Effectively conserving a species' genetic variation ex situ: the case of *Fraxinus excelsior* in the UK







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Royal Botanic Gardens, Kew UK National Tree Seed Project Millennium Seed Bank Project



Multiple Purposes: breeding, reforestation/ restoration, preserve endangered flora, basic study of ecology, unknown future use

Challenge: gather a sample of genetic diversity from a plant species' native distribution



Why genetic diversity

- evolutionary potential
- new traits in breeding
- ecosystem function, stability



Clark (2010) Science Bailey (2009) Proc B

Why genetic diversity

- evolutionary potential
- new traits in breeding
- ecosystem function, stability

- USA Endangered Species Act
- Canadian Species at Risk Act
- Convention on Biological Diversity (CBD)
- Global Strategy for Plant Conservation
- UN FAO Forestry Global Plan of Action

"Having a seed bank of ecologically and genetically diverse, source-identified native seed <u>keeps</u> <u>our options open</u>"

Seed Sourcing for Restoration in an Era of Climate Change

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What, where, how to sample

How many

- populations
- maternal plants
- seeds

What spatial distribution

- among populations
- within populations



Efficiency: Limited money, resources, personnel, space, and time (especially for living collections)

Effectiveness: Desire to avoid missing genes that may be lost in wild plants

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Common problem!

How to make an efficient collectionminimum sample size

Brown & Marshall 1975, 1991, 1995
– 50 samples, every population

$$P[A_1, A_2] = 1 - (1 - p_1)^n - (1 - p_2)^n + (1 - p_1 - p_2)^n$$

How to make an efficient collectionminimum sample size

Brown & Marshall 1975, 1991, 1995
– 50 samples, every population

About 60% of protocols use this recommendation of 50 plants

How to make an efficient collectionminimum sample size

Simplifying assumptions:

- No genetic structure
- No spatial patterns
- Always random mating

Overall assumption: All plants are the same

How to make an efficient collectionminimum sample size

Simplifying assumptions:

- No genetic structure
- No spatial patterns
- Always random mating

Assumptions are a part of science...

Which assumptions are too unrealistic? How do they affect outcomes? Calls for trait-based protocols

CPC 1991

Way 2003

Guerrant 2004, 2014

Hoban et al 2015



Hoban, Fraga, Richards, Strand & Schlarbaum. 2015. Biological Conservation. "Developing quantitative seed sampling protocols using simulations" How to quantitatively guide collections, based on traits: geographic distribution, dispersal, rarity type, ecology, form, reproductive biology?







Naomi Fraga Rancho Santa Ana BG

Chris Richards, USDA

Allan Strand, C of Charleston

Scott Schlarbaum, Tennessee

Hoban & Schlarbaum. 2014. Biological Conservation.

"Optimal sampling of seeds from plant populations for ex situ conservation ..."

Hoban, Fraga, Richards, Strand & Schlarbaum. 2015. Biological Conservation. "Developing quantitative seed sampling protocols using simulations"

Hoban & Strand. 2015. Biological Conservation. "Ex situ conservation seed sampling can be improved ..."



Heliconia acuminata Herbaceous S America

Open-access data Dryad Spatial and genetic

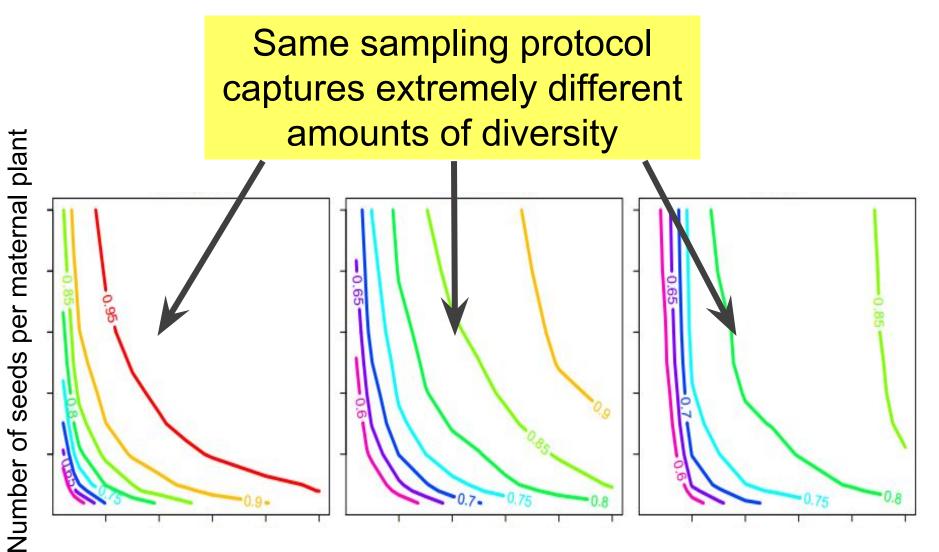




How to sample different species

Dysoxylum malabaricum Tree/ shrub India

> *Quercus lobata* Tree California



Number of maternal plants

A given sampling protocol will capture different amounts of diversity for different species...

Much of our current sampling is suboptimal

Different sampling design for different species- simulations and/or genetic data can help

Fraxinus excelsior in the UK

Case study high priority species

- 11% of area cover of deciduous trees
- 14% of standing volume
- 126 million trees



Fraxinus excelsior in the UK

• Ash is an especially urgent case study





www.kew.or

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Case study high priority species

SPOTTING ASH DIEBACK

Once infected, an ash tree will usually show some or all of the following symptoms



Long, diamond-shaped dark lesions often appear where a branch joins the trunk and around the base of dead shoots.

