DEVELOPING METHODOLOGIES FOR THE GENETIC CONSERVATION OF UK CROP WILD RELATIVES

by

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Abstract

Modern crop varieties contain limited genetic diversity. Demand from an expanding human population requires crop yields to increase substantially, however, a low capacity for adaptation may leave crops susceptible to environmental change, threatening future food security. Crop wild relatives (CWR) are a valuable, but threatened, genetic resource containing more genetic diversity than their cultivated relatives that can be utilised by plant breeders to improve the resilience of new crop varieties. This thesis researches methodologies to facilitate systematic, active conservation of CWR and their genetic diversity within a national context using the UK and its devolved administrations as an exemplar. Methods involve the development of CWR inventories, gap analyses, assessment of CWR habitat preferences, a case study seeking to justify the establishment of the first UK CWR genetic reserve on The Lizard Peninsula, Cornwall and finally, exploration of the use of next-generation sequencing as a tool for targeting collection of accessions to fill genetic gaps in genebanks. A key success of this research was the involvement of stakeholders, bridging the gap between research and practice. The methods used can now be applied to other countries and can be integrated into European and global conservation planning, contributing to improved future food security.

“It always seems impossible until it’s done”

Nelson Mandela

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List of abbreviations

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AONB	Area of Outstanding Natural Beauty
ASSI	Area of Special Scientific Interest
BSBI	Botanical Society of Britain and Ireland
CBD	Convention on Biological Diversity
CF	Change factor
CS	Countryside Survey
CWR	Crop wild relative
CWRIS	Crop Wild Relative Information System
ELC	Ecogeographic Land Characterisation
ESPC	European Strategy for Plant Conservation
GIS	Geographical Information System
GP	Gene Pool
GRS	Geographical representativeness score
GSPC	Global Strategy for Plant Conservation
IBERS	Institute of Biological, Environmental and Rural Sciences
ICARDA	International Center for Agricultural Research in the Dry Areas
IPCC	Intergovernmental Panel on Climate Change
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
IUCN	International Union for the Conservation of Nature
JNCC	Joint Nature Conservation Committee
LNR	Local Nature Reserve
LRC	Local Record Centre
MSB	Millennium Seed Bank
NE	Natural England

NERC	Natural Environment and Rural Communities
NGS	Next-generation sequencing
NNR	National Nature Reserve
NP	National Park
NRW	Natural Resources Wales
NSA	National Scenic Area
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PGR	Plant genetic resource
PGRFA	Plant genetic resources for food and agriculture
PLINC	Plant Link Cymru
RAPD	Random amplification of polymorphic DNA
RFLP	Restriction fragment length polymorphism
SAC	Special Area of Conservation
SNH	Scottish Natural Heritage
SNP	Single nucleotide polymorphism
SPA	Special Protection Area
SSSI	Site of Special Scientific Interest
TG	Taxon Group
UK	United Kingdom of Great Britain and Northern Ireland
VCCC	Vice County Census Catalogue
VIF	Variance inflation factor

Declaration

Chapter 2 has been published and Chapter 5 has been accepted for publication. The work presented in Chapters 3, 4, 6 and Appendix 1 has been submitted for publication. Chapter 5 was primarily written by Dr. Susan Jarvis, however substantial contributions were made by me, the work sits consistently within the body of work represented in the rest of the thesis, and the chapter would not make sense without the contribution of Dr. Susan Jarvis. Dr. Susan Jarvis has agreed for me to use her contributions to the text here, and she has signed this declaration, which indicates our relative contributions below. The wording of all chapters and the Appendix is largely identical to the manuscripts prepared for publication, however, Chapter 5 notwithstanding, all other chapters were written by me and represent my own work.

Chapter 2:
Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J., Ford-Lloyd, B. and Maxted, N. (2015) Enhancing the conservation of crop wild relatives in England. *PLOS One* 10: e0130804. doi: 10.1371/journal.pone.e0130804

Chapter 3:
Fielder, H., Smith, C., Ford-Lloyd, B. and Maxted, N. (submitted b) Enhancing the conservation of crop wild relatives in Scotland. *Journal for Nature Conservation*.

Chapter 4:
Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J, Burrows, C., Woodman, J., Smith, C., Ford-Lloyd, B. and Maxted, N. (submitted c) Enhancing the conservation of crop wild relatives in the United Kingdom. *Botanical Journal of the Linnean Society*.

Chapter 5:
Jarvis, S., Fielder, H., Hopkins, J.J., Maxted, N. and Smart, S. (in press) Distribution of crop wild relatives of conservation priority in the UK landscape. *Biological Conservation*. **See further note below.**

Chapter 6:
Fielder, H., Brotherton, P., Hopkins, J., Hosking, J., Ford-Lloyd, B. and Maxted, N. (submitted d) A genetic assessment of the crop wild relatives of the Lizard Peninsula, Cornwall and its suitability to become the first UK genetic reserve. *Journal of Applied Ecology*.

Appendix 1:
Fielder, H., Burrows, C., Woodman, J., Ford-Lloyd, B. and Maxted, N. (submitted a) Enhancing the conservation of crop wild relatives in Wales. *New Journal of Botany*.

I contributed to the text in Chapter 5 as follows:

- Introduction (section 5.2): Paragraphs 1, 2, 3 (second sentence), 4, 5 (second sentence), 6 (first sentence), hypotheses 1 and 4
- Methods (section 5.3): Paragraphs 1 (sentences 6, 7 and 8) and 5 (sentences 2, 3, 4 and 5)
- Discussion (section 5.5): Paragraphs 5 (sentences 2 and 3) and 6
- Conclusion (section 5.6)

All other parts of the text in Chapter 5 were contributed by Dr. Susan Jarvis.

Signed:

17/06/2015

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CHAPTER 1.

Introduction

1.1 What is food security?

Food security is a state that ‘exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life’ (FAO, 2010). There are many strands to this definition, both societal and scientific, which pose fundamental challenges to the global community in achieving a state of food security for an increasing population (United Nations, 2011). These include challenges relating to food production and its resilience particularly in light of climate change, the global distribution of food supply to address poverty and hunger, and the mitigation of the impact of intensive agricultural systems on biodiversity and ecosystems (Government Office for Science, 2011). Though all three challenges are crucial to achieving food security, it is the former challenge of improving the resilience of food production that is the core concept underlying this thesis with particular regard to crop wild relative (CWR) conservation and use for crop improvement.

The primary objective of the work presented in this thesis was to research methodologies to facilitate systematic, active conservation of CWR, and their genetic diversity, within a national context using the United Kingdom of Great Britain and Northern Ireland (UK) and its devolved administrations as an exemplar. CWR are a plant genetic resource (PGR) whose largely untapped pool of genetic diversity can be utilised by the plant breeding community to breed novel varieties, with improved biotic and abiotic stress tolerance, improved yield and reliance on fewer resource inputs, into modern crop varieties (Maxted and Kell, 2009; FAO, 2010). Their potential, particularly in light of climate change, to contribute to food security is vast and consequently, their conservation and use should be a priority in all parts of the world.

1.2 The food production challenge

Many of the food crops on which humans now depend were first domesticated approximately 10,000 years ago when hunter-gatherers made the transition to farming communities (Vaughan *et al*., 2007). Over the last century there has been a much faster transition moving away from the farming of traditional, farmer selected, crop varieties to the farming of modern crop cultivars that are high-yielding; a change that reached its peak during the 'Green Revolution' in the 1970s (Tanksley and McCouch, 1997; Esquinas-Alcázar, 2005). The primary driver behind this shift in agricultural production systems was the need to meet the demands of a rapidly increasing human population, a challenge that is just as relevant now with the global population expected to reach nine billion people by 2050 (United Nations, 2011).

Both domestication from wild species and the shift from traditional to modern crop varieties have caused bottlenecks that have led to a loss of genetic diversity in modern crops, as illustrated in Fig. 1.1 (Tanksley and McCouch, 1997). This narrowing of the genetic base of crops has resulted in the farming of modern crop varieties that are genetically uniform, often grown over large areas in monocultures where yield and quality have been valued over genetic variability, making crop varieties more susceptible to environmental stresses (Henry, 2014). Though breeding for agronomically beneficial traits has shown to have significantly increased crop yields in the past, it has led to a state of genetic vulnerability in the majority of crops with some extreme consequences (Godfray *et al*., 2010; FAO, 2010). For example, the breeding of hybrid corn in the USA by selecting for 'T' cytoplasmic male sterility, producing high yielding but uniform varieties with a narrow genetic base (Tatum, 1971). In the late 1960s, it was found that all lines with 'T' cytoplasmic male sterility were susceptible to Southern corn leaf blight *Helminthosporium maydis* Nisikdo and Miyake and in 1970 a major epidemic swept the USA with some regions experiencing losses of more than 50% of total yields (Tatum, 1971). The first report on the state of the world's plant genetic resources for food and agriculture (PGRFA) defines genetic vulnerability as the 'condition that results when a widely planted crop is uniformly susceptible to a pest, pathogen or environmental hazard as a result of its genetic constitution, thereby creating a potential for widespread crop losses' (FAO, 1997). The variation in modern varieties can only be expanded by the utilisation of the wider diversity found in PGR (Tanksley and McCouch, 1997).

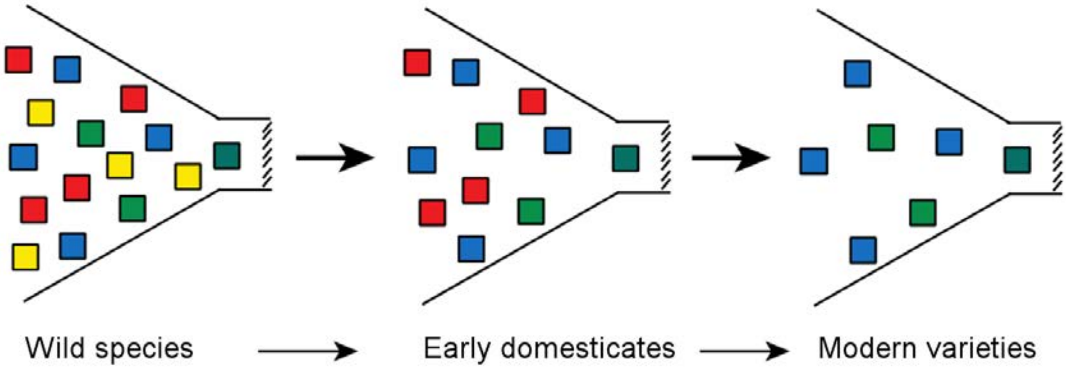

Figure 1.1 Genetic bottlenecks experienced by crops, resulting from domestication and the breeding of modern crop varieties. Coloured squares illustrate allelic variation in wild populations lost through domestication and breeding (Source: Tanksley and McCouch, 1997).

Pests, pathogens and environmental hazards are likely to become increasingly prevalent due to the impacts of climate change. The fifth assessment report from the Intergovernmental Panel on Climate Change (Stocker *et al.*, 2013) shows that global mean surface temperatures have been rising since the late 19th century. In the mid-to-high northern hemisphere, the last 30 years have been the warmest of the last 800 years and data suggest, with a 95–100% probability, that human activities have contributed to over 50% of the increases recorded in global mean surface temperatures between 1951 and 2010 (Stocker *et al.*, 2013). Future projections suggest that temperature increases will be a continuing trend to 2100 and beyond

(Stocker *et al*., 2013). Changes in patterns of precipitation have also been observed and future projections reveal that low latitudes are likely to experience less precipitation whereas high latitudes are likely experience increases (Stocker *et al*., 2013). Sea level rises, driven by warming oceans and the melting of ice sheets and glaciers from land masses, have occurred at an estimated rate of 3.2mm yr^{-1} between 1993 and 2010 and will continue to rise under future climate scenarios. In addition, more extreme weather patterns are predicted with increased frequency worldwide (Stocker *et al*., 2013). In Europe this is likely to manifest itself through heat waves, more frequent and intense precipitation and more frequent storms (Stocker *et al*., 2013).

All of these trends will require greater access to a wider pool of genetic diversity in food production systems to enable adaptation to novel conditions and resilience (FAO, 2015). Extreme temperatures are likely to have a detrimental effect on yield. This was demonstrated by Schlenker and Roberts (2009) in corn, soybean and cotton where even under the slowest warming climate scenario, yields are expected to reduce by between 30–46%. However, some studies have shown that projected temperature increases may actually contribute to increased crop yields, this has been demonstrated for sugar beet in England and potato yields in Scotland (Jaggard *et al*., 2007; Gregory and Marshall, 2012). Despite this potential benefit, modern crop varieties will also have to contend with a range of other abiotic stresses including water stress either through drought or flooding as a result of changing weather patterns, salinisation due to rising sea levels around coastal agricultural land, irrigation and forest clearance (Flowers and Flowers, 2005) and will need to have the capacity to survive extreme weather events. With the projected changes in temperature and weather patterns, the threat from pests and pathogens is also likely to increase. Evans *et al*. (2008) investigated the likely shift in distribution of the pathogen, phoma stem canker *Leptosphaeria maculans*

(Desm.) Ces. & de Not; a worldwide disease of *Brassica* crops, causing significant losses in yield. In the UK, the disease is most severe in the south with a much lower impact in northern areas; however over the next 20 years climate change models demonstrated that the range and severity of this disease will increase (Evans *et al*., 2008). The effects of globalisation, which increase the numbers of introductions of non-native pests and pathogens, jeopardise food security (Anderson *et al*., 2004). Dependence on finite resources, such as phosphorus in fertilizers, to improve crop yields also highlights the need for improved genetic diversity in crop varieties to create cultivars that use resource inputs more efficiently (Wissuwa *et al*., 2015).

The genetic vulnerability of modern crop cultivars, together with threats to agriculture through climate change, salinisation, globalisation and resource limitations puts food production in a precarious situation where future food security is uncertain. To compound this, Khoury *et al*. (2014) demonstrate that food supplies across the world are becoming increasingly homogenous, resulting in more people relying on fewer, genetically vulnerable, crop species and varieties. These issues are recognised in the majority of country reports collated for the second report of the state of the world's PGRFA, where there are clear concerns over the uniformity of crop cultivars, and emphasis on the need for increased genetic diversity in agriculture to adapt to changing conditions and ensure food security for the future (FAO, 2010).

1.3 Genetic resources: an ecosystem service

The Millennium Ecosystem Assessment published in 2004, was intended to investigate how ecosystem change is impacting human well-being in a global context (MEA, 2004). Through this work, the concept of 'ecosystem services' was also introduced, identifying the ways in which biodiversity and ecosystems benefit humankind. Ecosystem services were grouped into four classifications: supporting services (e.g. water and nutrient cycles), provisioning services (e.g. food and fuel), regulating services (e.g. pollination and pest/disease regulation) and cultural services (e.g. recreation) (MEA, 2004). Plant genetic resources were listed in the MEA (2004) as a provisioning ecosystem service.

The Convention on Biological Diversity (CBD) defines genetic resources as ‘any material of plant, animal, microbial or other origin containing functional units of heredity, of actual or potential value’ (CBD, 1992). Put simply this encompasses any living taxon containing genes that are, or could potentially be, of use to humans. According to Jackson and Ford-Lloyd (1990), PGR consist of the ‘total genetic diversity of cultivated species and their wild relatives’ and they are likely to have an agricultural value. PGRFA are those particularly associated with food production and include modern crop cultivars, landraces, traditional varieties and CWR (Hawkes *et al*., 2000). Wild relatives of crop plants are likely to contain biotic and abiotic stress resistant or tolerant traits that can be used in plant breeding to widen the genetic diversity in modern crop cultivars (Redden, 2013). However, CWR are quite often overlooked in terms of their potential for use and value in food production and security (Ford-Lloyd *et al*., 2011).

1.4 Defining a CWR

In a broad sense, a CWR can be defined as any plant belonging to the same genus as a crop (Maxted *et al*., 2006). Using this definition Kell *et al*. (2008) demonstrated that of the total flora in the region of Europe and the Mediterranean, approximately 80% of taxa could be considered as CWR. This high percentage shows that it is important to use a more selective definition of a CWR that considers the degree of relatedness between a crop and its wild relatives. This helps directly infer their relative use as gene donors for crop improvement and also enables targeted conservation, where resources are limited, of priority species rather than all genera.

In 1971, Harlan and de Wet devised the Gene Pool (GP) concept, which classifies crops and their related species into: GP1a containing the cultivated forms of the crop; GP1b the wild and weedy forms of the crop where there is no barrier to gene transfer into crops; GP2 which contains more distantly related species 'from which gene transfer to the crop is possible but difficult using conventional breeding techniques' (Maxted and Kell, 2009); and GP3 'which includes the species from which gene transfer to the crop is impossible, or if possible requires sophisticated techniques such as embryo rescue, somatic fusion or genetic engineering' (Maxted and Kell, 2009) (Fig. 1.2).

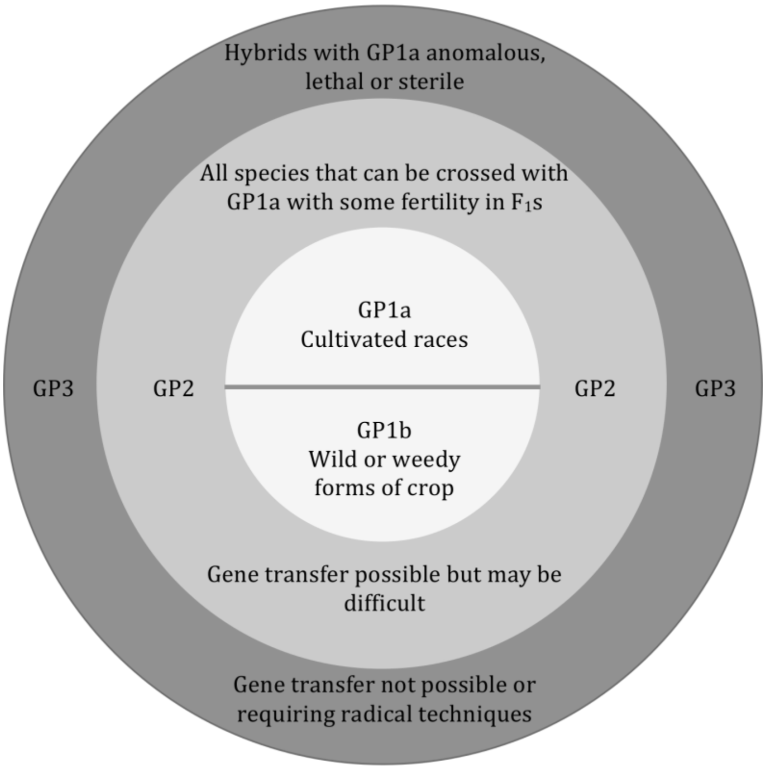

Figure 1.2 The Gene Pool concept showing that wild relatives toward the centre (GP1b and GP2) of the diagram are more closely related to the crop (GP1a) than those towards the edge (GP3) (Source: Harlan and de Wet, 1971).

Data regarding the ease of gene transfer between crops and CWR is primarily restricted to the most globally valuable crops, which are also the best studied e.g. wheat, potato, rice and maize (Maxted *et al*., 2006; Vincent *et al*., 2013). This meant the GP concept could not be applied for the majority of crops and therefore led to the publication of the Taxon Group (TG) concept in 2006, which can be used to define CWR through inference of their genetic relatedness to a crop using their classification (Maxted *et al*., 2006) as follows:

TG1a – crop

TG1b – same species as crop

TG2 – same series or section as crop

TG3 – same subgenus as crop

TG4 – same genus

TG5 – same tribe but different genus to crop

Combining these two concepts, a CWR can be defined as a taxon belonging to either GP1b or 2 or belonging to TG1b to 4 of a particular crop (Maxted *et al*., 2006).

1.5 The value of CWR and their utilisation

The use of genes from CWR in crop improvement has been steadily increasing since the 1940s (Hajjar and Hodgkin, 2007; Maxted and Kell, 2009). Maxted and Kell (2009) found that 2% of citations in the literature describing CWR use in plant breeding were published prior to 1970, whereas 38% had been published after 1999. The numbers of citations and examples of the use of CWR relating to 29 global priority crops are illustrated in Fig. 1.3, with results indicating that CWR have predominantly been used to improve staple crops such as wheat, rice and barley (Maxted and Kell, 2009). This suggests a need to expand research into a wider range of crops and their associated CWR to draw further beneficial traits from their largely untapped gene pools.

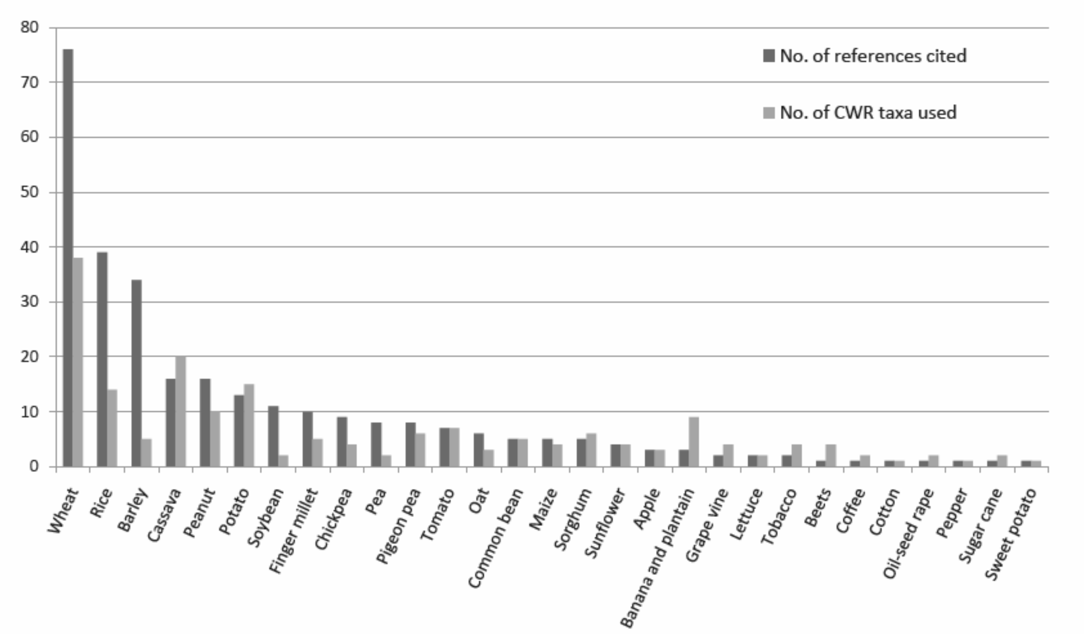

Figure 1.3 Total numbers of references that have reported the identification and transfer of beneficial traits from 185 CWR to 29 global priority crops, also showing the number of CWR taxa utilised per crop (Source: Maxted and Kell, 2009).

Their increasing utilisation has prompted many studies to estimate the value of their contributions to improving crop varieties. Pimentel *et al.* (1997) calculated that CWR contribute $115 billion per year globally to increased crop yield. In America alone, it was calculated that CWR contribute more than US$350 million per year in terms of improvements in yield and quality (Prescott-Allen and Prescott-Allen, 1986). Furthermore, Phillips and Meilleur (1998) estimated that if endangered CWR were to be lost, approximately $10 billion annually in wholesale farm value would be also be lost as a result. A more recent valuation of the potential benefit of using CWR to breed improved commercial crop varieties has been carried out by Price Waterhouse Cooper in collaboration with Kew’s Millennium Seed Bank (MSB). They estimate that the potential value of using CWR to improve MSB’s 29 priority crops, those listed in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2001), will increase from $42 billion in 2013 to $120 billion in 2021 (PwC, 2013). This valuation considers only 29 priority crops rather than CWR as a whole, suggesting that a full valuation of the potential of all globally important CWR to contribute to crop development would be much greater. Though the methods of estimation and valuation differ between studies, it is clear that CWR are a highly valuable genetic resource.

The majority of examples of crop improvement using traits from CWR have focused on pest and disease resistance. Hajjar and Hodgkin (2007) found that of 100 traits bred from a range of 60 wild species into 13 crops, over 80% conferred pest and/or disease resistance. Furthermore, 56% of citations (relating to the use of CWR in 29 priority crops) reviewed by Maxted and Kell (2009) were related to pest or disease resistance traits. For example, a cross, and subsequent backcrosses, between wild wheat relative *Aegilops peregrina* (Hack.) Maire & Weiller and cultivated wheat *Triticum aestivum* L. was carried out by Marais *et al.* (2008). This led to the spontaneous translocation of a segment of a chromosome containing a gene (*Lr59*) conferring resistance to mixed leaf rust (*Puccinia triticina* Eriks.) in cultivated wheat. In another example, a dominant gene (*Er3*) was identified in the wild species *Pisum fulvum* Sm. that provides resistance to powdery mildew (*Erysiphe pisi* DC.), a disease that results in severe yield losses in cultivated pea *Pisum sativum* L. (Fondevilla *et al*., 2007). Successful hybridisation between these wild and cultivated pea species and the resulting production of plants resistant to powdery mildew demonstrated the potential of using *P. fulvum* in breeding programmes (Fondevilla *et al*., 2007).

