Investigating honey bee foraging using DNA metabarcoding

Laura Jones

National Botanic Garden of Wales
Bangor University

BeeCraft

The Informed Voice of British Beekeeping

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The National Botanic Garden of Wales is dedicated to the **research and conservation of biodiversity**, to sustainability, lifelong learning and the enjoyment of the visitor.

Honey Bees are Under Threat

Honey bees contribute to pollination of crops and wild habitats

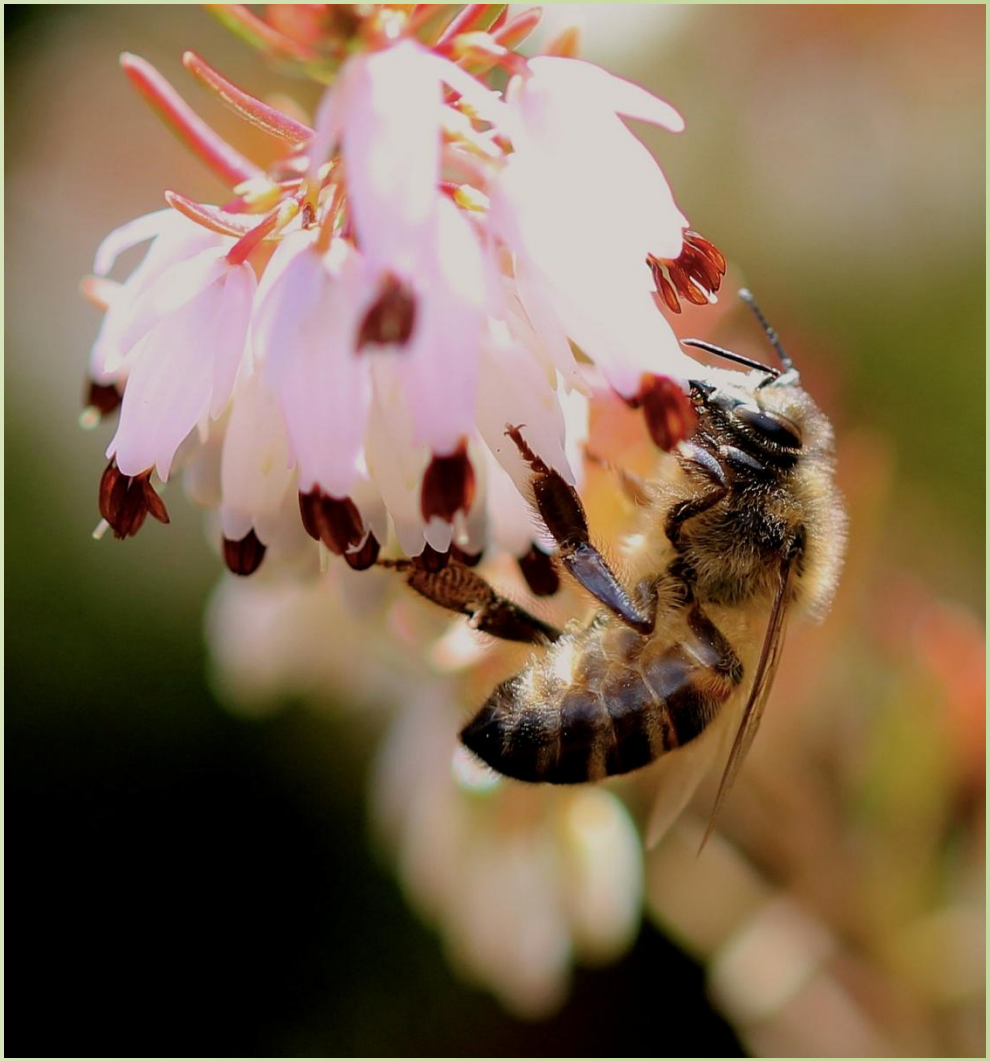
- Threatened by pests and diseases, apicultural mismanagement, pesticides,
- Lack of suitable foraging habitat

Honeybee Nutritional Requirements

- Honeybees require nectar, pollen and water.
- Nectar provides carbohydrates for energy and trace elements
- Pollen provides protein, lipids and other vitamins and minerals.

Nectar Content

- Nectar found in flowers can vary in sugar type, concentration and volume.
- Trace elements
- Can contain secondary plant compounds e.g. caffeine, nicotine



Pollen Nutrition

- Pollen varies in its protein content, amino acid composition, lipid levels.
- Pollen diversity affects disease tolerance and longevity in honeybees.
- In nearly all cases a single pollen diet is insufficient.

Foraging Habitat

- Honeybees are described as super-generalists.
- But beekeepers know that honeybees have particular plants that they target.
- Advice is to provide a wide range of forage.
- But what are the BEST plants for healthy honeybees and why.

