Using DNA technology to monitor the movement of wildlife without invasive tracking and tagging systems

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Animal movements – a cental issue in animal ecology

- Resource utilization
- Territorial defense
- Dispersal and migration - connectivity and gene flow

- How can these issues be adressed from non-invasive genetic sampling?

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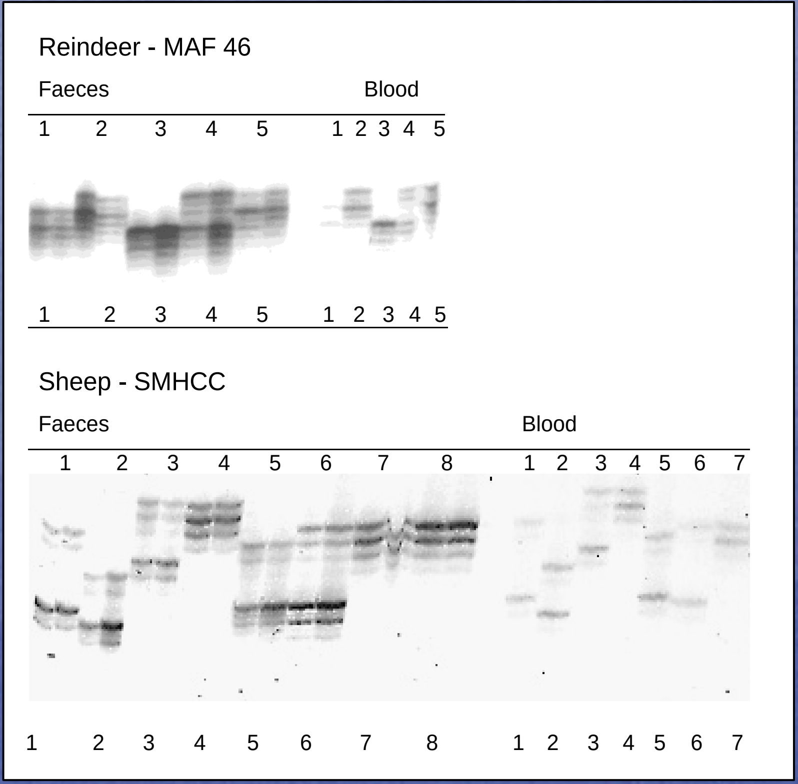
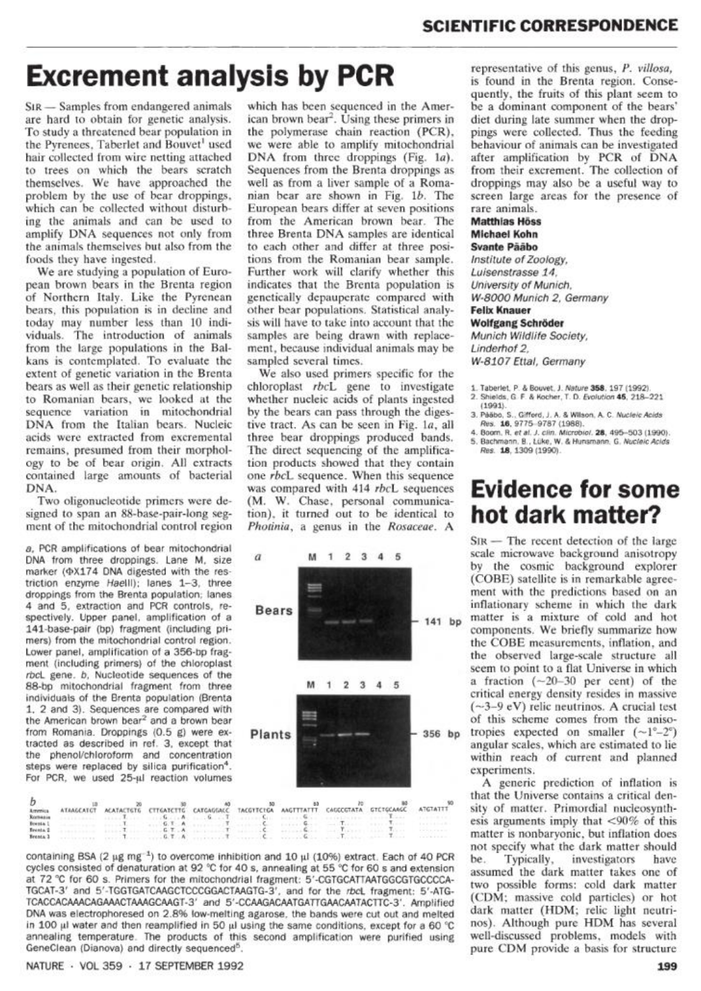
1995 – MSc studies on Swayne's hartebeest – pioneering methodological work





Excrement analysis by PCR

SIR — Samples from endangered animals are hard to obtain for genetic analysis. To study a threatened bear population in the Pyrenees, Taberlet and Bouvet[1] used hair collected from wire netting attached to trees on which the bears scratch themselves. We have approached the problem by the use of bear droppings, which can be collected without disturbing the animals and can be used to amplify DNA sequences not only from the animals themselves but also from the foods they have ingested.

We are studying a population of European brown bears in the Brenta region of Northern Italy. Like the Pyrenean bears, this population is in decline and today may number less than 10 individuals. The introduction of animals from the large populations in the Balkans is contemplated. To evaluate the extent of genetic variation in the Brenta bears as well as their genetic relationship to Romanian bears, we looked at the sequence variation in mitochondrial DNA from the Italian bears. Nucleic acids were extracted from excremental remains, presumed from their morphology to be of bear origin. All extracts contained large amounts of bacterial DNA.

Two oligonucleotide primers were designed to span an 88-base-pair-long segment of the mitochondrial control region which has been sequenced in the American brown bear[2]. Using these primers in the polymerase chain reaction (PCR), we were able to amplify mitochondrial DNA from three droppings (Fig. 1*a*). Sequences from the Brenta droppings as well as from a liver sample of a Romanian bear are shown in Fig. 1*b*. The European bears differ at seven positions from the American brown bear. The three Brenta DNA samples are identical to each other and differ at three positions from the Romanian bear sample. Further work will clarify whether this indicates that the Brenta population is genetically depauperate compared with other bear populations. Statistical analysis will have to take into account that the samples are being drawn with replacement, because individual animals may be sampled several times.

We also used primers specific for the chloroplast *rbc*L gene to investigate whether nucleic acids of plants ingested by the bears can pass through the digestive tract. As can be seen in Fig. 1*a*, all three bear droppings produced bands. The direct sequencing of the amplification products showed that they contain one *rbc*L sequence. When this sequence was compared with 414 *rbc*L sequences (M. W. Chase, personal communication), it turned out to be identical to *Photinia*, a genus in the *Rosaceae*. A representative of this genus, *P. villosa*, is found in the Brenta region. Consequently, the fruits of this plant seem to be a dominant component of the bears' diet during late summer when the droppings were collected. Thus the feeding behaviour of animals can be investigated after amplification by PCR of DNA from their excrement. The collection of droppings may also be a useful way to screen large areas for the presence of rare animals.

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1. Taberlet, P. & Bouvet, J. *Nature* **358**, 197 (1992).
2. Shields, G. F. & Kocher, T. D. *Evolution* **45**, 218–221 (1991).
3. Pääbo, S., Gifford, J. A. & Wilson, A. C. *Nucleic Acids Res.* **16**, 9775–9787 (1988).
4. Boom, R. *et al. J. clin. Microbiol.* **28**, 495–503 (1990).
5. Bachmann, B., Lüke, W. & Hunsmann, G. *Nucleic Acids Res.* **18**, 1309 (1990).

a, PCR amplifications of bear mitochondrial DNA from three droppings. Lane M, size marker (ΦX174 DNA digested with the restriction enzyme *Hae*III); lanes 1–3, three droppings from the Brenta population; lanes 4 and 5, extraction and PCR controls, respectively. Upper panel, amplification of a 141-base-pair (bp) fragment (including primers) from the mitochondrial control region. Lower panel, amplification of a 356-bp fragment (including primers) of the chloroplast *rbc*L gene. *b*, Nucleotide sequences of the 88-bp mitochondrial fragment from three individuals of the Brenta population (Brenta 1, 2 and 3). Sequences are compared with the American brown bear[2] and a brown bear from Romania. Droppings (0.5 g) were extracted as described in ref. 3, except that the phenol/chloroform and concentration steps were replaced by silica purification[4]. For PCR, we used 25-μl reaction volumes containing BSA (2 μg mg^{-1}) to overcome inhibition and 10 μl (10%) extract. Each of 40 PCR cycles consisted of denaturation at 92 °C for 40 s, annealing at 55 °C for 60 s and extension at 72 °C for 60 s. Primers for the mitochondrial fragment: 5′-CGTGCATTAATGGCGTGCCCCATGCAT-3′ and 5′-TGGTGATCAAGCTCCCGGACTAAGTG-3′, and for the *rbc*L fragment: 5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′ and 5′-CCAAGACAATGATTGAACAATACTTC-3′. Amplified DNA was electrophoresed on 2.8% low-melting agarose, the bands were cut out and melted in 100 μl water and then reamplified in 50 μl using the same conditions, except for a 60 °C annealing temperature. The products of this second amplification were purified using GeneClean (Dianova) and directly sequenced[5].