More recently, traits in CWR for abiotic stress tolerance have been more thoroughly investigated, with 13% of citations reviewed by Maxted and Kell (2009) relating to such traits. Lakew *et al*. (2011) tested the drought tolerance of a range of introgressed lines of barley when exposed to nine environments with variable water availability. These breeding lines originated from a cross between wild *Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell. and cultivated barley *Hordeum vulgare* L., the former known to have a higher capacity to tolerate drought (Grando *et al*., 2001). The range of responses to the environments to which these barley lines were exposed led Lakew *et al*. (2011) to conclude that *H. vulgare* subsp. *spontaneum* is likely to be a good source of drought tolerance genes that can be further investigated and used in barley breeding programmes to produce varieties more resilient to predicted changes in climate. Another key example of a recent success in improving abiotic stress tolerance of a crop using traits from CWR is that of rice. In 2013 the International Rice Research Institute released details of a successful interspecific hybridisation between the CWR *Oryza coarctata* Roxb. and the cultivated rice variety IR56 of *Oryza sativa* L., using embryo rescue techniques. *O. coarctata* is able to tolerate twice the concentration of salt compared to cultivated varieties, and this cross will no doubt have a vast impact on improved yields of cultivated rice once a commercial variety has been developed and released (IRRI, 2013). Taking this research further still, Garg *et al*. (2014) has sequenced the transcriptome of *O. coarctata* and identified 15,158 genes linked to salinity and submergence tolerance. This vast array of genes found in a single wild relative in relation to a single abiotic stress provides some idea of the range of useful genes and beneficial traits that are yet to be identified and characterised in CWR. Advances in biotechnology such as sequencing and embryo rescue techniques are having a significant impact on plant breeding, particularly in aiding the characterisation of CWR germplasm to find genes of interest and facilitating the use of more distantly related CWR, including those in the tertiary gene pool of a crop (e.g. *Lens*: Davies *et al*., 2007; Alliaceae: Chuda and Adamus, 2009; *Cajanus*: Mallikarjuna *et al*., 2011; *Vaccinium*: Ehlenfeldt and Ballington, 2012; *Oryza*: Garg *et al*., 2014).

Above are just a few examples of a vast literature on crop improvement using traits from CWR. Examples of other types of beneficial traits used from CWR include those for improved yield, improved quality, cytoplasmic male sterility, fertility restorers and husbandry improvement (Maxted and Kell, 2009). Ultimately, the value of CWR can be considered in terms of their potential economic contribution to the agricultural industry but also, and perhaps more importantly, the innate value of the pool of genetic diversity and potentially useful traits within CWR that could help to mitigate the impact of climate change or other unforeseen problems facing food production, and that will be lost if these genetic resources are not preserved for the future.

1.6 Threats facing CWR

1.6.1 The biodiversity crisis

CWR are a component of biodiversity and differ from wild plants only in that they are related to one or more crop species. The CBD describes biodiversity as 'the variability among living organisms from all sources...and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems' (CBD, 1992). To the present day this remains the favoured definition (Mace *et al.*, 2012) as it highlights the three distinctive levels of diversity: genetics, species and ecosystems. Biodiversity is threatened at all three of these levels. The Millennium Ecosystem Assessment (MEA, 2004) found that the impact of humans on the environment has meant that the vast majority of ecosystems worldwide have been significantly changed requiring species adaptation and migration to ensure survival. Species diversity was shown to be in decline, with species extinction rates having increased by up to one thousand times the expected background rate (MEA, 2004). Considering only species of well-studied higher taxa, between 12% and 52% are threatened according to the International Union for Conservation of Nature (IUCN) red list criteria (MEA, 2004; IUCN, 2012). Genetic diversity has also declined across the globe, particularly within domesticated species (MEA, 2004). An assessment of the factors driving these losses of biodiversity, such as habitat destruction, over-exploitation, invasive species and climate change, concluded that in the majority of cases the impact of these factors was either ongoing or increasing (MEA, 2004). These results are supported in a study by Butchart *et al*. (2010), which demonstrated that there has been no reduction in the rate of global biodiversity loss over the last four decades and that the CBD 2010 target 'to achieve by 2010 a significant reduction of the current rate of biodiversity loss' was missed (CBD, 2002). This is likely to be due to the increasing pressures being exerted on the environment due to the unsustainable rate of global human population growth (Loreau *et al*., 2006). Though the profile of biodiversity and its ongoing decline was raised following the United Nations Earth Summit conference in Rio de Janeiro in 1992, when the CBD was ratified, many years later biodiversity as a whole remains undervalued (Loreau *et al*., 2006). It is imperative that more is done to maintain biodiversity and improve conservation efforts. It is clear that although legislation for conservation exists, it is insufficient without action, crucially this must stem from improved communication between research and policy makers (Loreau *et al*., 2006; Butchart *et al.*, 2010).

1.6.2 Focus on genetic diversity

Genetic diversity has been studied to a lesser extent than species and ecosystem diversity (Hargreaves, 2011) but it is this level of biodiversity that is widely accepted as imperative for populations to persist (Frankel and Soulé, 1981; Gilpin and Soulé, 1986), and in the case of CWR it has the added value of providing traits to expand the limited genetic diversity in modern crop varieties. Natural selection is a non-random process whereby populations adapt to cope with environmental change, resulting in a change in population allele frequencies and higher numbers of individuals able to tolerate the environmental change i.e. they have an increased evolutionary fitness (Bijlsma and Loeschcke, 2012). The capacity of a population to adapt is positively correlated with the amount of extant genetic variation and it follows that with limited genetic variation, natural selection will also be limited (Reed and Frankham, 2003; Blows and Hoffmann, 2005).

The processes of inbreeding and genetic drift (together known as 'genetic erosion') both act to reduce the genetic diversity of populations, the former due to breeding between related individuals and the latter due to the random loss of alleles in populations where not all individuals contribute equally to the next generation, both processes result in increased homozygosity (Charlesworth and Charlesworth, 1987; Bijlsma and Loeschcke, 2012). With low genetic diversity, individual and population fitness is reduced because there is less capacity for adaptation to changing environments, leading to a higher risk of extinction (Höglund, 2009). This is particularly concerning in light of the predicted effects of climate change (section 1.2).

Habitat destruction and land use change are exacerbating this issue as both lead to increasingly fragmented and isolated habitats and populations, between which gene flow is limited (Bijlsma and Loeschcke, 2012). This results in small populations that are particularly susceptible to genetic erosion and therefore, extinction. The effects of genetic drift have been shown to have a larger effect as population sizes become smaller, resulting in a higher rate of allele loss, particularly of rare alleles (Bijlsma and Loeschcke, 2012). As stress resistant/tolerant genes often occur at low frequencies (McNair, 1997) this will greatly affect the capacity of populations to persist under changing environments and is a concern for maintaining valuable traits in CWR. In addition, Bijlsma *et al.* (2000) demonstrate that the probability of extinction of a small population significantly increases due to a higher rate of inbreeding and is further increased when exposed to environmental stress. This suggests that environmental change, particularly the combined effects of climate change and habitat destruction, will have a strong and detrimental impact on the adaptive capacity of populations; the impact is also likely to be greater for outbreeding species (Bijlsma *et al.*, 2000). As a result, active conservation of CWR to maintain sufficient population sizes and genetic variation is crucial to ensure their long-term persistence, their ongoing adaptation and their availability for use for crop improvement. An additional factor that may result in a higher susceptibility of CWR genetic diversity is the association of some populations with disturbed, early-successional plant communities (Grime, 1977; Maxted and Kell, 2009). These habitats are likely to be more susceptible to the impacts of anthropogenic change, experiencing higher levels of stress than perennial, mid to late-successional plant communities as they are not usually considered for inclusion in conservation designations (JNCC, 2013).

1.7 How can CWR be systematically conserved?

Despite recognition of the threats facing CWR and their importance in crop improvement, they remain undervalued and poorly conserved (Ford-Lloyd *et al.*, 2011). Conservation priorities have most often fallen into two categories: conservation of rare/threatened species and conservation of crop varieties (Maxted and Kell, 2009). CWR fall between these two branches of conservation and so have often lacked necessary attention (Maxted, 2003). This has resulted in some piecemeal approaches to CWR conservation where more comprehensive and systematic conservation methodologies would be appropriate, such as the model presented by Maxted *et al.* (2007) in Fig. 1.4. The model illustrates the process required for the systematic conservation of the full diversity of CWR at a national level. The methodological approaches to CWR conservation outlined in sections 1.7.1 to 1.7.5 below highlight how key aspects of this model can be achieved.

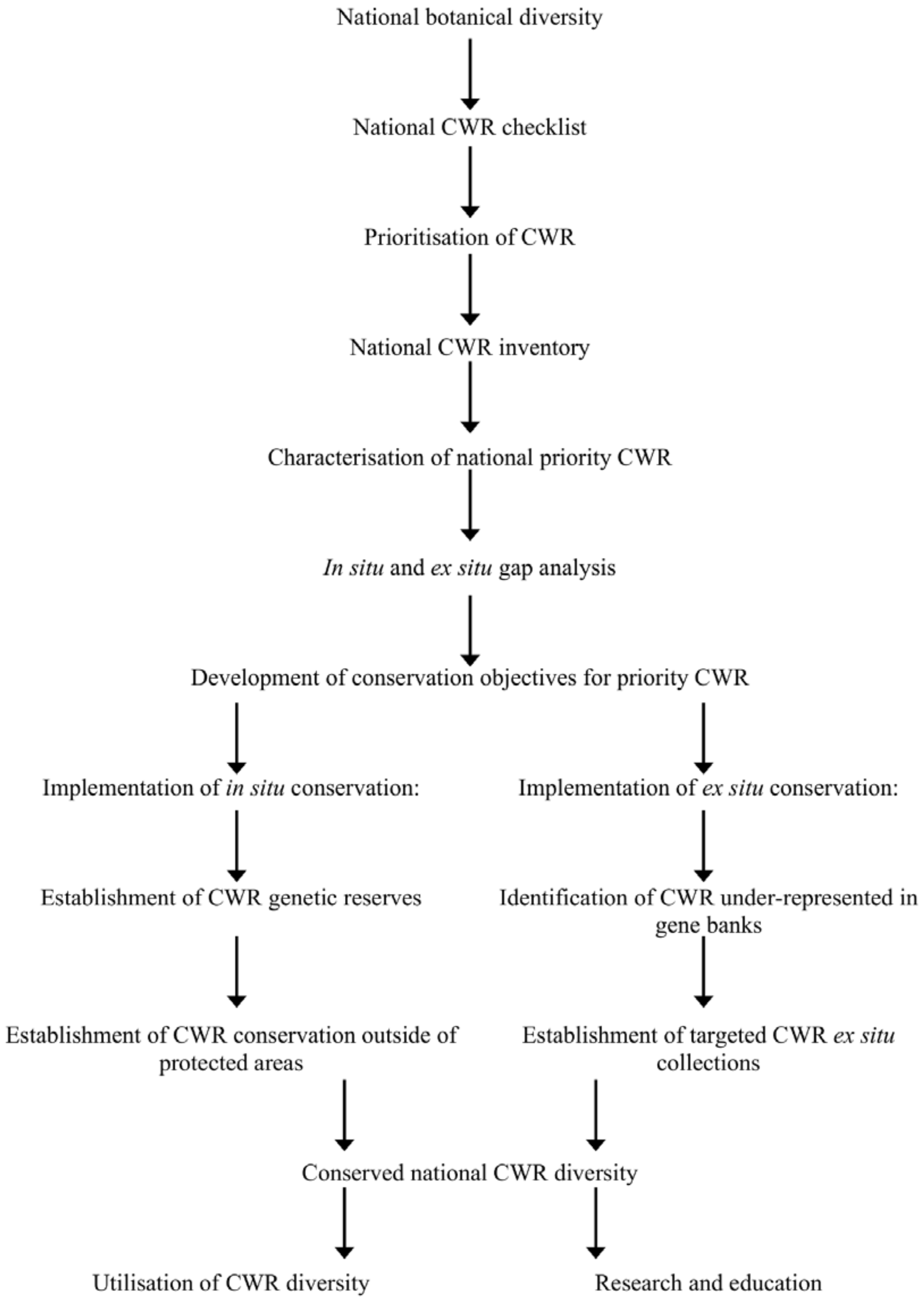

Figure 1.4 Model for systematic conservation of national CWR diversity (Modified from: Maxted *et al*., 2007).

1.7.1 CWR checklists and inventories

The development of a national checklist and inventory for CWR acts as a starting point and foundation for the establishment of appropriate national CWR conservation actions. A checklist is a full list of all of the CWR taxa and their authorities occurring within a defined geographic area (Maxted *et al*., 2013a). It reveals the breadth of CWR diversity across the geographic area and can be developed by, 1) identifying the genera of cultivated crops within the study area and 2) matching the genera to a list of the flora of the same area, a method used by Kell *et al*. (2015) in China and previously developed by Kell *et al*. (2005; 2008) to produce a CWR Catalogue for Europe and the Mediterranean. This catalogue is a valuable resource enabling European countries to extract national CWR checklists, despite this Iriondo *et al*. (2015) reports that only about 10 checklists are actively being worked on within Europe and globally this figure is no higher than 20.

An inventory is a subset of the checklist, consisting of CWR taxa that are a priority for active conservation; inventories will also contain ancillary information about each taxon (Maxted *et al*., 2013a). It is highly unlikely that the resources and finance will be available to establish active conservation management and monitoring for all taxa in a checklist, so a process of prioritisation based on select criteria is necessary to create an inventory of priority CWR on which to focus conservation actions (Maxted *et al*., 1997a; Magos Brehm *et al*., 2010; Kell *et al*., 2015; Iriondo *et al*., 2015). The taxa listed in an inventory will be those included in downstream gap analyses. More and more inventories of priority CWR are now being developed, taking vital steps towards implementation of CWR conservation. These include, among others, a global inventory of priority CWR containing 1,667 taxa related to 173 global priority crops (Vincent *et al*., 2013), an inventory of CWR in the USA containing 821 taxa related to 63 major agricultural crops (Khoury *et al*., 2013) and a number of inventories from

European countries such as, Spain (Rubio Teso *et al*., 2013), Finland (Fitzgerald, 2013), Italy (Landucci *et al*., 2014) and Cyprus (Phillips *et al*., 2014) containing 580, 209, 129 and 178 taxa respectively.

A wide range of criteria can be used for prioritisation and these are outlined in detail in Maxted *et al*. (1997a) and Magos Brehm *et al*. (2010). Kell *et al*. (2015) highlight the three criteria that are the most relevant when determining priority CWR for conservation planning: 1) socio-economic use of the related crop, 2) the threat status of the CWR and 3) the closeness of the genetic or taxonomic relationship between the crop and its wild relative, useful in determining the ease of use of the CWR (Ford-Lloyd *et al*., 2008; Maxted and Kell, 2009). These three criteria have all been used in the Spanish, Finnish, Italian and Cypriot inventories. Amongst other criteria including: native status of the CWR, distribution and endemicity, legislation and the centre of diversity of the CWR (Rubio Teso *et al*., 2013; Fitzgerald, 2013; Landucci *et al*., 2014; Phillips *et al*., 2014). Though the application of criteria differs markedly between studies, the most important outcome is that the final list of priority CWR must consist of taxa that the stakeholders in the geographical region of study are willing to conserve.

1.7.2 Gap analysis

The concept of gap analysis was first outlined by Margules (1989) as a means of evaluating the effectiveness of existing conservation efforts and identifying areas where certain aspects of biodiversity were either absent or under-represented. It was principally concerned with investigating habitat-based conservation but since then has increasingly been considered for studies concerning taxonomic and genetic diversity in PGR. Maxted *et al*. (2008a) proposed the following four-step methodology, specifically adapted to facilitate PGR gap analyses:

1. Circumscription of target taxon and target area
2. Assessment of natural diversity (taxonomic diversity, genetic diversity, ecogeographic diversity, threat assessment)
3. Assessment of current conservation strategies (*in situ* and *ex situ*)
4. Reformulation of conservation strategies according to identified gaps in current conservation

Since a study by Jones *et al*. (1997), which sought to identify gaps in genebank collections of wild forms of *Phaseolus vulgaris* L., the volume of gap analysis publications focused on conservation of PGR has significantly increased (*in situ*: Parra-Quijano *et al*., 2003, 2007; Rodrigues *et al*., 2004; Hijmans and Spooner, 2001 and *ex situ*: Jarvis *et al*., 2005; Ghamkhar *et al*., 2007; Grenier *et al*., 2001; see Parra-Quijano *et al*., 2012a for a full review). More recently Landucci *et al*. (2014) have conducted a gap analysis case study for two priority *Brassica* species occurring within Italy, *B. incana* Ten. and *B. montana* Pourr. Results showed that not all populations of these economically important CWR are sufficiently protected either in the wild or as back-up collections in genebanks. Khoury *et al*. (2015) published an *ex situ* gap analysis study of sweet potato *Ipomoea batatas* (L.) Lam. CWR in the Americas. Findings highlighted that 78.6% of the CWR analysed were a high priority for further collection of accessions to fill gaps in genebanks and that the species hotspots of central Mexico to Central America and the southeastern USA would be ideal locations for collecting missions. From these studies is it clear that gap analysis is an important tool for informing CWR conservation planning and can be easily applied to any geographic area and any group of CWR taxa.

1.7.3 Complementary in situ and ex situ conservation

Conservation is principally carried out using either *in situ* or *ex situ* approaches. *In situ* conservation approaches can generally be defined as those which maintain target biological diversity within its natural habitat or, in terms of agricultural species, within the habitat where their distinctive traits and adaptations were developed (CBD, 1992). Approaches can include genetic reserves, on-farm conservation or home garden management (Maxted *et al*., 2008b). In contrast, *ex situ* conservation approaches are those that conserve biological diversity outside of their natural habitat (CBD, 1992), with the most common approach for plants being the storage of dried seed germplasm at sub-zero temperatures in genebanks such as the MSB, Royal Botanic Gardens, Kew.

Previously, focus was most often directed at *ex situ* conservation approaches, but more recently the implementation of *in situ* conservation techniques has become more of a priority (Maxted *et al*., 2008b). Many advantages and disadvantages have been identified in relation to both approaches (Maxted *et al*., 1997b), perhaps the most crucial advantage being that *in situ* conservation maintains the dynamic biotic and abiotic interactions within their natural habitat enabling a taxon to respond and adapt to a changing environment. In contrast, *ex situ* conservation halts this completely so no further evolutionary development can take place, decoupling the plant from its natural habitat. This could limit the ability of the conserved material to survive if reintroduced to its natural habitat or to subsequently provide advantageous genes for crop improvement (Maxted *et al*., 1997b). Some species are intolerant of *ex situ* storage conditions and are termed ‘recalcitrant’, recalcitrant species however are easily conserved within an *in situ* environment or in *ex situ* field genebanks (Maxted *et al*., 1997b). Furthermore, *in situ* conservation can also allow a large number of taxa to be conserved within one protected area, including a target taxon or range of target taxa and their associated species, whereas *ex situ* conservation only allows the conservation of one taxon per accession without its associated species (Maxted *et al*., 1997b).

Considering the advantages discussed above, there seems to be a strong case to support the use of *in situ* techniques but equally, *ex situ* techniques do also have their own advantages. Germplasm can be stored *ex situ* securely and long-term, whereas *in situ* taxa could be seen as insecure. In the event of sudden change such as a natural disaster or urban development, part or all of a conserved population could be put at risk of extinction; *ex situ* conservation can overcome this drawback (Maxted *et al*., 1997b). As previously stated by Maxted *et al*. (1997b), conservation of CWR can be considered as pointless without the ability to use the conserved material for crop improvement. *Ex situ* conservation can allow easier access to material for use in breeding whereas material can be much harder to access from *in situ* approaches due to restrictions concerning access to land and permission to collect material.

It is clear that both approaches have their merits as well as certain drawbacks and as such it was emphasised in article 9 of the CBD (1992) that neither approach should be used independently of the other but each should complement the other. Greene *et al*. (2014) also agree that conservation strategies should integrate both approaches to achieve full conservation and use of CWR. This idea of complementary conservation enables continued evolutionary development of *in situ* populations, with *ex situ* accessions acting as an insurance against sudden loss of *in situ* populations, capturing a snapshot of alleles from populations that may otherwise be at risk of loss through genetic drift and providing a store of material that can be easily accessed for utilisation in plant breeding and crop improvement (Maxted *et al*., 1997b; Greene *et al*., 2014). However, PGR conservation efforts remain largely focused on *ex situ* approaches, with Maxted (2015) estimating that 99% of funding is spent on *ex situ* conservation; this is far from being complementary in practice. The concept of complementary conservation is adopted in this thesis.

1.7.4 Focus on genetic reserves

Genetic reserves are protected areas where the primary aim is to conserve the maximum range of genetic diversity of a species in the reserve, both within and among populations; such reserves must provide active and long-term management (Maxted, *et al*., 1997c; Maxted and Kell, 2009). The call for CWR genetic reserves has been clearly set out in target 9.1 of the European Strategy for Plant Conservation (ESPC), which states the requirement for the: 'establishment of 25 European crop wild relative genetic reserves covering the major hotspots of species and genetic diversity' (Planta Europa, 2008). It is widely recognised that genetic reserves should be established within existing protected areas and management plans amended to accommodate this (Maxted *et al*., 1997b; Heywood and Dulloo, 2006; Iriondo *et al*., 2008a; Hunter and Heywood, 2011). Maxted *et al*. (2008b), emphasise the potential benefits of basing CWR genetic conservation within existing protected areas as:

1. Existing protected areas are already associated with a long-term conservation ethos so are unlikely to be affected by stochastic change in the environment or in their management

2. It is relatively easy to amend current protected area management plans to incorporate genetic conservation of CWR, in some cases minimal amendments and just routine monitoring may be required

3. Establishment of new protected areas is not necessary, avoiding the potential costs of acquiring land for this purpose

In addition, this approach of conserving CWR within existing protected areas means that there is no additional regulatory burden on land owners, and also that the reserves can be established without new legislation.

Globally there already exists a number of CWR genetic reserves e.g. in Armenia, Turkey and Mexico (Maxted and Kell, 2009; Hunter and Heywood, 2011). In Europe some genetic studies have identified potential sites for their establishment such as for *Brassica* wild relatives in Italy (Branca *et al*., 2012) and *Beta patula* Aiton species in Madeira (Pinheiro de Carvalho *et al*., 2012; Frese *et al*., 2012) but few, if any, are thought to meet the minimum standards outlined in Iriondo *et al*. (2012). To meet the standards, a potential genetic reserve would have to have been selected based on systematic taxon prioritisation, a demographic survey and genetic diversity analysis. CWR within the reserve must be granted full, long-term protection through establishment of active management plans, ensuring there are no conflicts with the conservation of any other aspect of protected biodiversity, establishment of long-term monitoring plans and development of a procedure to ensure CWR material is made available for utilisation e.g. through complementary *ex situ* conservation in genebanks (Iriondo *et al*., 2012). The establishment of CWR genetic reserves that meet these standards would result in a significant improvement in the current state of *in situ* conservation for CWR. Essentially though, genetic reserve approaches should also be complemented by conservation outside of protected areas where many CWR populations are thought to exist (Maxted and Kell, 2009), ensuring the full range of CWR genetic diversity is preserved.

1.7.5 Genetic diversity analysis: molecular markers

As stated in section 1.6.2, conservation of genetic variation within CWR is crucial to ensure long-term persistence and ongoing adaptation in changing environmental conditions, as well as the presence of traits for use in crop improvement. As a result it is important that conservation efforts, both *in situ* and *ex situ* are informed by genetic diversity analyses (Schoen and Brown, 1993). Genetic diversity can be quantified using molecular markers, which reveal polymorphisms in DNA. Many molecular marker techniques have been developed over the past few decades. The ideal marker is one which is highly polymorphic, codominant (able to distinguish heterozygotes from homozygotes), abundant and distributed evenly throughout the genome whilst at the same time involving a simple, cost-effective technique that produces large amounts of high quality data, is easily reproducible and transferable across different laboratories (Vignal *et al*., 2002; Semagn *et al*., 2006). However, no single molecular marker has yet been developed that meets all of these criteria and so the most appropriate choice of marker depends on the aims of the specific study in question (Semagn *et al*., 2006).

Restriction fragment length polymorphisms (RFLPs), hybridisation-based markers, were widely used in the late 1980s due to their high reproducibility and codominant nature, but they are now obsolete due to their cost and the labour intensive technique involved (Mammadov *et al*., 2012). Polymerase chain reaction (PCR) based methods, including random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites have since become widely used. RAPD have been used successfully in conservation planning (e.g. to investigate the geographic distribution of genetic diversity in lentil CWR (Ferguson *et al*., 1998). However due to the low reproducibility of RAPD marker results, AFLPs and microsatellites are now more commonly used (e.g. Hargreaves *et al*., 2010; Adugna *et al*., 2013; Richards *et al*., 2014; Marfil, *et al*., 2015). AFLPs, first described by Vos *et al.* in 1995, combine the use of restriction enzymes and PCR. Though they are dominant in nature they are highly polymorphic, reproducible and distributed across the whole genome (Semagn *et al*., 2006). A significant additional benefit is that they do not require any prior sequence information so they can be used to quantify genetic diversity in any species; this is particularly useful in CWR studies where few species have known sequence information (Schløtterer, 2004). Microsatellites are short repeated sequences of 2–8 base pairs (Semagn *et al*., 2006). Similarly to AFLPs they are highly polymorphic and yield reproducible results but have the added advantage of codominance (Vignal *et al*., 2002). However, they do require prior sequence information to develop primers for amplification and this is an expensive and time-consuming process (Vignal *et al*., 2002). As such they are likely to be most appropriate in genetic diversity studies of major global crops.

