

# Microsatellites as a model for decision making in *in-situ* management of sea beets

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The sea beet (*Beta vulgaris* L. ssp. *maritima* (L.) Arcang.) was introduced into the Baltic Sea in the seventeenth century carried in ballast sand in ships coming from the Mediterranean area. The world distribution of sea beets is presented in figure 1, indicating that the Swedish west-coast is the boreal frontier for this species. Sea beet is regionally Red Listed in Sweden. Diversity studies of sea beets have been carried out in Denmark (Andersen et al. 2005), Germany (Driessen et al. 2001) and this work presents a preliminary study performed among populations in southern Sweden.

## Materials

Leaf samples of 13 sea beet populations were collected on the Swedish west coast as shown in the map (figure 2). Number of sampled individuals in the populations varied from 10 - 35. Accessions are shown in table 1.

**Table 1.** Collection sites of sea beet populations. *N<sub>pop</sub>* denotes the number of plants in the population, *N<sub>sample</sub>* the number of sampled individuals, and *N<sub>analysis</sub>* the number of plants analysed.

Pop #	Location	Province	N <sub>pop</sub>	N <sub>sample</sub>	N <sub>analysis</sub>	Area (m <sup>2</sup> )	Latitude/Longitude
10	Hamnerö *	Bohuslän	150	50	29	150	58°33'06"N 11°05'27"E
11	Gunnarsskär *	Bohuslän	400	50	30	100	58°17'02"N 11°21'18"E
12	Skutevik	Bohuslän	28	28	16	75	58°12'31"N 11°25'05"E
13	Håkansskär *	Bohuslän	1000	50	28	500	58°05'51"N 11°20'06"E
14	Tången	Bohuslän	23	23	17	30	58°05'07"N 11°29'26"E
9	Koholmen	Bohuslän	18	18	15	10	57°56'53"N 11°32'42"E
8	Stansvik	Bohuslän	11	11	10	20	57°56'16"N 11°34'45"E
5	Nidingen *	Halland	500	50	30	2500	57°17'11"N 11°54'13"E
7	Glommen	Halland	12	12	11	4	56°55'52"N 12°21'13"E
4	Torekov	Skåne	50	37	27	500	56°25'39"N 12°37'32"E
2	Mölle	Skåne	17	17	16	50	56°16'52"N 12°29'49"E
1	Viken	Skåne	50	33	22	100	56°08'28"N 12°34'46"E
3	Helsingborg	Skåne	12	8	6	30	56°02'39"N 12°29'49"E

## Methods

The collected samples were subjected to microsatellite analysis in three loci. A multiplex reaction of three microsatellite primer pairs, CA2 (Arnauld et al. 2003), GAA1 and BVM3 (Viard et al. 2002), was performed. The PCR-amplification products were separated on 4% polyacrylamide gels and the



**Figure 2.** The collection sites in South-west Sweden.

bands visualized by silver staining.

The banding patterns were interpreted as homozygotes (AA) or heterozygotes (AB) and analysed using the software package POPGENE (Yeh, 1997)

Mean expected heterozygosity over all loci is calculated according to Nei (1973), and the partitioning of the diversity, within and between populations was calculated as  $F_{ST}$  (Nei, 1987). Finally an UPGMA dendrogram is drawn based on genetic distance calculated after Nei (1972).

## Results and discussion

The number of alleles found, two in each locus, in this study was relatively low. The reason for this may be

- genetic drift in small populations
- founder effect and bottlenecks in marginal population
- hard selection under extreme conditions
- low sensitivity of the silver staining technique

The heterozygosity varied from 0.109 at Koholmen up to 0.500 at Tången, while the overall diversity for all loci and all populations was 0.368. Results are presented in figure 3.

The differentiation among populations expressed as the  $F_{ST}$  value ranged from 0.182 to 0.305 at the different loci with average of 0.218 indicating, that 21,8% of the diversity is found between the population while the remaining 78% is within the populations. This is a typical pattern for an out crossing wind pollinated species like beet (Hamrick and Godt, 1989). Low  $F_{ST}$  indicates high similarity between populations, which implies that relatively few populations needs to be conserved.

They can be selected using the following dendrogram (Figure 3).

