Conservation genetics
of some European reptiles

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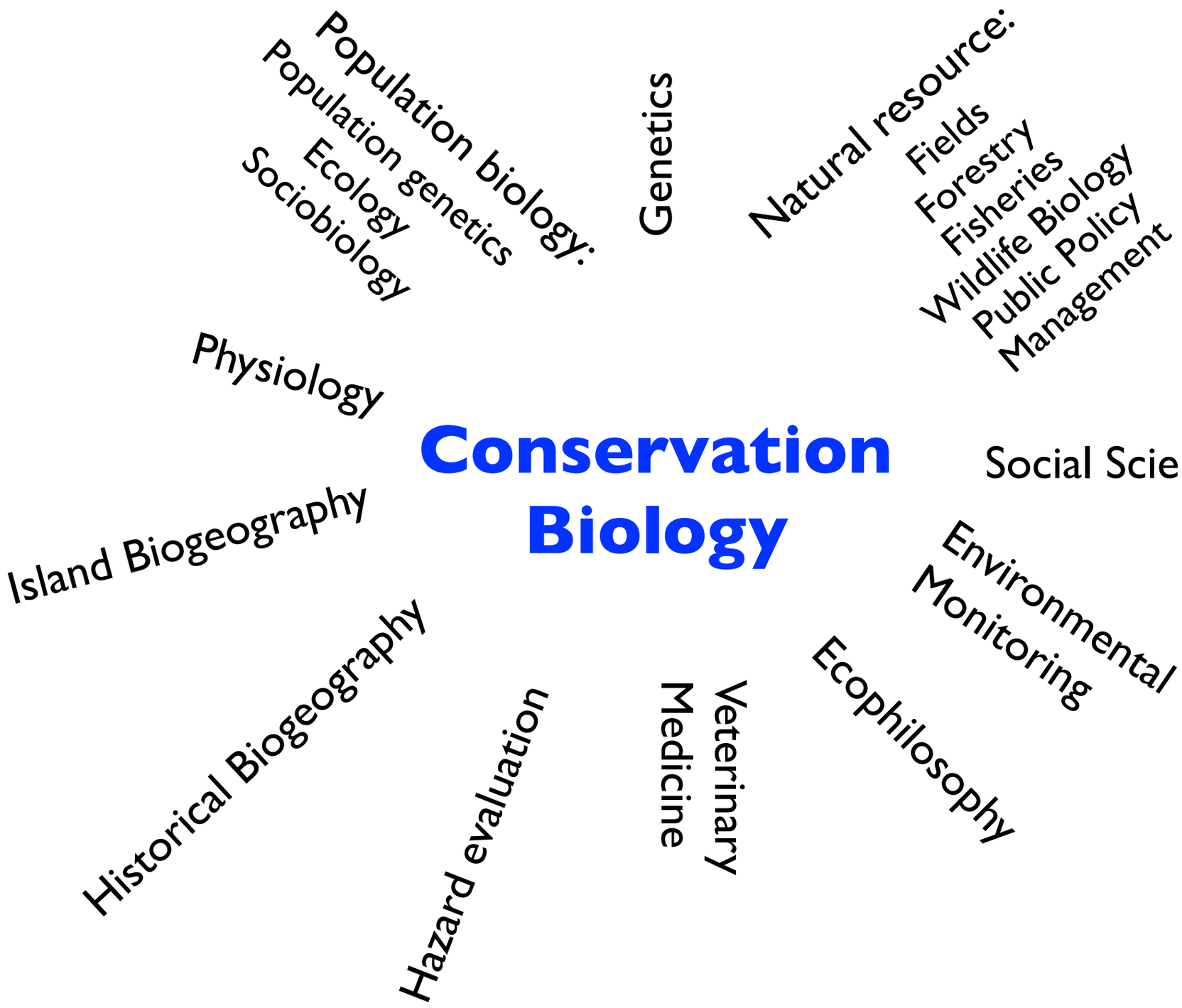
UNI BASEL

Introduction - *Conservation biology*



adapted from Soulé, 1985

Introduction - *Conservation genetics*

- how genetic analyses can help threatened species: some examples...
  - measure inbreeding / outbreeding depression
  - loss of genetic diversity
  - fragmentation of population and reduction of gene flow
  - genetic drift
  - define management unit
  - understand aspects of species biology important for their conservation

Introduction - *conservation genetics in reptiles*

- reptiles
  - low interest
  - limited number of studies compare to other vertebrates

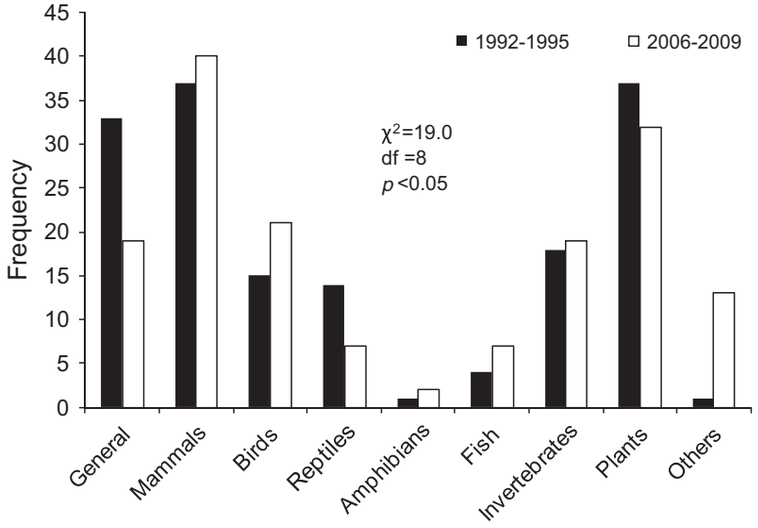
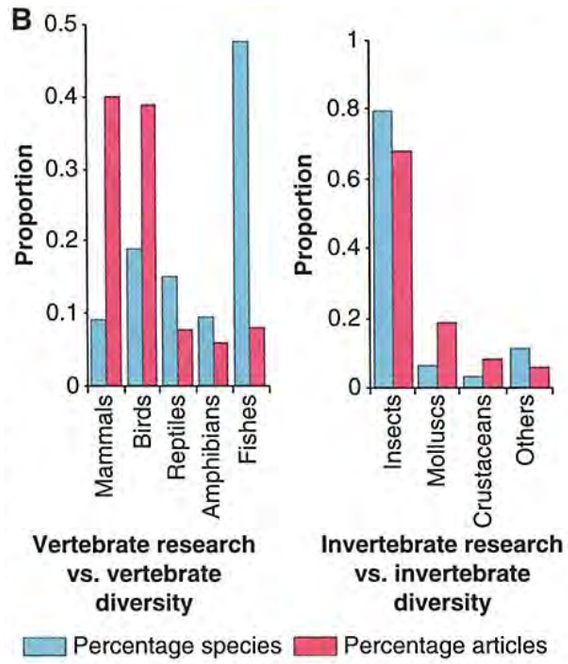


Fig. 2. Number of articles published on different taxa in four journals, based on a sample of 160 papers from each time frame. 'General' refers to papers without a main taxonomic focus, whereas 'Others' are represented by fungi, bacteria and other microorganisms. Pooling amphibians with reptiles (expected frequencies <5) and removing 'General': χ^2 = 13.4, df = 6, P < 0.05.



(A) Proportion of major organismal taxa in nature versus conservation literature. (B) Proportion of vertebrates and invertebrates in nature versus conservation literature.

- reptiles and conservation genetic studies
  - not easy to get enough samples
  - specific markers increase
  - number of studies increase

Griffiths & Dos Santos (2012) Biological Conservation

Clark & May (2002) Science

Introduction - *conservation genetics in reptiles*

several aspects

- Population genetics: comparison of species with a similar biology
  asp viper / adder

- Population genetics: comparison between locations
  smooth snake: UK, Alsace region, Belgium

- Landscape genetics
  slow worm

- environmental DNA

European Vipers - Vipera berus *and* Vipera aspis

- population genetics of the adder in the Jura Mountains

(*Vipera berus*)
E: adder
G: Kreuzotter
F: Vipère péliade

European Vipers - Vipera berus *and* Vipera aspis

- population genetics of the adder in the Jura Mountains

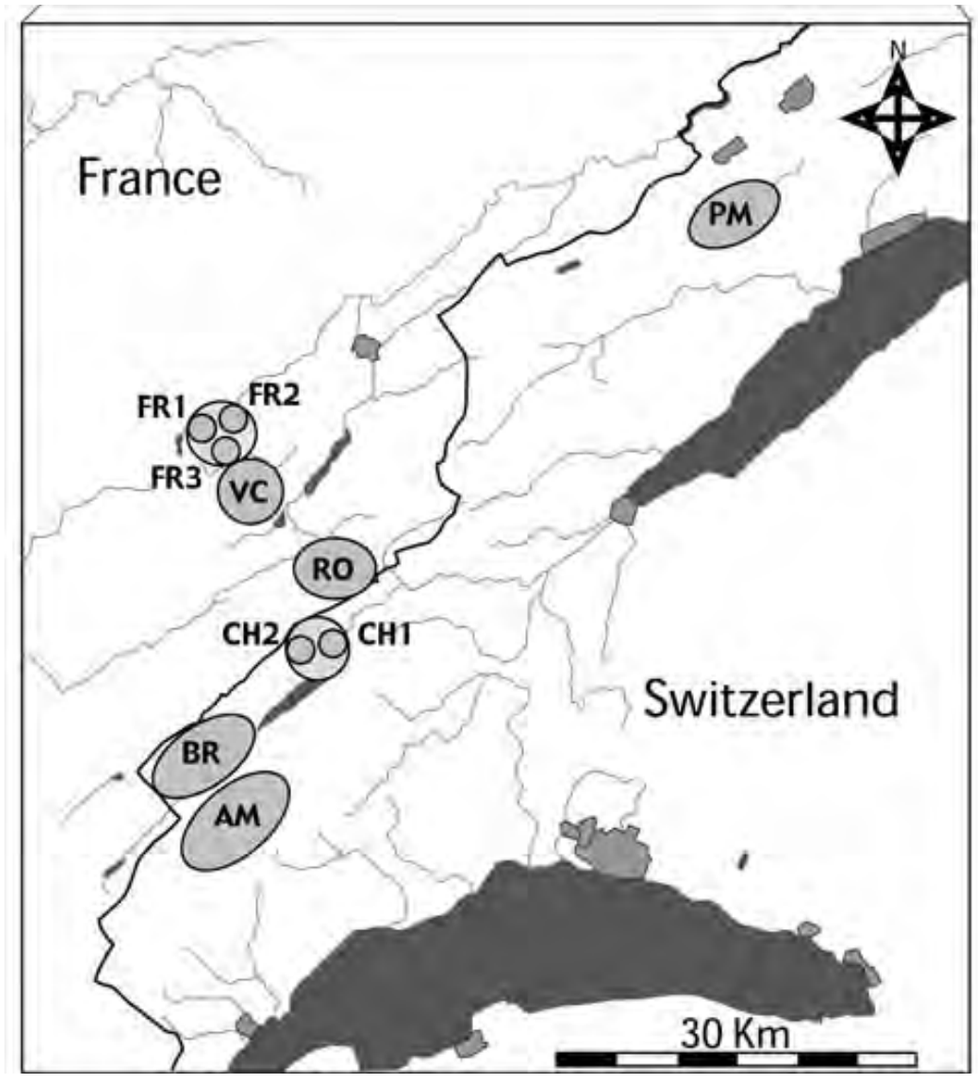


Fig. 1 Distribution of the sampled populations of *Vipera berus* Population codes correspond to Table 2. Population groups CH1-2 FR1-3, and MA1-2 contain geographically close (<3.5 km subpopulations

