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### RADseq suggests long-term persistence in Scania, southern Sweden

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# Tracing the origin of *Betonica officinalis*: RADseq suggests long-term persistence in Scania, southern Sweden

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## BACKGROUND & AIMS

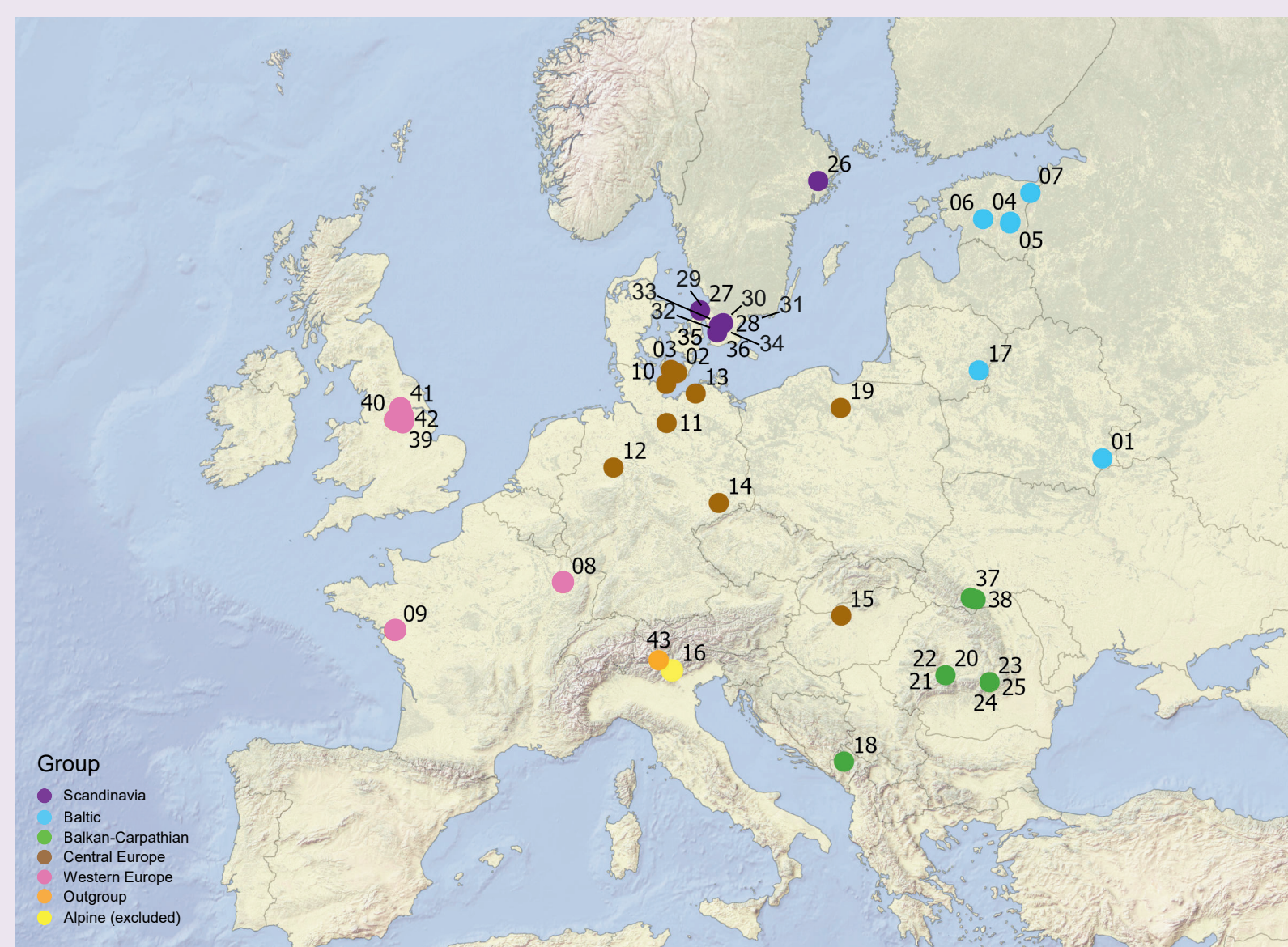
The **origin** of *Betonica officinalis* (Betony) in Sweden has long been debated as to whether it colonized naturally after **postglacial expansion** or was **introduced through human activity**. Many species recolonized northern Europe via land bridges across Denmark [1]. Betony could have reached Scania along these routes or been introduced later for medicinal use, after which it spread in the wild. It is one of Scandinavia's rarest herbs and among the region's oldest documented plants, **first recorded in 1534** in Scania [2]. Unique genetic variants and early flowering in Scandinavian populations suggest that Betony may have persisted in **isolation for a long time** [3-4]. The species has declined drastically due to the loss of traditionally managed coppice woodlands and hay meadows and is now considered **endangered** [5].