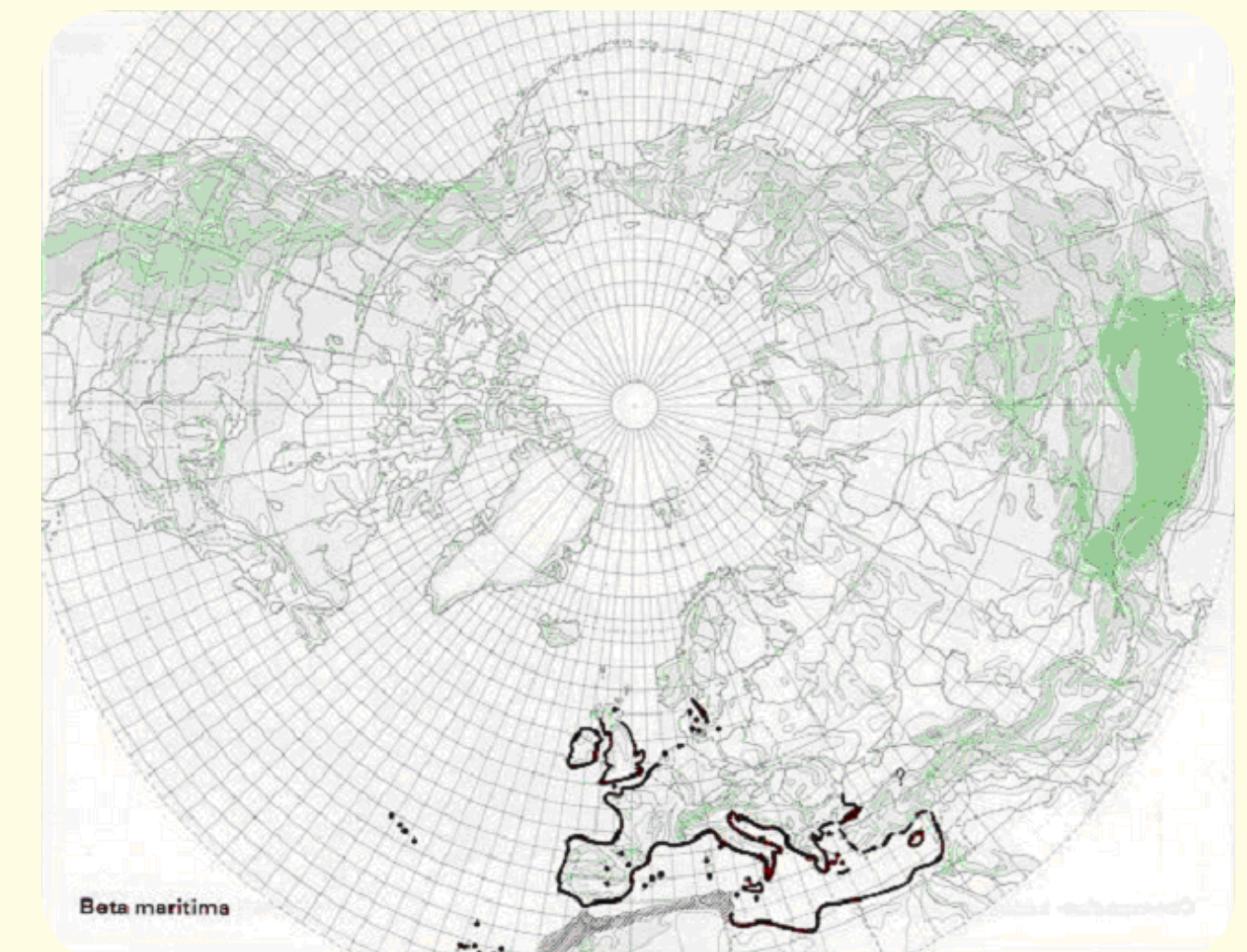
Three major clusters are identified and within each cluster some genetic distance between populations is seen.

The genetic distance ranged from 0.25% between Torekov and Viken to 27.7% between Torekov and Håkansskär

To decide how well the diversity of the Swedish beets is preserved the yellow spots in figure 2 and figure 3 represents populations growing in nature reserves and may thus be considered protected. The red spots, however, are outside and need additional attention

