Symbiosis WP2: Impacts of Road Landscaping on Gene Flow in Plants

Investigation of genetic diversity of hawthorn (*Crataegus monogyna)* populations in Ireland in context of road landscaping schemes.

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Project Aims

- **Develop molecular tools for investigation of genetic diversity of hawthorn materials used for roadside landscaping, whether of introduced or Irish provenance.**

- **Investigate use of hawthorn as an indicator of the effect of roadside landscape planting on intra-species genetic diversity**

- **Determine whether introduced hawthorn planting materials can impact on genetic diversity of pre-existing populations of hawthorn in Ireland**

What plant species to choose for genetic diversity analysis?

Hawthorn, *Crataegus monogyna.*
Family: *Rosaceae*

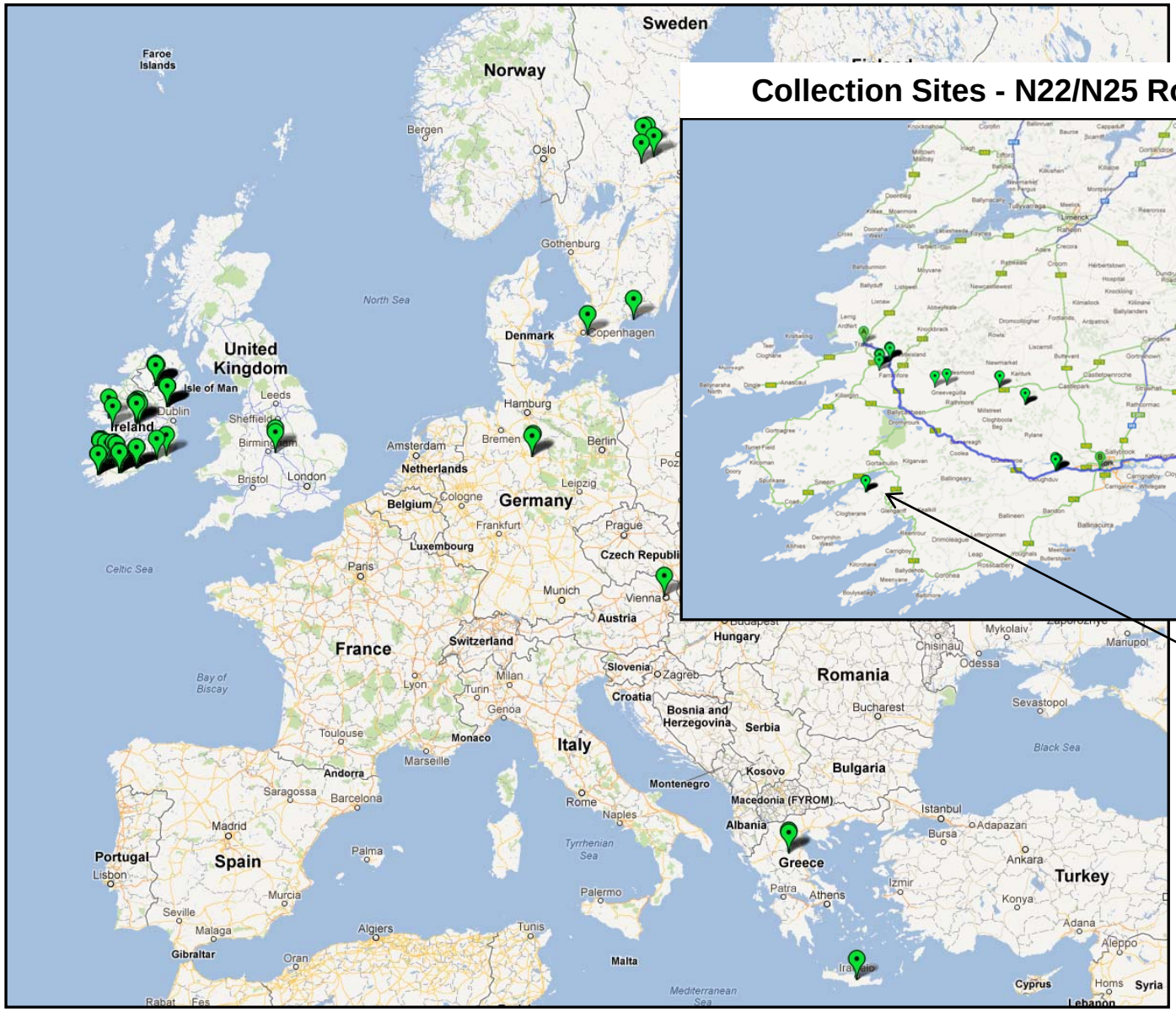
- **Why hawthorn (*Crateagus monogyna*)?**
- **Species with a broad distribution across Ireland**
- **Planted populations along newly constructed roads are of foreign origin (mainly from nurseries in the Netherlands)**
- **Possible polymorphism between the Irish stands of *C. monogyna* and the introduced *C. monogyna* genotypes**
- **Indicator species to assess hybridization and introgression between Irish and introduced genotypes**