The veins of leaves – usually pale – can turn brown.

Blackened, dead leaves. Dieback of twigs and branches in the crown of mature ash trees is common, with denser growth below.

Some stats

- First known in Poland 1992
- First UK observation- 2012
- Cannot be stopped or eradicated
- Focus is on building resistance and monitoring
- Currently exists in 57% of 10 km grid cells in England



UK National Tree Seed Project

To provide a national repository of plant material and knowledge

50 woody species in first phase; plans for 70 more species

Collections made by many volunteers in partnership with Woodland Trust, Forestry Commission, Wildlife Trusts, and others (2013-2018)







- 1. An accessible, genetically representative, national seed collection of UK native trees and shrubs
- 2. **Research to understand and overcome constraints** to ex situ conservation and use of UK tree species
- 3. To raise **public awareness** of ex situ conservation, to meet the challenges facing the conservation and management of UK trees



1. An accessible, genetically representative, national seed collection of UK native trees and shrubs







Sampling Strategy



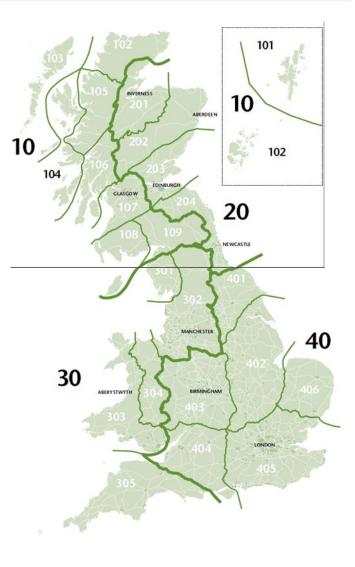
- >15 individuals
- Seed collected from across the canopy of each tree
- ~10,000 seeds per population

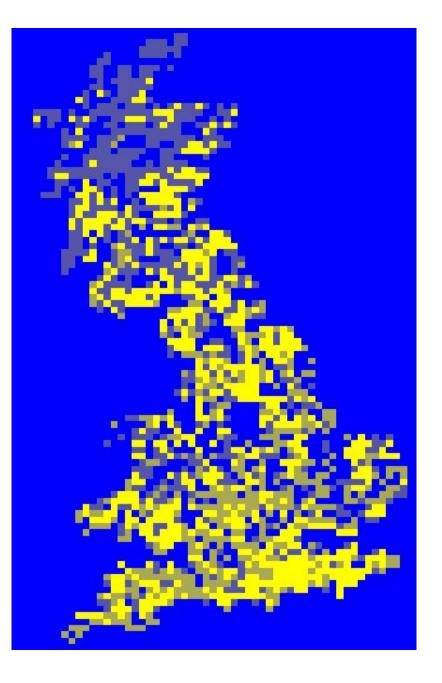
Each mother tree tagged and geo-referenced, and seed banked separately

No selection for any traits



"A species will be 'fully represented' when it has been collected from every seed zone (24) in which it occurs or from all genetically distinct populations where this is known"





Sampling Questions



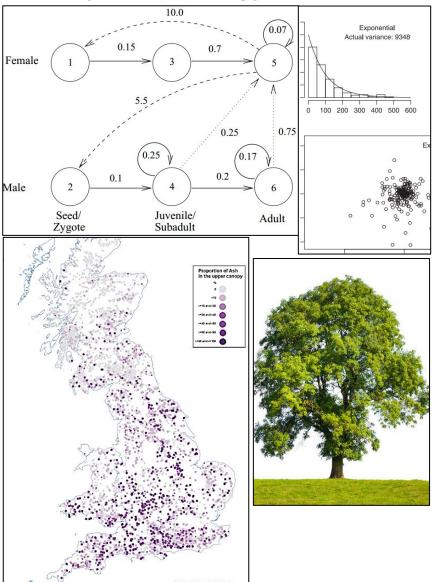
- Have we adequately captured the genetic diversity in the UK with 48 sites, 583 trees and 1.19 million seed collected?
- 2. How could sampling have been done differently?
- Going forward, should we sample from more populations, or return to known sites and collect from additional trees?
- 4. How good is the advice for collectors in the NTSP manual?
- 5. Are 50 individual trees needed?