Molecular markers known as single nucleotide polymorphisms (SNPs), the most abundant source of genetic variation in the genome, have been increasingly used over the last ten years (Mammadov *et al*., 2012; Ren *et al*., 2013). They are highly suitable for high-throughput automation, so can be used for studies involving sequencing (Mammadov *et al*., 2012). The cost of identifying SNPs through sequencing is decreasing and this suggests the use of this technology will soon become routinely affordable for population genetic studies to inform conservation planning (Schløtterer, 2004). SNPs can be identified in both coding and non-coding DNA sequences making it possible to study both genome-wide genetic diversity and also to focus on adaptive genetic diversity, which is of particular value in CWR conservation.

In terms of CWR conservation it is important that adaptive traits are conserved both to ensure their capacity to adapt to changing environments *in situ*, as well as to provide potentially useful traits for plant breeding. There is some debate as to whether genome-wide genetic diversity as revealed by molecular markers correlates with adaptive diversity. Reed and Frankham (2001) argue that there is no correlation, however there are also a number of studies suggesting a relationship (Schoen and Brown, 1993; Richardson *et al*., 2009; Johnson *et al*., 2011). Despite this, Reed and Frankham (2003) did find a significant, positive (though moderate) correlation between molecular heterozygosity and measures of evolutionary fitness, which demonstrates that molecular markers can indicate a population's fitness. Though adaptive diversity is most valuable in terms of CWR utilisation in plant breeding, the most pragmatic approach to their conservation is likely to be to preserve as much genetic diversity as possible. With more genetic diversity, populations have more capacity to adapt (Reed and Frankham, 2003; Blows and Hoffmann, 2005) so are more likely to persist under environmental stress. This also increases the likelihood of conserving adaptive variation. Equally, it is not possible to know the traits that may be brought under selection in future environmental conditions (Schoen and Brown, 1993) so the conservation of the maximum range of genetic diversity is a sensible approach. Though direct analysis of genetic diversity is always preferable, where such studies are not possible, the use of ecogeographic diversity as a proxy for genetic diversity can be used (Parra-Quijano *et al*., 2008).

1.8 The United Kingdom

1.8.1 Geography of the United Kingdom

The UK is formed of the devolved administrations of England, Wales, Scotland and Northern Ireland. The former three administrations make up the island of Great Britain. The UK has a total area of 242,000km^2, extending approximately 1,000km from the south coast of England to the north coast of Scotland (FAO, 2009). In mid-2013 the UK population was estimated to be 64.1 million, with England being home to 84% of the UK population and Scotland, Wales and Northern Ireland containing 8%, 5% and 3% respectively (ONS, 2014). The population is reported to have increased by more than 10 million people since 1964 (ONS, 2014).

The UK has a temperate climate where the temperature rarely exceeds highs of 32ºC or lows of -10ºC (FAO, 2009). Mean annual rainfall across the UK between 1981 and 2010 was recorded as 1,154mm, though in mountainous regions to the west and north it can exceed 1,600mm whereas in central and eastern areas of the UK it can be less than 800mm (FAO, 2009; Met Office, 2015). However, the impacts of climate change are likely to result in rising temperatures, particularly in the summer, less summer precipitation but more winter precipitation, rising sea levels and extreme weather events such as heat waves, flooding and drought occurring at greater frequencies (UKCP09, 2009).

1.8.2 Biodiversity of the United Kingdom

The UK National Ecosystem Assessment (UK NEA, 2011) identifies seven broad habitat types occurring within the UK. The most extensive of which is enclosed farmland covering 40% of land area in the UK, half of which is arable land, particularly in the east of the country, with the rest primarily consisting of nutrient rich grassland in wetter western regions (UK NEA, 2011). Other habitat types include mountains, moorlands and heaths (18% of UK land area), semi-natural grasslands, woodlands (managed plantations and both ancient and semi-natural woodlands), freshwaters (including openwaters, wetlands, floodplains, reed beds, fenland, bogs and marshes), coastal margins (including dunes, machair, saltmarsh, shingle, sea cliffs and coastal lagoons) and urban areas (7% of UK land area and containing 80% of the UK population) (UK NEA, 2011). Across all of these UK habitats (including marine), 30% of the ecosystem services they provide are reported as declining with others already in a degraded state (UK NEA, 2011).

The flora of the British Isles consists of approximately 4,800 taxa, including all vascular plants, (pteridopytes, gymnosperms and angiosperms), as well as native taxa, alien taxa and hybrids (Stace, 2010). Walker (2003) estimates that throughout the twentieth century, an average of one wild vascular plant was lost every two years in each UK county. According to the Vascular Plant Red Data List for Great Britain, 345 taxa (20%), out of 1,756 native and archaeophyte taxa assessed, are threatened based on IUCN red list criteria (Cheffings and Farrell, 2005). Archaeophytes are taxa introduced to the UK prior to the year AD 1500 (Stace, 2010). Hopkins and Maxted (2011) highlight the primary threats to CWR in the UK include changes in land use, the range of potential impacts of climate change and the impacts of non-native species leading to novel pests, diseases and invasive species. Each devolved administration also has its own list of species of principal importance, i.e. requiring conservation action due to value, rarity and/or threat but not based on IUCN criteria. The English and Welsh species of principal importance are listed in sections S41 and 42 of the Natural Environment and Rural Communities act 2006 respectively (NERC, 2013; Wales Biodiversity Partnership, 2015). The English list consists of 943 species, 152 of which are vascular plants. The Welsh list contains 557 species of which 78 are vascular plants. In Scotland, 1,947 species are listed under section 2 of the Nature Conservation (Scotland) Act 2004, 245 of which are vascular plants (Scottish Government, 2013a). Finally, Northern Ireland lists 414 species under section 3 of the Wildlife and Natural Environment Act (Northern Ireland) 2011 of which 78 are vascular plants (DOENI, 2011). All of these species lists include a handful of CWR each, suggesting that only a very small number of CWR taxa are considered in conservation planning in the UK but only in the context of being rare or threatened, this does not take into account the range of genetic diversity within each taxon or population, a key distinction between species and genetic conservation. A systematic approach to CWR conservation, including their genetic diversity, in the UK is required to protect these resources.

1.8.3 Policy context for UK CWR conservation

The UK, as a contracting party to the CBD (CBD, 1992), has a responsibility to protect its biodiversity and natural capital. PGR, including CWR, are a key component of biodiversity and natural capital, providing important ecosystems services, and therefore the UK is responsible for protecting its CWR. There is also a range of global, European and national policy instruments that set targets for the conservation of genetic resources and specifically, CWR. Target 9 of the Global Strategy for Plant Conservation (GSPC) is aiming for '70 per cent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species conserved' (CBD, 2010a). The CBD's Strategic Plan for Biodiversity 2011–2020 sets five 'Strategic Goals' including Goal C, 'to improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity' (CBD, 2010b). This includes Aichi Target 13 that states 'by 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimising genetic erosion and safeguarding their genetic diversity' (CBD, 2010b). The UK is also a contracting party to the ITPGRFA (FAO, 2001). Article five of the ITPGRFA encourages both *in situ* conservation of PGRFA including in protected areas and *ex situ* collection. The Second Global Plan of Action for PGRFA was established to support the ITPGRFA, helping to deliver its objectives (FAO, 2011). It includes a range of priority activities covering *in situ* conservation and management, *ex situ* conservation, the use of PGRFA and the establishment of sustainable means to achieve this (FAO, 2011).

At the European level, the ESPC (Planta Europa, 2008) expands Target 9 of the GSPC stating the need for the establishment of '25 European crop wild relative genetic reserves covering the major hotspots of species and genetic diversity' (Planta Europa, 2008). In 2011 the EU

Biodiversity Strategy 2020 was published (European Union, 2011). Action 9 of the strategy outlines the need for better targeting of biodiversity conservation through Rural Development programmes, specifically mentioning the importance of involving farmers to help protect genetic resources. More generally, action 10 highlights the need to conserve Europe's agricultural genetic diversity but there is no specific mention of CWR (European Union, 2011).

In the UK, most aspects of conservation policy are devolved. This has led to the publication of separate biodiversity strategies for each administration of the UK (England, Wales, Scotland and Northern Ireland). Though there are some differences between the strategies, the overall aims are to preserve biodiversity and national capital (Defra and JNCC, 2012); this encompasses all genetic resources, including CWR. In England, the Biodiversity Strategy 2020 states the need to conserve 'agricultural genetic diversity in cultivated plants, farmed animals and wild relatives' (Defra, 2011). Outcome 19 of the Environment Strategy for Wales states the need to halt the loss of biodiversity by 2026 including genetic diversity within species (Welsh Assembly Government, 2006). The Scottish Biodiversity Strategy with a recent update 'The 2020 challenge for Scotland's biodiversity' (Scottish Executive, 2004; Scottish Government, 2013b), acknowledges the need to preserve genetic diversity related to agricultural systems to ensure food security. The Northern Ireland Biodiversity Strategy looks to integrate conservation of biodiversity into policy development and a revised strategy is in development (DOENI, 2002; Defra and JNCC, 2012). A UK Biodiversity Framework 2012–2020 also exists (Defra and JNCC, 2012), which aims to complement the individual administration strategies to help coordinate efforts to achieve the Aichi targets (CBD, 2010b). Its success will require communication and coordination between Defra, other government departments, Joint Nature Conservation Committee and devolved administrations etc.

1.9 Thesis aims

It is clear that a comprehensive background of policy instruments exist at the global, European and national levels to encourage active and systematic conservation of CWR. Despite this, a lack of scientific knowledge regarding CWR and the best approaches to ensure the conservation of their full range of genetic diversity has restricted the implementation of CWR conservation in the majority of countries. The UK is no exception, where currently there is no provision for the conservation of CWR species or their genetic diversity. The overall aim of this thesis is to fill this knowledge gap by reviewing and planning CWR conservation in the UK. The following objectives were set to achieve this aim:

1. Create inventories of priority CWR related to human food and animal forage and fodder crops for the UK and its devolved administrations.
2. Perform *in situ* and *ex situ* gap analyses for priority CWR in the UK and devolved administrations and, based on the results, make recommendations as to how their conservation can be enhanced.
3. Assess the habitat preferences of UK priority CWR to further inform conservation planning.
4. Investigate the suitability of The Lizard Peninsula in Cornwall for the establishment of the first UK CWR genetic reserve.
5. Demonstrate the use of next-generation sequencing (NGS) in revealing genetic gaps in CWR genebank collections to enable more targeted collection to fill these gaps.

A major focus of this research was also to involve key stakeholders, encouraging their input in all aspects of the work in order to establish a strong link between research and practice.

Finally, the work completed in the UK and its devolved administrations is intended as an example that can be widely applied within and outside of Europe.

1.10 Thesis outline

To address each of the objectives outlined above and to achieve the overall aim of enhancing UK CWR conservation, this thesis is structured as follows:

1. Enhancing the conservation of crop wild relatives in England, Wales, Scotland and the UK as a whole (Chapters 2–4 – Fielder *et al*., 2015; submitted b; submitted c respectively and Appendix 1: Wales – Fielder *et al*., submitted a).

   Maxted *et al*. (2007) presented a preliminary analysis of UK CWR diversity, which highlighted the need for a full analysis and crucially, the need for close collaboration with relevant stakeholders in the UK responsible for conservation planning. The research in Chapters 2–4 and Appendix 1 present the results of this full analysis, including the development of inventories of priority CWR, *in situ* and *ex situ* gap analyses and formulation of recommendations to improve UK CWR conservation. This was carried out with input from statutory conservation agencies (Natural England, Natural Resources Wales and Scottish Natural Heritage) and takes a broad, multi-species approach to CWR conservation planning.

2. Distribution of crop wild relatives of conservation priority in the UK landscape (Chapter 5 – Jarvis *et al*., in press).

   The UK has an extremely well studied flora (Preston, 2002) but the habitat preferences of priority CWR in the UK are largely unknown. The Countryside Survey (CS), carried out across Great Britain (England, Wales and Scotland, excluding Northern

Ireland), provides an extremely detailed dataset of land cover, habitat classifications and species distributions for British vegetation (Carey *et al*., 2008). The CS was first carried out in 1978 and has since been repeated a further four times, the most recent survey taking place in 2007 (Carey *et al*., 2008). By extracting the 2007 CS data associated with the priority CWR listed in the UK inventory it was possible to identify habitat preferences for UK CWR in a series of 11,685 quadrats from 589 1km by 1km squares, a sample which is statistically representative of the wider UK countryside (Carey *et al*., 2008). With a more detailed understanding of habitat associations of CWR, *in situ* conservation actions can be more efficiently targeted in appropriate locations.

3. A genetic assessment of crop wild relatives on The Lizard Peninsula, Cornwall and its suitability to become the first UK genetic reserve (Chapter 6 – Fielder *et al*., submitted d).

   The Lizard was identified in the English and UK analyses in Chapter 2 and 4 as a potentially suitable site for a CWR genetic reserve, having high numbers of priority CWR. For this reason and because The Lizard already has a range of protected area designations associated with long-term conservation and a varied ecogeography, this study aimed to conduct a genetic analysis, using AFLPs, of a sample of the CWR that occur there to justifying its suitability as the first CWR genetic reserve in the UK, selected based on genetic data.

4. Next-generation sequencing: a powerful tool for targeting *ex situ* collection of crop wild relatives (Chapter 7).

Due to the under-representation of CWR accessions in genebanks, further collecting of germplasm is required. CWR collections should not only represent the range of taxa but should also cover the range of genetic diversity found in natural populations to maximise the diversity conserved and the range of traits available to plant breeders for crop improvement. Biotechnologies such as NGS can provide huge amounts of genetic information at ever-diminishing cost. In light of this, Chapter 7 aimed to demonstrate how NGS could be used routinely to identify SNP variation in existing CWR accessions to target CWR collecting missions to fill genetic gaps in genebanks e.g. the MSB, Royal Botanic Gardens, Kew. The model legume species *Medicago truncatula* Gaertn., which has a sequenced reference genome, was used in this study. Initial plans were to re-sequence only select genes of interest (rather than whole genome re-sequencing) relating to stress response pathways. Limitations outlined in section 8.3 highlight why this eventually became unfeasible. Though the setbacks experienced prevented the identification of SNPs and subsequent population genetic analysis, whole genome re-sequencing was successfully carried out resulting in a dataset ready for analysis.

CHAPTER 2.

Enhancing the conservation of crop wild relatives in England

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2.1 Abstract

Humans require resilient, rapidly renewable and sustainable supplies of food and many other plant-derived supplies. However, the combined effects of climate change and population growth compromise the provision of these supplies particularly in respect to global food security. Crop wild relatives (CWR) contain higher genetic diversity than crops and harbour traits that can improve crop resilience and yield through plant breeding. However, in common with most countries, CWR are poorly conserved in England. There is currently no provision for long-term CWR conservation *in situ*, and comprehensive *ex situ* collection and storage of CWR is also lacking. However, there is a commitment to achieve their conservation in England's Biodiversity Strategy and the UK has international commitments to do so as part of the Global Plant Conservation Strategy. Here, we identify a series of measures that could enhance the conservation of English CWR, thereby supporting the achievement of these national and international objectives. We provide an inventory of 148 priority English CWR, highlight hotspots of CWR diversity in sites including The Lizard Peninsula, the Dorset coast and Cambridgeshire and suggest appropriate sites for the establishment of a complementary network of genetic reserves. We also identify individual *in situ* and *ex situ* priorities for each English CWR. Based on these analyses, we make recommendations whose implementation could provide effective, long-term conservation of English CWR whilst facilitating their use in crop improvement.

2.2 Introduction

The most recent report by the Intergovernmental Panel on Climate Change (IPCC) suggests that crop yields will decrease by up to 2% per decade due to the negative impacts of climate change; with more severe forecasts expected beyond 2050 (Porter *et al*., 2014). This worrying statistic is compounded by another equally concerning prediction that the rise in the human population over the next 90 years (United Nations, 2011) will require global food production to increase by up to 70% (World Bank, 2008; Godfray *et al*., 2010). In order to ensure future food security, not only will crop yields need to increase significantly but the crops themselves will need to become progressively more resilient to changing conditions. As a result, plant breeders are now looking to crop wild relatives (CWR) and, more specifically, the largely untapped gene pool of genetic diversity within them as the key to tackling these issues through conventional plant breeding (Maxted *et al*., 2006).

CWR are the wild plants related to crops of socio-economic value, such as human food and animal forage and fodder crops as well as those used for medicinal, forestry, industrial and ornamental purposes etc. Though the conservation of CWR related to all plant-derived supplies is strongly encouraged, the focus of this paper is in the context of CWR related to human food and animal forage and fodder crops in order to address the pressing issue of food security. In contrast to their cultivated relatives, CWR 'have not passed through the genetic bottleneck of domestication' (Tanksley and McCouch, 1997; Maxted and Kell, 2009). As such, CWR harbour higher levels of genetic diversity and potentially contain a range of traits that could be used for crop improvement to increase the resilience and yield of modern crop varieties. The closeness of the relationship between a crop and its CWR can be defined in terms of the Gene Pool (GP) concept (Harlan and de Wet, 1971), where genes in CWR belonging to the primary Gene Pool (GP1b) of a crop can easily be transferred to the crop (belonging to GP1a). CWR in the secondary gene pool (GP2) can be crossed with the crop with some success but CWR in the tertiary gene pool (GP3) require biotechnological approaches to facilitate gene transfer (Harlan and de Wet, 1971). However, gene pool studies are often lacking, particularly for less studied crops, and so the Taxon Group (TG) concept relying upon traditional taxonomic analyses of relatedness can be employed to define this relationship (Maxted *et al*., 2006). In this case, TG1a corresponds to the crop/GP1a, TG1b denotes CWR belonging to the same species as the crop, TG2 denotes CWR belonging to the same section as the crop, TG3 being those belonging to the same subgenus and finally TG4 being those belonging to the same genus as the crop. Those CWR where gene transfer to a related crop is possible can contribute significantly to improving crop varieties, and their use in this way will become increasingly important (Tester and Langridge, 2010; Hopkins and Maxted, 2011). An extensive literature exists detailing examples of the use of CWR in crop improvement (Maxted and Kell, 2009). The introduction of *Cercospora* leaf spot and Rhizomania resistances from wild sea beet (*Beta vulgaris* L. subsp. *maritima* (L.) Arcang.) into cultivated sugar beet (Munerati, 1932; Lewellen *et al*., 1987), the transfer of corn leaf blight resistance from wild *Tripsacum dactyloides* (L.) L. into maize (Goodman *et al*., 1987) and more recently, the identification of the potential for wild barley (*Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell.) to improve the drought tolerance of cultivated barley (Lakew *et al*., 2011) are just a handful of examples of the use of CWR in plant breeding programmes for crop improvement. Recent estimates suggest that the potential for contribution of beneficial traits from CWR for 29 priority crops identified by the Millennium Seed Bank, Kew (including wheat, rice and potato) could alone amount to approximately $120 billion by 2021 (PwC, 2013).

Despite the recognition of their potential value in crop development, CWR are also highly threatened by factors which are impacting all wild plant species, such as the effects of habitat destruction, nutrient enrichment and climate change (Kell *et al*., 2012). In Britain it has been estimated that an average of one wild vascular plant was lost per county every two years throughout the twentieth century (Walker, 2003). This decline can be taken as a proxy for CWR decline, but worryingly CWR remain poorly conserved both *in situ* and *ex situ* globally and in the UK (Maxted, 2003; Maxted *et al*., 2008c; Hopkins and Maxted, 2011; Dias *et al*., 2012). This has led to a call for improved conservation of genetic resources such as CWR (Scholten *et al*., 2008; Hopkins and Maxted, 2011), working towards safeguarding their populations and the range of genetic diversity contained within.

The initial step required to improve CWR conservation is to identify which CWR taxa require improved protection. This can be achieved through the creation of a CWR checklist and inventory. A checklist simply consists of names of taxa and their authorities within the geographical region of study (Maxted *et al*., 2013a). It is likely to be unrealistic to conserve all CWR taxa in a checklist due to limitations of resources, time and money. Hence a prioritisation process is required, selecting appropriate criteria in order to create an inventory of CWR taxa. A CWR inventory consists of a prioritised list of CWR with ancillary information for each taxon (Maxted *et al*., 2013a). Production of CWR inventories is essential in development of conservation strategies, allowing a more focussed approach that targets taxa of highest priority in a given region of study (Lozano *et al*., 2012; Maxted *et al*., 2012).

Early European CWR checklists (e.g. by Zeven and Zhukovsky (1975), containing 430 plant genetic resource (PGR) species; Heywood and Zohary (1995), containing 206 CWR species and subspecies) tended to focus solely on the primary gene pools of the globally most important cultivated crops (Maxted *et al*., 2007). More recently a Crop Wild Relative

Catalogue for Europe and the Mediterranean was created, an inclusive checklist of 25,687 crop and CWR taxa occurring within Europe and the Mediterranean (Kell *et al*., 2005; Kell *et al*., 2008). As noted by Maxted *et al*. (2007), this checklist takes a more comprehensive view of CWR as it includes all European CWR taxa related to any socio-economically important crops, not just those used in food production. It also enables the extraction of CWR checklists for each European country via the Crop Wild Relative Information System, CWRIS (Kell *et al*., 2005).

Increasing numbers of national inventories of priority CWR are now being developed to encourage CWR conservation worldwide. Recently, prioritised CWR inventories for Venezuela, Benin and the USA have been developed (Berlingeri and Crespo, 2012; Idohou *et al*., 2012; Khoury *et al*., 2013). In Europe, CWRIS has been used as a starting point for the creation of a national inventory of CWR for Portugal (Magos Brehm *et al*., 2008) as well as inventories for Finland, Spain, Italy and Cyprus (Fitzgerald, 2013; Rubio Teso *et al*., 2013; Panella *et al*., 2014; Phillips *et al*., 2014). Although all of these inventories have been developed in a similar manner there are key differences in the approaches taken, particularly in terms of the criteria used in prioritising CWR. The selection of appropriate criteria is largely dependent on the specific requirements of the geographical region of study, those undertaking the inventory and the available information on which to base the prioritisation.

Once priority CWR have been identified for any geographical area of study it is then necessary to carry out a ‘gap analysis’. This is a process whereby the extent of current conservation efforts for priority taxa are examined and decisions made as to where further conservation efforts are necessary to ensure the long-term persistence of populations and the genetic diversity within them, using both *in situ* and *ex situ* approaches (Maxted *et al*., 2008a). A number of gap analyses have now been successfully carried out for a range of

CWR taxa in diverse locations. This has led to the identification of conservation priorities for wild soybean (*Glycine* Willd.) relatives in Australia (González-Orozco *et al*., 2012), beans (*Vigna* Savi) in Africa (Maxted *et al*., 2004), beans (*Phaseolus* L.) throughout North, Central and South America (Ramírez-Villegas *et al*., 2010), and national conservation priorities for Finland, Spain, Italy and Cyprus (Fitzgerald, 2013; Rubio Teso *et al*., 2013; Panella *et al*., 2014; Phillips *et al*., 2014).

Despite significant progress being made across Europe, no conservation priorities for CWR in the UK have yet been identified. Approximately eight percent of European CWR occur within the UK (Kell *et al*., 2008). These include wild relatives of economically important crops such as sugar beet and *Brassica* crops (e.g. cabbage, broccoli and Brussels sprouts) which are of particular commercial interest and which highlight the international role that the UK can play in the conservation of CWR (Hopkins and Maxted, 2011).

Previous CWR inventories for the UK have predominately consisted of short lists of minor crops with wild UK populations rather than the wild relatives themselves (MAFF, 1995; Franks, 1999; Maxted *et al*., 2007). Since then Defra (2011) have committed to conserving ‘agricultural genetic diversity in cultivated plants, farmed animals and wild relatives’ as part of their Biodiversity 2020 strategy for England. This follows on from commitments to the Convention on Biological Diversity’s Global Strategy for Plant Conservation as well as target 13 of the Strategic Plan for Biodiversity which states that ‘by 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, [will be] maintained, and strategies [will] have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity’ (CBD, 1992; 2010a; 2010b). This is reiterated in the European Strategy for Plant Conservation (Planta Europa, 2008) and Europe’s own Biodiversity 2020 strategy (European Union, 2011). Such policy documents are now providing the impetus for national conservation organisations within the UK to move genetic conservation of CWR higher up their agenda.

The objective of the current study was to support the achievement of these national and global commitments to conserve CWR in England. Conservation within the UK is managed separately within each of the devolved administrations of the UK, with some aspects coordinated by the Joint Nature Conservation Committee (JNCC). As such it was deemed appropriate to devise conservation priorities for England, Wales and Scotland separately with the involvement of the relevant statutory organisations in each country (Fielder *et al.*, submitted a; submitted b). In this way it could be ensured that the priorities identified were supported in each respective country. The University of Birmingham and Natural England (NE) developed the conservation priorities identified in the current study for English CWR jointly. Targets for CWR conservation are here identified and recommendations made as to how systematic, active and long-term conservation can be achieved in England to secure these valuable resources.

