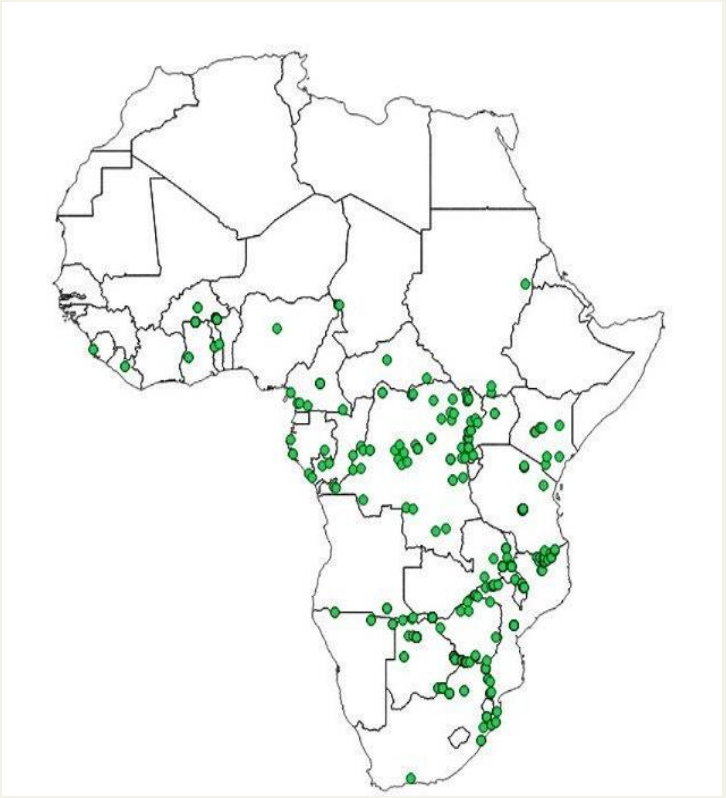
Assigning elephant ivory with stable isotopes: pitfalls, caveats and risks

Stefan Ziegler*°

*WWF Germany; °Evolutionary Ecology Group, Goethe University Frankfurt, Germany

Not a new concept, but…

- Earlier studies were often restricted by low numbers of reference samples.
- More than 500 references from 54 African elephant range states collected.
- Assignment tests to validate the potential of isotope markers for provenance testing quantitavely.
- Highly conservation relevant.



Methodology

Ivory samples:

- 500 samples from 25 African elephant and 6 Asian elephant range states.
- Source: museums, CITES Management Authorities and trophy hunters.

Geochemical analysis:

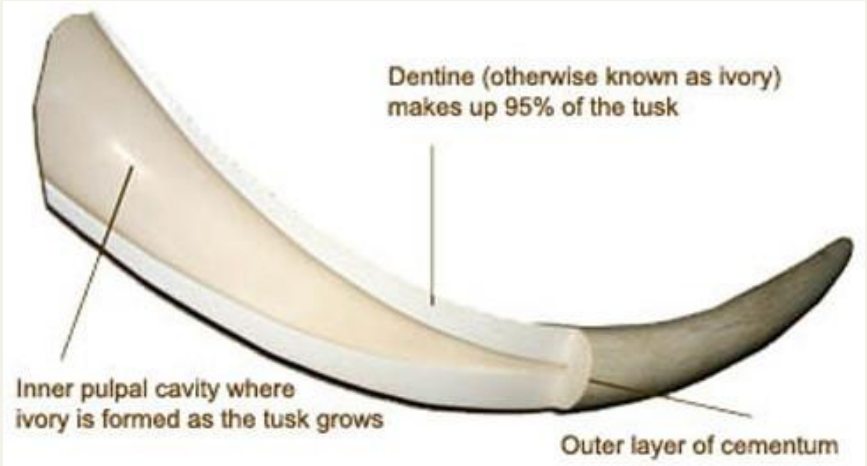
- Stable isotope analysis of D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$.

GIS platfom:

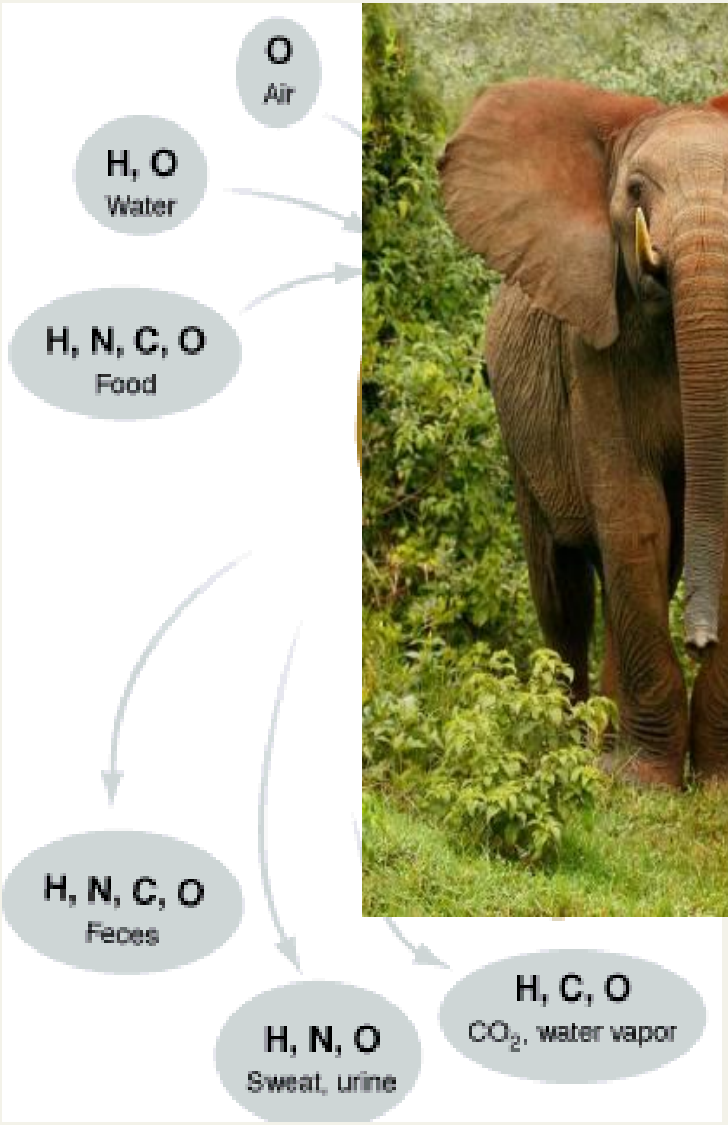
- Superimposing georeferenced isotopic measurements with layers of climate, elevation and vegetation (resolution 50km grid).

Sample collection

- Sampling from the most proximal end of the tusk.
- As this is the youngest part of the tusk, it is assumed that the isotopic signal reflects the environment where the animal lived just before its death.
- Bulk measurements of powdered ivory due to limited amount of reference material from museums and trophy hunters.



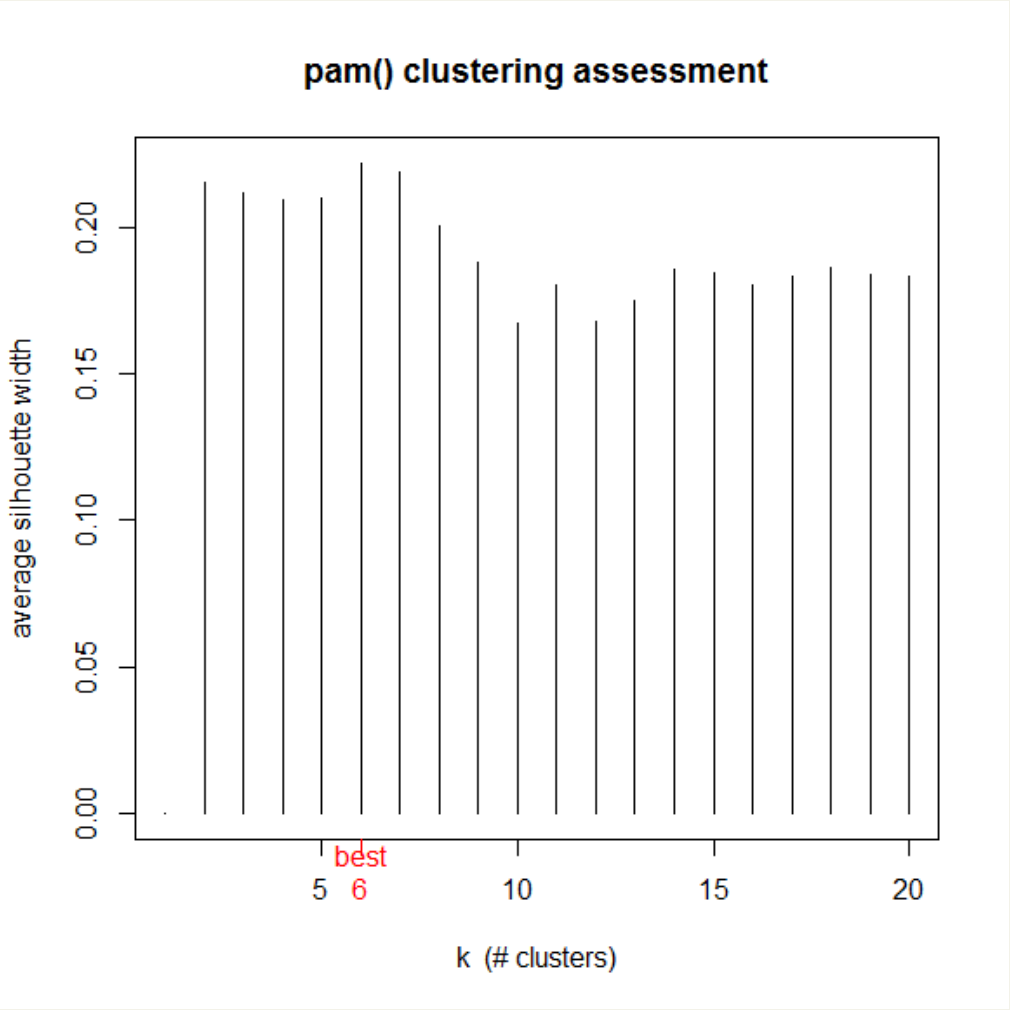
What are you interested in?



