Trace elements methods and its usage for studying bird migration

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Trace element, history

- Measurement of elements in feathers to obtain mineral profiles as an indicator of the geographical location in which the feathers were grown

- First attempts for using it to infer geografical origin were in waterfowl (Devine and Peterle 1968, Hanson and Jones 1976)
- Parish et al. (1983) distinguished three natal populations of Peregrine Falcons by measuring five trace elements in feathers.

Trace element, history

- Early studies of non-passerines have investigated the role of age, sex and moulting locality on variations in elemental composition (Kelsall and Burton 1979, Bortolotti and Barlow 1988) and demonstrated that the mineral profile of feathers varies micro-geographically (Bortolotti et al. 1990)

- Problems raised
  - considerable intrapopulation variation by age and sex
  - trace element profiles not differed among disparate populations
    - (Bortolotti et al. 1989, 1990)
  - Early analytical technologies needed large sample size

Trace elements, now

- Recent development of ICP technology (ICP-OES, ICP-MS) let us to investigate more than 40 elements from one small feather samples

- More chance
  - Find site specific markers
  - Larger sample size (number of sampled individuals)
  - Investigate intrapopulation variation

  - Test this technology for studying migration

- Attempts to revitalise this methods (Szép et al. 2003, Donovan et al 2006)
  - Testing former questions with recent tools
  - Usage for recent problems

Trace element profile of feather and its moulting area

Chemical composition of the given feather depend on the:

- Moulting and non moulting area(s)
  - Soil
  - Water
  - Air
  - Vegetation
- Individual
  - Habitat usage
  - Diet
  - Age
  - Sex
  - Condition
  - Physiology
  - Molting

Chemical elements in the feathers from

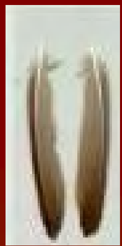
- Internal sources
  - Through bloodstream during the short period of growth (moult)
- External sources
  - Atmospheric deposition
  - Waxes from the uropygal gland

Level of chemical elements from internal sources depend:

- **Area of the moulting, due to diet**
- Accumulated amount of the elements in the body (e.g. Hg)
- Moulting pattern
- Pigmentation of the given feathers
- Other species, age, sex and condition dependent physiological processes

Level of chemical elements from external sources depend:

- Area of the moulting
- Areas of living after the moulting
- Time and exposition for deposition
- Time and exposition for leaching
- Species, age, sex and condition dependent physiological processes influenced waxes of uropygial gland



Basic steps to use this method?

1. Collecting feathers (full size) moulted in the area (breeding/wintering) we want to know

2. Collect and store the feather to avoid contamination (sealed plastique bag, label outside the bag)

3. Prepare the feather samples for chemical analysis
   - ⋆ Cleaning the surface of the feather (water and/or organic solvent, ultrasonic washing)
   - ⋆ Control samples for detecting potential contanmination during the preparation of the feather samples
   - ⋆ Dissolving the feathers (Mixture of HNO_3 and H_2O_2, microwave digestion)
   - ⋆ Drying out the samples and preparing to measurment

4. Determine elemental content of these small-volume samples with ICP-OES or ICP-MS
   - ⋆ Standards for each measured elements within the range of the element concentration in the studied feathers
5. Statistical analysis of the measured values

Investigation of sand martin (Riparia riparia) in Europe

- Long distance migrant species
- Breeding in colonies
- Parents collecting food for young birds whithin 1 km radius of the colonies
- Adults moulting in the African wintering area
- During the moulting In Africa, they stay around a wintering roost

Studied sites

- Continental scale
  - Hungary, Denmark, UK, Spain
- Regional scale
  - Varying distance within Hungary (between 4-250 km)
- Local scale
  - Within one colony at Rakamaz in Hungary
- Habitat scale
  - Close colonies with different habitats (Rakamaz- riverside forest, medows, Tímár- grain and corn fields, 4 km)

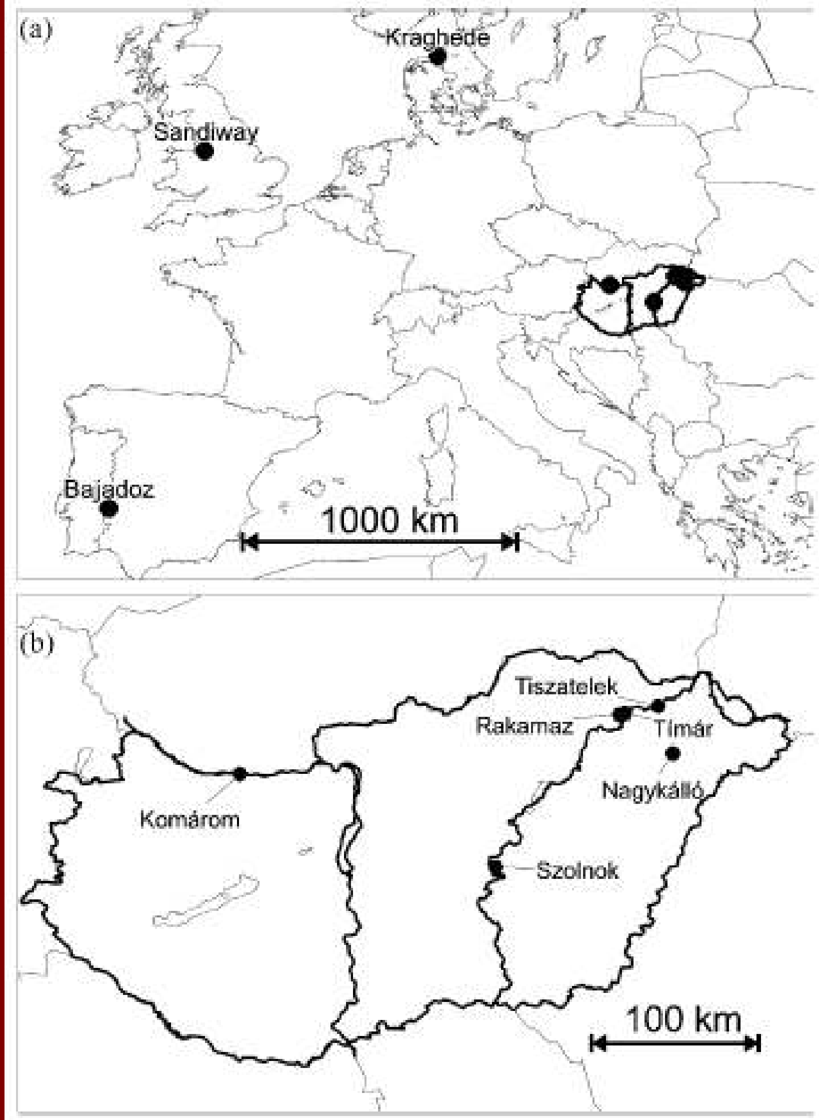
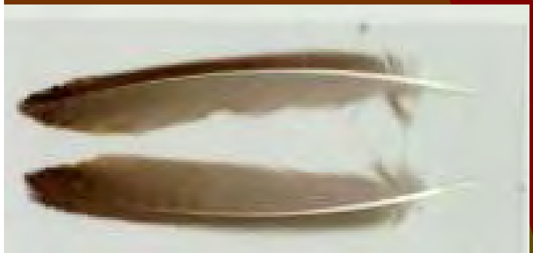


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

Studied sand martin feathers in Europe

- A pair of the second outermost tail (T5) feathers
  - Hungarian juvenile – moulted in Hungary by juvenile birds at different colonies in Hungary
  - British juvenile – moulted in UK by juvenile birds at one colony in UK
  - African adult – moulted in Africa by adult birds belong to different European breeding areas
  - Hungarian adult – newly moulted feathers after removal of African moulted ones from adult birds at colonies in Hungary

Methods of chemical analysis



- Trace element
  - Measurement of 23 chemical elements by ICP-OES technique from one feathers

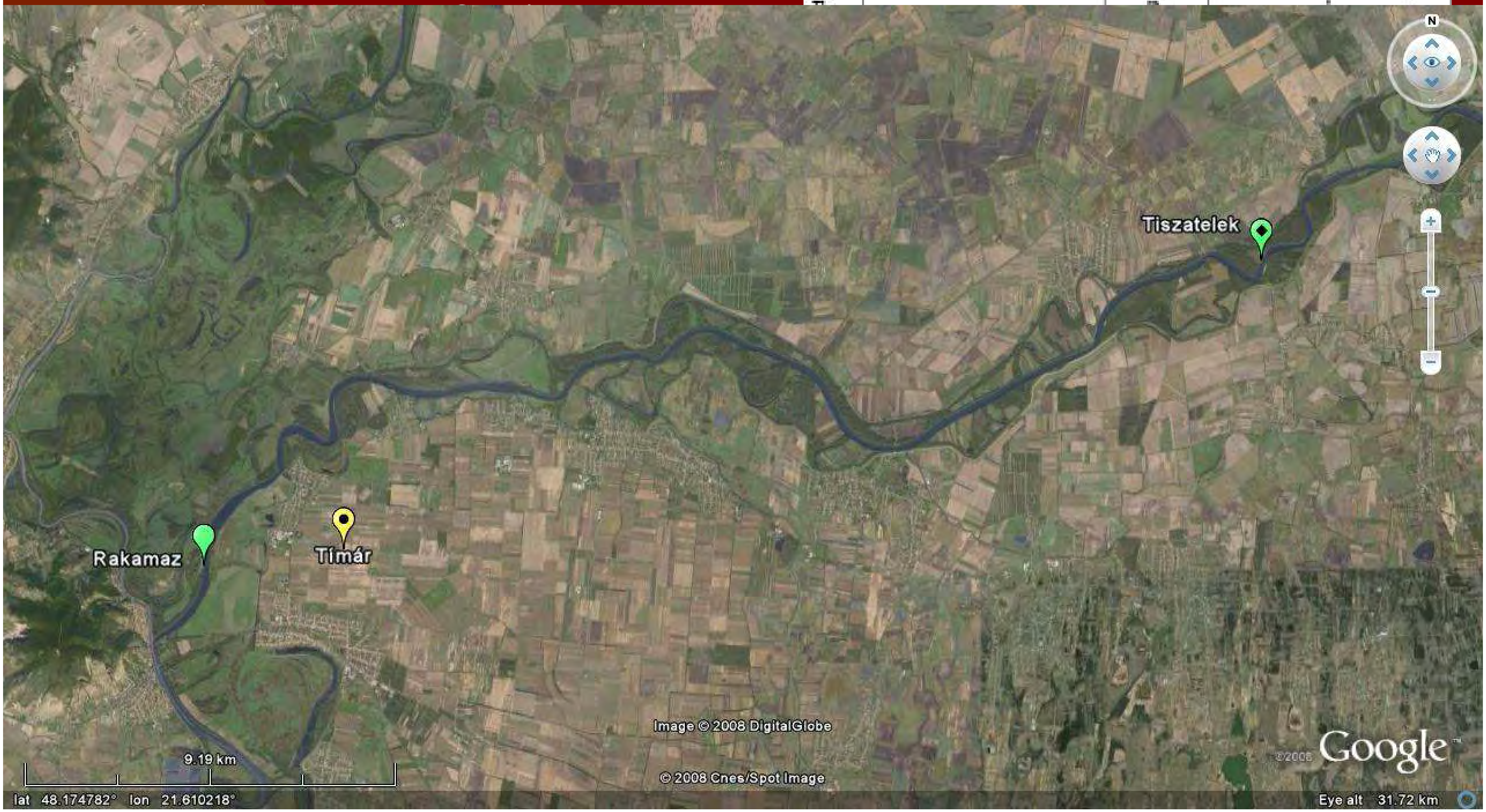
    As, Cd, Mg, Mn, Mo, Se, Sr, Ca, Co, Fe, Zn, Li, P, Ti, V, Ag, Cr, Ba, Hg, Pb, S, Ni, Cu
  - Usage of specific cleaning/handling method for preparation and of small sample size (Vallner et. al 1999)

Methods of statistical analysis

- Multivariate ANOVA (MANOVA) for studying differences among groups based on stable isotopes or trace elements
- T-test (independent, paired) for comparing two groups for one specific variable
- Discriminant Analysis
  - Classifying the samples with known origin on the base of the measured stable isotope or trace element contents
  - Cross-validating the samples on the base of the classification coefficients

Is there a difference in composition of juvenile feathers among European breeding populations?

Trace elements

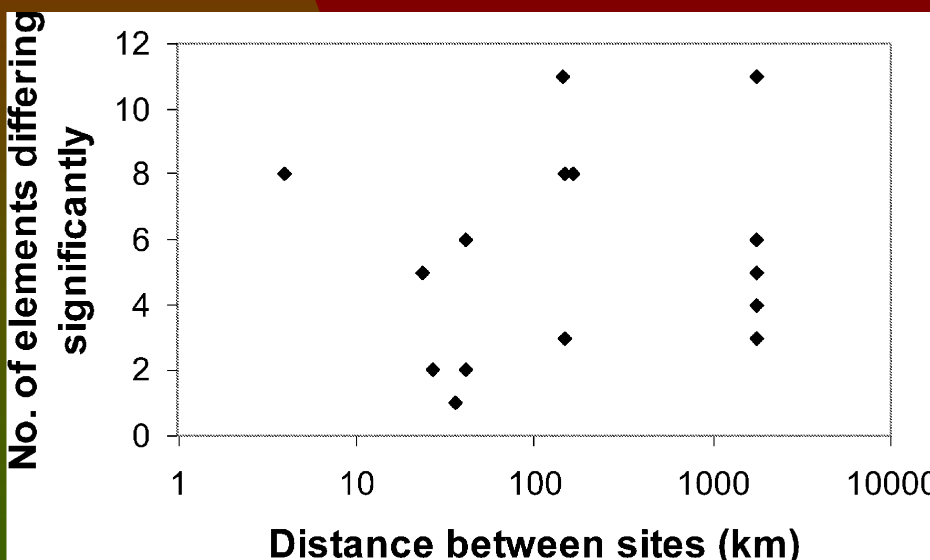


Is there a difference in composition of juvenile feathers among European breeding populations?

Trace elements

Yes (P<0.001; MANOVA)

Geographical distance alone did not predict the number of elements that were significantly different between sites



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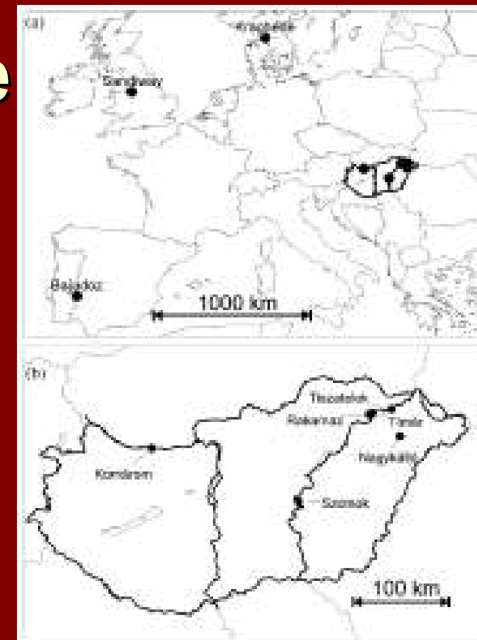
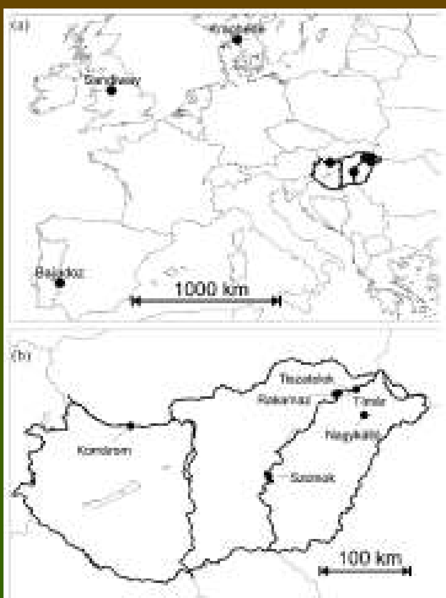


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

Classifying samples on the base of juvenile feathers

Trace elements

- Four functions with high Eigen values, explained 94.7% of the variance
- The first two functions explained 86.6%



