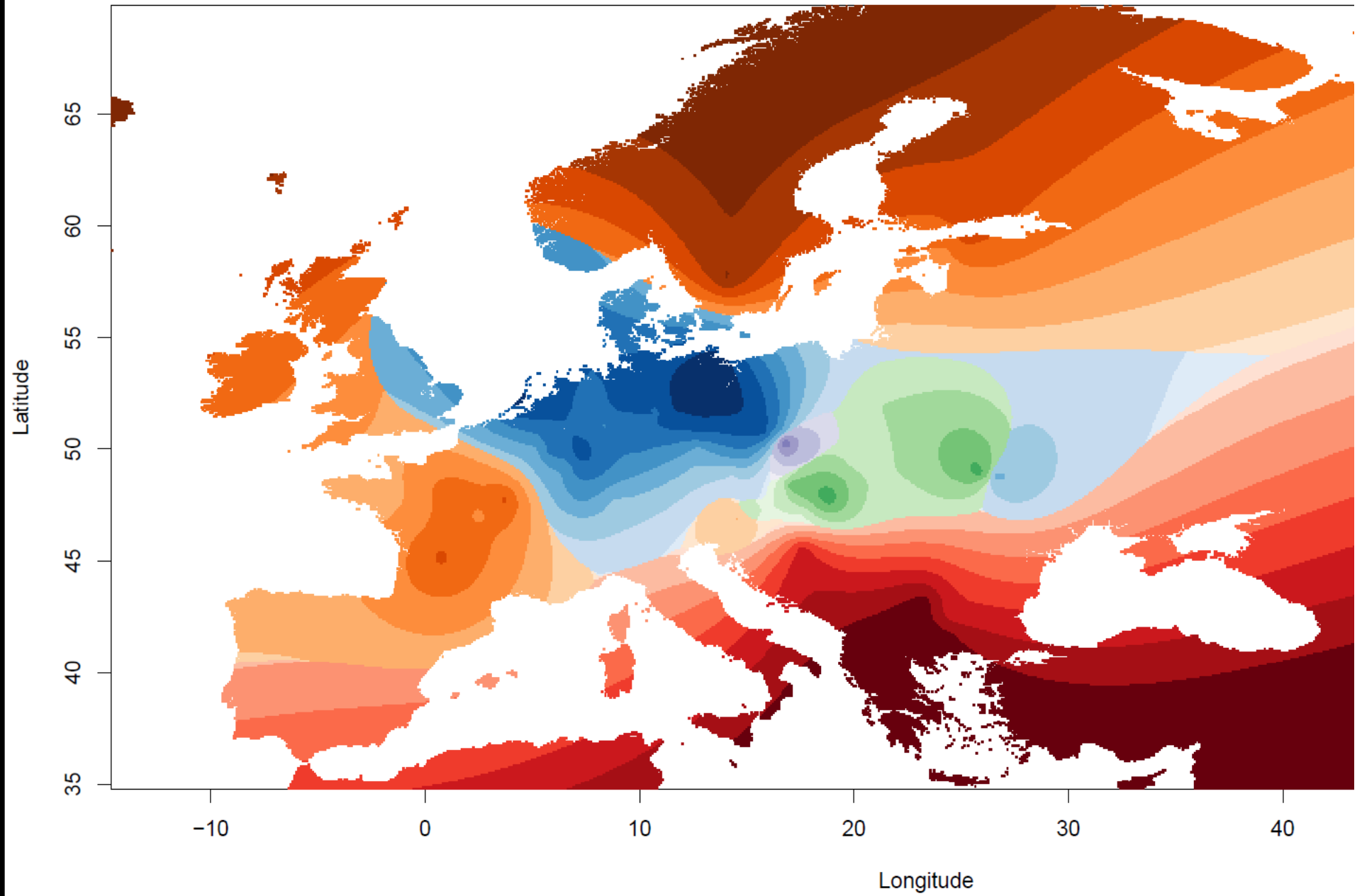
Ancestry coefficients, K=3



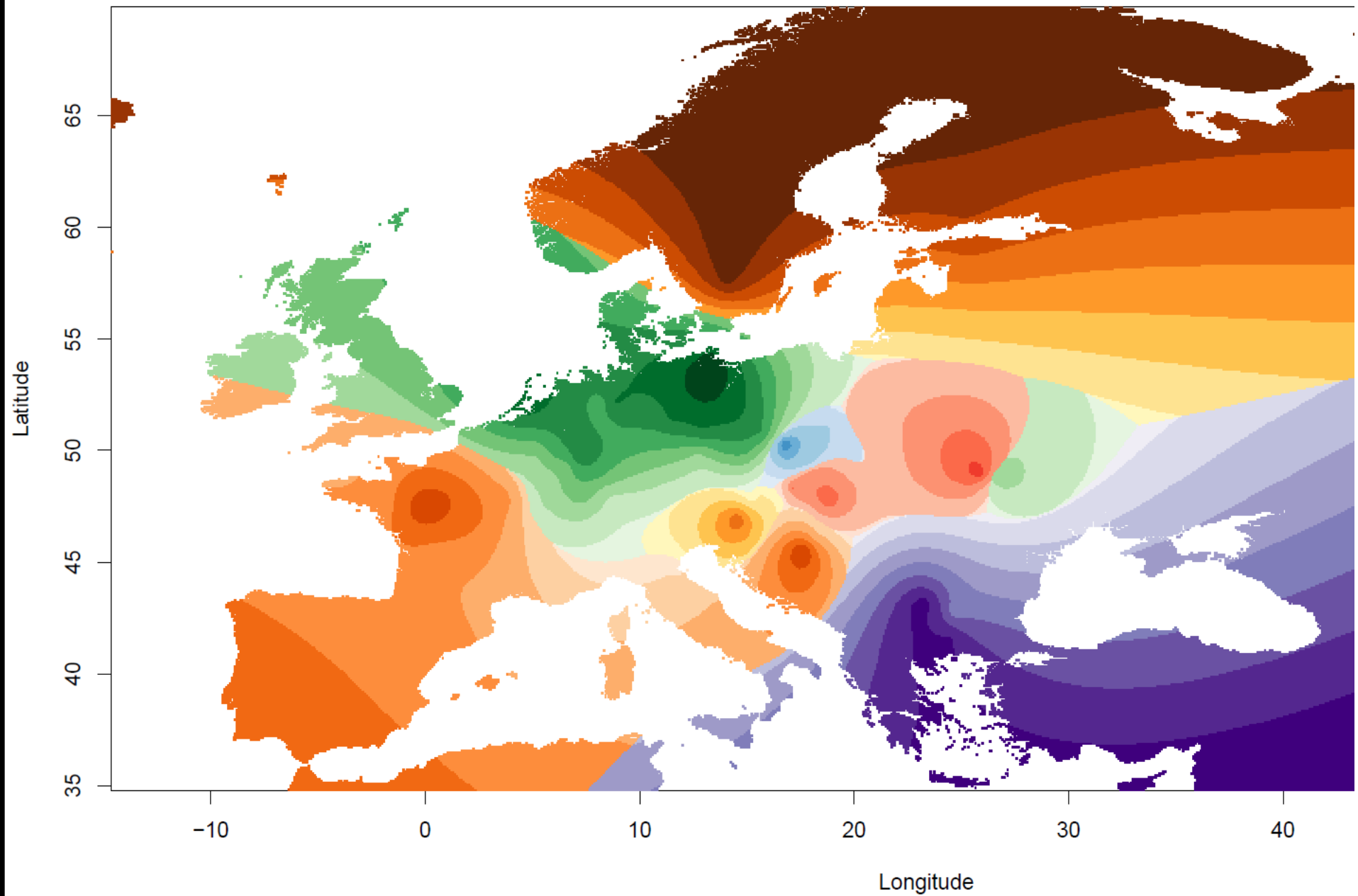
Ancestry coefficients, K=4



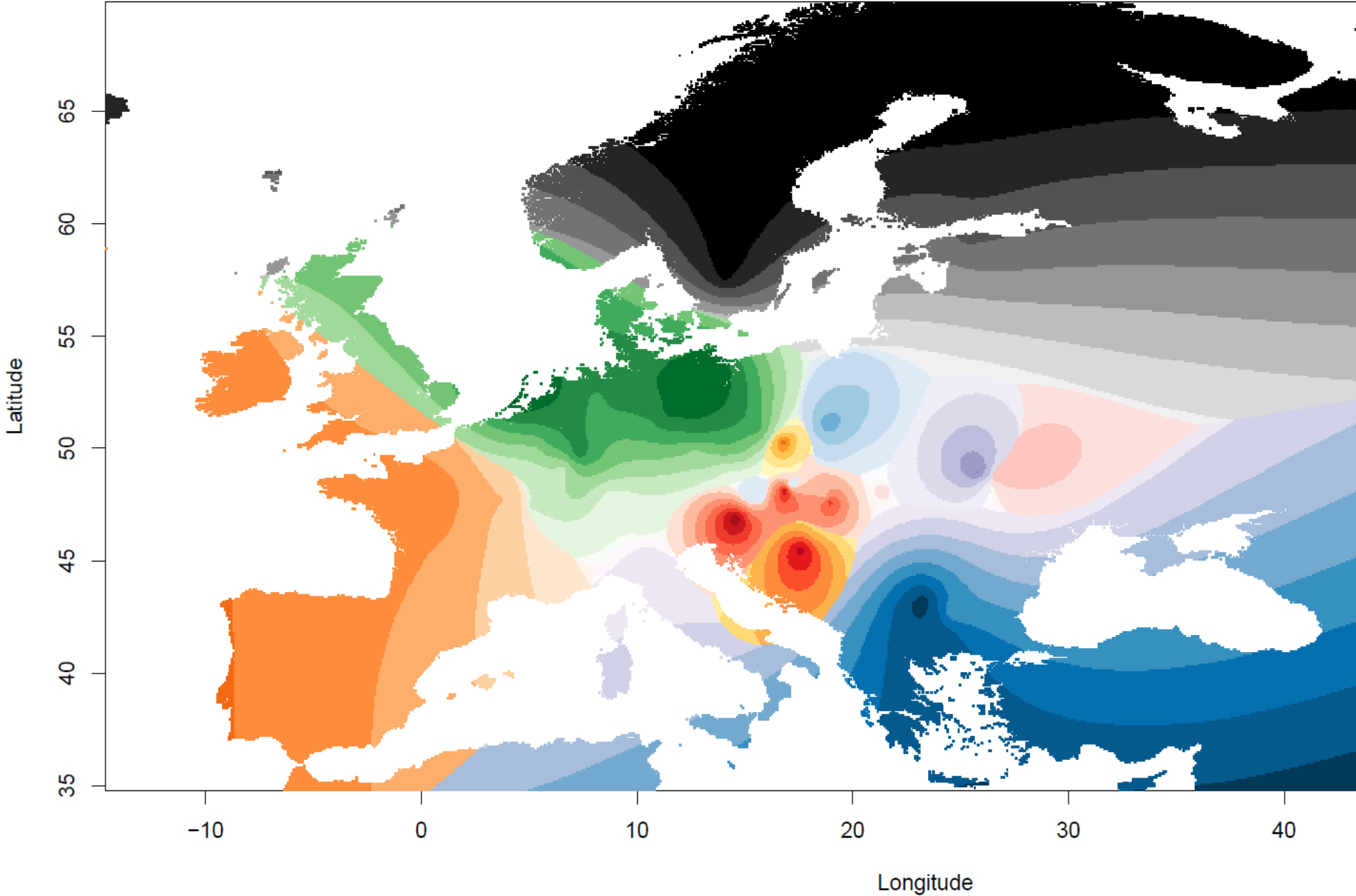
Ancestry coefficients, K=5



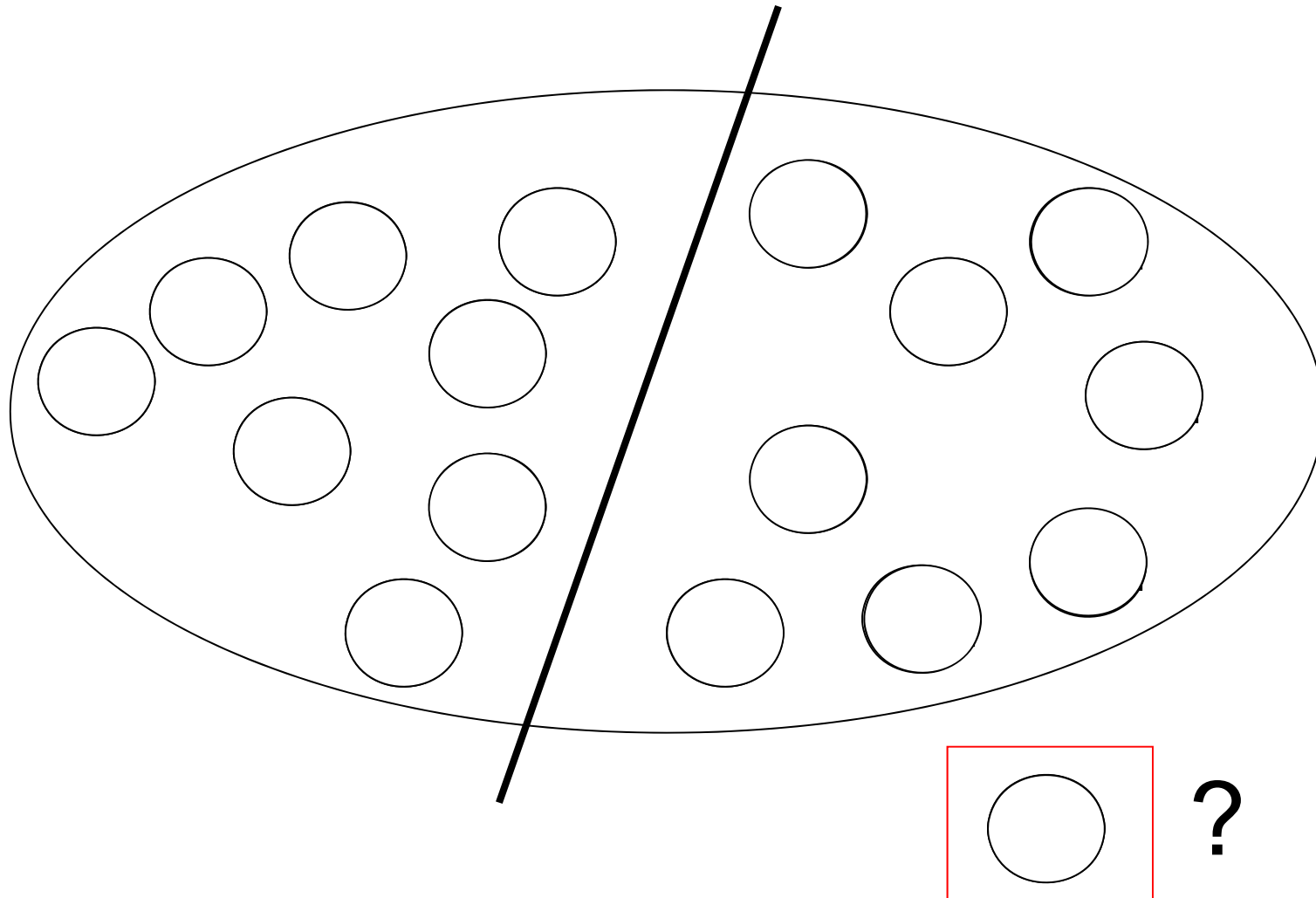