Botanical overview of *Betonica officinalis* showing the whole plant, a close-up flower and herbarium specimens representing the dwarf and common meadow ecotypes.

Here, RADseq was used to:

- 1 Characterize genetic structure across Europe
- 2 Assess whether Scandinavian Betony is native or introduced
- 3 Provide a genomic baseline for conservation



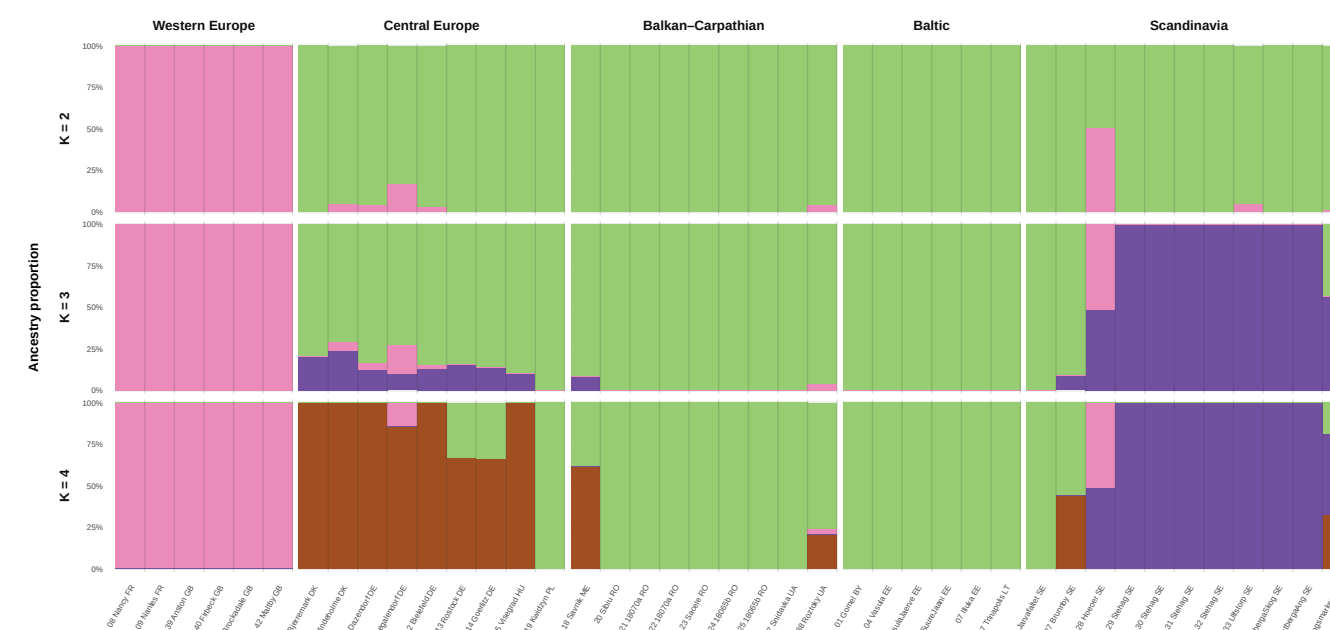
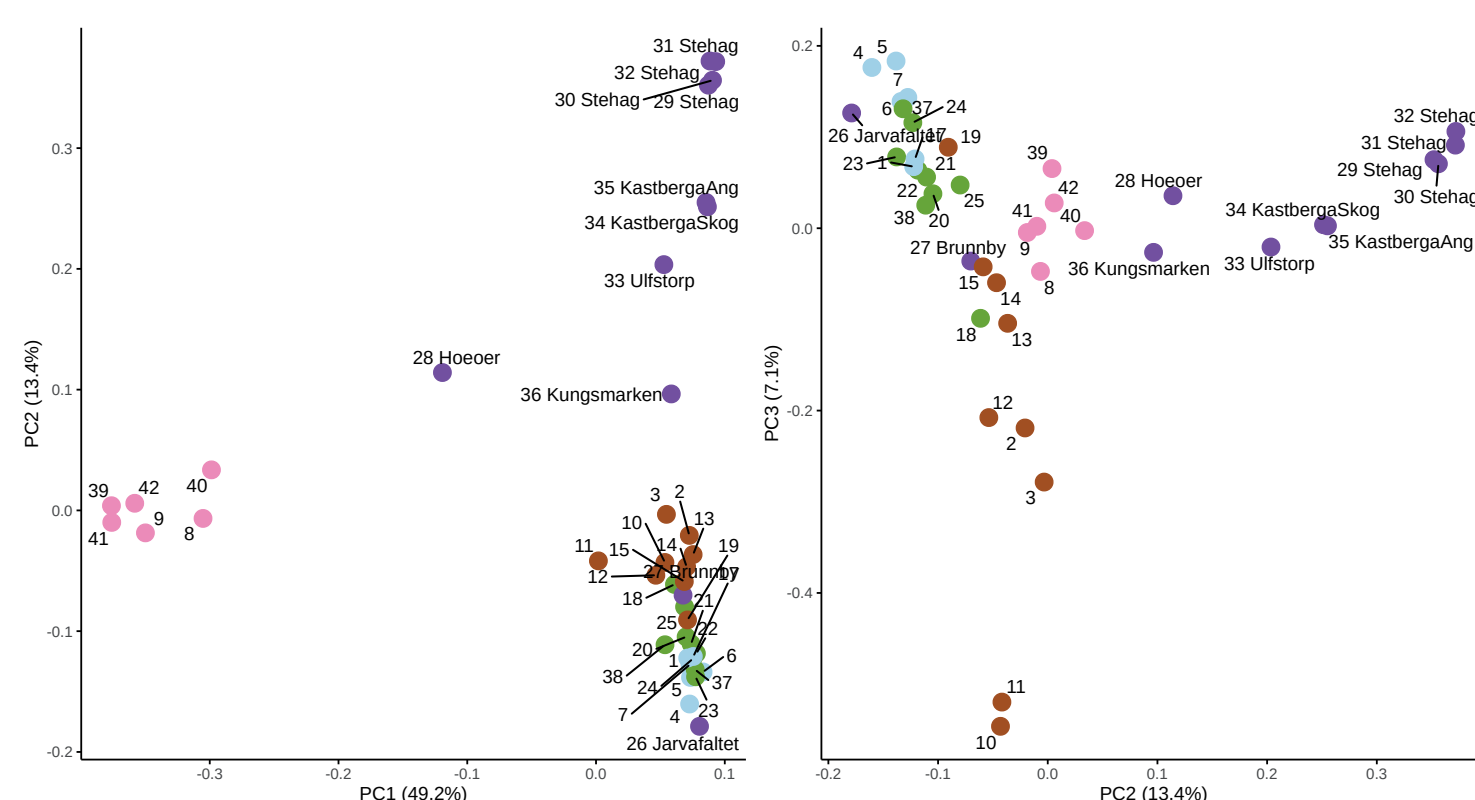
## METHODS