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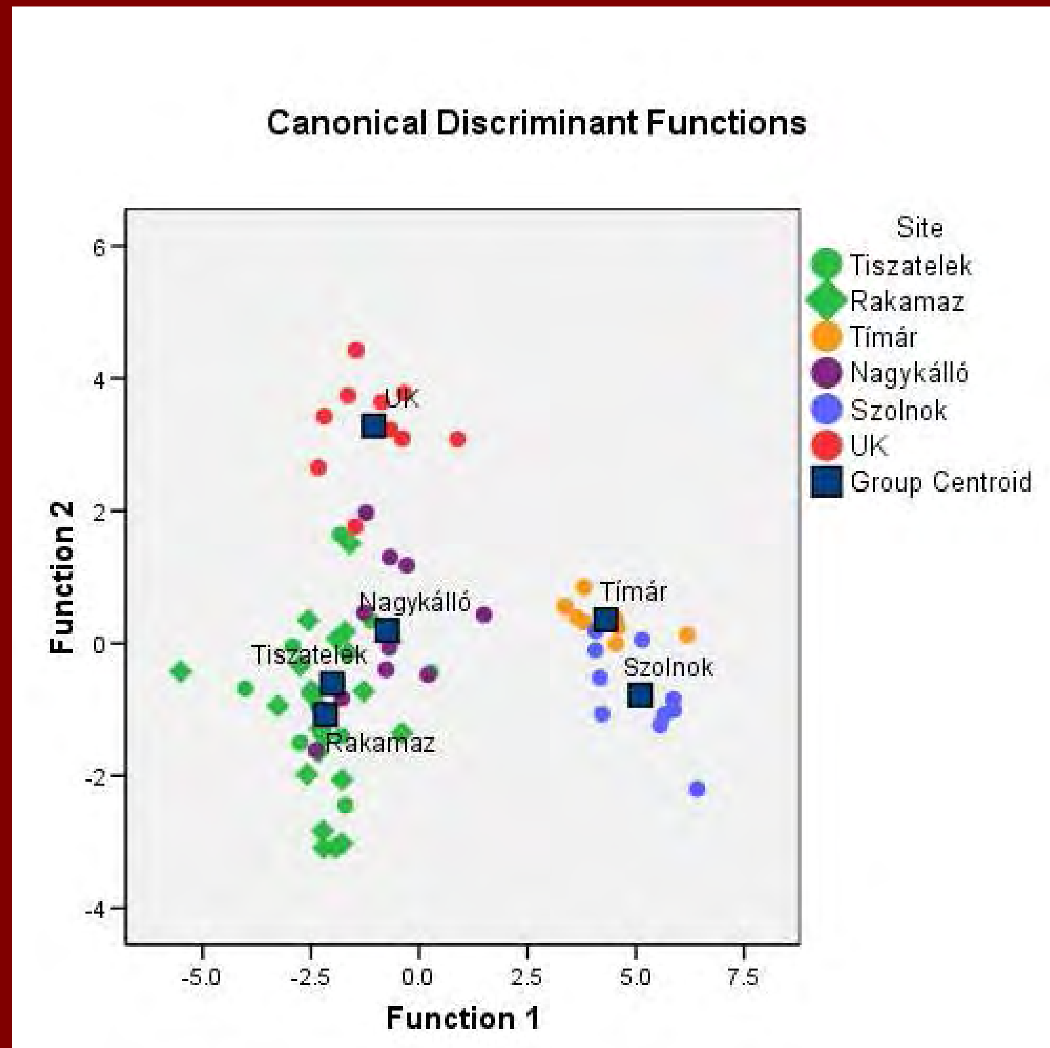

Discriminant Analysis

- Performs linear discriminant analysis for two or more groups. The goal of discriminant analysis is to classify cases into one of several mutually exclusive groups based on their values for a set of predictor variables
- In the analysis phase, a classification rule is developed using cases for which group membership is known
- In the classification phase, the rule is used to classify cases for which group membership is not known

Classifying samples on the base of juvenile feathers

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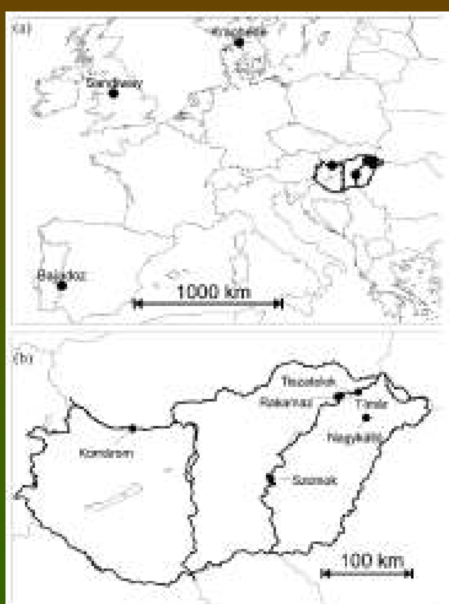

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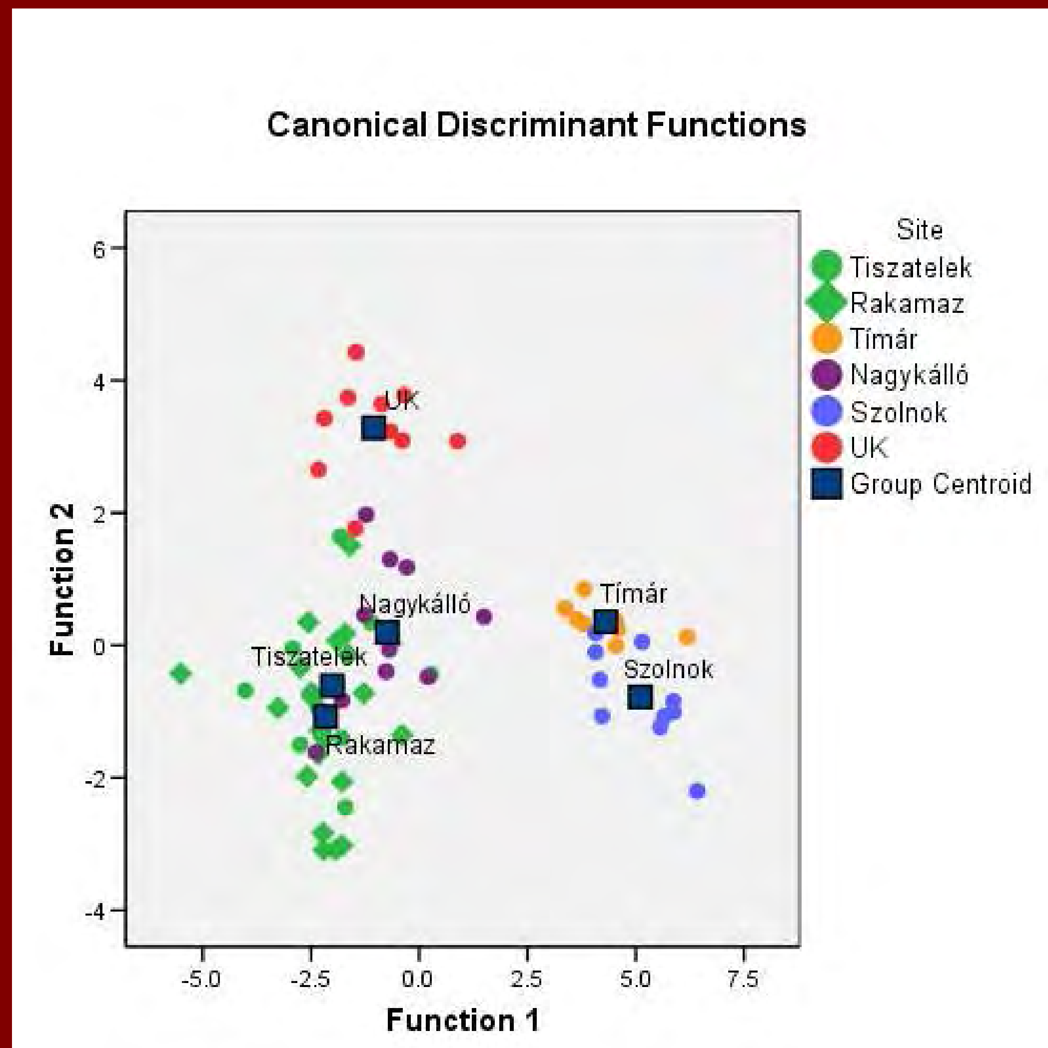
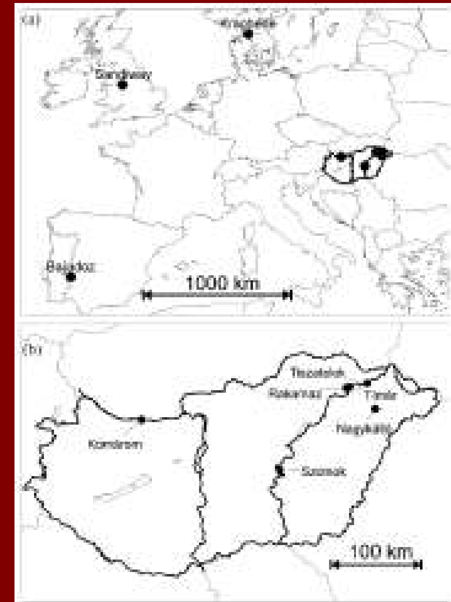


Classifying samples on the base of juvenile feathers



Trace elements

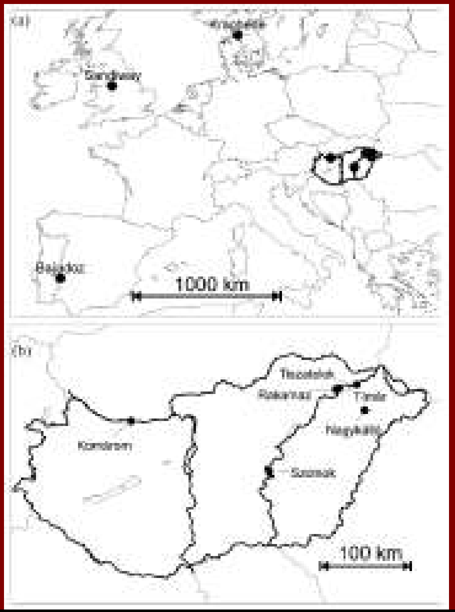
- 61.4 % of the know origin feathers were correctly classified using the cross-validation by the SPSS

Site	Predicted group membership (%)						Sample size
	Tiszatelek	Rakamaz	Tímár	Nagykálló	Szolnok	UK	
Tiszatelek	40.0	40.0	0	10.0	0	10.0	10
Rakamaz	13.6	68.2	0	9.1	4.5	4.5	22
Tímár	0	0	87.5	0	12.5	0	8
Nagykálló	0	40.0	0	20.0	10.0	30.0	10
Szolnok	0	0	20.0	0	70.0	10.0	10
UK	0.0	0	0	20.0	0	80.0	10

Discriminant Analysis

- The cross-validation is done by treating *n*–1 out of *n* observations as the training data set to determine the discrimination rule and using the rule to classify the one observation left out

Classifying samples on the base of juvenile feathers



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Nagykálló	0	40.0	0	20.0	10.0	30.0	10
Szolnok	0	0	20.0	0	70.0	10.0	10
UK	0.0	0	0	20.0	0	80.0	10

Is there a difference in the moulting areas in Africa among the studied European breeding populations on the base of adult feathers ?

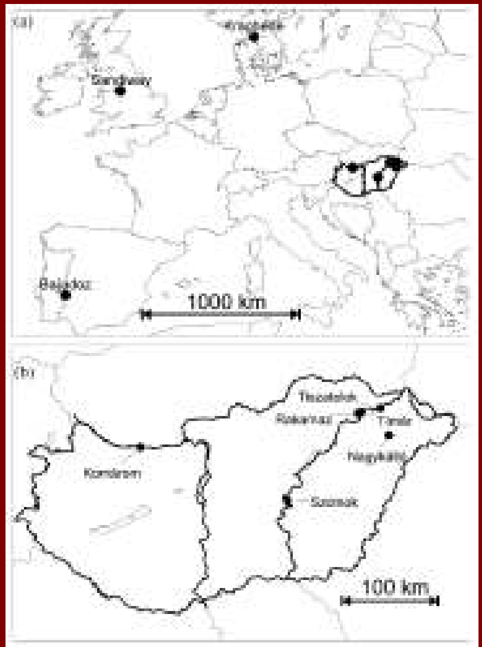


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

- Trace elements
  - Yes ($P<0.001$; MANOVA)
- There are significant differences for the 18 elements among populations

Classifying samples of adult feathers of known breeding populations moulted at the unknown wintering areas

Trace element

- Four functions with Eigenvalues higher than one which four functions explained 97.7 % of the variance

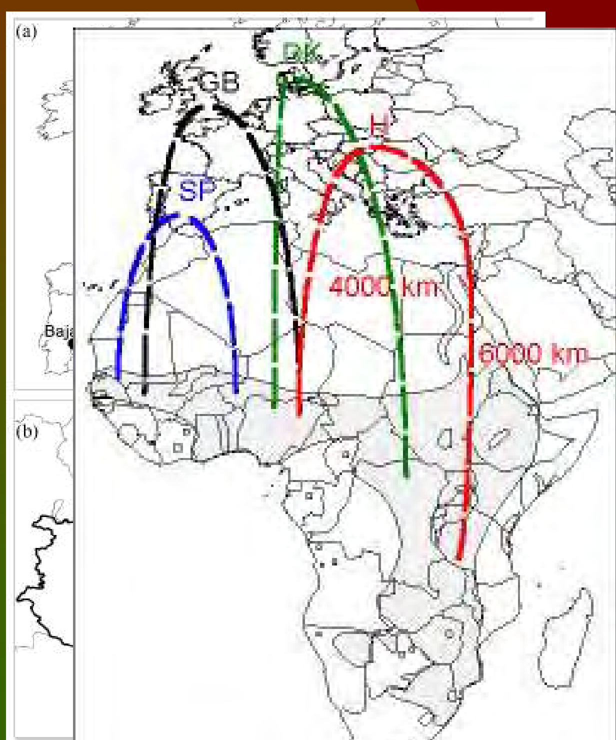
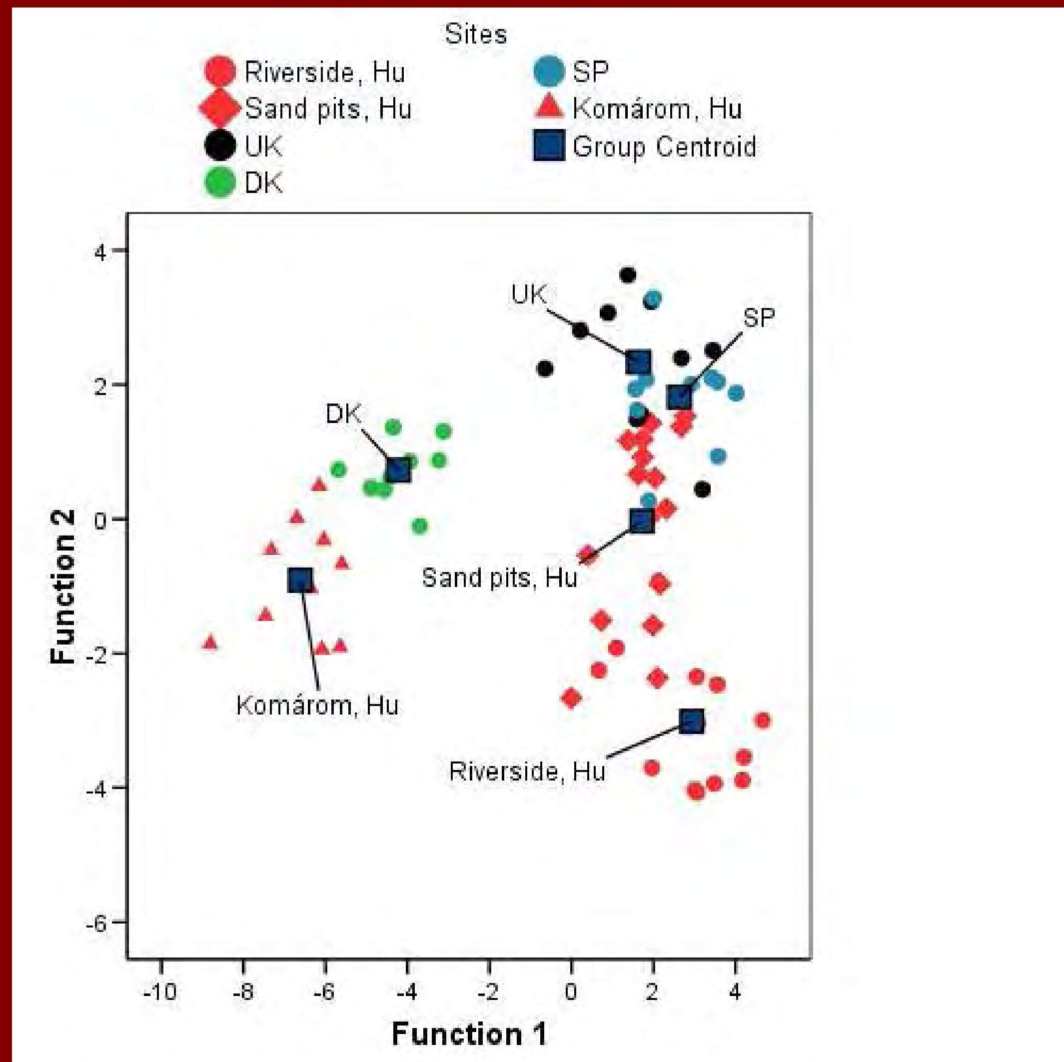
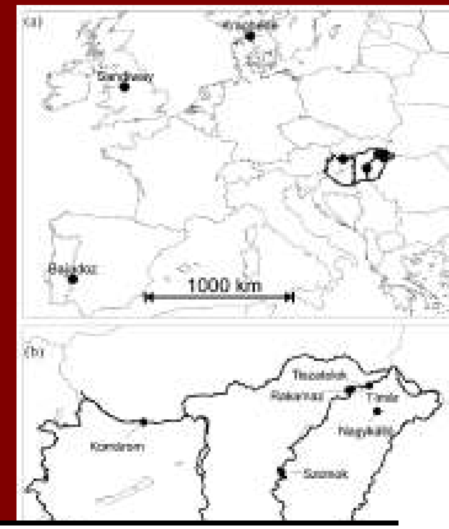


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.



