Collecting Genetic Variation on a Small Island[1]

S. Kallow[2] and C. Trivedi[2]

Abstract

Genetic variation is the most powerful factor in ensuring the long term success of trees and forests in times of change. In order to protect against loss of genetic variation from threats, including pests and diseases and climate change, the Royal Botanic Gardens, Kew, is developing a national tree seed collection for the United Kingdom.

This paper describes the authors methodologies and experiences of developing the national collection: firstly, forming species target lists using plant health risk and conservation assessments; then, developing national sampling strategies based on the distribution of target species in biogeographic zones; and, finally, how collecting strategies were designed to capture and preserve genetic variation within populations.

Additionally, we discuss social and ecological factors taken into account when developing and implementing the sampling program. These include phylogeography; the history of woodland management, ownership and fragmentation; and introgression risks from plantation and garden escapees. Finally citizen participation is discussed.

Introduction

The Royal Botanic Gardens, Kew launched the United Kingdom National Tree Seed Project (UKNTSP) in 2013 as an *ex situ* seed conservation initiative. The UKNTSP's stated aim is: 'To provide a national repository of plant material and associated knowledge, for the purposes of long term conservation, and to make these resources available to users, in order to better understand and manage tree and shrub species in the United Kingdom landscape.'

In this paper we set out our key considerations and experiences in targeting, sampling and collecting seed for the project.

Developing Target Lists

Native Species

The United Kingdom woody flora consists of 139 native species, 11 archeophytes, (naturalized non-native species introduced prior to 1500), and 57 neophytes (naturalized and introduced after 1500). Native species are considered to be those that recolonized the British Isles between the last ice age and the separation of the British Isles from mainland Europe, around 8 to 10,000 years ago; or those that speciated in the United Kingdom after this period. Re-colonization occurred from glacial refugial populations from Spain, Italy and the Balkans, in the main (Newton et al. 1999).

Prioritization

To prioritize native species for seed banking in the project timeframe, a target list was developed, using a scoring and ranking system. Impacts from invasive species, including pests and diseases, is one of the key drivers for change affecting forest genetic resources (FAO 2014). Plant health, therefore, was a primary factor in our prioritization. We used the United Kingdom Plant Health Risk Register (DEFRA 2014) to prioritize species most at risk. This tool adopts a risk assessment approach to potential plant health threats to hosts, by assessing impact and likelihood and setting out mitigation approaches. 'Unmitigated risk'

---

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

[2] Royal Botanic Gardens, Kew, Millennium Seed Bank, Wakehurst Place, Haywards Heath, Sussex, RH17 6TN, UK. Corresponding author: S.Kallow@kew.org.

assessments for plant health risks were totalled for each host, which were then ranked accordingly. Wider threats to species were scored using Great Britain Red List status (Cheffings et al. 2005). Species were again ranked, those with a higher 'Extinction Risk' threat status received greater priority. Finally, as a measure of potential ecological impact at a landscape-scale, 'prevalence in the landscape' was also used to rank targets, using the PLANTATT database (Hill et al. 2004); species that had wider distributions received greater priority. For each ranking 'Plant health risk' was used as a secondary factor in ranking if species received the same score, for example both *Pinus sylvestris* and *Acer campestre* have a Red List status of 'Least Concern', but *P. sylvestris* received a higher rank in this category because there is a greater 'Plant health risk' for this species. See table 1 for a list of target taxa and prioritisation data.