Investigating the Plants Honeybees Use

- Observation of bees on flowers
- Analyse pollen returned to the hive using pollen traps
- Analyse honey – melissopalynology
- Microscopy
- DNA

What do they eat?

What's in this?

DNA can be used where morphological I.D. is not possible.

What does it pollinate?

Is this legal?

DNA Barcoding

- Building a database of all species.
- Internationally agreed regions of DNA for different taxonomic groups.
- Open science.

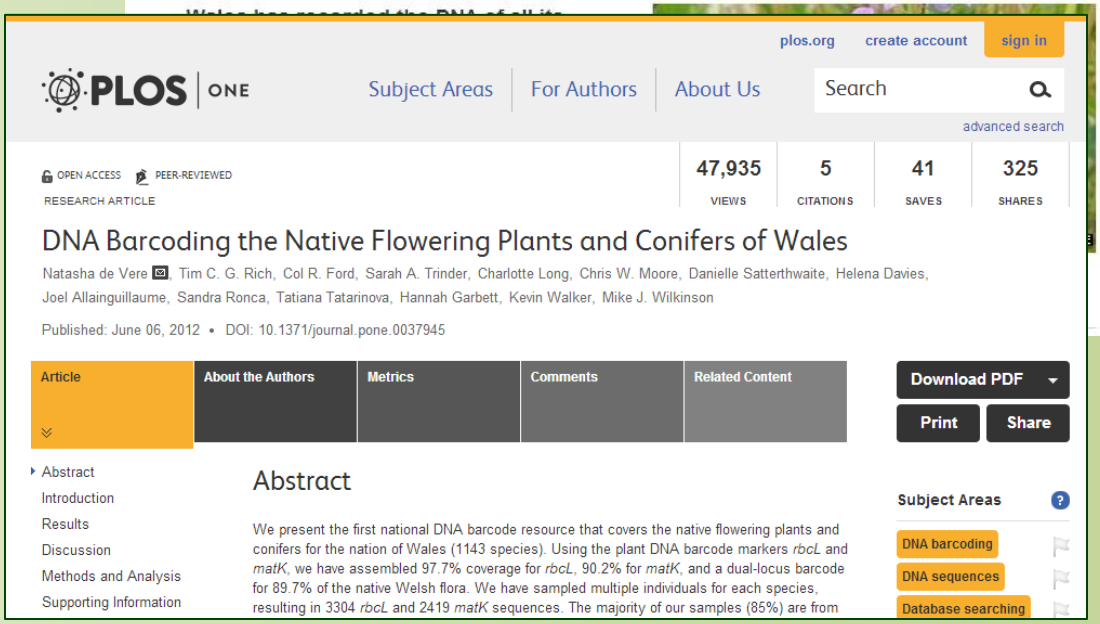
>Cotoneaster_cambricus

AGAGACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAA
ATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATTTTG
GCAGCATTTCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCA
GGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTA
TGGACTGACGGTCTTACCAGTCTTGATCGTTACAAAGGTCGATGCTACC
ACATCGAGCCTGTTGCTGGAGAAGAAAGTCAATTTATTGCTTATGTAGC
TTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTT
CCATTGTAGGTAATGTGTTTGGGTTCAAGGCCCTGCGCGCTCTACGTCT
GGAGGATTTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAGGGCCCG
CCTCATGGTATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGCCGC
CCTCTATTGGGATGTACTATAAAACCAAAATTGGGGTTATCCGCTAAGA
ATTACGGTAGAGCAGTTTATGAATGTC


Canadian Centre for DNA Barcoding
SPONGE
BeBoL
Belgian Network for DNA Barcoding of Life
TreeBOL
DNABARCODING FLORAKRUGERUJ
BARCODE OF LIFE
NBOL
THE NETHERLANDS
polarbarcode
BanglaBOL
A DNA BARCODING FO
FISH-BOL
BARCODE BULLETIN
Vol. 2, No. 2 - July 2011
All Leps Barcode of Life
A BARCODING
010101010101001
NorBOL
Norwegian Barcode of Life
ADELAIDE 2011