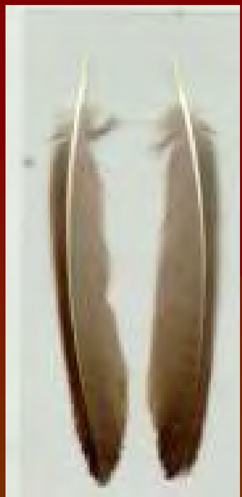
Classifying samples of adult feathers of known breeding populations moulted at the unknown wintering areas



Trace element

- 73.9 % of the know origin feathers were correctly classified, it is better than one can expect by chance by chance ($P<0.001$; Press'Q)

Site	Predicted group membership (%)						
	Riverside, Hungary	Sand pits, Hungary	UK	Denmark	Spain	Komárom, Hungary	Sample size
Riverside, Hungary	76.9	7.7	7.7	0	7.7	0	13
Sand pits, Hungary	25.0	56.3	12.5	0	6.3	0	16
UK	20.0	10.0	70.0	0	0	0	10
Denmark	0	0	0	100.0	0	0	10
Spain	0	20.0	0	0	80.0	0	10
Komárom, Hungary	0	0	10.0	20.0	0	70.0	10



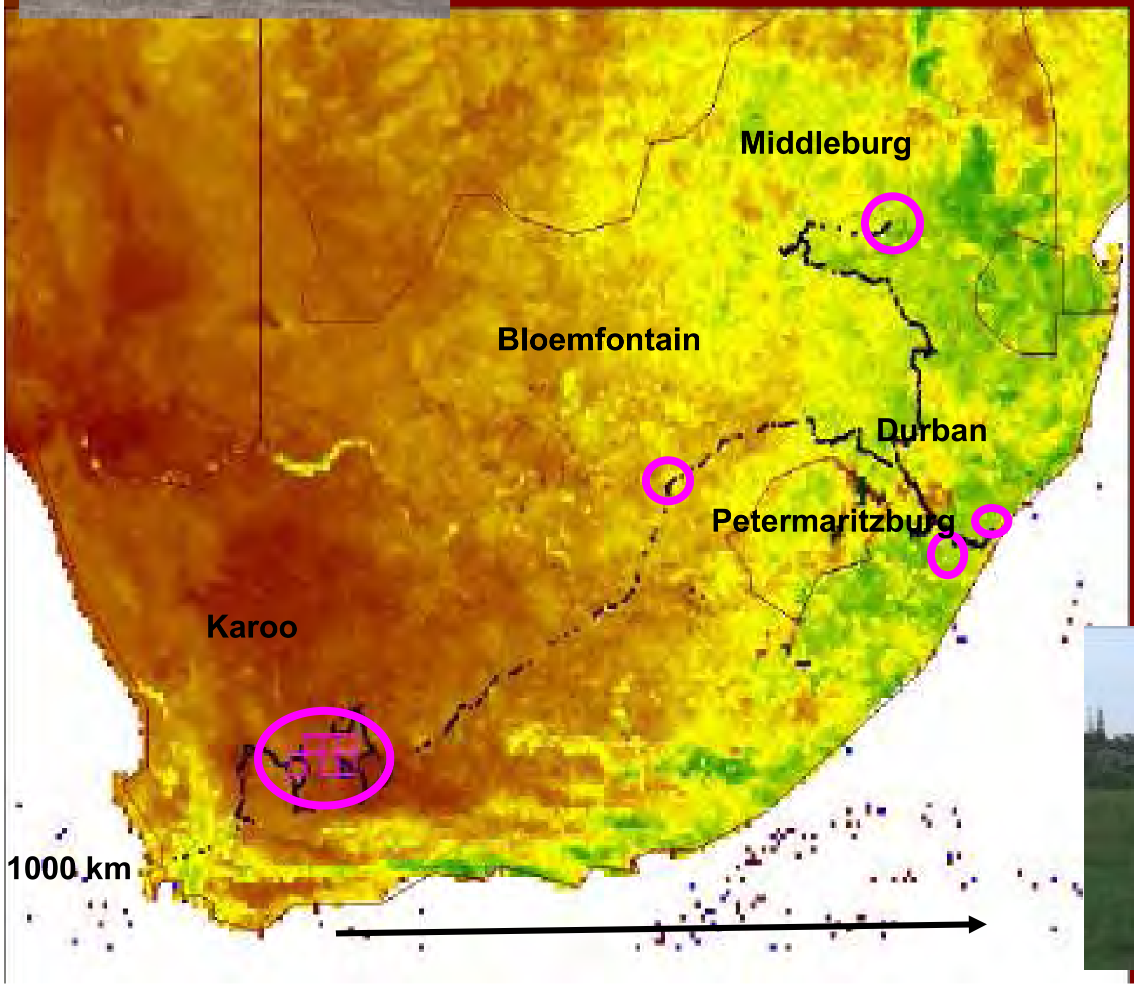
Trace elements – questions tested (Szép et al. 2003)

- Is there a difference in composition of juvenile feathers among European breeding populations? Yes
- Is there a difference in composition of juvenile feathers among nests in a single colony? No
- Is there a difference in composition of juvenile feathers between years in a single colony? No
- Does composition of feathers grown by adults and juveniles in the same areas differ? Yes
- Is there a difference in composition of adult feathers grown by the same individuals in Africa and in Europe? Yes
- Does composition of adult feathers grown in Africa and juvenile feathers grown in Hungary differ? Yes
- Does composition of adult feathers grown in Africa and juvenile feathers grown in UK differ? Yes
- Is there a difference among European breeding populations in composition of adult feathers grown in Africa? Yes
- Does sex have an effect on composition of feathers, and does this effect vary among populations? No

Studied barn swallow and sand martin feathers in South Africa

- Swallows in the roost foraging within a ~50 km radius area
- A pair of freshly moulted first primary feather (P1) collected
- At each roosts from 10 adults and 10 juveniles were sampled

Investigation of barn swallows feathers in the South African wintering grounds in 2003



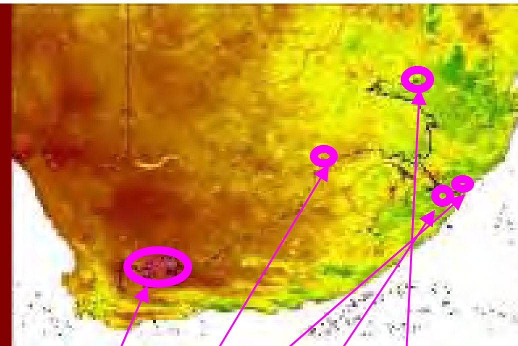
Studies sites (roosts)

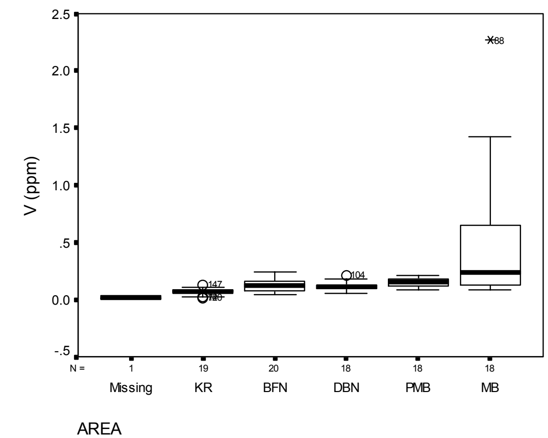
- Continental-regional scale
  - Varying distance within the country (between 60-1300 km)
- Habitat scale
  - Semi-desert (Karoo)
  - Subtropical (Durban)

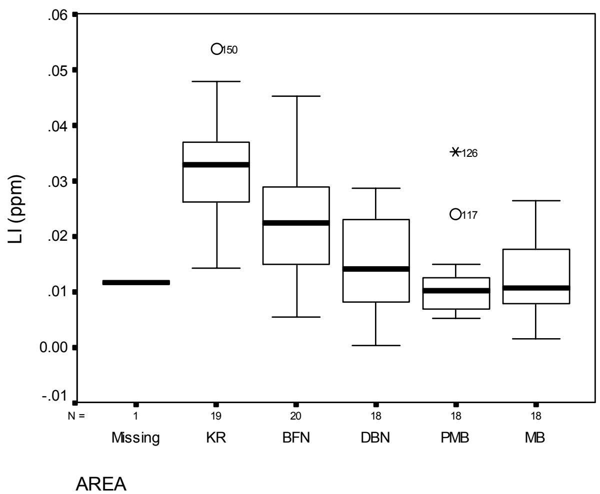
Is there differences among the roosts in the wintering area?

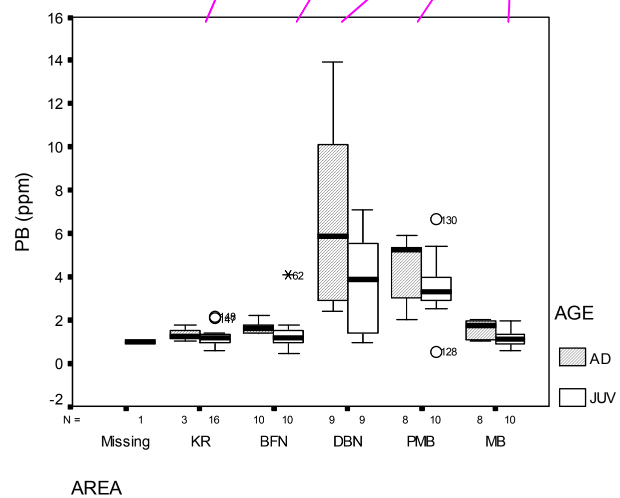
Trace elements

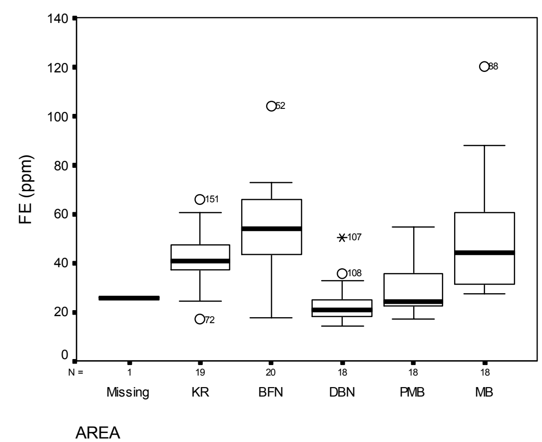
YES (P<0.001; MANOVA)
Significant differences: Mg, Mn, Sr, Ca, Li, Ti, V, Ba, Pb and Cu

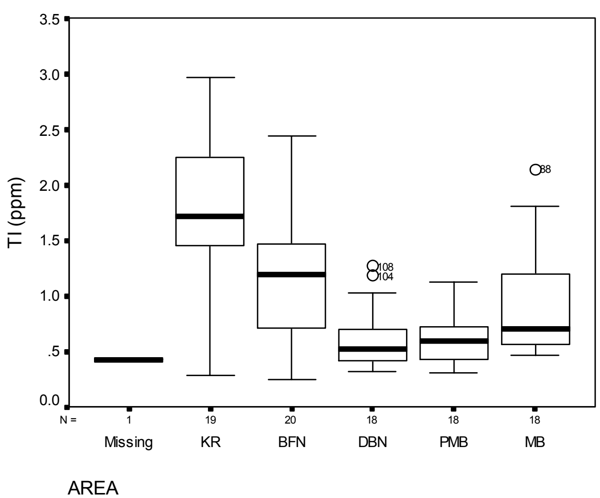


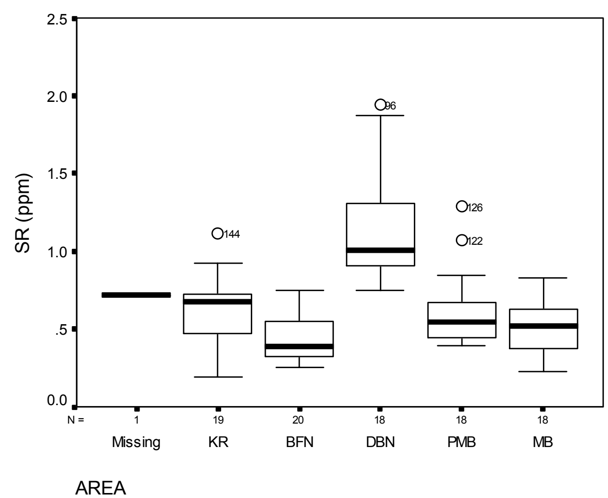


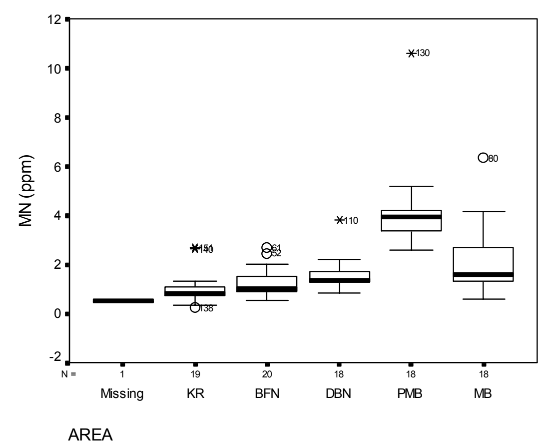




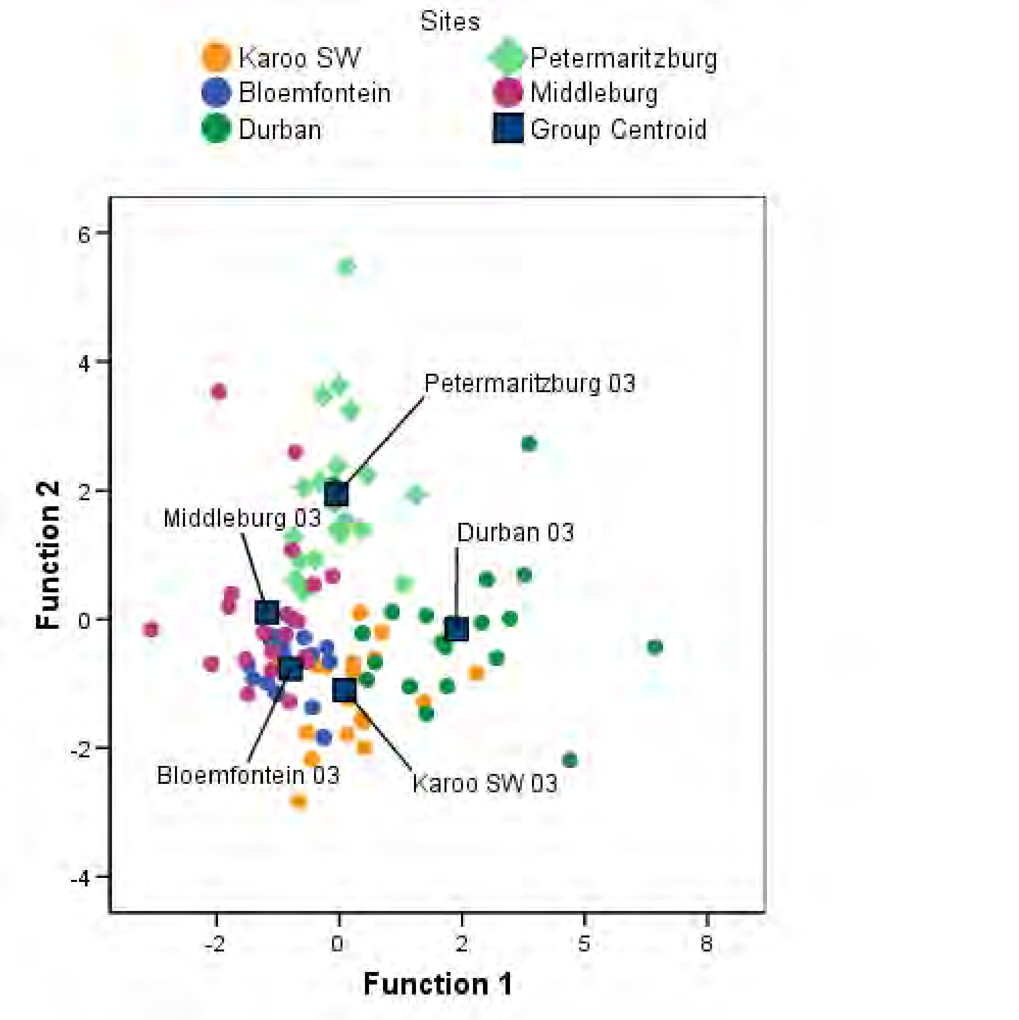


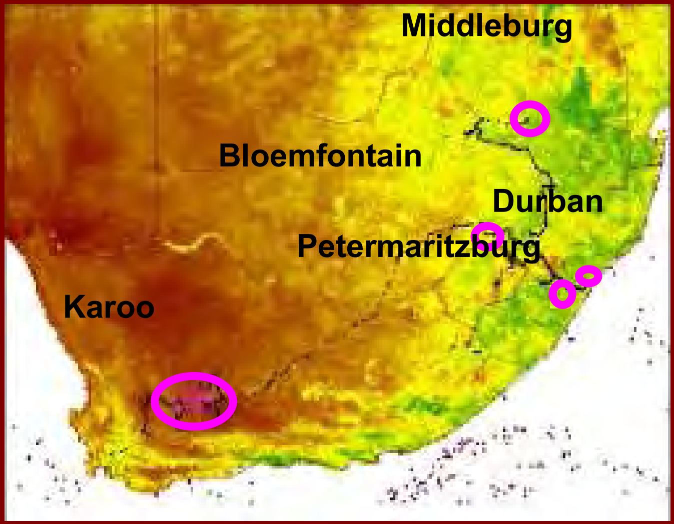






Classifying samples on the base of feathers collected at the roosts in South Africa