Methodology

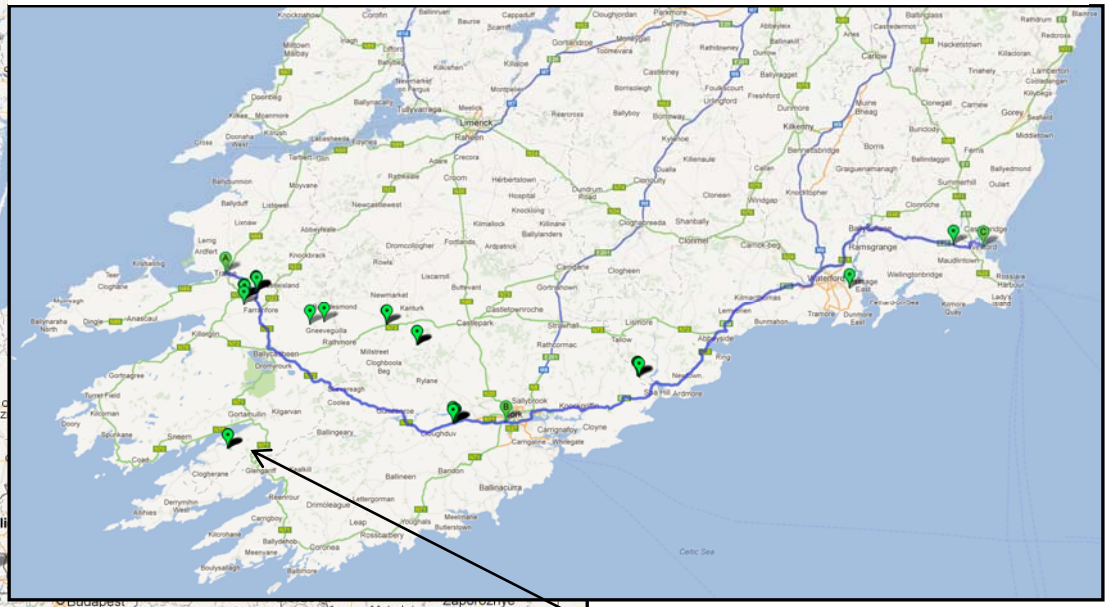
- Sampling strategy
- Collection of berries and/or leaves from individual hawthorn trees (within each population)
- DNA extraction
- Nuclear molecular marker development (novel nuclear microsatellite SSR markers for hawthorn)
- Chloroplast genome molecular marker development
- Use of molecular markers to investigate genetic diversity relationships within and between all populations of hawthorn
- Data analysis - determination of molecular diversity within and between the populations