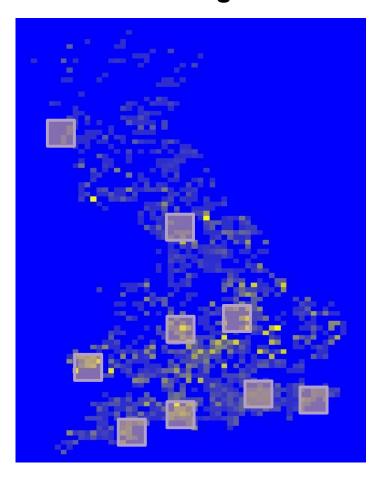
Case study for one species

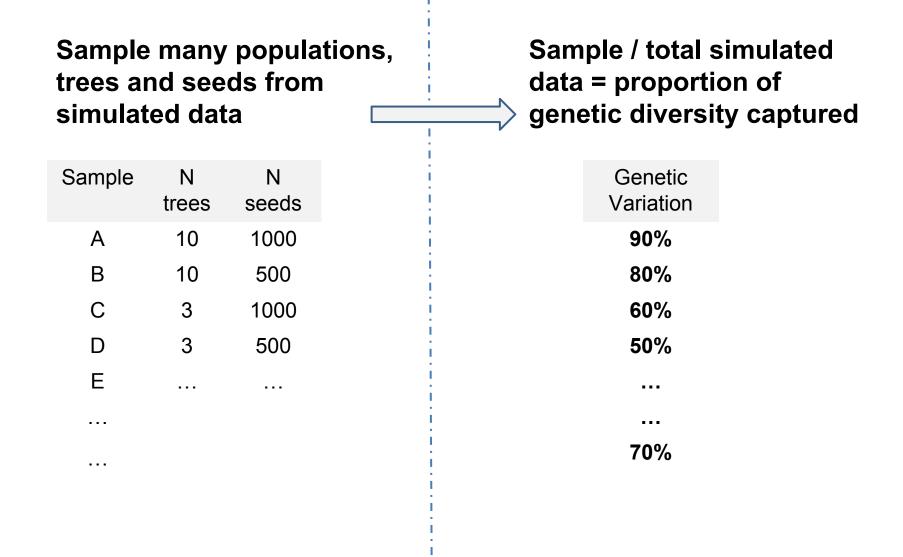
How we test sampling strategies

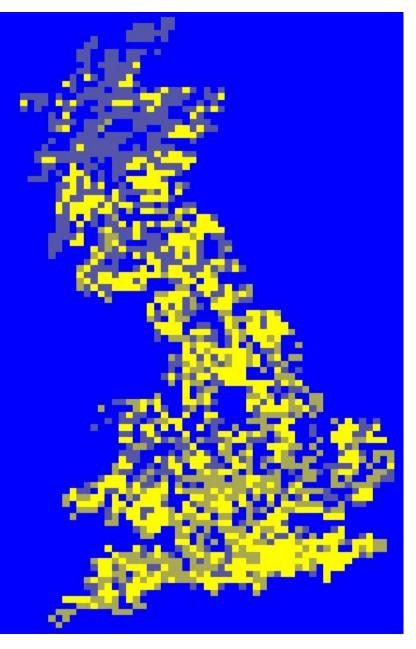
Demographic-genetic model built on data and theory, tree biology



Simulation of the model produces data files of distribution of trees and their genes







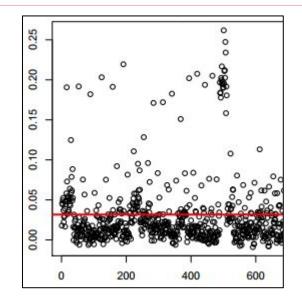
Parameters of this model result in genetic differentiation that is observed in a real genetic

Molecular biodiversity and population structure in common ash (*Fraxinus excelsior* L.) in Britain: implications for conservation

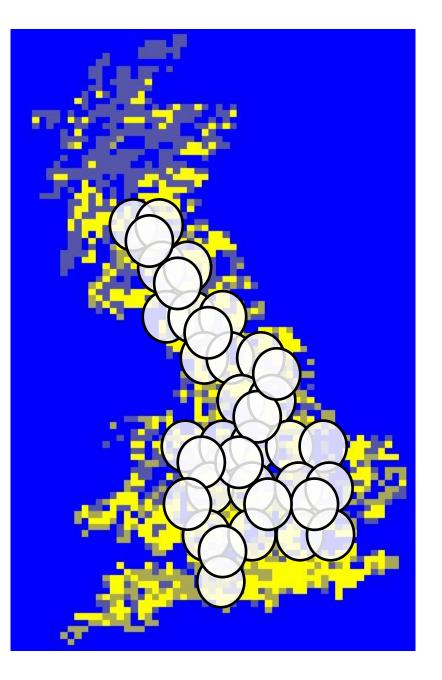
study (FST etc.)

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*East Malling Research, New Road, East Malling, Kent ME19 6BJ, UK, †Forest Research, Northern Research Station, Rosl Midlothian EH25 9SY, UK