Ancestry coefficients, K=6



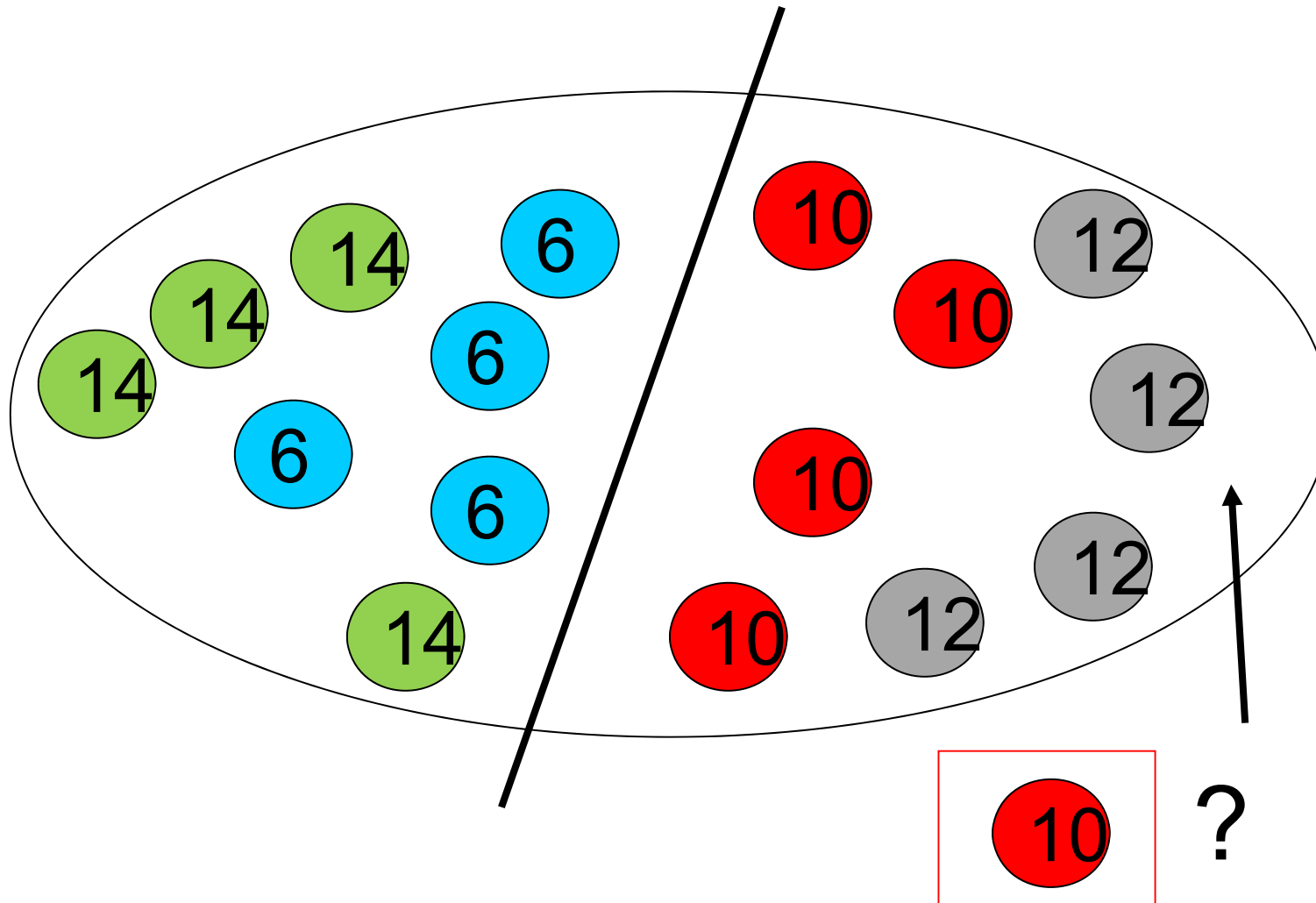
Ancestry coefficients, K=10



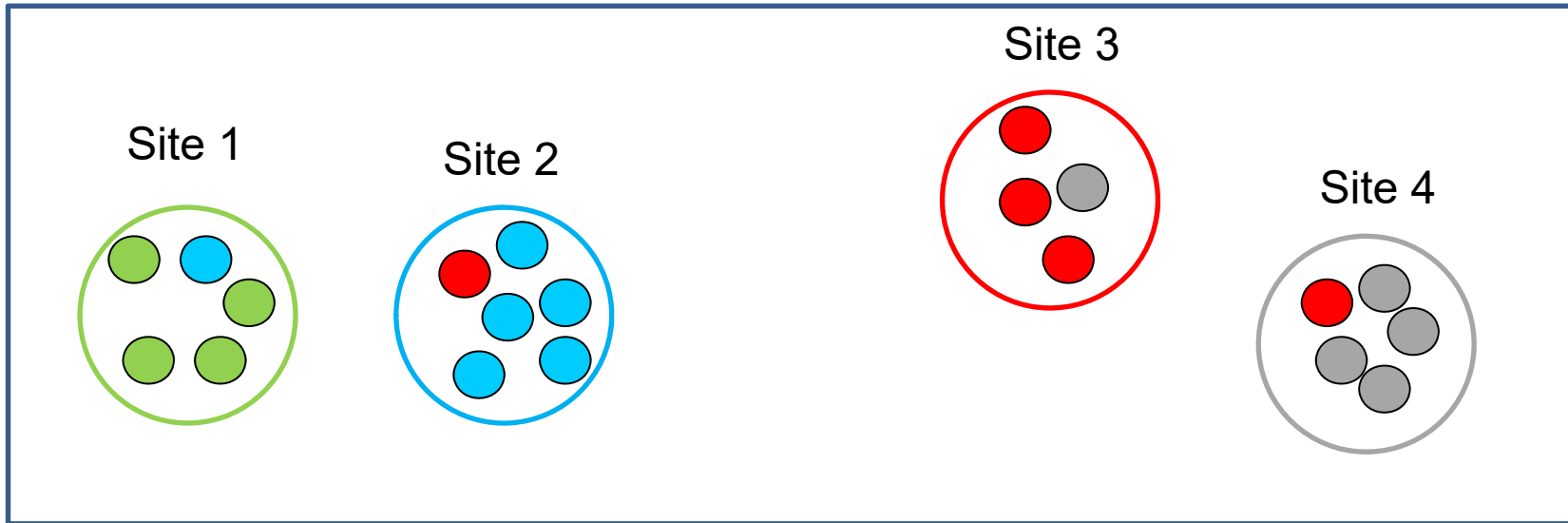
GENETIC ASSIGNMENT



GENETIC ASSIGNMENT

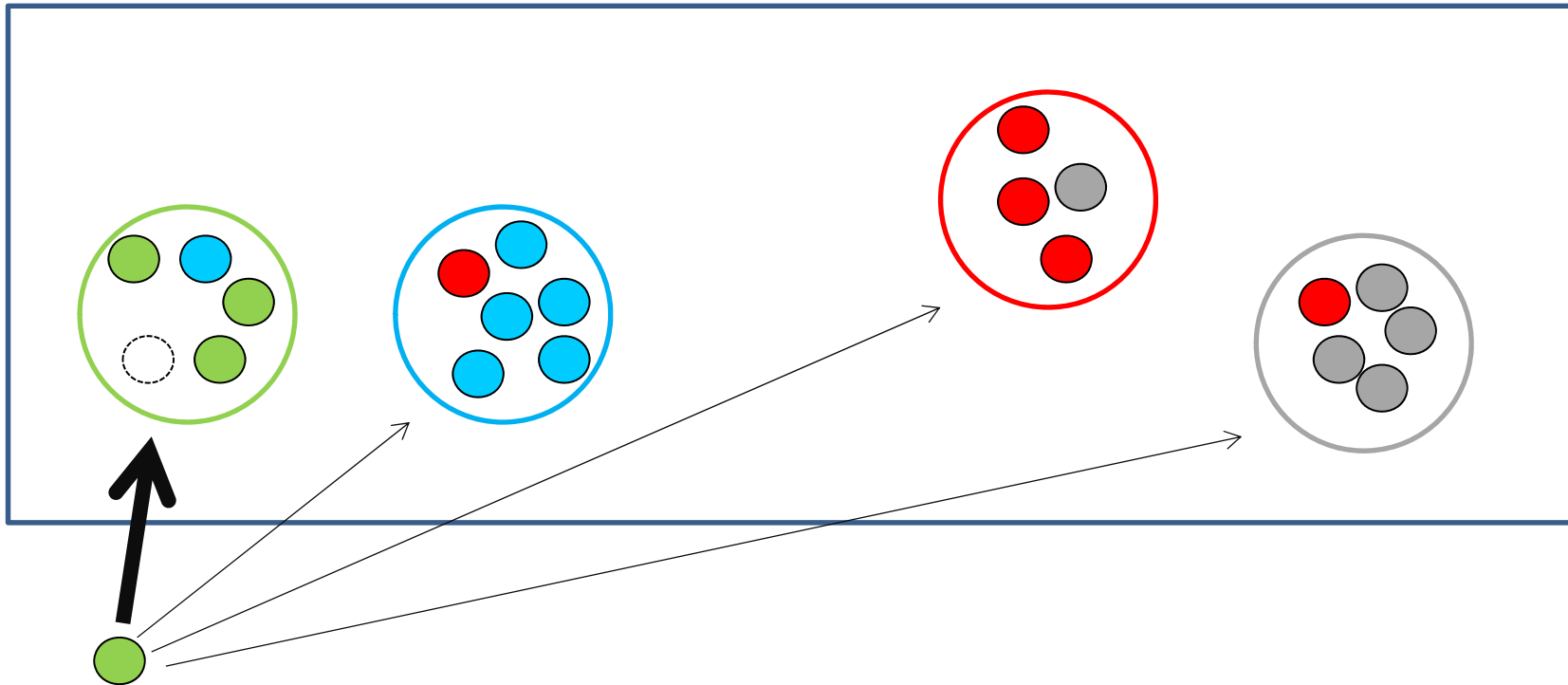


GENETIC ASSIGNMENT