Hawthorn flowers, berries and tree

Sampling of populations & samples across Ireland, UK and continental Europe

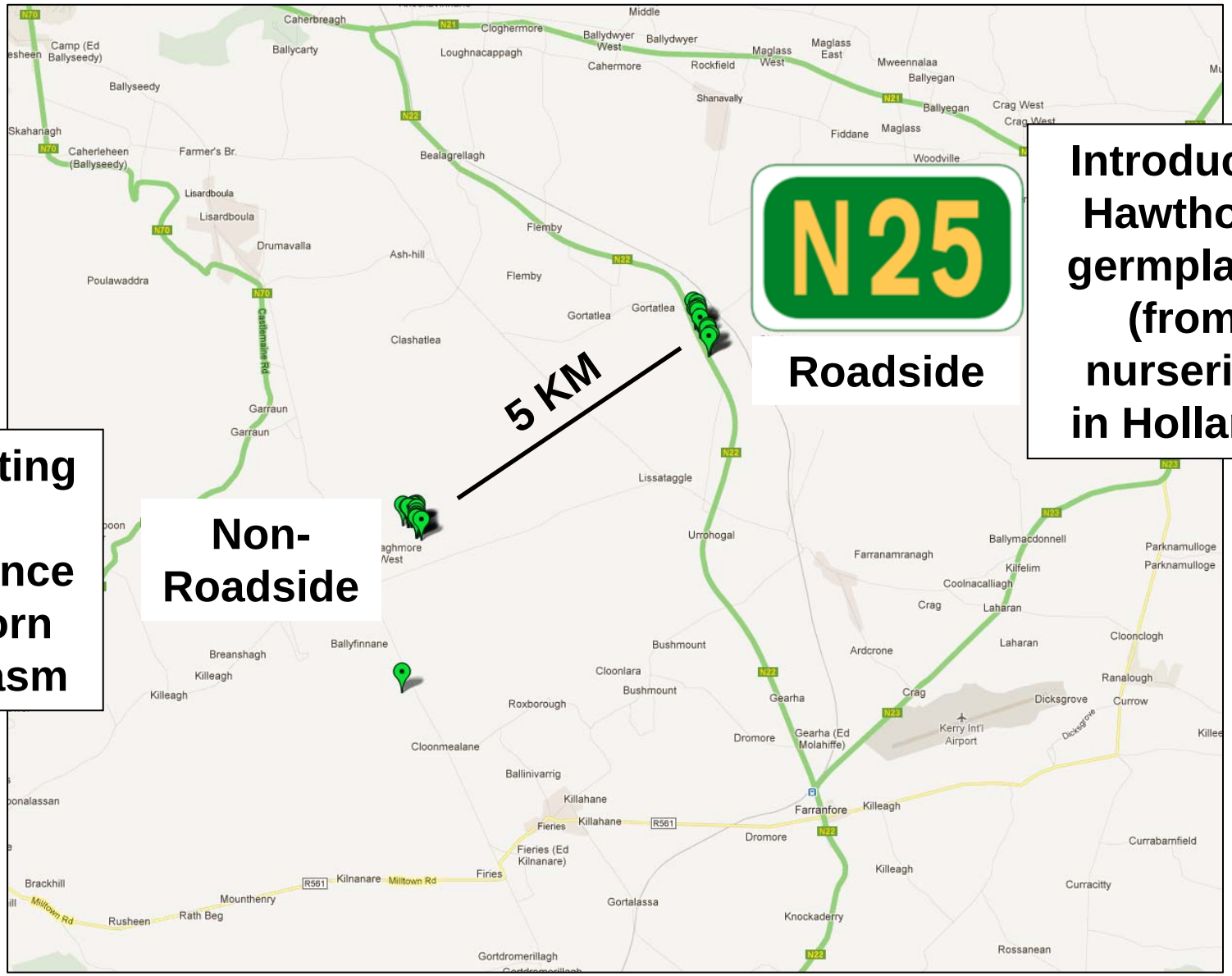


Collection Sites - N22/N25 Road (Tralee to Wexford)



Uragh Wood Nature Reserve

Road Sampling Strategy



Key to the hawthorn populations analyzed

Cork RS = Roadside Samples, Carrigrohane, Cork
Cork IS = Inside Road Samples, Glunishine, Cork

East RS = Roadside Samples, Cork, Waterford, Wexford
East IS = Inside Road Samples, Cork, Waterford, Wexford

West RS = Roadside Samples, Tralee, Kerry
West IS = Inside Road Samples, Tralee, Kerry

U-F = Fairy trees & Uragh Wood Nature Reserve, Kerry

OI = Other parts of Ireland (e.g. Offaly, Galway, Clare, Monaghan, Meath)

INT = e.g. UK, Germany, Greece, Austria, Denmark

Sweden = Swedish samples

Development of novel molecular markers for hawthorn genetic diversity assessment

- **Prior to EPA project no molecular markers (SSRs, microsatellites) available for hawthorn (*Crataegus monogyna*)**

- **EPA Project identifies six nuclear microsatellites marker loci (SSRs) that can be used for genetic diversity analysis of hawthorn**