Table 1—Prioritization list of target taxa

Taxon name	Unmitigated risk total score	Risk rank	Red list GB[a]	Red list rank	No. 10 km^2 in GB	Distri-bution rank	Native status[b]	Native rank	Total rank
Acer campestre	745	25	LC	68	1389	37	N	62	192
Alnus glutinosa	420	46	LC	85	2478	8	N	80	219
Betula nana	591	45	LC	84	125	77	N	79	285
Betula pendula	591	44	LC	83	2293	21	N	78	226
Betula pubescens	651	30	LC	72	2399	15	N	66	183
Buxus sempervirens	54	111	DD	13	2	112	N	108	344
Carpinus betulus	281	51	LC	90	1488	35	N	84	260
Cornus sanguinea	182	56	LC	95	1179	41	N	89	281
Crataegus laevigata	153	58	LC	97	597	59	N	91	305
Crataegus monogyna	233	54	LC	93	2496	7	N	87	241
Erica vagans	16	115	LC	116	6	99	N	110	440
Fagus sylvatica	280	52	LC	91	2397	16	N	85	244
Fraxinus excelsior	407	47	LC	86	2459	11	N	81	225
Ilex aquifolium	183	55	LC	94	2353	17	N	88	254
Juniperus communis	147	59	LC	98	1020	46	N	92	295
Juniperus communis subsp. *hemisphaerica*	87	65	CR	1	2	107	N	98	271
Lonicera periclymenum	0	120	LC	119	2622	1	N	115	355
Malus sylvestris	1175	19	LC	62	2023	26	N	56	163
Pinus sylvestris	2114	2	LC	46	65	83	N	39	170
Prunus avium	2225	1	LC	45	2136	24	N	38	108
Prunus padus	1901	4	LC	48	1089	44	N	41	137
Prunus spinosa	1901	3	LC	47	2308	20	N	40	110
Pyrus cordata	642	31	VU	32	9	94	NA	128	285
Rubus idaeus	914	22	LC	65	2425	13	N	59	159
Sambucus nigra	4	118	LC	118	2457	12	N	113	361
Sorbus admonitor	72	87	EN	24	1	122	NE	22	255

Sorbus anglica	72	99	NT	41	12	93	NE	34	267
Sorbus aria	72	106	LC	109	341	67	N	103	385
Sorbus arranensis	72	96	VU	36	1	130	NE	31	293
Sorbus arvonicola	72	67	CR	3	1	113	NE	3	186
Sorbus aucuparia	*72*	103	LC	106	2472	9	N	100	318
Sorbus bristoliensis	72	88	EN	25	1	123	NE	23	259
Sorbus cambrensis	72	84	EN	21	2	109	NE	19	233
Sorbus cheddarensis	72	68	CR	4	1	114	NE	4	190
Sorbus cuneifolia	72	89	EN	26	1	124	NE	24	263
Sorbus devoniensis	72	101	LC	104	32	89	NE	36	330
Sorbus domestica	72	76	CR	12	4	101	N	99	288
Sorbus eminens	72	77	EN	14	9	95	NE	12	198
Sorbus eminentiformis	72	85	EN	22	2	110	NE	20	237
Sorbus eminentoides	72	69	CR	5	1	115	NE	5	194
Sorbus evansii	72	90	EN	27	1	125	NE	25	267
Sorbus greenii	72	91	EN	28	1	126	NE	26	271
Sorbus herefordensis	72	92	EN	29	1	127	NE	27	275
Sorbus lancastriensis	72	100	NT	42	9	96	NE	35	273
Sorbus leighensis	72	93	EN	30	1	128	NE	28	279
Sorbus leptophylla	72	81	EN	18	3	104	NE	16	219
Sorbus leyana	72	66	CR	2	2	108	NE	2	178
Sorbus margaretae	72	79	EN	16	5	100	NE	14	209
Sorbus minima	72	97	VU	37	1	131	NE	32	297
Sorbus parviloba	72	70	CR	6	1	116	NE	6	198
Sorbus porrigentiformis	72	102	LC	105	30	90	NE	37	334
Sorbus pseudofennica	72	98	VU	38	1	132	NE	33	301
Sorbus pseudomeinchii	72	71	CR	7	1	117	NE	7	202
Sorbus richii	72	86	EN	23	2	111	NE	21	241
Sorbus rupicola	72	107	LC	110	98	81	N	104	402
Sorbus rupicoloides	72	72	CR	8	1	118	NE	8	206
Sorbus saxicola	72	73	CR	9	1	119	NE	9	210
Sorbus spectans	72	94	EN	31	1	129	NE	29	283
Sorbus	72	82	EN	19	3	105	NE	17	223

stenophylla									
Sorbus stirtoniana	72	74	CR	10	1	120	NE	10	214
Sorbus subcuneata	72	95	VU	35	4	103	NE	30	263
Sorbus torminalis	*72*	105	LC	108	*573*	60	N	102	375
Sorbus vexans	72	80	EN	17	4	102	NE	15	214
Sorbus whiteana	72	83	EN	20	3	106	NE	18	227
Sorbus wilmottiana	72	75	CR	11	1	121	NE	11	218
Taxus baccata	57	110	LC	113	1881	28	N	107	358
Tilia cordata	72	104	LC	107	896	51	N	101	363
Tilia platyphyllos	72	108	LC	111	84	82	N	105	406
Ulmus glabra	659	29	LC	71	2338	18	N	65	183