Combining the heterozygosity information, with the similarity of populations

**Figure 1.** The world distribution of sea beet (from Hul-tén and Fri-es 1986)

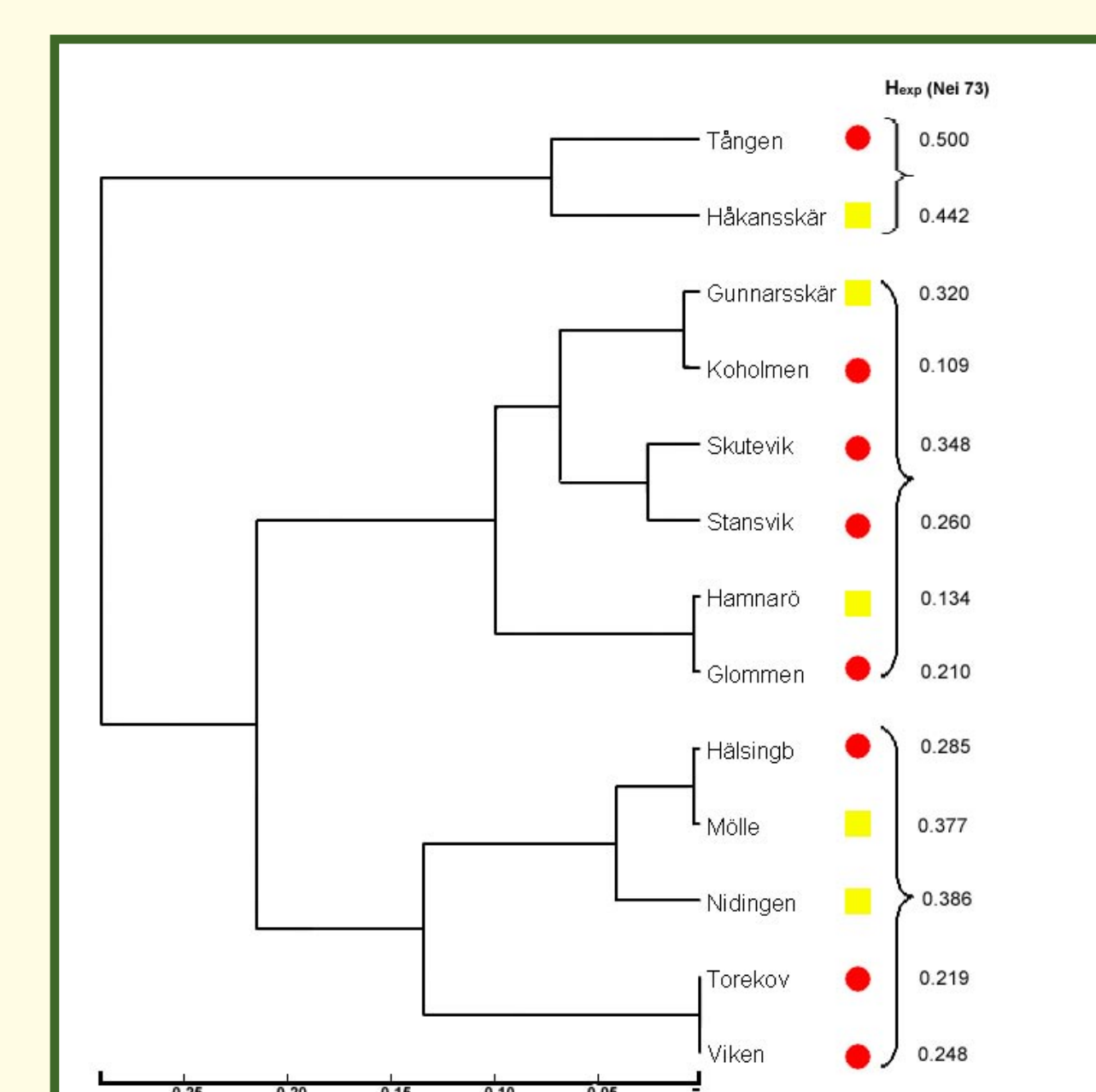


and the protections status – figure 3, provides a tool to prioritize which populations to conserve.

- It is interesting to note that it is not the most diverse populations that are preserved.
- That a population exists in a protected area does not guarantee its preservation, some of the visited areas were primarily bird protection areas and nobody pays attention to the sea beets, except for the sheep.
- If none of the populations in a cluster is in a protected location or a superior population is unprotected, collection and *ex-situ* conservation may be an alternative.

The study and results presented here are based on only three microsatellite loci, and therefore may not reflect the true situation. However, the potential use of the microsatellite analysis for making priorities and strategies for conservation of populations of plant genetic resources is well illustrated.

Plans for a regional project among Denmark, Germany and Sweden will be proposed, where common markers and techniques will be used. Furthermore, the project will comprise morphological characterizations and evaluations for agriculturally interesting traits.



**Figure 3** A UPGMA Dendrogram Based on Nei's (1972) Genetic distance Method. The color indication of protection status from figure 2 is transferred to this illustration.