Evidence for some hot dark matter?

SIR — The recent detection of the large scale microwave background anisotropy by the cosmic background explorer (COBE) satellite is in remarkable agreement with the predictions based on an inflationary scheme in which the dark matter is a mixture of cold and hot components. We briefly summarize how the COBE measurements, inflation, and the observed large-scale structure all seem to point to a flat Universe in which a fraction (~20–30 per cent) of the critical energy density resides in massive (~3–9 eV) relic neutrinos. A crucial test of this scheme comes from the anisotropies expected on smaller (~1°–2°) angular scales, which are estimated to lie within reach of current and planned experiments.

A generic prediction of inflation is that the Universe contains a critical density of matter. Primordial nucleosynthesis arguments imply that <90% of this matter is nonbaryonic, but inflation does not specify what the dark matter should be. Typically, investigators have assumed the dark matter takes one of two possible forms: cold dark matter (CDM; massive cold particles) or hot dark matter (HDM; relic light neutrinos). Although pure HDM has several well-discussed problems, models with pure CDM provide a basis for structure

PhD studies in Uppsala 1999-2002

Development of large-scale DNA-based monitoring of Scandinavian carnivores

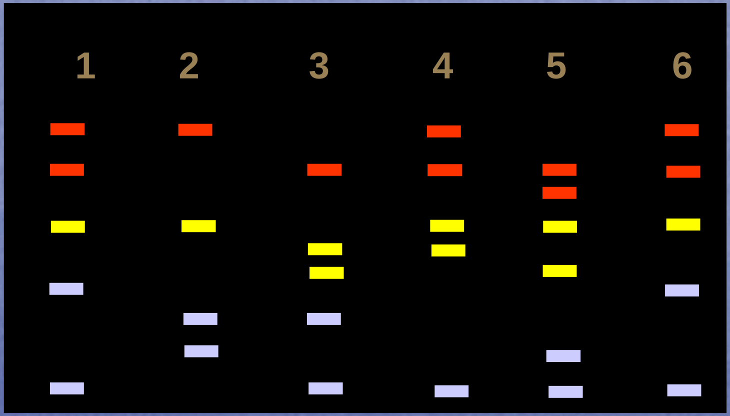
(1) Sample collection in the field - scats, hair, urine, blood remains

(2) Laboratory analyses

(3) DNA profiles

DNA-based monitoring Scandinavian carnivores

DNA-profiles from the scat samples provide unique ID-codes (1=6), that can be traced back to certain individuals in the target population(s)



Yearly sample collection allow us to follow the same individuals during a period of several years

mapping⇒

- home range and territories
- reproduction
- dispersal and migration patterns
- population size and - dynamics
- genetic structure; levels of isolation

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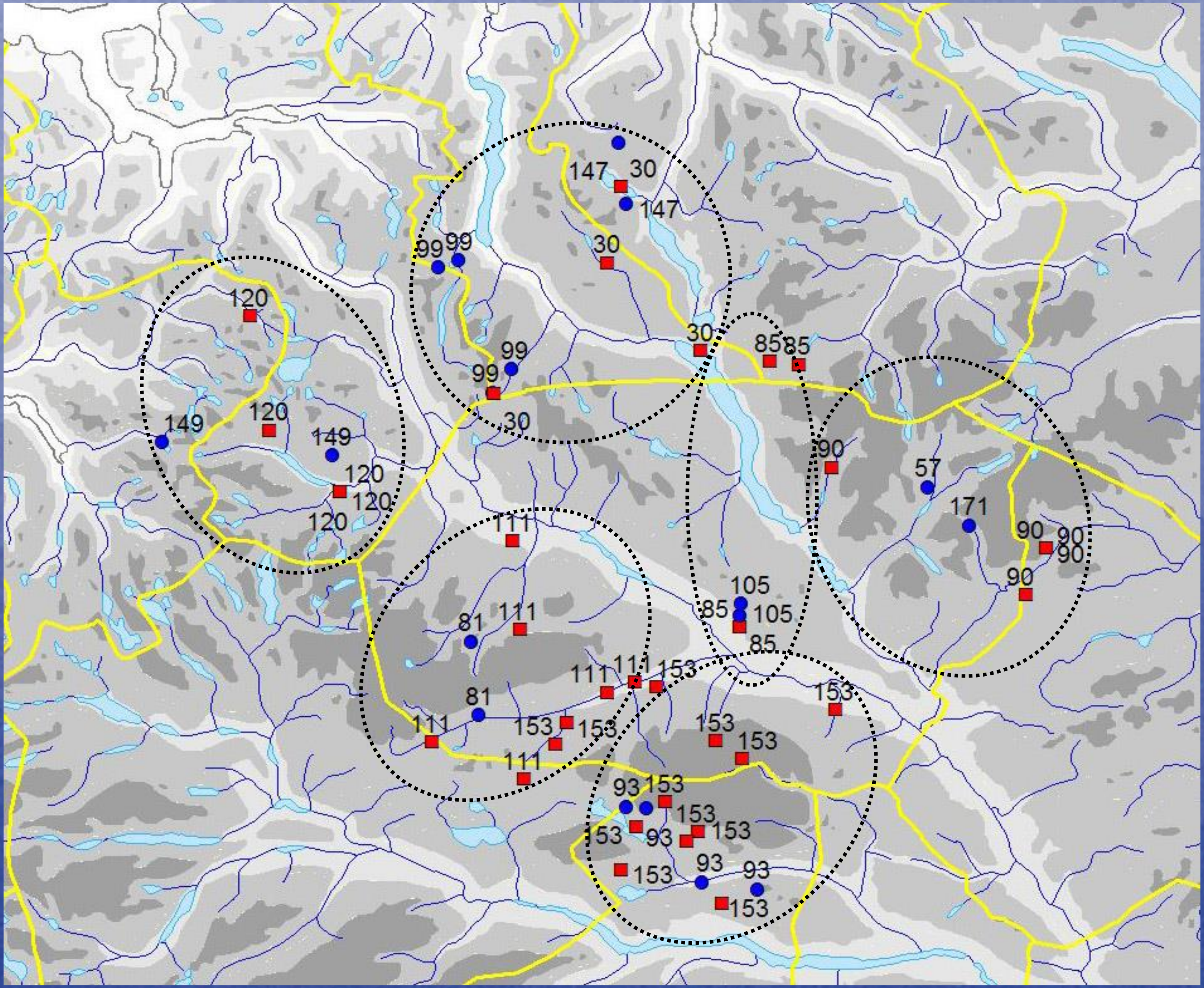
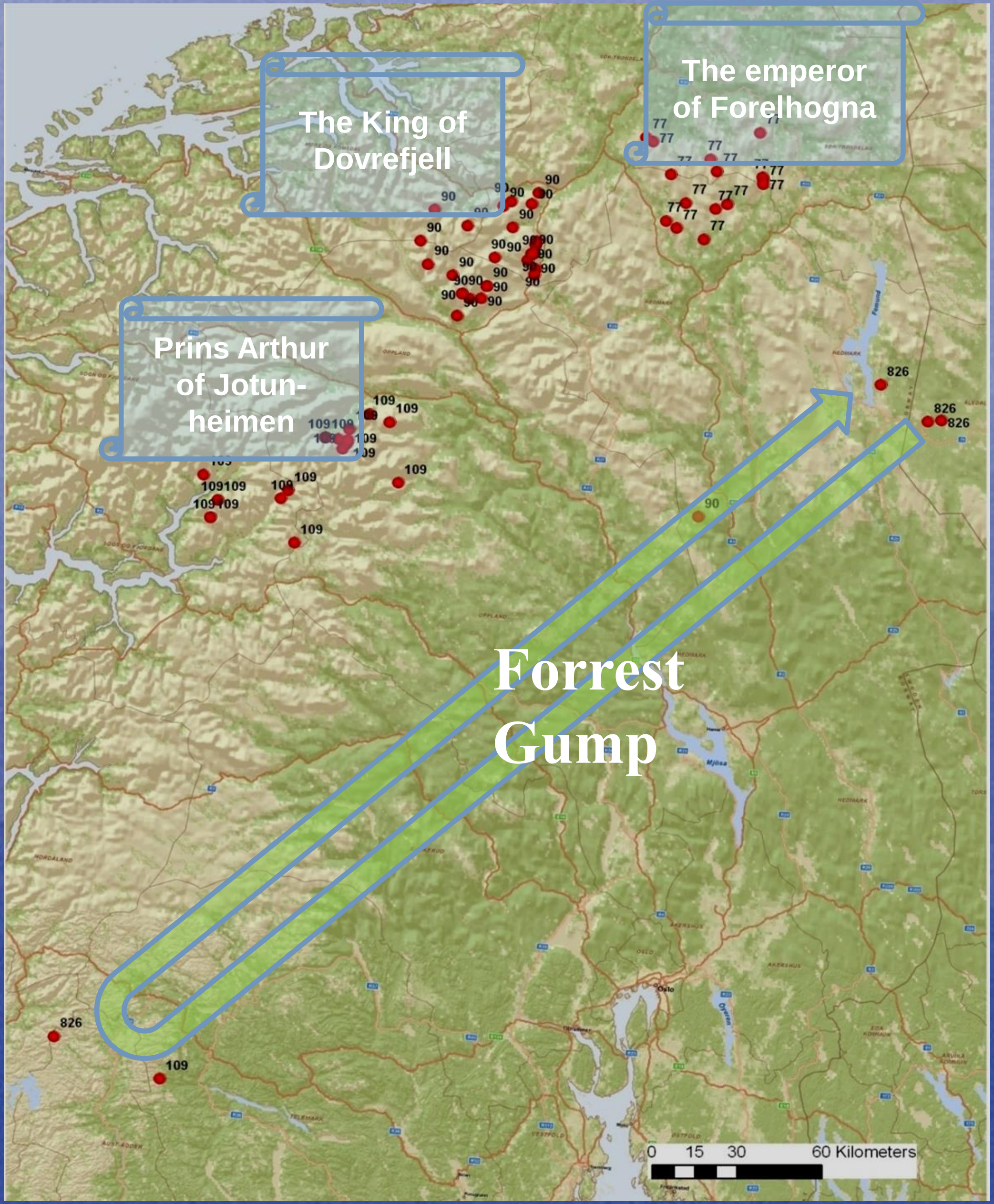
Mapping territorial wolverines