Ursenbacher et al. (2009) Conservation Genetics

European Vipers - Vipera berus *and* Vipera aspis

- population genetics of the adder in the Jura Mountains

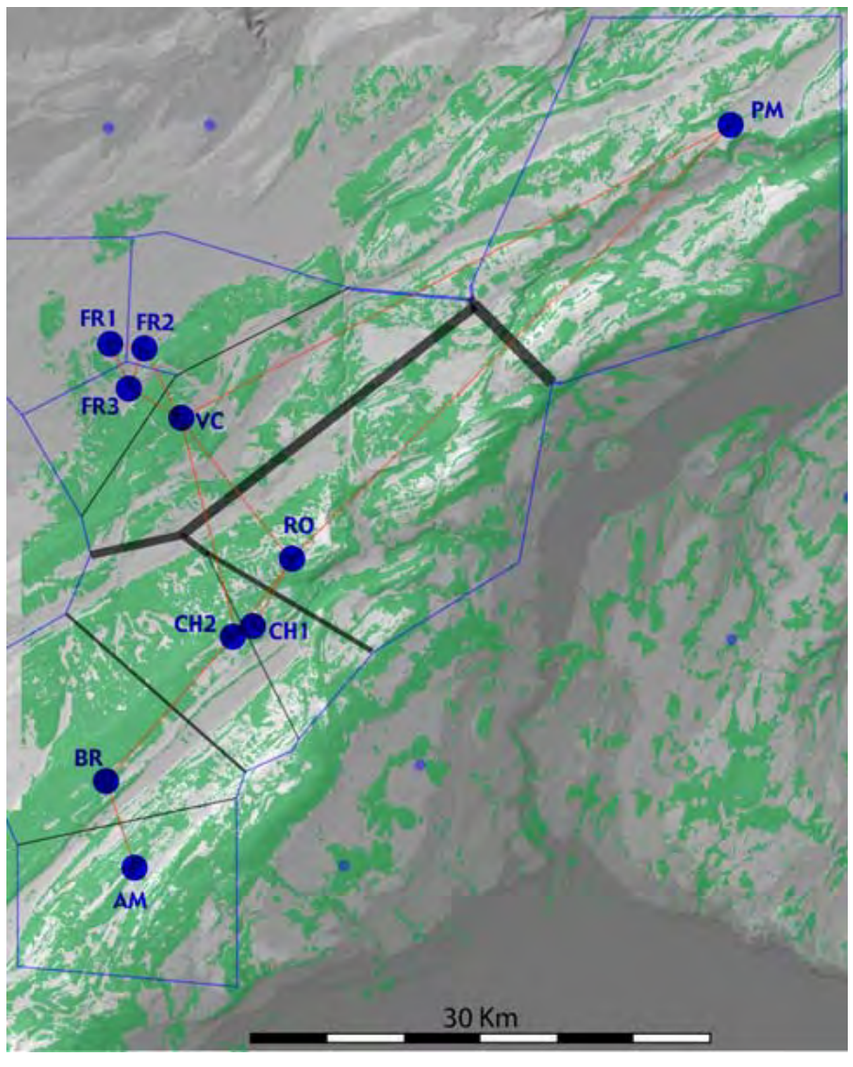


Fig. 5 Barrier locations between sampled populations estimated by BARRIER (Manni et al. 2004), where thickness is proportional to divergence between populations. Blue lines correspond to the Voronoï tessellation whereas green patches correspond to forest area and grey scale to altitude. To avoid divulging the exact location of remnant populations, the network resulting from the BARRIER analysis has been slightly shifted. Populations correspond to map in Fig. 1

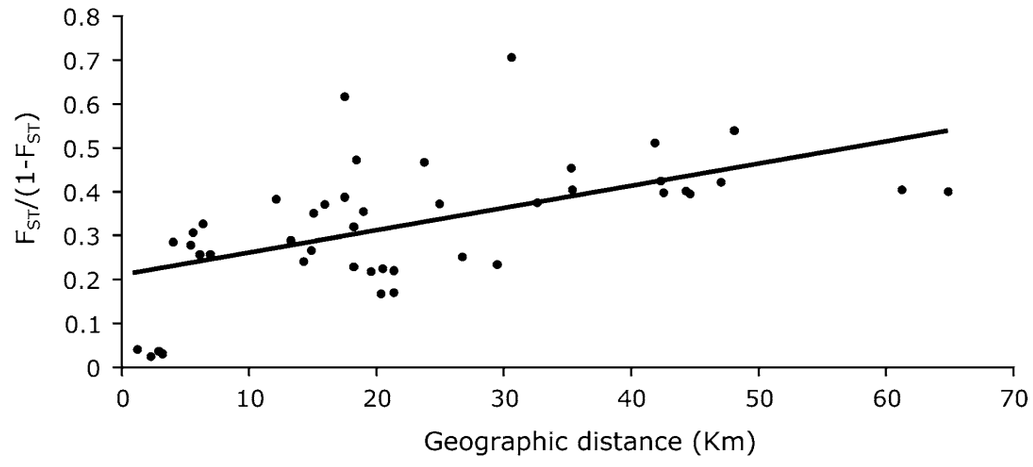
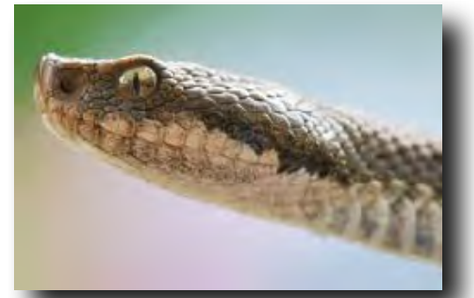


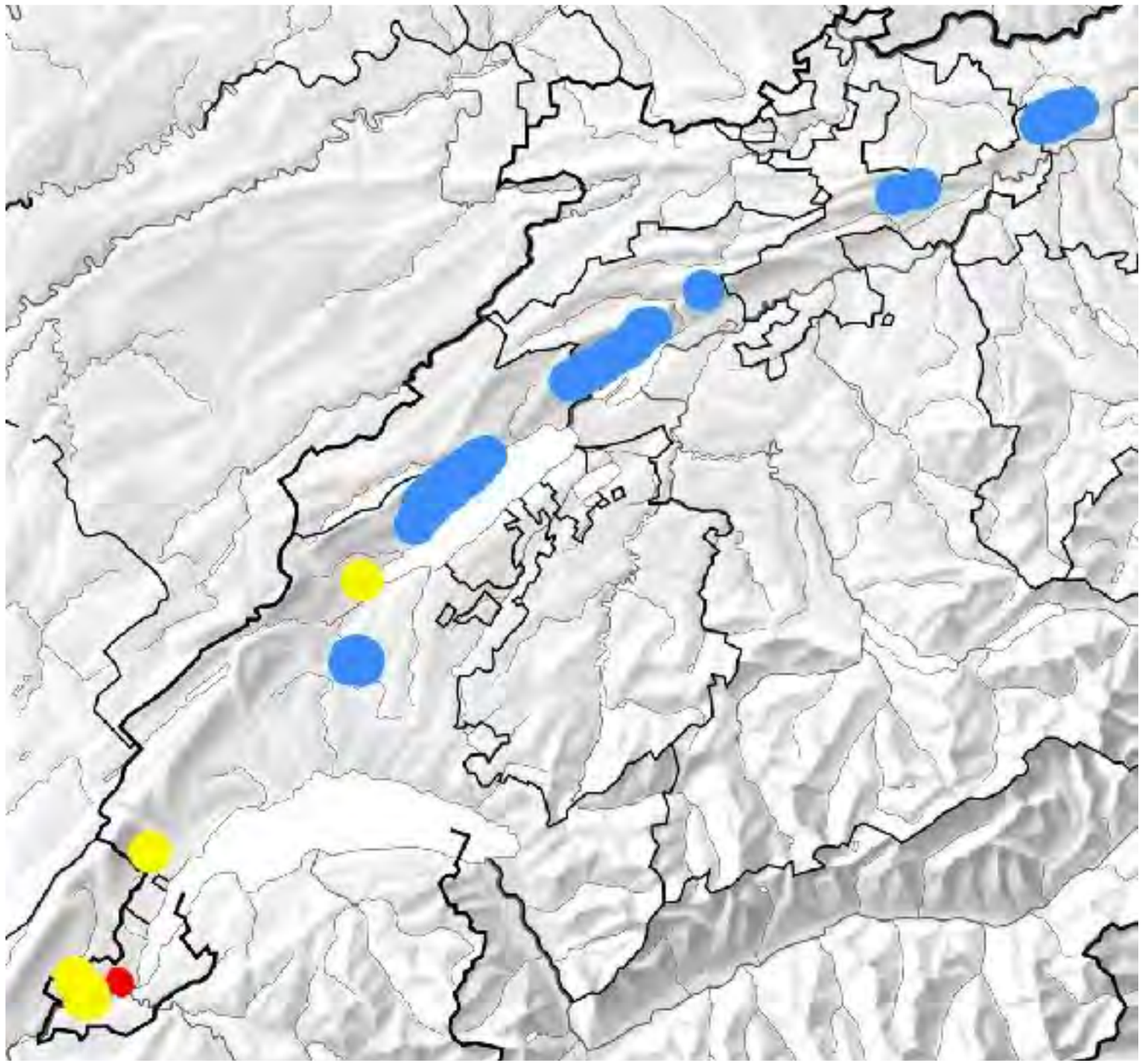
Fig. 3 Isolation by distance in ten Jura Mountains populations of *Vipera berus* ($r^2 = 0.33$, $P < 0.01$). Comparisons are pairwise between all populations