2.3 Materials and methods

2.3.1 CWR checklist and inventory

A checklist of English CWR was developed by matching a UK checklist of CWR (derived from CWRIS (Kell *et al.*, 2005; Maxted *et al.*, 2007) with a checklist of the English flora, extracted from the Vice County Census Catalogue (VCCC) (Stace *et al.*, 2003). The taxonomy in the English CWR checklist was then standardised against the British flora (Stace, 1997). The final checklist contains 1,471 CWR taxa including those related to all socio-economic crops (e.g. human food, animal forage and fodder, medicinal, forestry, industrial, ornamental). The CWR checklist includes native, archaeophyte, neophyte and casual taxa and represents 35% of the total English flora (again, including all native and introduced taxa). The checklist is available at the Plant Genetic Resources Diversity Gateway (http://pgrdiversity.bioversityinternational.org), and see Supplementary Table 2.1a.

The usual approach to identifying conservation priorities for vascular plants (e.g. considering rarity, threat and rate of decline) is not appropriate in the case of CWR. For CWR the focus is shifted towards those taxa that are the most useful or valuable in terms of their potential contribution to developing improved crop varieties, and in this particular case, food security. As a result, it is criteria such as the economic value of the crop with which they are related and the closeness of the genetic or taxonomic relationship between CWR and crop that infer which CWR should be considered a priority for conservation. Prioritisation of the CWR checklist was based on five criteria listed below, the selection of which aimed at the identification and conservation of genetic resources most likely to support resilience in global food production. Criteria one and two were first applied to the CWR checklist to identify a pool of native and archaeophyte CWR that were most relevant to food security considerations. The remaining three criteria (criteria 3 to 5) were applied in turn to this CWR pool. A CWR was required to meet the conditions of just one of criteria 3 to 5 to be listed in the final inventory of priority CWR for England.

1. Use of the related crop – CWR related to crops used as a human food source or for animal feed (forage/fodder) were prioritised due to their being the CWR most relevant to ensuring future food security. ‘Use’ data were extracted from GRIN Taxonomy for Plants (USDA, ARS, National Genetic Resources Program, 2015).

2. Native status – Priority was assigned to taxa classified as native or archaeophyte in England. Data regarding native status was extracted from the VCCC (Stace *et al.*, 2003).

3. Economic value of the related crop – A list of economically important human food crops was generated by extracting crop production quantity data (million tonnes) at the global, European and UK levels between 2007 and 2011 from FAOSTAT (2013), crop production value at producer price data between 2007 and 2011 (millions of euro) from Eurostat (2013) and crop production at market prices data (£ million) at the UK level between 2006 and 2010 from Defra (2010). Data representing a period of five years were extracted to reflect recent value and current trends in agriculture. However, when long-term trends were considered (period of 20 years), identical crop lists were obtained. Wild relatives of all crops with data relating to any of these statistics were prioritised. Equivalent economic values for forage and fodder crops were unavailable; a similar problem was experienced by Kell *et al.* (2012).

4. Degree of relatedness to the crop – Using the Gene Pool and Taxon Group concepts described above, priority was assigned to taxa in GP1b and GP2 and TG1b, TG2 and TG3 of their related crop. Where sub-generic taxonomic classifications were not available, CWR were assigned to TG4. Taxa only occurring in GP1a or TG1a were not prioritised as they are the cultivated forms of the taxa for which wild populations were not recorded as present in England. Data were extracted from the Harlan and de Wet inventory of globally important CWR taxa (Vincent *et al.*, 2013). It was commonly observed that a single CWR would be related to more than one crop. In these cases, the lowest GP/TG that it belonged to was selected, and prioritisation based on this number.

5. Recent change in population range – Priority was assigned to CWR whose population ranges have declined between two recent survey years (1987 and 2004) according to Change Factor (CF) data. CF data take into account the differing range sizes of each species and corrects the data to enable interspecific comparisons to be made (Braithwaite *et al*., 2006). Any CWR with a negative CF value (i.e. below the threshold of zero) indicated population decline and was prioritised.

2.3.2 In situ *gap analysis*

Occurrence data records for taxa in the final English inventory of priority CWR were extracted from the Botanical Society of Britain and Ireland (BSBI) distribution database (BSBI, 2013). These raw data were filtered to produce a 'clean' dataset of records. Records listed as 'doubtful' or 'unconfirmed' were excluded, as were any records dated older than 1970. Records lacking both coordinates and location descriptions were removed from the dataset and all records that had a precision lower than tetrad level ($2km^2$) were also excluded. The reliability of the final gap analysis results is directly related to the accuracy and quality of data input into the analysis (Maxted *et al*., 2008a; Ramírez-Villegas *et al*., 2010). The coordinates for all occurrences in the final dataset were recorded in decimal degrees for compatibility with mapping software.

In situ gap analysis was undertaken using the mapping softwares ArcMap 10.0 and DIVA-GIS 7.5.0 (ESRI, 2011; Hijmans *et al*., 2012). Country boundary files were obtained from DIVA-GIS (www.diva-gis.org). Using methods described by Hijmans *et al*. (2012) and Scheldeman and van Zonneveld (2010) the following GIS functions were carried out:

- Taxon richness and observation richness – to determine hotspots of taxon diversity and to identify any sources of data bias, using the 'point to grid' function in DIVA-GIS with a grid cell size of 0.1 degrees.

- Complementarity analysis – using the 'reserve selection' function in DIVA-GIS to select potential sites for CWR genetic reserves. An iterative method was used where the first selected site contains the highest number of taxa, the second site was selected on the basis that it contained the next highest number of taxa excluding those contained in the first site etc. (Rebelo, 1994). A grid cell size of 0.1 degrees was used. Complementarity results were then further explored to determine which taxa are represented in five or more complementary grid squares. Conserving at least five geographically distinct populations of a taxon decreases the likelihood that it will be lost in the face of stochastic change or through human influence (Brown and Briggs, 1991; Dulloo *et al*., 2008).

- Identification of *in situ* conservation actions required for each priority CWR – to identify the extent to which CWR taxa are passively conserved *in situ* using spatial join tools in ArcMap 10.0. Boundary shape files for protected areas were obtained from NE (2014). These included: Sites of Special Scientific Interest (SSSI), Special Areas of Conservation (SAC), Areas of Outstanding Natural Beauty (AONB), National Nature Reserves (NNR), Local Nature Reserves (LNR), National Parks, Country Parks, Ramsar sites, Special Protection Areas (SPA) and Biosphere Reserves. Each CWR was then categorised into priority levels according to how well represented they are in protected areas. A protected area with at least five occurrences was considered an appropriate threshold above which CWR are well represented. This is likely to cover 90–95% of common alleles for any particular species in a protected area (Brown and Briggs, 1991), ensuring a range of genetic diversity is conserved per species using a practical and achievable method.

- Priority 1 – Poorly represented in protected areas (Less than five protected areas contain five or more occurrence records of the CWR)
- Priority 2 – Poorly represented in SSSIs but well represented in other protected areas i.e. SACs, AONBs, NNRs, LNRs, National Parks, Country Parks, Ramsar sites, SPAs and Biosphere Reserves (Less than five SSSIs contain five or more occurrence records of the CWR but five or more other protected areas contain at least five occurrence records)
- Priority 3 – Well represented in SSSIs (Five or more SSSIs contain at least five occurrence records of the CWR)

For each priority level, recommendations were made for improved *in situ* conservation of the CWR (Supplementary Table 2.2a). CWR listed under Section 41 (S41) of the 2006 Natural Environment and Rural Communities (NERC) Act of rare and threatened species were also identified (NERC, 2013).

2.3.3 Ex situ *gap analysis*

Accession data for priority English CWR were obtained from the UK National Plant Inventory (UKNPI, 2013) and the Millennium Seed Bank, Kew. Data from these two sources were combined and any accessions lacking data for latitude and longitude fields were georeferenced where possible by comparing their written location description with both the UK Grid Reference Finder (http://www.gridreferencefinder.com) and the Gazetteer of British Place Names (http://www.gazetteer.org.uk).

In order to carry out an *ex situ* gap analysis, the following steps were carried out:

- CWR were listed according to the number of *ex situ* accessions stored in genebanks. A minimum threshold was set at five stored accessions (Brown and Briggs, 1991; Dulloo *et al*., 2008), above which CWR are considered sufficiently represented in *ex situ* collections but below which further collection is required. A minimum of five accessions was deemed more practical and achievable in the field than other more ambitious thresholds e.g. Brown and Marshall's recommendation to collect accessions from a minimum of 50 populations (Brown and Marshall, 1995).

- For all taxa with accession data, the geographic coverage of the accessions and occurrences were compared for each taxon. Using the 'circular area statistic', a geographical representativeness score (GRS) was calculated for each taxon (GRS is the proportion of occurrence data covered by accession data for each taxon, expressed as a percentage) (Ramírez-Villegas, 2010). The lower the GRS value, the higher the taxon in terms of its level of priority for collection. A GRS of 30% or less is generally viewed as a threshold, below which further *ex situ* collection of the taxon is advisable (Ramírez-Villegas, 2010; Phillips *et al*., 2014).

- Based on these two *ex situ* results, CWR were categorised into *ex situ* priority levels, 1–6, according to the number of accessions stored *ex situ* per taxon and the GRS value. The highest priority being assigned to CWR for which there are currently no accessions. Conservation actions were recommended according to each priority level (Supplementary Table 2.2a).

- Priority 1 – No accessions
- Priority 2 – Has accessions but none are georeferenced/location data restricted
- Priority 3 – Fewer than five accessions and GRS lower than 30%
- Priority 4 – Fewer than five accessions but GRS greater than 30%
- Priority 5 – Greater than or equal to five accessions but GRS lower than 30%
- Priority 6 – Greater than or equal to five accessions and GRS greater than 30%

2.4 Results

2.4.1 CWR inventory

The English national inventory of priority CWR contains 148 taxa (126 species and 22 subspecies), representing 10% of the taxa listed in the English CWR checklist. A summary of the inventory is displayed in Table 2.1 (and see full inventory at the Plant Genetic Resources Diversity Gateway, http://pgrdiversity.bioversityinternational.org, and Supplementary Table 2.1b). Of the 148 priority CWR, 76% are related to food crops whilst the remaining 24% are related only to forage or fodder crops. The English inventory contains 13 plant families, with Poaceae, Brassicaceae and Fabaceae containing the most genera (16, 7 and 7 respectively). The three genera with the highest taxon richness are *Trifolium* L. (clovers, 18 taxa), *Vicia* L. (vetches, 12 taxa) and *Chenopodium* L. (goosefoots, 11 taxa).

Table 2.1 Summary of inventory containing 148 priority CWR in England.

Family	Genera	Species	Infra-specific taxa	Native status
Apiaceae	3	2	4	N
Asteraceae	2	4		A & N
Brassicaceae	7	9	4	A & N
Chenopodiaceae	3	13	1	A & N
Corylaceae	1	1		N
Ericaceae	1	5		N
Fabaceae	7	38	5	A & N
Geraniaceae	1	1		N
Grossulariaceae	1	3		N
Liliaceae	2	9		A & N
Linaceae	1	2	1	N
Poaceae	16	28	4	A & N
Rosaceae	6	11	3	A & N
Totals	**51**	**126**	**22**	

A = Archaeophyte, N = Native.

Through the application of criteria for prioritisation of CWR, 34 food crops with native or archaeophyte CWR within England were of economic value based on agricultural statistics (Defra, 2010; Eurostat, 2013; FAOSTAT, 2013), including sugar beet, barley, onions, apples and various brassica crops. Crops of economic value according to production at market prices data from Defra (2010) are illustrated in Fig. 2.1. All of the native and archaeophyte CWR in England associated with these 34 economically valuable crops were listed in the English inventory of priority CWR (77 CWR in total). In terms of the genetic relationships between priority CWR and their associated crops, little over a quarter of taxa (26%) had available Gene Pool classifications. Within this, 16.89% were classified as GP1b and just 5.41% and 4.05% in GP2 and GP3 respectively. The remaining taxa (74%) were classified using the Taxon Group concept.

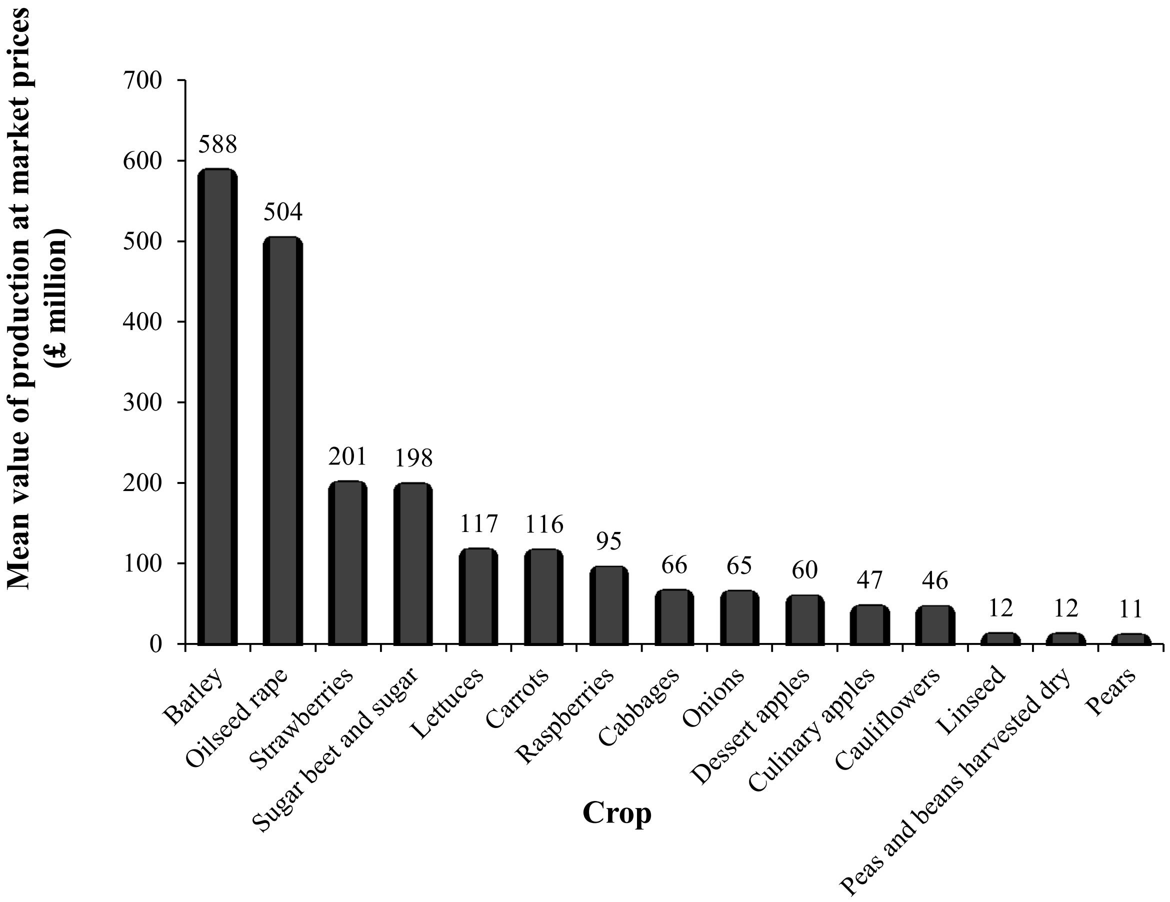

Figure 2.1 Economic value of English crops. Mean value of production at market prices (£ million) in England between 2006 and 2010 (Defra, 2010) for socio-economic crops with native or archaeophyte CWR occurring within England.

Almost half (43%) of English priority CWR had a negative CF showing that their populations have declined between surveys carried out between 1987 and 2004. The most extreme changes are in white clover (*Trifolium repens* L., -84), annual meadow-grass (*Poa annua* L., -68) and wood vetch (*Vicia sylvatica* L., -52). In contrast only 28% were shown to be increasing and a further 28% had no available data. According to the newly published Vascular Plant Red List for England (Stroh *et al*., 2014), 14% of priority CWR taxa in England are threatened. Both upright goosefoot (*Chenopodium urbicum* L.) and alpine cat's-tail (*Phleum alpinum* L.) are listed as being Critically Endangered with a further five taxa listed as Endangered and 14 listed as Vulnerable (Table 2.2).

Table 2.2 Threatened taxa listed in the English CWR inventory.

Taxon	Red List Status	Criterion
Chenopodium urbicum L.	CR	A2c AOO trend
Phleum alpinum L.	CR	D
Chenopodium vulvaria L.	EN	A2c AOO trend
Lactuca saligna L.	EN	B1ac(iv) + B2ac(iv)
Chenopodium murale L.	EN	A2c AOO trend
Pyrus cordata Desv.	EN	D
Trifolium bocconei Savi	EN	A2ac AOO trend; D
Asparagus prostratus Dumort.	VU	D1
Apium inundatum (L.) Rchb.f.	VU	A2c AOO and EOO trend
Cichorium intybus L.	VU	A2c AOO trend
Trifolium fragiferum L.	VU	A2c AOO trend
Trifolium ochroleucon Huds.	VU	A2c AOO trend
Vicia lutea L.	VU	A2c AOO trend
Vicia orobus DC.	VU	D1
Allium sphaerocephalon L.	VU	D1; D2
Chenopodium bonus-henricus L.	VU	A2c AOO trend
Chenopodium glaucum L.	VU	A2c AOO trend
Hordeum marinum Huds.	VU	A2c AOO trend
Medicago minima (L.) Bartal.	VU	A2c AOO trend
Trifolium strictum L.	VU	D2
Vicia parviflora Cav.	VU	A2c AOO trend

VU = Vulnerable; EN = Endangered; CR = Critically Endangered; A2c = reduction in population size based on trend in Area of Occupancy (AOO) or Extent of Occurrence (EOO); B1ac(iv) = EOO less than 5000km^2 and highly fragmented or in no more than 5 locations and extreme fluctuations in number of locations; B2ac(iv) = AOO less than 500km^2 and highly fragmented or in no more than 5 locations and extreme fluctuations in number of mature individuals; D = restricted population size (less than 50 mature individuals if CR and less than 250 mature individuals if EN); D1 = Very restricted population of less than 1000 mature individuals; D2 = Very restricted population based on Area of Occurrence or number of locations). Data Source: (Stroh *et al.*, 2014).

2.4.2 In situ *gap analysis*

Pastinaca sativa L. subsp. *sylvestris* (Mill.) Rouy & E. G. Camus was the only priority CWR in England with no occurrence records of sufficient quality, despite being listed as native in the British flora (Stace, 2010). It is likely that records for this taxon have, until now, been treated as wild parsnip (*Pastinaca sativa* L.) for which there are many more occurrence records (3,763). Occurrences recorded only to species level may belong to other subspecies and varieties. For this reason, the inclusion of all *P. sativa* records could have introduced inaccuracies to the dataset. This highlights the importance of up-to-date and specific recording to the sub-specific level. In total, 679,521 occurrence data points relating to 147 taxa were included in the *in situ* gap analysis. Small cranberry (*Vaccinium microcarpum* (Turcz. ex Rupr.)) and eastern parsnip (*Pastinaca sativa* L. subsp. *urens* (Req. ex Godr.) Čelak) were found to have the fewest occurrence records with just one each, (again, the latter is likely to have been recorded as *P. sativa*), whereas cock's foot (*Dactylis glomerata* L.) was found to have the highest number of occurrence records (28,793).

Taxon richness analysis of the 147 CWR taxa revealed a number of CWR hotspots throughout England, particularly focussed in the south and east of the country (Fig. 2.2a). These include sites in Cornwall, the Dorset coast, Somerset, Norfolk and Bedfordshire. However, some recording bias is apparent in Bedfordshire (Fig. 2.2b). A new flora was published for this county in 2011 (Boon and Outen, 2011), which could account for the recording bias detected in this area.

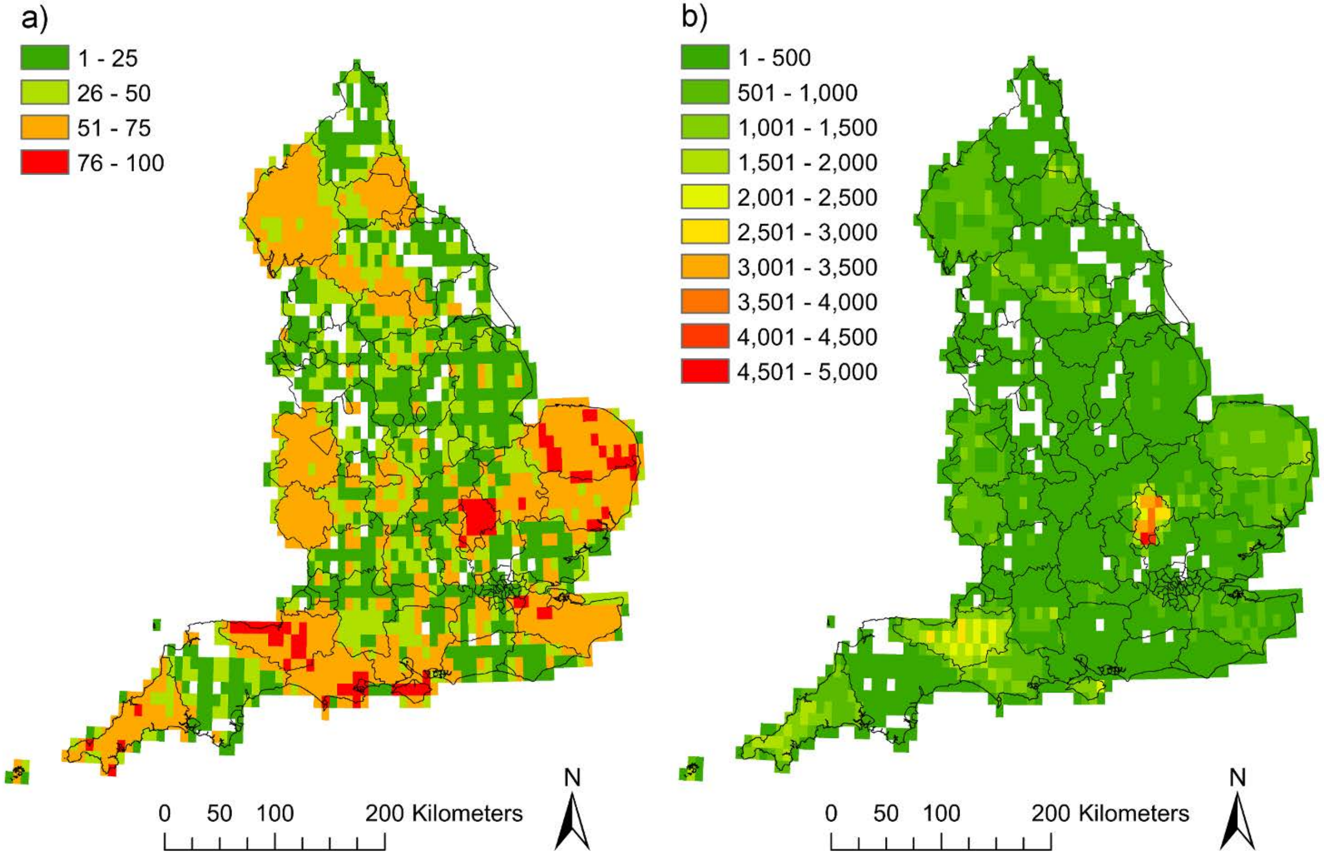

Figure 2.2 Richness analysis. a) Taxon richness. b) Observation richness. Both a) and b) include all 147 taxa with occurrence data points in the English CWR inventory and a grid square size of 0.1 degrees.

The complementarity analysis shows that a total of 15 grid squares (each measuring 0.1 degrees square or approximately 11km^2) are sufficient to contain at least one occurrence of all priority CWR taxa included in the analysis (Fig. 2.3). The highest priority grid square, containing the highest number of CWR (94), is located in Purbeck on the south coast of Dorset. The second and third priority grid squares are located on The Lizard Peninsula in Cornwall and in the south of Cambridgeshire respectively. The Lizard grid square contains 75 CWR, 14 of which do not occur in the highest priority grid square. The grid square in Cambridgeshire contains a total of 80 CWR, 10 of which do not occur in either of the preceding grid squares (Dorset and The Lizard). Together, the top three priority grid squares cover over 80% of all English priority CWR. The percentage of additional CWR contributed by each grid square is illustrated in Fig. 2.4. Other priority grid squares were located across the full range of the country, from Cornwall through the midlands to Cumbria and Northumberland. All 15 grid squares overlap with a range of protected areas including SSSIs, AONBs and NNRs. However, these designations do not necessarily provide any protection or active conservation for CWR. In addition, it was found that 53% of priority CWR were recorded in at least five of the 15 complementarity grid squares.