[a] Red list Great Britain (GB): Not Evaluated (NE), Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically endangered (CR).
[b] Native status: Native (N), Native endemic (NE), Native or alien; Native status doubtful (NA).

Practical and Technical Considerations

The Millennium Seed Bank Seed Information Database (Royal Botanic Gardens, Kew 2008) was used to identify the seed storage behavior of target species. Recalcitrant *Quercus* species, and short lived Salicaceae were removed from the target list, as these cannot be dried and stored under conventional seed bank conditions or require careful post-harvest handling, not considered possible when using volunteer seed collectors for much of the collecting. Similarly, taxonomically complex woody taxa including most *Rubus* and *Rosa* species were removed to reduce problems of identification. The *Sorbus* micro-species were agreed as targets as, while taxonomically complex, they are well-recorded in the United Kingdom, have limited distribution, and there was an expert available to collect them. All *Ulmus*, apart from *U. glabra*, were removed because of the on-going effects of the Dutch elm disease (caused by species of *Ophiostoma*) outbreak which limits the ability of elm trees to reach maturity. *Ulmus glabra* is less susceptible than other *Ulmus* to Dutch elm disease, and has a greater propensity for sexual reproduction and therefore seed production. The resulting ranked target list was used as the basis for a project target list of 70 taxa which was agreed upon following further discussions by the project advisory group, a group of professionals from both the forestry and conservation sector brought together for oversight and technical advice.

Sampling Strategy

National Level

Unfortunately, most published research dealing with the genetic diversity and structure of United Kingdom woody species is only at the European scale, providing an insufficient basis for a national sampling strategy. The Forestry Commission (the United Kingdom government department responsible for forestry), has developed a seed zone map to guide seed transfer under the Forest Reproductive Material (Great Britain) Regulations 2002 legislation implemented under the EC Directive 1999/105. The map divides Britain into four broad regions of provenance and 24 smaller native seed zones; each zone is further divided by elevation above and below 300 m (Herbert et al. 1999) (fig. 1). Zones are based on biogeographic factors, such as watersheds and climate as well as man-made barriers which may influence dispersal and gene-flow, such as major roads. We decided to use the seed zone map as a framework for our sampling strategy, aiming for a seed collection in each seed zone where there is a native population for each of our target species, and an additional collection over 300 m, where this is possible. This standardised approach was adopted across all target taxa.

The native distribution of each target taxa was mapped against each seed zone using the Atlas of the British and Irish Flora (Preston et al. 2002), excluding the introduced distribution, based on historical pollen records and archaeological findings. Unfortunately, elevation analysis is not provided by the Atlas. The open access data from the Shuttle Radar Topography Mission (Jarvis et al. 2008), and records from the Botanical Society of Britain and Ireland database (BSBI 2016) were used to estimate the elevation of specific records. Estimates of occurrence over 300 m in seed zones were then confirmed on the ground by partners in seed zones 105, 108, 109 and 204, to validate the desk-based analysis, in order to produce a target list which is both vertically and horizontally distributed. This resulted in a total of 680 target seed collections.