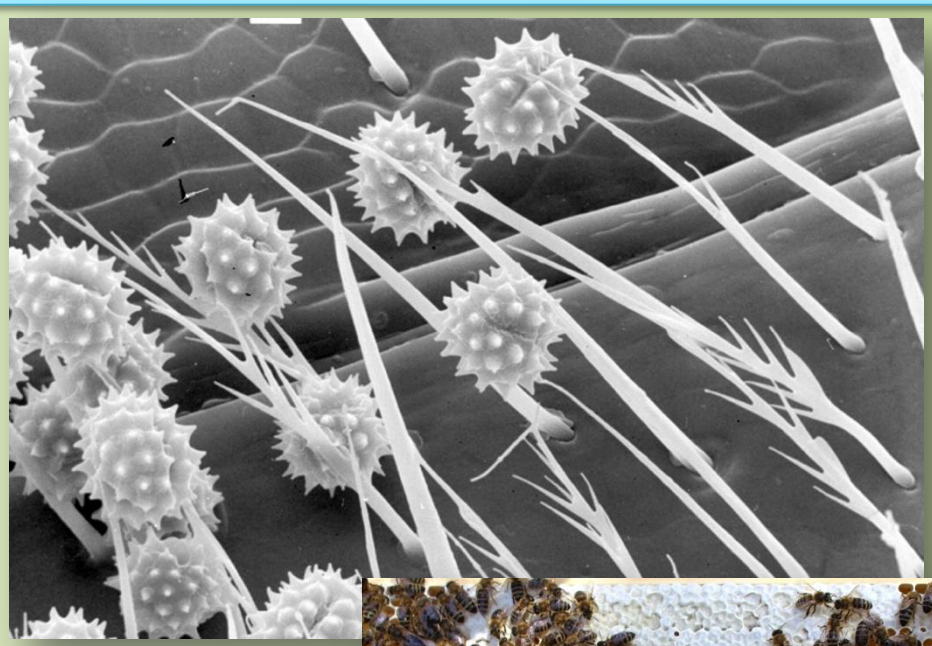
Barcode Wales & Barcode UK

- Barcode Wales: 1st nation in the world to DNA barcode their native flowering plants
- Barcode UK: Built on Barcode Wales to DNA barcode all of the native flowering plants of the UK
- Develop applications that utilise this research platform



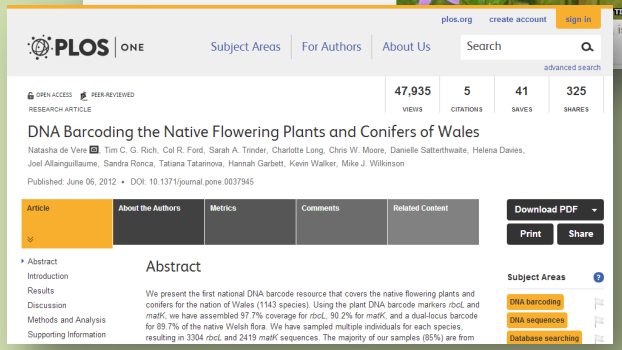
DNA Metabarcoding for Pollen I.D.

- Floral visitation can be tracked by DNA metabarcoding pollen collected by insects.
- Pollen can be retrieved from the bodies of insects, from pollen loads or honey.



DNA Metabarcoding

1. Retrieve pollen
2. Extract DNA
3. Amplify using *rbcL* DNA barcode
4. Sequence DNA on Illumina Mi-Seq
5. Compare to Barcode UK reference library



Aims and methods

What plants are honey bees foraging on throughout the season when offered a diverse floral resource?

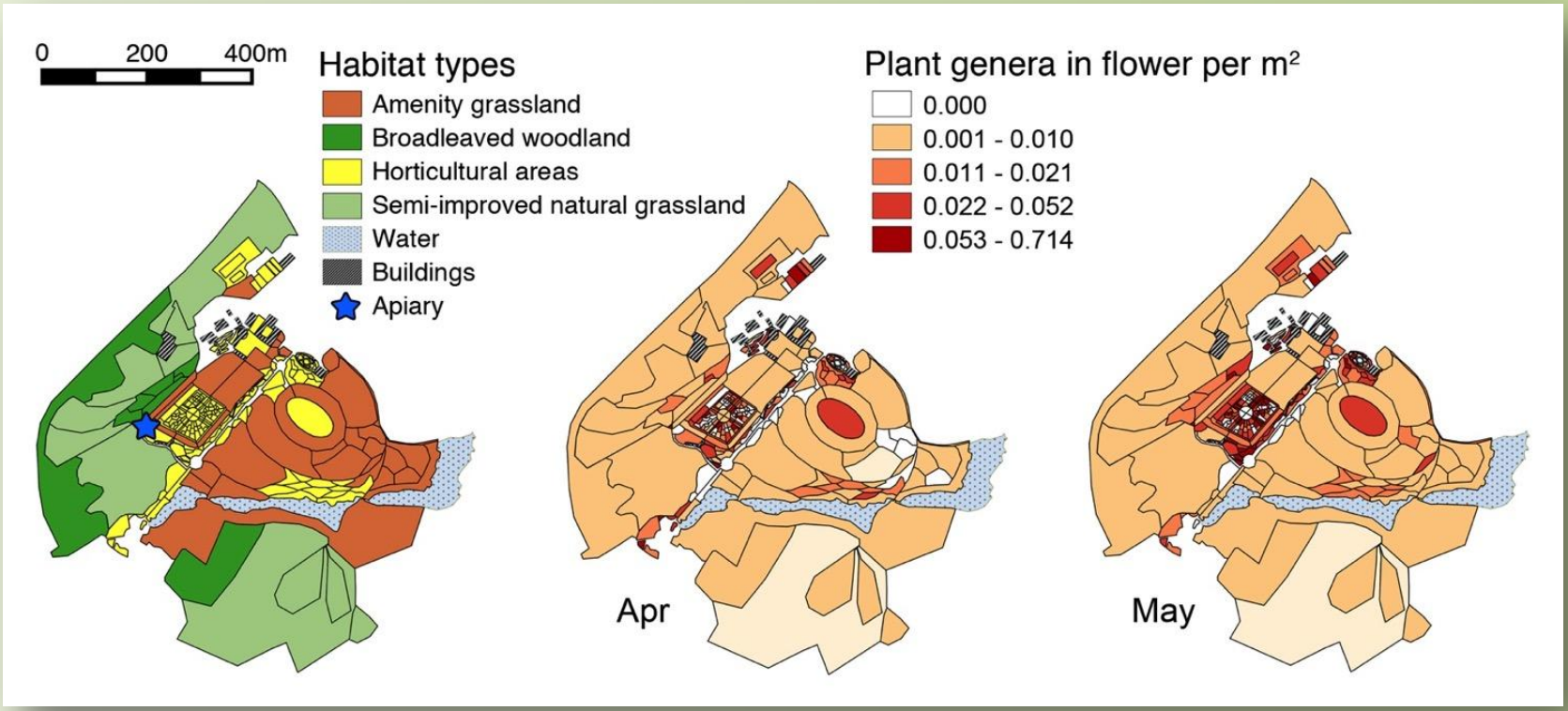
- Use DNA metabarcoding to characterise the floral composition of honey from hives set in study area.
- Survey study area for available flowering species.
- Compare what plant species honey bees are using with what plant species are available