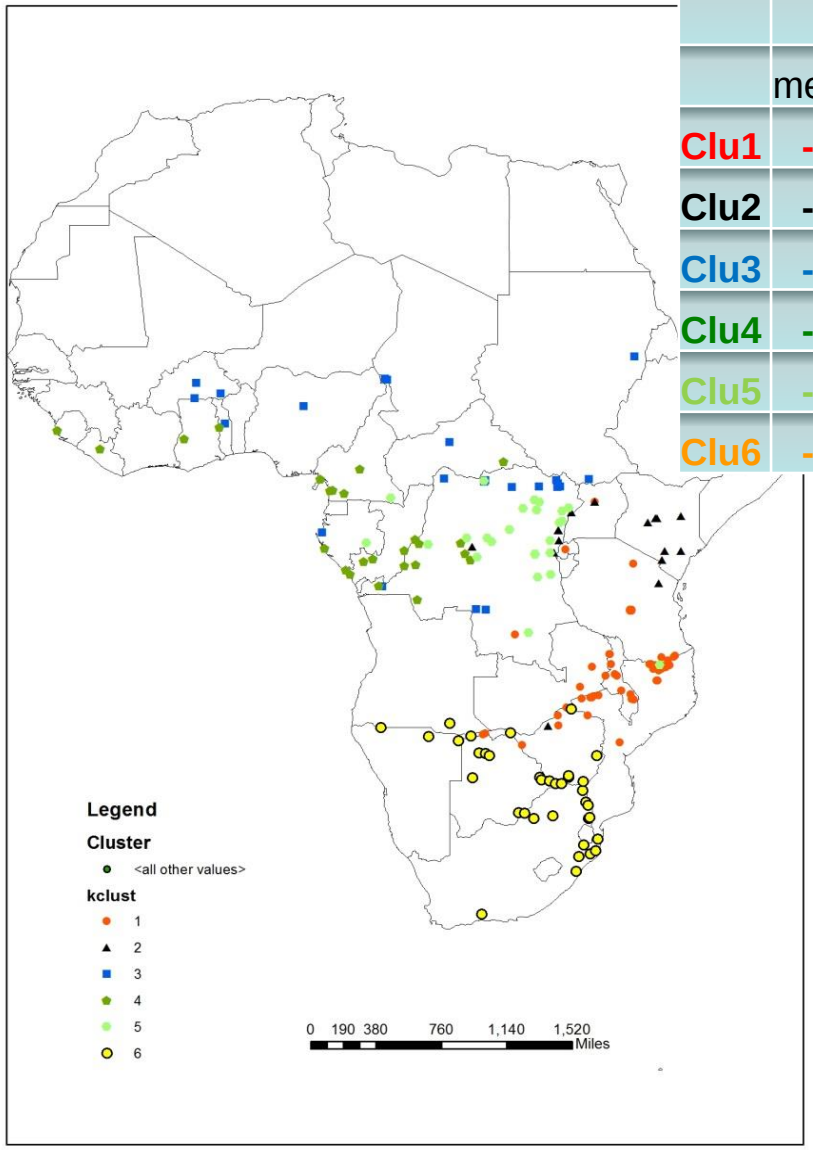
- ➔ Is there geographic structure in the data?
- ➔ How accurate is the nominal assignment framework?
- ➔ Which environmental/ ecological factors are accountable for the variation in data?
- ➔ How does age of the tusks influence isotope ratios?

Cluster analysis

- Normalized data were examined to find “natural” groupings in multivariate space.
- PAM and kmeans algorithm with Euclidian distance as dissimilarity measure.
- Optimal no. of clusters = six clusters.



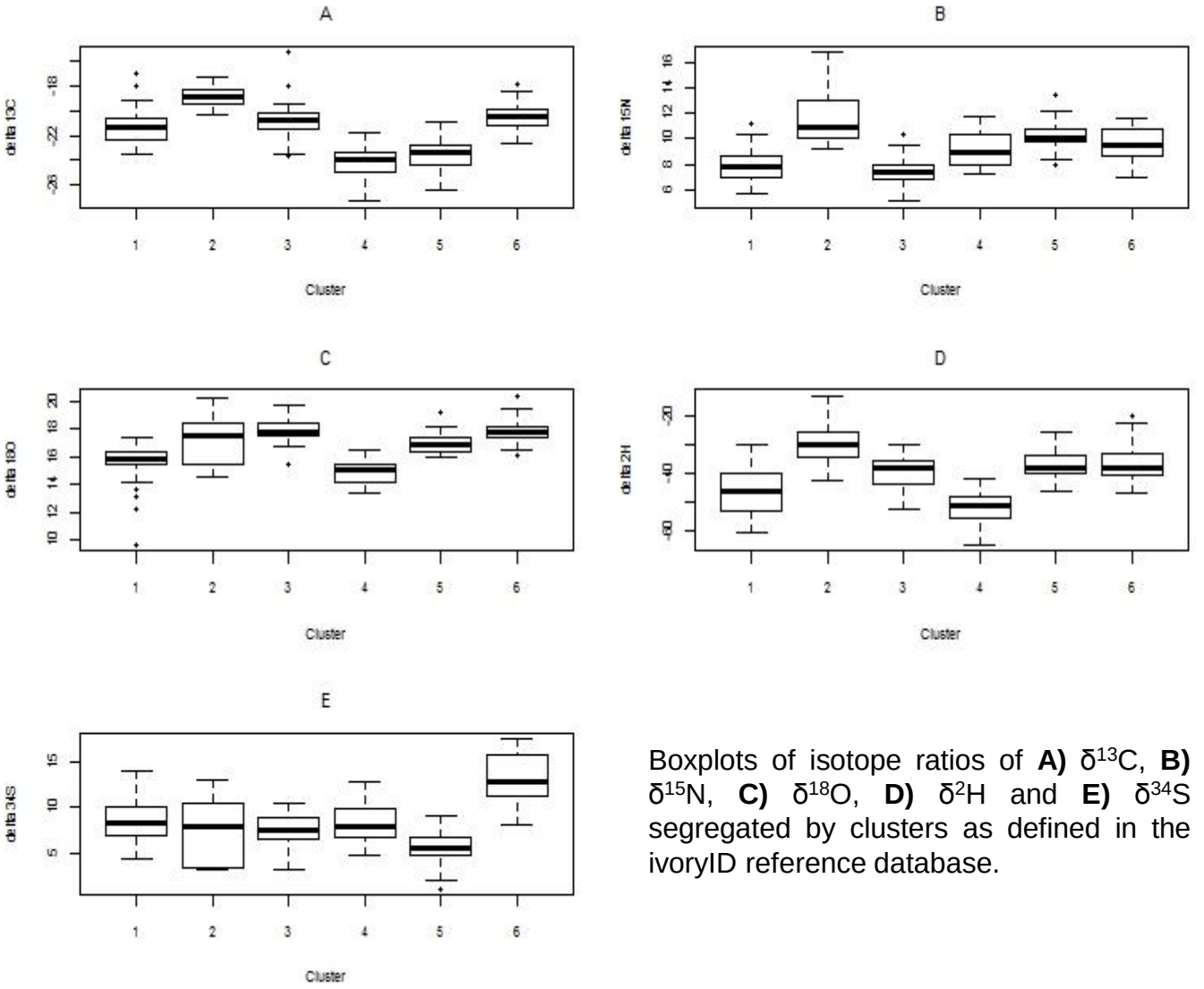
Geographic structure in data



	$\delta^{13}C$		$\delta^{15}N$		$\delta^{2}H$		$\delta^{34}S$	
	mean	SD	mean	SD	mean	SD	mean	SD
Clu1	-21.3	1.33	7.9	1.15	-49.9	7.90	8.4	2.24
Clu2	-18.8	0.82	11.7	2.16	-29.8	8.22	7.3	3.52
Clu3	-20.8	1.75	7.4	1.22	-39.9	6.34	7.7	1.85
Clu4	-24.2	1.50	9.3	1.40	-52.4	6.90	8.2	2.10
Clu5	-23.4	1.40	10.0	1.20	-37.0	6.00	5.6	2.00
Clu6	-20.5	1.04	9.6	1.21	-36.8	6.33	13.1	2.58