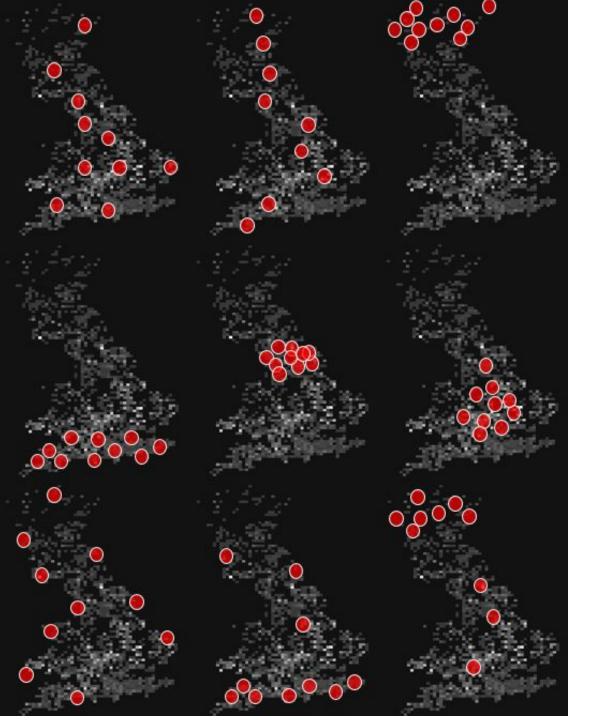


Results will be preliminary and we have to further refine our model



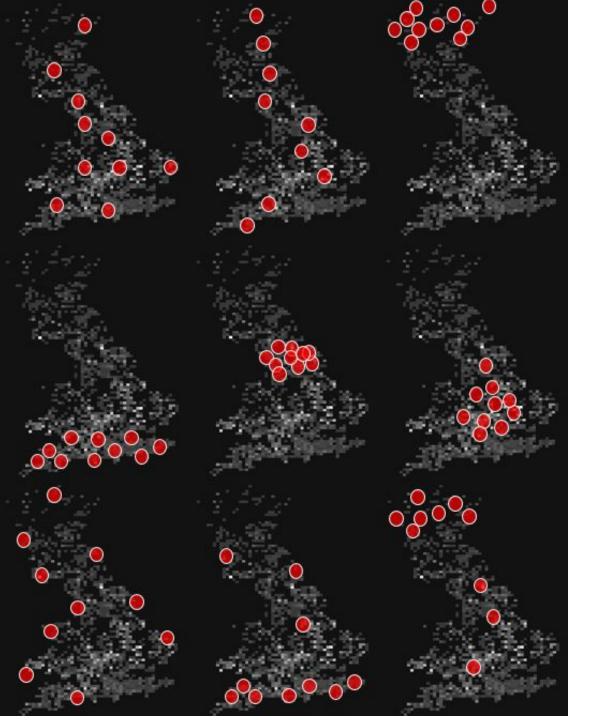


Percent captured in different allele categories		
Global alleles (all)	Globally rare alleles	Locally common alleles
91.3%	77.9%	96.1%



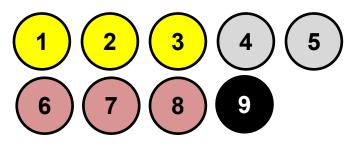


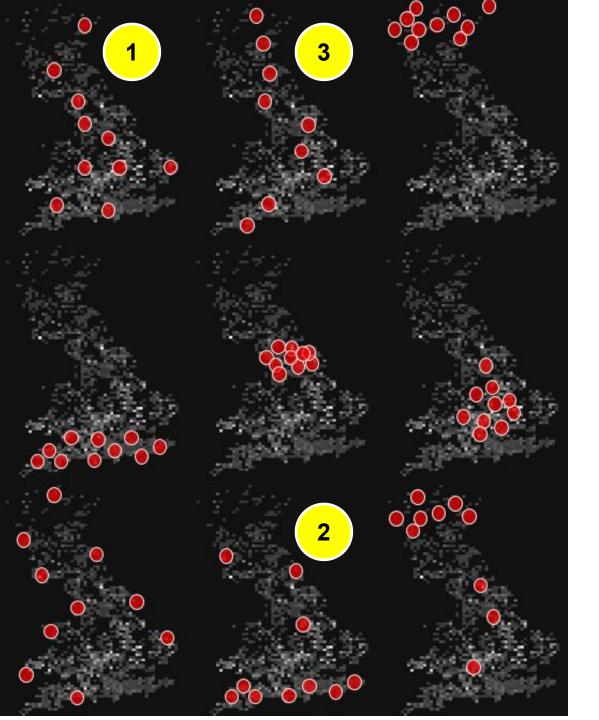
Some possible sampling strategies... let's rank them





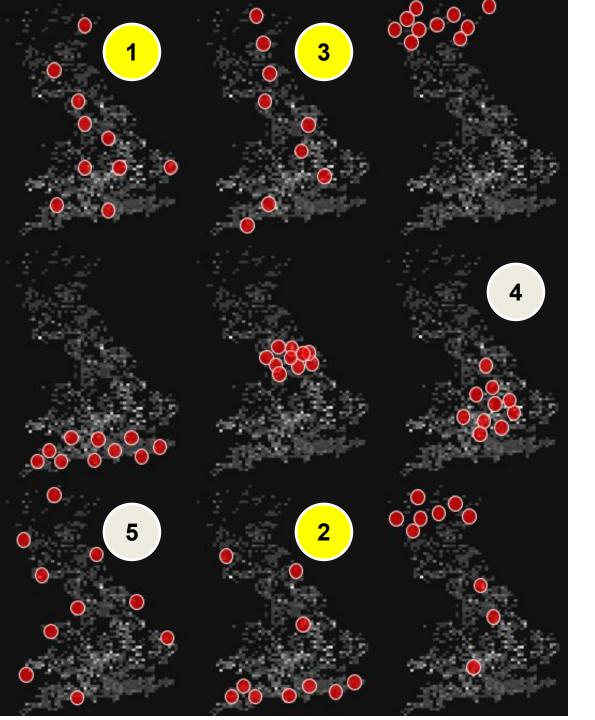
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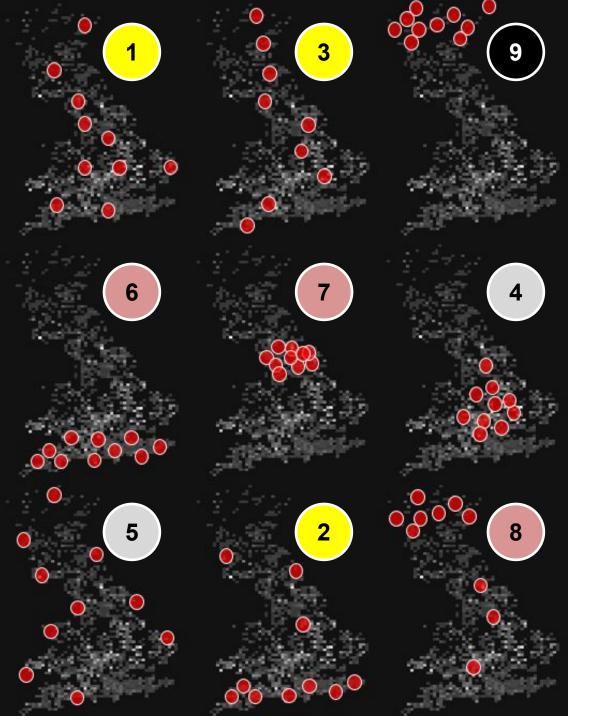