Study site: National Botanic Garden of Wales

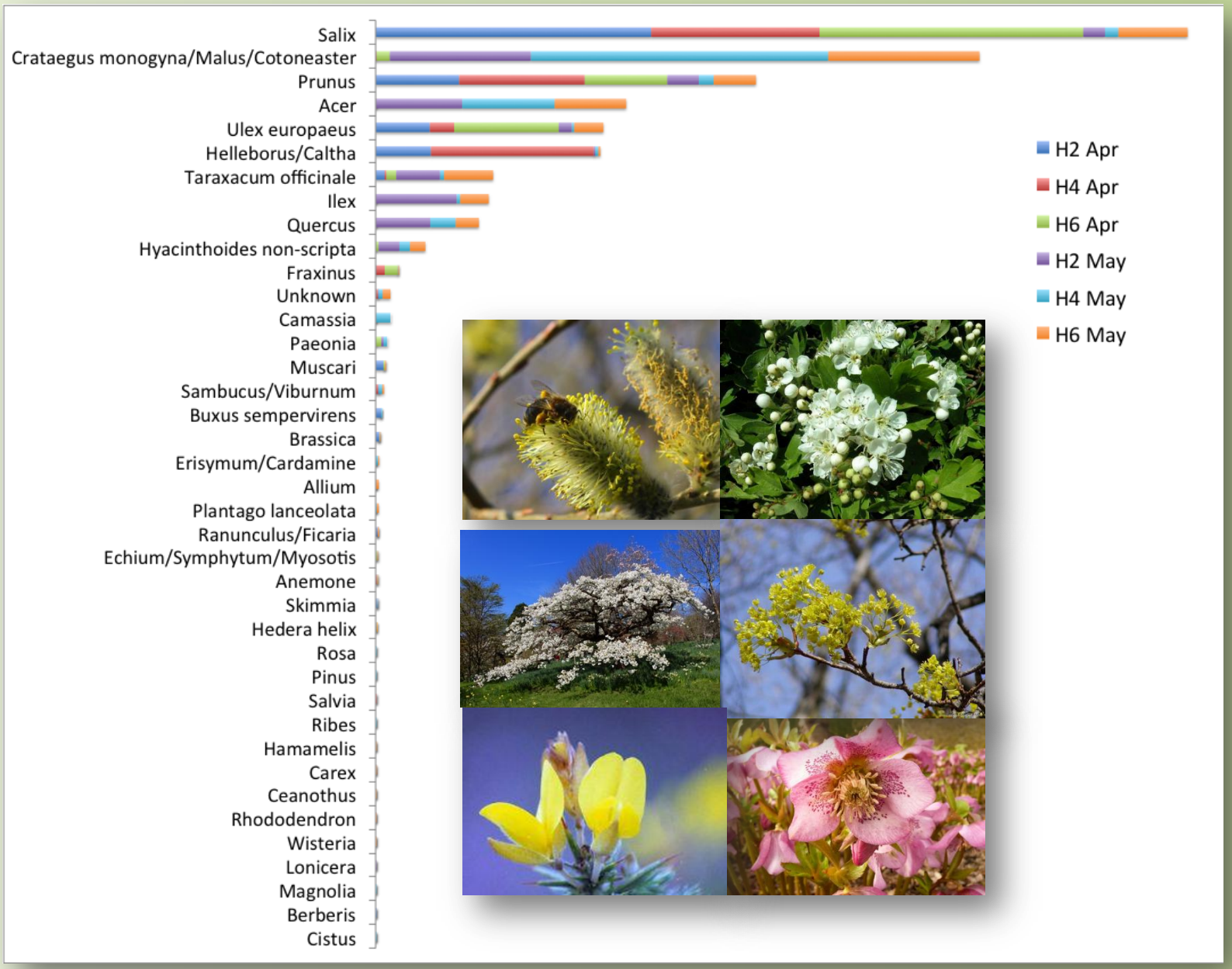
- Contains 8000 plant taxa, diverse horticultural resource
- Surrounded by NNR, native habitat
- Mapped all the plants in flower monthly
- Collected honey samples throughout the season
- DNA metabarcoding of honey Illumina Mi-Seq *rbcL*