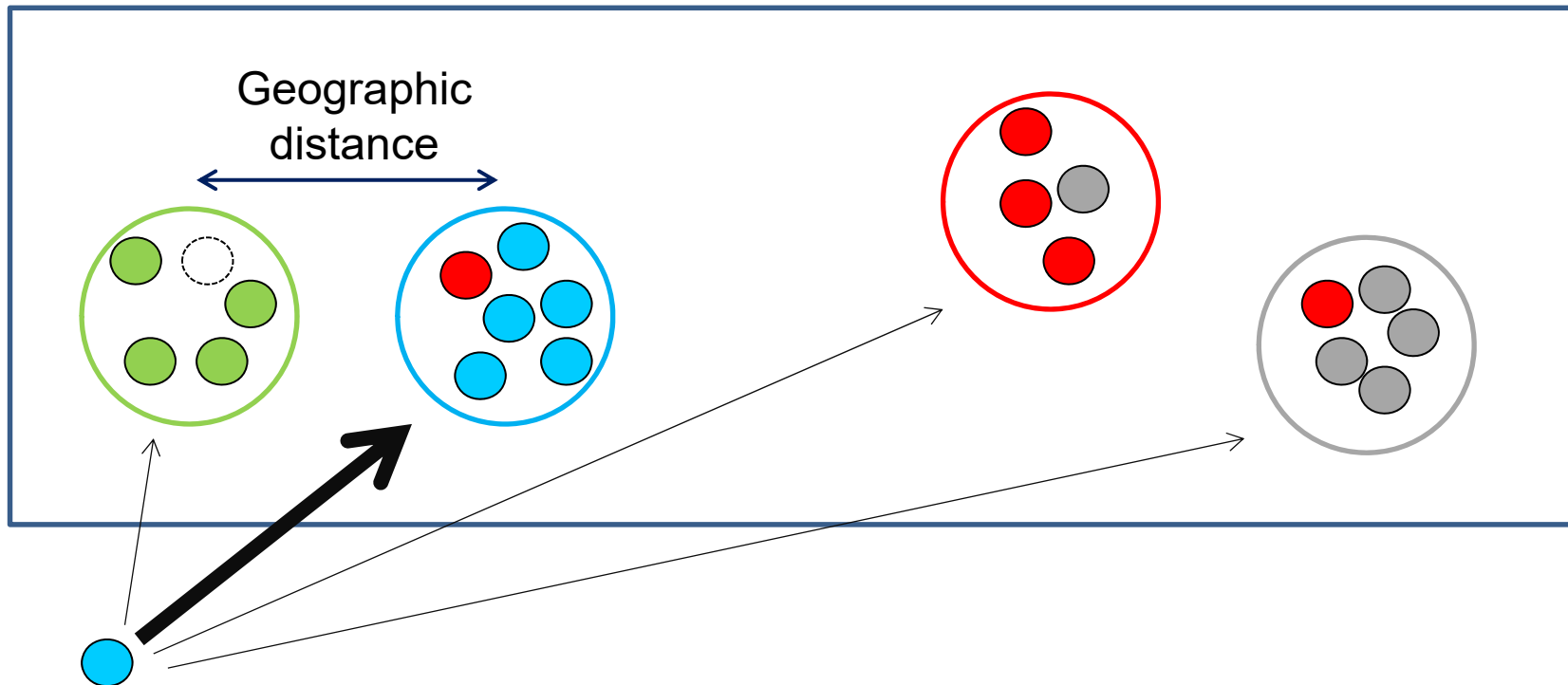
4 sites
20 samples

GENETIC ASSIGNMENT



Reassignment to the correct site

GENETIC ASSIGNMENT

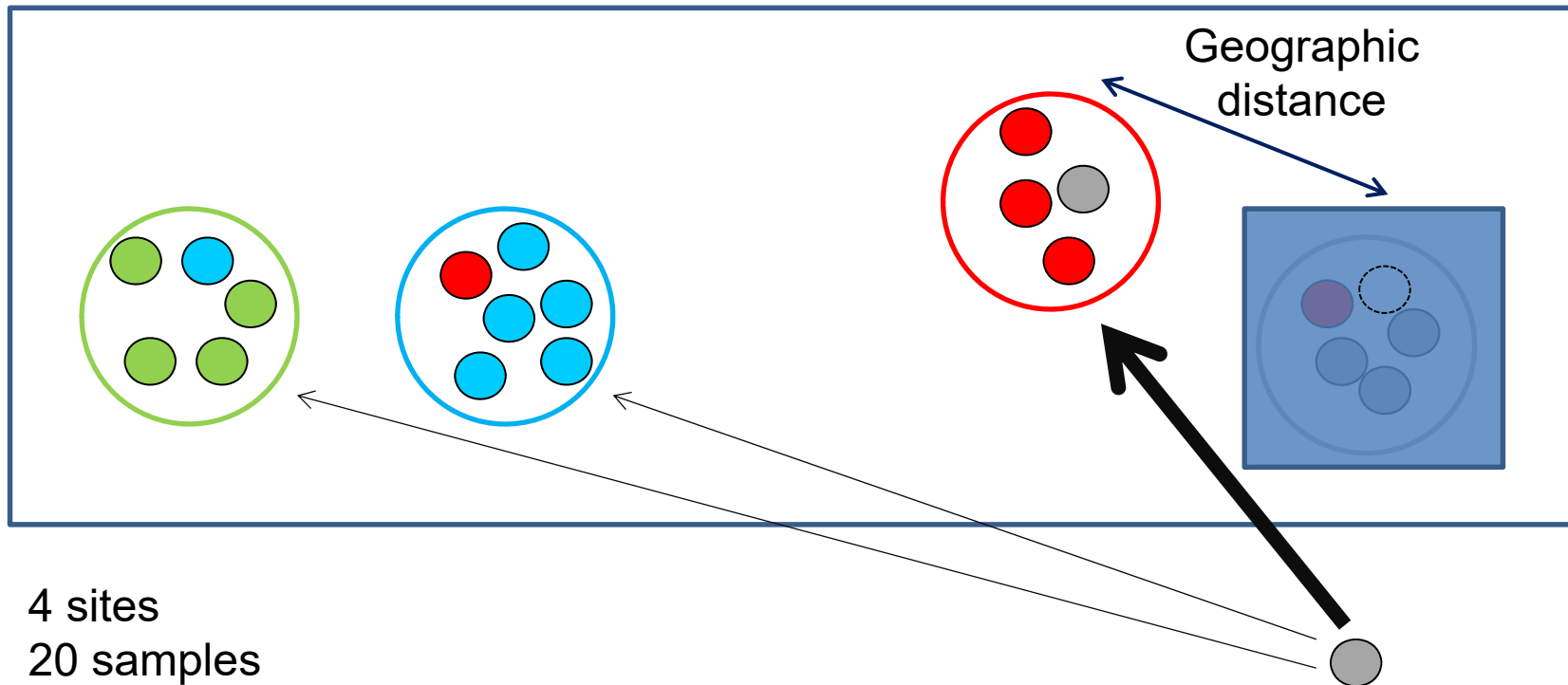


Reassignment to the wrong site