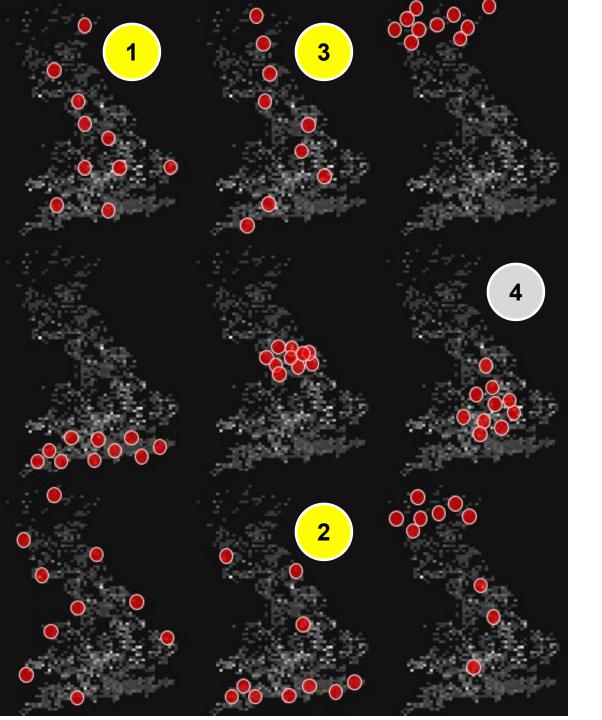
Assuming a moderate effort of about 20 sites







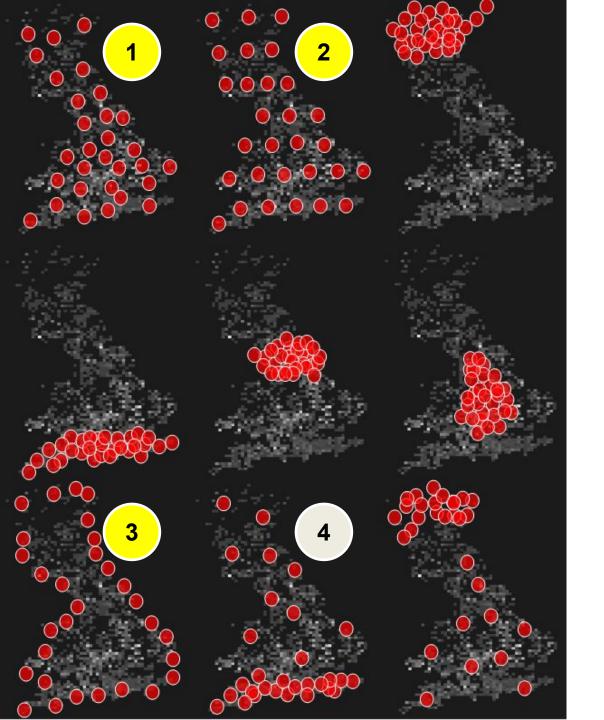






If can only sample few populations (5-10) due to logistical constraints, sample in south and core