Created with Inspire 1 drone

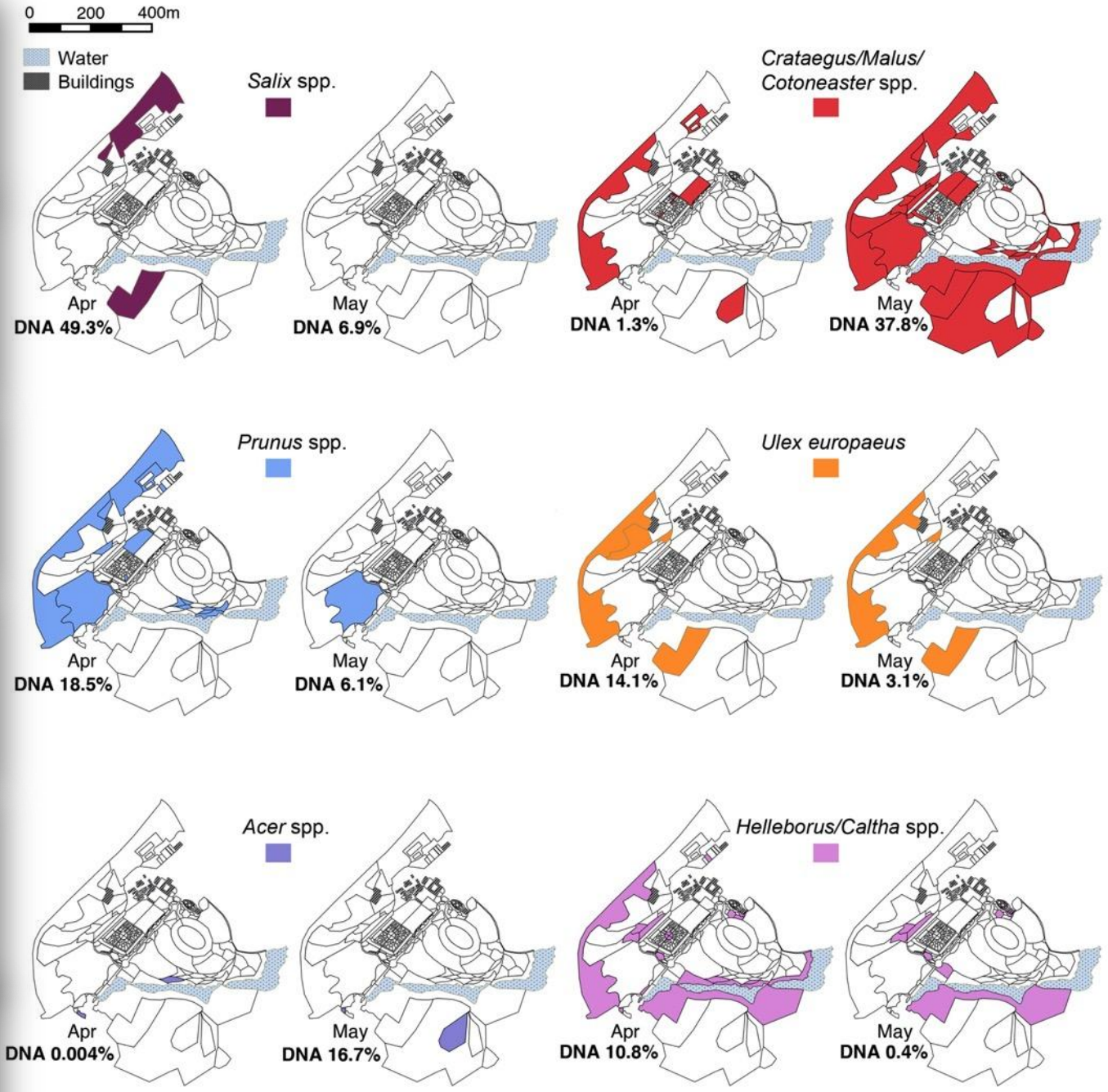
National Botanic Garden of Wales
Spring Landscape