Home ranges and movements



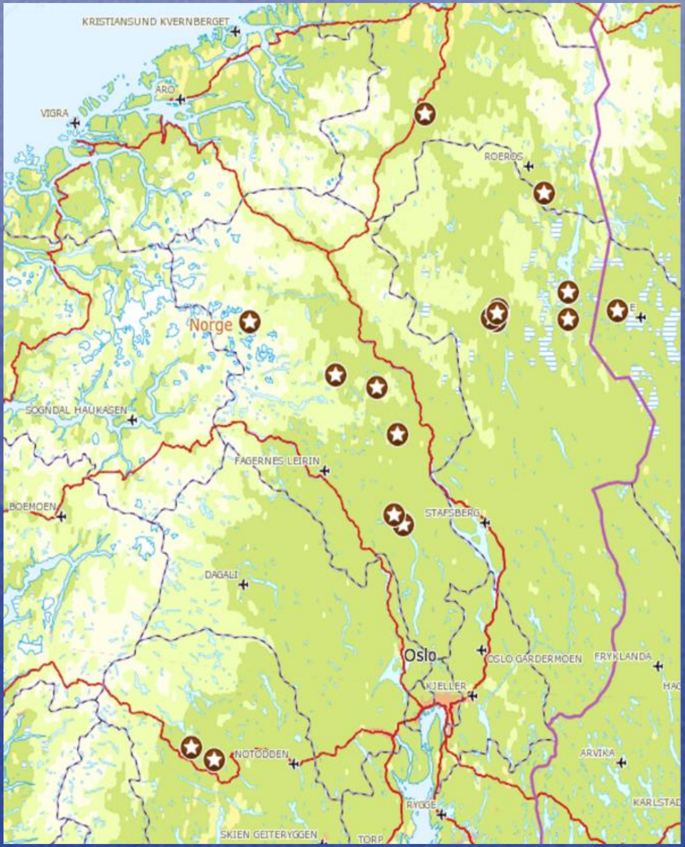
The King of Dovrefjell

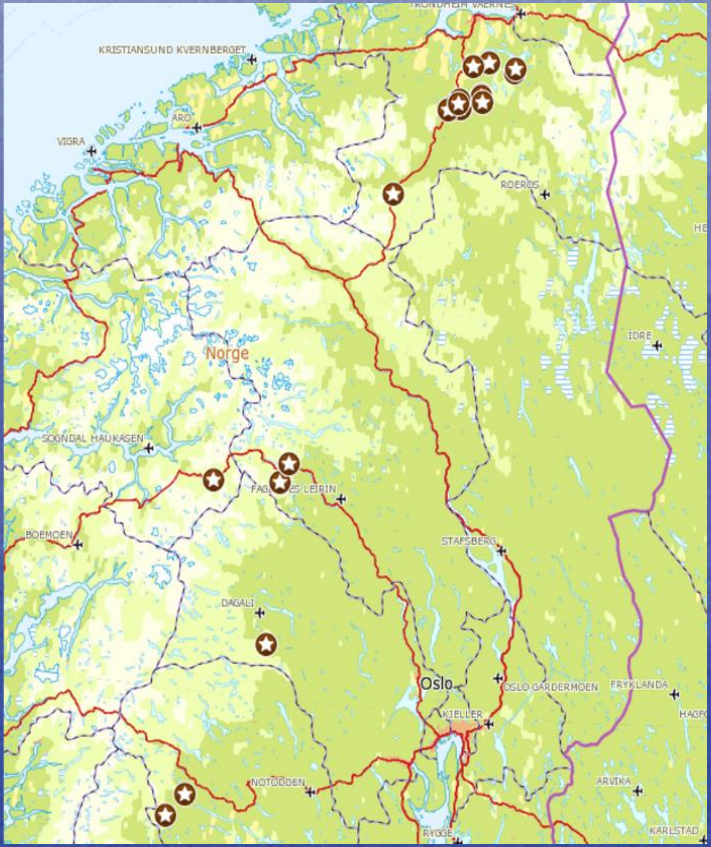
The emperor of Forelhogna

Prins Arthur of Jotun-heimen

Forrest Gump

The brown bear – males travel over vast areas





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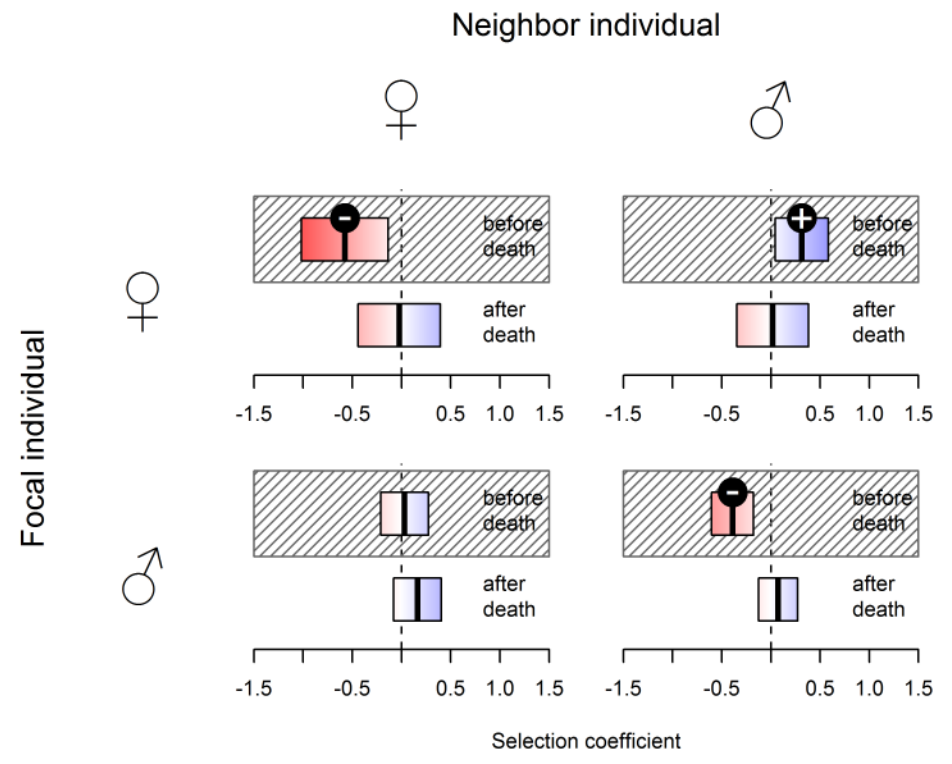
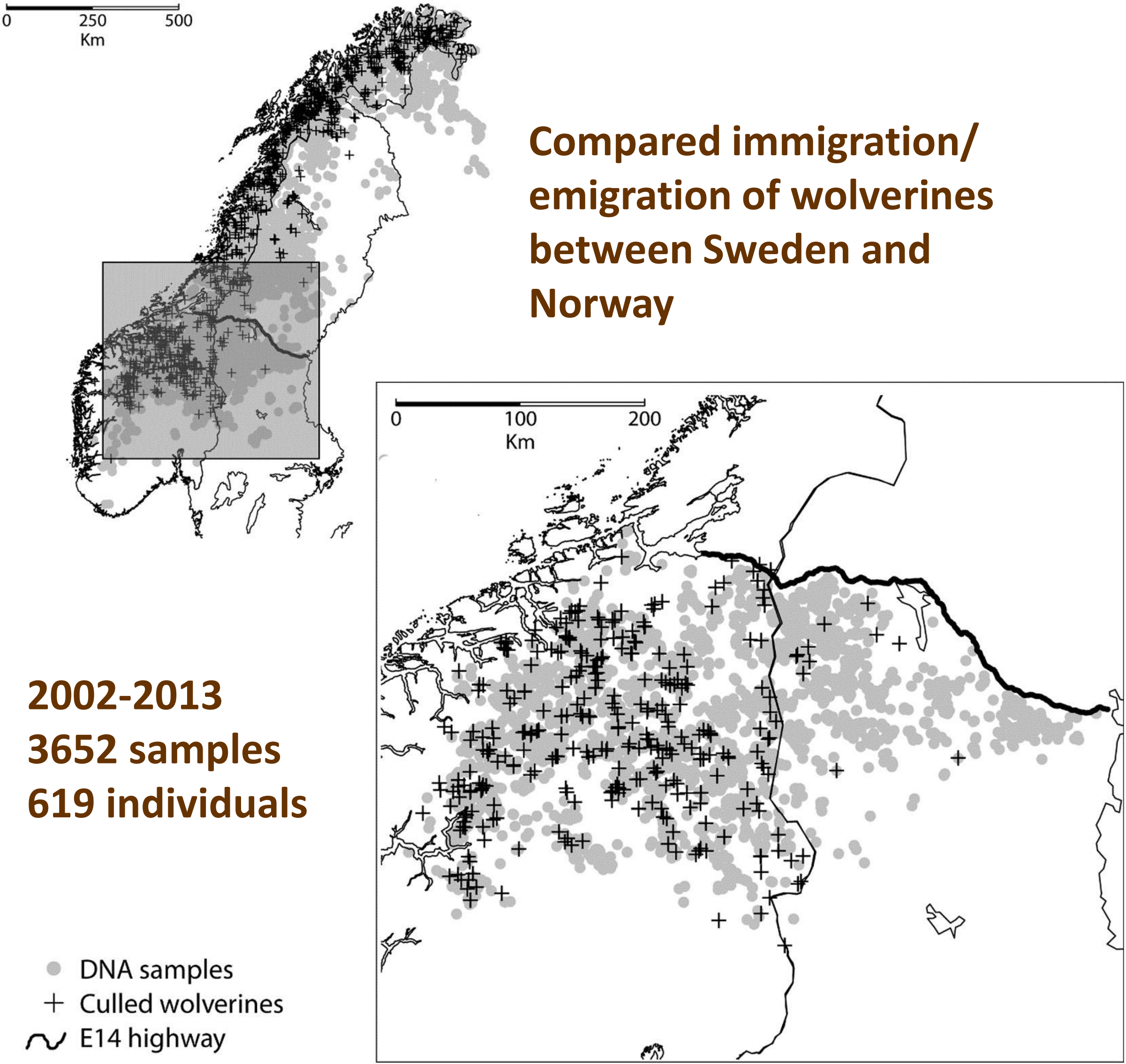
Is it possible to address territorial behaviour from scat samples?