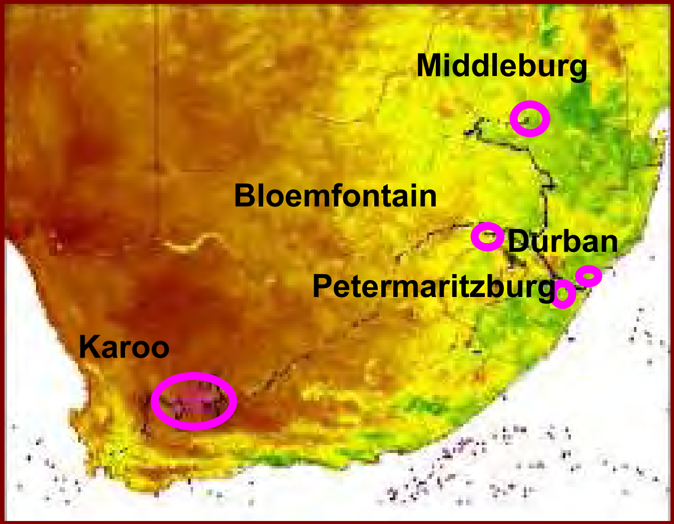




Trace elements

- Two functions with Eigen value higher then one, which are explains 81.2 % of the variance

Classifying samples on the base of feathers collected at the roosts in South Africa

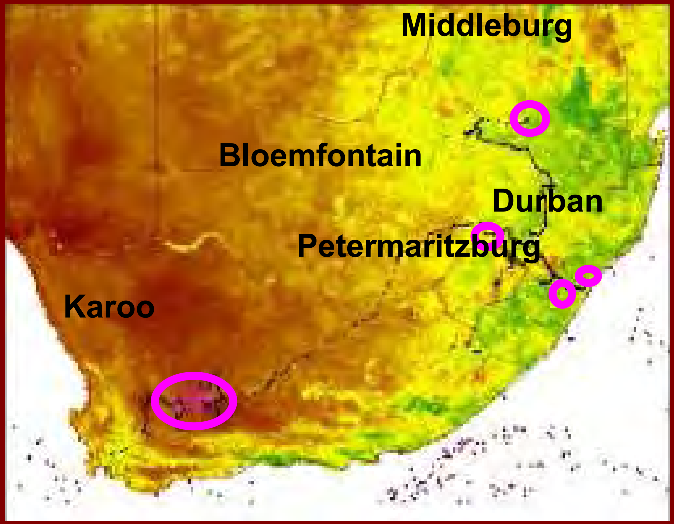


Trace elements

- 60.0% of the know origin feathers were correctly classified using the cross-validation by the SPSS
- This value is significantly higher than one can expect it by chance (P<0.001; Press'Q) .

Site	Predicted Group Membership (%)					Sample size
	Karoo SW	Bloemfontein	Durban	Pietermaritzburg	Middleburg	
Karoo SW	50.0	30.0	15.0	0.0	5.0	20
Bloemfontein	15.0	75.0	5.0	0.0	5.0	20
Durban	30.0	0.0	65.0	5.0	0.0	20
Pietermaritzburg	0.0	10.0	10.0	75.0	5.0	20
Middleburg	15.0	30.0	0.0	20.0	35.0	20

Comparison feathers moulted by sand martin and barn swallow at the same roosts at Durban in South Africa



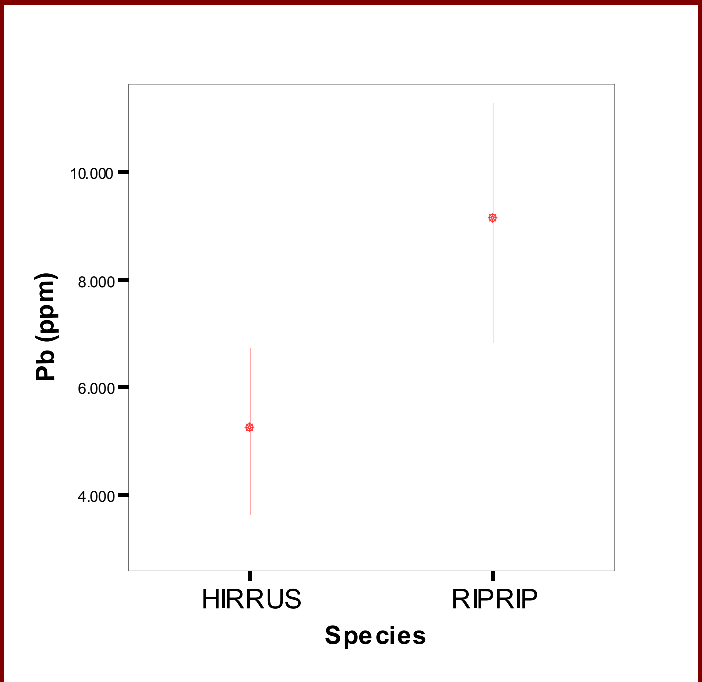
Trace elements

- Significant difference (P=0.017; MANOVA)

◆Eight elements has significantly different levels (P<0.03)

◆Higher concentration were in the feathers of sand martin from As, Mg, Sr, V, Pb, S

◆Concentration were higher in the feathers of barn swallow for the Ca and Zn



Donovan et al. (2006) study in US

- Study of 7 species at 27 sites (Eastern Bluebird, Ovenbird, Pine Warbler, Prairie Warbler, Eastern Towhee, Tree Swallow and Wood Thrush)
- Collecting feathers of juveniles
- Use of ICP-MS and ICP-OES method

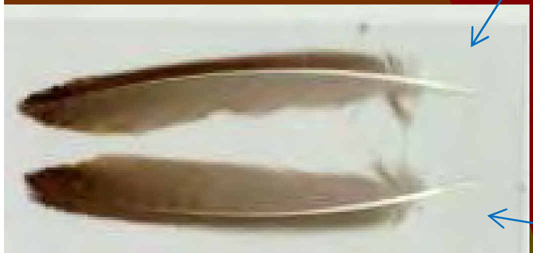
- Both ICP-MS and ICP-OES analyses separated species within a site with fairly high accuracy, though the discriminating elements varied with site.
- Within a species, natal feather locations were not identified with high accuracy on the basis of feather elements.

- Base-map of element signatures across the eastern United States was ineffective at predicting feather-element values at sample sites.

COMPARISON OF TRACE ELEMENT AND STABLE ISOTOPE APPROACHES TO THE STUDY OF MIGRATORY CONNECTIVITY: HIRUNDINE SPECIES BREEDING IN EUROPE AND WINTERING IN AFRICA

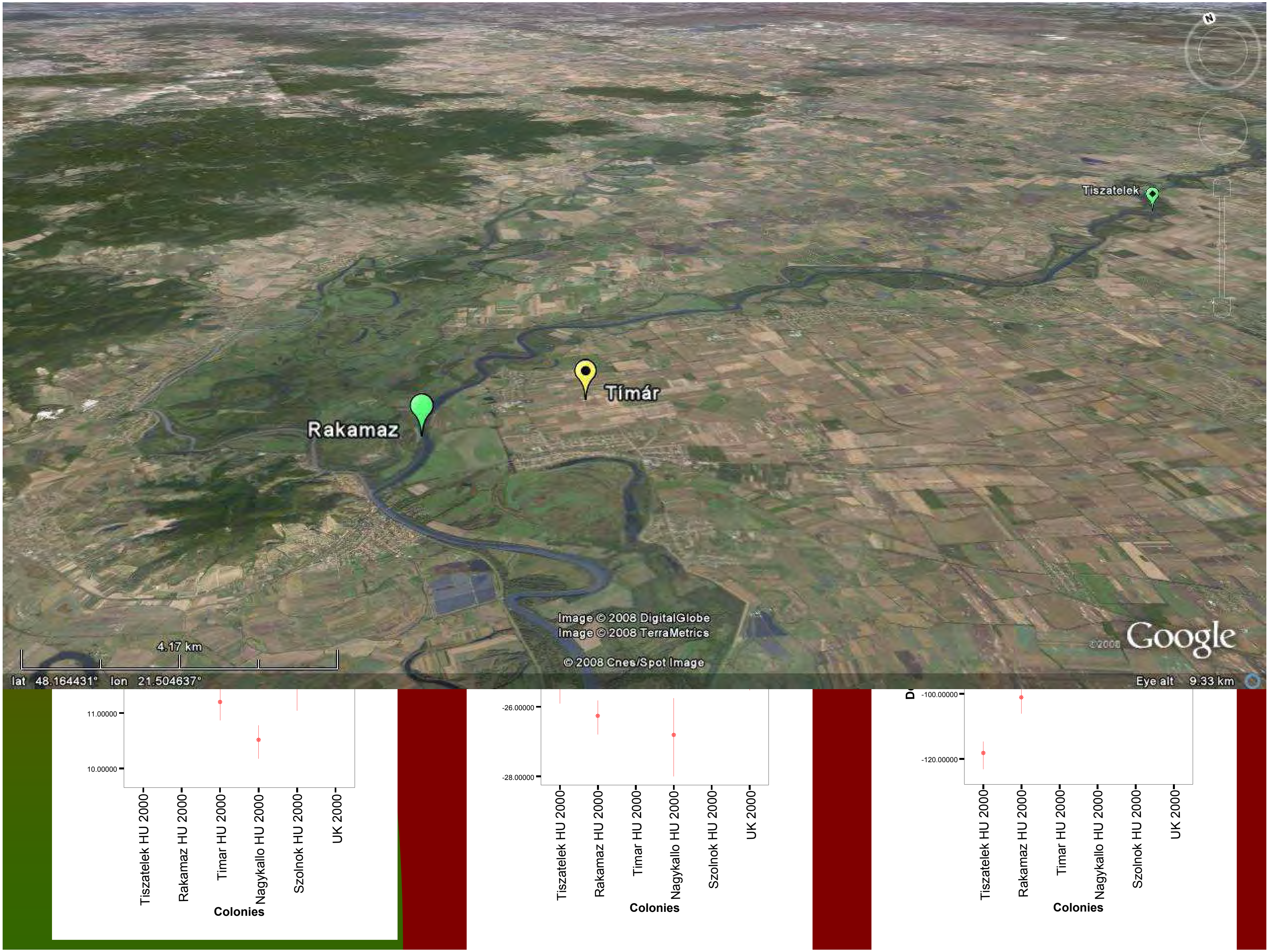
Tibor Szép, College of Nyíregyháza, Hungary
Keith Hobson, Canadian Wildlife Service, Canada
Judit Vallner, College of Nyíregyháza, Hungary
Steven Piper, Univ. Kwazulu-Natal, South Africa
Béla Kovács, Univ. Debrecen, Hungary
Zoltán D. Szabó, Univ. Babes-Bólyai, Romania
Anders P. Møller, Univ. Paris, France

Methods of chemical analysis



- Pair of the collected feather was used
- Stable isotope
  - C^{13} and N^{15} for all samples, Deuterium only for the sand martin collected in Europe
  - by Keith Hobson in Canada
- Trace element
  - Measurement of 23 chemical elements by ICP-OES technique from one feathers

    As, Cd, Mg, Mn, Mo, Se, Sr, Ca, Co, Fe, Zn, Li, P, Ti, V, Ag, Cr, Ba, Hg, Pb, S, Ni, Cu
  - Usage of specific cleaning/handling method for preparation and of small sample size (Vallner et. al 1999)
  - by the Hungarian team

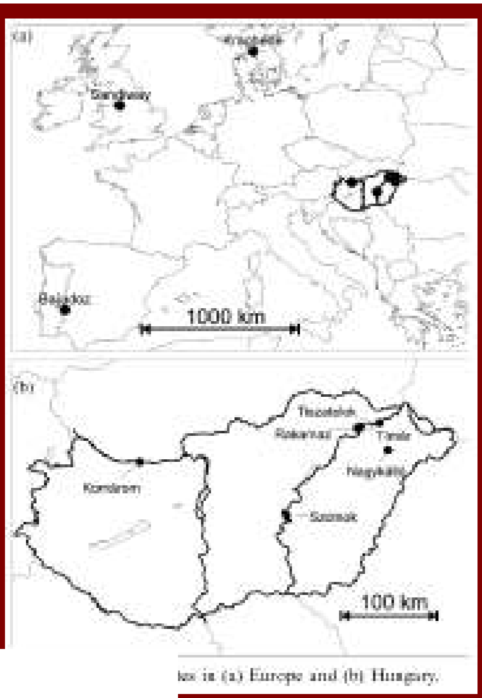

Tiszatelek
Tímár
Rakamaz
Image © 2008 DigitalGlobe
Image © 2008 TerraMetrics
© 2008 Cnes/Spot Image
Google
4.17 km
lat 48.164431° lon 21.504637°
Eye alt 9.33 km
11.00000
10.00000
-26.00000
-28.00000
-100.00000
-120.00000
Tiszatelek HU 2000
Rakamaz HU 2000
Timar HU 2000
Nagykallo HU 2000
Szolnok HU 2000
UK 2000
Colonies

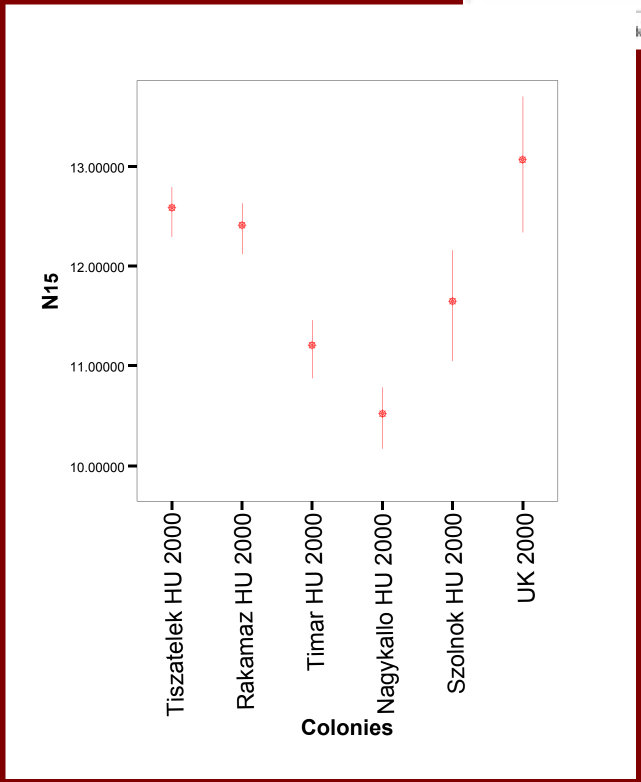
Is there a difference in composition of juvenile feathers among European breeding populations?