(*Vipera aspis*)
E: Asp viper
G: Aspisviper
F: Vipère aspic

European Vipers - Vipera berus *and* Vipera aspis

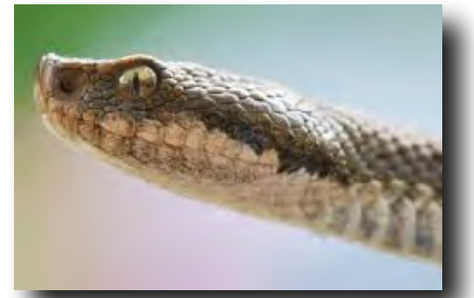


- population genetics of the asp viper in the lowland of Switzerland

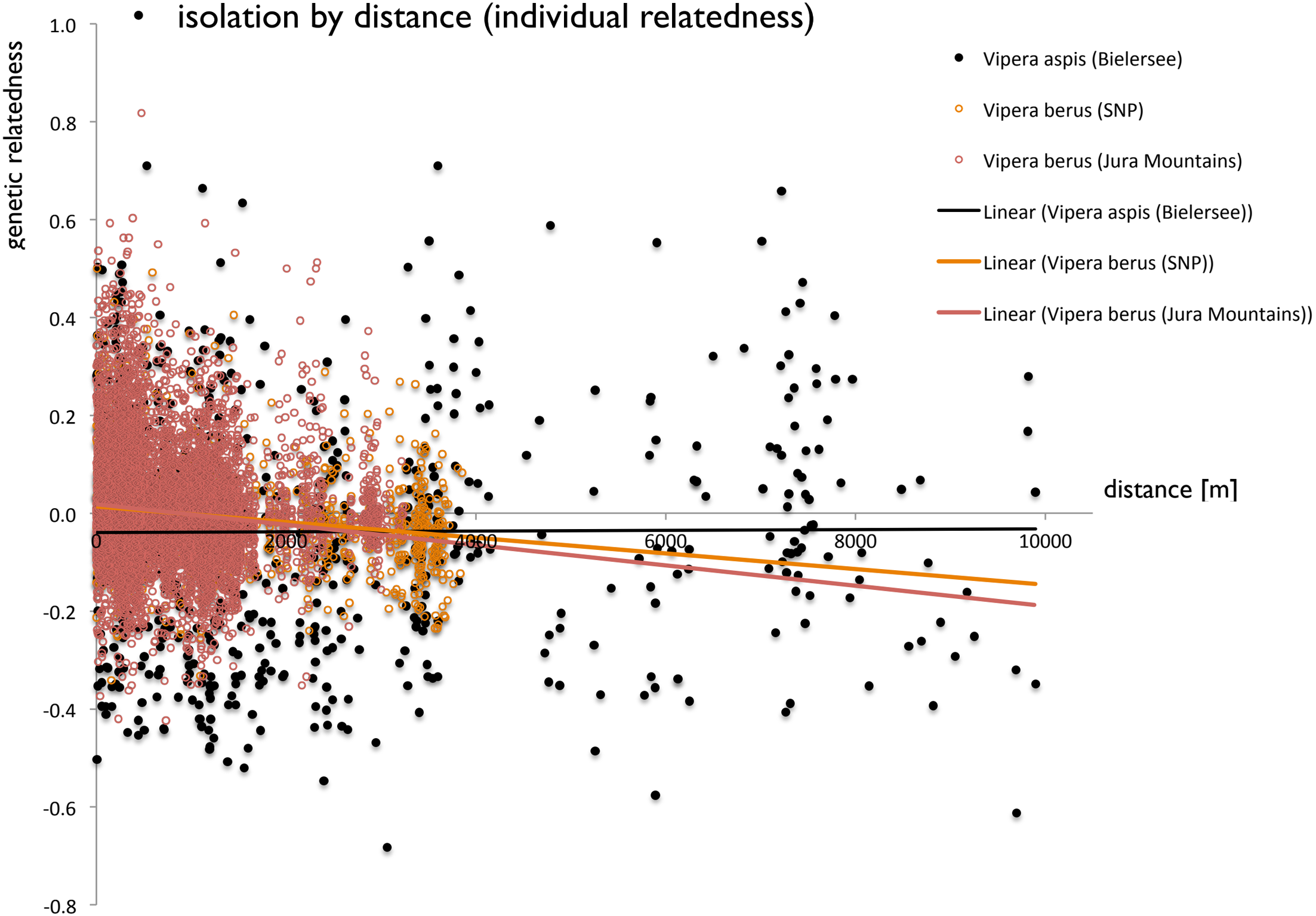


Silvia Geser's PhD thesis, University of Basel

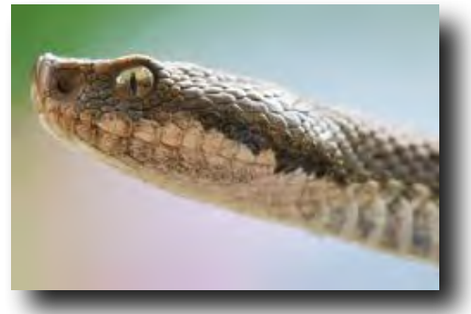
European Vipers - Vipera berus *and* Vipera aspis



- isolation by distance (individual relatedness)



European Vipers - Vipera berus *and* Vipera aspis



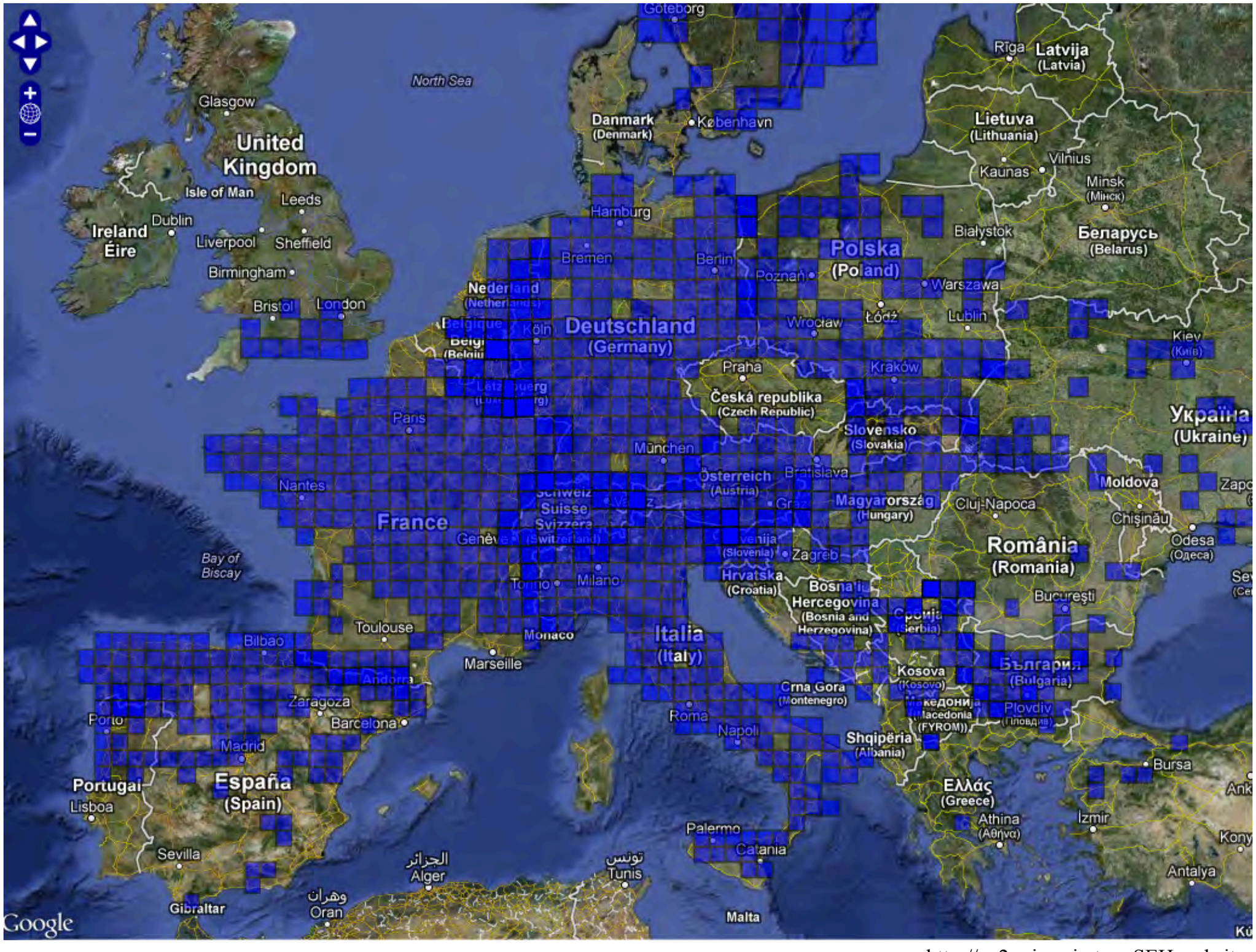
- "similar species"
- Asp viper
  - very limited genetic differentiation
  - high gene flow if corridors occur
- Adder
  - strong genetic differentiation
  - lack of gene flow even at a low distance

➡ complete different behavior

The smooth snake - *comparison between 3 locations*

(Coronella austriaca)
E: smooth snake
G: Schlingnatter
F: Coronelle lisse

The smooth snake - *comparison between 3 locations*



http://na2re.ismai.pt SEH website

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in UK

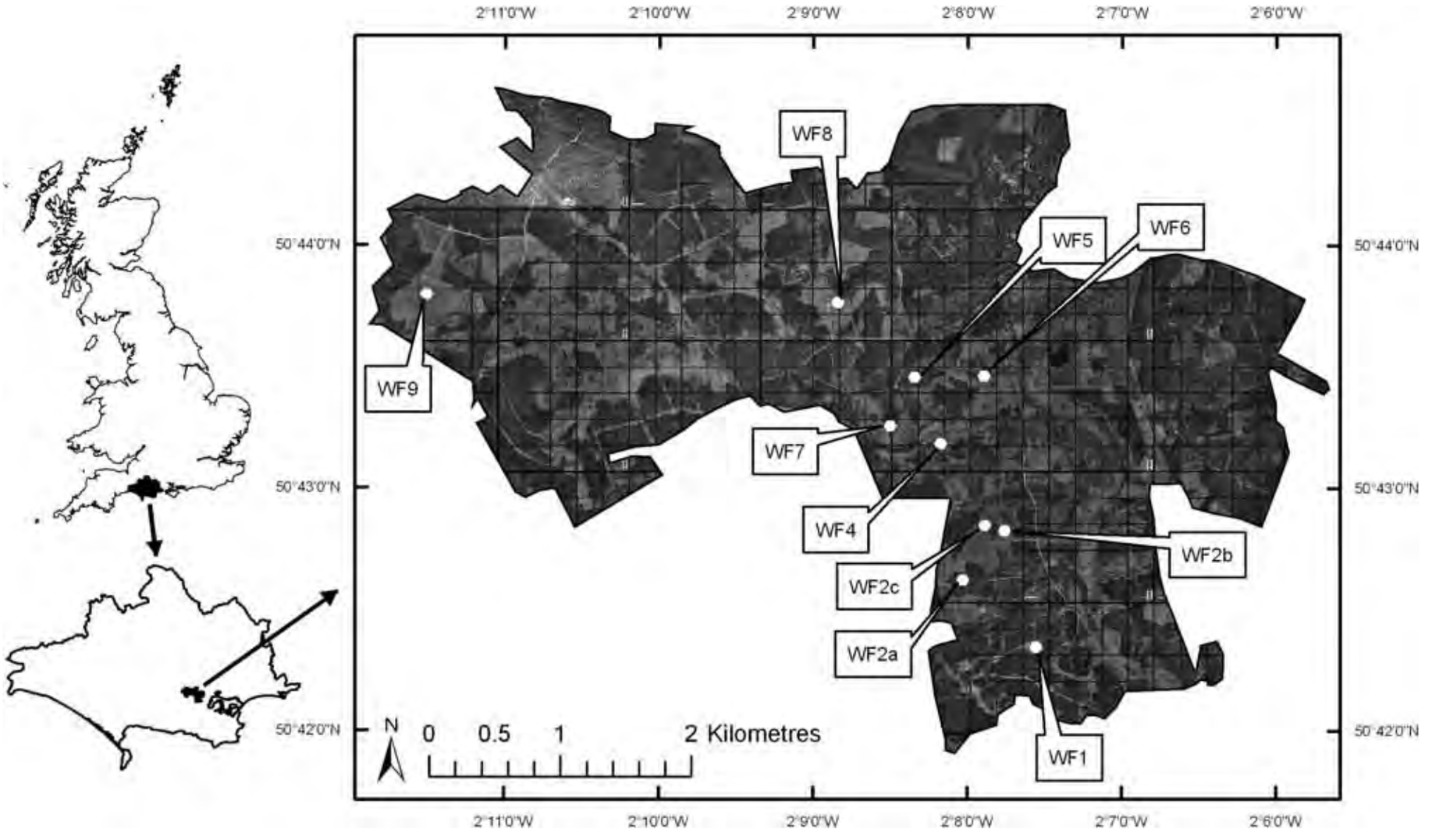
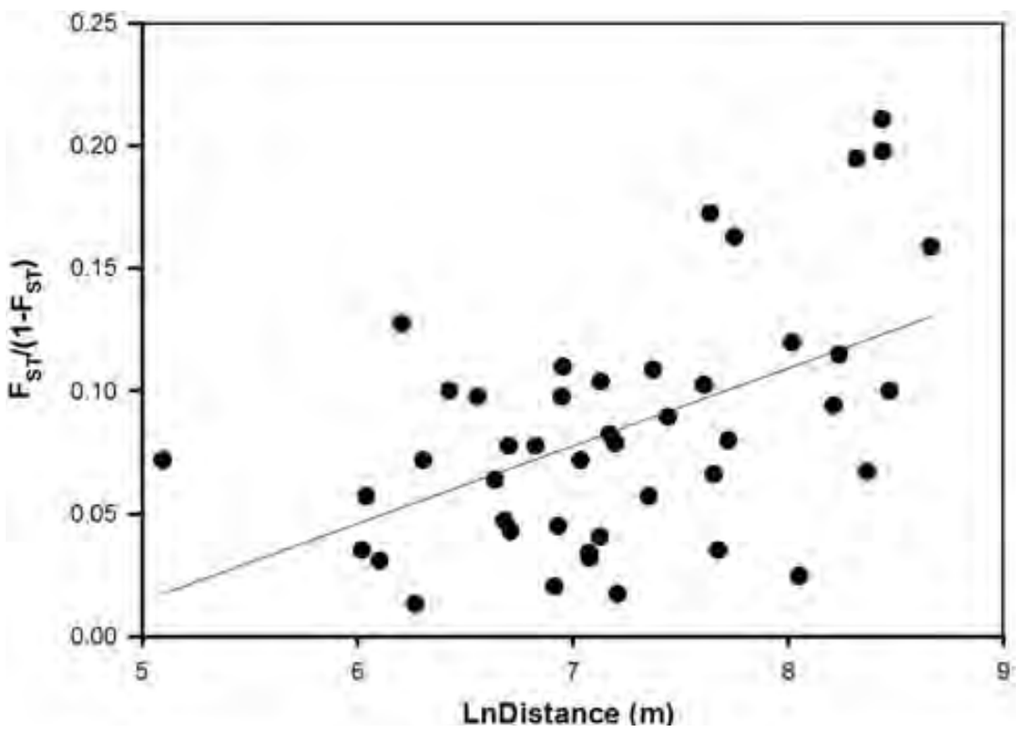