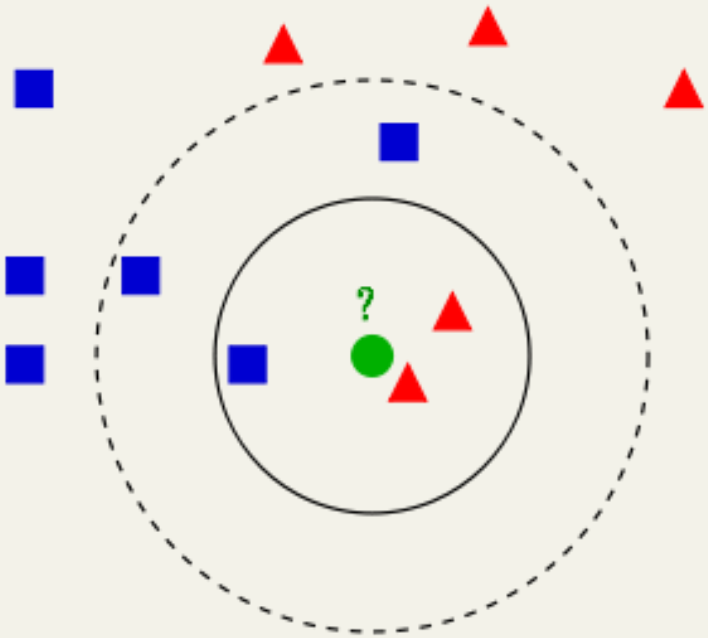
- Southeast Africa
- Albertine Rift/ Kenya
- Savanna/rain forest transition zone
- Coastal west and central Africa
- Congo Basin
- Southern African plateau

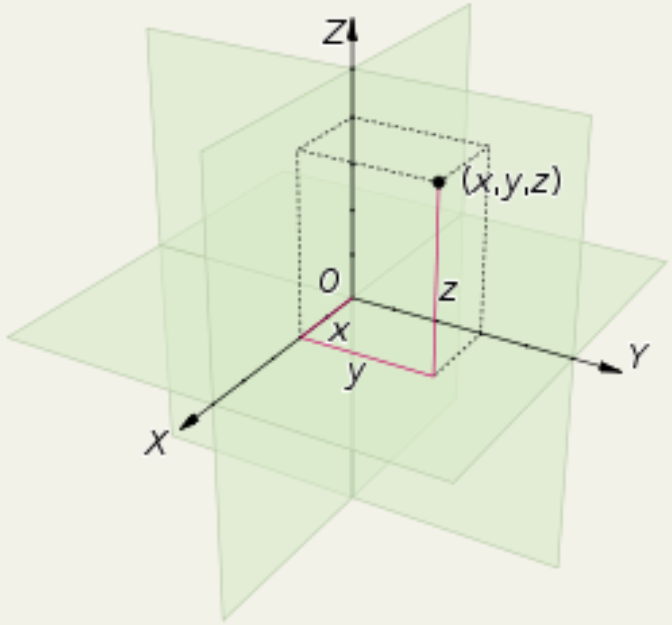
Isotope ratios by cluster



Boxplots of isotope ratios of **A)** $\delta^{13}C$, **B)** $\delta^{15}N$, **C)** $\delta^{18}O$, **D)** $\delta^{2}H$ and **E)** $\delta^{34}S$ segregated by clusters as defined in the ivoryID reference database.

K-nearest neighbor (*k*-NN) rule





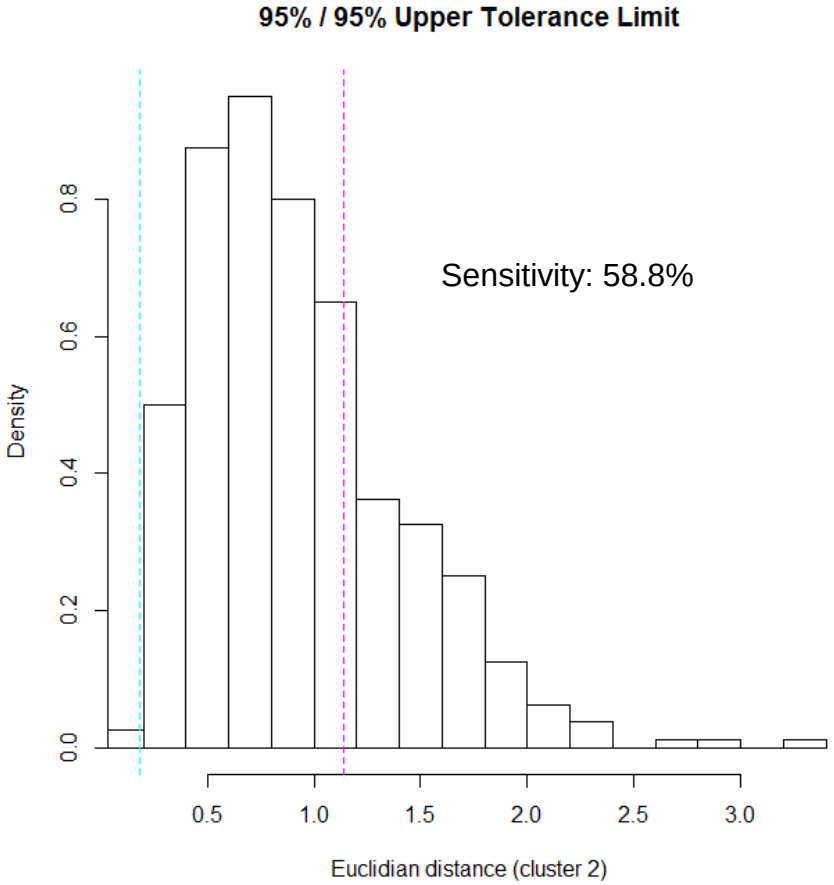
Rationale: samples with low Euclidian distance belong to the same class. The *k*-NN rule classifies the vector to the class that appears most frequently among its *k* nearest neighbors.

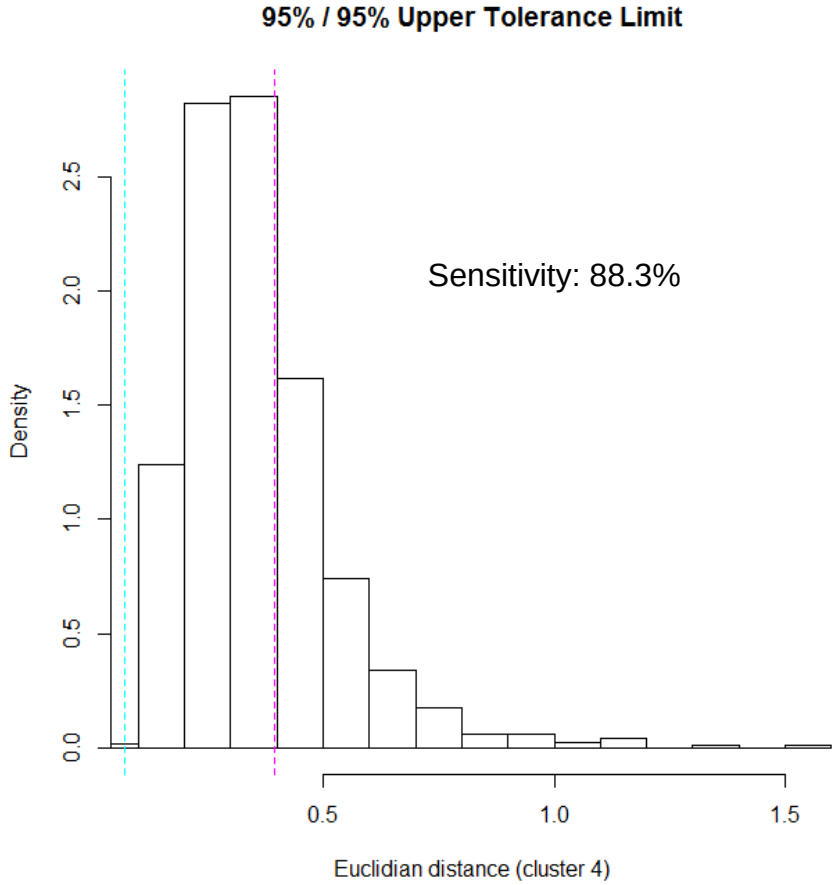
Cross-validation with rnorm- simulated data (*k*-NN, 41 sites; n=41,000)

		Cluster						
		1	**2**	**3**	**4**	**5**	**6**	Specificity
Prediction	Cluster	South-east Africa	Albertine Rift/ Kenya	Savanna /rain forest transition zone	Coastal west and central Africa	Congo Basin	Southern African plateau	
	n =	13,000	2,000	4,000	6,000	3,000	13,000	
	1	9970	300	225	595	60	1575	90.2%
	2	130	1175	125	0	70	100	98.9%
	3	270	165	3405	25	35	195	98.1%
	4	735	25	20	5295	80	10	97.5%
	5	175	210	70	75	2710	70	98.4%
	6	1720	125	155	10	45	11050	92.7%
	Sensitivity	76.7%	58.8%	85.1%	88.3%	90.3%	85.0%	
	Accuracy	85.9%	97.0%	96.9%	96.2%	97.8%	90.2%	