**42 individuals** from 35 European sites (11 from Sweden) were analysed using RADseq. Reads were processed *de novo* in **Stacks**, resulting in **~9,600 high-quality SNPs**. Population structure, phylogenetic relationships, genetic diversity and isolation by distance were evaluated among regions.

## RESULTS

### Genetic structure

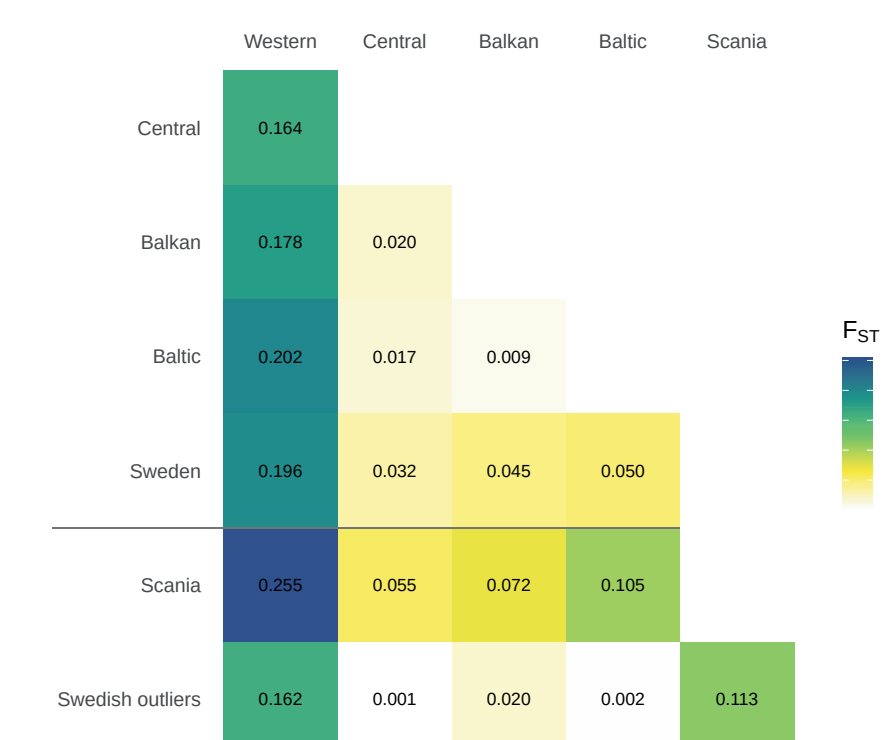
Clear geographic structure was observed across Europe, with western populations strongly separated from other regions and most Swedish populations were distinct from the continental ones. Within Sweden, Scanian sites formed a slightly dispersed cluster. Three samples outside this cluster shared ancestry with populations from other European regions.



PCA and admixture (K 2-4) analyses showing genetic structure across Europe.

### Genetic diversity and differentiation

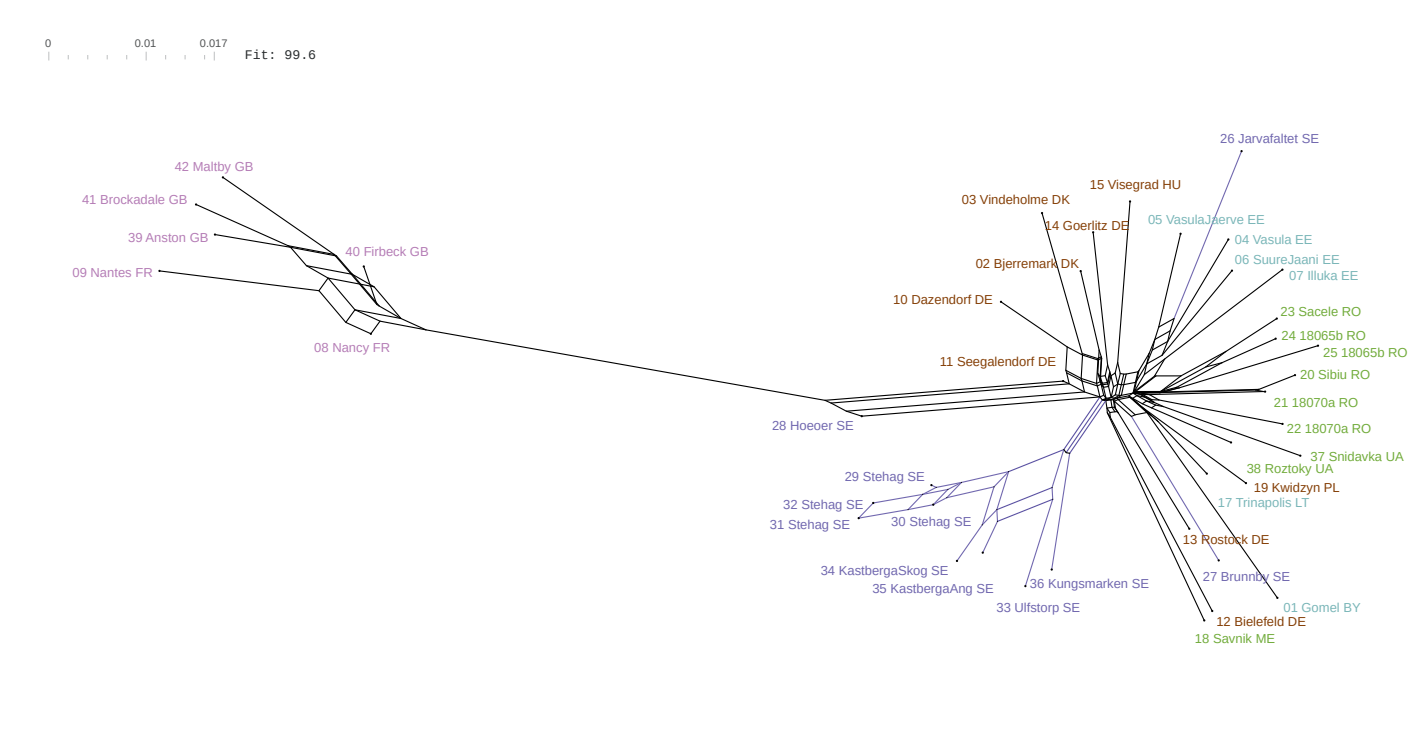
Core Scania had the lowest heterozygosity ( $H_0 = 0.062$ ) and highest inbreeding ( $F_{IS} = 0.49$ ). Differentiation was strongest between western Europe and Scania ( $F_{ST} = 0.26$ ) and moderate between Scania and Baltic or central Europe ( $F_{ST} \approx 0.05-0.10$ ). Most genetic variance (56%) occurred among regions.