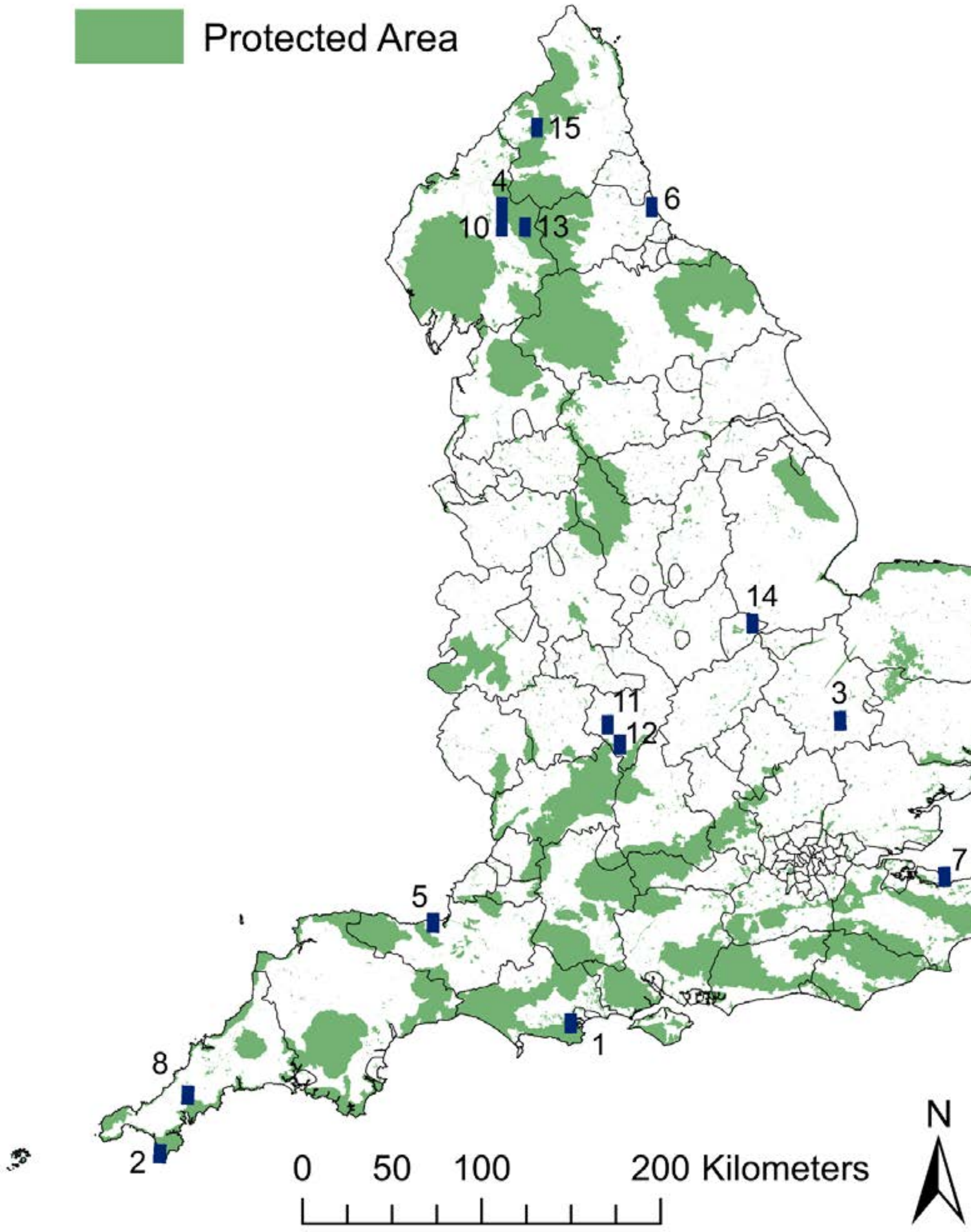

Figure 2.3 Complementarity analysis. The locations of all 15 priority grid squares/candidate sites recommended for CWR genetic reserves.

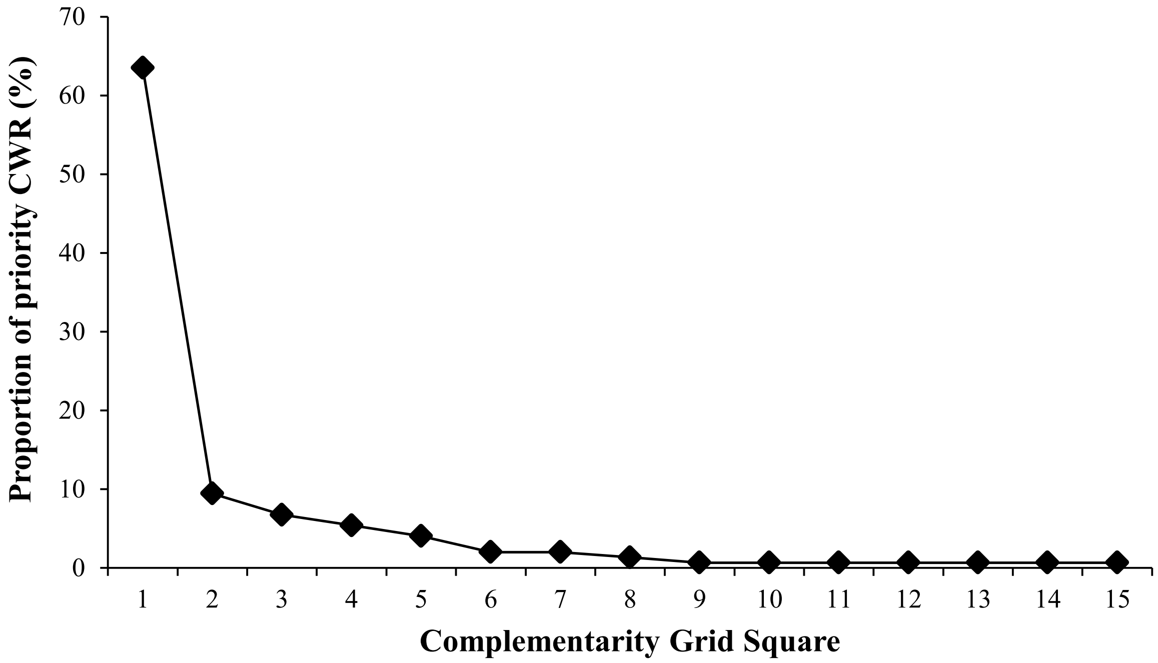

Figure 2.4 Complementarity analysis. The percentage of additional priority CWR contained within each of the 15 priority grid squares/candidate sites recommended for CWR genetic reserves.

In situ conservation actions required for each priority CWR were identified by assigning a priority level to each CWR. Results show that 34 CWR (23%) are poorly represented in protected areas in England (Priority 1) highlighting the need for enhanced conservation for these taxa. An additional 32 CWR (22%) are poorly represented in SSSIs but are well represented in other protected area designations (Priority 2). The remaining 82 CWR are well represented in SSSIs (Priority 3). Recommended actions for enhancing the conservation of each priority group of CWR are detailed in Supplementary Table 2.2a. A further six CWR are listed under section 41 of the NERC act of rare and threatened species. These are wild asparagus (*Asparagus prostratus* Dumort.), upright goosefoot (*C. urbicum*), stinking goosefoot (*Chenopodium vulvaria* L.), least lettuce (*Lactuca saligna* L.), plymouth pear (*Pyrus cordata* Desv.) and sea barley (*Hordeum marinum*). Actions required to conserve all six of these taxa are outlined under the NERC act, however all actions are listed as 'yet to start' except for three associated with *P. cordata* which are 'in progress'.

2.4.3 Ex situ *gap analysis*

Sixty-five priority CWR (44%) have no *ex situ* accessions stored within UK genebanks. These taxa were assigned the highest level of priority (Priority 1). Amongst the taxa with no accessions are upright goosefoot (*C. urbicum*) and alpine cat's-tail (*P. alpinum*), both of which are listed as Critically Endangered in England. Of the 83 CWR with accessions, perennial rye-grass (*Lolium perenne* L.) had the most (202). A further 22 CWR also had more than the advised minimum of five accessions (Brown and Briggs, 1991; Dulloo *et al*., 2008) with next highest number of accessions belonging to plymouth pear (*P. cordata*) with 49. A total of 687 accessions exist for English priority CWR, 50% of which are stored at the Millennium Seed Bank, Kew. A total of 279 accessions are stored at the Genetic Resources Unit, IBERS at Aberystwyth University and a further 57 are stored at Warwick Genetic Resources Unit. Seven accessions lack holding institution data. The majority of accessions (92%) have fully georeferenced passport data. See Supplementary Table 2.3 for the number of accessions stored in *ex situ* collections per priority CWR.

Geographical representativeness analysis revealed that only two taxa have a GRS score above the threshold of 30% (Ramírez-Villegas *et al*., 2010). These are two species with very restricted abundance and range in England, plymouth pear (*P. cordata*) and round-headed leek (*Allium sphaerocephalon* L.), suggesting that these are the only priority CWR for which *ex situ* collections are representative of their *in situ* range. The majority of taxa (69) had a GRS score below 5%. The relationship between the geographic coverage of *in situ* occurrence data and *ex situ* accession data is illustrated in Fig. 2.5. It is clear that forage CWR tend to have higher numbers of accessions stored *ex situ* than food CWR, but also that for the majority of priority CWR taxa in England GRS percentages are extremely low. This indicates clear gaps in *ex situ* collections for priority CWR in England.

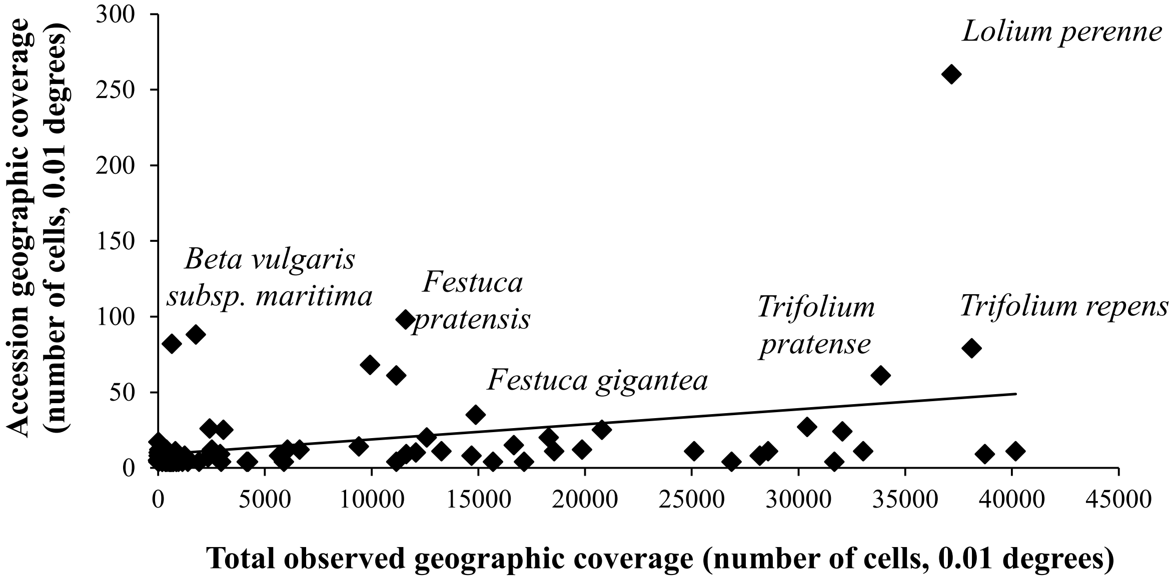

Figure 2.5 Geographic coverage of accession data. Taxa falling above the line show those with a GRS score higher than the mean GRS across all taxa, the accessions for these taxa cover a larger proportion of the taxon's native range in England.

On combining the number of accessions and GRS results, it was found that only plymouth pear (*P. cordata*) is currently well represented in *ex situ* collections (i.e. assigned priority level 6). In addition, 22 CWR were listed in priority level 5 (having greater than five accessions but with a GRS lower than 30%). Over a third of CWR were listed as priority level 3 (36%) with between one and four accessions and a GRS below 30%. The majority of CWR were assigned to priority level 1 (44%), with no accessions. See Supplementary Table 2.2 for a full list of assigned priority levels and recommended actions per priority level required to enhance *ex situ* collections.

2.5 Discussion

2.5.1 Prioritisation to create a CWR inventory

In England, conservation actions through Red Lists, site designations, species protection measures and other mechanisms have primarily been focussed upon nationally local and rare taxa. However this does not result in conservation of all CWR taxa and even less so the genetic diversity within them. For example, it has resulted in considerable efforts aimed at the conservation of wild asparagus (*A. prostratus*) (King *et al*., 2007; King and Edwards, 2007) but little attention paid to conservation of potentially more important germplasm in taxa such as sea beet (*B. vulgaris* subsp. *maritima*) and cabbage (*Brassica oleracea* L.). Assessment and specific conservation planning for all CWR (food, forage, fodder, forestry, medicinal, industrial etc.) is therefore an important adjunct to more traditional conservation assessments and actions if CWR and their genetic diversity are to be adequately conserved. The English inventory of 148 priority CWR was developed based on criteria selected through consultation with Natural England. It identifies priorities for CWR conservation with a focus on the genetic resources most likely to be valuable to global food security. In addition to the current study, further work should be carried out to ensure comprehensive conservation of all other valuable plant-derived supplies and their wild relatives.

Identifying priority CWR based on meaningful criteria is the first step in planning for CWR conservation. Criteria selected to produce the English CWR inventory are similar to those selected in the development of CWR inventories in other countries. Three key criteria are most commonly cited: CWR native status, economic value of the related crop and degree of relatedness to an associated crop (Venezuela (Berlingeri and Crespo, 2012); USA (Khoury *et al*., 2013); Finland (Fitzgerald, 2013) and Spain (Rubio Teso *et al*., 2013)), the latter two in particular showing the emphasis is frequently on criteria relevant to global food security. Other more distinct criteria have also been used. One such example can be seen in the CWR inventory for Cyprus where any CWR whose centre of diversity is within Cyprus, the Near East or the Middle East were prioritised, a decision taken through consultation with Cypriot stakeholders and focussing on conserving areas with the highest levels of CWR genetic diversity (Phillips *et al*., 2014). The selection of criteria can also be heavily influenced by available data. In England, the criterion 'change in population range' was included due the need to focus conservation efforts on CWR that are declining, and due to the availability of data looking at the change in distributions in plant species in Britain between two survey years (1987 and 2004) (Braithwaite *et al*., 2006).

The selection and application of criteria for prioritisation of CWR is application specific and must be considered in terms of their degree of relevance to national and international conservation needs and priorities (Maxted *et al*., 1997a; Magos Brehm *et al*., 2010). The process should involve key national stakeholders and conservation organisations to ensure their support for any conclusions drawn from such analysis but should equally consider whether the process addresses international policy targets e.g. Aichi target 13 (CBD, 2010b). Though the use of different criteria can change the pool of CWR listed as priority, if the criteria selected are appropriate to the project objectives and respond to policy then the various approaches should not compromise the achievement of effective conservation of CWR.

2.5.2 In situ *gap analysis*

There are currently very few examples of active *in situ* conservation of CWR in the UK. Only six priority CWR in England (Supplementary Table 2.2b) are recognised as threatened and are therefore listed on country or UK conservation priority lists (Cheffings *et al*., 2005; NERC, 2013) and just two of these six species have long-term and active conservation plans (wild asparagus – *A. prostratus* and plymouth pear – *P. cordata*) (Jackson, 1995; King *et al*., 2007; King and Edwards, 2007). This shows there is a need for a concerted effort to enhance *in situ* conservation in England for CWR.

The results of the complementarity analysis revealed 15 grid squares across England that together are sufficient to conserve all priority English CWR, though for this to be possible active conservation of CWR populations and genetic diversity would need to be established within each of these sites. Each complementarity square overlaps with at least one protected area and over half of English priority CWR are well represented in SSSI protected areas. A further 22% are well represented in other protected area designations. Though presence within protected areas may offer a level of passive protection (Maxted *et al*., 1997b; Maxted *et al*., 2008b) it is important that there is specific monitoring and management for CWR to ensure their long-term survival. It may also be the case that the existing management of a protected area may conflict with the needs of the CWR, for example, on The Lizard Peninsula in Cornwall where there is concern over the impact of the level of cattle grazing in coastal sites on already threatened populations of wild asparagus (*A. prostratus*, Steve Townsend pers. comm.). This emphasises the need for incorporating active CWR conservation, ideally in the form of genetic reserves where conservation of genetic diversity is a priority, into existing protected area planning to enable CWR monitoring and appropriate management. Further analysis revealing the number of hectares of each protected area designation overlapping the 15 grid squares identified here has been carried out in 2015 by Natural England (unpublished data) with a view to justifying the inclusion of CWR in the management plans of existing protected area designations. This can be a highly effective method for achieving active CWR conservation as it avoids the high start-up costs of acquiring land for a new reserve and it may only require minimal adjustments to existing management plans (Maxted and Kell, 2009). Standards for the establishment of CWR genetic reserves have been outlined by Iriondo *et al.* (2012).

The complementarity analysis method used in the current study (Rebelo, 1994) is a valuable tool that gives a broad picture of which sites in a country or region are suitable for *in situ* CWR conservation. It is important to note that the priority grid square boundaries do not denote the recommended outline of CWR genetic reserves, rather they indicate broad-scale areas which will require more detailed investigation and ground-truthing in order to identify more specific, fine-scale locations appropriate for *in situ* conservation of CWR both inside and outside of protected areas (Fitzgerald, 2013). Part of this fine-scale selection of priority CWR populations and sites should consider the individual priority levels assigned to each CWR (Supplementary Table 2.2) but should also involve an assessment of the genetic diversity within target CWR (Frankel *et al*., 1995; Magos Brehm *et al*., 2012). With this data available, it ensures that conserved populations are representative of the range of genetic diversity that exists across a delineated area and that the selected populations are also complementary to one another. This is an ideal that has been achieved on The Lizard Peninsula in Cornwall where the range of genetic diversity in eight CWR was assessed and populations were identified as priority based on this data (Fielder *et al*., submitted d).

Traditional conservation of rare and threatened plants generally takes place within designated protected areas. CWR however could also benefit from an approach that targets their conservation outside of protected areas. It is widely accepted that CWR tend to be associated with pre-climax communities and areas experiencing anthropogenic change (Maxted and Kell, 2009) and though such habitats are present within protected areas, habitats outside protected areas are likely to experience more human-induced change. This in turn could be exposing CWR to a range of different selection pressures, which could lead to adaptation of novel and potentially beneficial traits that could be exploited in crop development. A recent study by Jarvis *et al*. (in press) has been able to provide strong evidence that UK CWR show preferences for linear landscape features such as field margins and road verges. Further, the current study revealed that 23% of priority English CWR are poorly represented within the existing protected area network. As such, it should be seen as a priority that alongside the establishment of genetic reserves for *in situ* conservation of CWR inside protected areas, there should be an additional focus on monitoring and management of CWR populations outside of protected areas. This approach would be most effective if undertaken with the approval and involvement of appropriate landowners and land managers.

2.5.3 Ex situ *gap analysis*

There are clear gaps in *ex situ* CWR collections with just over half of the English priority CWR having any stored accessions at all. Of the taxa with accessions, very few have more than one. Ideally, it is recommended that at least five different populations are stored *ex situ* in genebanks to ensure that the collections represent the range of genetic diversity found within and among populations *in situ* (Brown and Briggs, 1991). From the results of the current gap analysis it is clear that this is a target of which English collections are currently falling short with only 16% of English priority CWR having greater than five stored accessions.

The GRS method for analysing the geographic coverage of *ex situ* accessions has been used successfully in previous studies (Maxted *et al*., 2008a; Ramírez-Villegas *et al*., 2010; Phillips *et al*., 2014), providing a broad picture of the representativeness of collections. In the case of

England, it is apparent that most collecting effort has so far been focussed on forages. There has been a particular focus on the collection of perennial rye-grass (*L. perenne*) accessions at IBERS, Wales where extensive research has been carried out on this species due to its high importance as forage in temperate areas (Skøt *et al*., 2007). Ramírez-Villegas *et al*. (2010) advise that a GRS result above a threshold of 30% suggests a CWR taxon is adequately conserved *ex situ* (though comparative genetic diversity studies are still required to validate this). Using GRS in combination with the total numbers of accessions per CWR provides a useful means of categorising CWR into priority levels to imply the urgency of further collecting.

In previous GRS studies herbarium specimens have been used to infer the full species distribution across a country or continent, providing a useful comparison of *in situ* distributions and gene bank representativeness (Maxted *et al*., 2008a; Phillips *et al*., 2014). In contrast, the use of herbarium specimens was not necessary in England due to the volume of field occurrence data available, directly showing species distributions across the country. However, the comparison of these occurrence records to the relatively few *ex situ* accessions suggests that extensive collecting would be necessary to achieve the 30% threshold for all CWR in England. In such cases where there is such a discrepancy between the volumes of available *in situ* and *ex situ* data, it may be more appropriate to consider each taxon on a case-by-case basis and to employ genetic or ecogeographic methodologies. In this way it would be possible to see more clearly whether further collecting is necessary.

Ex situ collections should be considered as a backup of material, which should be representative of populations conserved *in situ* (Maxted and Kell, 2008). In addition, it is necessary that CWR accessions are stored in genebanks to enable plant breeders and other users to access this material for use in crop improvement. Therefore, it is a prerequisite that *ex* *situ* collections represent the range of genetic diversity found within and among *in situ* CWR populations (Parra-Quijano *et al*., 2008). This can be achieved by proxy through collection of material from the full ecological and geographic range of each CWR, though direct analyses of genetic diversity using molecular markers will always be preferable and should be carried out where possible. The inclusion of genetic diversity and/or ecogeographic diversity data in genebank databases would take large steps towards improving the completeness of *ex situ* CWR collections (Rubio Teso *et al*., 2013).

2.5.4 Recommendations for enhancing CWR conservation in England

- The CWR inventory should be regularly reviewed (e.g. once every ten years) with the involvement of key stakeholders, providing the opportunity for: 1) national and international priorities to be reviewed according to policy, climate change and other factors such as pests and diseases, invasive species, pollution etc. 2) appropriate prioritisation criteria to be re-evaluated and 3) incorporation of more up-to-date data. Moreover, prioritisation of all other categories of CWR in England (not just those relating to human food or animal forage/fodder crops) should be undertaken.
- The *in situ* gap analysis results presented here should be used as a guide for enhancing CWR conservation within England. It is encouraged that existing protected areas (including, but not limited to SSSIs, SACs and NNRs) fully integrate CWR conservation into species and habitat management plans. Where possible these protected areas should strive to meet the agreed standards for CWR genetic reserves (Iriondo *et al*., 2012), particularly where they overlap with the 15 complementarity squares. Work is already underway on The Lizard Peninsula, Cornwall and in Purbeck, Dorset to achieve this.

- CWR conservation should also be encouraged outside of protected areas. Relevant landowners and land managers should be given the opportunity to agree to the quality standards for CWR genetic reserves, with the aim of establishing long-term CWR conservation. Governmental incentives (e.g. through the Rural Development Programme) could be provided to encourage this. Appropriate management of farmed semi-natural habitats such as hedgerows, grasslands and coastlands, as well as low intensity management of field margins and buffer strips would benefit CWR (Hopkins and Maxted, 2011).

- To maximise the level of genetic diversity conserved *in situ* for priority CWR, at least five populations representing their full geographic range in England should be conserved per CWR. Where possible direct genetic analyses of populations of all priority CWR (in all categories) is encouraged. Initial focus should be on CWR assigned to *in situ* priority level 1 (Supplementary Table 2.2).

- Accurate records of the locations of all CWR throughout England are required, ideally along with assessments of population sizes and densities. All demographic data should be made available in an online database (e.g. NBN Gateway or BSBI distribution database). The data should be widely accessible and easy to update with new records.

- *Ex situ* collections need to be representative of *in situ* genetic diversity within and among populations of all CWR categories. To address this, there should be a renewed effort to improve the completeness of *ex situ* collections of English CWR by ensuring a minimum of five accessions representative of the geographic range of each CWR are stored in genebanks. Where possible genetic analyses should also be undertaken to achieve this goal. Initial focus should be on CWR in *ex situ* priority level 1

(Supplementary Table 2.2). This threshold is not intended to be prescriptive but should be a seen as a minimum, above which the specific life histories of taxa are also considered to ensure the maximum range of diversity is conserved within the accessions maintained *ex situ*.

- Existing accessions should also be assessed for regeneration ability and to ensure they are being maintained according to genebank 'best practices' to enable long-term viability and use.
- The relationship between accessions held in long-term storage and population genetic changes over time should be explored and the appropriate intervals for updating *ex situ* collections determined per species to capture the genotypic evolution of *in situ* populations over time.
- Accessions should have at least one duplicate stored in a geographically distant genebank to reduce the likelihood of material being lost in any unforeseen circumstances.
- Genebanks should undertake greater promotion of the conserved CWR material available to researchers, pre-breeders and plant breeders for use in crop improvement, encouraging their use in the development of new crop varieties.

2.6 Conclusion

Conservation in England of the 148 CWR identified as priority in the context of food security is currently incomplete. This paper presents recommendations for enhancing their conservation based on both national and international priorities. The methodology described is applicable to all types of CWR (not only those with a role in improving food security) and can be used to achieve comprehensive coverage of all wild relatives. Through the integration of CWR into existing protected area management plans and establishment of genetic reserves in CWR hotspots, *in situ* CWR conservation can be simply but effectively improved. Together with a representative back up of material stored in genebanks this will provide effective, long-term monitoring and management of English CWR whilst facilitating their use in crop improvement. In this way, active and long-term conservation of English CWR can be established, contributing to European and global efforts to underpin future food security.

CHAPTER 3.