Figure 1 Localities of the 10 arrays of artificial *refugia* (white hexagons) within Wareham Forest, Dorset, from which individual *Coronella austriaca* were sampled. Darker coloured areas indicate stands of closed canopy *Pinus* spp., whereas lighter coloured areas include heathland, clearfelled stands and young *Pinus* spp. plantations.

Pernetta et al. (2011) Heredity

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in UK



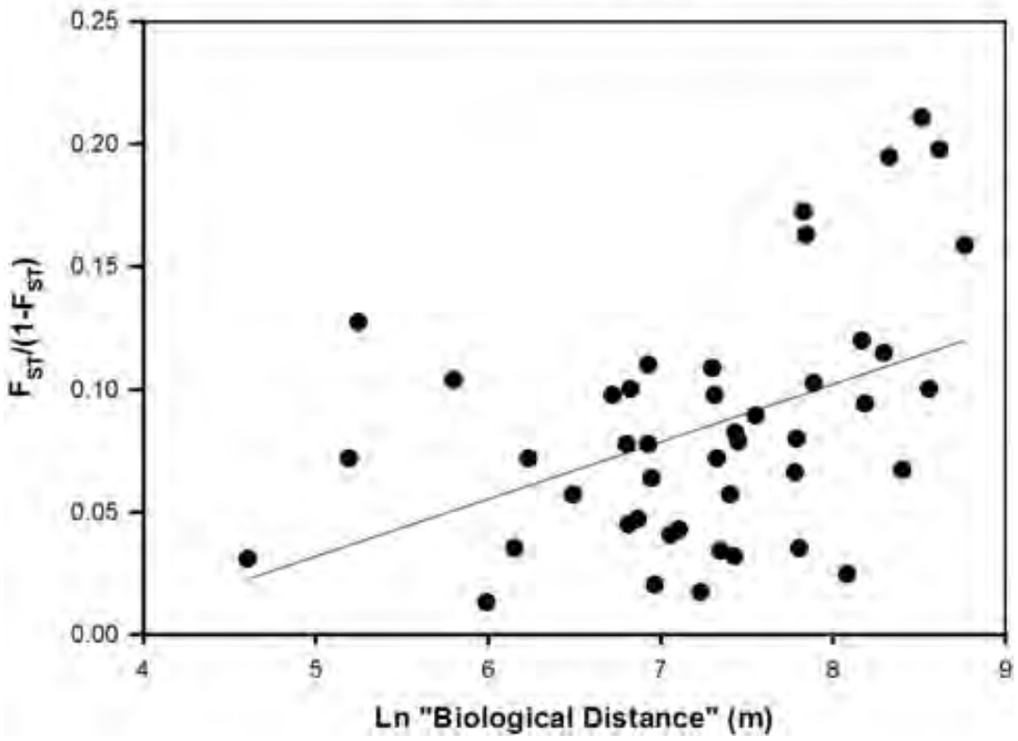
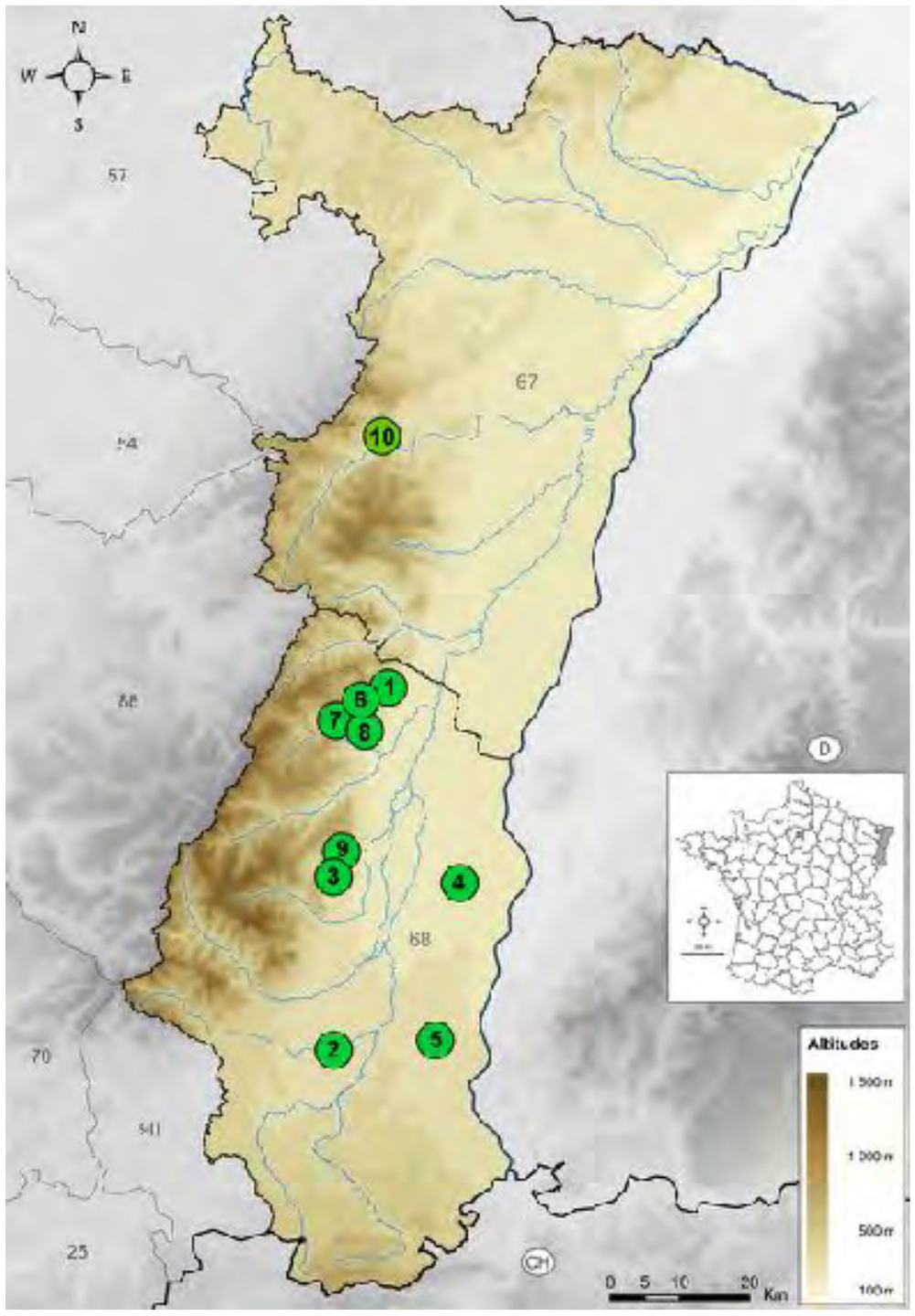


Figure 2 Relationship between pairwise F_{ST} values and (**a**) the straight-line distance ($r_S = 0.511$, $P = 0.003$), and (**b**) the 'biological' distance ($r_S = 0.445$, $P = 0.005$) between smooth snake-sampling sites in Wareham Forest.