Where ash is most abundant- majority of genetic variants

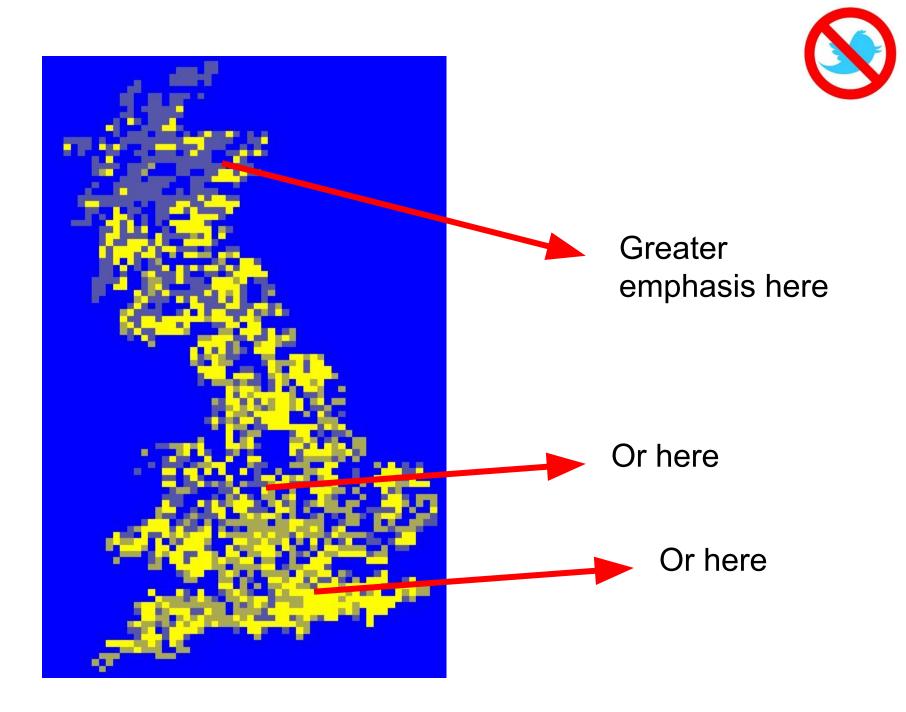




If I can sample large numbers (25-50) of populations, sample widely including the edges

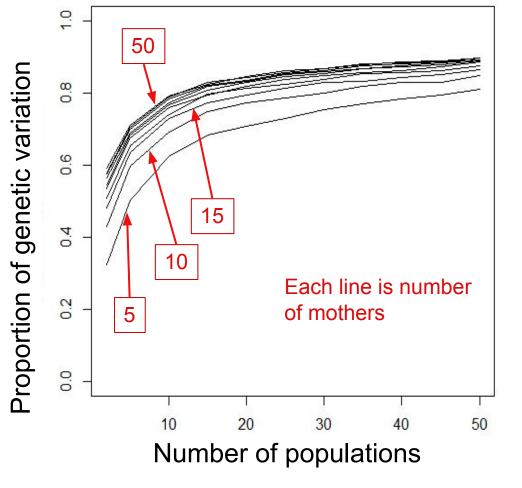
Where the rest of the genetic variation isalleles drift in more isolated populations, which are rare/ absent in core of range

As predicted! Lesica and Allendorf, 1995

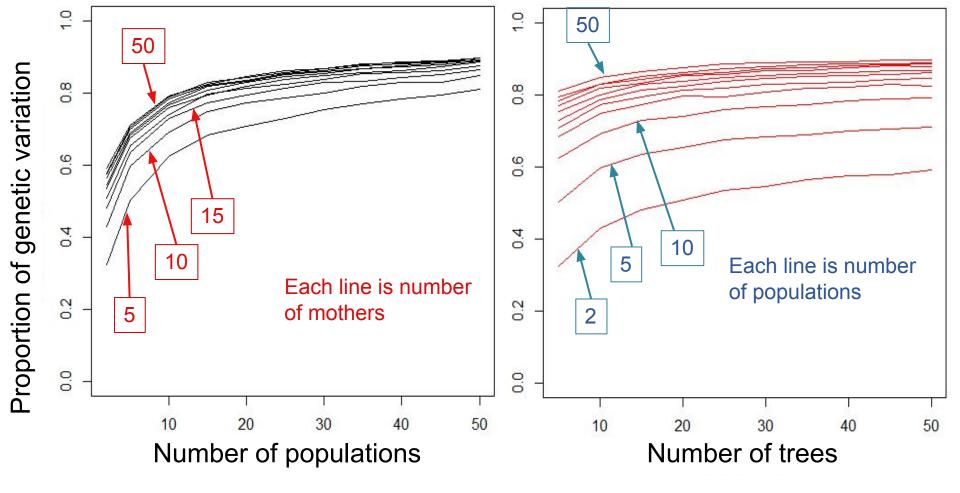




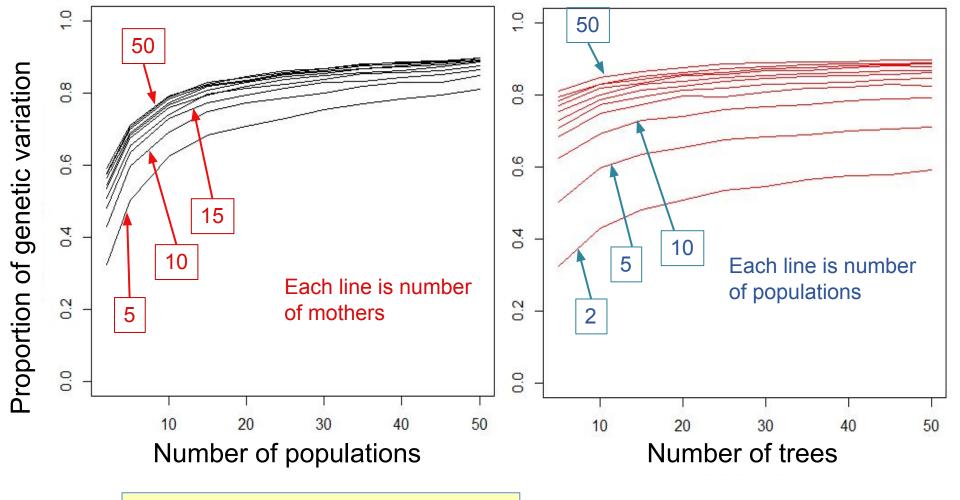
How many populations, trees and seeds?