Enhancing the conservation of crop wild relatives in Scotland

The work presented in this chapter has been submitted to the

Journal for Nature Conservation

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Author contributions:

Conceived and designed the study: **HF**, CS, BFL, NM

Data collation and preparation: **HF**

Performed analysis: **HF**

Interpreted results: **HF**

Wrote the paper: **HF**

Critically reviewed the paper: **HF**, CS, BFL, NM

3.1 Abstract

The impact of climate change upon the agricultural industry is predicted to severely reduce crop yields, leading to a global state of food insecurity. This will be compounded by the necessity to feed a rapidly expanding human population. Crop wild relatives (CWR), with a wider gene pool than domesticated crops, may offer a means of mitigating this situation through the use of novel traits (e.g. stress tolerant or disease resistant traits) in crop improvement. The United Kingdom is a contracting party to international policy such as the Strategic Plan for Biodiversity of the Convention on Biological Diversity, which is committed to securing these valuable resources. However, as in the majority of countries around the world, there are as yet no long-term commitments to either *in situ* or *ex situ* conservation of CWR at UK or devolved level. This study has produced an inventory of 120 priority CWR within Scotland, has identified potential sites suitable for their *in situ* conservation (most notably a site to the west of Glasgow close to the Loch Lomond and Trossachs National Park) and has also prioritised these CWR in terms of the urgency for *ex situ* collection and storage in genebanks. Finally, recommendations have been made as to how active, long-term conservation of CWR in Scotland could be established to secure this resource *in situ* whilst ensuring accessibility of genetically diverse material in genebanks for use by plant breeders in crop improvement.

3.2 Introduction

Global mean surface temperatures are predicted to increase by between 0.3°C and 4.8°C by 2100 (Collins *et al*., 2013). Due to this and other effects of climate change, crop yields are predicted to decrease by an average of 2% per decade until 2050 with forecasts becoming more severe during the latter half of the century (Porter *et al*., 2014). Agricultural crops have limited genetic diversity having been through generations of breeding to obtain high yielding, uniform crop varieties (Tanksley and McCouch, 1997). This has left crops highly susceptible to biotic and abiotic stresses associated with climate change. This is an issue compounded by estimates that the human population is set to reach 9 billion people by 2050 (United Nations, 2011). This presents the significant challenge of providing global food security for an increasing population by improving the resilience of agricultural systems in the face of climate change (Government Office for Science, 2011).

Crop wild relatives (CWR) are wild plant species that are related to crops. They offer genetic resources that can be harnessed in crop breeding. The relationship between a crop and its wild relative can be defined using the Gene Pool (GP) Concept (Harlan and de Wet, 1971), which classifies CWR according to the ease of transfer of genes/traits from CWR to a crop (GP1a – cultivated crop varieties, GP1b – CWR which are easily crossable with crops in GP1a, GP2 – where is it possible to breed genes from CWR to crop though efforts are not always successful and GP3 – where more advanced biotechnologies are required to achieve successful trait transfer from CWR to crop). Similar classifications are possible based on the taxonomic relationships between CWR and crops and can be useful where experiments into genetic relationships are yet to take place. This classification is called the Taxon Group (TG) Concept (Maxted *et al*., 2006) where TG1a contains the cultivated crop variety, TG1b includes any

CWR of the same species as the crop, TG2 contains CWR in the same taxonomic section as the crop, TG3 includes those in the same subgenus and TG4 contains those in the same genus. Never having been through the process of domestication, CWR have a much wider gene pool and potentially contain traits that could be bred into existing crop varieties to improve their resilience to a range of stresses (Tanksley and McCouch, 1997). For example, traits from *Hordeum bulbosum* L. (a wild relative of barley) have been utilised as a source of resistance to many diseases including leaf rust, powdery mildew (Zhang *et al*., 2001) and scald (Pickering *et al*., 2006), all of which result in severe yield losses in susceptible cultivated barley varieties. It has been calculated that the potential value of breeding novel and beneficial traits from CWR into just 29 food crops identified by the Millennium Seed Bank, Kew could be approximately $120 billion by 2021 (PwC, 2013).

Despite their clear economic value, CWR themselves, as with all wild plant species, are also threatened by a range of factors including habitat destruction and climate change (Kell *et al*., 2012). In recognition of their potential value and the threats to which they are exposed, the conservation of CWR and of the range of genetic diversity within them is increasingly becoming a priority. As a result they are being included in global and regional conservation policy (Convention on Biological Diversity (CBD, 1992), Global Strategy for Plant Conservation (CBD, 2010a), Strategic Plan for Biodiversity (CBD, 2010b), International Seed Treaty (FAO, 2001), European Strategy for Plant Conservation (Planta Europa, 2008) and European Biodiversity 2020 Strategy (European Union, 2011). Priority CWR and recommended conservation actions for CWR are now starting to be identified in a range of countries in response to policy documents (e.g. Benin (Idohou *et al*., 2012); USA (Khoury *et al*., 2013); Finland (Fitzgerald, 2013); Spain (Rubio Teso *et al*., 2013); Italy (Panella *et al*., 2014)). Within the UK however, active conservation of CWR has not yet been established. In light of this, recent research effort has focussed on identifying priority CWR and finding effective means of establishing active conservation, both *in situ* and *ex situ*, for these plants in England and Wales (Fielder *et al*., 2015; submitted a). The current study addresses these same issues within the context of Scotland. These three studies are each part of one wider study that aims to encourage the implementation of active CWR conservation across the UK.

The agricultural industry plays a vital role in the economy of Scotland. According to Scotland's State of the Environment Report (Critchlow-Watton *et al*., 2014), 9% of Scotland's agricultural land area is specifically used for crop production and is located primarily in the east of the country. Though this may seem a small percentage, the contribution of this land area to the economic output of agriculture in Scotland far exceeds this value, contributing 34%. The most commonly grown crops in Scotland are barley, wheat, oilseed rape, oats, potatoes and strawberries of which CWR of barley, oilseed rape, oats and strawberries are present within Scotland. No CWR of wheat or potatoes are present within Scotland as these crops originate from the Middle East and South America respectively (Vavilov, 1926). A total of 12% of UK cereal production originates from Scotland (CCRA, 2012). A further 21% of agricultural land area is used as managed grassland supporting livestock through grazing (with the remaining agricultural land used for rough grazing) (Critchlow-Watton *et al*., 2014). This highlights the value not only of crops grown as a human food source but also of forage and fodder crops for livestock farming.

The UK Climate Change Impact Programme (UKCP09, 2009) has outlined the expected long-term effects of climate change across the UK. It is predicted that Scotland will experience increasingly hotter, drier summers and milder, wetter winters (UKCP09, 2009). This in turn is predicted to have some beneficial effects on agriculture, such as a longer growing season and the ability to grow a wider range of crops. However, a range of deleterious effects are also likely, including but not limited to decreased yields due to drought in summer, the increased incidence of pests and diseases that are able to survive through milder winters and the spread of invasive species (Critchlow-Watton *et al.*, 2014). Crops in Scotland could be further threatened due to their being cultivated in coastal locations, leaving them susceptible to rising sea levels (CCRA, 2012). In order to maintain or ideally increase crop yields over time it will be necessary for them to become more resilient and stress tolerant in response to the predicted changes in climate. This presents a strong case for the conservation of CWR in Scotland and the subsequent utilisation of their genetic diversity to breed new crop varieties able to withstand these changes.

The UK is a contracting party to the Aichi targets under the CBD Strategic Plan (CBD, 2010b), which explicitly state the need for the conservation of crops' genetic diversity and of their wild relatives. The Scottish Biodiversity Strategy (Scottish Executive, 2004) and the recently published 2013 update, 'The 2020 challenge for Scotland's biodiversity' (Scottish Government, 2013b) set out Scotland's strategy for protecting biodiversity and harnessing nature to improve Scotland's prosperity and welfare. It acknowledges genetic diversity as an important element of Scotland's natural capital and recognises the contribution it can make towards maintaining the robustness of food production. While conservation of landraces (traditional crop varieties) is being undertaken through the Scottish Landrace Protection Scheme (Green *et al.*, 2009), at present no conservation actions are being undertaken for conserving CWR in Scotland.

The objective of the current study was to identify ways to enhance CWR conservation within Scotland. It first aimed to inventory Scotland's CWR, identifying those present in the country and those of particular conservation priority, and then to perform a gap analysis investigating the extent of their current protection both *in situ* and *ex situ*. This process was carried out with advice from Scottish Natural Heritage (SNH). Based on the results of the study it was possible to make recommendations for providing active management of CWR in Scotland, which ultimately would ensure the persistence of CWR populations, making them available as a resource for use in crop improvement.

3.3 Materials and methods

3.3.1 CWR checklist and inventory

To produce a checklist of all CWR present within Scotland, a checklist of all CWR in the UK (extracted from the Crop Wild Relative Catalogue for Europe and the Mediterranean, Kell *et al.,* 2005) was matched against a checklist of the Scottish flora derived from the Vice County Census Catalogue (VCCC, Stace *et al*., 2003). The taxonomy of the resulting Scottish CWR checklist was harmonised with the British flora (Stace, 1997). The checklist contained all native and introduced taxa as well as CWR related to crops of all socio-economic uses (including human food, animal forage and fodder, medicinal, forestry, industrial etc.), amounting to a total of 1,259 CWR taxa and representing 43% of the Scottish flora. The Scottish checklist is available at the Plant Genetic Resources Diversity Gateway (http://pgrdiversity.bioversityinternational.org) and Supplementary Table 3.1a.

Unlike the traditional approach to prioritising vascular plants for conservation, which usually focuses on criteria relating to rarity and threat at the species level, the focus for prioritising CWR is on identifying those that can potentially contribute most to the improvement of crop varieties and in this case, food security. With this in mind, six criteria were selected through a consultation process with SNH for the prioritisation of the CWR checklist to produce an inventory containing priority CWR most likely to contribute to plant breeding. The six criteria were applied to the checklist in a step-wise process. First, criteria 1 and 2 were applied to identify a pool of CWR which were either natives or archaeophytes and which were related to human food or animal forage and fodder crops; these being the CWR most likely to have a role in increasing food security. The remaining criteria (3 to 6) were then applied in turn. Any CWR that met one of these criteria were included in the final Scottish inventory of priority CWR. The criteria were as follows:

1. Use of the related crop – Data stating the socio-economic uses of crops were extracted from GRIN Taxonomy for Plants (USDA, ARS, National Genetic Resources Program, 2015). Crops used as human food sources or for animal forage or fodder were identified and all CWR related to these crops were listed, due to their potential role in improving food security.

2. Native status – Any CWR listed as native or an archaeophyte according to the VCCC (Stace *et al*., 2003) were identified from the list of CWR generated by criterion one.

3. Economic value of the related crop – CWR belonging to the same genus or within the same Gene Pool as economically valuable crops were prioritised. Crops were categorised as economically valuable if they were listed in agricultural statistics (FAOSTAT, 2013 – production quantity in million tonnes at the global, European and UK levels; Eurostat, 2013 – crop production value at producer prices in millions of euros; Defra, 2010 – crop production at market prices in million pounds). This method identified human food crops of value but data concerning the value of forage and fodder crops could not be found. This lack of forage and fodder data is an issue also identified by Kell *et al*. (2012).

4. Degree of relatedness to the crop – CWR categorised into GP1b, GP2, TG1b, TG2 or TG3 of a related crop, according to the Harlan and de Wet inventory of globally

important CWR (Vincent *et al.*, 2013), were prioritised. Any taxa in GP1a or TG1a with no wild populations are cultivated varieties and therefore were not prioritised. If a CWR was related to more than one crop, the crop to which it is most closely related (i.e. with the highest GP/TG classification) was used as the basis for prioritisation.

5. Red list threat assessment – CWR classified as threatened according to the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005), the European Red List of Vascular Plants (Bilz *et al.*, 2011) or the IUCN Red List of Threatened Species (IUCN, 2012) were prioritised. These included any CWR listed as Critically Endangered, Endangered, Vulnerable or Near Threatened.

6. Other conservation designations – The number of conservation designations assigned to each CWR in addition to the three red data list threat assessments mentioned above were extracted from the Conservation Designations for UK Taxa database (JNCC, 2011). CWR that did not meet any of criteria 3 to 5 but were listed as having one or more designations, excluding designations based on IUCN red list criteria, were prioritised.

3.3.2 In situ *gap analysis*

Data records indicating occurrences of each priority CWR in Scotland were extracted from the BSBI distribution database (BSBI, 2013). All confirmed records with full passport information, recorded from 1970 onwards and with a precision of tetrad level (2km by 2km square) and above were selected for the *in situ* gap analysis. The software ArcMap 10.0 and DIVA-GIS 7.5.0 (ESRI, 2011; Hijmans *et al.*, 2012) were used to carry out the spatial analysis of CWR occurrence records. A shape file depicting Scotland and its counties was obtained by extracting Scotland from a shape file of the United Kingdom, downloaded from

DIVA-GIS (www.diva-gis.org). The following three analyses were performed according to methods described by Scheldeman and van Zonneveld (2010) and Hijmans *et al*. (2012):

- Taxon richness and observation richness – CWR taxon hotspots and areas with high numbers of observation records were determined using DIVA-GIS. The richness analysis used a 'point to grid' function and a grid square size of 0.1 degrees.
- Complementarity analysis – A series of grid squares (0.1 degrees in size) were selected from across Scotland that contained all of the priority Scottish CWR within as few grid squares as possible. These squares indicate sites with potential for the establishment of genetic reserves for CWR. The 'reserve selection' function in DIVA-GIS was used to perform this analysis. It first selects the grid square with the most CWR and then selects each additional square in turn based on the number of additional CWR that can be covered by that square (i.e. the number of CWR not present in any of the preceding squares) (Rebelo, 1994). A minimum of five different populations of each CWR should be conserved *in situ* to ensure protected populations represent a range of genetic diversity and also that the likelihood of the taxon persisting is increased (Brown and Briggs, 1991; Dulloo *et al*., 2008). In light of this, the CWR present in five or more squares were identified.
- Incidence of priority CWR within protected areas – a comparison of occurrence record locations and the boundaries of Scottish protected areas was performed using 'spatial join' tools in ArcMap 10.0. Boundary shape files were downloaded from the Scottish Natural Heritage website (http://gateway.snh.gov.uk/natural-spaces/index.jsp). The following protected areas were used in the analysis: Sites of Special Scientific Interest (SSSI), Special Areas of Conservation (SAC), National Scenic Areas (NSA), National

Nature Reserves (NNR), Local Nature Reserves (LNR), National Parks (NP), Country Parks, Ramsar sites and Special Protection Areas (SPA).

3.3.3 Ex situ *gap analysis*

Similarly to Fielder *et al*. (2015), to investigate the completeness of *ex situ* collections for priority Scottish CWR, accession data were extracted from the UK National Plant Inventory (UKNPI, 2013) and were also provided by the Millennium Seed Bank, Kew. If any accessions required georeferencing, longitude and latitudes were obtained using the UK Grid Reference Finder (www.gridreferencefinder.com) and the Gazetteer of British Place names (www.gazetteer.org.uk). The *ex situ* gap analysis was then performed in three stages as described:

- The total number of accessions stored per priority Scottish CWR was determined. CWR with five or more accessions, as a back up for populations protected *in situ*, were considered sufficiently collected whereas those with fewer require additional collecting effort (Brown and Briggs, 1991).
- A geographical representativeness score (GRS) was calculated per priority CWR using the 'circular area statistic' (Ramírez-Villegas *et al*., 2010). GRS represents the percentage of occurrence data that is also stored *ex situ* for each CWR. For CWR with a GRS greater than or equal to 30%, *ex situ* collections adequately represent their *in situ* range, whereas those with a GRS lower than 30% require further collecting of accessions from across the taxon's full range (Ramírez-Villegas *et al*., 2010; Phillips *et al*., 2014).

- A priority level was then assigned to each CWR based on the two preceding results (i.e. number of accessions and GRS). CWR with no accessions were categorised as being highest priority. Priority levels and associated conservation recommendations are given in full in Table 3.1.

Table 3.1 *Ex situ* priority levels and recommended actions required to ensure gaps in *ex situ* collections are sufficiently filled.

Priority Level	Criteria	Action required
1	No accessions	Highest priority for collection; Ensure minimum of five accessions are collected representing the full range of the CWR
2	Has accessions but none are georeferenced or the location data is restricted	High priority for collection; Georeference existing accessions; Further collection of accessions which are representative of CWR range required
3	Fewer than five accessions and GRS lower than 30%	Increase accessions to a minimum of five; ensure representative of range
4	Fewer than five accessions but GRS greater than 30%	Increase accessions to a minimum of five; confirm that current stored accessions are representative of CWR range
5	Greater than or equal to five accessions but GRS lower than 30%	Improve geographic representativeness of accessions through further targeted collection
6	Greater than or equal to five accessions and GRS greater than 30%	Sufficiently represented in *ex situ* collections

3.4 Results

3.4.1 CWR inventory

The Scottish national inventory of priority CWR contains 120 taxa (102 species and 18 subspecies). This represents 9.53% of the 1,259 CWR taxa present in Scotland, as listed in the Scottish CWR checklist. A summary of the inventory is shown in Table 3.2 (the full inventory is available at the Plant Genetic Resources Diversity Gateway (http://pgrdiversity.bioversityinternational.org), also see Supplementary Table 3.1b). The inventory contains 78 taxa that are related to crops used as a human food source and an additional 42 taxa, which are related only to forage and/or fodder crops. The 120 CWR taxa represent a total of 14 plant families, of which Poaceae, Brassicaceae and Fabaceae contain the most genera (17, 8 and 6 respectively). The genera with the highest taxon richness are *Vicia* L. (vetches, 11 taxa), *Trifolium* L. (clovers, 8 taxa) and *Chenopodium* L. (goosefoots, 8 taxa).

Table 3.2 Summary of inventory of 120 priority CWR in Scotland.

Family	No. of genera	No. of species	No. of infra-specific taxa	Native status
Apiaceae	2	1	2	N
Asteraceae	2	3		A & N
Brassicaceae	8	8	4	A & N
Chenopodiaceae	2	8	1	A & N
Corylaceae	1	1		N
Ericaceae	1	5		N
Fabaceae	6	23	3	A & N
Geraniaceae	1	3		A & N
Grossulariaceae	1	2		N
Liliaceae	2	5		A & N
Linaceae	1	1	1	N
Plantaginaceae	1	2		N
Poaceae	17	30	4	A & N
Rosaceae	5	10	3	A & N
Totals	**50**	**102**	**18**	

A = Archaeophyte, N = Native.

Agricultural statistics extracted from FAOSTAT (2013), Eurostat (2013) and Defra (2010) showed that a total of 34 food crops have either native or archaeophyte CWR within Scotland that are of economic value. According to Eurostat data (Fig. 3.1), barley and rape seed are the crops with the highest mean production value in Europe (producer price from 2007 to 2011) with values of 8,520 and 6,587 million Euros respectively. Other listed crops of value include apples, peaches and pears as well as other brassica crops e.g. cauliflower. These statistics are similar to those for England (Defra, 2010) where barley and rape seed are also shown as having the highest production value (market prices from 2006 to 2010). Data at the global scale (FAOSTAT, 2013) also mirror this result, showing that barley and brassicas are again of high value (being among the five highest values for production quantity between 2007 and 2011). A total of 63 CWR taxa were identified as economically valuable based on the crops identified by these agricultural statistics, and were listed in the Scottish inventory of priority CWR.

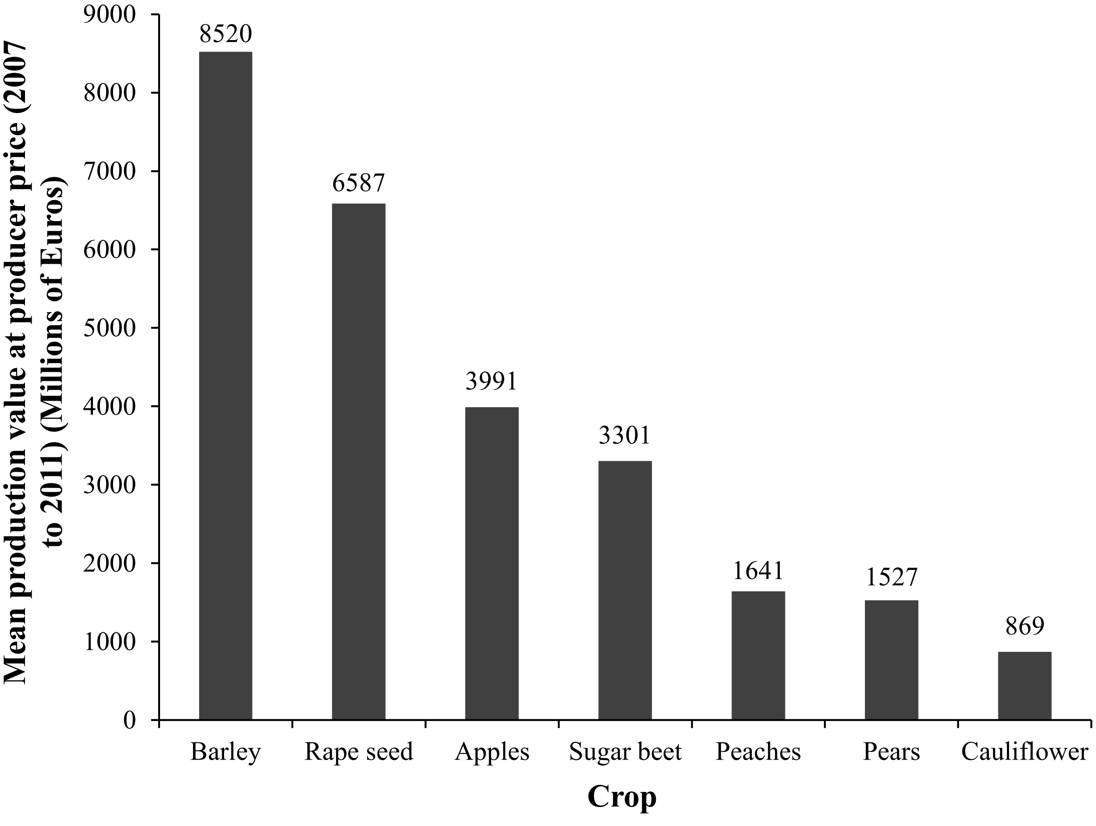

Figure 3.1 European production values at producer price in millions of Euros between 2007 and 2011 of crops with native or archaeophyte CWR occurring within Scotland (Data source: Eurostat, 2013).

Little under a quarter (24%) of priority CWR were classified using the Gene Pool concept, with 17% within GP1b and just four percent in GP2 and three percent in GP3. The remaining 76% of taxa were classified using the Taxon Group concept, with the vast majority (35%) of taxa classified as TG4 (Fig. 3.2).

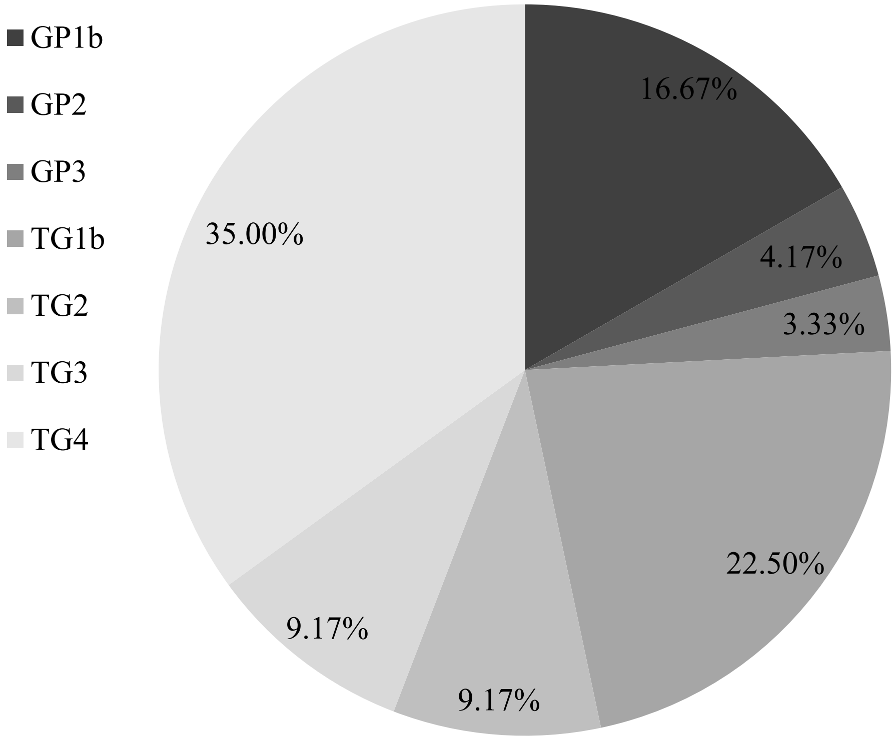

Figure 3.2 Proportions of priority CWR classified in GP1b to 3 and TG1b to 4 in the Scottish CWR inventory, indicating their degree of relatedness to their associated crops.