Error can be estimated (percentage, distance and direction)

GENETIC ASSIGNMENT

If the site of origin was not sampled ...

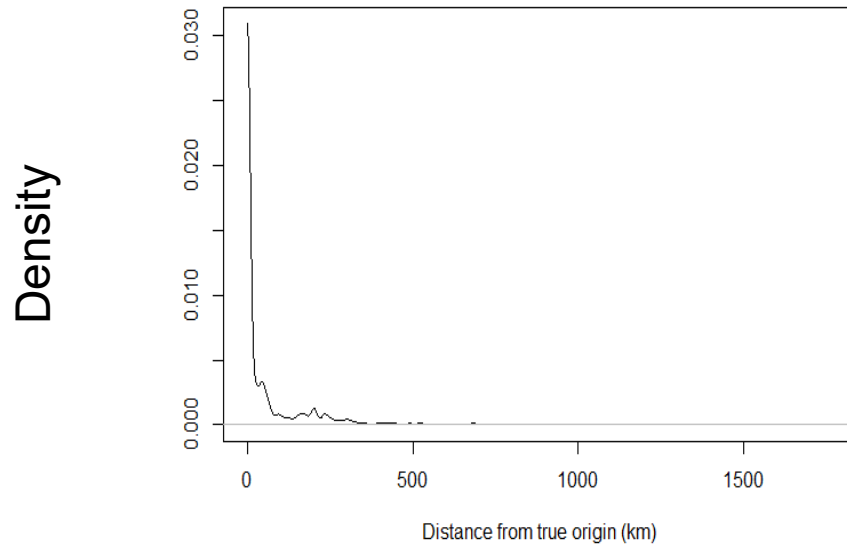


4 sites
20 samples

Reassignment to the wrong site (systematically)
Error in distance can be estimated