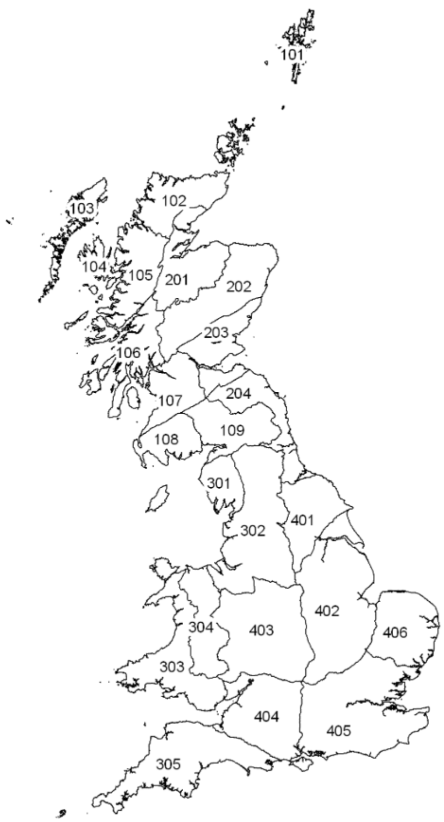

Figure 1—Native seed zones in Great Britain (Herbert et al. 1999).

This sampling strategy is a highly-effective way of sampling both common and rare locally adapted alleles (Hoban and Schlarbaum 2014), which are ecologically and economically important for conservation. Additionally, this form of sampling allows for conservation of the spatial structure of populations, which provides important data for research and restoration use of the collections. However, as we are using a standard strategy for all species which is based upon assumptions of genetic diversity and structure, rather than actual data, the strategy risks missing important species-specific factors and potentially over or under sampling.

One further implication of our sampling approach is that some of the populations collected from are at the northwestern edge of their ranges. Range-edges have profound implications on seed collecting programs, for example, marginal population may have decreased population density or reduced seed production (Garcia et al. 2000, Rasmussen and Kollmann 2004). Anecdotally, both the number of trees and number of seeds collected for the project have been less at range edges. Such peripheral populations may be adapted to the extremes possible for the species, and are, therefore, important sources of evolutionary potential, especially in the context of climate change (Fady et al. 2016, Havens et al. 2015).

The north and west are also the most deforested regions of the United Kingdom; therefore, also reducing seed sample sizes. The project is therefore re-assessing how to maximize sampling in these regions.

Sampling From Appropriate Sites

To meet our project aim, it is important that seeds are sampled from autochthonous, locally-adapted populations. As the United Kingdom is one of the least forested countries in Europe, with around 12 percent forest cover in contrast to averages in Europe of 45 percent and the United States of 40 percent (World Bank 2016a), this can be a challenge. Average woodland size (including exotic plantations), is only 7.9 ha in the United Kingdom overall, and only 4.9 ha in England (Watts 2006). The forest which once covered Britain was cleared for agriculture, mainly in the Bronze and Iron Age (Rackham 2001). The remaining ‘ancient semi-natural woodland’ (defined as having a continuous woodland cover since the earliest maps of 1600), is highly fragmented and now accounts for only 2 percent of land cover (Atkinson and Townsend 2011). The remaining woodlands also have a long history of management, usually by coppicing and favouring ‘useful’ species (Rackham 2001). There is, therefore, a high probability that any sampled population is not strictly autochthonous. There is an inevitable risk, therefore, that seed collected for the project may result from planted or naturalized trees originating from other parts of the United Kingdom or abroad, and in some cases form hybrid progeny. Indeed, the challenge is so great that even when we hand-pollinated native black poplars (*Populus nigra* subsp. *betulifolia*) and excluded pollen using bags, DNA analysis showed several resulting progeny had been pollinated by exotic, but abundant hybrid poplar (*Populus* x *euramericana*) (Gargiulo, personal communication, 2016). Resources such as the Native Woodland Survey of Scotland (Forestry Commission Scotland 2016), the Ancient Woodland Inventory (Natural England 2015), and sites protected under the Wildlife and Countryside Act (1981), and the EC Habitats Directive have been invaluable in guiding seed collectors to autochtonous populations. While every effort has been made to collect from such populations, we recognize that sampling is of the current genepool, which includes introduced material.