Of the 120 priority CWR taxa, 10.8% (13 taxa) are recorded as being threatened according to the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005) and these taxa are listed in Table 3.3. Ten taxa are listed as Vulnerable, including Scottish small-reed *Calamagrostis scotica* (Druce) Druce, which is endemic to Scotland. Purple milk-vetch *Astragalus danicus* Retz. and stinking goosefoot *Chenopodium vulvaria* L. are listed as Endangered and upright goosefoot *Chenopodium urbicum* L. as Critically Endangered. In addition, Scandinavian small-reed *Calamagrostis purpurea* (Trin.) Trin. is classified as Data Deficient. A further 17 CWR taxa were identified as priority based on their being assigned additional conservation designations (Table 3.4). Eight taxa are listed on the Biodiversity List for Scotland as species of principal importance for conservation. A further seven taxa are listed as being nationally rare or scare, occurring in fewer than 100 hectads (10km by 10km squares) across the UK. Scandinavian small-reed *C. purpurea* subsp. *phragmitoides* (Hartm.) and sea pea *Lathyrus japonicus* Willd. subsp. *maritimus* (L.) P.W. Ball are the only two taxa listed as both rare/scarce and are on the Scottish Biodiversity List.

Table 3.3 Red list status and the threat criteria met for CWR in the Scottish inventory.

CWR	Red List Status	Criterion
Chenopodium urbicum L.	CR	A2c AOO and EOO trend
Astragalus danicus Retz.	EN	A2c AOO trend
Chenopodium vulvaria L.	EN	A2c AOO and EOO trend
Allium oleraceum L.	VU	A2c EOO trend
Astragalus alpinus L.	VU	D2
Bromus secalinus L.	VU	A2c AOO trend
Calamagrostis scotica (Druce) Druce	VU	D1+2
Calamagrostis stricta (Timm) Koeler	VU	A2c AOO trend
Chenopodium bonus-henricus L.	VU	A2c AOO trend
Chenopodium glaucum L.	VU	A2c AOO and EOO trend
Poa flexuosa Sm.	VU	D1
Poa glauca Vahl.	VU	A2c AOO trend
Vicia bithynica (L.) L.	VU	A2c EOO trend
Calamagrostis purpurea (Trin.) Trin.	DD	N/A

VU = Vulnerable; EN = Endangered; CR = Critically Endangered; DD = Data Deficient; AOO = Area of occupancy; EOO = extent of occurrence; A2c = reduction in population size based on trend in AOO or EOO; D1 = Restricted population of less than 1000 mature individuals; D2 = Restricted population based on AOO or number of locations (Data source: Cheffings and Farrell, 2005).

Table 3.4 CWR in the Scottish inventory with conservation designations.

CWR	**Conservation designation**
Alopecurus borealis Trin.	Rare and scarce species (not based on IUCN criteria)
Alopecurus myosuroides Huds.	Biodiversity Lists – Scotland
Bromus hordaceus L. subsp. *ferronii* (Mabille) P.M. Sm.	Rare and scarce species (not based on IUCN criteria)
Bromus hordaceus L. subsp. *thominei* (Hardouin) Braun-Blanq.	Rare and scarce species (not based on IUCN criteria)
Calamagrostis epigejos (L.) Roth	Biodiversity Lists – Northern Ireland
Calamagrostis purpurea (Trin.) Trin. subsp. *phragmitoides* (Hartm.)	Biodiversity Lists – Scotland; Rare and scarce species (not based on IUCN criteria)
Crambe maritima L.	Biodiversity Lists – Northern Ireland
Erodium maritimum (L.) L'Hér.	Biodiversity Lists – Scotland
Erodium moschatum (L.) L'Hér.	Biodiversity Lists – Scotland
Festuca arenaria Osbeck	Rare and scarce species (not based on IUCN criteria)
Lathyrus japonicus Willd. subsp. *maritimus* (L.) P.W. Ball	Biodiversity Lists – Scotland; Rare and scarce species (not based on IUCN criteria)
Lepidium campestre (L.) W.T. Aiton	Biodiversity Lists – Scotland
Plantago media L.	Biodiversity Lists – Scotland
Poa alpina L.	Rare and scarce species (not based on IUCN criteria)
Poa bulbosa L.	Rare and scarce species (not based on IUCN criteria)
Rorippa islandica (Oeder ex Gunnerus) Borbás	Rare and scarce species (not based on IUCN criteria)
Trifolium micranthum Viv.	Biodiversity Lists – Scotland

(Data source: JNCC, 2011)

3.4.2 In situ *gap analysis*

After the exclusion of all occurrence records that did not meet the quality standards outlined in the method above, no occurrence records remained for eight of the 120 priority Scottish CWR. As a result the following taxa were unable to be included in the *in situ* gap analysis: garden asparagus *Asparagus officinalis* L., Scandinavian small-reed *C. purpurea* subsp. *phragmitoides*, upright goosefoot *C. urbicum*, stinking goosefoot *C. vulvaria*, wall barley *Hordeum murinum* L. subsp. *murinum*, sea pea *L. japonicus* subsp. *maritimus*, perennial flax *Linum perenne* L. subsp. *anglicum* (Mill.) Ockendon and white mustard *Sinapis alba* L.

subsp. *alba*. However, records did exist for the five subspecies when a search for data was performed at the species level (e.g. 23 records existed for *C. purpurea* rather than *C. purpurea* subsp. *phragmitoides*). In such cases, these records were not included in the analysis due to uncertainty as to which subspecies these data belonged. This left a total of 112 CWR taxa in the *in situ* gap analysis with 90,277 high quality occurrence data records within Scotland. Seven of the 112 taxa had only one occurrence record whereas sweet vernal-grass *Anthoxanthum odoratum* L. had the highest number of records (5,356).

Taxon richness analysis of the 112 CWR revealed a number of hotspots throughout Scotland with a high concentration in central and eastern areas (Fig. 3.3a). A significant hotspot is located on the border between Renfrewshire and West Dunbartonshire to the west of the city of Glasgow and stretching into the Loch Lomond and Trossachs National Park. Another key hotspot lies around the Firth of Forth in the east, with other hotspots located in the county of Perthshire and Kinross and further north in Moray and the Highlands, overlapping with the Cairngorms National Park. An analysis of observation richness reveals two small localities that could be subject to bias with higher numbers of observations compared to the rest of the country (Fig. 3.3b). The first area is located in West Lothian, between the cities of Edinburgh and Glasgow, and the second area is located in Clackmannanshire. In contrast, there are areas of Scotland, particularly in the Highlands, that show much less recording has taken place.

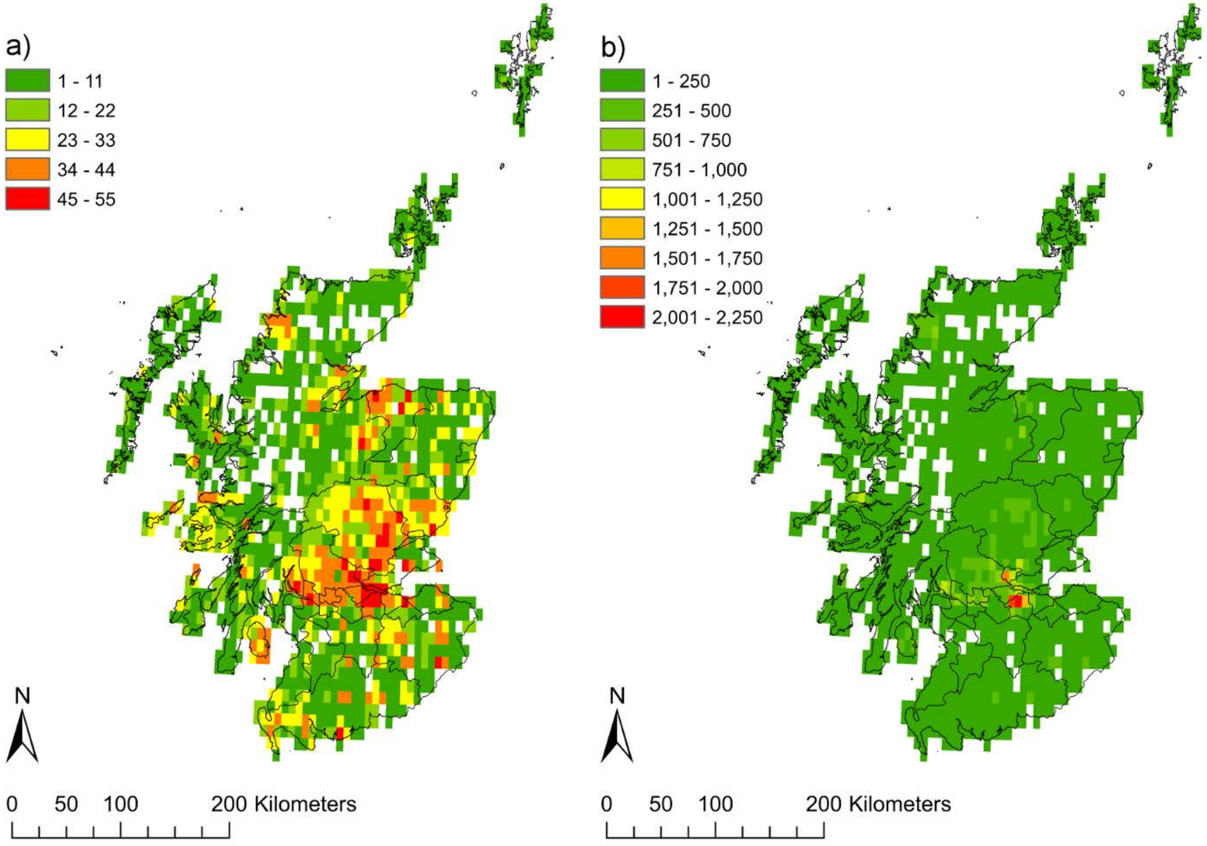

Figure 3.3 Richness analysis for the 112 priority CWR in the Scottish inventory with occurrence data points using a grid square size of 0.1 degrees. a) Taxon richness and b) Observation richness.

The complementarity analysis of the 112 CWR taxa for which there were high quality occurrence records shows that a total of 22 grid squares (each 0.1 degree squares or approximately 11km by 11km) are necessary to conserve 109 priority CWR taxa (the reserve selection tool in DIVA-GIS did not allocate any grid squares to cover the single occurrence records for fig-leaved goosefoot *Chenopodium ficifolium* Sm., sea carrot *Daucus carota* L. subsp. *gummifer* (Syme) Hook. f. or bulbous meadow-grass *Poa bulbosa* L.). This equates to 91% of the priority CWR in the Scottish inventory. The 22 grid squares are primarily located in an arc from Glasgow in the west to Dundee on the east coast but also include sites in the far south west of Scotland, in the Outer Hebrides and the eastern reaches of the Highlands (Fig. 3.4). The first three grid squares together conserve 61% of the 120 priority CWR taxa. Grid square one contains 55 taxa in total and is located to the west of the city of Glasgow. Grid square two contains 31 CWR, 11 of which do not occur in grid square one and is located within the city of Edinburgh. Grid square three contains 22 CWR, seven of which do not occur in either grid squares one or two and is located in the Highlands in close proximity to the counties of Moray and Aberdeenshire. The percentage of the 120 priority CWR represented in each grid square is shown in Fig. 3.5. Further analysis of the complementarity results showed that 50 out of the 120 priority CWR taxa have been recorded as present in five or more of the 22 complementarity squares, with white clover *Trifolium repens* L. present in the most (19 out of 22). However, this also shows that 70 CWR are recorded in fewer than five squares.

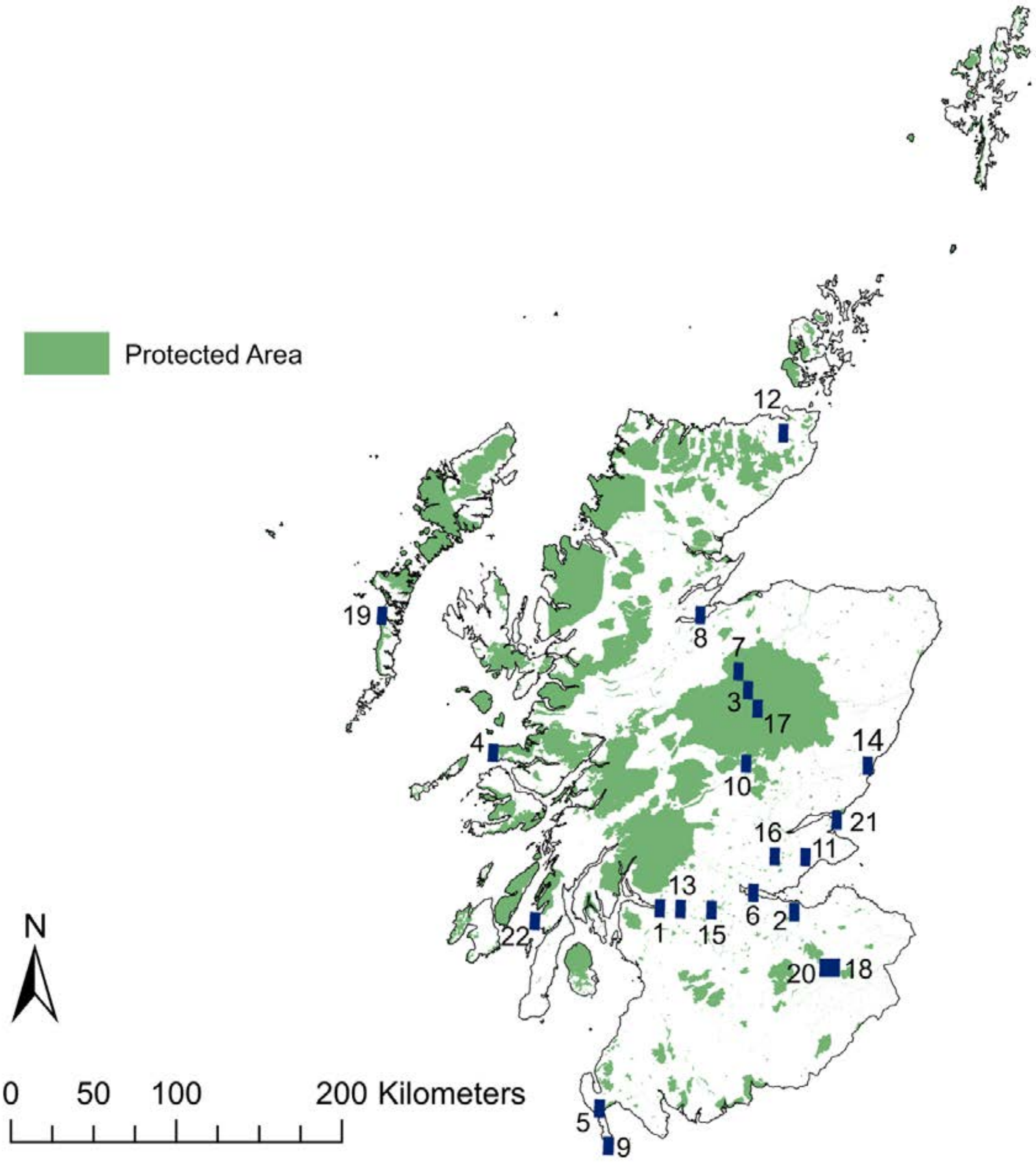

Figure 3.4 Complementarity analysis for the 112 priority CWR in the Scottish inventory with occurrence data points showing the 22 grid squares identified as suitable candidate sites for establishing *in situ* conservation (e.g. genetic reserves within existing protected areas and also protection outside of protected areas).

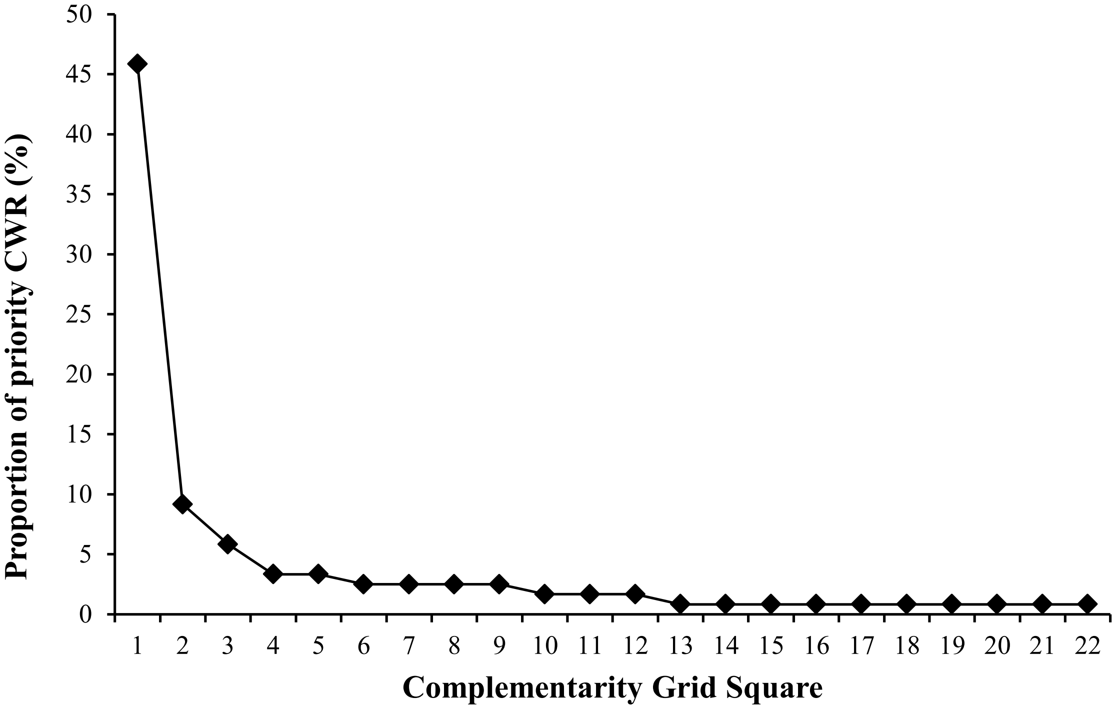

Figure 3.5 Percentage of additional CWR contained within each of the 22 priority grid squares (i.e. CWR that are not present in any of the preceding grid squares).

Considering protected areas in Scotland, it was found that all 22 complementarity grid squares overlap with a range of different protected areas including SSSIs, SACs, NSAs, LNRs and NPs. Four of the squares overlap with the Cairngorms National Park. By comparison of occurrence records with locations of protected areas it was found that 105 (88%) of priority CWR had at least one occurrence record within a protected area. Furthermore, 85 of these CWR have records located within five or more protected areas. However, only 34% of all 90,277 records in the *in situ* gap analysis are located within protected areas.

3.4.3 Ex situ *gap analysis*

Only 40 accessions are stored in genebanks for the 120 priority CWR in Scotland, 23 of which are stored at the Genetic Resources Unit, IBERS at Aberystwyth University and the remaining 17 of which are stored at the Millennium Seed Bank, Kew. Furthermore, these 40 accessions represent only 11 of the priority CWR and are primarily grasses (Table 3.5). Perennial rye-grass *Lolium perenne* L. has the most accessions (14), all of which are stored at IBERS, Wales. Of these 11 CWR only one has a GRS greater than the recommended threshold of 30% and this is Scottish small-reed *C. scotica*. This CWR is endemic to Scotland, despite this, it only has one stored accession but in proportion to its restricted range this equates to a high GRS.

Table 3.5 Number of accessions and associated GRS percentage for all 11 priority Scottish CWR with accessions originating from Scotland.

CWR	Accessions	GRS (%)
Lolium perenne L.	14	1.13
Trifolium repens L.	8	0.30
Astragalus alpinus L.	3	24.24
Rorippa islandica (Oeder ex Gunnerus) Borbás	3	12.50
Vaccinium uliginosum L.	3	1.19
Dactylis glomerata L.	2	0.12
Poa glauca Vahl.	2	4.17
Vaccinium vitis-idaea L.	2	0.27
Calamagrostis scotica (Druce) Druce	1	50.00
Lathyrus sylvestris L.	1	11.43
Poa pratensis L.	1	0.09

The number of accessions and GRS percentages were combined to categorise the 120 CWR into priority levels inferring urgency for further collection and storage of accessions (Supplementary Table 3.2). It is clear that none of the Scottish priority CWR are currently well represented in *ex situ* collections as no taxa were assigned the lowest priority (level 6).

All priority CWR lacking accessions (109) were assigned the highest priority (level 1) where urgent collection of accessions is necessary. The lowest priority CWR were perennial rye-grass *L. perenne* and white clover *T. repens* both with greater than five accessions but a GRS below 30% (level 4). *C. scotica* was the only taxon with a GRS greater than 30% however, with only one accession this taxon would benefit from further collecting to represent its range of genetic diversity. The remaining eight CWR were assigned a priority level of 3 (i.e. they have fewer than five accessions stored *ex situ* and a GRS value which is below 30%) and further collection of seed representative of the range of each taxon is recommended.

3.5 Discussion

Traditional conservation practices have focussed on protecting rare or threatened species. However, this approach to conservation is not adequate to ensure the full protection of all CWR. To comprehensively conserve CWR there must be a focus on conserving the range of genetic diversity within species rather than just ensuring the survival of the species as a whole. The potential value to the agricultural industry of traits that may be present in CWR (PwC, 2013), not just within those that are rare, justifies the inclusion of these resources in appropriate conservation planning as an adjunct to traditional approaches. Efforts to conserve CWR should look to identify those requiring protection and where their conservation is currently lacking and should then ensure active monitoring and management across each species' range (Maxted *et al.*, 2008a).

Prioritisation of Scottish CWR was based on criteria selected jointly by The University of Birmingham and Scottish Natural Heritage. Many inventories of priority CWR have now been developed both within Europe (e.g. Finland – Fitzgerald, 2013; Spain – Rubio Teso *et al.*, 2013; Italy – Panella *et al.*, 2014; Cyprus – Phillips *et al.*, 2014; Czech Republic – Taylor *et al*., in prep) and globally (e.g. Venezuela – Berlingeri and Crespo, 2012; Benin – Idohou *et al*., 2012; USA – Khoury *et al*., 2013). Although the ultimate goal of each country to ensure CWR are effectively conserved is the same, no two countries have yet used exactly the same prioritisation method. A wide range of different criteria can be used for prioritisation including: native status, economic value, distribution, legislation and threat (Maxted *et al*., 1997a; Magos Brehm *et al*., 2010). Differences also exist between criteria selected for prioritising English, Welsh and Scottish CWR. For example, in Scotland and England conservation recommendations for food, forage and fodder CWR were the focus (Fielder *et al*., 2015) however in Wales, tree species were also selected due to the concern of representatives in Natural Resources Wales for the threats the forestry industry is now facing, particularly in terms of the emergence of new pests and diseases (Fielder *et al*., submitted a). The process of selecting criteria should be an objective and inclusive process whereby the criteria most appropriate to addressing global policy targets are selected, but equally there should be opportunity for input from national stakeholders in selecting nationally relevant criteria as well. In this way stakeholder support can be gained for the conservation of any priority CWR identified through this process, increasing the likelihood that CWR conservation will be established and maintained in the long-term.

There is a lack of active CWR conservation in Scotland. This is a situation echoed in England and Wales where CWR conservation is largely limited to efforts to maintain wild asparagus populations (King and Edwards, 2007; King *et al*., 2007). Despite the presence of a number of red listed CWR in Scotland and further CWR listed on the Biodiversity List of Scotland, as yet there is no conservation effort for these species in the context of their being CWR. For example the Scottish small-reed *C. scotica* is the only endemic priority CWR in the Scottish inventory. With only one population present in the Highlands and being classified as

Vulnerable in the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005) it is important that this population is managed appropriately to preserve its full geographical distribution and ultimately its full range of genetic diversity.

Hotspot and complementarity analyses are valuable and widely used tools for determining areas with high numbers of CWR taxa and more specifically, areas that together could form a genetic reserve network sufficient to conserve priority CWR (Hijmans *et al*., 2012; Rubio Teso *et al*., 2013 *inter alios*). This approach enables a national and multi-taxon view of CWR conservation rather than focussing on each individual taxon in turn. This is particularly appropriate for widespread CWR, such as red currant *Ribes rubrum* L. and red clover *Trifolium pratense* L., as they are unlikely to be granted the same level of attention as rare and threatened species. The Scottish hotspot analysis suggests that the lowlands in central and Eastern Scotland are richer in CWR than the mountainous north and west. A trend supported by Jarvis *et al*. (in press) who demonstrated that CWR (particularly those most closely related to forage and fodder crops) tended to be associated with agricultural habitats and landscape features. Some association of CWR with coastal regions in the east is also evident, particularly around the Firth of Forth. Some CWR in the UK are known to be associated with coastal habitats (Preston *et al*., 2002) and as such, the predicted rise in sea level around Scotland (of between 0.12 to 0.18m by 2050; CCRA, 2012) may threaten CWR such as sea kale *Crambe maritima* L., sea pea *Lathyrus japonicus* subsp. *maritimus*.

The network of selected grid squares (or potential *in situ* conservation sites for CWR) identified by the complementarity analysis shows the same association with central and eastern areas of Scotland and together these locations are sufficient to cover the majority of Scotland's priority CWR. This analysis allows efficient conservation planning by focussing in on the fewest areas necessary to cover the most priority CWR. As discussed by Fielder *et al*.

(2015) and Fitzgerald (2013) the complementarity squares do not denote exact boundaries of suggested genetic reserves but rather they indicate general locations that should be further investigated for their suitability for *in situ* CWR conservation, whether inside or outside of existing protected areas. Findings by Jarvis *et al.* (in press), who carried out the first assessment of habitat preferences for UK CWR, will help to focus CWR conservation further by locating suitable habitats (particularly linear landscape features) within complementarity squares.