δ15N

Values were highest in the UK, although that value did not differ significantly from the very distant sites (1700 km away, along the river Tisza (Tiszatelek and Rakamaz)

There was a significant difference between the two closest sites (Rakamaz and Tímár, 4 km apart; P = 0.001)





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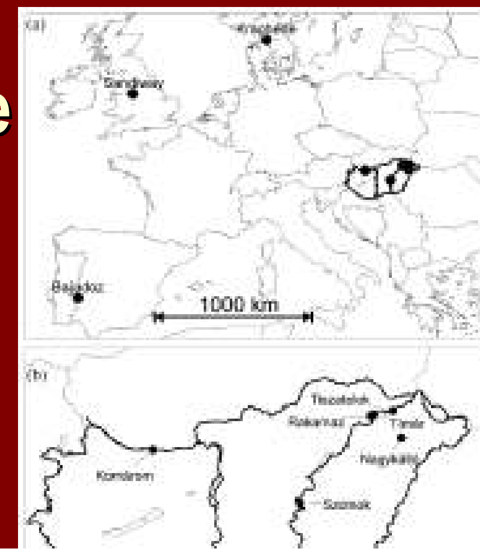
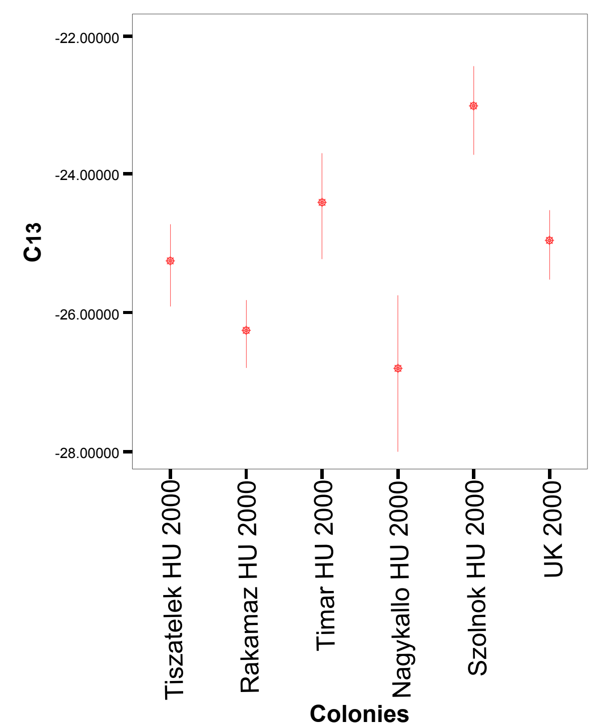


$\delta^{13}C$

There was no significant difference among the very distant sites (1700 km away, UK vs. Sites along the river Tisza (Tiszatelek and Rakamaz)

There was a significant difference between the two closest sites (Rakamaz and Tímár, 4 km apart, P = 0.004)



Is there a difference in composition of juvenile feathers among European breeding populations?

δD

The highest level was in the UK, significantly larger than the colonies along the river and nearby sand pits (Tiszatelek, Rakamaz, Tímár) ($P < 0.001$)

There was a significant difference between the two closest sites (Rakamaz and Tímár, 4 km apart; $P = 0.001$)

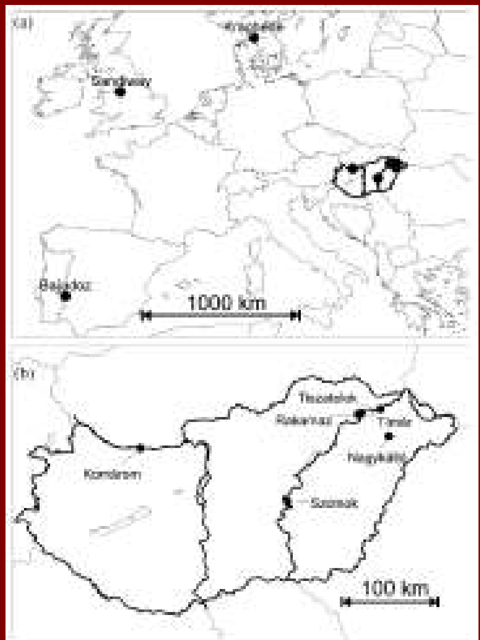
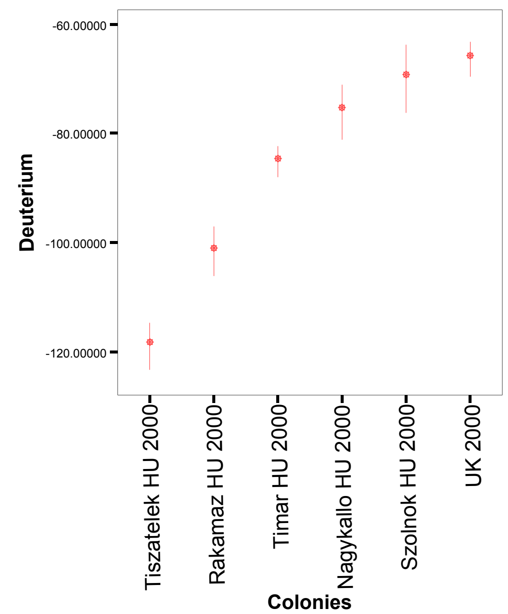
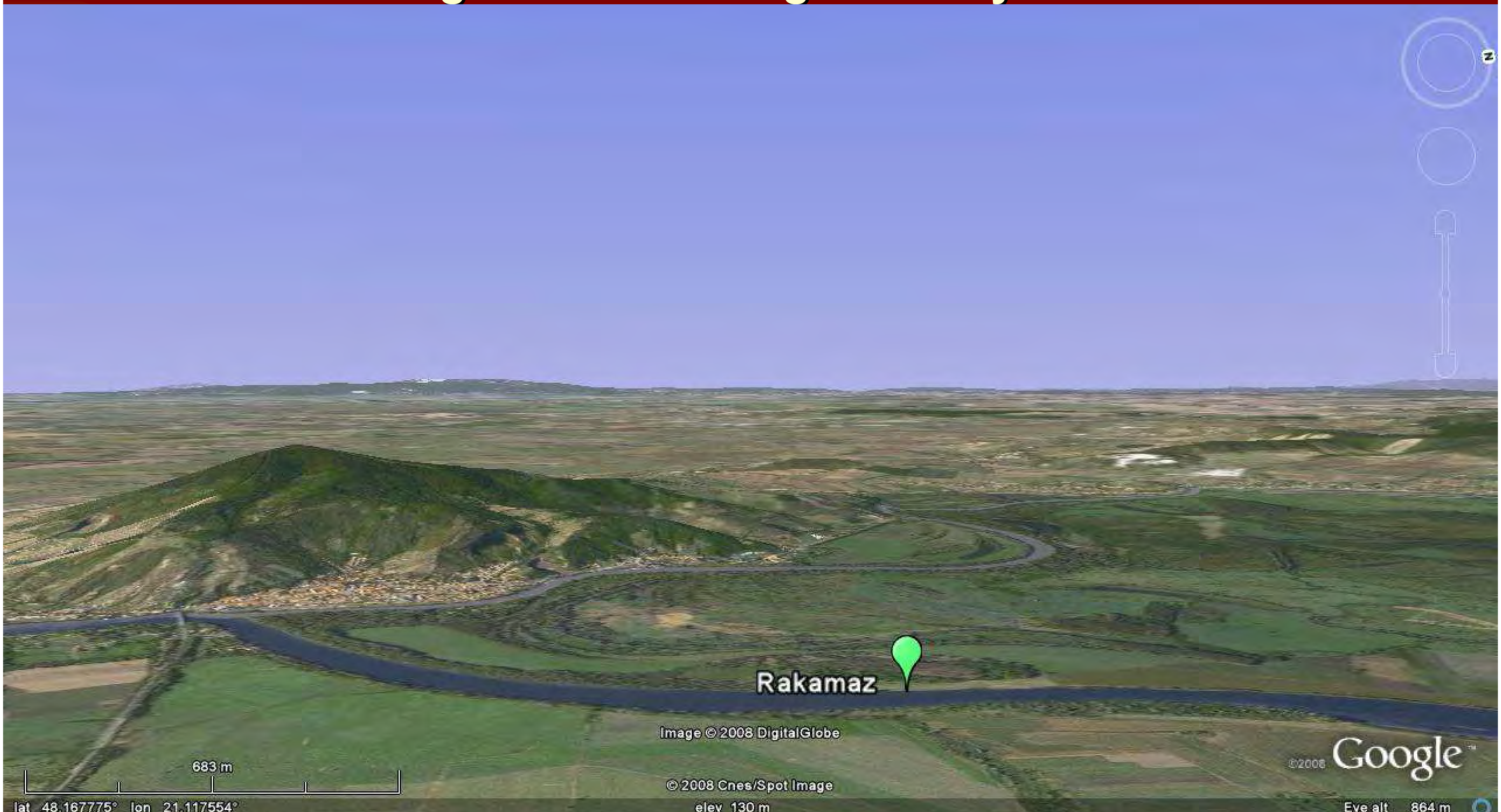
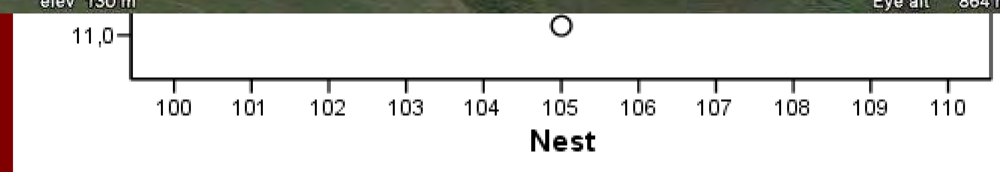


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.



Is there a difference in composition of juvenile feathers among nests in a single colony?





Is there a difference in composition of juvenile feathers between years in a single colony?

Comparing feathers collected at Tiszatelek colony at 1996 and 2000

Trace elements

No (P=0.306; MANOVA)

Stable isotopes

Yes (P<0.001; MANOVA)

δ13C and δD levels were lower in 2000 (P<0.01)

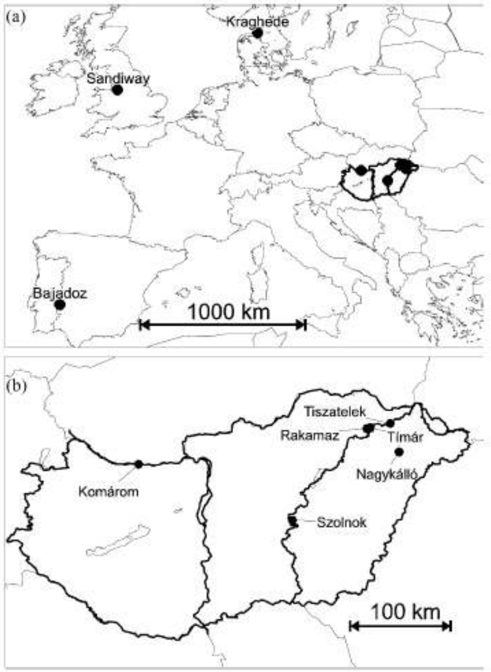
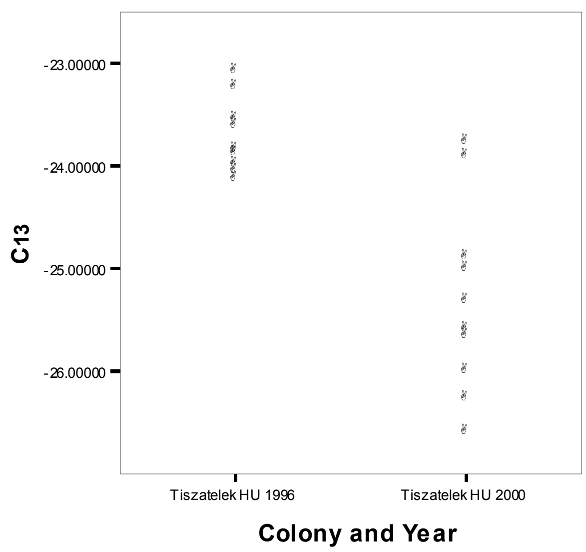
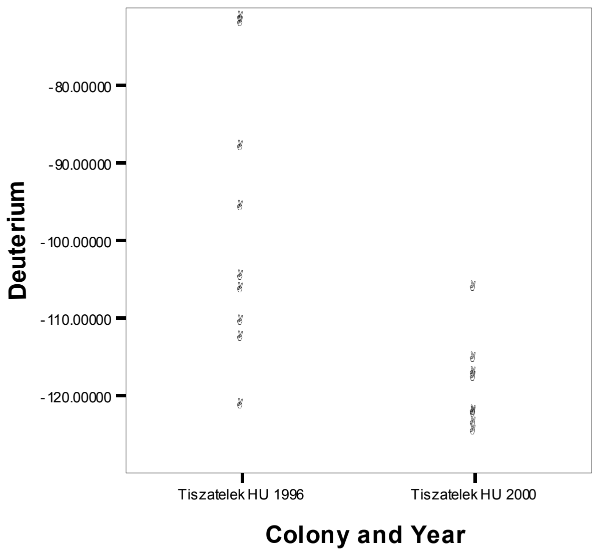


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.





Does the composition of feathers moulted in the breeding areas by adults males and adult females differs?

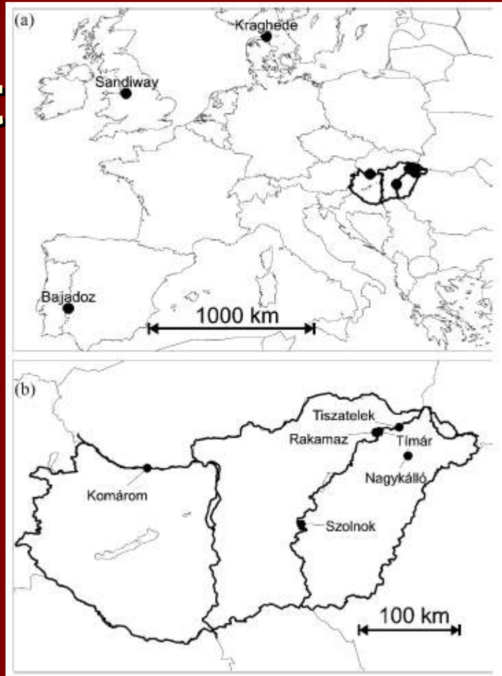


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

Trace elements

No (P=0.33; MANOVA)

Stable isotopes

No (P=0.25; MANOVA)

		Mean	N	SE
male	N^{15}	12.501	9	.349
female	N^{15}	12.293	9	.203
male	C^{13}	-24.569	9	.310
female	C^{13}	-24.763	9	.290
male	Deuterium	-67.567	8	7.071
female	Deuterium	-64.366	8	6.808

Classifying samples on the base of juvenile feathers

Stable isotopes

- Two functions with high Eigen values, explained 90.7% of the variance

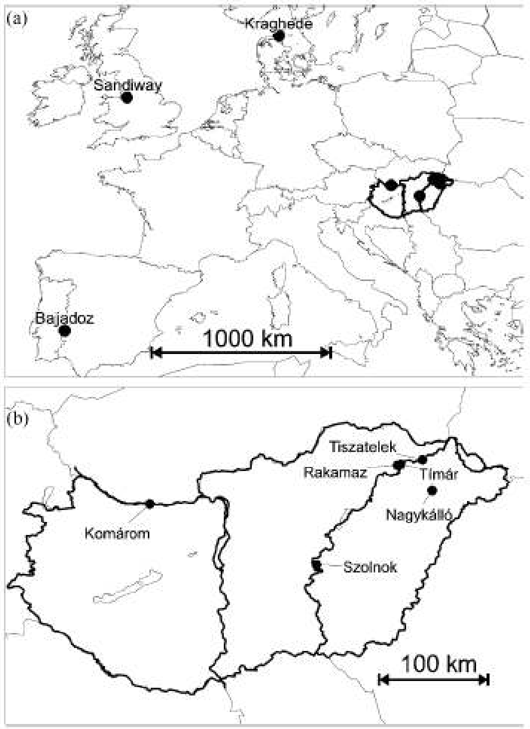
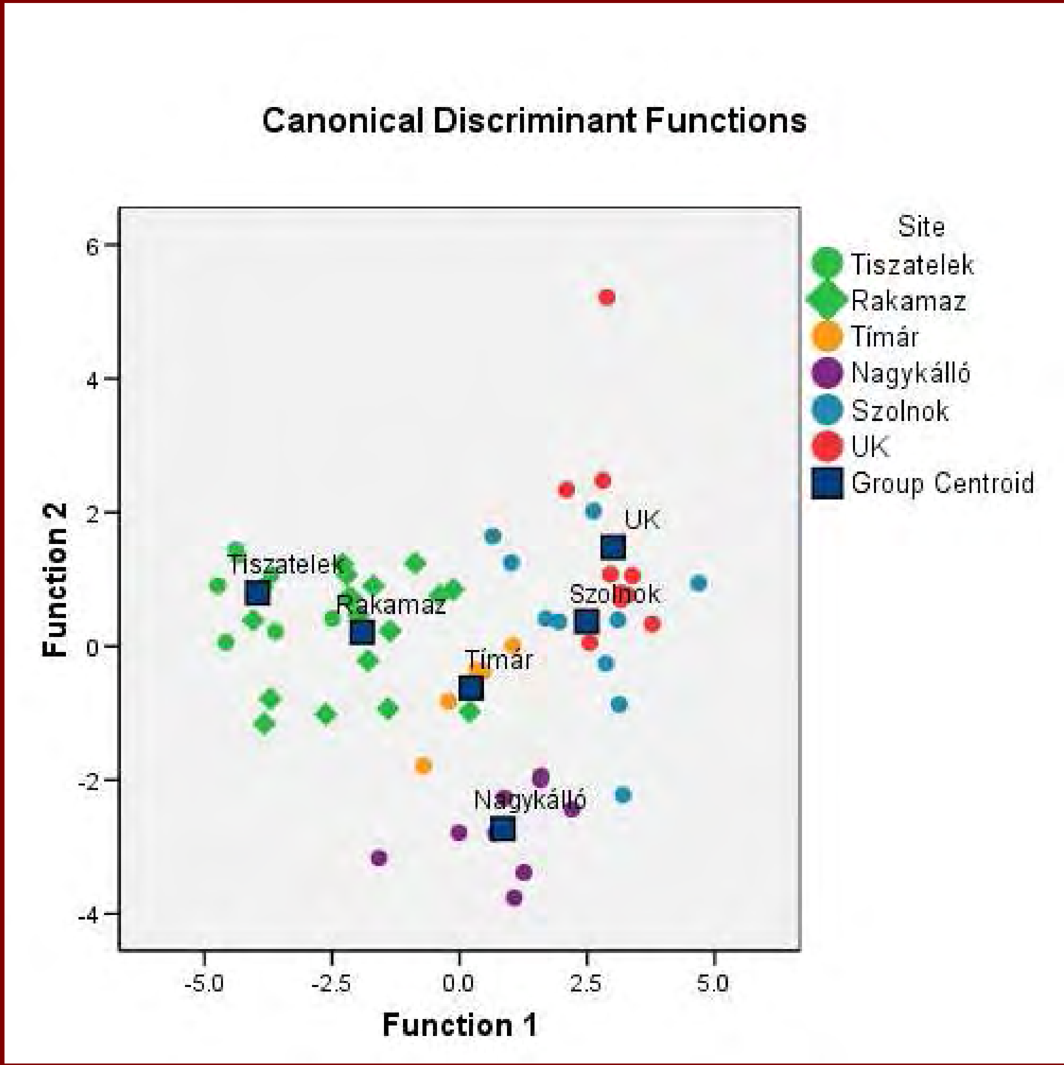


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.