DNA: % seq in April and May honey for 3 Hives



Family	Taxa	Status	Habitat	Growth
Salicaceae	Salix	B	W	W
Rosaceae	Crataegus/Malus/Cotoneaster	B	WH	W
Rosaceae	Prunus	B	WH	W
Fabaceae	Ulex europaeus	N	W	W
Sapindaceae	Acer	B	WH	W
Ranunculaceae	Helleborus/Caltha	B	GH	H
Asteraceae	Taraxacum officinale	N	G	H
Aquifoliaceae	Ilex aquifolium	N	W	W
Fagaceae	Quercus	N	W	W
Asparagaceae	Hyacinthoides non-scripta	N	WG	B
Oleaceae	Fraxinus	N	W	W
Paeoniaceae	Paeonia	A	H	H
Asparagaceae	Camassia	A	H	B
Asparagaceae	Muscari	A	H	B
Adoxaceae	Sambucus/Viburnum	B	WH	W
Buxaceae	Buxus sempervirens	A	H	W
Brassicaceae	Brassica	B	H	H
Brassicaceae	Erisymum/Cardamine	B	H	H
Amaryllidaceae	Allium	A	H	B
Plantaginaceae	Plantago lanceolata	N	G	H
Ranunculaceae	Ranunculus/Ficaria	B	G	H
Boraginaceae	Echium/Symphytum/Myosotis	B	GH	H
Rutaceae	Skimmia	A	H	W
Ranunculaceae	Anemone	A	H	B
Araliaceae	Hedera helix	N	W	W
Rosaceae	Rosa	B	WH	W
Pinaceae	Pinus	A	W	W
Hamamelidaceae	Hamamelis	A	H	W
Grossulariaceae	Ribes	A	H	W
Cyperaceae	Carex	N	G	H
Lamiaceae	Salvia	A	H	H
Caprifoliaceae	Lonicera	B	WH	H
Ericaceae	Rhododendron	A	H	W
Rhamnaceae	Ceanothus	A	H	W
Fabaceae	Wisteria	A	H	W
Magnoliaceae	Magnolia	A	H	W
Berberidaceae	Berberis	A	H	W
Cistaceae	Cistus	A	H	W
Liliaceae	Tulipa	A	H	B


0 200 400m
Water
Buildings
Salix spp.
Apr
DNA 49.3%
May
DNA 6.9%
Crataegus/Malus/
Cotoneaster spp.
Apr
DNA 1.3%
May
DNA 37.8%
Prunus spp.
Apr
DNA 18.5%
May
DNA 6.1%
Ulex europaeus
Apr
DNA 14.1%
May
DNA 3.1%
Acer spp.
Apr
DNA 0.004%
May
DNA 16.7%
Helleborus/Caltha spp.
Apr
DNA 10.8%
May
DNA 0.4%

Honey bee use compared to availability

	April 2015	May 2015	April & May combined
Number of plant families in flower	**80**	**85**	**96**
Number of families recorded in honey (all hives)	**18 (23%)**	**29 (34%)**	**33 (34%)**
Number of plant genera in flower	**291**	**360**	**437**
Number of genera recorded in honey (all hives)	**31 (11%)**	**45 (13%)**	**49 (11.2%)**

- Close range access to high diversity of native and horticultural plants
- Honey bees used a small proportion of genera available

Discussion

- Foraging choices:
  - Abundance of plants at a landscape level
  - Nectar and pollen quality
- Consistent with observations of honey bees using a small number of nectar and pollen-rich species to supply majority of nutritional needs
- Native habitat: hedgerows and woodland important spring forage
- Long tail of other species suggests honey bee diet may be supplemented with less intensively utilised species