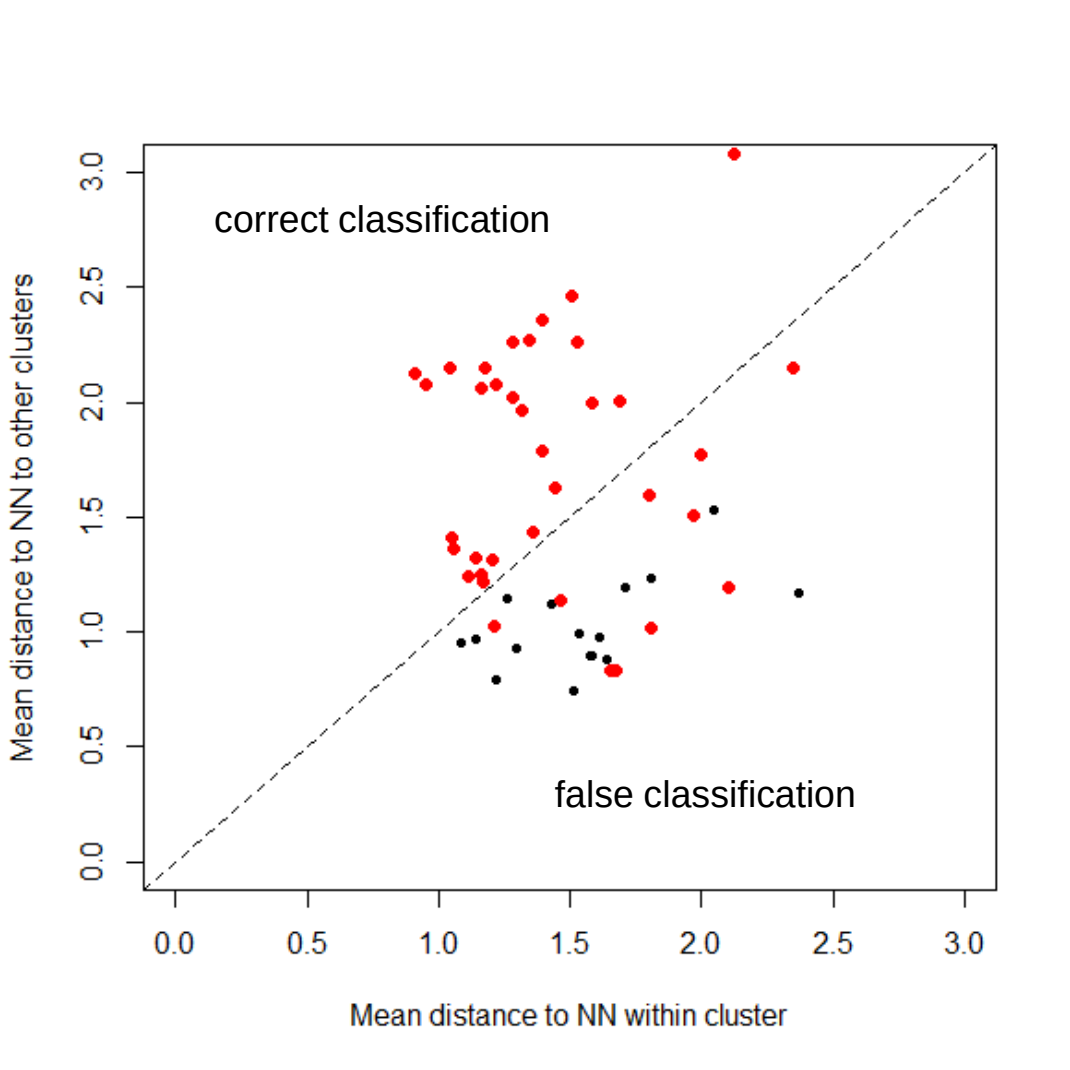
Within cluster variation

(*k*-NN leave-one-out cross validation; *k*=5)



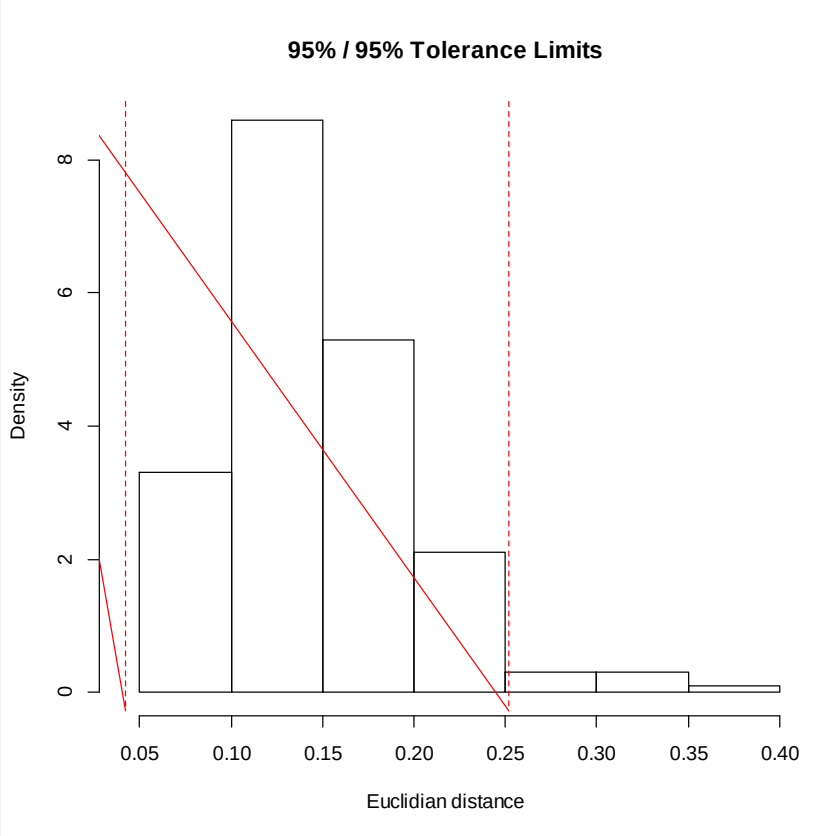


Within cluster variation

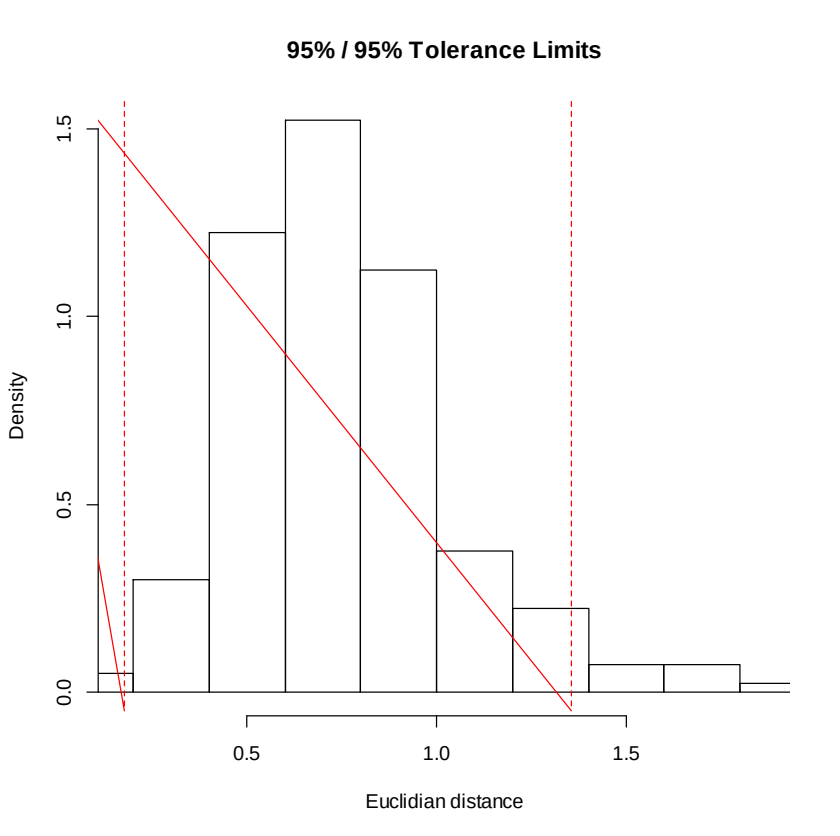


Within site variation

(*k*-NN leave-one-out cross validation; *k*=5)