Pernetta et al. (2011) Heredity

The smooth snake - *comparison between 3 locations*

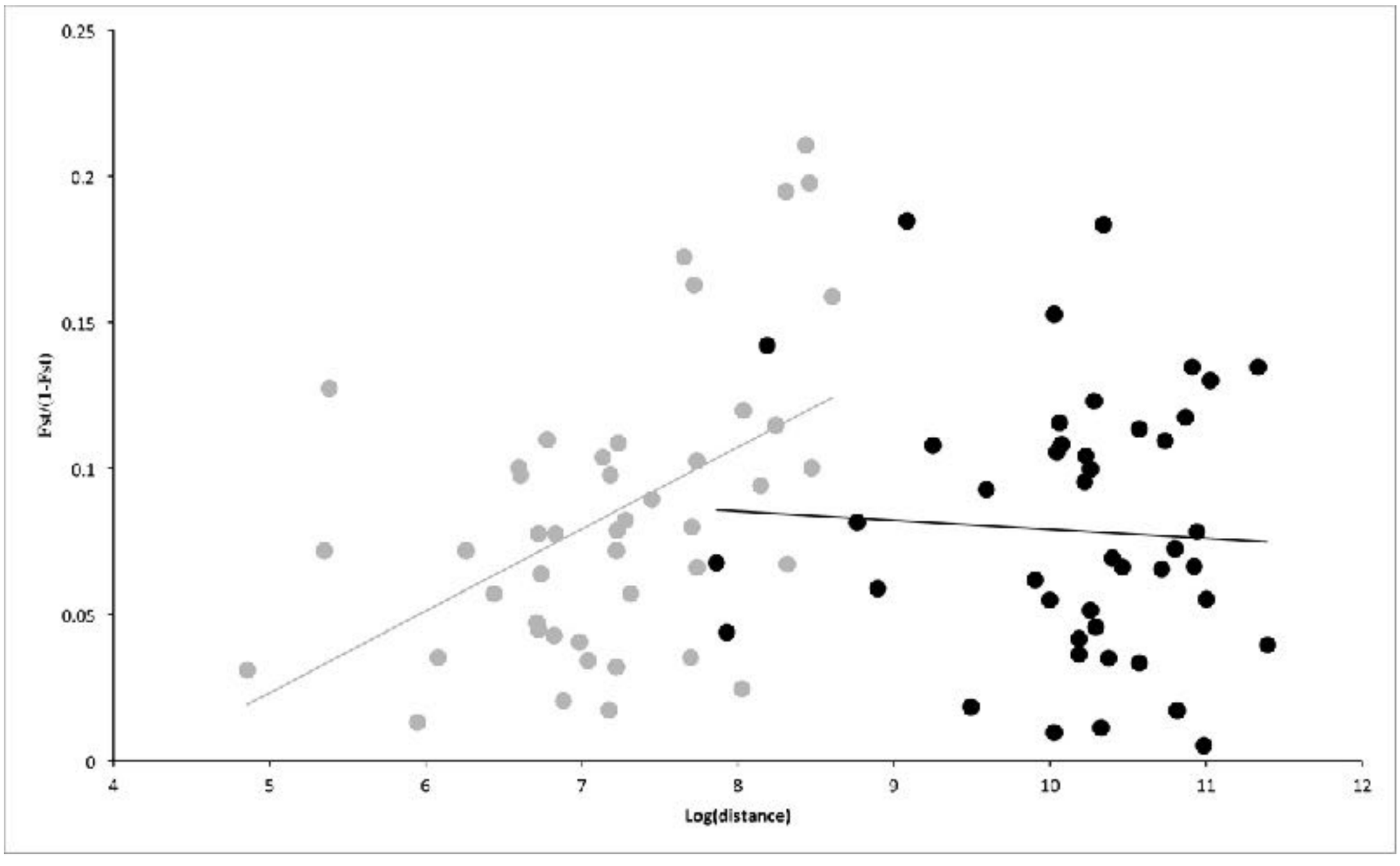
- population genetics of the smooth snake in the Alsace region



Vacher & Ursenbacher (submitted) Amphibia-Reptilia

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in the Alsace region



Vacher & Ursenbacher (submitted) Amphibia-Reptilia

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in Belgium

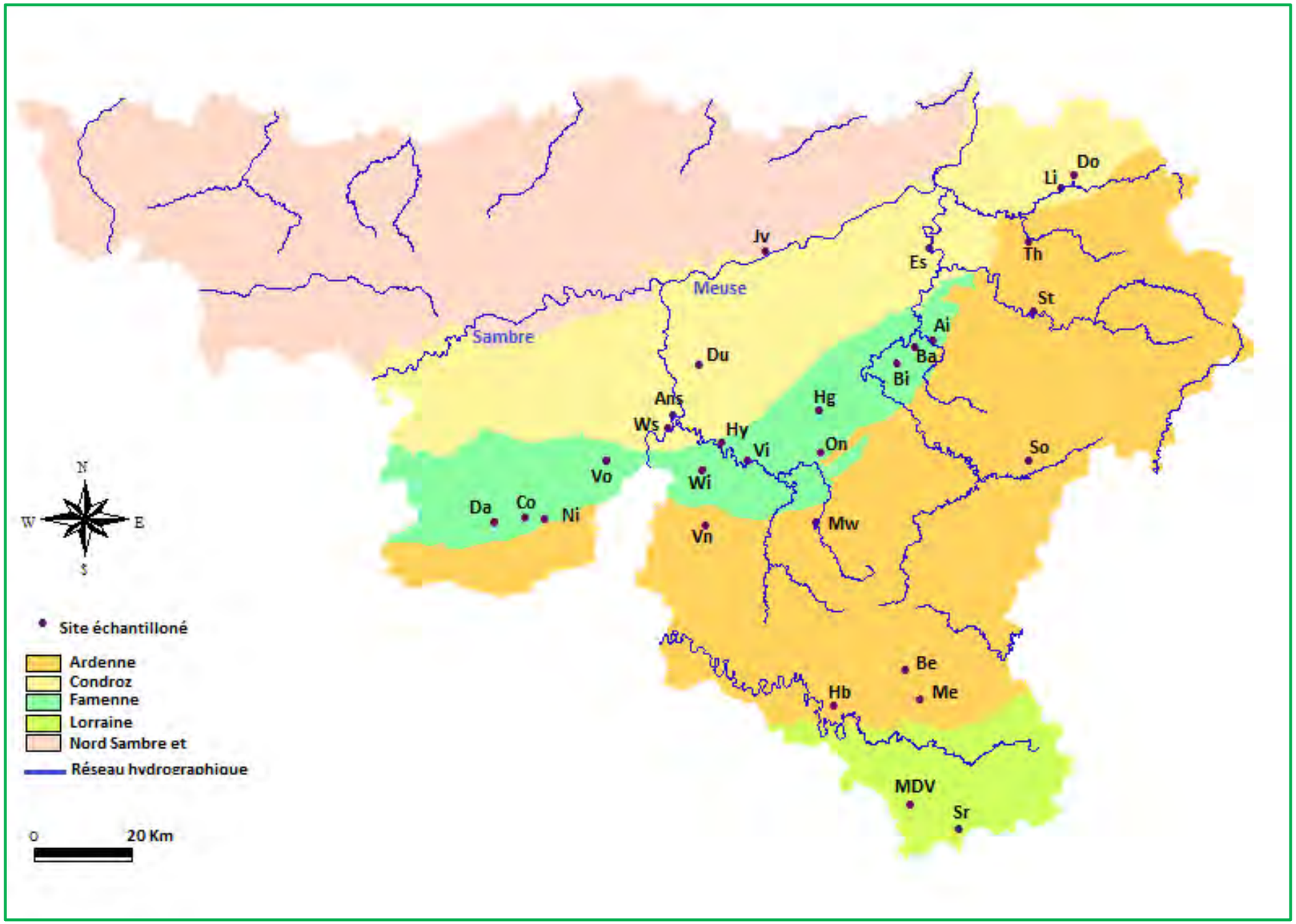


Figure 8: Carte des 5 éco-régions de Wallonie. Les sites d'échantillonnages sont représentés par un point violet.

Julie Cauwenberg: MSc work

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in Belgium

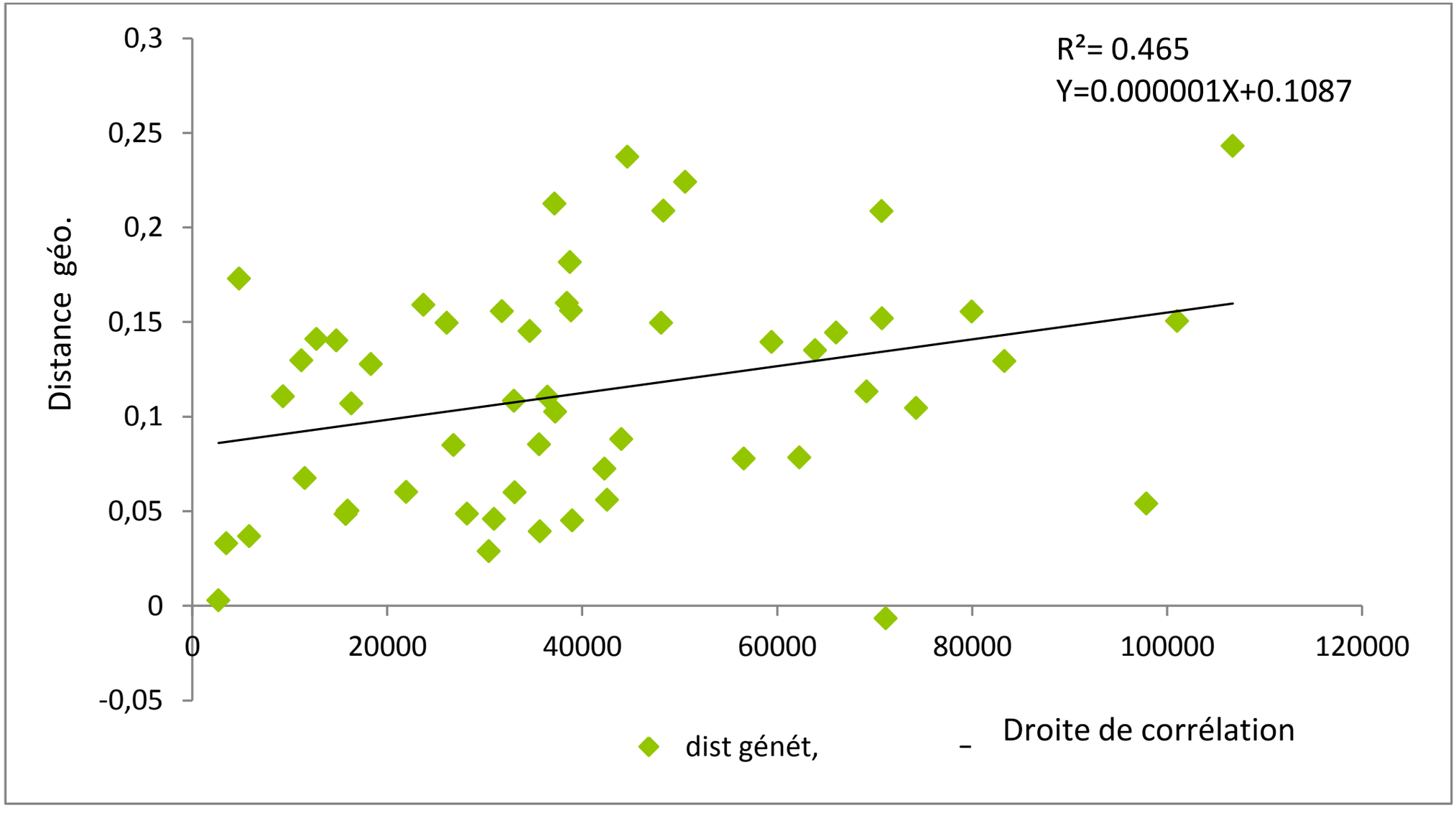


Figure 11: Corrélation entre les matrices de distances géographiques et les matrices de distances génétiques (Fst et Fst corrigés) obtenues par Excell.

Julie Cauwenberg: MSc work

The smooth snake - *comparison between 3 locations*

- population genetics of the smooth snake in UK
  - isolation by distance
  - high F_{ST} value already at a low distance: F_{ST} = 0.10 for populations 3 km away
- population genetics of the smooth snake in Alsace region
  - no isolation by distance
  - mean F_{ST} < 0.10 even for populations more than 60 km away
- population genetics of the smooth snake in Belgium
  - isolation by distance
  - F_{ST} = 0.10 for populations about 20 km away
- different results:
  - ?? central / northern part of the distribution area
  - ?? differences: habitat, connectivity, history, ...