Excess homozygosity in inbred Irish hawthorn populations

Population	Na	Ne	Na Freq. >=5%	No. P.A.	Ho	He	F	N
Cork RS	7.17	5.27	5.33	1	0.45	0.68	0.32	9.00
East RS	7.67	5.80	5.33	1	0.52	0.77	0.32	9.00
West RS	8.17	5.28	5.33	1	0.41	0.80	0.49	10.50
Cork IS	6.00	4.62	6.00	0	0.50	0.77	0.34	7.17
West IS	8.50	4.93	5.00	1	0.43	0.78	0.45	12.67
U-F	5.30	3.91	5.33	0	0.41	0.63	0.35	7.33
OI	10.00	6.00	6.17	2	0.45	0.82	0.45	23.00
INT	5.00	4.20	5.00	1	0.71	0.73	-0.04	4.33
Sweden	6.17	5.11	6.17	1	0.40	0.78	0.48	5.83

Na- total number of alleles
Ne- effective number of alleles
Na freq>5%- number of alleles freq >0.05
N- Sample size

Ho- observed heterozygosis
He- expected heterozygosis
F- Coefficient of inbreeding

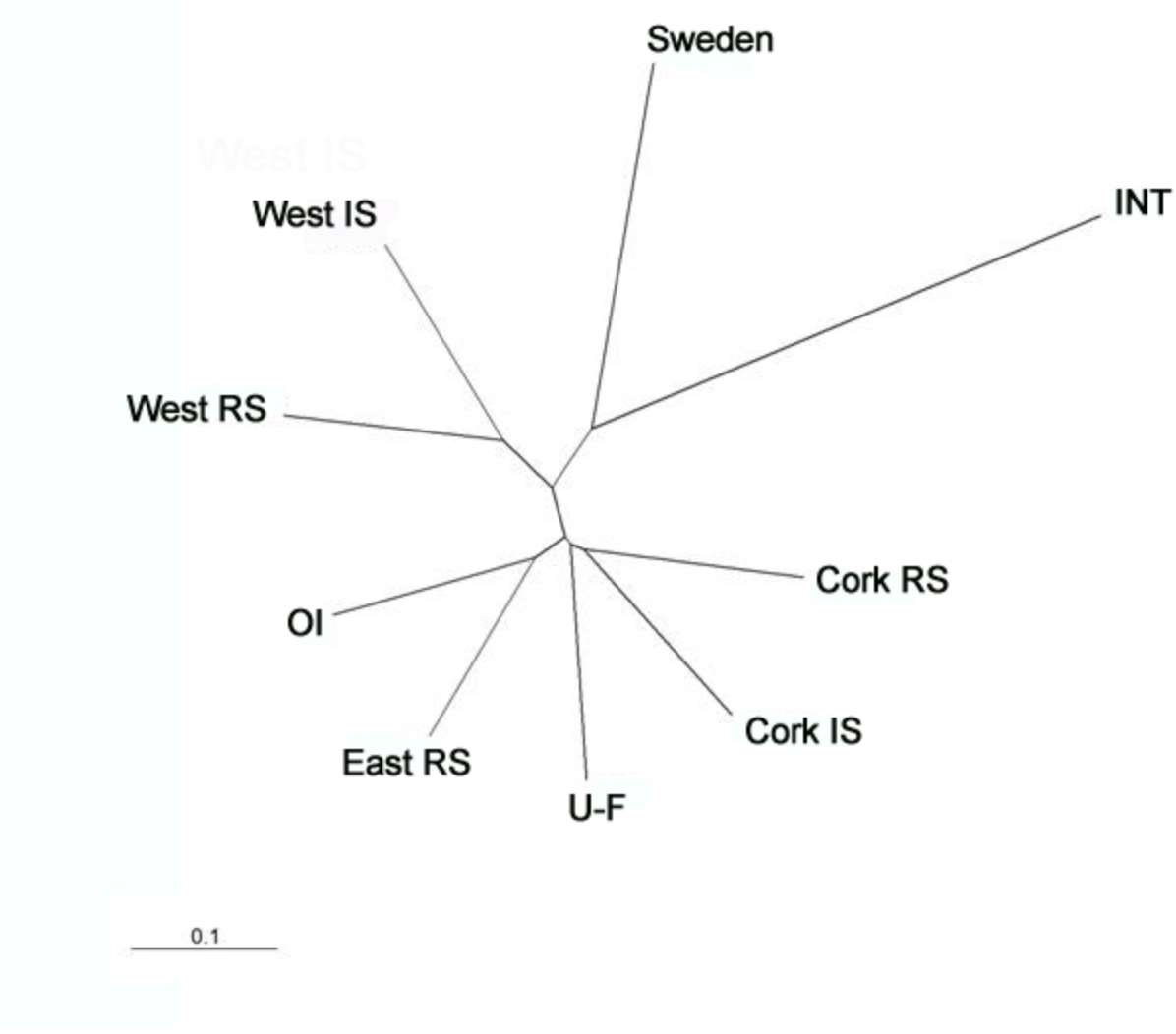
Most of the genetic variation detected is within hawthorn populations rather than between populations