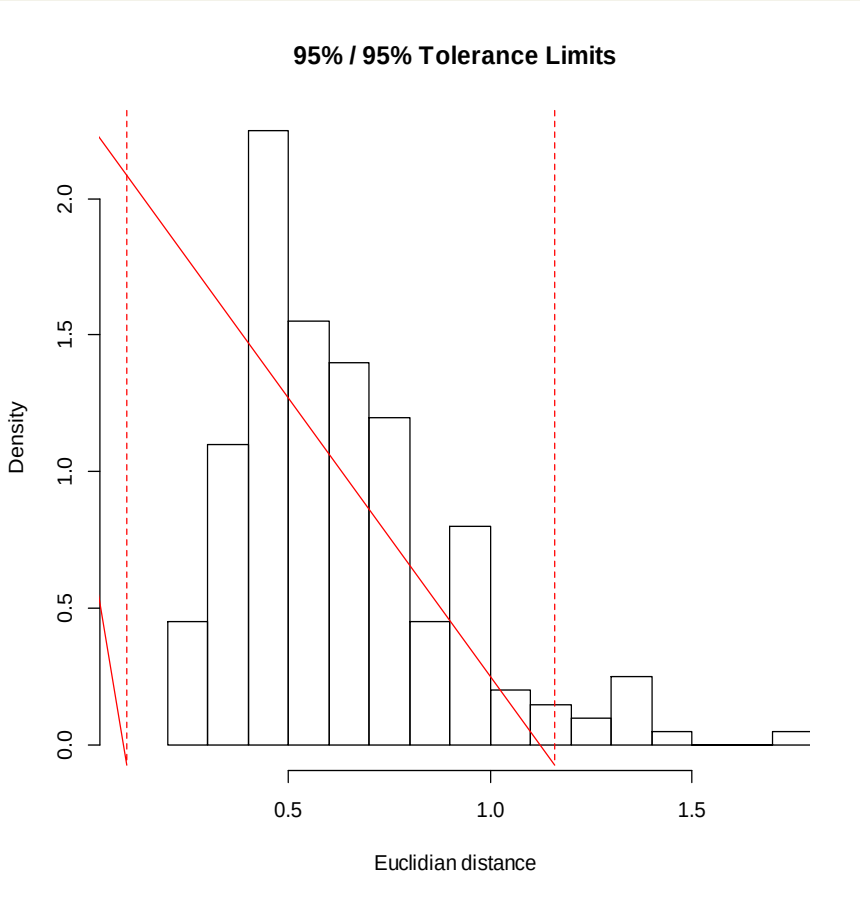


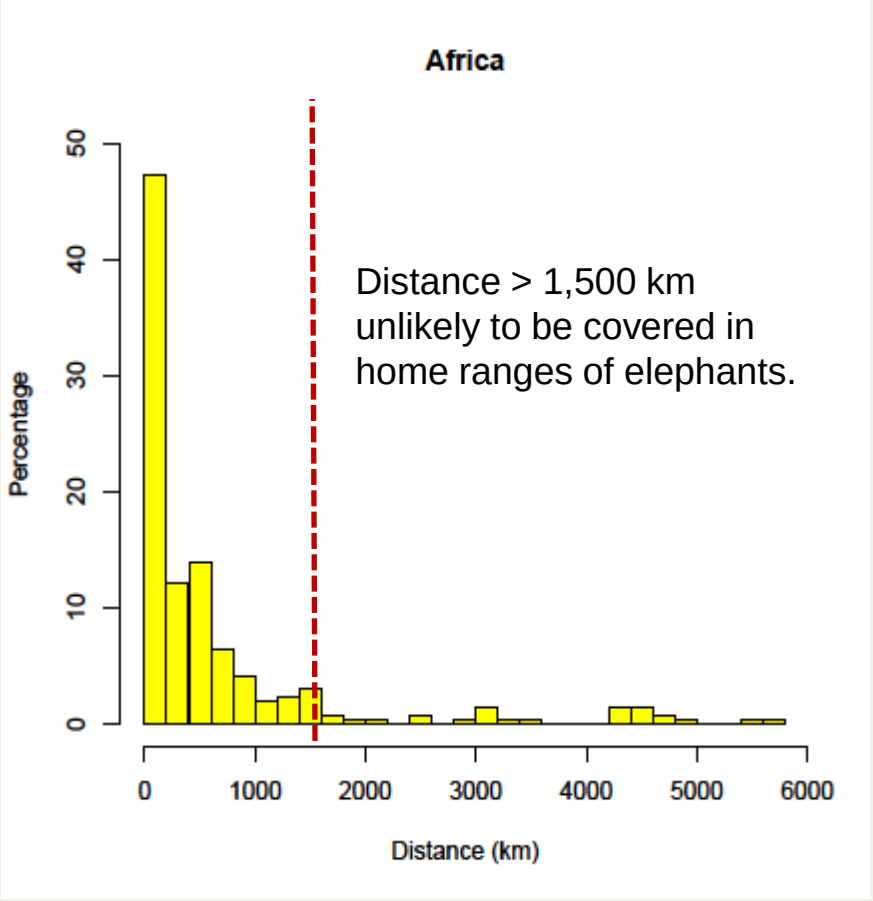
Site: ZA8
Sensitivity: 97.0%



Site: MW2
Sensitivity: 12.0%

Within site variation



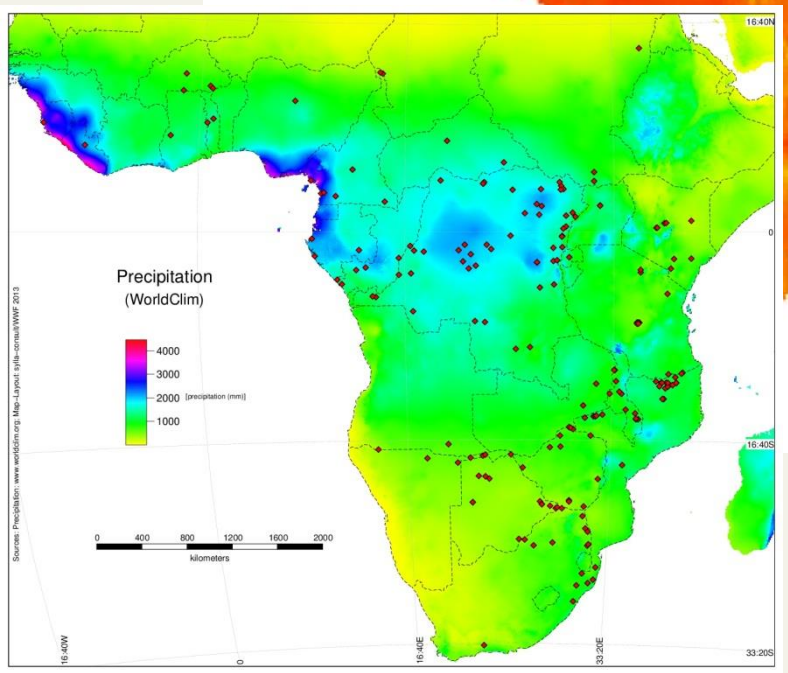


Site BW3: Sensitivity: 38.5%, but 82% of all samples are assigned to sites within 500 km.

50% of all samples within 244 km, and 83% within 750 km of their place of origin

Factors that may influence within cluster and within site variation

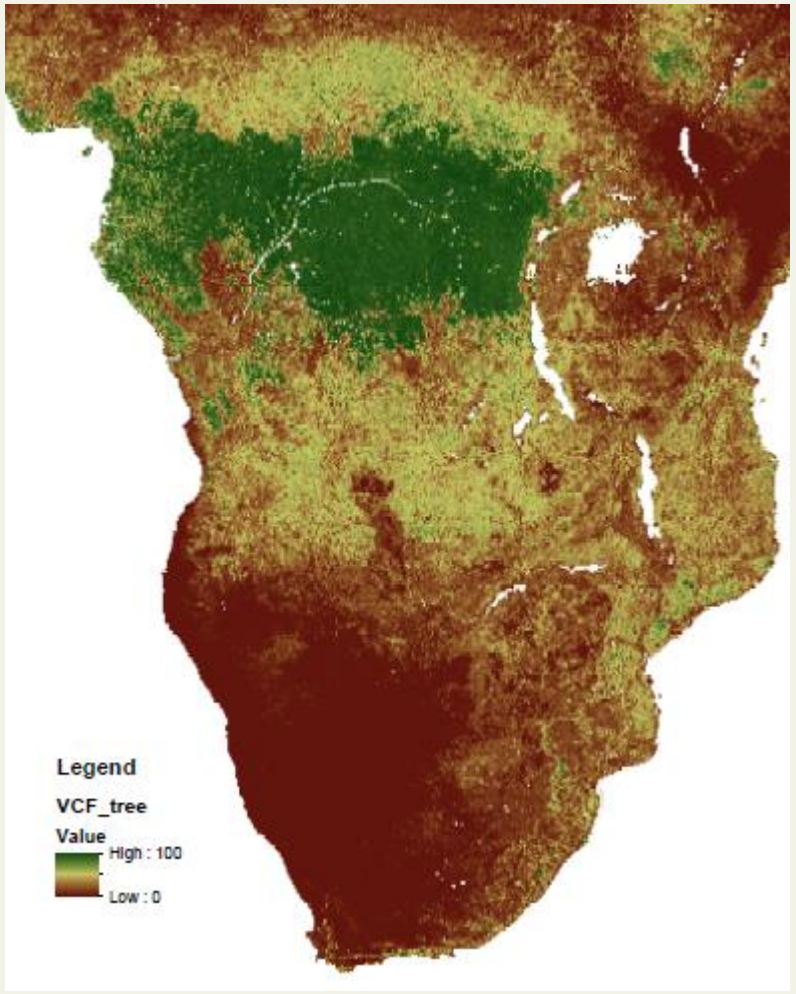
Elevation (dem_mean)
Temperatur (temp_mean)
Annual precipitation (prec_mm)
Soil moisture index (smos_mean)



Factors that may influence within cluster and within site variation

MODIS Vegetation Continuous Fields (VCF)

The Vegetation Continuous Fields collection contains proportional estimates for vegetative cover types: **woody vegetation** (vcft), **herbaceous vegetation** (vcfh), and **bare ground** (vcfb).