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

(Anguis fragilis)
E: slow worm
G: Blindschleiche
F: Orvet

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

- combining population genetics and Landscape information (GIS)
- aims: understand the landscape elements that reduce/increase the gene flow

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

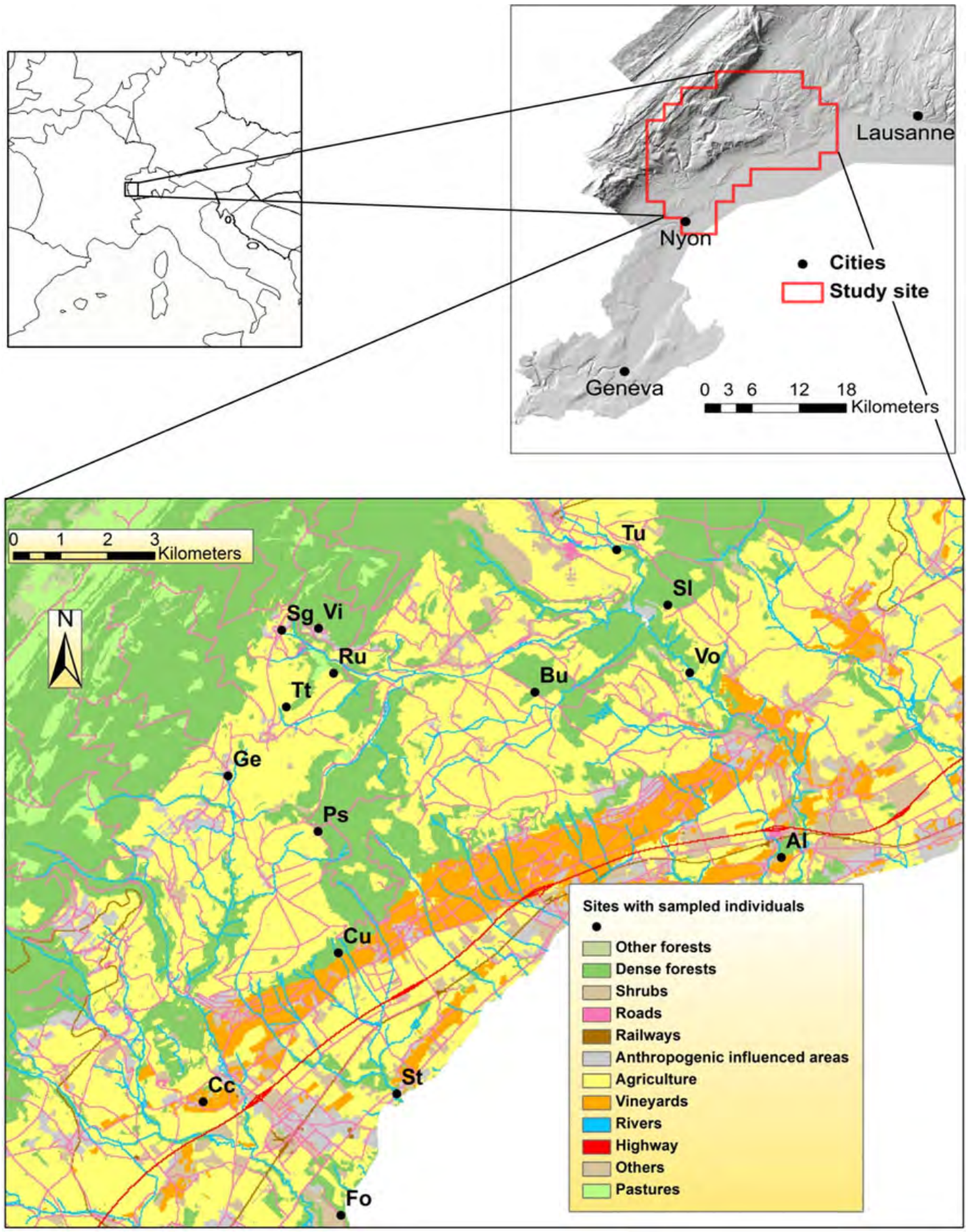


Fig. 1 Repartitioning of the sampled sites in Switzerland (between Lausanne and Geneva) with the landscape elements used for the landscape genetic analysis. The *black dots* represent sites where slow worms were sampled. Abbreviations correspond to those in Table 3. (Color figure online)

Geiser et al. (2013) Conservation Genetics

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

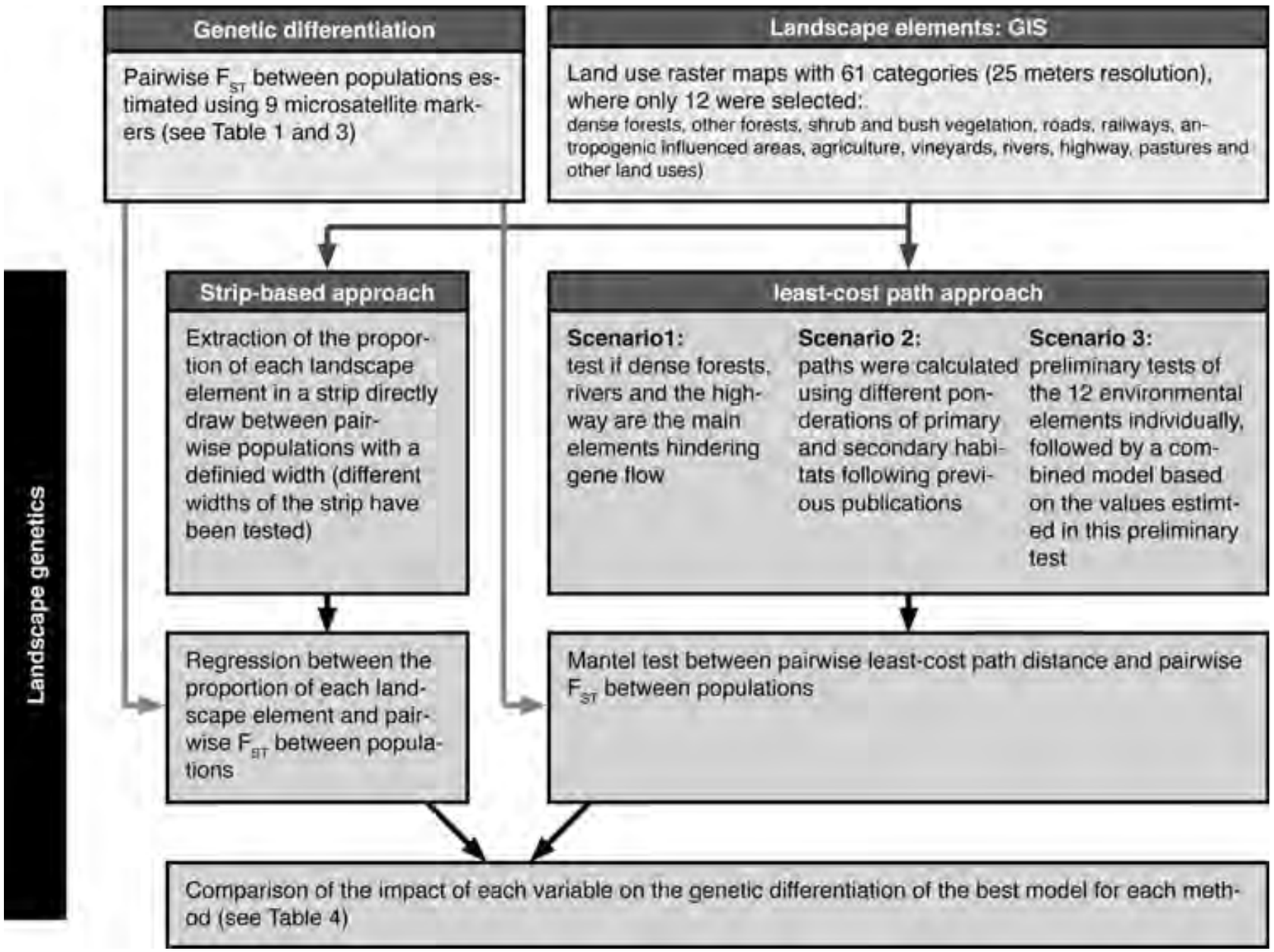


Fig. 2 Summary of the different landscape genetic approaches used in this study: the strip-based approach following that of Emaresi et al. (2011) on the *left* and the least-cost path method on the *right*

Geiser et al. (2013) Conservation Genetics

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

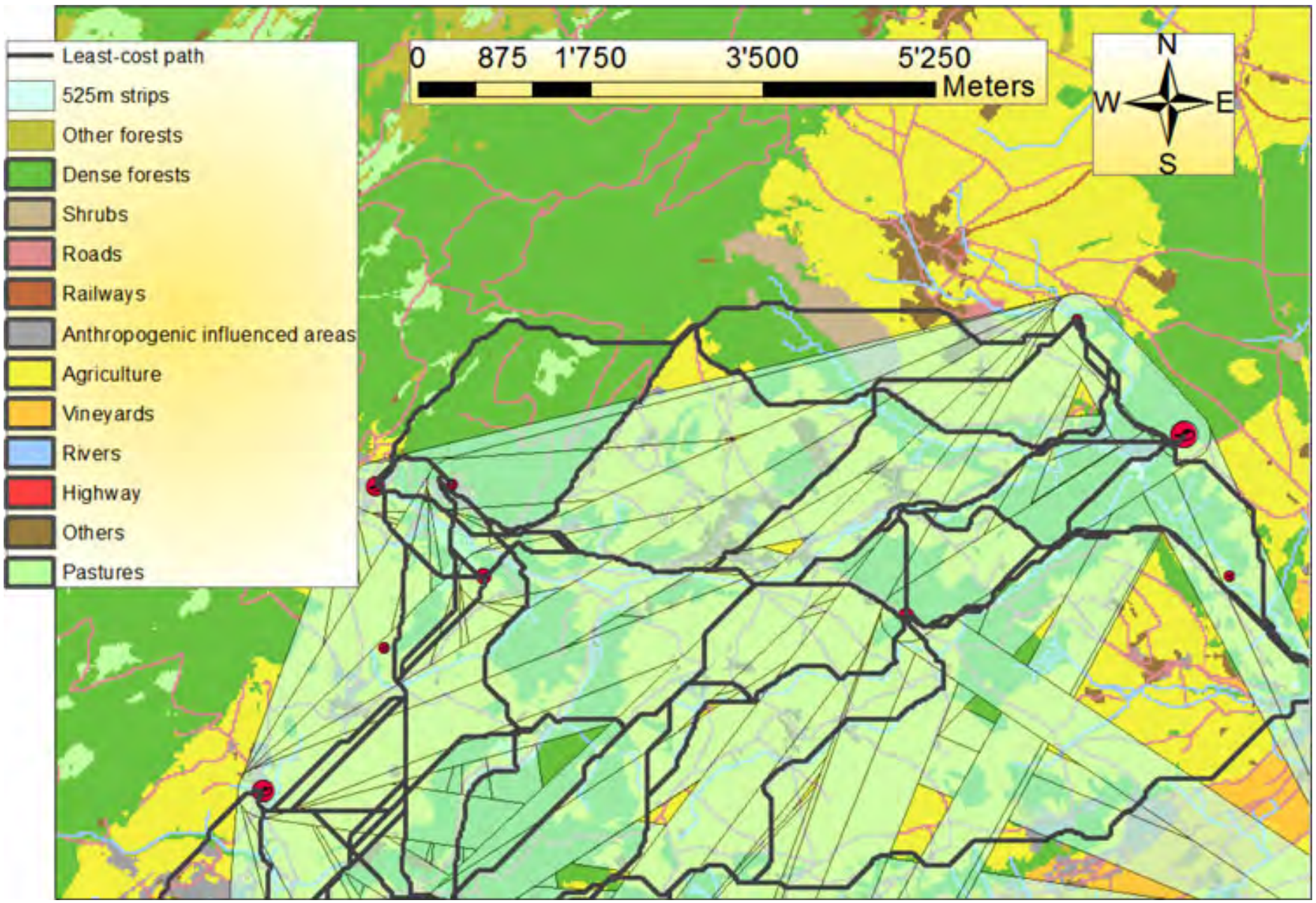


Fig. 3 A part (northern part) of the studied area with pairwise least-cost paths (*black lines*) of the best model and the pairwise straight 525-m wide strips of the best model (*light green stripes*). (Color figure online)