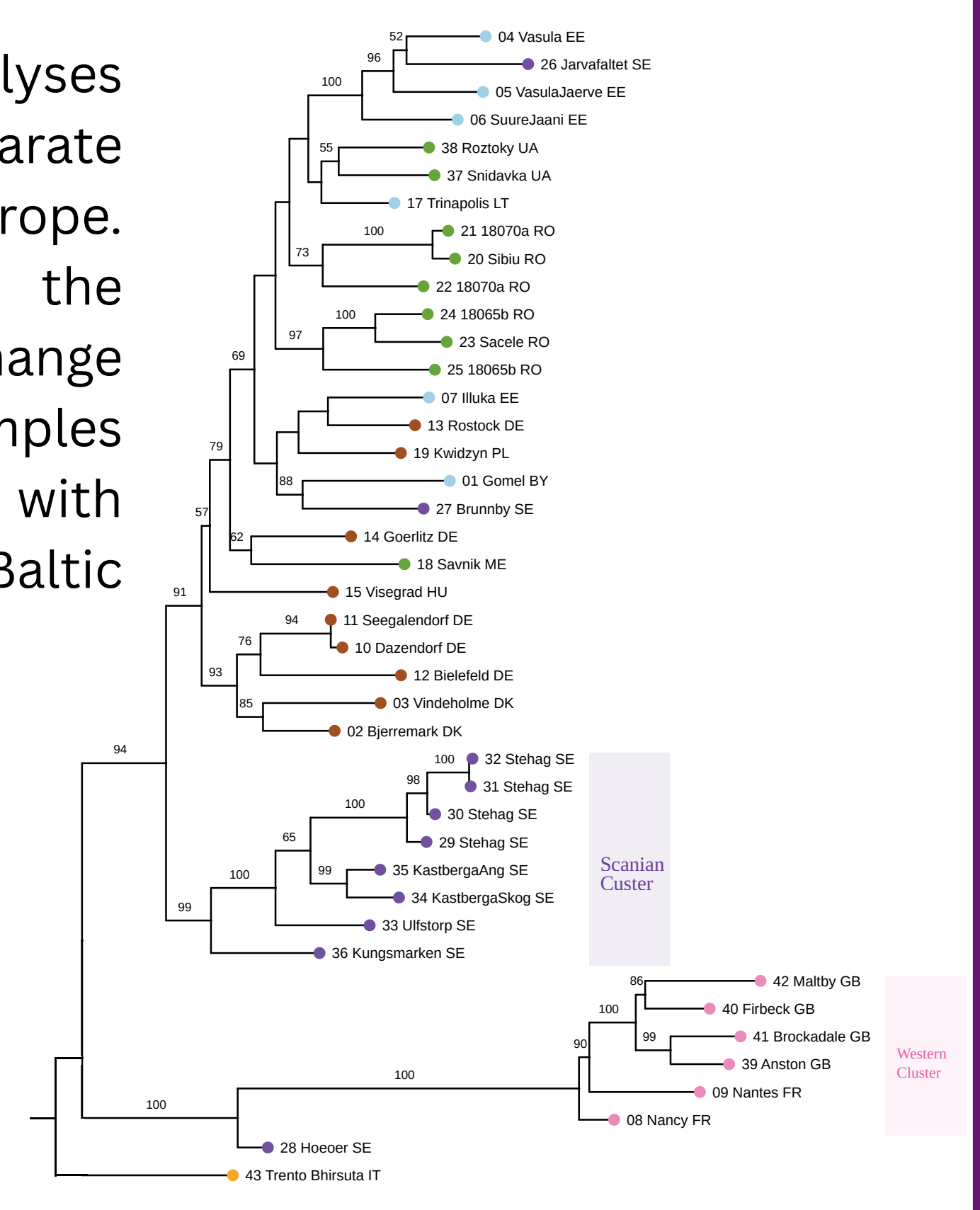
Pairwise genetic differentiation ( $F_{ST}$ ) between regions.