Bischof et al 2016 Ecology and Evolution 6, 1527-1536

Patterns of emigration/immigration in a managed population

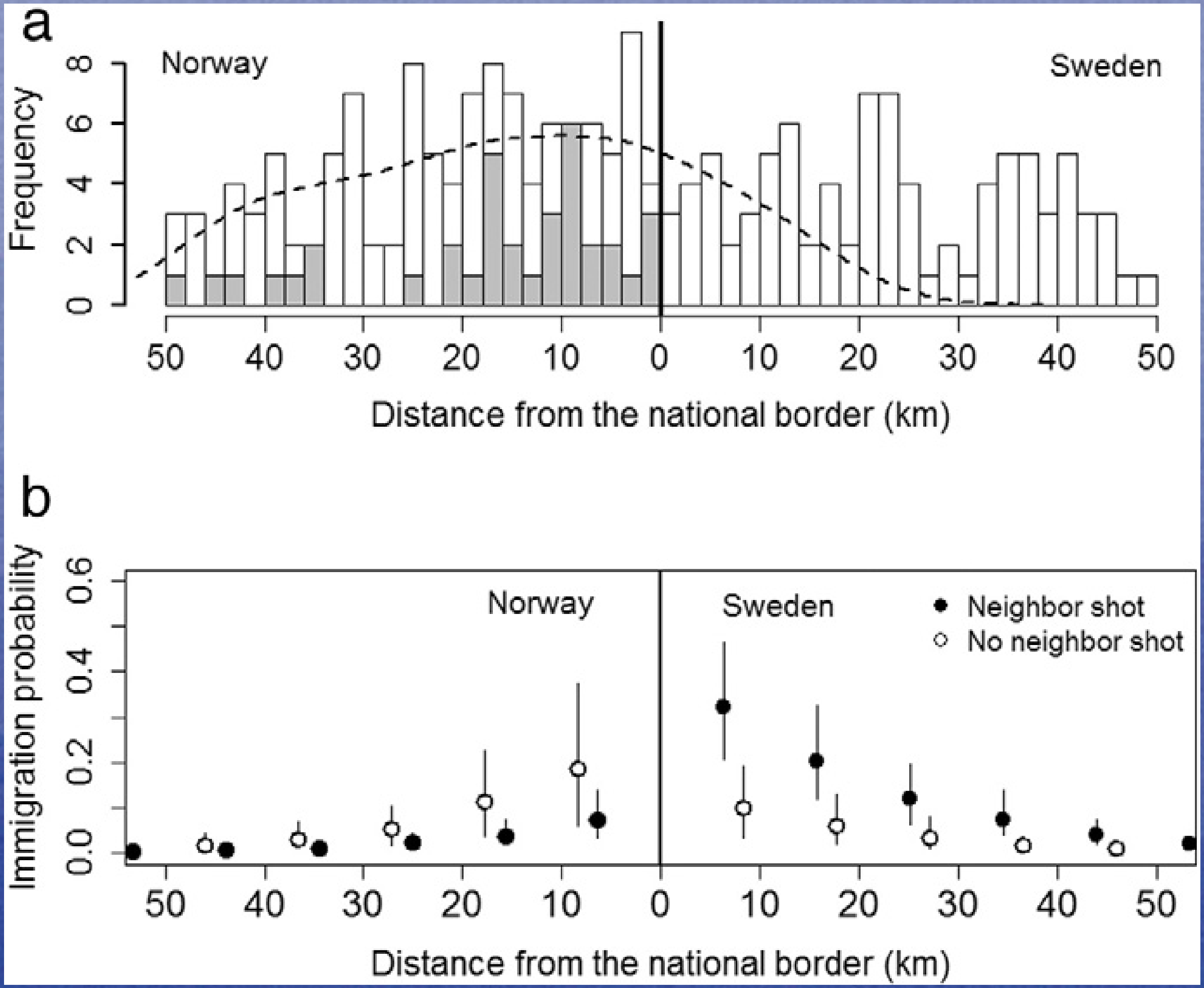


Compared immigration/ emigration of wolverines between Sweden and Norway

2002-2013
3652 samples
619 individuals

Gervasi et al. 2015 (*Biological Conservation* 191, 632-639)