- **Amova performed to detect the sources of variation within and between hawthorn population groups**

Source of variation	df	SS	MS	Est. Var. (%)	Variance (%)	P-Value
Among *C. monogyna*	8	92.553	11.569	0.346	4	0.001
Within *C. monogyna*	102	758.826	7.439	7.439	96	0.001
Total	110	851.378	19.00	7.786	100	0.001
Stat	Value	P(rand>=data)				
PhiPT	0.044	0.001				

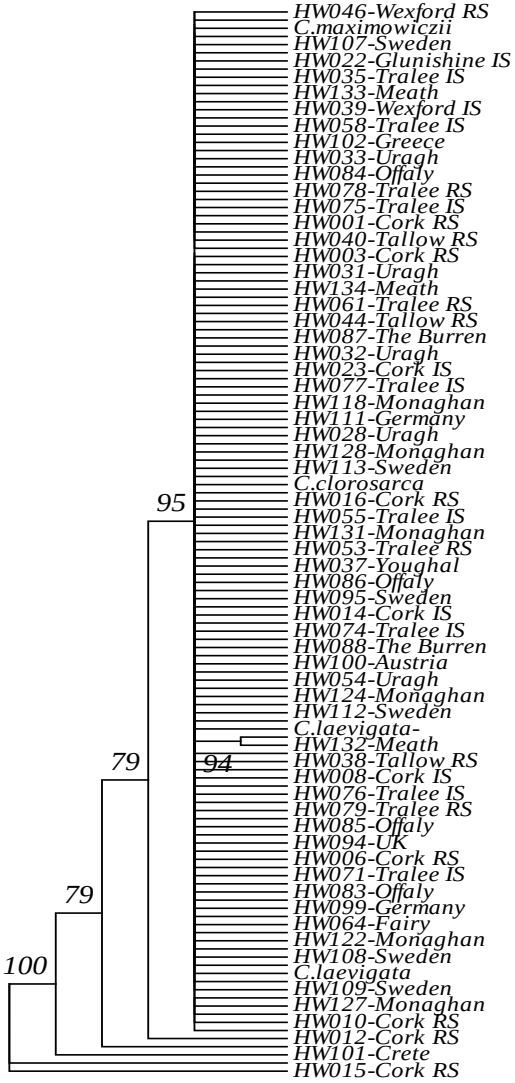
At the resolution of the molecular markers used there are no significant differences in genetic diversity between any of the populations of hawthorn analysed in this study (i.e. roadside vs inside road, foreign vs Irish)

Low level of differentiation between the hawthorn population groups analyzed



Chloroplast loci across all hawthorn populations analysed display lack of genetic diversity

- **Founder event at European scale?**
- **Evolution: recent radiation of hawthorn?**
- **Irish populations are not distinct**



Neighbour Joining, derives from sequences of rps16 and LSC region of the chloroplast of 128 individuals of hawthorn *C. monogyna*, two individuals of *C. laevigata* and *C. chlorosarca*

Conclusions

- **Successful development of novel molecular markers (microsatellites, SSRs) for hawthorn (*C. monogyna)***
- **Genetic diversity analysis of Irish hawthorn populations indicates significant excess of homozygotes & indicated that populations were inbred and displayed low genetic variability**
- **No significant genetic diversity differences between any of the population groups analysed i.e. roadside vs inside and road and foreign (planted or sourced) versus Irish provenance**
- **Genetic diversity does not provide a basis for limiting roadside management to planting hawthorn of Irish provenance**

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