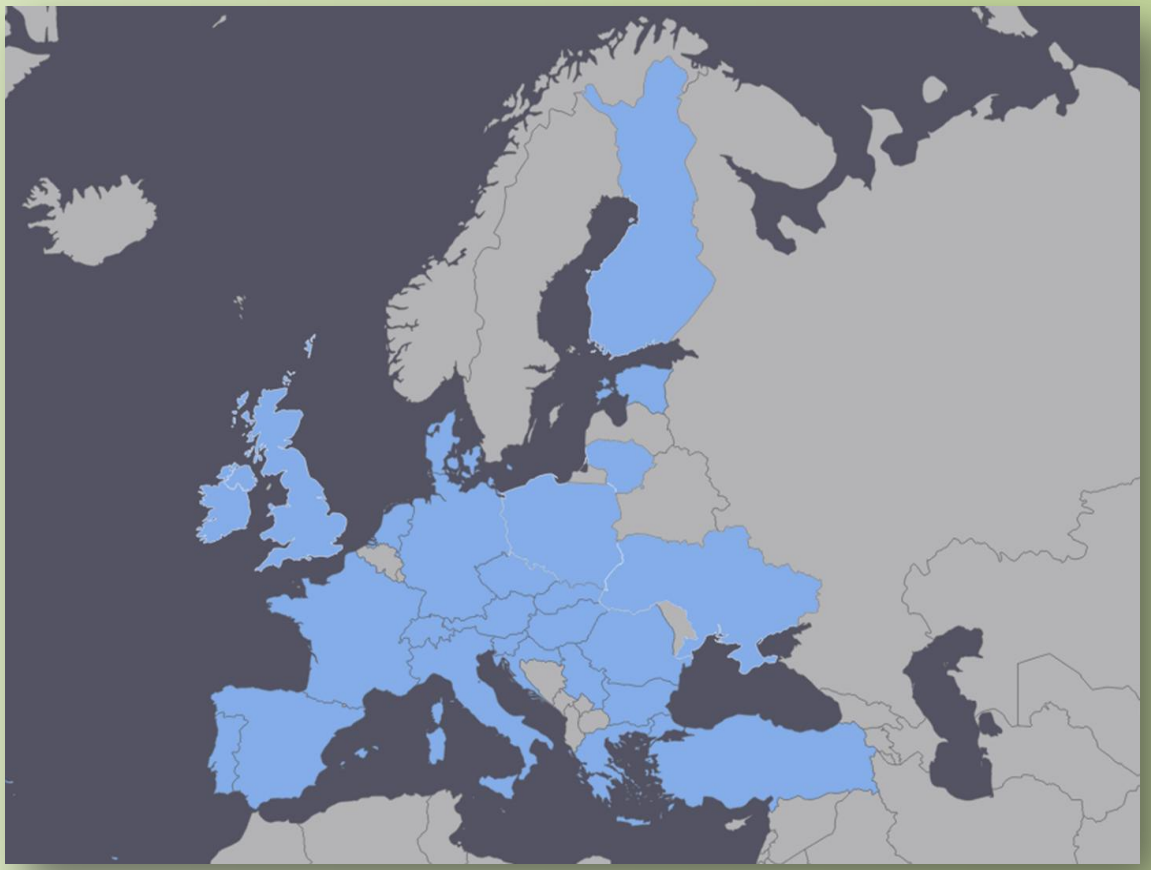
Why these Plants?

- Is the pattern the same every year?
- What makes these plants important?
- What happens on a wider scale in Wales and the UK?

- Sample honey in the Garden each month after multiple years.
- **Collect honey and hive health information from beekeepers throughout Wales/UK.**

Honey Bee Plants in Europe

- Which plants do honey bees use in Europe?
- Analysed 377 scientific papers: 301 honey, 76 pollen.
- Found 1354 different types of plant, but a small number of species are commonly seen.



% of papers

Castanea sativa	34		
Eucalyptus sp.	32	Cistus sp.	14
Rubus sp.	31	Taraxacum officinale	14
Tilia sp.	28	Acer sp.	13
Salix sp.	27	Carduus sp.	12
Prunus sp.	26	Taraxacum sp.	12
Quercus sp.	25	Cyanus segetum	12
Echium sp.	24	Zea mays	12
Robinia pseudoacacia	24	Lavandula sp.	12
Erica sp.	23	Rumex sp.	12
Trifolium repens	23	**Apiaceae**	11
Trifolium sp.	23	**Asteraceae**	11
Helianthus annuus	21	Centaurea sp.	11
Citrus sp.	20	Papaver sp.	11
Plantago sp.	19	Thymus sp.	11
Brassica napus	19	Calluna vulgaris	11
Brassicaceae	18	Crataegus monogyna	11
Brassica sp.	17	**Fabaceae**	10
Lotus sp.	16	Rosmarinus officinalis	10
Poaceae	15	Campanula sp.	10
Rosaceae	15	Hedera helix	10
Trifolium pratense	15	**Liliaceae**	10
Vicia sp.	15	Allium sp.	10
Pinus sp.	15	Lavandula stoechas	10

DNA metabarcoding of 20 honey samples

- 20 honeys collected throughout Wales and the UK.