Main points:

- Bigger gain for adding more populations than more trees
- Diminishing returns in both cases, after 20-30

Two genetically equivalent collections



- 5 trees from each of 35 populations
- (175 total trees sampled)

35 trees each from 10 populations

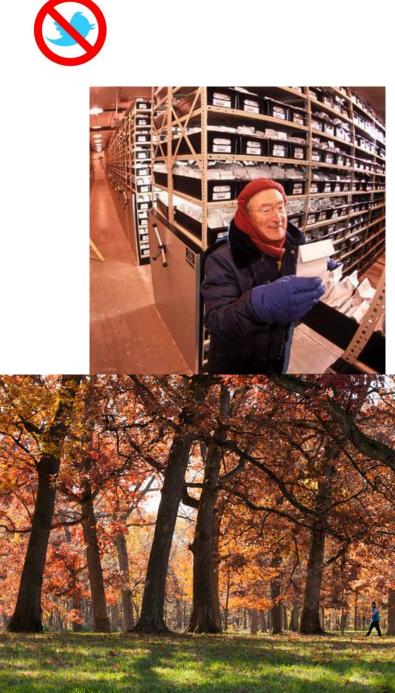
• (350 total trees sampled)

Where are your logistical constraints?

Two genetically equivalent collections

- 5 trees from each of 35 populations
- (175 total trees sampled)
- 35 trees each from 10 populations
- (350 total trees sampled)
- Where are your logistical constraints?







How could UKNTSP have been more efficient?





Number of maternal trees collected from	1-5	5-10	10-15	16-20	21-25	>25
Number of MSB sites sampling that number of trees	17	4	12	9	4	3

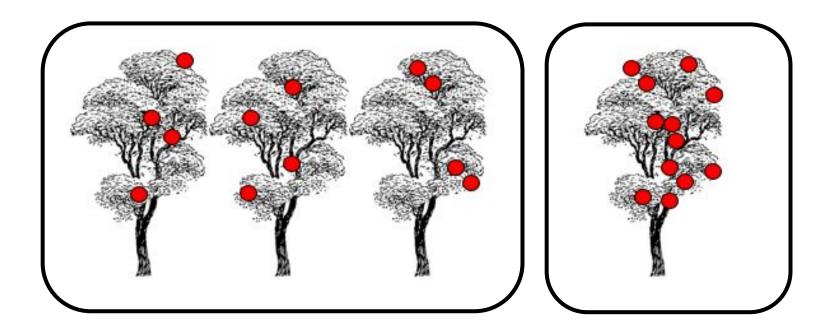


How could UKNTSP have been more efficient?

53% of sites sampled ten or fewer trees37% of sites sampled five or fewer28% of sites sampled three or fewer14% of sites only sampled one tree

A lot of tree collections collect from only few maternal plants

Trees are harder to access Produce a lot more seed Makes sense for the collector Collectors do get more seed per plant

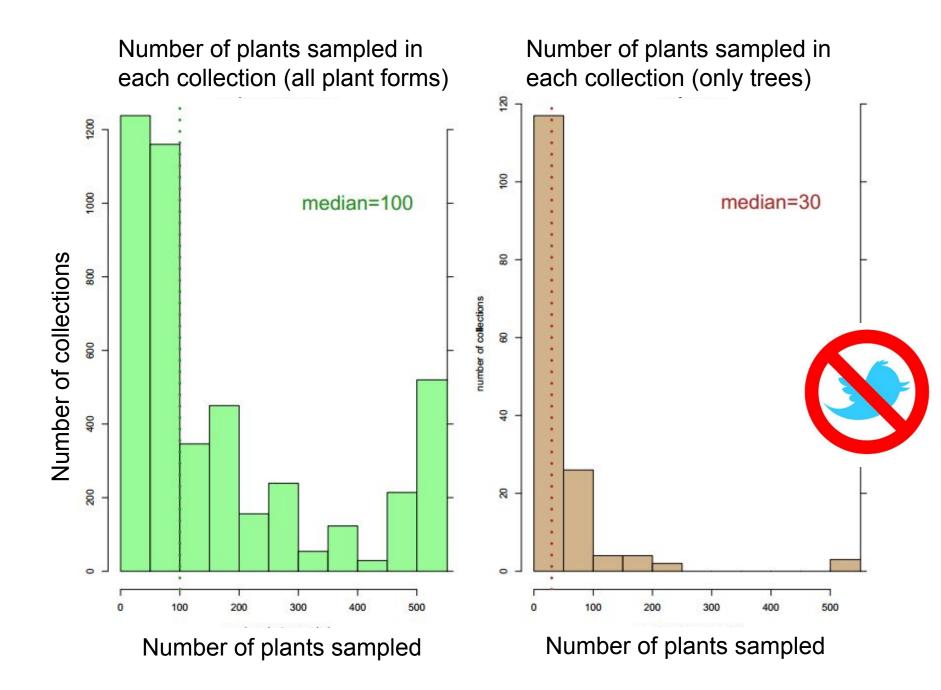






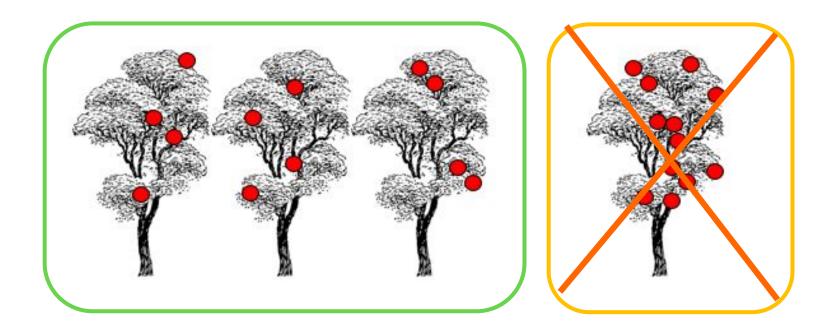
- Michael Way
- Analysis of >5000 collections
- From North America from 1970s to present
- Royal Botanic Gardens Kew, Millennium Seed Bank Partnership

Hoban & Way. 2016 Samara. "Improving the sampling of seeds for conservation..."