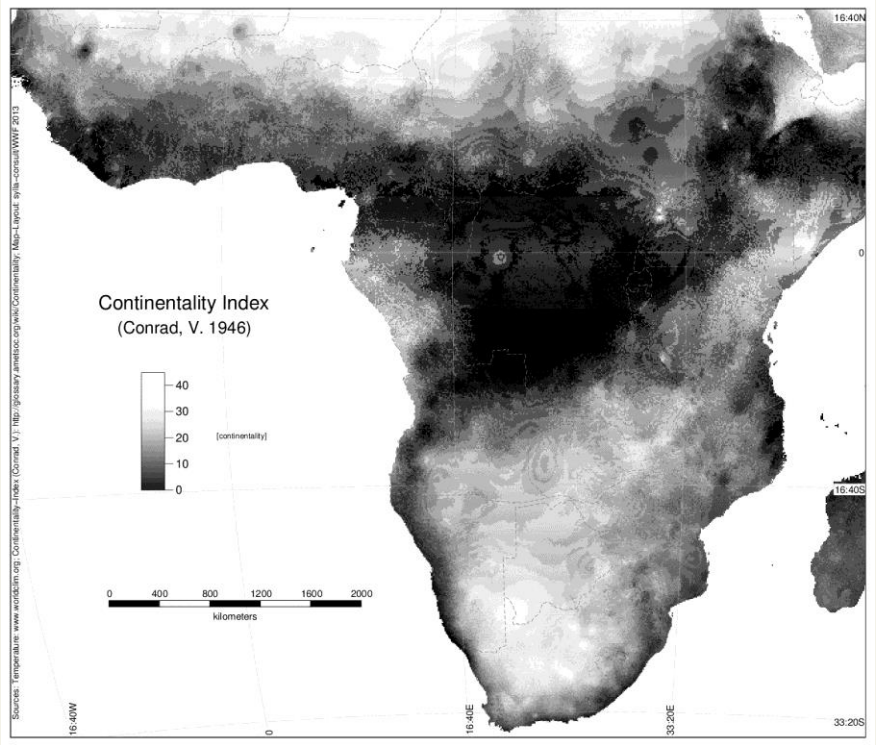


Factors that may influence within cluster and within site variation

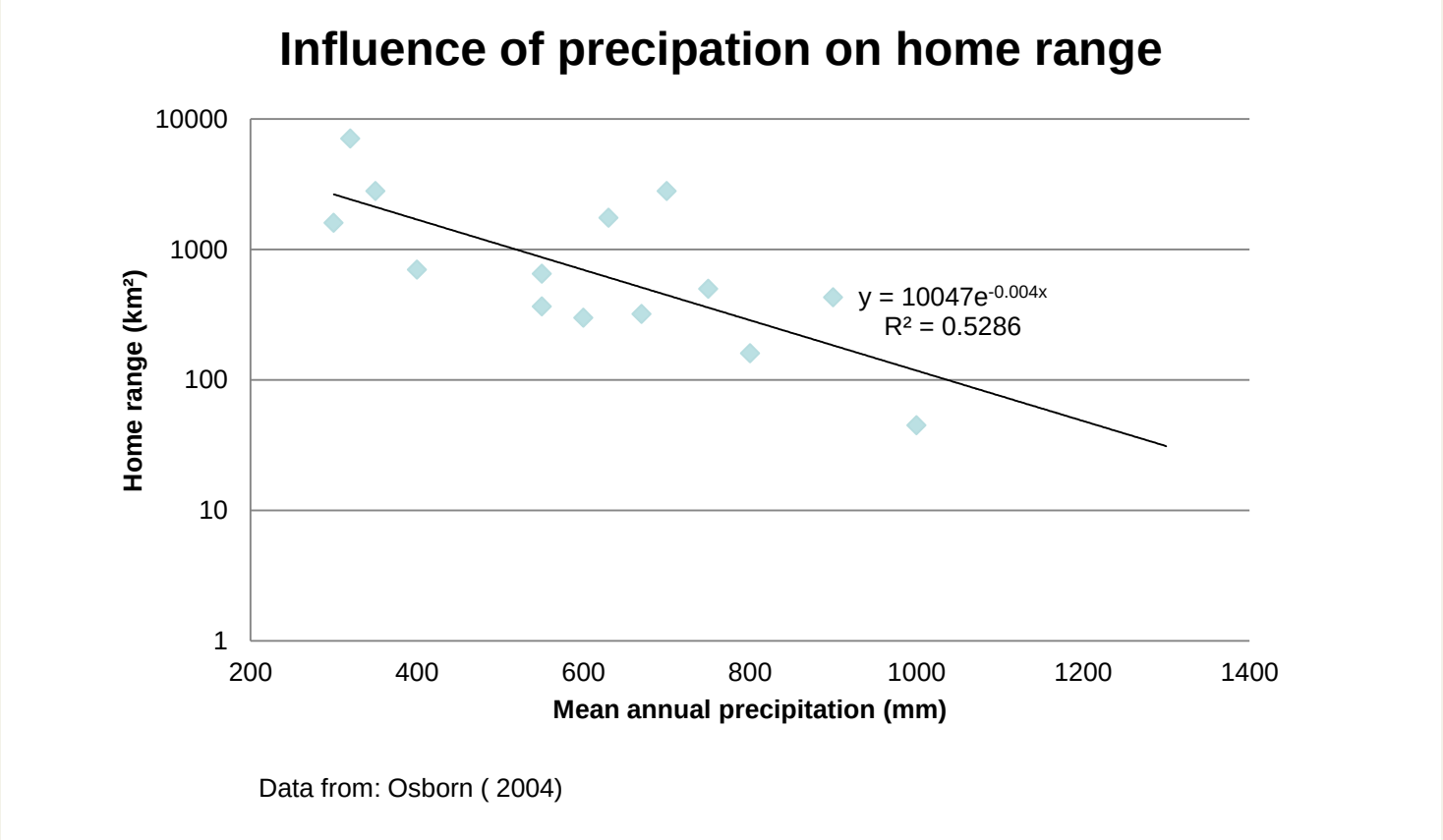
Conrad‘s index (cont_mean):

$$K = \frac{a\,A}{\sin(\varphi + 10)} - b$$

K: index of continentality (%)
A: range of annual temperature
a and b: constants, (a = 1.7; b = 14)
φ: latitude