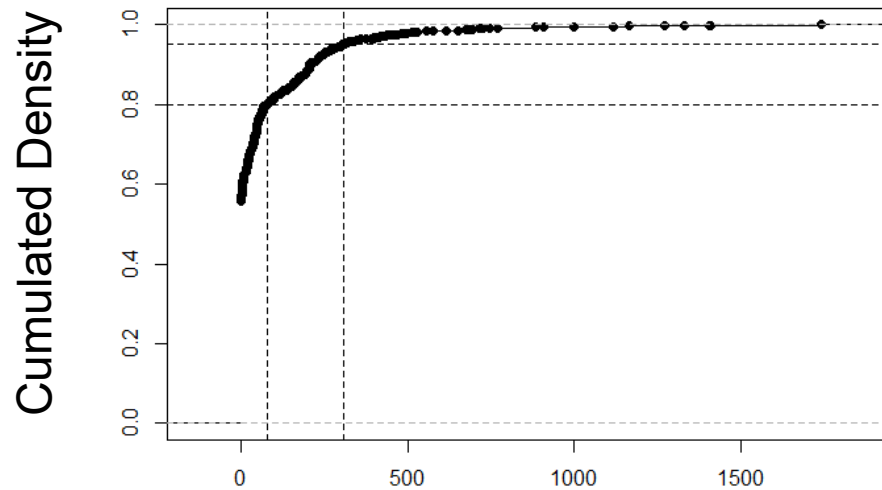
DAPC using the leave-one out procedure

GENETIC ASSIGNMENT



55 % of samples are correctly identified to their original site

80% of the samples assigned within 80 km



95% of samples assigned within 310 km

BAT MOVEMENT

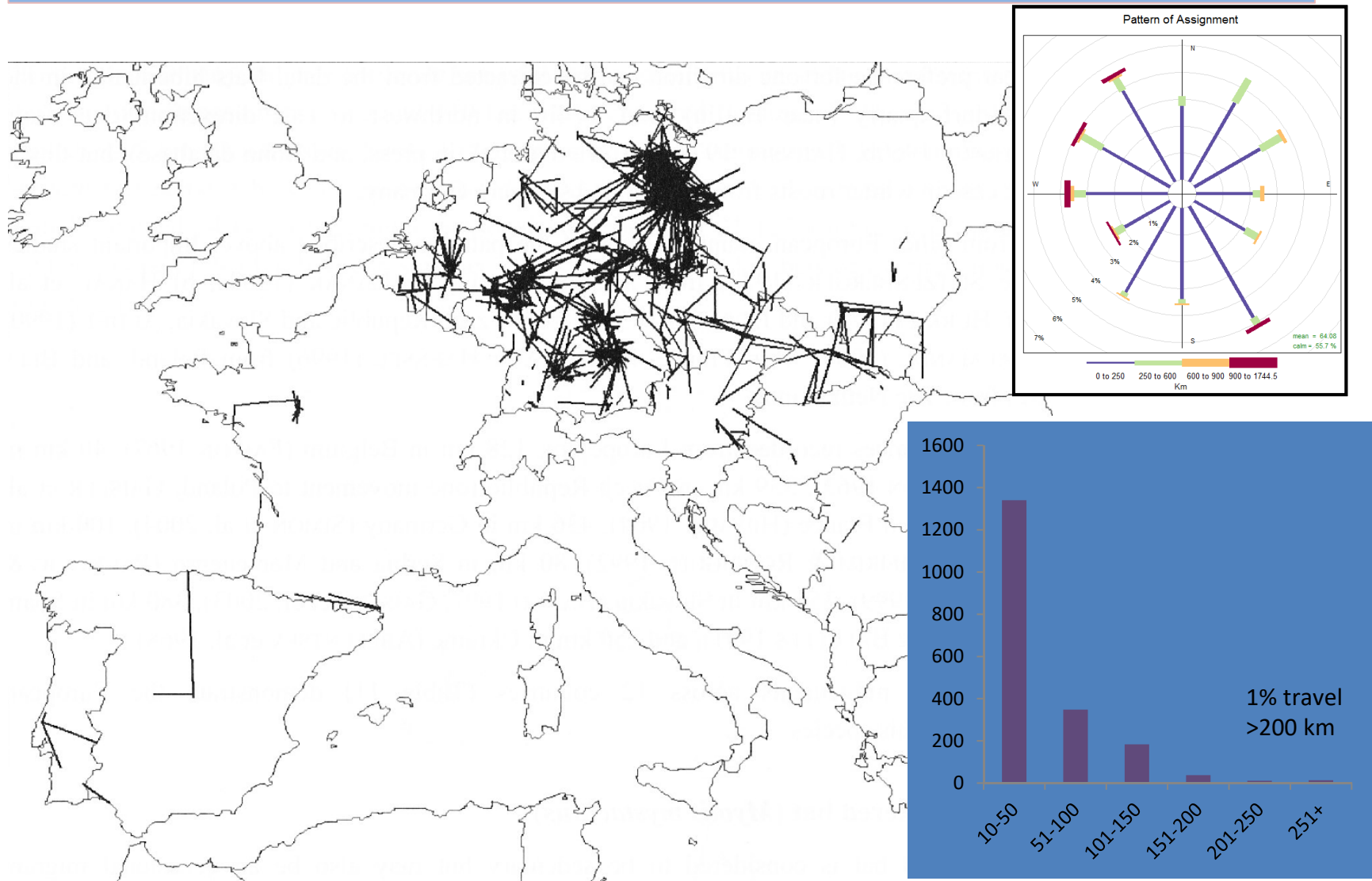
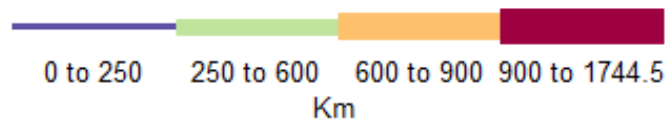
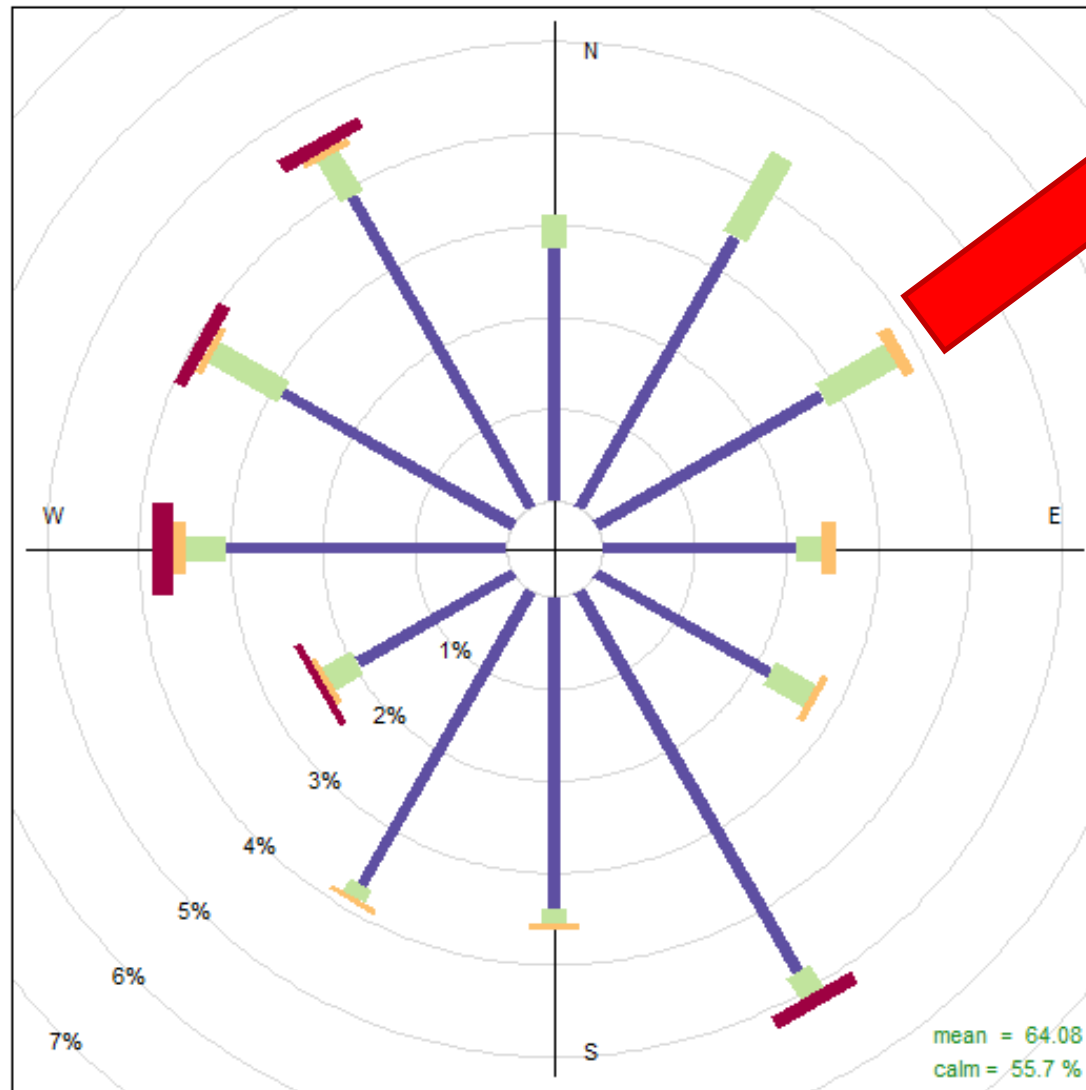


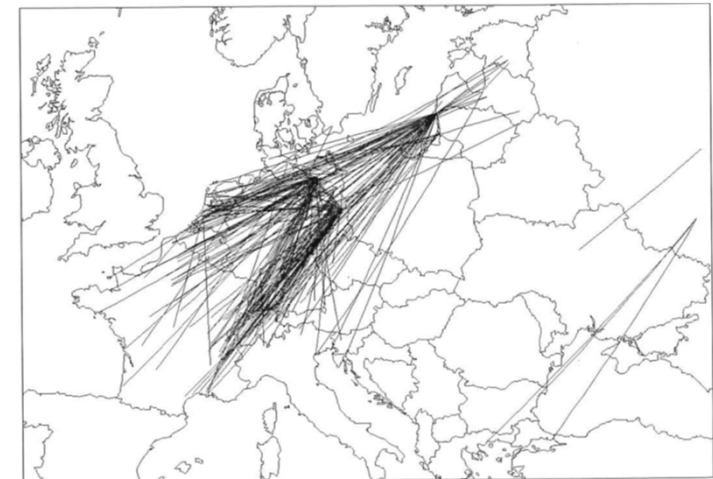
Fig. 19. Documented long-distance movements of *Myotis myotis* in Europe (n=3273).