Compensatory immigration



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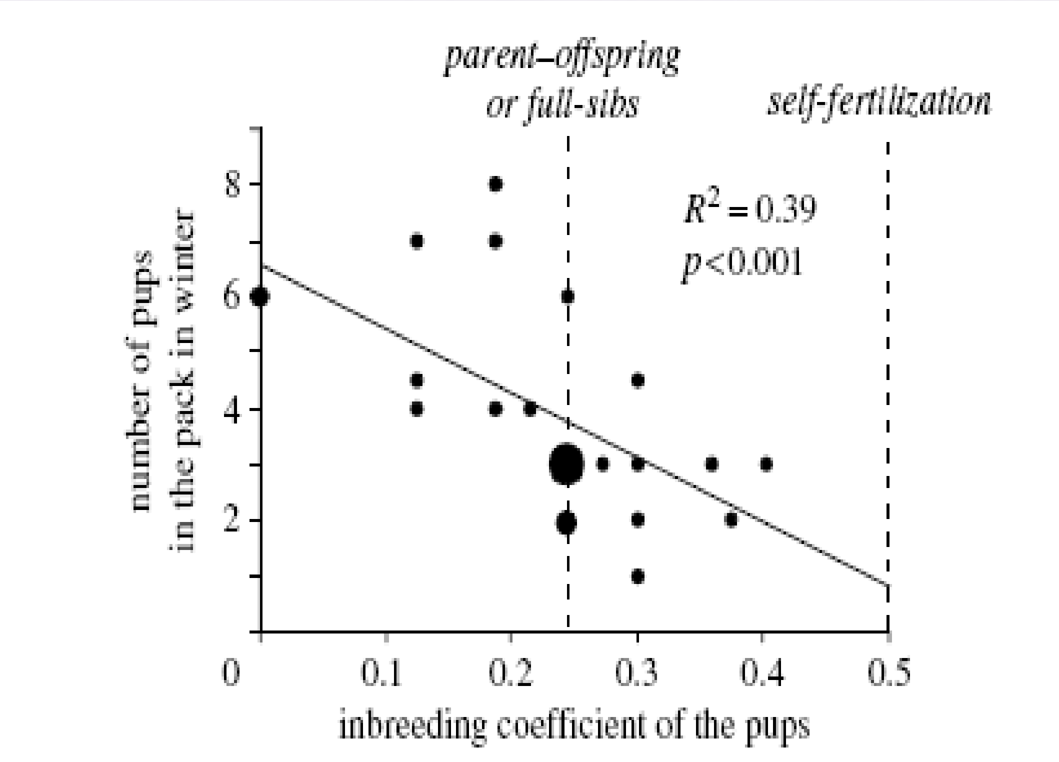
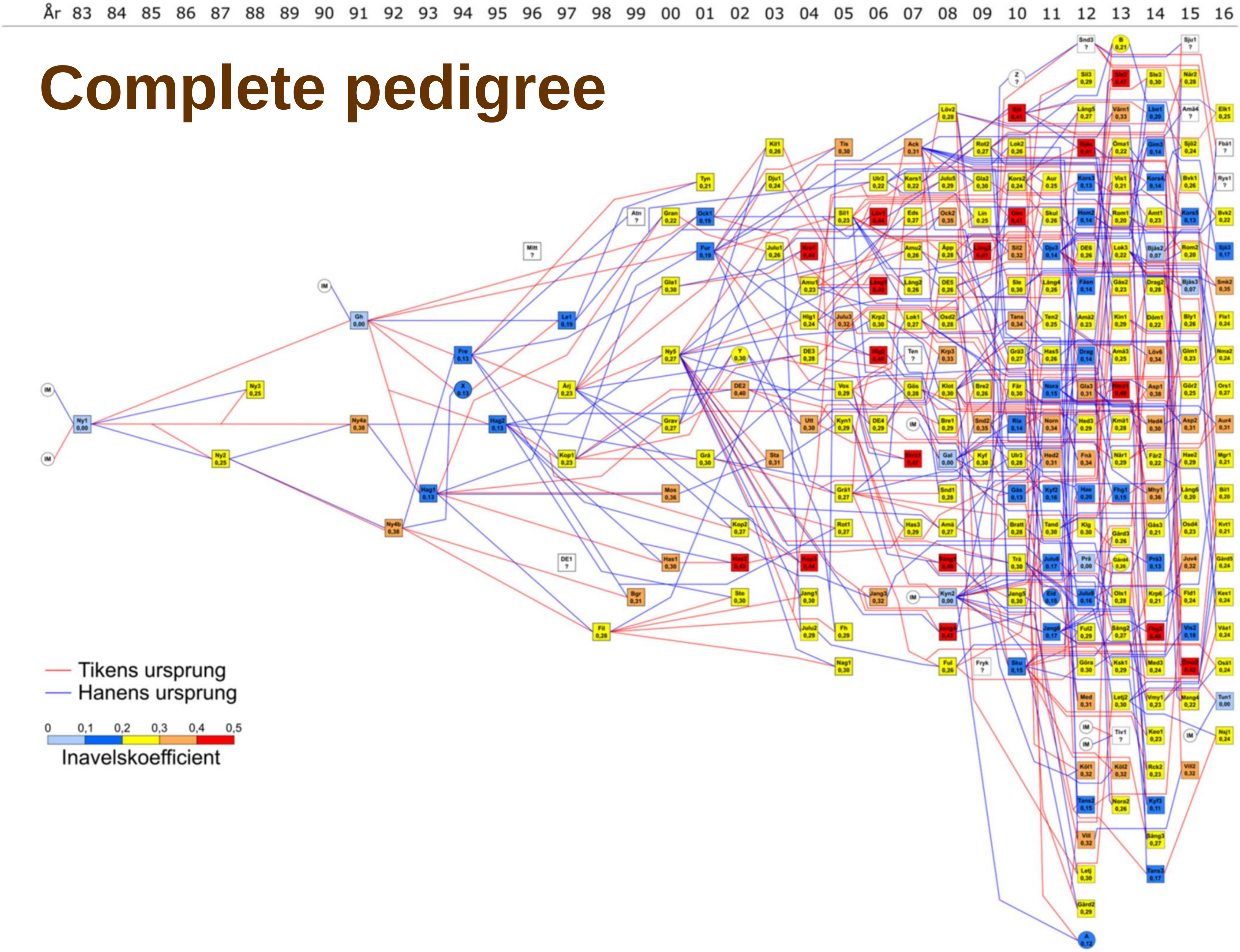
Inbreeding depression in the Scandinavian wolf popultion

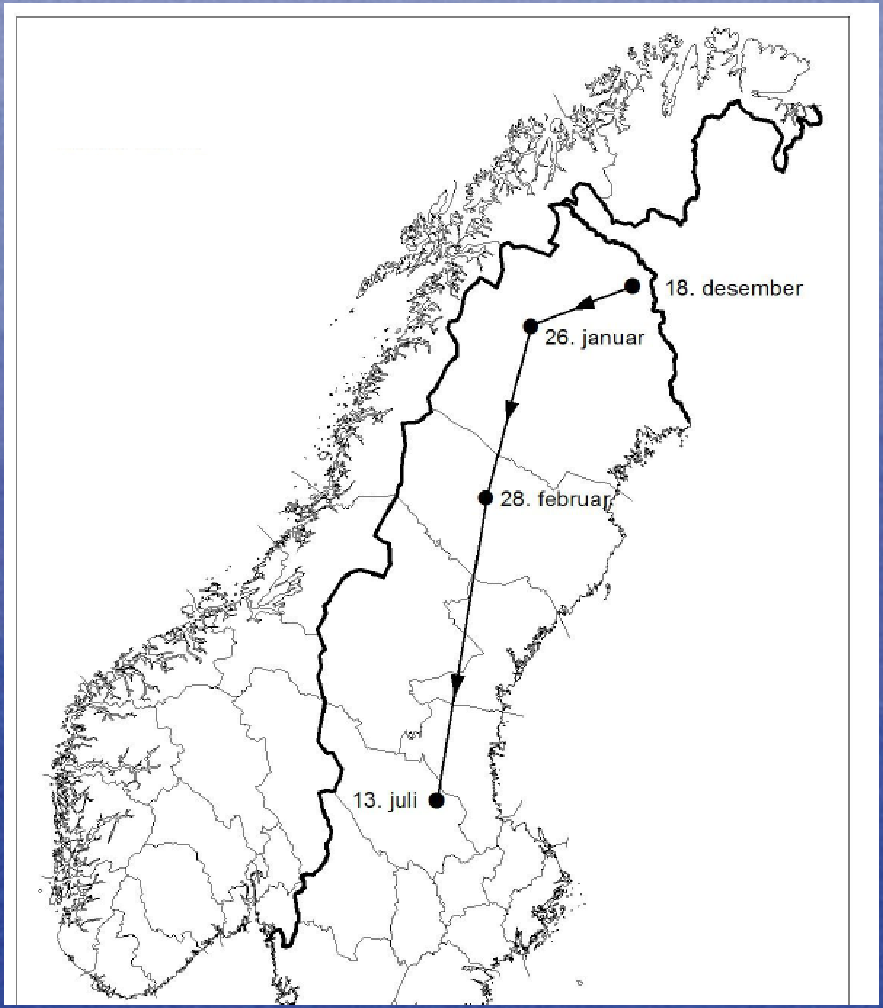


Liberg et al 2005 Biology Letters 1, 17-20

Complete pedigree



Detection of new immigrants and other genetic important individuals is highly important in the monitoring of the population