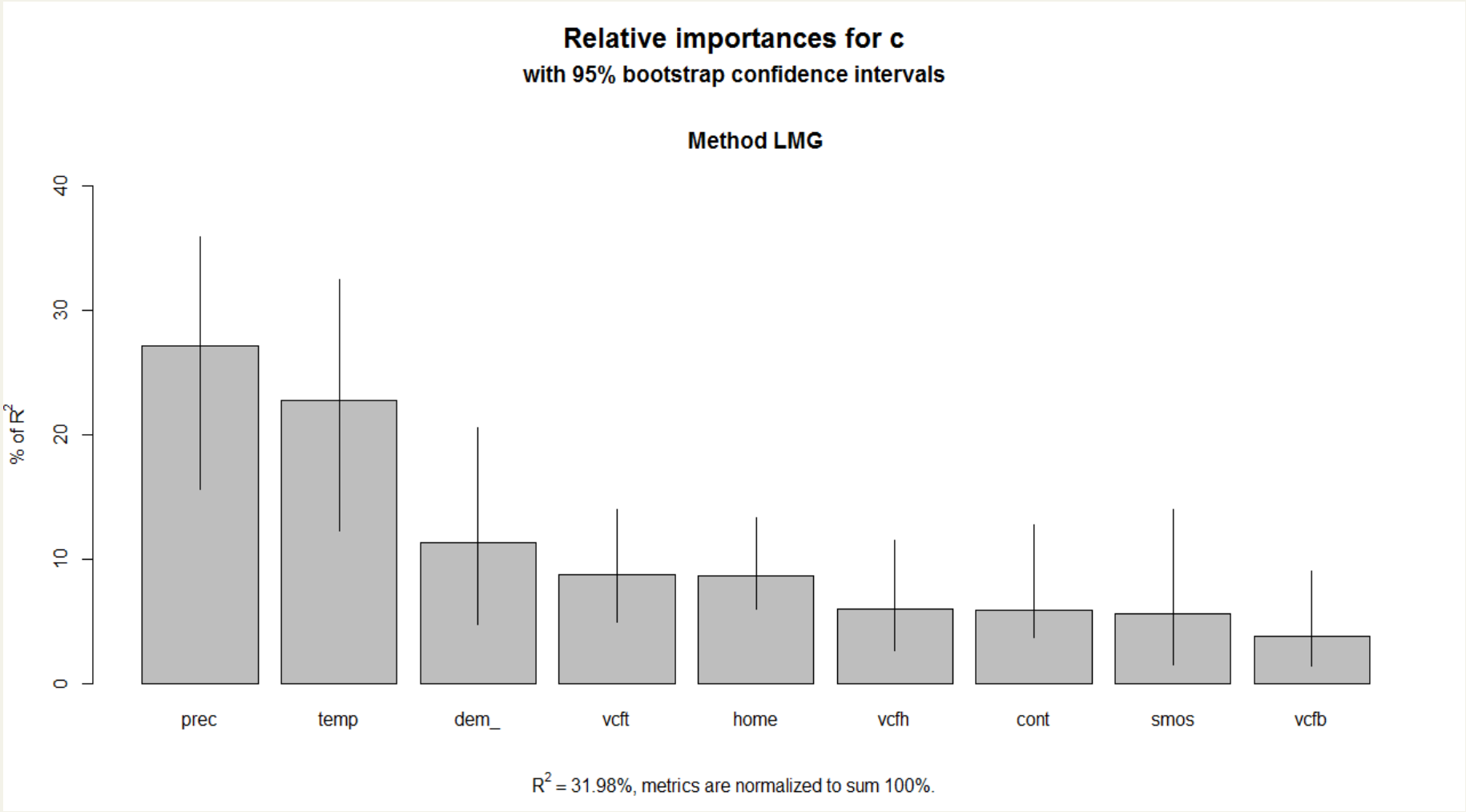


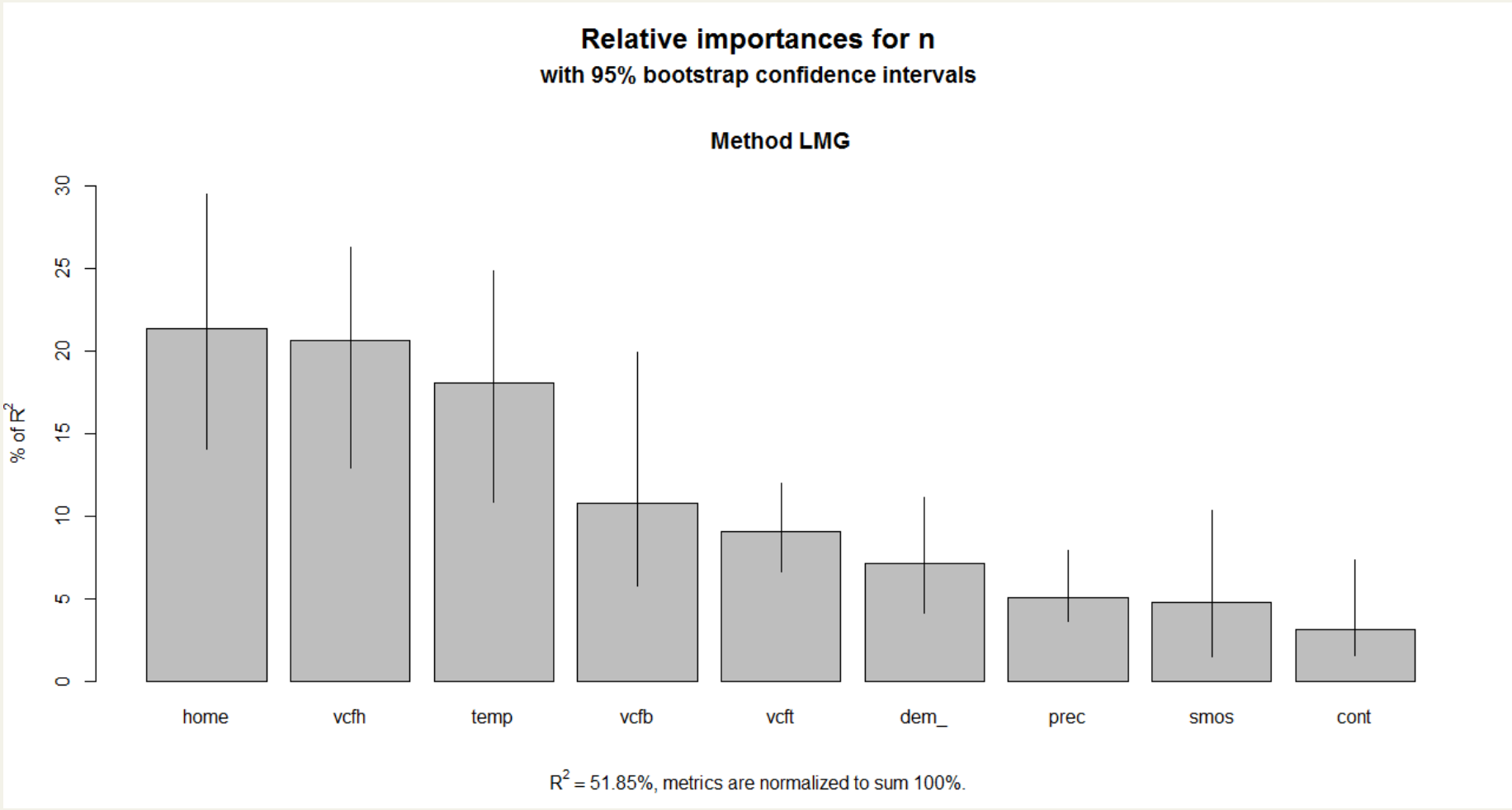
Factors that may influence within cluster and within site variation



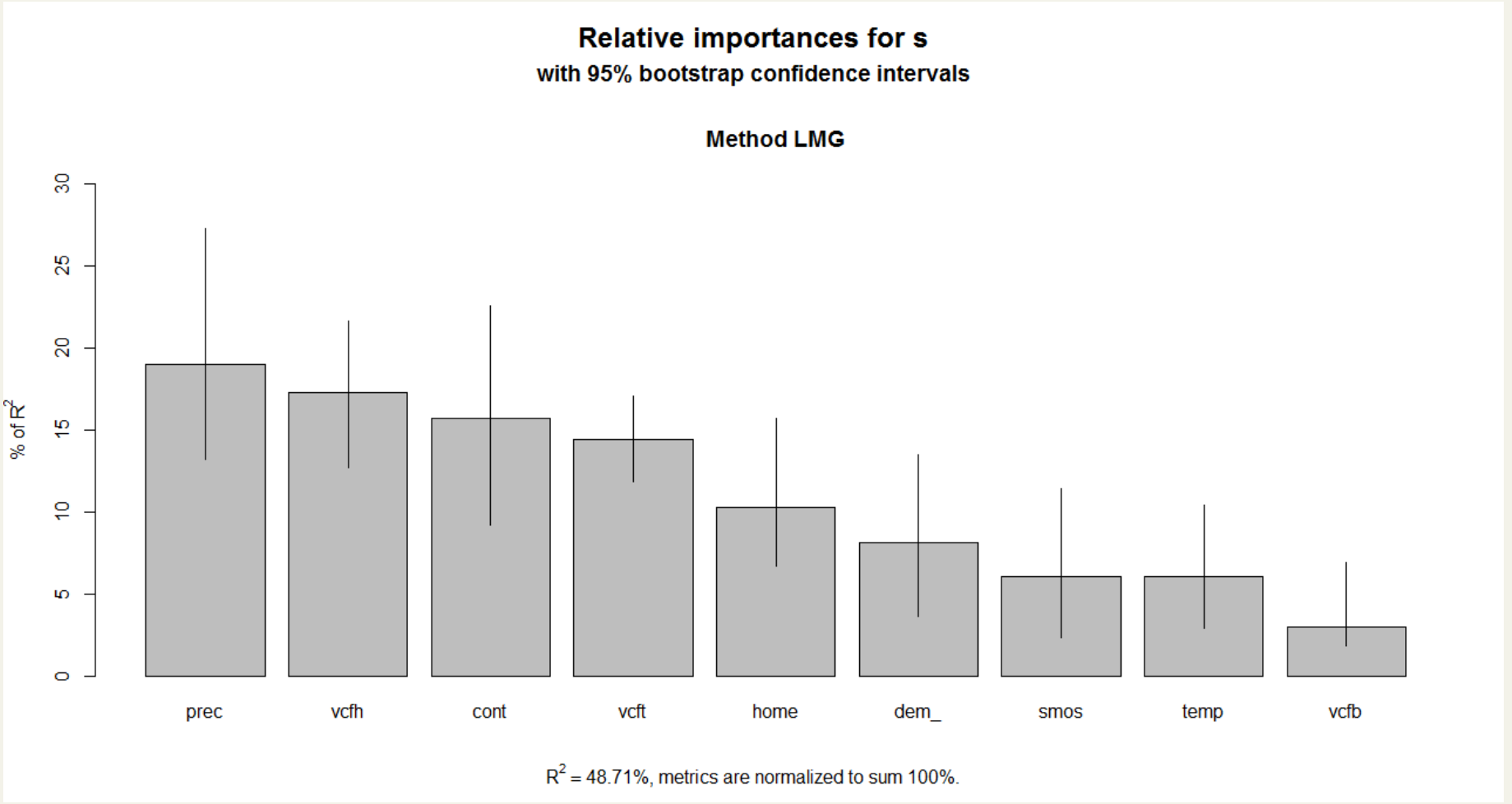
Data from: Osborn (2004)

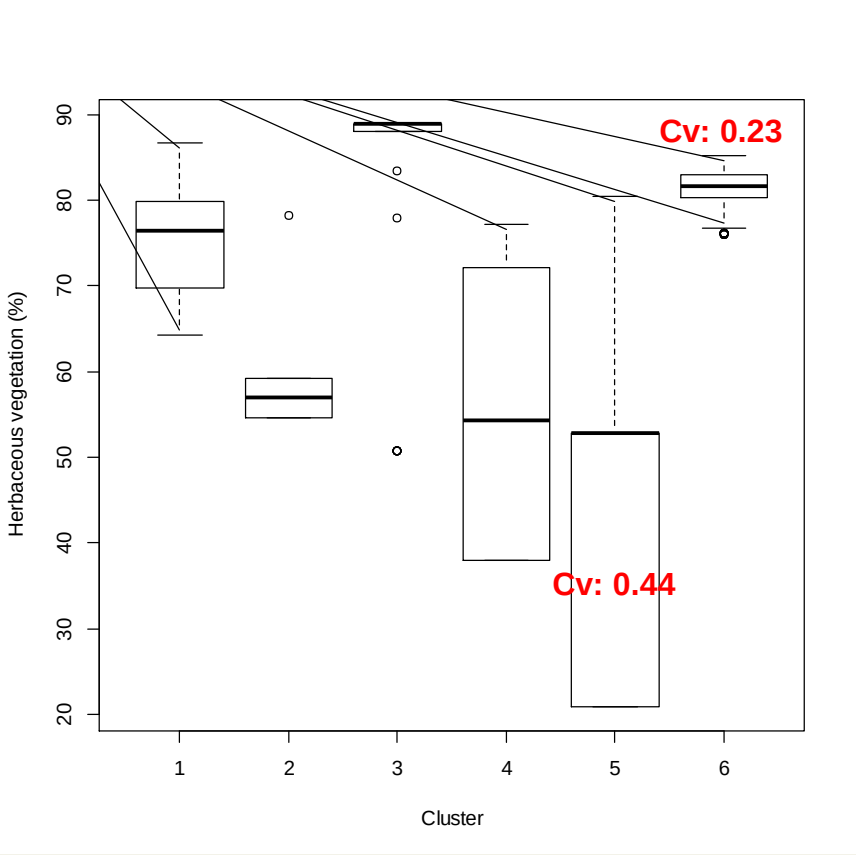
```
Call:
lm(formula = c ~ dem_mean + temp_mean + vcft_mean + vcfh_mean +
    vcfb_mean + cont_mean + smos_mean + prec_mm + home.range..km.,
    data = a)

Residuals:
    Min      1Q  Median      3Q     Max
-4.5227 -0.9567 -0.1567  0.8493  4.7493

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    -1.816e+01  3.111e+00  -5.836 1.46e-08 ***
dem_mean        1.484e-04  3.775e-04   0.393  0.69444
temp_mean      -1.619e-01  5.522e-02  -2.932  0.00364 **
vcft_mean       2.472e-02  2.525e-02   0.979  0.32840
vcfh_mean       2.591e-02  2.483e-02   1.044  0.29752
vcfb_mean       3.075e-02  1.596e-02   1.927  0.05504 .
cont_mean      -2.020e-02  1.791e-02  -1.128  0.26024
smos_mean       3.879e+00  1.823e+00   2.128  0.03420 *
prec_mm        -2.098e-03  4.686e-04  -4.478 1.10e-05 ***
home.range..km. -3.630e-04  2.161e-04  -1.680  0.09410 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.484 on 283 degrees of freedom
Multiple R-squared: 0.3198,     Adjusted R-squared: 0.2982
F-statistic: 14.78 on 9 and 283 DF,  p-value: < 2.2e-16
```