Hutterer *et al.* 2005

PATTERNS OF MIS-ASSIGNMENT



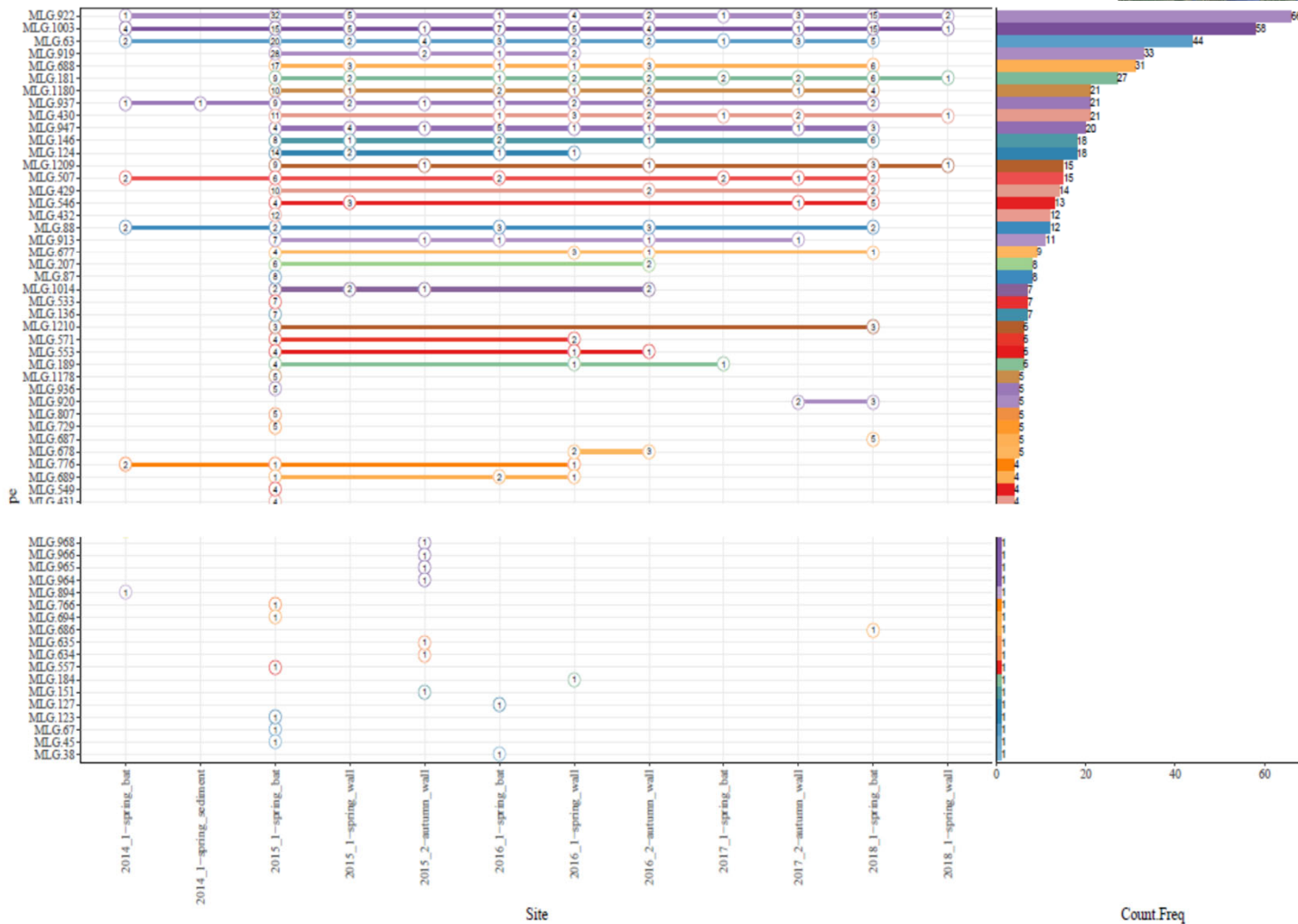
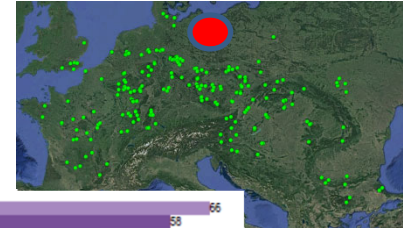
Spring
migration



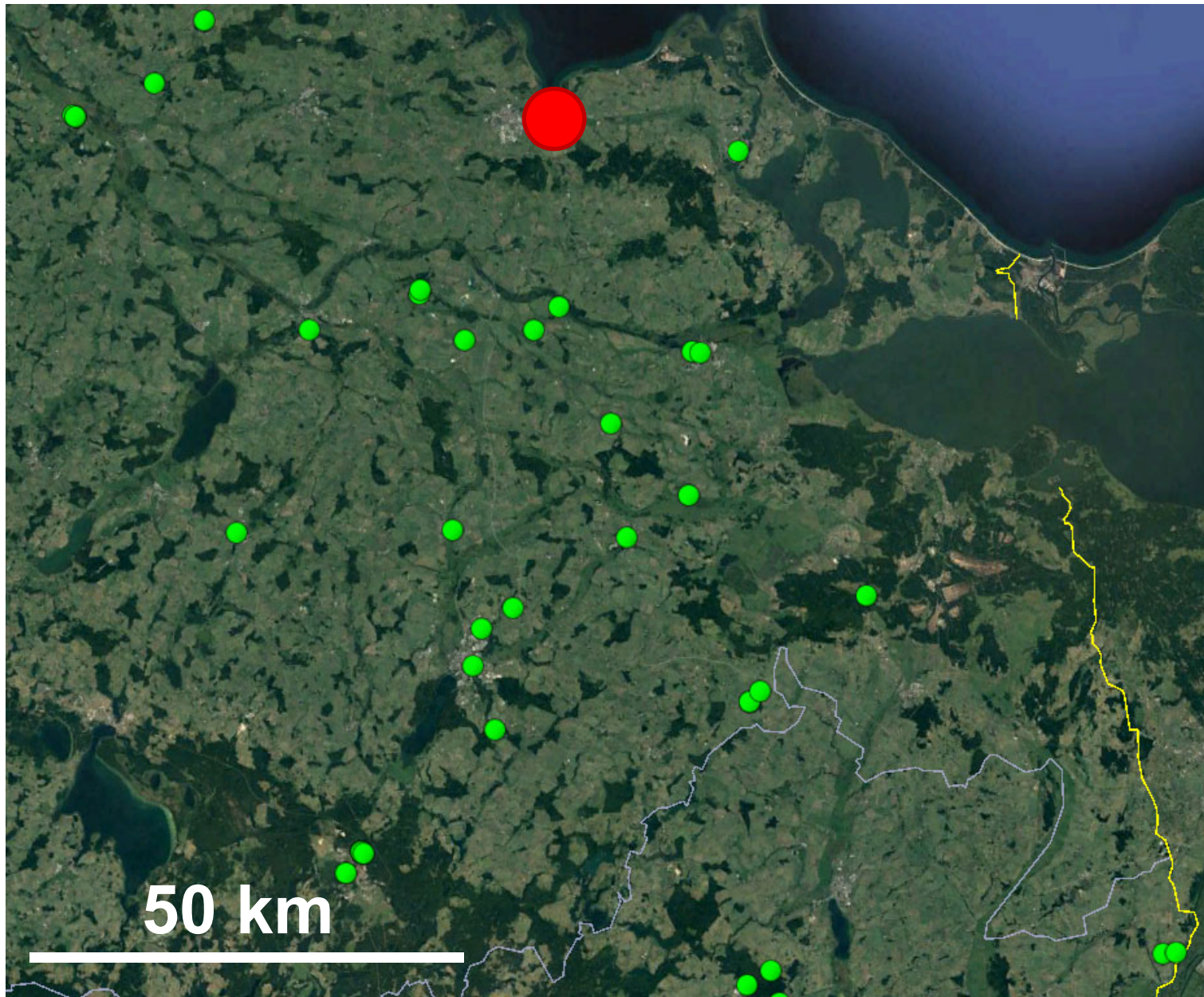
Hutterer *et al.* 2005 (*P. nathusii*)

IS SEXUAL REPRODUCTION HAPPENING?

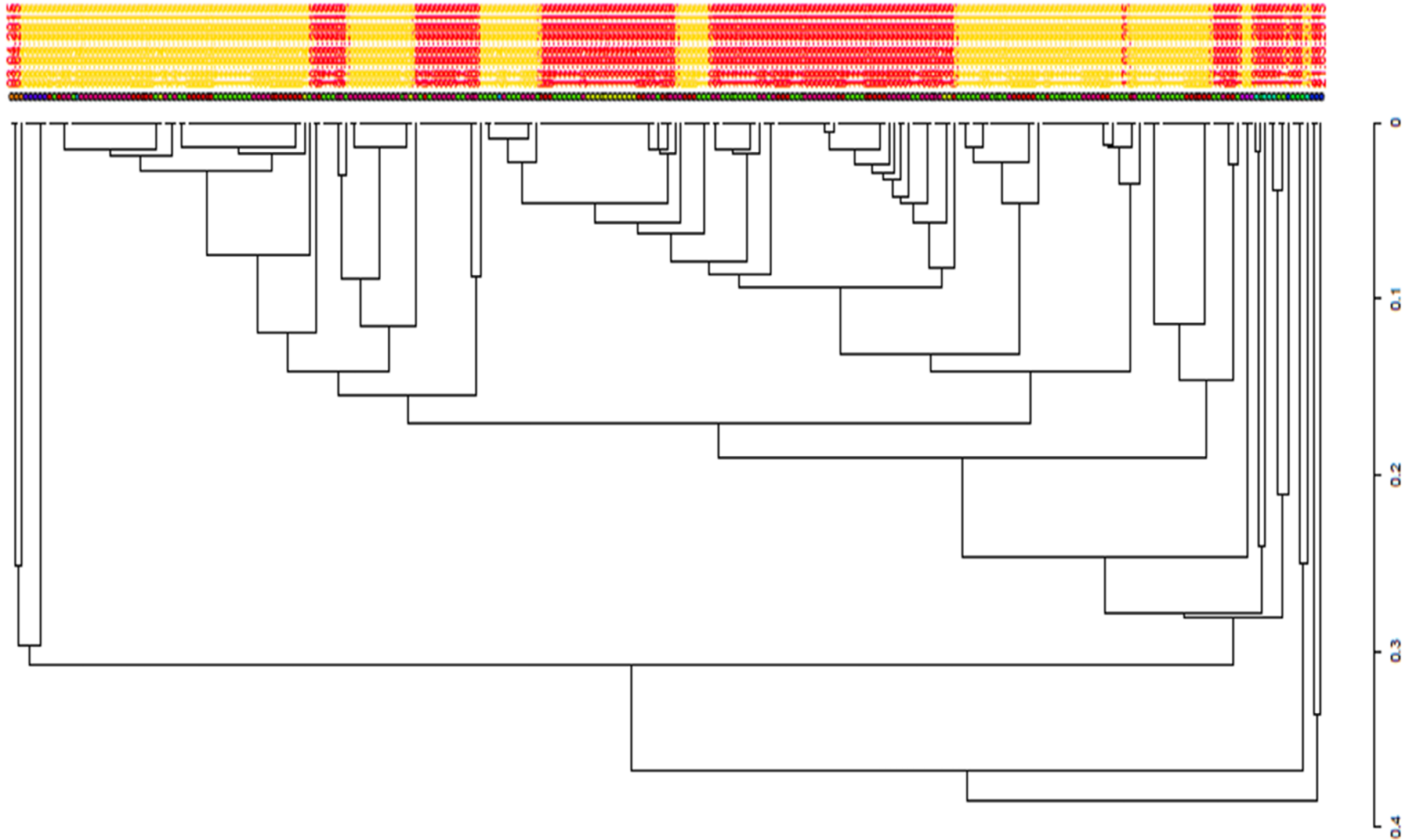
N=1,800 isolates (5 years temporal sampling, 1 site, NE Germany)
MLG=150



IS SEXUAL REPRODUCTION HAPPENING?