Classifying samples on the base of juvenile feathers

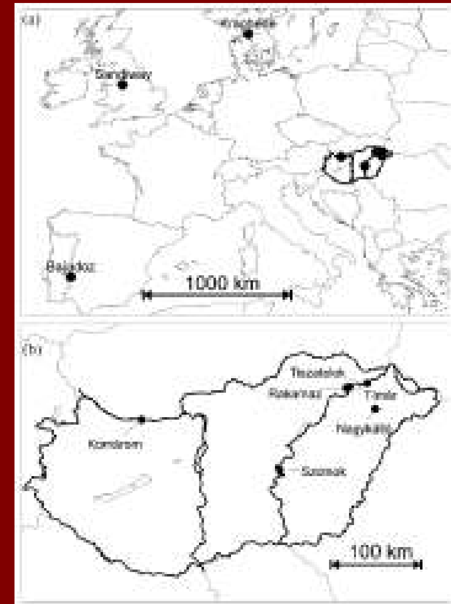


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

Stable isotopes

- 85 % of the know origin feathers were correctly classified using the cross-validation procedure of the SPSS

Site	Predicted group membership (%)						Sample size
	Tiszatelek	Rakamaz	Tímár	Nagykálló	Szolnok	UK	
Tiszatelek	87.5	12.5	0	0	0	0	8
Rakamaz	11.8	82.4	0	5.9	0	0	17
Tímár	0	16.7	66.7	0	16.7	0	6
Nagykálló	0	11.1	0	88.9	0	0	9
Szolnok	0	0	0	0	100.0	0	10
UK	0	0	0	0	20.0	80.0	10

Is there a difference in the moulting areas in Africa among the studied European breeding populations on the base of adult feathers ?

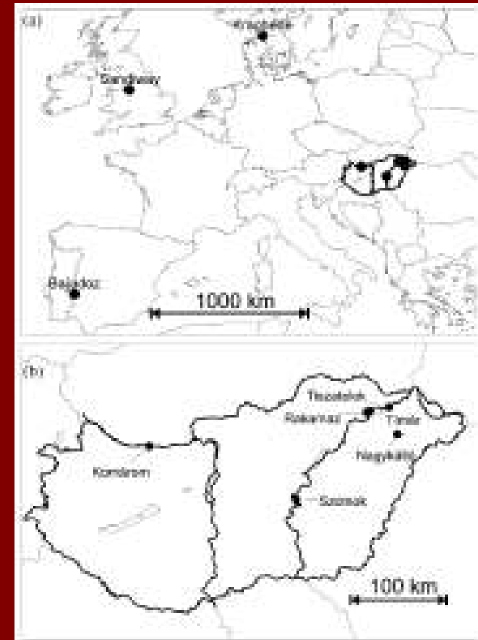


Fig. 1. Sampling sites in (a) Europe and (b) Hungary.

Trace elements

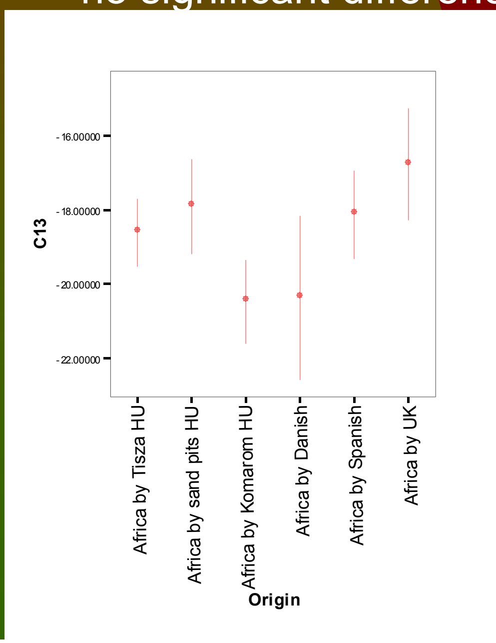
Yes ($P<0.001$; MANOVA)

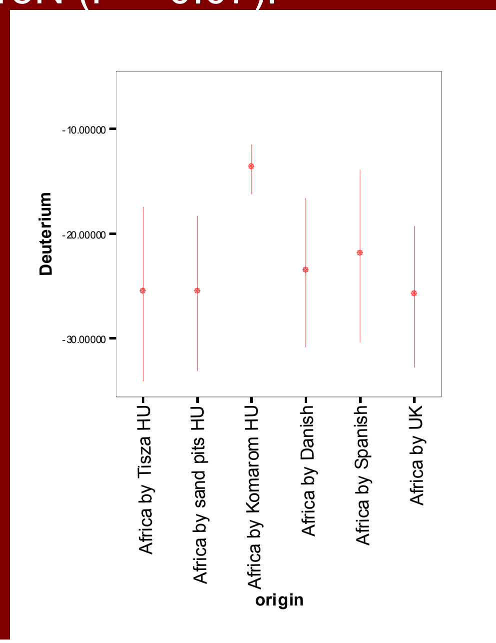
There are significant differences for the 18 elements among populations

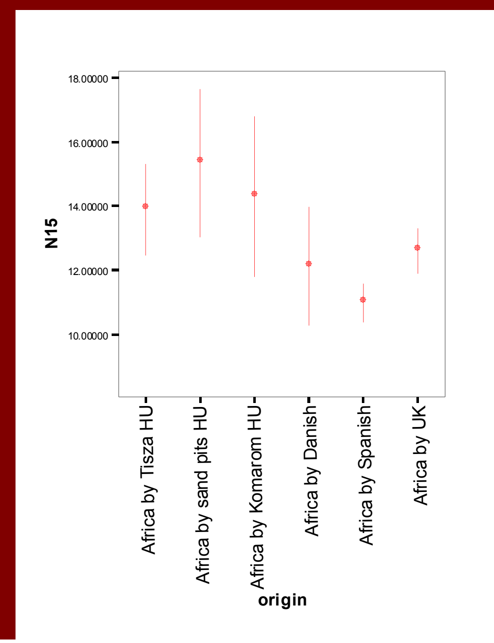
Stable isotopes

Yes ($P=0.019$; MANOVA)

Stable isotope values differed significantly among localities for δ13C ($P = 0.01$) and δD ($P = 0.023$), while there was no significant difference for δ15N ($P = 0.07$).



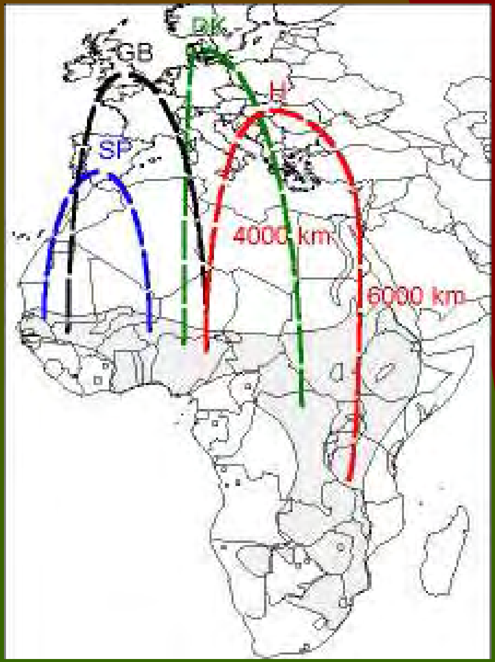


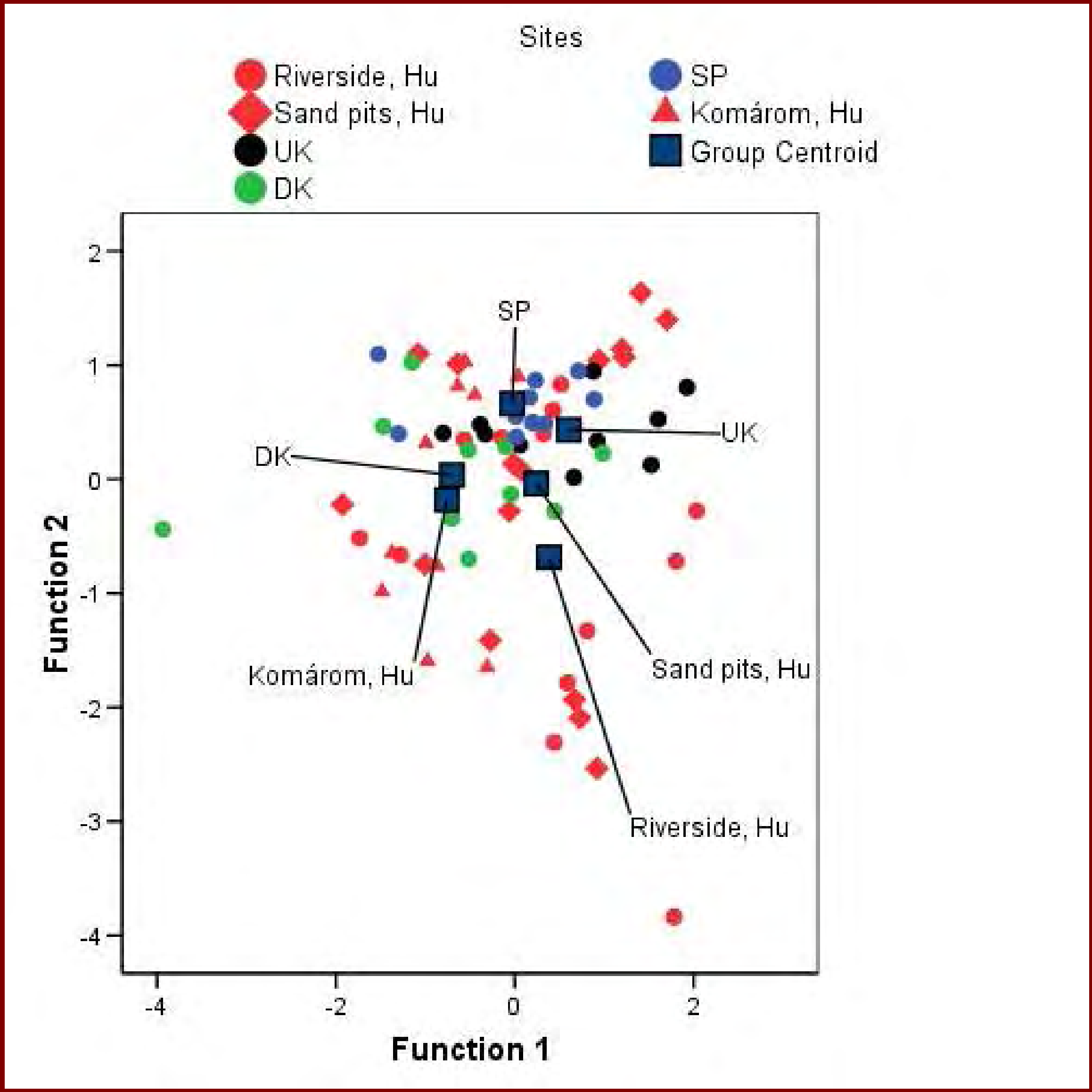


Classifying adult feathers of known breeding populations moulted at the unknown wintering areas

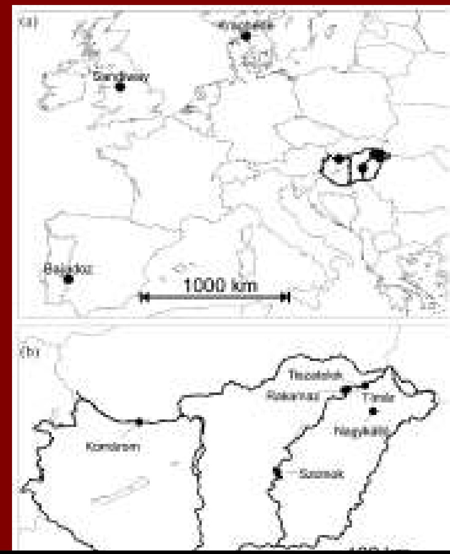
Stable isotopes

- Nor of any functions had Eigen values higher than one





Classifying samples on the base of adult feathers of known breeding populations moulted at the unknown wintering areas



Stable isotopes

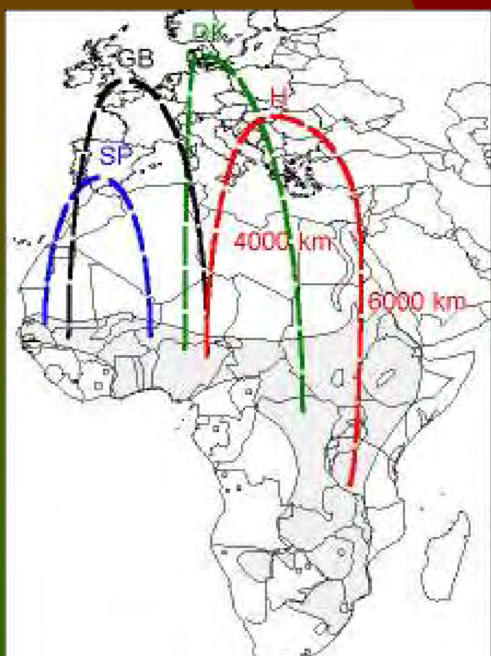
- 38 % of the know origin feathers were correctly classified, it is better than one can expect by chance by chance (P<0.001; Press'Q)