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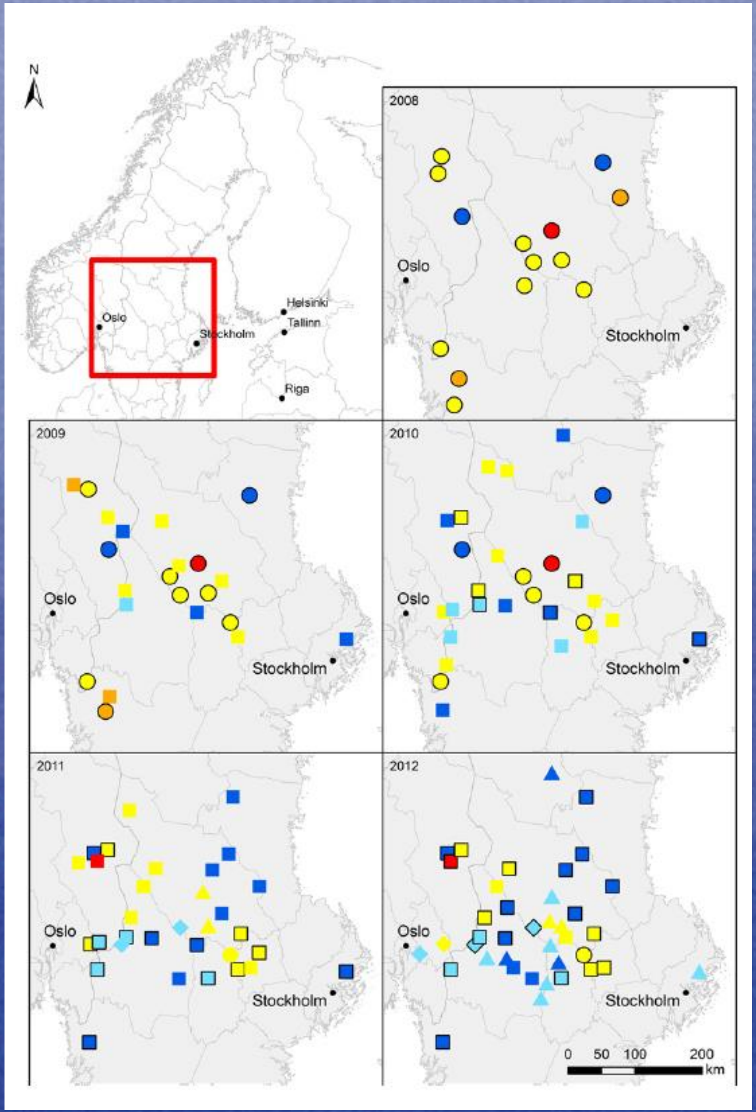
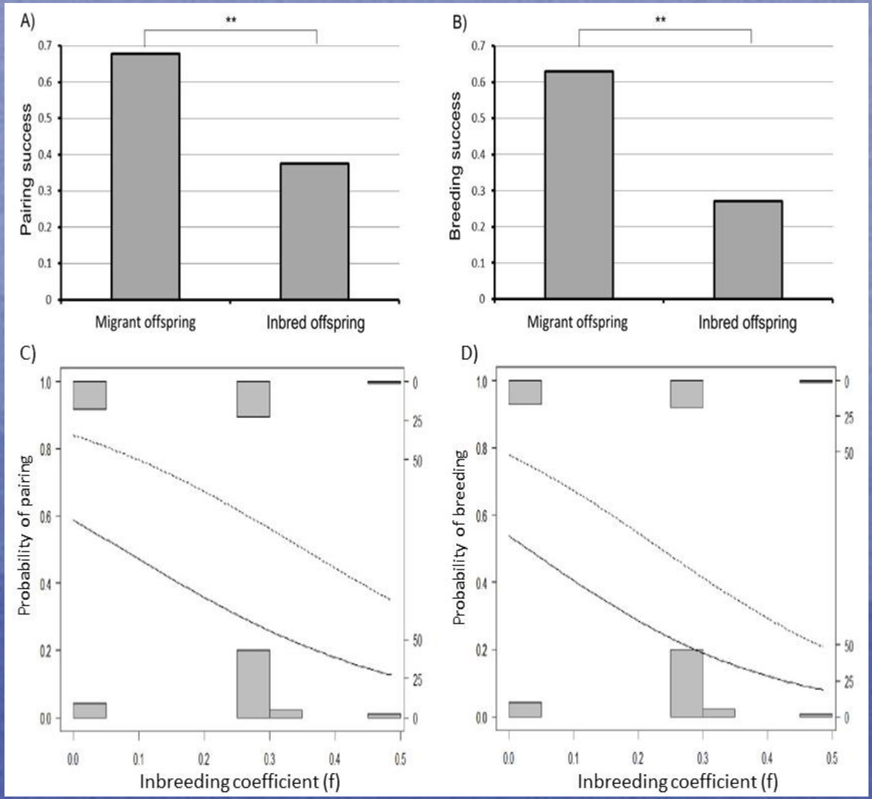
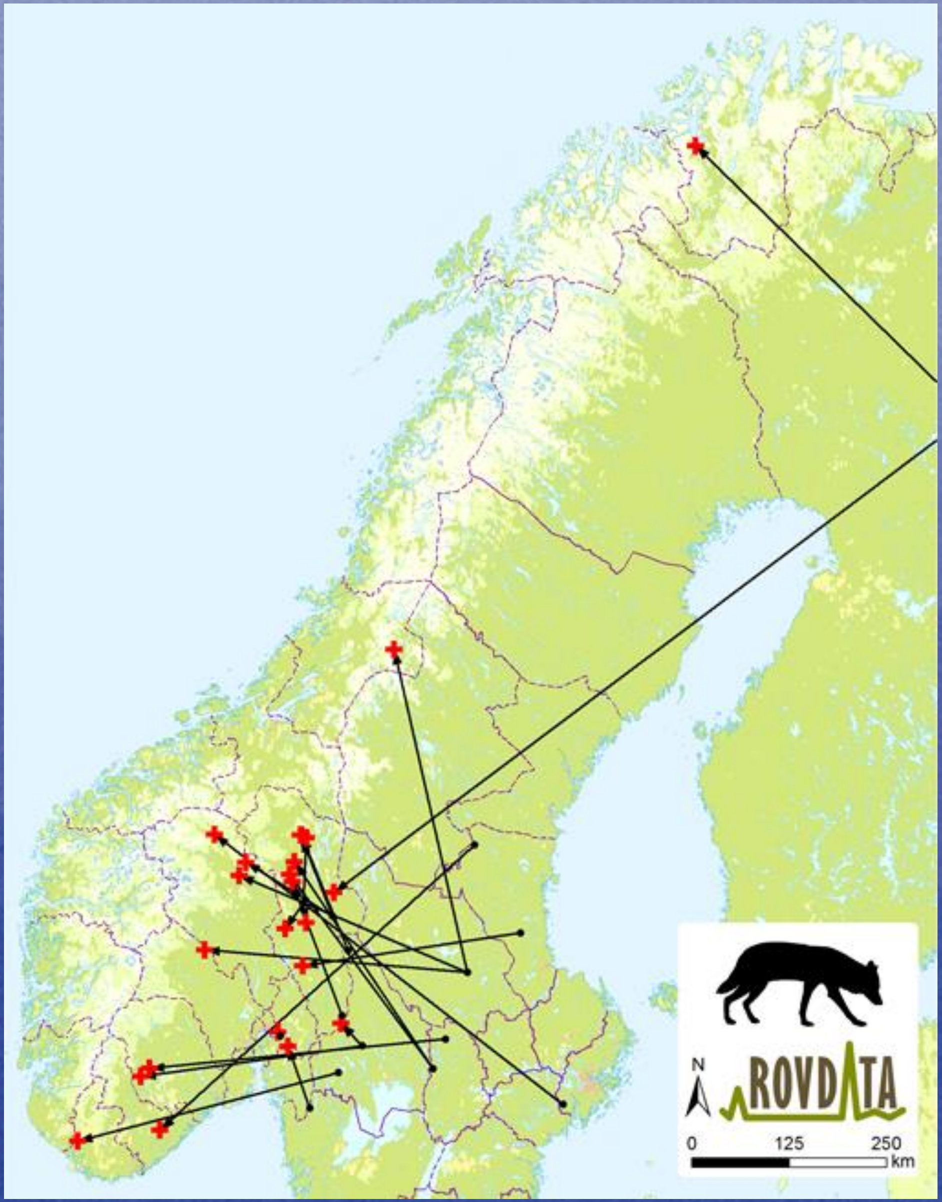
Monitoring the reproductive success of offspring to the immigrants