Geiser et al. (2013) Conservation Genetics

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

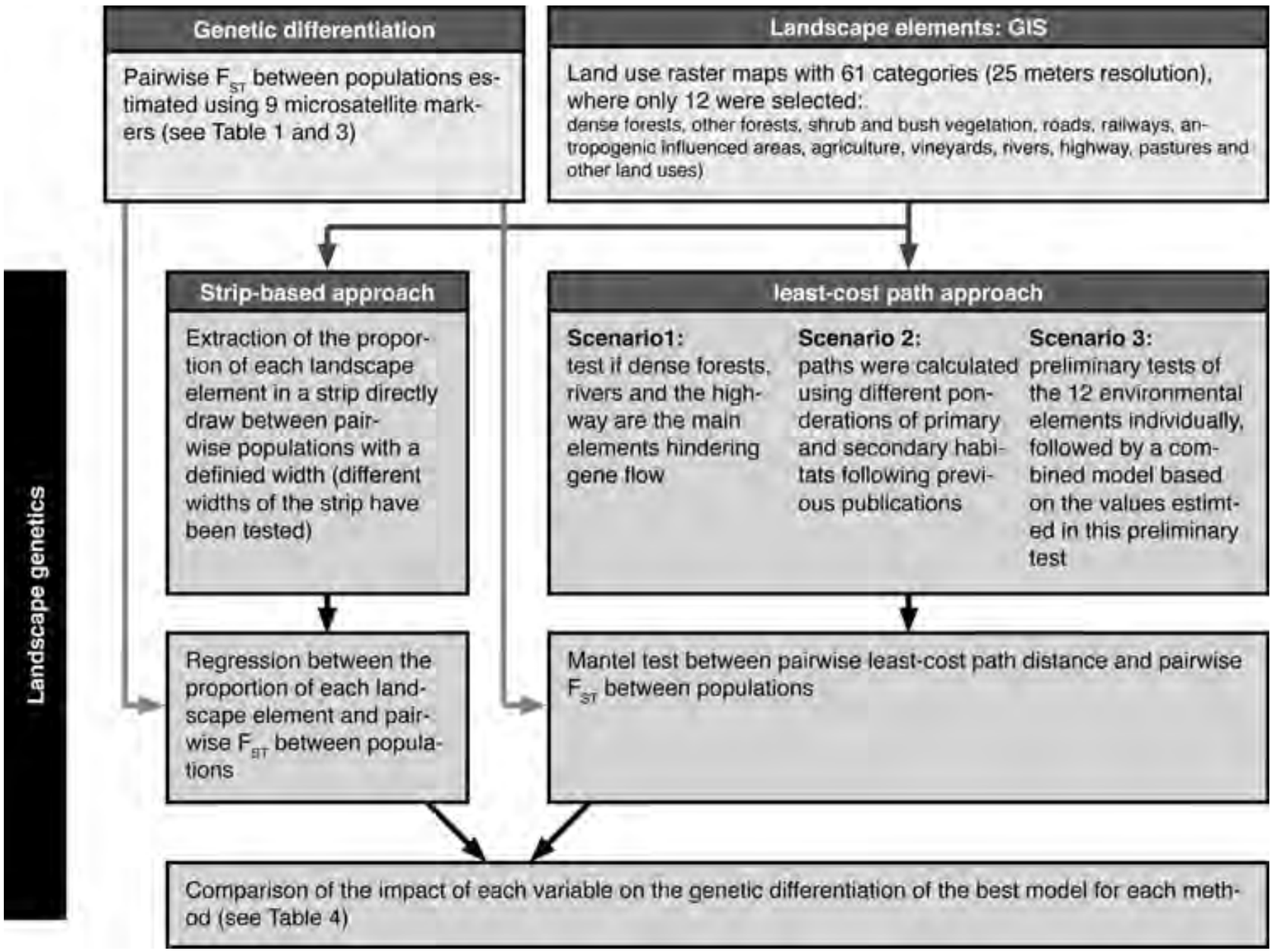


Fig. 2 Summary of the different landscape genetic approaches used in this study: the strip-based approach following that of Emaresi et al. (2011) on the *left* and the least-cost path method on the *right*

Geiser et al. (2013) Conservation Genetics

Landscape Genetics - *example with the slow worm (*Anguis fragilis*)*

Table 4 Comparison of the environmental elements negatively impeding gene flow in the least-cost path and strip-based approaches

Variable	Least-cost modelling	Strip-based approach
Railway	24 %	20 %
Highway	13 %	15 %
Other land use	No	15 %
Anthropogenic-influenced areas	No	13 %
Agriculture	No	No
Dense forests	<1 %	No
Other forests	No	10 %
Roads	24 %	10 %
Rivers	13 %	9 %
Vineyards	24 %	8 %

Environmental elements favouring gene flow were not taken into account when assessing their role in fragmentation in the two approaches. They are consequently noted with a "No"

Geiser et al. (2013) Conservation Genetics

Environmental DNA

genetic material from living organisms that can be detected by sampling the non-living environment

taking water/soil samples

extract whole DNA

amplify whole DNA with a custom primer set

sequencing / next generation sequencing (depending on the project)

useful for

detection of rare species

genetic diversity in soils

...

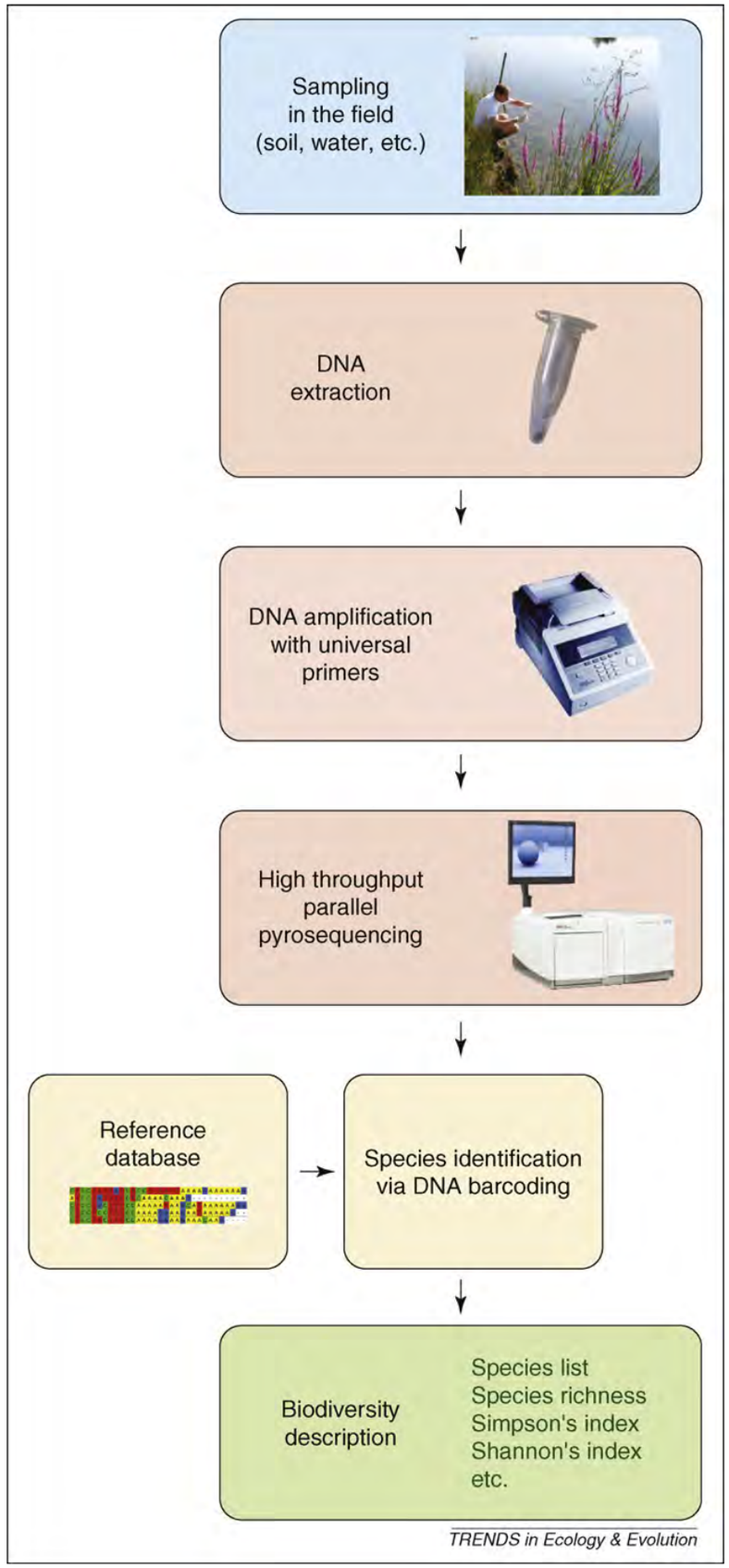


Figure 1. Methodology for analyzing biodiversity from environmental samples based on next-generation DNA sequencers. After collecting environmental samples in the field, extracting DNA and amplifying with universal primers that target very short DNA fragments (less than 150 base pairs), hundreds or thousands of amplified DNA molecules are sequenced using next-generation sequencers (Box 3). Using a reference DNA database, the taxa these sequences come from are identified and used to estimate different biodiversity parameters.

Valentini et al. (2009) TREE

Environmental DNA - *detection of invasive samples*

detection of an invasive frog (*Rana catesbeiana*)

- water samples (15 ml)
- extraction and amplification (PCR) with specific primers (about 80 bp amplified), using a multiple-tube approach (3-5 tubes/sample)
- electrophoresis and sequencing
- positive amplification for most samples even if the density of bullfrog is limited

Table 1. Rate of bullfrog detection in water samples.

pond	bullfrog presence and relative abundance	water samples positives at least once	positive PCRs
1	yes-low	2/3	2/9
2	yes-low	3/3	6/9
3	yes-low	2/3	2/9
4	yes-high	3/3	8/9
5	yes-high	3/3	6/9
6	yes-high	3/3	8/10
7	no	0/3	0/9
8	no	0/3	0/9
9	no	0/3	0/15

Ficetola et al. (2008) Biology Letters

Environmental DNA - *detection of rare species*

detection of two species in a stream

- low density of the species
- fast-moving streams
- filtered water samples from five streams with varying densities
- Idaho giant salamanders: 5/5
  Rocky Mountain tailed frogs:4/5
- variability of detection ability (early spring / in early fall)