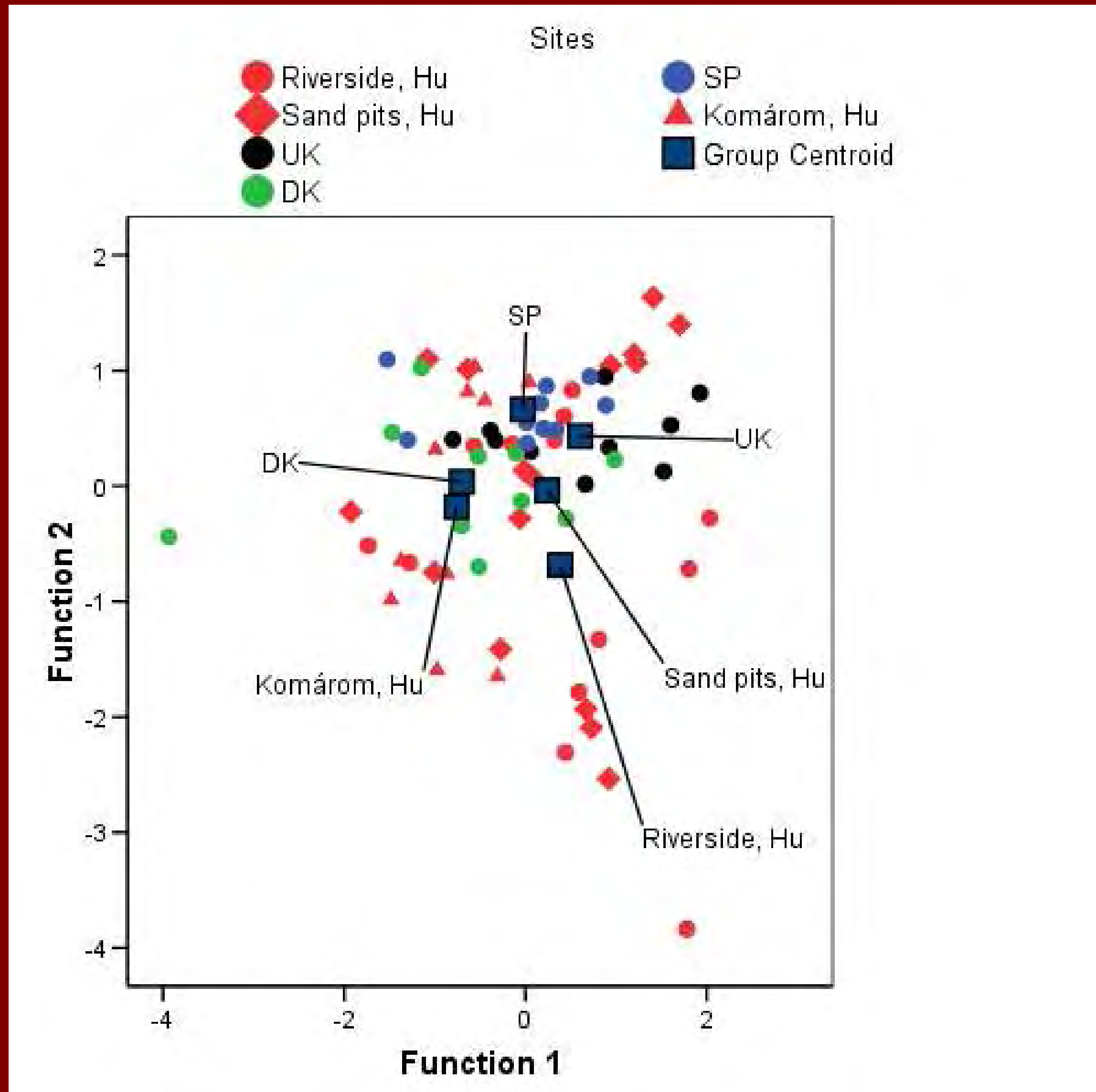
Site	Predicted group membership (%)						Sample size
	Riverside, Hungary	Sand pits, Hungary	UK	Denmark	Spain	Komárom, Hungary	
Riverside, Hungary	30.8	46.2	0	0	7.7	15.4	13
Sand pits, Hungary	25.0	18.8	31.3	6.3	12.5	6.3	16
UK	0	90.0	10.0	0	0	0	10
Denmark	0	50.0	0	20.0	10.0	20.0	10
Spain	0	80.0	0	10.0	0	10.0	10
Komárom, Hungary	0	30.0	0	0	20.0	50.0	10

Classifying adult feathers of known breeding populations moulted at the unknown wintering areas

- More than 110 thousand sand martin ringed in the studied Hungarian population since 1986 – no any African recoveries

- UK, there are several hundreds recoveries from Western Africa

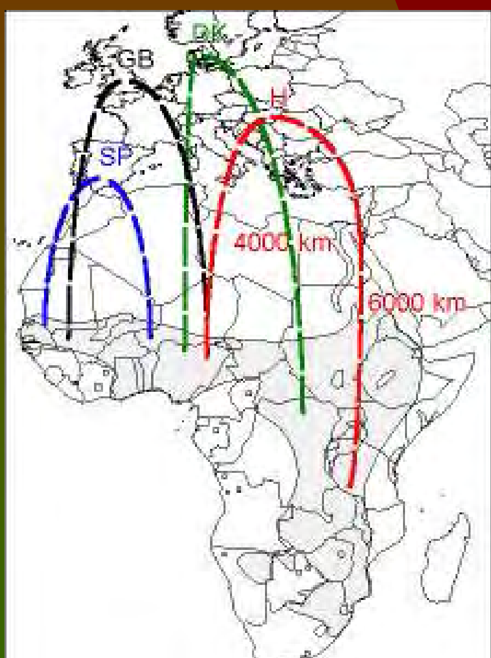


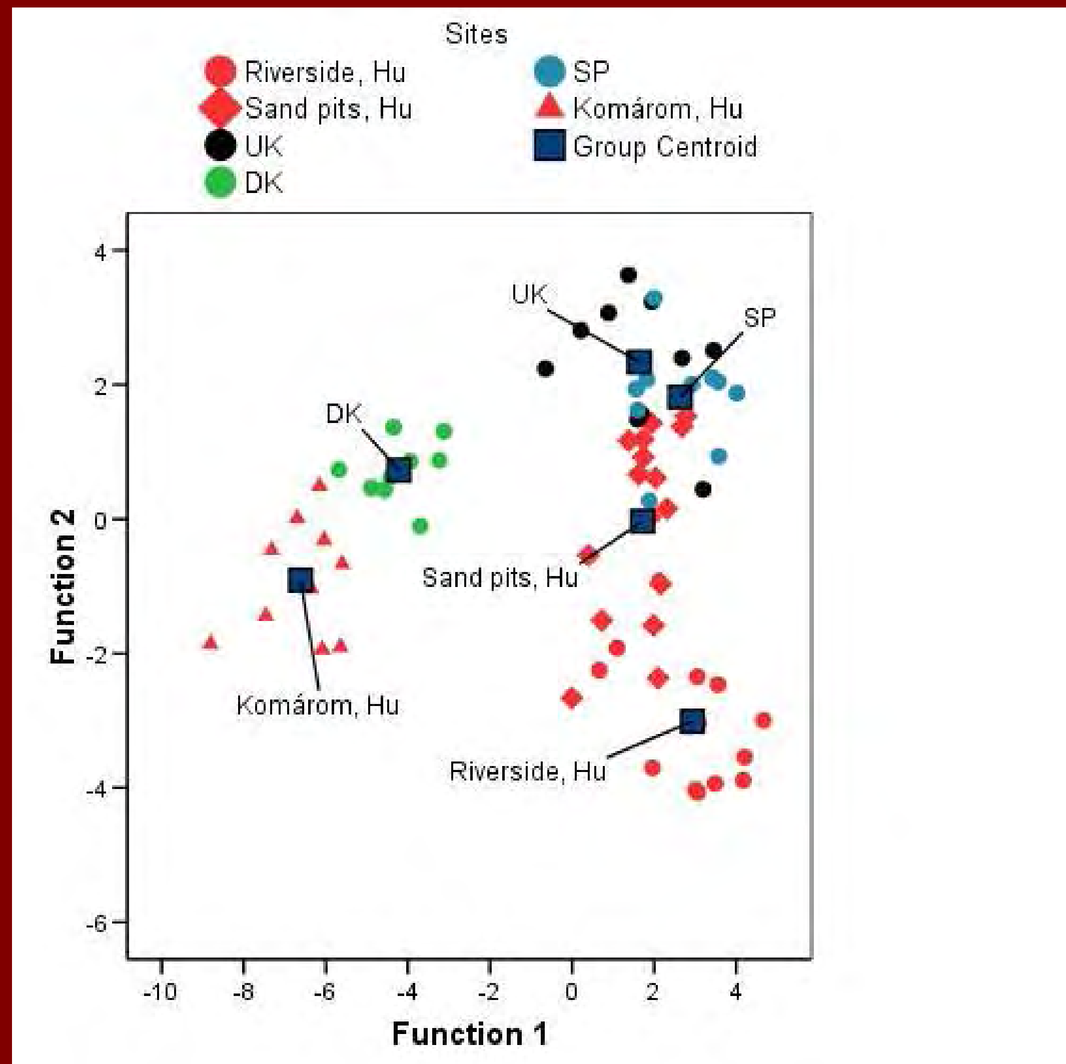


Classifying samples of adult feathers of known breeding populations moulted at the unknown wintering areas

Trace element

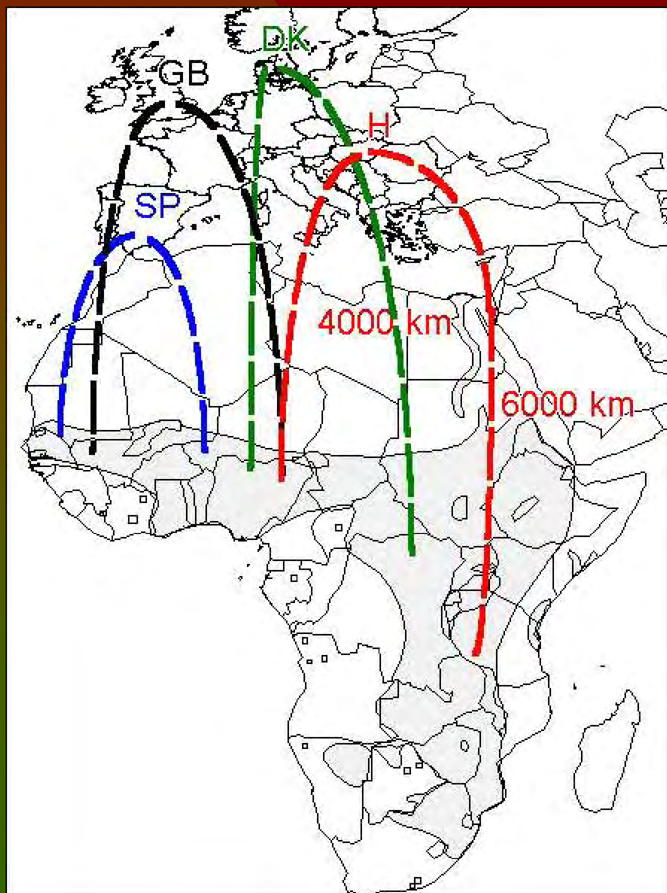
- Four functions with Eigenvalues higher than one which four functions explained 73.9 % of the variance

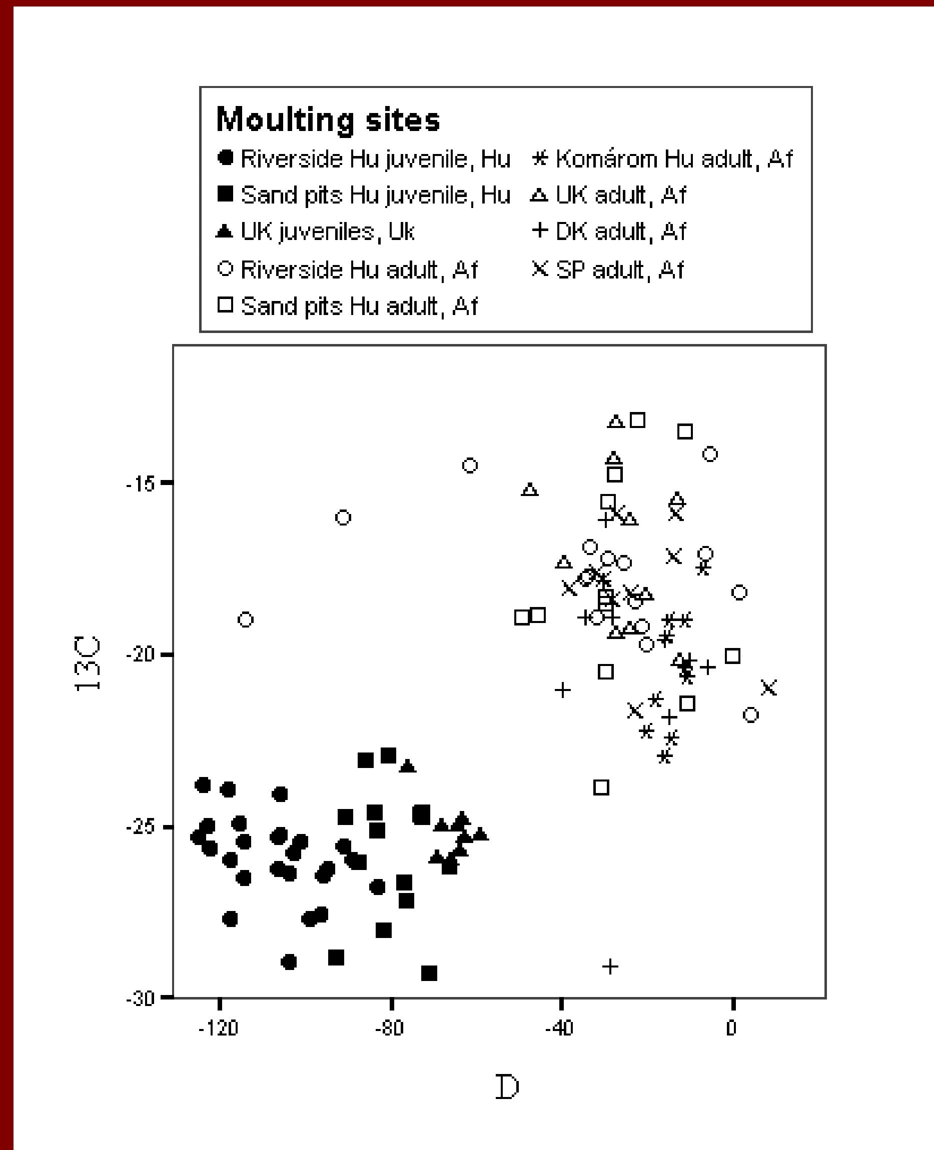




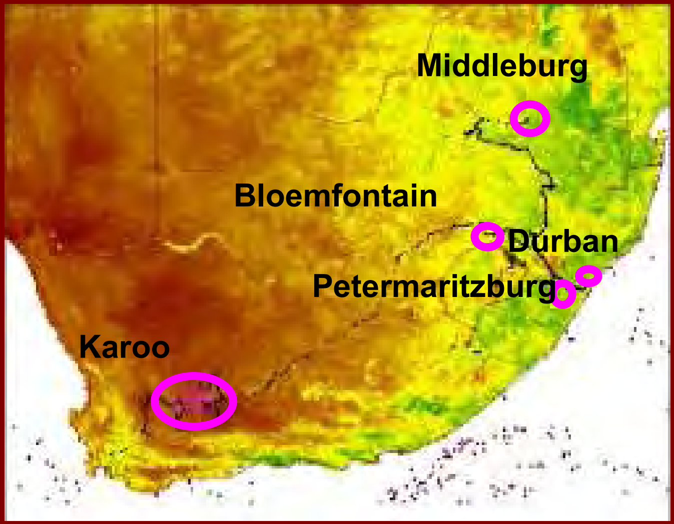
Stable isotopes profile of feathers moulted in Europe and Africa

- Obvious difference between feathers moulted in Europe vs. Africa



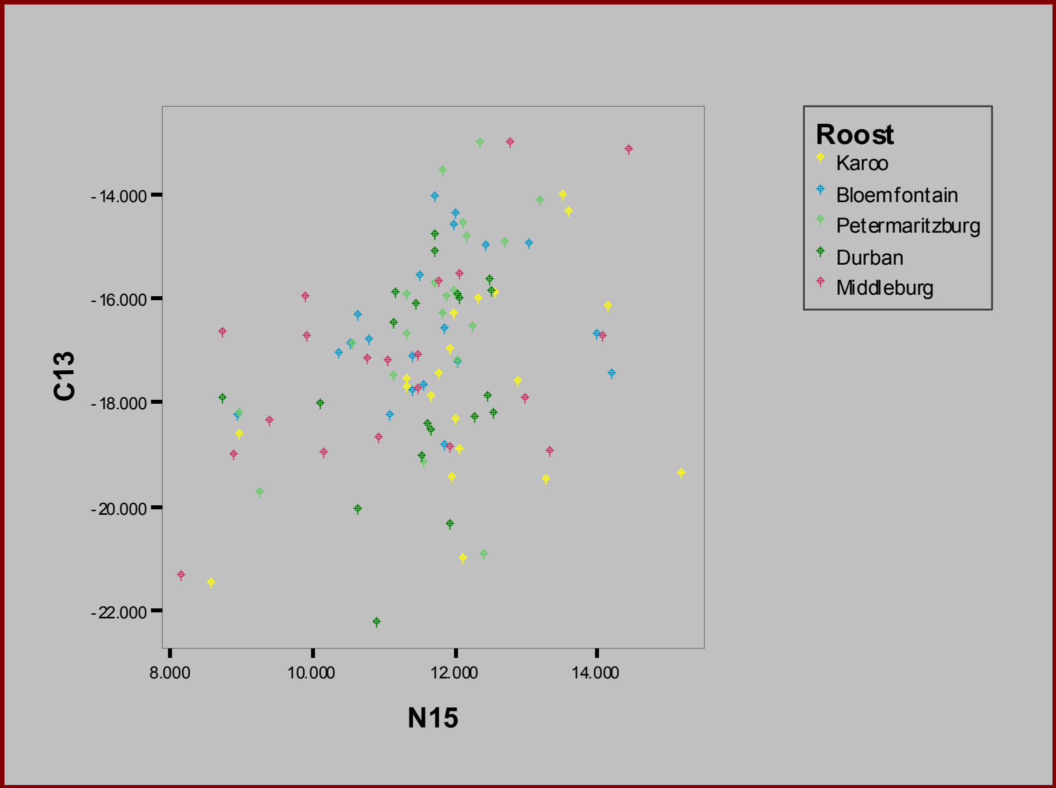


Is there differences among the roosts in the wintering area?