IS THERE SEXUAL REPRODUCTION?

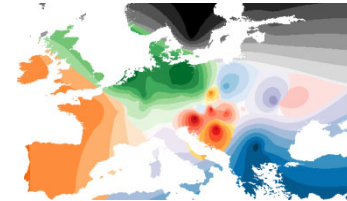


N=283, MAT1-1=127, MAT1-2=156

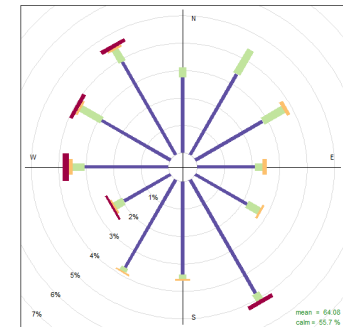
CONCLUSIONS & PERSPECTIVES

-The use of genetic data is an extremely powerful tool to elucidate the population structure in Europe,

➤ **The population structure is very strong,**

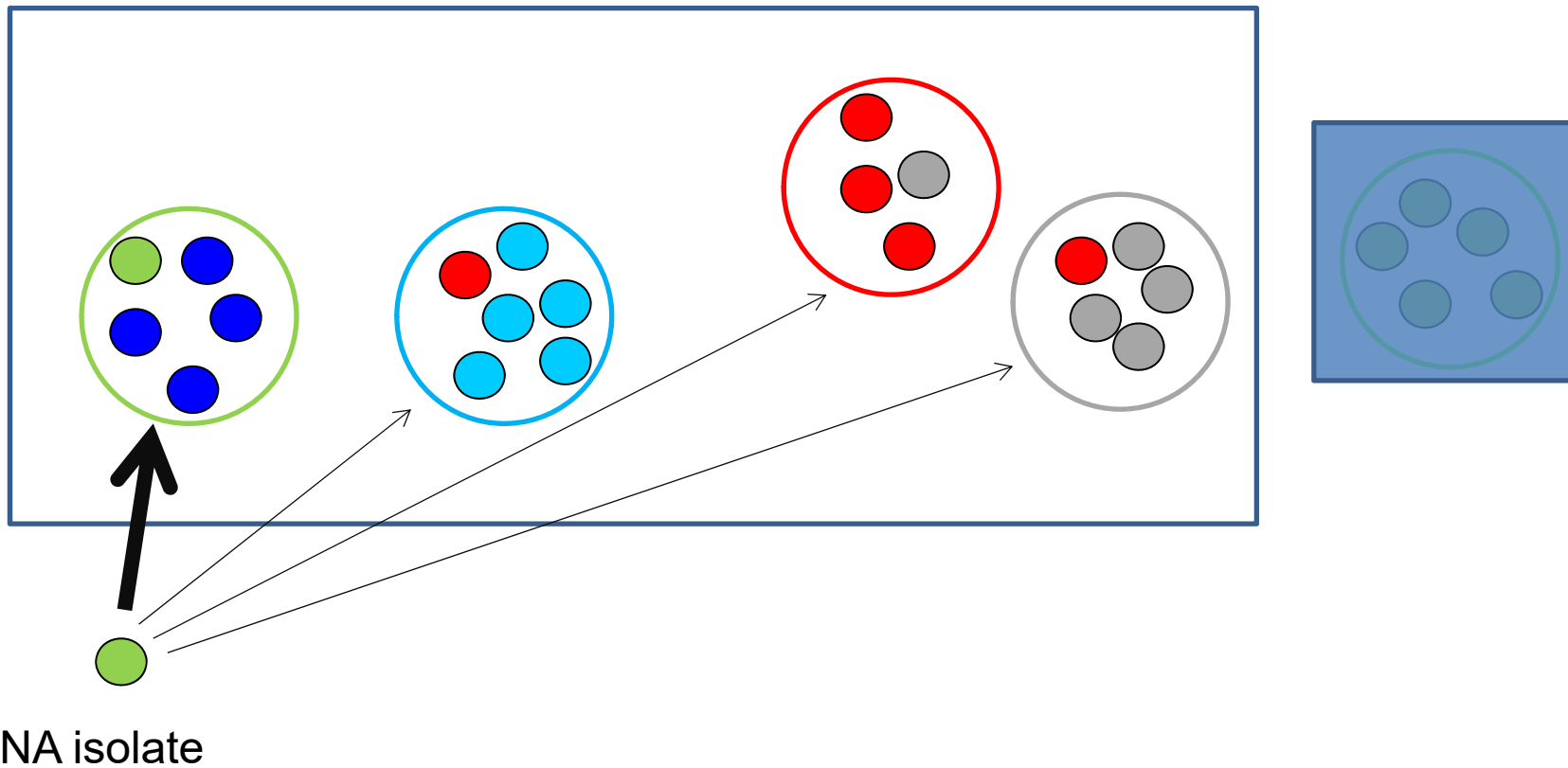


➤ **The fungus moves relatively little (likely via bats) but some long distance movements are suspected (via humans?),**

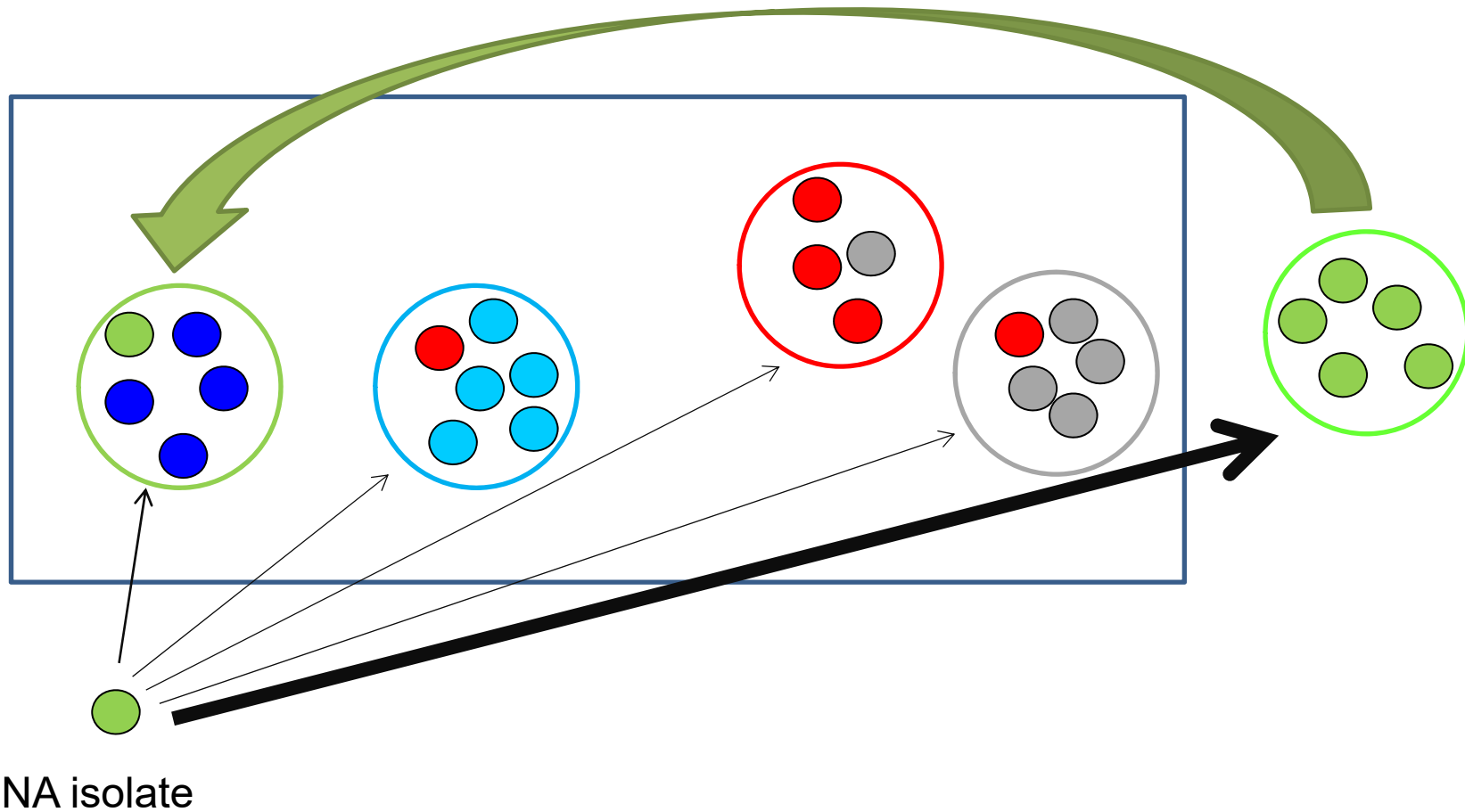


➤ **There is a risk that a recombinant becomes virulent and could kill European bats although evaluating precisely the level of risk is extremely challenging, -> though the higher the number of long distance movements, the higher this risk is!**

AN WHAT ABOUT THE EU ORIGIN OR THE INTRODUCTION TO NA?