Åkesson et al. 2016 Mol Ecol 25, 4745-4756

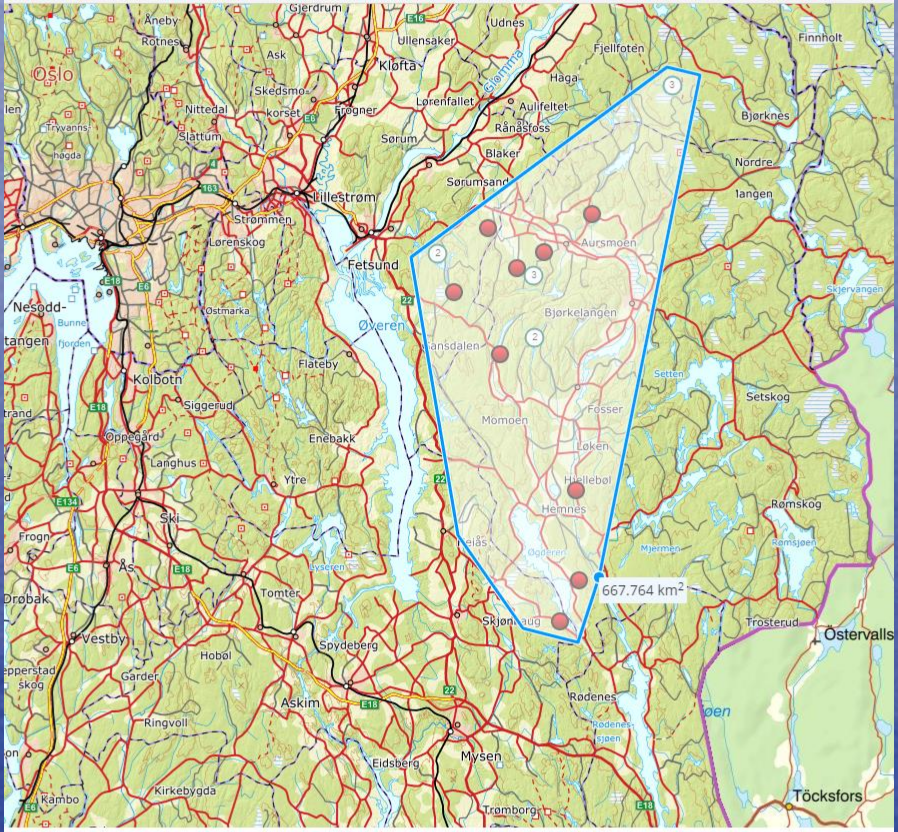
Dispersal and migration patterns



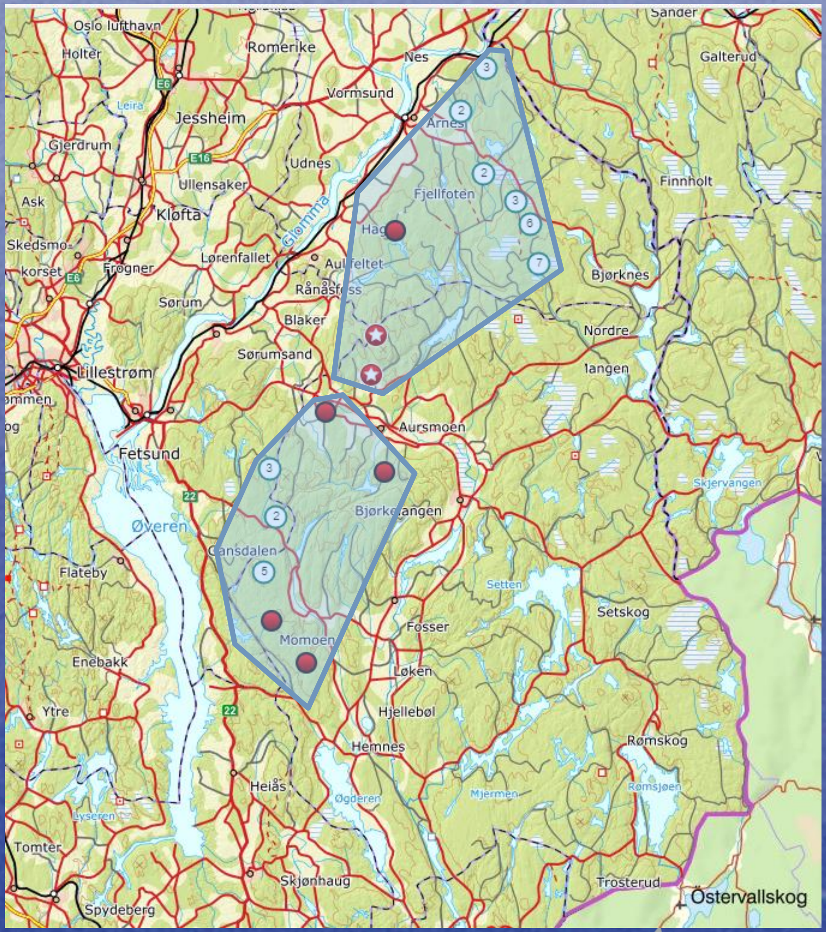
- Scandinavian wolves tend to disperse towards the center of the population
- Move further on if they do not find a partner and/or vacant territory

Territory dynamics

Aurskog winter 19-20



Aurskog and Svarthus winter 20-21



Metapopulation dynamics in the Scandinavian arctic fox population

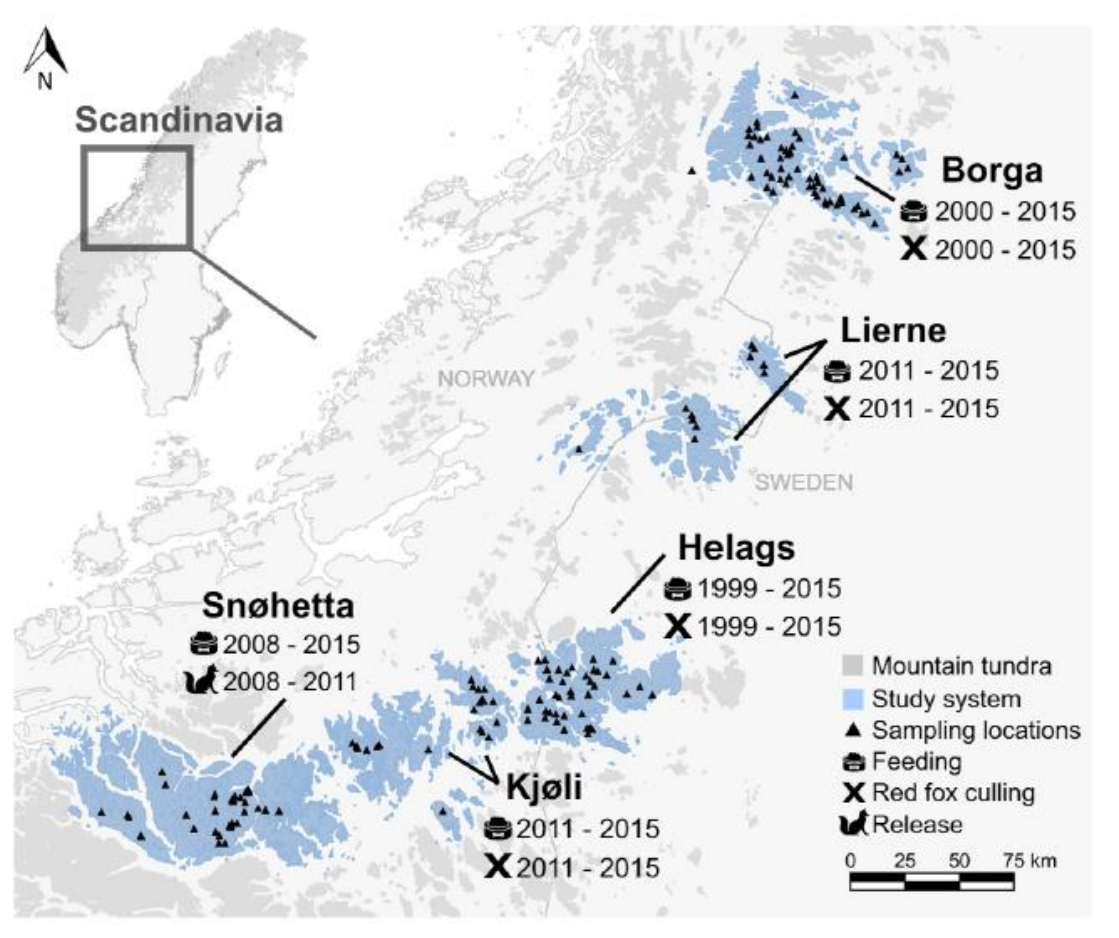



Photo: SNO

- Study area: 65,222 km^2
- 2,667 faeces and hair samples collected in the field (2008-2015)
- 290 tissue samples from Sweden from marked pups