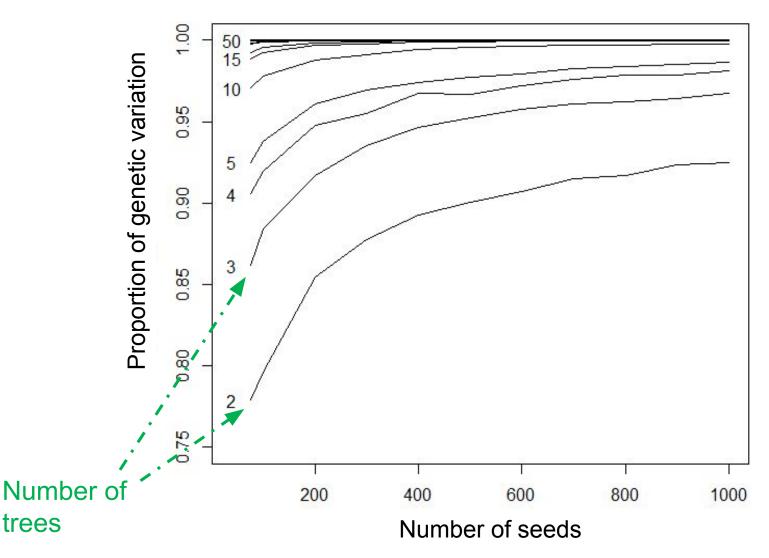
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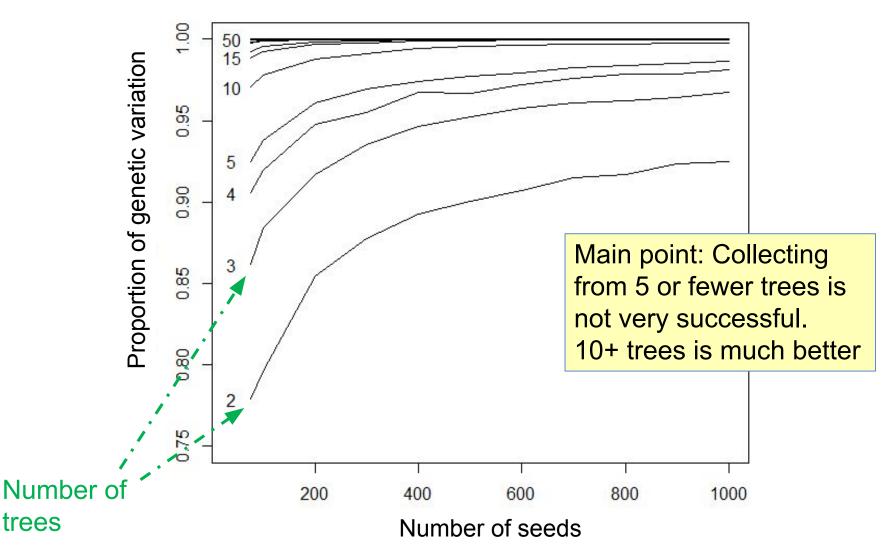


How could UKNTSP have been more efficient?





How could UKNTSP have been more efficient?





Overall seedlot can be much smaller if collectors can visit many trees

Two genetically equivalent collections

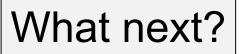
- 3 trees and 100 seeds per tree
- (3000 total seeds)
- 30 trees with 10 seeds per tree
- (300 total seeds)

Summary of findings for ash

- MSB has likely captured at least one copy of the vast majority of alleles within the UK native range
- Random and stratified sampling are best, followed by a focus on southern populations, and range edges
- Sampling more populations better than sampling more trees
- Protocols in UKNTSP seed collecting manual are sound: visiting each "seed zone", sampling from as many trees as possible
- Fewer than 50 trees (10 to 20) are ok if many seeds (1000) are collected per tree; *can sample less in range edge*



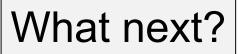
We can do this for other priority species (threatened, economically or culturally important)





End goal would be a software/ tool that can:

Assist with sample planning based on species traits, different genetic goals, collection management, economic costs





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Assist with sample planning based on species traits, different genetic goals, collection management, economic costs

But how much does a species need? How much do we need?

Discussions: Rafael Zenni, lab of Paul Armsworth, lab of Joe Bailey, Dan Schoen, Jeanne Romero-Severson, Oscar Gaggiotti, Giorgio Bertorelle, Mike Bruford, Andrew Hipp, Drew Duckett, Jill Hamilton, Patrick Griffith

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Visits: Royal Botanic Gardens Kew, Rancho Santa Ana Botanic Garden, USDA National Center for Genetic Resource Preservation



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This and additional presentations available at http://nativeseed.info