Sampling Strategy at the Seed Zone

As a citizen science project working with a wide range of, mostly voluntary sector, partner organizations, we had to be realistic and pragmatic when setting an achievable sample size. Standard population sampling guidelines advise collection from either 30 randomly chosen individuals in a fully out-crossing species, or 59 random individuals in a self-fertilizing species (Brown and Marshall 1995) in order to collect 95 percent of the non-rare alleles present in a population. For many of our targets, collecting from this number of individuals in a population is not possible, because of the limited numbers of accessible seed-bearing plants. Even when populations were large enough, practical experience in the first year of the project demonstrated that collecting from 15 individuals in a day represented good effort. We therefore agreed to aim for sampling 10,000 seeds from at least this many individuals, where possible. This number of seeds is enough for some to be used for standard MSB germination tests, and allows for others to be available for distribution to researchers and conservation projects, while maintaining a sizeable sample in the seed bank.

In order to maximize the genetic diversity of the sample, collections were taken throughout the population, and over 50 m apart where possible; and seeds were collected from across the canopy at various places, to maximize the number of pollen sources. Individual mother trees were georeferenced using a GPS and were also physically tagged with a project aluminium tree tag. The seed from each mother tree was collected and stored separately in order to maximize the potential use of the collections for research and breeding. On every occasion, herbarium specimens accompanied a collection, for identification. In some cases tissue samples were collected for dry storage in silica gel, these can be used for DNA extraction and genomic analysis.

Building a Network of Seed Collectors

Although the United Kingdom is one of the least forested and most densely populated countries in Europe, with 267 people per km^2 compared to an European Union average of 116, and United States of 35 people per km^2 (World Bank 2016b), many people have easy access to local woodlands. In fact, 65 percent of the population live within 4 km of a 20 ha woodland (The Woodland Trust 2010). The link between health and wellbeing and access to natural environments, such as woodlands, is increasingly being valued (DEFRA 2012). It was important for the project, therefore, to develop an approach which capitalised on local community engagement in woodlands and forests, by working with conservation non-governmental organizations (NGOs). Working with land-owning NGOs also increased access to collecting sites, particularly useful as state ownership of woodlands and forests in the United Kingdom is also relatively low, at around 28 percent (DEFRA 2012).

Since the project began in 2013, seed collecting partnerships have been developed with over 30 organizations. Small local groups, for example Cree Valley Community Woodland Trust in Dumfries and Galloway, Scotland, or Suffolk Wildlife Trust, England, have collected the full target list in their respective seed zones. Locally-based NGOs, with access to woodland and existing experience in seed collecting with a team of engaged volunteers, are an incredible resource for the UKNTSP. Partners like this are able to monitor the availability and maturity of seed for collection without having to travel long distances. We also worked with the Forestry Commission, which led on collecting ash (*Fraxinus excelsior*), yew (*Taxus baccata*) and juniper (*Juniperus communis*) right across the United Kingdom.

In order to communicate the sampling strategy and collecting methodology to NGOs and volunteers, we produced a seed collecting manual (Kallow 2013), and ran training sessions across the United Kingdom each year. We developed a standard memorandum of collaboration and grant agreement in order to provide funding to partners and to agree the terms of ownership and transfer of seed collections, herbarium vouchers and data. We devised a new landowner consent form for use across the project partnership and made consent agreements with statutory agencies for collecting from protected sites. Working with these NGOs has also provided an opportunity to raise public awareness of forest conservation and *ex situ* conservation.

To conclude, the UKNTSP took into account scientific knowledge, existing national infrastructures, and engaged with local communities to build a seed repository which provides an important resource of genetic material and knowledge to help the conservation and management of trees and forests for the future.

Acknowledgments

Funding for the United Kingdom National Tree Seed Project has been kindly provided by the players of People's Postcode Lottery. Thank you to Michael Way, Christopher Cockel and Ted Chapman (Royal Botanic Gardens, Kew) for comments on this paper.

Literature Cited

Atkinson, S.; Townsend, M. 2011. The state of the UK's forests, woods and trees: perspectives from the sector. Grantham, England: Woodland Trust.

Brown, A.H.D.; Marshall, D.R. 1995. A basic sampling strategy: theory and practice. In: Guarino, L.; Ramanatha Rao, V.; Reid, R., eds. Collecting plant genetic diversity: technical guidelines, Wallingford, UK: CAB International: 75–91.

Botanical Society of Britain and Ireland [BSBI]. 2016. BSBI distribution database. http://bsbidb.org.uk/. (3 May 2017).