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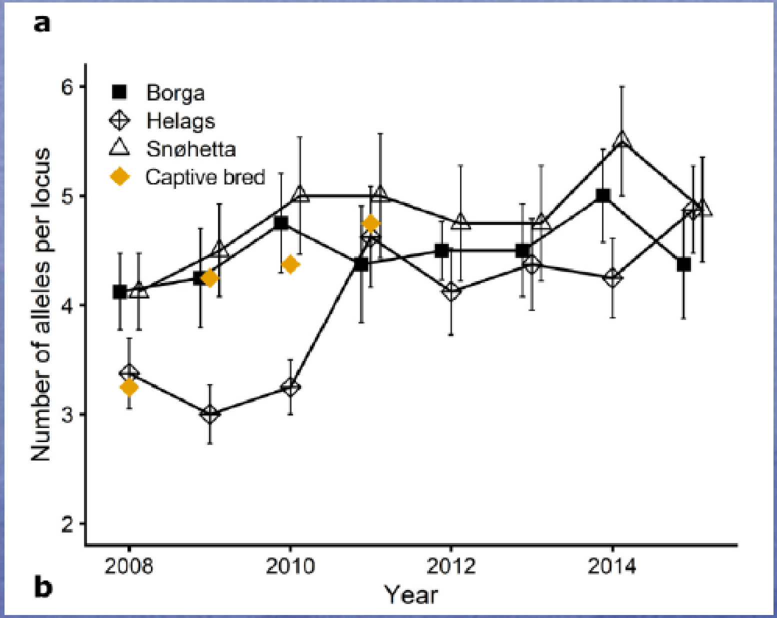
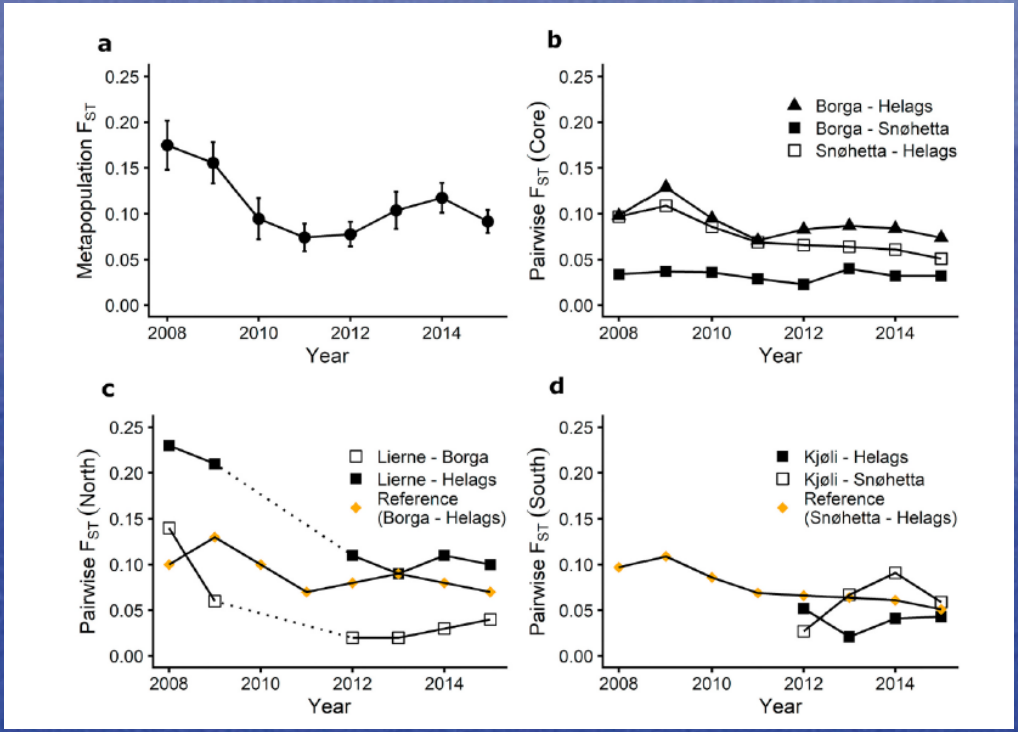
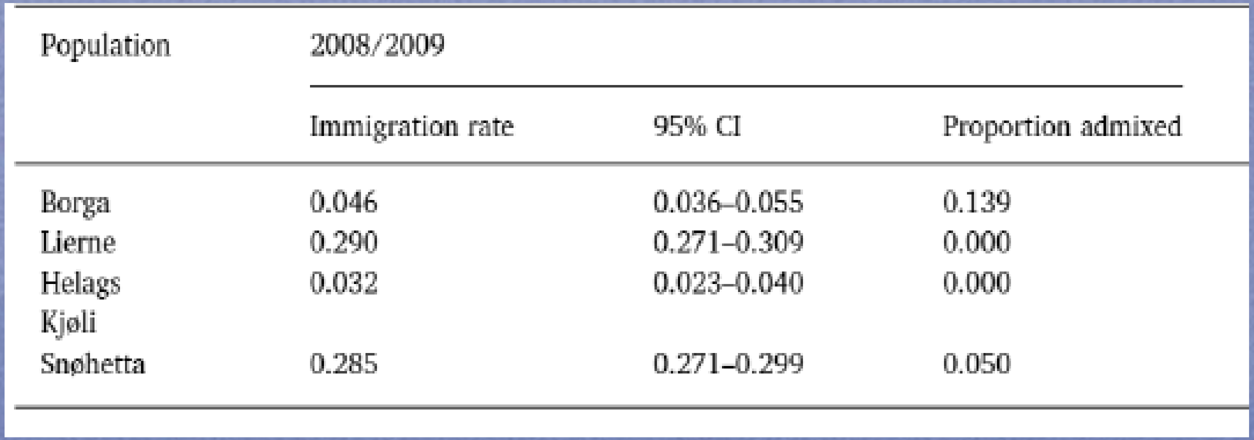
Patterns of genetic variation

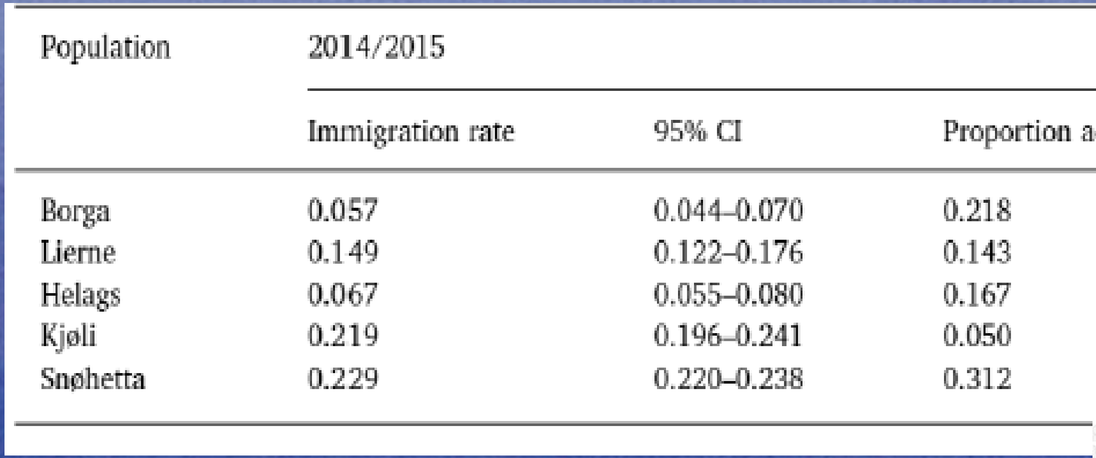




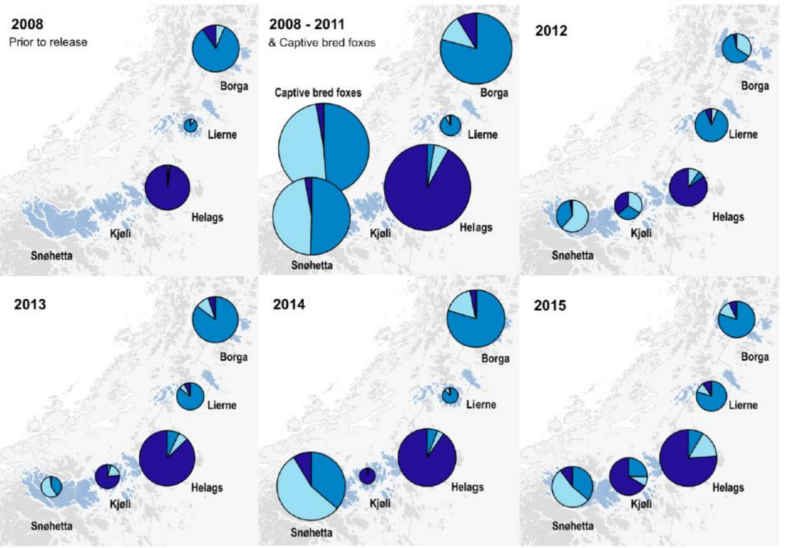
Genetic structure and dispersal patterns



Population	2008/2009		
	Immigration rate	95% CI	Proportion admixed
Borga	0.046	0.036–0.055	0.139
Lierne	0.290	0.271–0.309	0.000
Helags	0.032	0.023–0.040	0.000
Kjøli			
Snøhetta	0.285	0.271–0.299	0.050



Population	2014/2015		
	Immigration rate	95% CI	Proportion admixed
Borga	0.057	0.044–0.070	0.218
Lierne	0.149	0.122–0.176	0.143
Helags	0.067	0.055–0.080	0.167
Kjøli	0.219	0.196–0.241	0.050
Snøhetta	0.229	0.220–0.238	0.312



International projects

Chetri, M., M. Odden, K. Sharma, O. Flagstad, and P. Wegge. 2019. 'Estimating snow leopard density using fecal DNA in a large landscape in north-central Nepal', *Global Ecology and Conservation*, 17.

Flagstad, O., N. M. B. Pradhan, L. G. Kvernstuen, and P. Wegge. 2012. 'Conserving small and fragmented populations of large mammals: Non-invasive genetic sampling in an isolated population of Asian elephants in Nepal', *Journal for Nature Conservation*, 20: 181-90.

Flagstad, O., P. O. Syvertsen, N. C. Stenseth, J. E. Stacy, I. Olsaker, K. H. Roed, and K. S. Jakobsen. 2000. 'Genetic variability in Swayne's hartebeest, an endangered antelope of Ethiopia', *Conservation Biology*, 14: 254-64.

Wegge, P., R. Shrestha, and O. Flagstad. 2012. 'Snow leopard Panthera uncia predation on livestock and wild prey in a mountain valley in northern Nepal: implications for conservation management', *Wildlife Biology*, 18: 131-41.

Samarbeid og kunnskap for framtidas miljøløsninger