### Phylogenetic relationships

Maximum-likelihood and NeighborNet analyses supported a distinct Scanian lineage separate from continental and western Europe. Moderate reticulation occurred within the Scanian cluster, indicating gene exchange among local populations. The outlying samples (Höör, Brunnby, Järvafältet) grouped with western Europe, central Europe and the Baltic region, respectively.



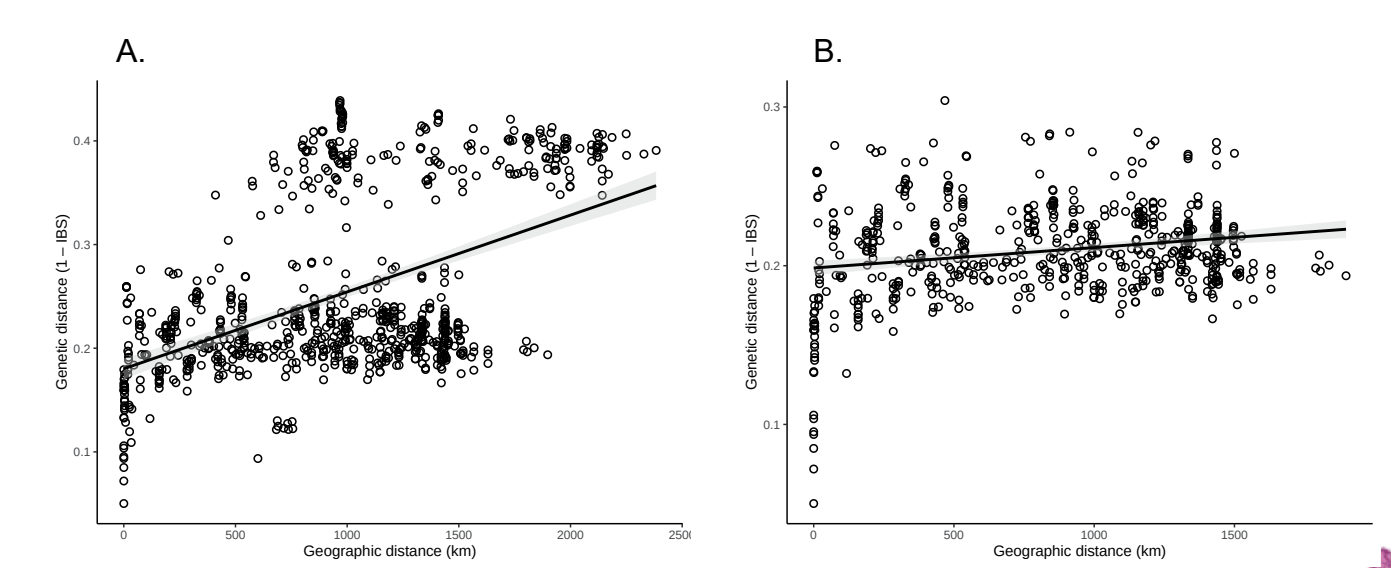
NeighborNet network showing reticulation within Scania and clear separation from other European regions.



Maximum-likelihood tree showing a distinct Scanian lineage, separated from western and continental Europe.

### Isolation by distance

IBD was significant across all samples ( $r = 0.49$ ,  $p < 0.0001$ ) and strongest within Scania ( $r = 0.83$ ,  $p = 0.0001$ ).



Isolation-by-distance (IBD) relationships with (A) all samples and (B) excluding western populations.

## CONCLUSIONS

- Low diversity, high inbreeding, strong structure → **long-term isolation**
- Cohesive Scanian lineage → **likely locally adapted, postglacial persistence**
- Outlying Swedish sites → **mixed ancestry, independent introductions**

## Conservation Implications

- Prioritize core Scanian populations as key conservation units
- Preserve local lineages and genetic diversity
- Maintain habitats and connectivity to reduce isolation



### Acknowledgements

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