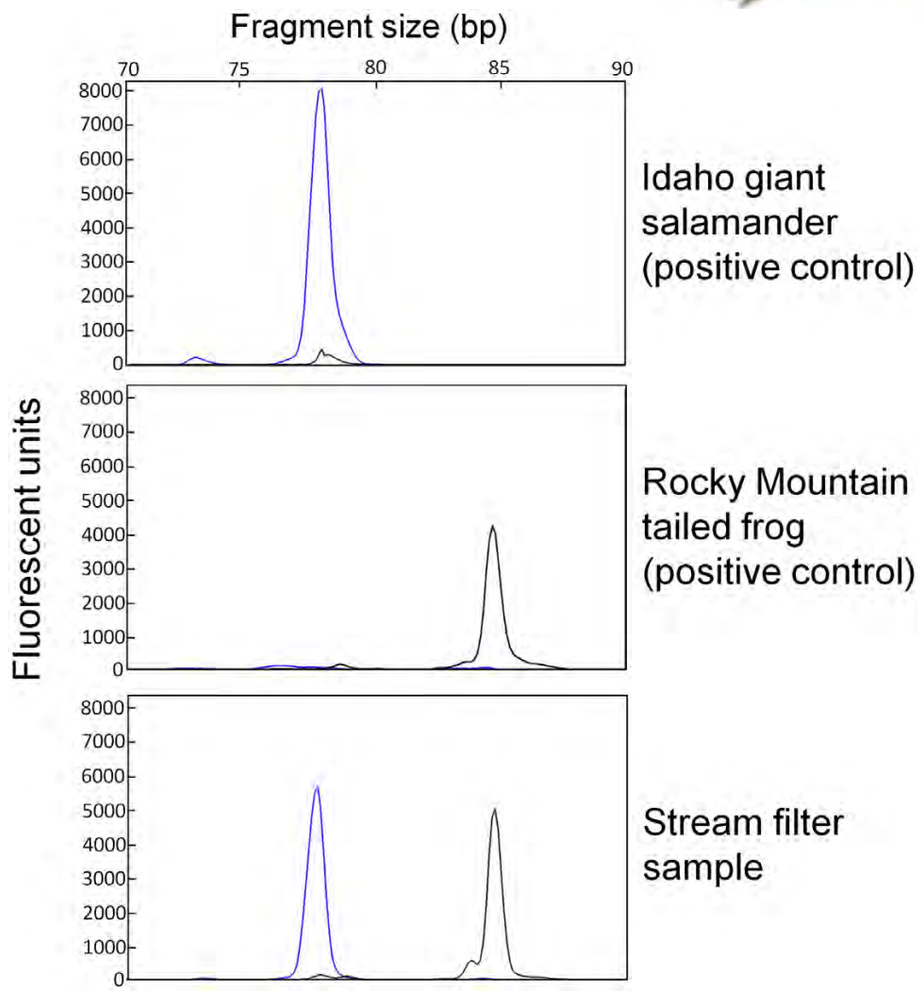


Figure 1. Electropherograms of species-specific PCR amplification of DNA in positive controls and stream water. The blue peak indicates the species-specific fragment for Idaho giant salamanders (*Dicamptodon aterrimus*) and the black peak indicates the species-specific fragment for tailed frogs (*Ascaphus montanus*). All reactions were diluted to produce these images.
doi:10.1371/journal.pone.0022746.g001

Goldberg et al. (2011) PLoS ONE

Environmental DNA - *quantitative evaluation*

relation between eDNA and species density

- eDNA quantified with qPCR
- rough evaluation of the amphibian diversity
- positive correlation between eDNA quantification and density evaluation (*P. fuscus*: $P < 0.01$, $R^2 = 0.68$; *T. cristatus*: $P < 0.05$; $R^2 = 0.40$, Pearson's product–moment correlation)
-

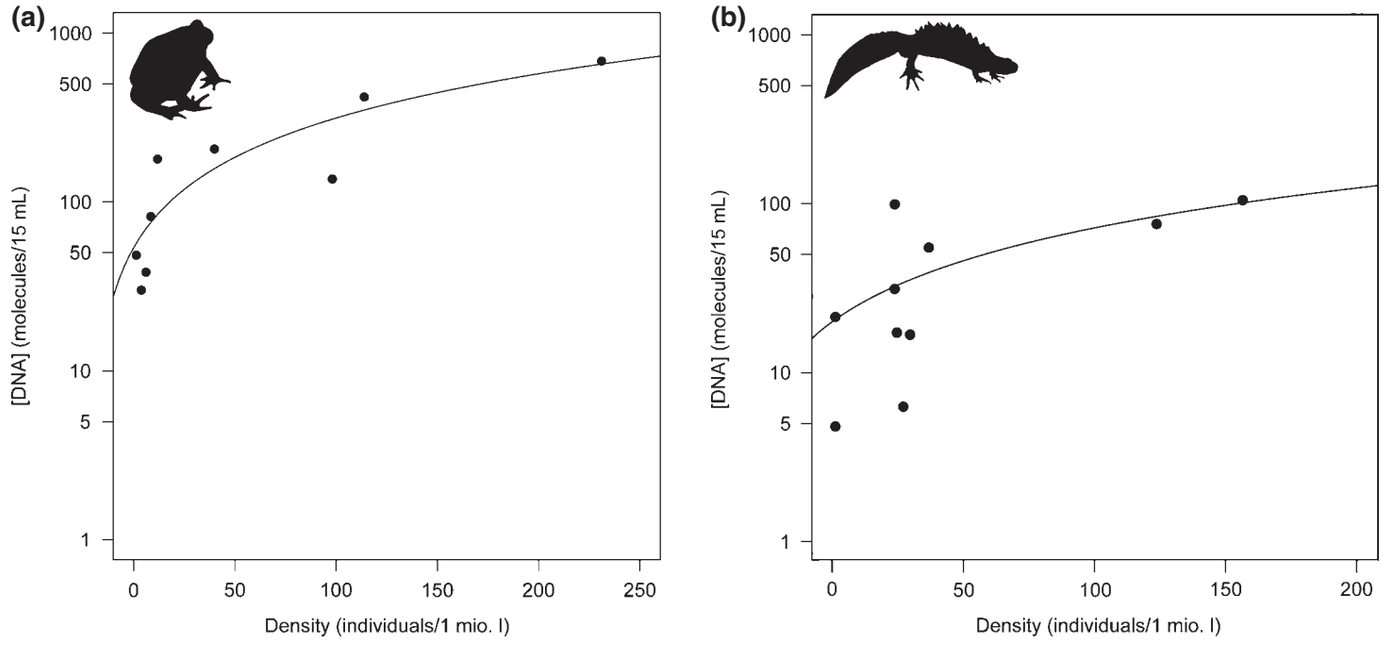


Fig. 3 Environmental DNA quantification in natural ponds with *Pelobates fuscus* ($n = 9$) (a) and *Triturus cristatus* ($n = 10$) (b). Pearson's product moment correlation between average number of DNA molecules and estimated population size in each pond. The line shows linear regression, a: $R^2 = 0.68$, $P < 0.01$; b: $R^2 = 0.40$, $P < 0.05$.

Thomsen et al. (2011) Molecular Ecology

Environmental DNA - *persistence of eDNA in water*

eDNA: how long the DNA persist in the water

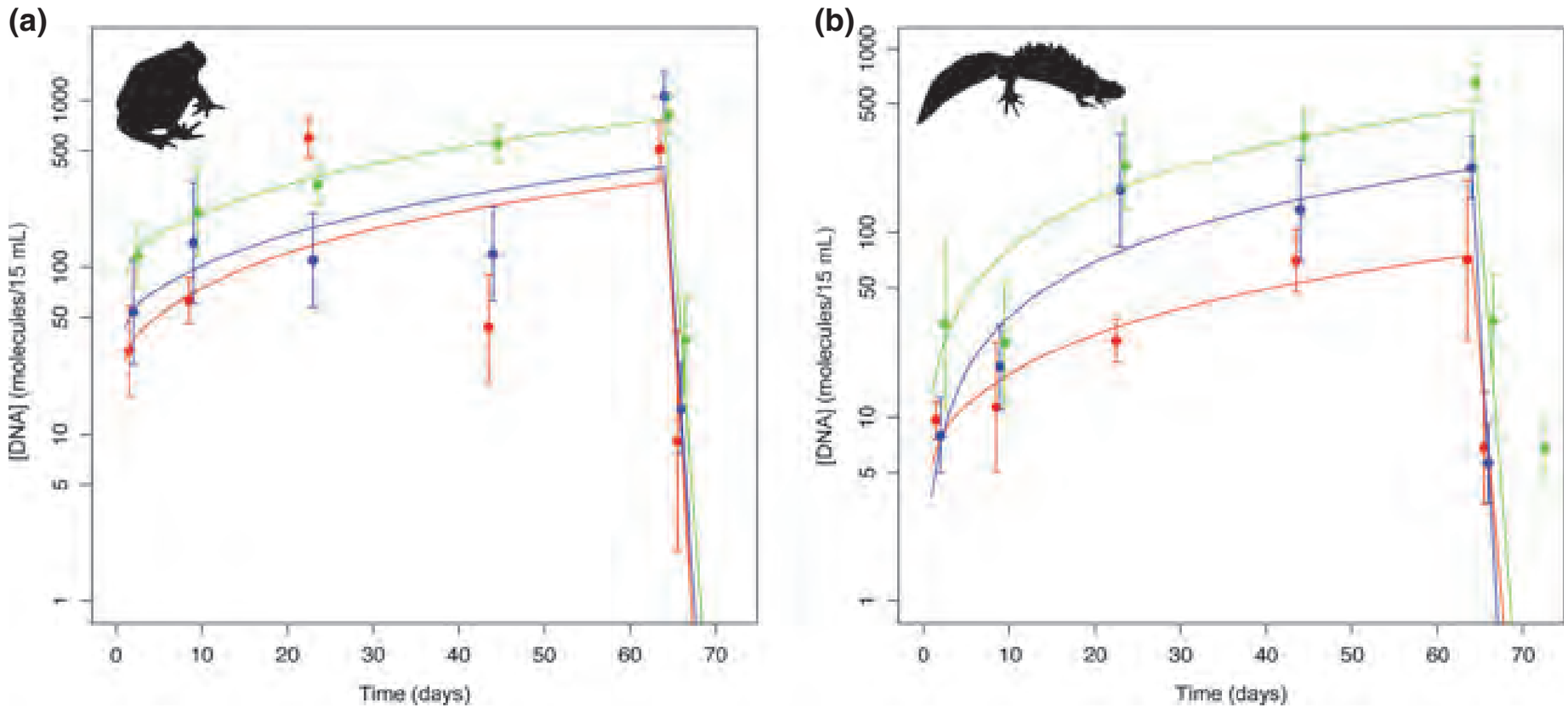


Fig. 4 Environmental DNA quantification in controlled mesocosm experiment with *Pelobates fuscus* (a) and *Triturus cristatus* (b). Means + 2 × SE of DNA molecules in water samples from freshwater containers with 1 (red), 2 (blue) or 4 (green) individuals in 80 L. After a control sample was taken, animals were introduced at time $t = 0$ and samples were taken at 2, 9, 23, 44, 64, 66, 73, 79 and 112 days. Animals were removed at $t = 64$ (after sampling). The lines show a differential equation model fitted to the data (see Materials and methods section), a: $R^2 = 0.29$ (red), 0.50(blue), 0.61(green); b: $R^2 = 0.49$ (red), 0.67 (blue), 0.62 (green).

Thomsen et al. (2011) Molecular Ecology

genetic tools in conservation

- genetics: interesting tools to answer several questions
  - global genetic structure
    - species
    - ESU
  - population history
    - phylogeny / phylogeography
    - bottleneck detection
  - population structure
    - population differentiation / migration
    - inbreeding, inbreeding depression
  - forensic
    - e.g. illegal hunting
  - better understanding of the biology of the species
    - pedigree, paternity assignment
    - ....

General conclusions

- genetics: interesting tools to answer several questions

BUT

- must be combined with other approaches
    - biology of the studied species
    - history
    - Population viability analysis (PVAs)
        - as well as combining with e. g. genetic impacts of inbreeding
    - ...

for conservation, genetic is just an additional tool