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WHITE NOSE SYNDROME/DISEASE

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ORIGINAL DEFINITION (Reeder & Turner, 2008; Veilleux, 2008)

✓ **Set of symptoms, including visible fungal growth on skin surfaces, depletion of fat reserves, altered torpor patterns and aberrant winter behaviour,**

OTHER DEFINITION (Meteyer et al., 2009)

✓ **Fungal hyphae form cup-like epidermal erosions and ulcers in the wing membrane with involvement of underlying connective tissue.**

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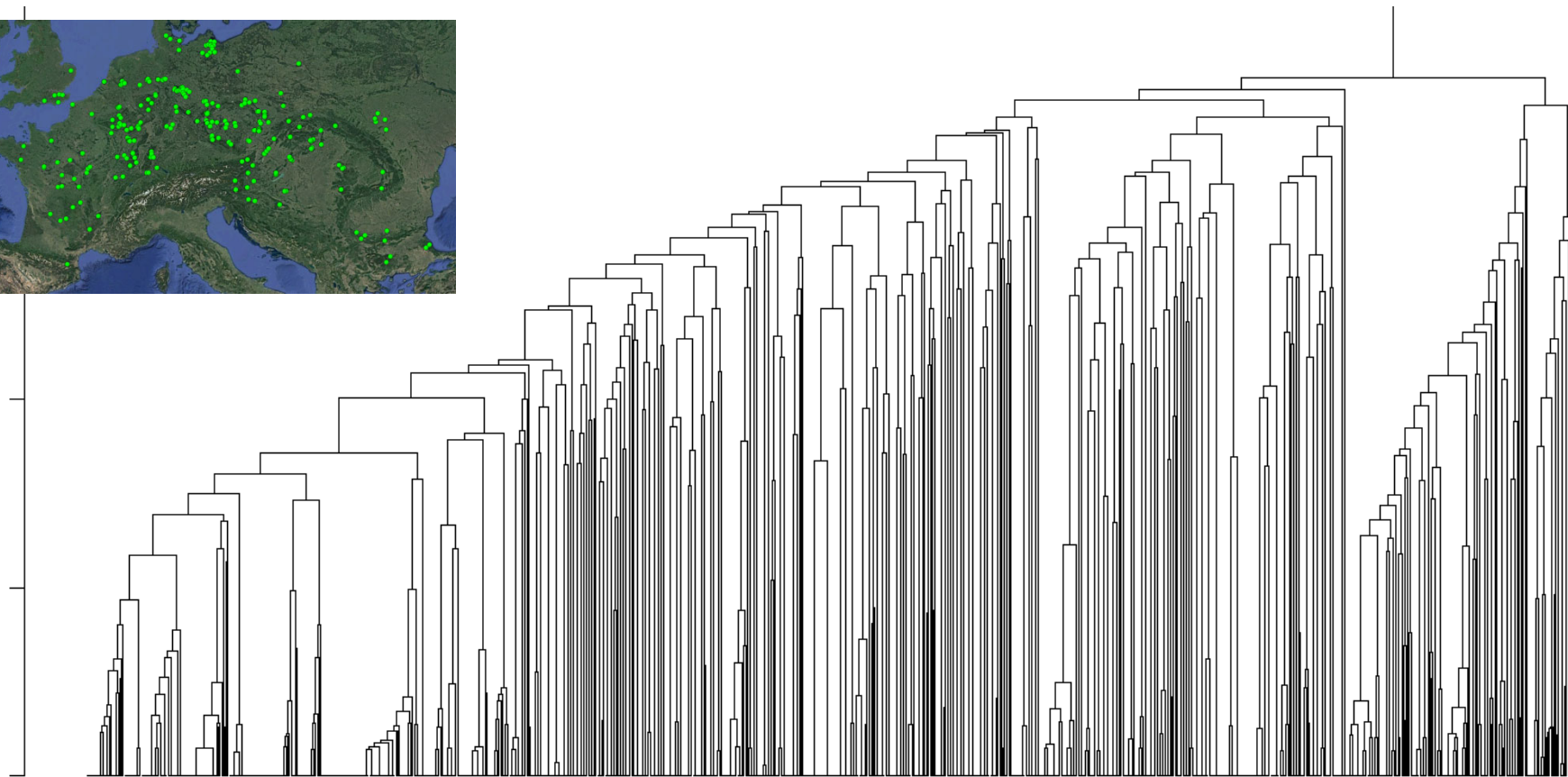
WHITE NOSE DISEASE (diagnosed via Histopathology)

✓ **Pd fungal hyphae form cup-like epidermal erosions and ulcers in the wing membrane with involvement of underlying connective tissue.**

Frick, Puechmaille & Willis, 2016. *In* Bats in the Anthropocene: Conservation of bats in a changing world. Springer.

FURTHER GENETIC EVIDENCE (MICROSATS)

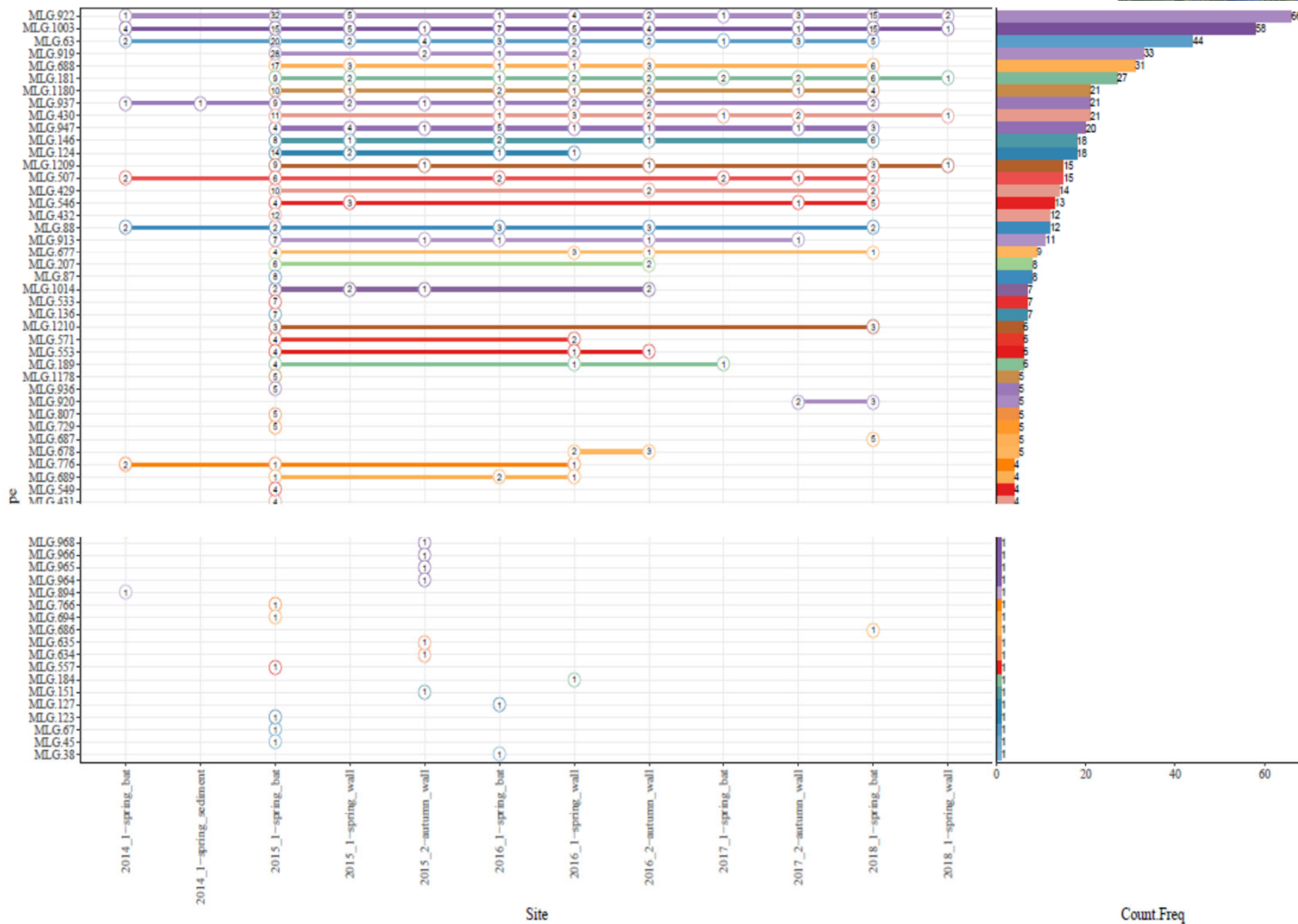
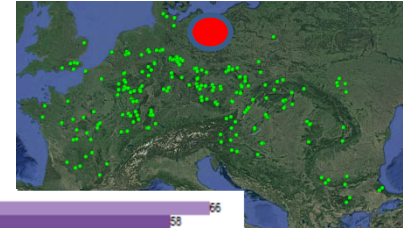
N=3,000 isolates (125 sites, 26 countries)
MLG=1208



Mostly reproduces clonally  (genetically 100% identical)

TEMPORAL SAMPLING (MICROSATS)

N=1,800 isolates (5 years temporal sampling, 1 site, NE Germany)
MLG=XXX



**Next Generation Sequencing:
38 sites, ca. 1 million sequences
1,500 Operational Taxonomic Units
0.2 gram of sediment ->600 OTU and no less
than 100 (mean=240).**