Honey I.D.	Number of taxa detected
H1	18
H2	14
H4	13
H6	19
H7	15
H8	21
H9	23
H11	18
H13	14
H14	17
H15	1
H16	4
H17	15
H18	16
H19	1
H20	31
H21	18
H22	1
H23	10
H24	18

Taxa	%	Taxa	%
Taraxacum officinale	**85**	*Bellis*	15
Trifolium repens	**65**	*Crepis*	15
Rubus fruticosus	**60**	*Calluna vulgaris*	15
Trifolium	**55**	*Ononis*	15
Rosa	**55**	*Quercus*	15
Prunus	**50**	*Digitalis / Antirrhinum / Veronica*	15
Cirsium	40	*Epilobium*	15
Brassica napus	40	*Festuca*	15
Sambucus / Viburnum	40	*Poa pratensis*	15
Solidago	35	*Filipendula ulmaria*	15
Impatiens glandulifera	35	*Ribes*	15
Centaurea	25	*Erigeron*	10
Brassica	25	*Cornus*	10
Polygala	25	*Hydrangea*	10
Oxalis	25	*Philadelphus*	10
Crataegus monogyna	25	*Fabaceae*	10
Malus	25	*Lathyrus*	10
Hypochaeris	20	*Trifolium pratense*	10
Brassica oleracea	20	*Vicia sativa*	10
Erysimum	20	*Castanea sativa*	10
Escallonia	20	*Centaurium*	10
Ulex	20	*Geranium*	10
Persea	20	*Euphrasia*	10
Salix	20	*Chamerion angustifolium*	10
Holcus lanatus	20	*Agrostis capillaris*	10
Cotoneaster	20	*Arrhenatherum elatius*	10
Sorbus	20	*Athyrium filix-femina*	10
Saxifraga	20		

Taxa most frequently found in 20 honeys

Conclusions so far…

- Honey bees use a wide number of plants BUT only a small number are frequently used.
- In the spring the commonly used plants are native or near native trees, shrubs and some herbs, often found in deciduous woodlands and hedgerows.
- Garden plants are used at lower levels and only a small fraction of those available.
- It is important that honey bees have access to native habitat.
- BUT if a few plants provide most resources, why use the other plants – are they providing the nutrients required for a balanced diet?

Bee Garden


PEILLIO - BETH SY'N MYND YMLAEN?
POLLINATION - WHAT'S IT ALL ABOUT?
HUNAN-BEILLIO
SELF-POLLINATION
CROESBEILLIO
CROSS-POLLINATION
PAILL AR ADAIN Y GWYNT
LOVE IS IN THE AIR

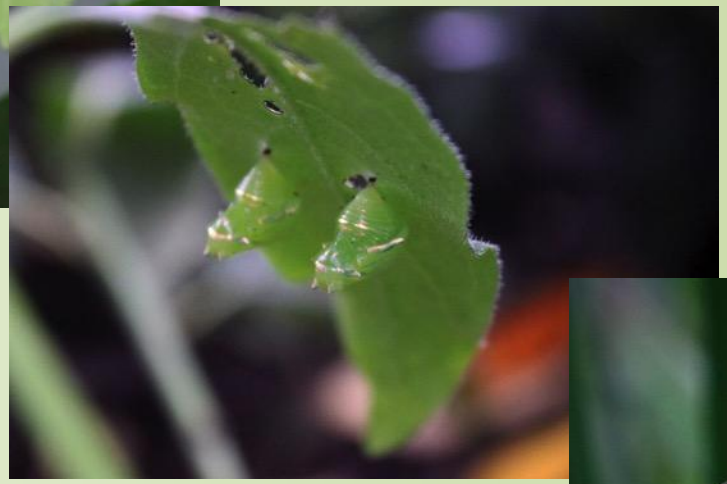
Art-Science

PLAS PILIPALA
BUTTERFLY HOUSE

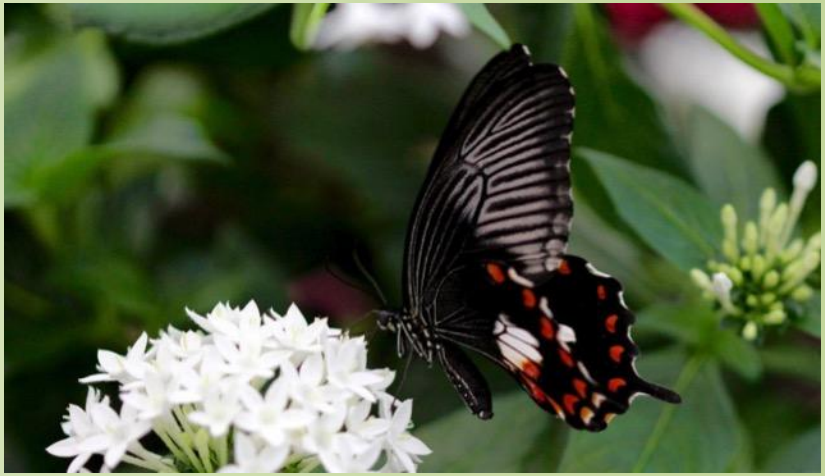
AR AGOR NAWR OPEN NOW

National Botanic Garden of Wales
Gardd Fotaneg Genedlaethol Cymru

Butterflies breeding: Glasswings, *Greta oto*



Butterflies breeding:
Papilio polyetes, Common Mormon



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