Cheffings, C.M.; Farrell, L., eds; Dines, T.D.; Jones, R.A.; Leach, S.J. [et al.]. 2005. The vascular plant red data list for Great Britain. Peterborough: Joint Nature Conservation Committee.

Department for Environment, Food and Rural Affairs [DEFRA]. 2012. Independent panel on forestry – final report. Department for Environment Food and Rural Affairs, UK.

Department for Environment, Food and Rural Affairs [DEFRA]. 2014. UK plant health risk register. Department for Environment, Food and Rural Affairs, UK. https://secure.fera.defra.gov.uk/phiw/riskRegister/ (3 May 2017).

Fady, B; Aravanopoulos, F.A.; Alizoti, P.; Mátyás, C.; von Wühlisch, G. [et al.]. 2016. Evolution-based approach needed for the conservation and silviculture of peripheral forest tree populations. Forest Ecology and Management. 375: 66–75.

Food and Agriculture Organization [FAO]. 2014. The state of the world's forest genetic resources. Rome: Food and Agriculture Organization, United Nations.

Forestry Commission Scotland 2014. Native woodland survey of Scotland. http://scotland.forestry.gov.uk/supporting/strategy-policy-guidance/native-woodland-survey-of-scotland-nwss. (3 May 2017).

García, D.; Zamora, R.; Gómez, J.M.; Jordano, P.; Hódar, J.A. 2000. Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. Journal of Ecology. 88: 435–446.

Havens, K.; Vitt, P.; Still, S.; Kramer, A.T.; Fant, J.B.; Schatz, K. 2015. Seed sourcing for restoration in an era of climate change. Natural Areas Journal. 351: 122–133.

Herbert, R.; Samuel, S.; Patterson G.S. 1999. Using local stock for planting native trees and shrubs. Forestry Commission Practice Note 8. Edinburgh: Forestry Commission.

Hill, M.O.; Preston, C.D.; Roy, D.B. 2004. PLANTATT. Attributes of British and Irish plants: status, size, life history, geography and habitats. Huntingdon, Cambridgeshire: Centre for Ecology and Hydrology.

Hoban, S.; Schlarbaum, S. 2014. Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. Biological Conservation. 177: 90–99.

Jarvis, A.; Reuter, H.I.; Nelson, A.; Guevara, E. 2008. Hole-filled SRTM for the globe. Version 4. CGIAR-CSI SRTM 90m Database. http://www.webcitation.org/6ndSoZQWJ. (3 May 2017).

Kallow, S. 2013. UK national tree seed project: seed collecting manual. Kew, UK: Royal Botanic Gardens.

Natural England 2015. Ancient woodlands (England). http://www.magic.gov.uk/MagicMap.aspx. (3 May 2017).

Newton, A.C.; Allnutt, T.R.; Gillies, A.C.M.; Lowe, A.J.; Ennos, R.A. **1999**. Molecular phylogeography, intraspecific variation and the conservation of tree species. TREE. 14: 140–145.

Preston, C.D.; Pearman, D.A.; Dines, T.D., eds. 2002. New atlas of the British and Irish flora: an atlas of the vascular plants of Britain, Ireland, the Isle of Man and the Channel Islands. Oxford: Oxford University Press.

Rackham, O. 2001. Trees and woodland in the British landscape. London: Phoenix Press.

Rasmussen, K.K.; Kollmann, J. 2004. Poor sexual reproduction on the distribution limit of the rare tree *Sorbus torminalis*. Acta Oecologica. 25: 211–218.

Royal Botanic Gardens, Kew 2008. Seed information database SID. Version 7.1. http://data.kew.org/sid/. (3 May 2017).

The Woodland Trust 2010. Space for people: targeting action for woodland access. https://www.woodlandtrust.org.uk/publications/2010/07/space-for-people/. (3 May 2017).

Watts, K. 2006. British forest landscapes the legacy of woodland fragmentation. Quarterly Journal of Forestry. 100: 273–279.

World Bank. 2016a. Forest area % of land area. http://data.worldbank.org/indicator/AG.LND.FRST.ZS. (3 May 2017).

World Bank. 2016b. Population density people per sq. km of land area. http://data.worldbank.org/indicator/EN.POP.DNST. (3 May 2017).