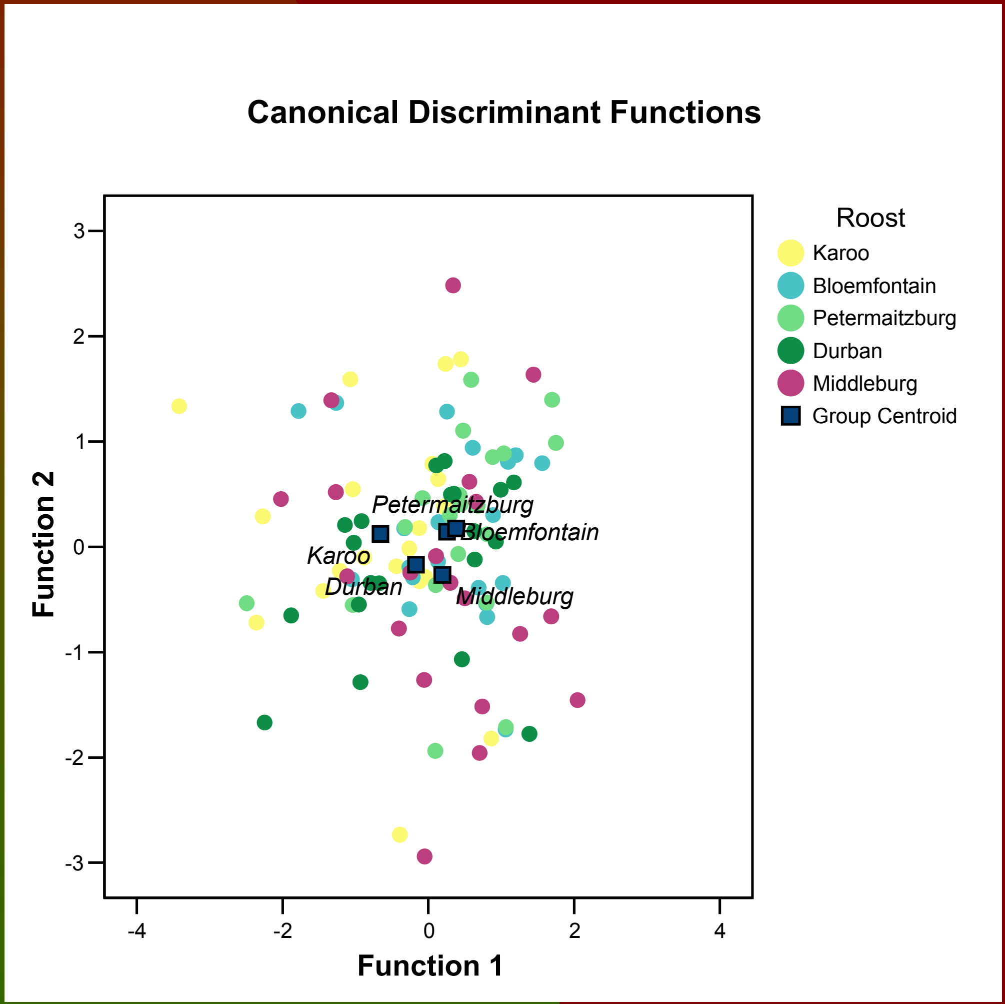


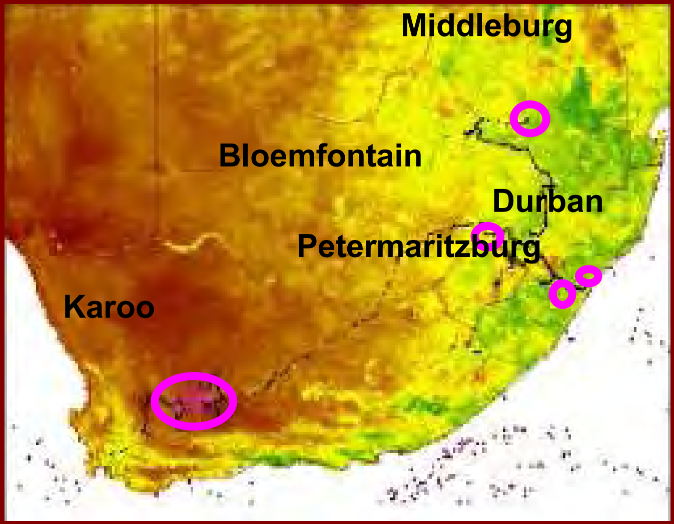
Stable isotopes

- No (P=0.11; MANOVA)
  - ◆Nor of the studied isotopes has significant difference among the roosts (P>0.1; ANOVA)



Classifying samples on the base of feathers collected at the roosts in South Africa

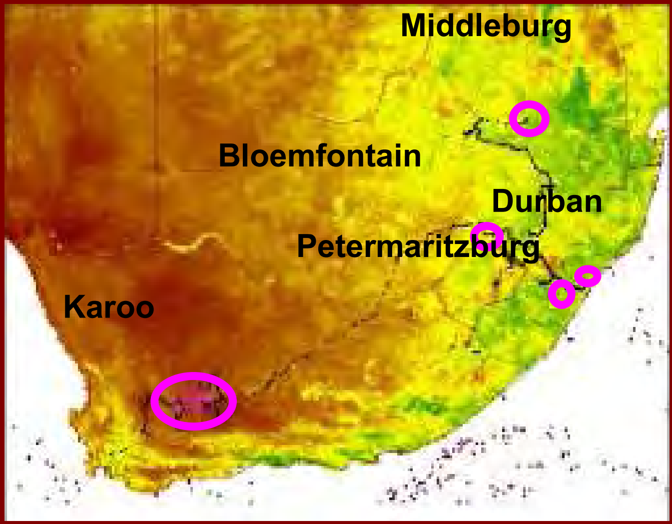




Stable isotopes

- No functions with Eigen value higher than one

Classifying samples on the base of feathers collected at the roosts in South Africa

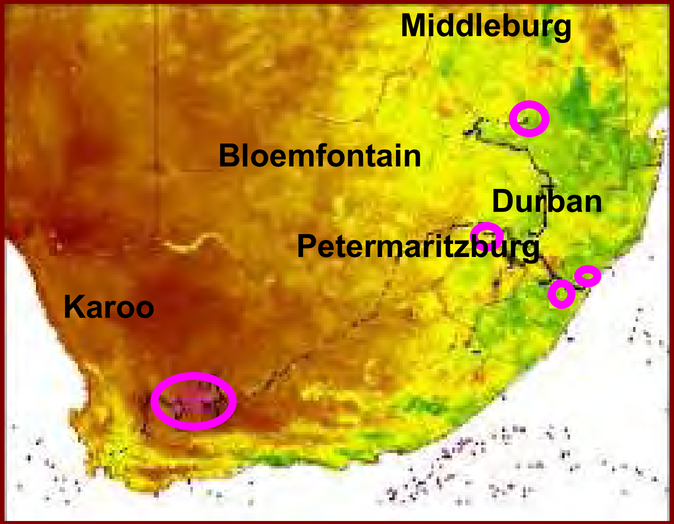


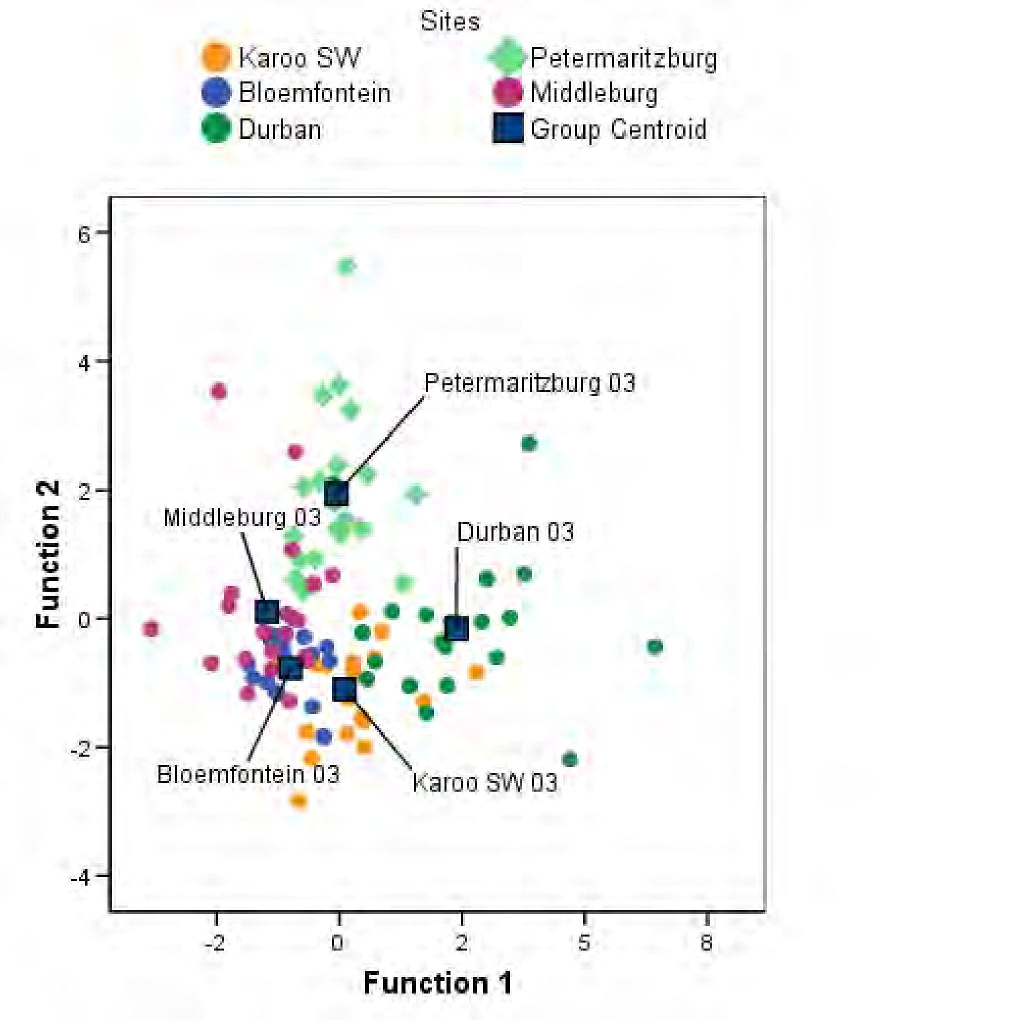
Stable isotopes

- Only 29 % of the know origin feathers were correctly classified using the cross-validation by the SPSS.
- This value is higher than one can expect it by chance (P<0.001; Press'Q).
- There was large overlap among the sites on the base of the classification.

Site	Predicted Group Membership (%)					Sample size
	Karoo SW	Bloemfontein	Durban	Pietermaritzburg	Middleburg	
Karoo SW	40.0	15.0	25.0	10.0	10.0	20
Bloemfontein	20.0	5.0	15.0	35.0	25.0	20
Durban	40.0	5.0	5.0	40.0	10.0	20
Pietermaritzburg	15.0	10.0	0.0	55.0	20.0	20
Middleburg	20.0	0.0	15.0	25.0	40.0	20

Classifying samples on the base of feathers collected at the roosts in South Africa

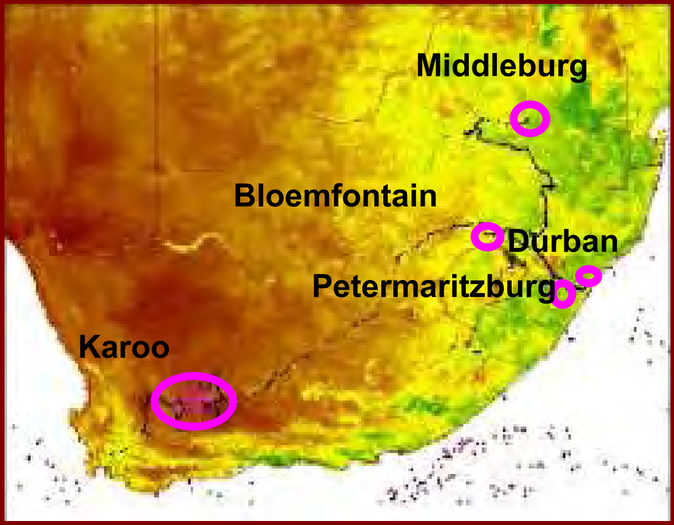




Trace elements

- Two functions with Eigen value higher then one, which are explains 81.2 % of the variance

Classifying samples on the base of feathers collected at the roosts in South Africa



Trace elements

- 60.0% of the know origin feathers were correctly classified using the cross-validation by the SPSS
- This value is significantly higher than one can expect it by chance (P<0.001; Press'Q) .

Site	Predicted Group Membership (%)					Sample size
	Karoo SW	Bloemfontein	Durban	Pietermaritzburg	Middleburg	
Karoo SW	50.0	30.0	15.0	0.0	5.0	20
Bloemfontein	15.0	75.0	5.0	0.0	5.0	20
Durban	30.0	0.0	65.0	5.0	0.0	20
Pietermaritzburg	0.0	10.0	10.0	75.0	5.0	20
Middleburg	15.0	30.0	0.0	20.0	35.0	20

Comparison feathers moulted by sand martin and barn swallow at the same roosts at Durban in South Africa

Stable isotopes

- No difference (P>0.4; t-test)

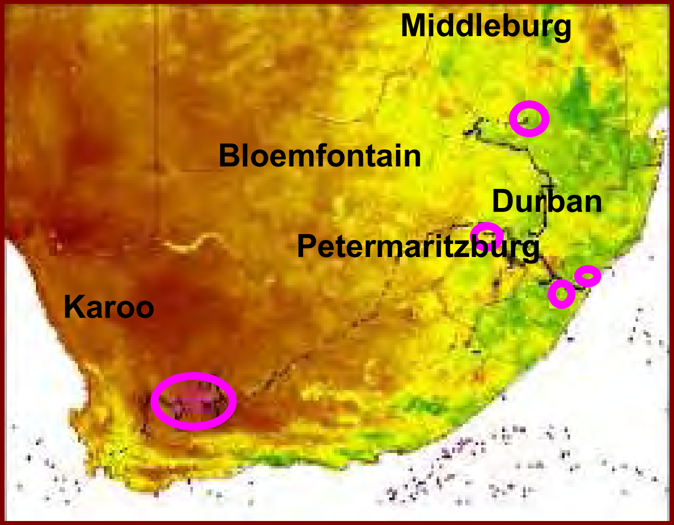
Trace elements

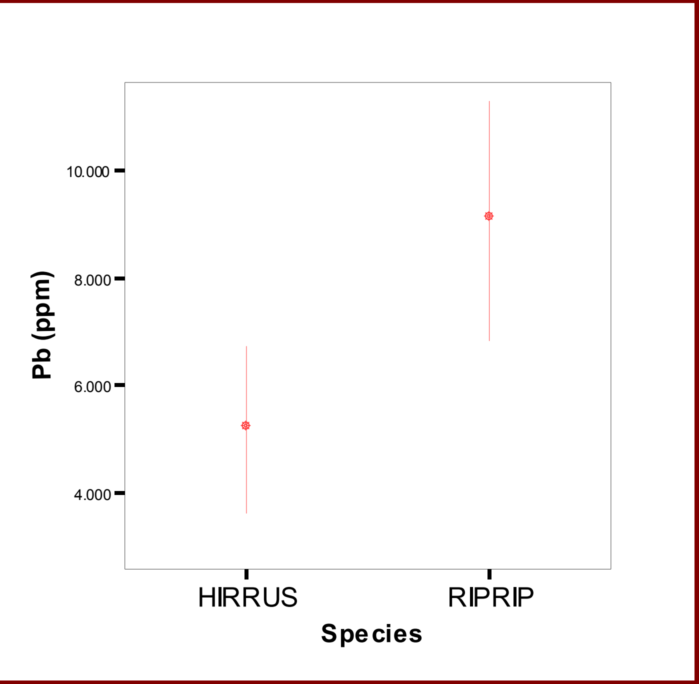
- Significant difference (P=0.017; MANOVA)

  ◆Eight elements has significantly different levels (P<0.03)

  ◆Higher concentration were in the feathers of sand martin from As, Mg, Sr, V, Pb, S

  ◆Concentration were higher in the feathers of barn swallow for the Ca and Zn





Conclusions

- Trace elements has high spatial resolution in both the breeding and both the wintering areas
  - Spatial resolution below the regional level (<50 km)
  - Best applied to those species that breed in colonies or winter in roosts in a limited number of sites that can be catalogued by elemental profile
  - Opportunity for individual level of investigation of the usage of same wintering/migration areas

- Stable isotopes has high spatial resolution in the breeding area but not in the wintering area
  - Needs of considering year effects in the classification
  - Spatial resolution varying largely (between 4 km – over 1000 km)
  - High habitat sensitivity

- Spatial interpretation of trace element and stable isotope data needs more attention

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