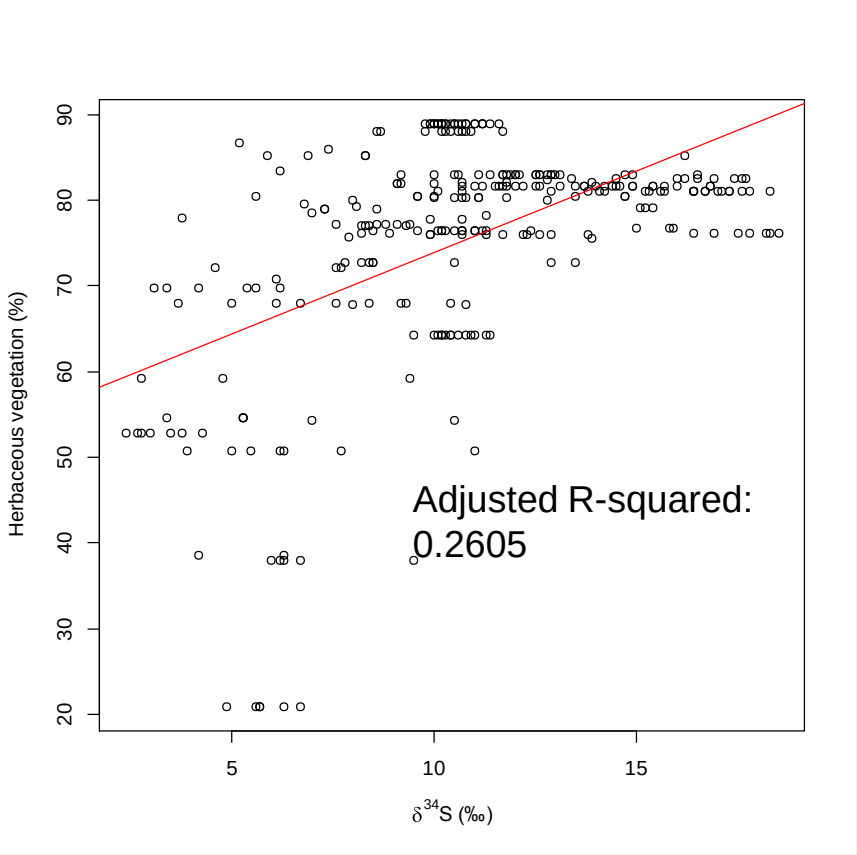

Relative importances for c
with 95% bootstrap confidence intervals
Method LMG
% of R2
0
10
20
30
40
prec
temp
dem_
vcft
home
vcfh
cont
smos
vcfb
R2 = 31.98%, metrics are normalized to sum 100%.



Higher ratio of $\delta^{15}N$ can be expected in drier elephant habitats due to effects of nutritional stress (van der Merwe, 1988; Vogel et al., 1990).

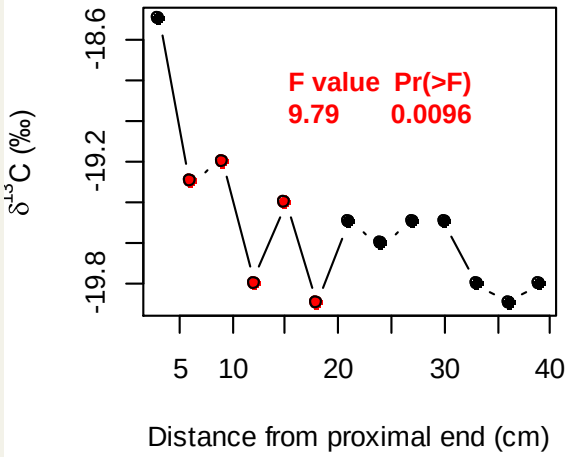

Relative importances for s
with 95% bootstrap confidence intervals
Method LMG
% of R2
0
5
10
15
20
25
30
prec
vcfh
cont
vcft
home
dem_
smos
temp
vcfb
R2 = 48.71%, metrics are normalized to sum 100%.

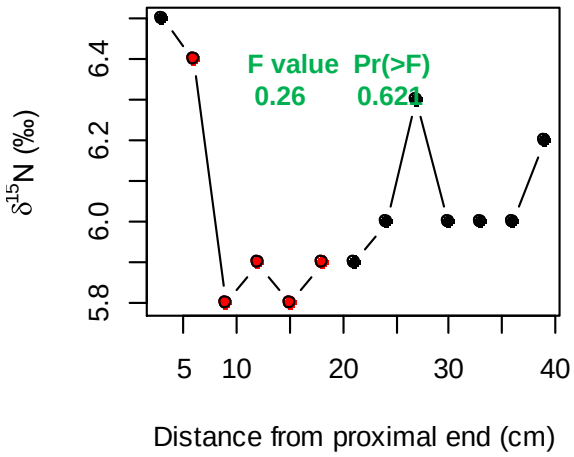




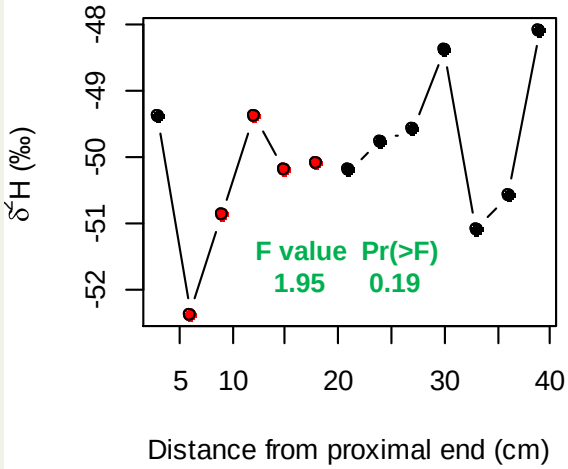
On a C_4 diet, likely to be low in digestible protein, $\delta^{34}S$ fractionation was +4‰, which could be the result of sulfur recycling from body proteins in addition to dietary sulfur intake (Richards et al., 2003).

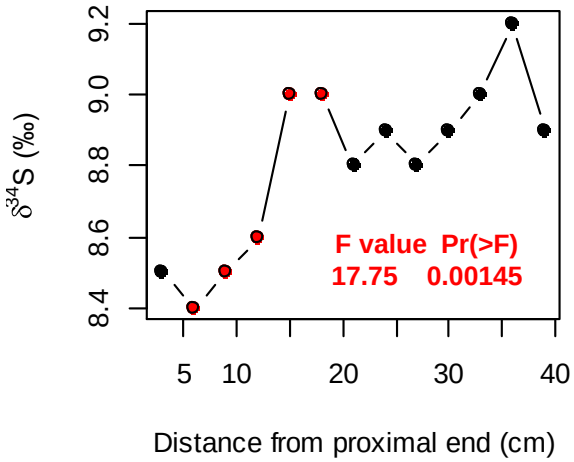
Individual within tooth isotopic variability





Black dots - assignment to site in Malawi; **red dots** – assignment to site in Burkina Faso. **Distance is 4,492 km** !!!





Conclusion

- **Exploratory data mining** (cluster analysis, *k*-NN) useful to detect **geographic structure** of isotope ratios.
- **Boundary areas** of ivory isoscapes are prone to **overlap**.
- **Sensitivity** of assignment test is moderate to good (cluster dependent); **Specificity** (testing against “false positives”) is fairly high.
- **Euclidian distance imprint** is promising proxy to estimate within cluster/ site variability and thus, the risk of miss-classifications.
- **Quantitative assessment** of environmental/ ecological factors can be used to predict variability of isotope ratios and develop continuous assignment frameworks.
- **Pronounced individual within tooth variability** is thought to be accountable for trans-regional miss-classifications.

Thank you!

This project is funded through the Federal Agency for Nature Conservation (BfN) with a grant from the Federal Ministry of Environment, Nature Conservation and Nuclear Safety (BMU). Several European museums and collections as well as numerous trophy hunters provided ivory samples. We are particularly thankful to the continuous support of the International Council for Game and Wildlife Conservation (CIC). The CITES Management Authorities of Botswana, Burkina Faso, Malawi, Mozambique and South Africa supported this project with the provision of samples from their ivory stockpiles.

Further information: stefan.ziegler@wwf.de