According to the 2020 challenge for Scotland's biodiversity, 18% of Scotland's land area falls within the boundaries of a nature conservation site (Scottish Government, 2013b). The current study found that approximately one third of priority CWR occurrence records are located within these nature conservation sites. It should be noted that despite the incidence of CWR within protected areas there can be no guarantee of the provision of active and appropriate management of CWR in these locations (Hunter and Heywood, 2011). To achieve this will require the inclusion of CWR monitoring and management into existing conservation planning, this may often require only minimal changes to current practices but should address and meet all of the expected standards for CWR genetic reserves. Sites should be determined having been through a scientific process that identifies CWR present and carries out both demographic surveys and genetic diversity studies enabling the design of reserves capturing the maximum range of CWR genetic diversity (Iriondo *et al*. 2012; Fielder *et al*., submitted d). Establishment of CWR conservation outside of protected areas is a novel challenge that would require commitments from landowners and land managers to monitor and manage CWR and to resolve any conflicts in land management to ensure CWR and their genetic diversity is protected. Furthermore, there would be a requirement for funding mechanisms to be put in place to support land managers to preserve CWR. This may be possible through the inclusion of an option for the preservation of agrobiodiversity as part of the Scotland Rural Development Programme, which delivers Pillar II of the EU Common Agricultural Policy (SRDP, 2015).

Ex situ collections of CWR from Scotland are severely lacking. The Millennium Seed Bank, Kew aims to collect and store accessions for all native plants in the UK (Stephanie Miles, pers. comm.). In the context of traditional plant conservation, if an accession for a particular species has been collected from one UK administration (e.g. England or Wales) there may be no urgency to collect further material from Scotland. In the context of CWR this is not true. Due to the need to conserve a large range of genetic diversity in CWR, collections should span the range of their distribution to incorporate ecogeographic extremes or, where genetic diversity analyses have been carried out, collections should directly reflect this. To reach this objective, Brown and Briggs (1991) suggest that a minimum of five accessions should be stored *ex situ* per taxon as a back up of populations protected *in situ*. Due to Scotland's distinctive landscape and climate (Critchlow-Watton *et al*., 2014), CWR there are likely to contain genetic diversity not found elsewhere in the UK. This provides a strong argument for ensuring priority Scottish CWR are well represented in genebanks. The priority level assigned to each CWR in the Scottish inventory reveals the taxa in most urgent need of collection and enables a more focussed approach to be taken.

3.5.1 Recommendations for enhancing CWR conservation in Scotland

- The CWR identified as priority in the Scottish inventory should be reviewed at regular intervals. A time scale of 10-year intervals may be appropriate. The review process should consider relevant policy, national stakeholder opinion and should address existing and novel threats to CWR and their genetic diversity. The review process is also an opportunity to include more up-to-date prioritisation data.

- Work towards establishing active *in situ* CWR conservation by focussing on those present in the network of complementarity squares and the suitable habitats within them. Initial efforts should be concentrated on the square containing the most CWR (the site west of Glasgow).
- Include CWR monitoring and management in existing conservation planning for protected areas, ensuring the standards for CWR genetic reserves are met to secure a long-term commitment to the protection of the species and their genetic diversity.
- Investigate the potential for conservation outside of protected areas in habitats less likely to be a priority within nature conservation sites, e.g. field margins and road verges (Jarvis *et al.*, in press).
- Aim to conserve a minimum of five *in situ* populations of each CWR and to carry out genetic diversity analyses where feasible to ensure that the populations are representative of the range of genetic diversity within CWR in Scotland.
- Continue to obtain occurrence records from the field that are of high quality (specific location data) and record associated population data where possible. Ensure data continues to be widely accessible.
- Collect a minimum of five accessions per priority CWR and store them *ex situ*. CWR assigned to priority level one should be considered in most urgent need of collection.
- The relationship between accessions held in long-term storage and population genetic changes over time should be explored and the appropriate intervals for updating *ex*

situ collections determined per species to capture the genotypic evolution of *in situ* populations over time.

- Maintain all accessions in UK genebanks with duplicates stored in a separate genebank where possible.
- Genebanks should undertake greater promotion of the conserved CWR material available to researchers, pre-breeders and plant breeders for use in crop improvement, encouraging their use in the development of new crop varieties.

3.6 Conclusion

Conservation of priority CWR in Scotland is incomplete due to a lack of active *in situ* and *ex situ* conservation efforts for these taxa. The inclusion of CWR monitoring and management into existing conservation planning in established protected areas (and also into appropriate sites outside of protected areas) would vastly improve CWR protection *in situ*. A renewed effort to collect accessions from all priority CWR would also vastly improve the completeness of *ex situ* collections from Scotland. Using a complementary *in situ* and *ex situ* approach, CWR in Scotland can be maintained in their natural habitats enabling on going adaptation to a changing climate. At the same time, storage of this material and the genetic diversity within can ensure it is available for use in crop development. These efforts would contribute to the UK's global and European commitments to conserve agricultural diversity and associated wild relatives (CBD, 1992; 2010a; 2010b; FAO, 2001; Planta Europa, 2008; European Union, 2011), and enhance the resilience of food production.

CHAPTER 4.

Enhancing the conservation of crop wild relatives in the United Kingdom

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4.1 Abstract

Crop wild relatives (CWR) harbour a wider range of genetic diversity than is found within modern crop cultivars. Beneficial traits identified within CWR can be bred into crops to improve their resilience in the face of climate change, aiding global efforts to improve future food security. Despite the UK's commitments to improve the conservation of agricultural genetic diversity outlined in international policy documents such as the Aichi targets of the CBD Strategic Plan for Biodiversity, there are currently no active, long term measures in place in the UK to conserve these valuable resources. Here a UK inventory of 223 priority CWR is presented. *In situ* gap analysis of these CWR reveals that although they are well represented in the current protected area network, this only amounts to potential for passive conservation. Potential sites for the establishment of active *in situ* CWR conservation are presented. *Ex situ* gap analysis identifies large gaps in the completeness of current collections of UK CWR that will need to be filled. Finally, recommendations are outlined, the implementation of which would provide active *in situ* conservation of UK CWR with a complete and complementary back up of *ex situ* accessions available for use in crop improvement. The recommendations presented in the current study would be most effective if integrated with approaches outlined in a wider study of CWR conservation in each of the devolved administrations in the UK (England, Scotland and Wales). By establishing active and long term conservation of CWR at both devolved and UK level, these resources and their associated genetic diversity can be secured and utilised.

4.2 Introduction

The transition from farming landraces and traditional crop varieties to the farming of modern crop cultivars has resulted in a significant loss of agricultural genetic diversity (Tanksley and McCouch, 1997; van de Wouw *et al.*, 2010). This has led to concerns over the capacity of these modern crops to withstand and adapt to predicted changes in climate (HLPE, 2012). Susceptibility to climate change, together with a rising demand for high yielding crops in light of a rapidly increasing human population (United Nations, 2013), threatens future food security. One key approach in improving this outlook is to introduce beneficial traits from the wider gene pool of the wild relatives of crop plants, to improve their resilience to change.

Crop wild relatives (CWR) are wild plants related to crops and that contain a wider range of genetic diversity, as they have not been through any process of domestication (Maxted *et al.*, 2006). CWR differ from other wild plants only in that they are related to one or more crop species. The closeness of this relationship determines the ease by which the CWR and crop can be crossed, and genes or traits transferred from wild relative to cultivar through plant breeding (Harlan and de Wet, 1971). CWR can be classified using the Gene Pool (GP) concept proposed by Harlan and de Wet (1971) where the crop is categorised as GP1a, its closest wild relatives (where there is no barrier to gene transfer) are classified as GP1b, CWR where gene transfer to the crop is possible but more challenging are classified as GP2 and CWR where more advanced biotechnology is required for successful gene transfer are assigned to GP3. Gene pool classifications exist for many globally important crops (Vincent *et al.*, 2013). However, for other crops these crossing experiments often have not been carried out. For these cases, Maxted *et al.* (2006) propose the Taxon Group (TG) concept where CWR are classified based on their taxonomic relationship to a crop. TG1a includes the crop itself, TG1b includes CWR of the same species as the crop, TG2 includes CWR within the same section of a genus as the crop, TG3 includes CWR in the same subgenus as the crop and TG4 includes any CWR otherwise in the same genus as the crop.

There is an extensive literature on the use of CWR in crop development. One example can be seen in the successful transfer of resistance to the fungal disease ‘powdery mildew’ from wild Abyssinian cabbage *Brassica carinata* A. Braun into cultivated brassica crops such as broccoli and cabbage (Tonguç and Griffiths, 2004). Another example is the use of wild oat *Avena sterilis* L. as a source of over 40 genes conveying resistance to crown rust disease enabling the development of resistant oat (*Avena sativa* L.) cultivars (Hoffman *et al*., 2006). The transfer of beneficial traits such as disease resistance and stress tolerance can have a significant economic impact on agriculture. One estimate by Price Waterhouse Cooper suggests that the development of improved cultivars of 29 key food crops through the transfer of novel traits from their CWR could be valued at approximately $120 billion by the year 2021 (PwC, 2013).

CWR, as with all wild plants, are vulnerable to climate change (Jarvis *et al*., 2008) and are poorly conserved both *in situ* and *ex situ* (Maxted, 2003; Dias *et al*., 2012). In response to this, the conservation of these valuable taxa and the genetic diversity within them should be a priority. This will facilitate their use in crop improvement, responding to a need for varieties with increased resilience to conditions predicted under a changing climate as well as addressing factors such as globalisation leading to increased spread of disease (Anderson *et al*., 2004), depletion of finite phosphate resources (Wissuwa *et al*., 2015) and salinisation of agricultural systems (Flowers and Flowers, 2005). International and regional policy documents are calling for improved conservation of agricultural genetic diversity, including CWR (Convention on Biological Diversity – CBD, 1992; Strategic Plan for Biodiversity –

CBD, 2010b; Biodiversity 2020 strategy – European Union, 2011). In recent years, this has encouraged the publication of national CWR conservation strategies where the current state of CWR conservation is assessed and recommendations for its improvement outlined (E.g. Finland – Fitzgerald, 2013; Spain – Rubio Teso *et al*., 2013; Italy – Panella *et al*., 2014; Cyprus – Phillips *et al*., 2014).

The UK contains approximately eight percent of Europe's CWR (Kell *et al*., 2008), including wild plants related to human food crops, animal forage and fodder crops as well as forestry species, medicinal and ornamental commercial plants. As a contracting party to the CBD Strategic Plan for Biodiversity (CBD, 2010b) the UK is committed to establishing active conservation for agricultural genetic diversity. This is stated in Aichi Target 13: 'By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity' (CBD, 2010b). Currently though, no active conservation is being undertaken for CWR in the UK. A preliminary analysis of UK CWR diversity was published by Maxted *et al*. (2007), which raised awareness of the need for a full analysis and recognised the necessity for closer collaboration with the appropriate statutory agencies of the UK. The UK is formed of the devolved administrations of England, Wales, Scotland and Northern Ireland. Most UK conservation policy is devolved with each administration having its own biodiversity strategy with commitments to conserve genetic diversity (Defra, 2011; Welsh Assembly Government, 2006; Scottish Government, 2013b; DOENI, 2002). Inventories of priority CWR and recommendations for their active and systematic conservation have been developed individually for England, Wales and Scotland

(Fielder *et al*., 2015; submitted a; submitted b) with input from Natural England (NE), Natural Resources Wales (NRW) and Scottish Natural Heritage (SNH) respectively.

The objective of the current study was to further the work completed for each administration in enhancing CWR conservation with input from NE, NRW and SNH. This study presents an inventory of priority CWR at the national (whole UK) level, assesses their current level of conservation across the country and finally provides recommendations as to how to improve the completeness of both *in situ* and *ex situ* CWR conservation in the UK. This study is not intended to supersede recommendations made at the administration level but rather as an adjunct to them to further improve the completeness of UK CWR conservation. This integrated bottom-up approach to conservation ensures CWR of value to an individual administration are conserved using locally available policy instruments, together with those of national importance. Facilitation of national CWR conservation will then contribute to European and global CWR initiatives. The focus of the current study is on food security but it should be noted that conservation of other use groups of wild relatives should not be overlooked and could form the basis of further studies of UK genetic resources.

4.3 Materials and methods

4.3.1 CWR checklist and inventory

A checklist of all CWR occurring within the UK was extracted from the Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell *et al.,* 2005) and the taxonomy harmonised with the British flora (Stace, 2010). The checklist contains a total of 2,109 CWR taxa (both native and introduced) related to agricultural, horticultural, forestry, ornamental, medicinal and aromatic crops (Supplementary Table 4.1a). According to Stace (2010) there are approximately 4,800 plant taxa in the British Isles, therefore approximately 44% of the British flora can be defined as CWR. It is likely to be impractical to effectively conserve over two thousand plant taxa, including many which are only casual, i.e. introduced but not yet naturalised (Stace, 2010 defines a naturalised plant as one that has been introduced, has established and is self-perpetuating. Plants naturalised prior to 1500 AD are known as archaeophytes whereas plants naturalised after 1500 AD are known as neophytes). As a result, a process of prioritisation of the checklist was necessary to create an inventory of priority CWR containing only those that are in more urgent need of active conservation.

The criteria selected for prioritisation were those that would identify the CWR that are likely to contribute most to the development of improved food and forage crop varieties. This contrasts with traditional approaches to plant conservation where the focus is primarily on rare or threatened species. Criteria were selected jointly by NE, NRW, SNH and the University of Birmingham and were applied to the checklist in turn. Criteria one and two were first applied to identify a subset of CWR containing only taxa that are native, archaeophyte and neophyte, i.e. no casuals (introduced and not yet naturalised), related to human food or animal forage and fodder crops. Subsequently, any of these CWR meeting the remaining criteria (3–6) were prioritised and listed in the UK inventory of priority CWR. Prioritisation criteria seek to balance agricultural value of the related crop with requirement for conservation due to decline and are as follows:

1. Use of the related crop – identification of all CWR related to crops used for human food or animal forage and fodder according to GRIN Taxonomy for Plants (USDA, ARS, National Genetic Resources Program, 2015).

2. Native status – identification of all native, archaeophyte and neophyte CWR and exclusion of casuals (Stace, 2010).

3. Economic value of the related crop – identification of economically valuable crops according to global, European and UK production quantities (FAOSTAT, 2013), crop production value at producers prices (Eurostat, 2013) and crop production at market prices (Defra, 2010). Any crop assigned a value by any of these sources above was considered economically valuable. Any CWR within the same genus or gene pool as these economically valuable crops were prioritised.

4. Degree of relatedness to the crop – prioritisation of CWR within GP1b, GP2 or TG1b to 3 of their related crop according to Vincent *et al*. (2013). CWR related to more than one crop were prioritised based on the crop to which they were most closely related.

5. Threat assessment – prioritisation of any threatened (vulnerable, (VU); endangered, (EN); or critically endangered, (CR)), near threatened (NT), data deficient (DD) or extinct in the wild (EW) CWR in the IUCN Red List of Threatened Species (IUCN, 2012), European Red List of Vascular Plants (Bilz *et al*., 2011) or the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005).

6. Other conservation designations – prioritisation of CWR with any conservation designations other than a red listing category according to the Conservation Designations for UK Taxa database (JNCC, 2011).

4.3.2 In situ *gap analysis*

UK occurrence data was extracted from the BSBI distribution database (BSBI, 2013). Records were filtered to produce a dataset with only confirmed records from 1970 onwards (so sites are likely to still be current), with a precision equal to or greater than 2km by 2km. Records with no passport data (information detailing accurate geographic locations and taxonomic classifications of the occurrence records) or which were clearly inaccurate (e.g.

occurring in the sea) were excluded. Spatial analyses were carried out using ArcMap 10.0 and DIVA-GIS 7.5.0 (ESRI, 2011 and Hijmans *et al*., 2012). A UK boundary shapefile was obtained from DIVA-GIS (www.diva-gis.org). The following three *in situ* analyses were carried out according to methods outlined in Scheldeman and van Zonneveld (2010) and Hijmans *et al*. (2012):

1. Richness analysis – Identification of CWR taxon hotspots and areas of high recording effort in DIVA-GIS using the ‘point to grid’ tool to count the numbers of taxa/records within each 0.2 by 0.2 degree grid square across the UK.

2. Complementarity analysis – Selection of the optimal number and distribution of grid squares (0.2 by 0.2 degrees) across the UK required to conserve all priority UK CWR, using the ‘reserve selection’ tool in DIVA-GIS. This tool first selects the grid square with the highest number of CWR taxa and then selects a second grid square containing the next highest number of taxa, excluding those already covered by the first square. This process of grid square selection is iterated until all CWR taxa are represented in at least one grid square, according to the method described by Rebelo (1994). Brown and Briggs (1991) and Dulloo *et al*. (2008) suggest a minimum of five distinct populations should be conserved to improve the chances of persistence and range of genetic diversity protected for each plant taxon. As a result, the number of grid squares in which each priority CWR occurs was also then identified.

3. Incidence of priority CWR within protected areas – Investigation into the extent of potential passive protection already received by UK priority CWR using ‘spatial join’ tools in ArcMap 10.0 to find the numbers of occurrence records within and outside of UK protected areas. Protected area boundaries included: Sites of Special Scientific

Interest (SSSI), Areas of Special Scientific Interest (ASSI), Special Areas of Conservation (SAC), Areas of Outstanding Natural Beauty (AONB), National Scenic Areas (NSA), National Nature Reserves (NNR), Local Nature Reserves (LNR), National Parks (NP), Country Parks, Ramsar sites and Special Protection Areas (SPA) and were obtained from: NE (2014), NRW (2012), SNH (2012) and DOENI (2012).

4.3.3 Ex situ *gap analysis*

UK genebank accession data were obtained from the UK National Plant Inventory (UKNPI, 2013) and the Millennium Seed Bank, Royal Botanic Gardens, Kew. Accessions lacking longitude and latitude coordinates were georeferenced using the UK Grid Reference Finder (www.gridreferencefinder.com) and the Gazetteer of British place names (www.gazetteer.org.uk). The following three *ex situ* analyses were carried out:

1. Count of accessions per priority UK CWR. Taxa with fewer than the minimum accepted level of five accessions require further collecting to reach this threshold (Brown and Briggs, 1991; Dulloo *et al*., 2008).
2. Calculation of a geographical representativeness score (GRS) for each priority CWR using the 'circular area statistic' (Ramírez-Villegas *et al*., 2010). A GRS indicates the proportion of the geographic range of occurrence data represented in genebanks and a score greater than 30% is considered adequate *ex situ* representation (Ramírez-Villegas *et al*., 2010; Phillips *et al*., 2014).
3. By combining these first two *ex situ* analyses, priority level were assigned to each priority CWR and recommendations made as to the actions to be taken to improve the completeness of *ex situ* accessions for UK CWR. Levels are as follows:

- Priority 1 – No accessions
- Priority 2 – Has accessions but none are georeferenced/location data restricted
- Priority 3 – Fewer than five accessions and GRS lower than 30%
- Priority 4 – Fewer than five accessions but GRS greater than 30%
- Priority 5 – Greater than or equal to five accessions but GRS lower than 30%
- Priority 6 – Greater than or equal to five accessions and GRS greater than 30%

4.4 Results

4.4.1 CWR inventory

The UK national inventory of priority food and forage/fodder CWR contains 223 taxa (192 species and 31 infra specific taxa) (Supplementary Table 4.1b). This represents 10.57% of all UK taxa in the national CWR checklist. The inventory includes 163 taxa related to human food crops (of which 80 taxa are also related to forage and fodder crops); a further 60 taxa are related only to forage and fodder crops. Seventeen plant families are represented in the inventory (Table 4.1). The family Poaceae contains the most genera (20) including *Avena* (oats) and *Hordeum* (barley). The family containing the most taxa is Fabaceae, which includes species within the genera: *Lathyrus* (vetchlings), *Medicago* (medics), *Trifolium* (clovers) and *Vicia* (vetches). A total of 142 of the inventory taxa are classified as native, 23 as archaeophytes and the remaining 58 are neophytes. Two CWR endemics are present in the UK, Scottish small-reed *Calamagrostis scotica* Druce (located at one site in the highlands of Scotland) and perennial flax *Linum perenne* L. subsp. *anglicum* (Mill.) Ockendon (occurring largely in the east of the UK).

Table 4.1 Summary of inventory of 223 priority CWR in UK.

Family	**No. of genera**	**No. of species**	**No. of infra-specific taxa**	**Native status**
Alliaceae	1	10	3	N; Neo
Amaranthaceae	3	13	1	N; A; Neo
Apiaceae	3	2	4	N
Asparagaceae	1	2		N; Neo
Asteraceae	2	5		N; A; Neo
Betulaceae	1	2		N; Neo
Brassicaceae	8	10	5	N; A; Neo
Ericaceae	1	6		N; Neo
Fabaceae	8	59	8	N; A; Neo
Fagaceae	1	1		Neo
Geraniaceae	1	3		N; A
Grossulariaceae	1	8		N; Neo
Juglandaceae	1	1		Neo
Linaceae	1	2	1	N
Moraceae	2	2		Neo
Poaceae	20	39	9	N; A; Neo
Rosaceae	6	27		N; Neo
Totals	**61**	**192**	**31**	

N = Native, A = Archaeophyte, Neo = Neophyte.

Through prioritisation of the UK CWR checklist, 123 food and forage/fodder taxa were identified as related to economically valuable crops according to agricultural statistics (Defra, 2010; FAOSTAT, 2013; Eurostat, 2013). Prioritised CWR were related to crops including sugar beet, brassicas, carrots, onions, peaches, lettuces, asparagus and raspberries among others. In terms of the closeness of the relationships between crops and their wild relatives, one third of CWR were within GP1b or TG1b. Twenty six percent of CWR were classified using the GP concept and 74% using the TG concept (Fig. 4.1). According to the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005), 26 food, forage or fodder CWR in the UK are threatened (Table 4.2). Upright goosefoot, *Chenopodium urbicum* L., is listed as Critically Endangered and a further four species are listed as Endangered (wild asparagus *Asparagus prostratus* Dumort., purple milk-vetch *Astragalus danicus* Retz., stinking goosefoot *Chenopodium vulvaria* L. and least lettuce *Lactuca saligna* L. The remaining 21 threatened taxa are listed as Vulnerable. In addition, interrupted brome *Bromus interruptus* (Hack.) Druce is Extinct in the wild. Portugal laurel *Prunus lusitanica* is listed as Vulnerable in the European Red List (Bilz *et al*., 2011) but has not been evaluated in Great Britain, as it is not native. A further 26 taxa were prioritised based on their having at least one additional conservation designation (Supplementary Table 4.2), for example, sea pea *Lathyrus japonicus* Willd. subsp. *maritimus* (Mill.) Ockendon is listed as Nationally Scarce and is also listed on the Scottish Biodiversity List of priority taxa (JNCC, 2011).

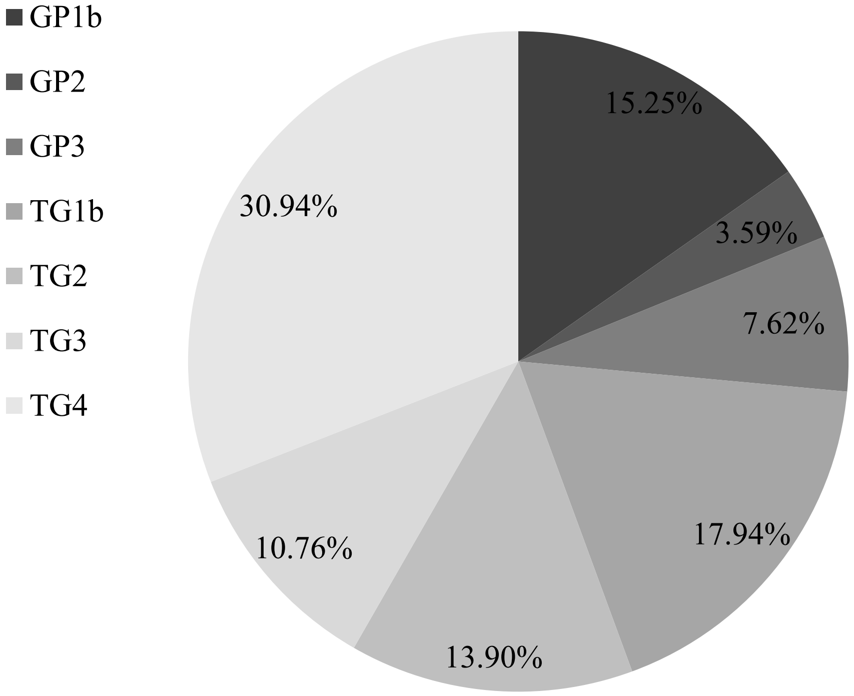

Figure 4.1 Percentages of each Gene Pool and Taxon Group classification assigned to CWR in the UK inventory showing the degree of relatedness to their associated crops.

Table 4.2 Red list status and the threat criteria met for CWR in the UK inventory.

CWR	**Red list category**	**Criterion**
Bromus interruptus (Hack.) Druce	EW	
Chenopodium urbicum L.	CR	A2c
Asparagus prostratus Dumort.	EN	C2a(i)
Astragalus danicus Retz.	EN	A2c
Chenopodium vulvaria L.	EN	A2c
Lactuca saligna L.	EN	A2c
Allium oleraceum L.	VU	A2c
Allium sphaerocephalon L.	VU	D2
Apium repens (Jacq.) Lag.	VU	D2
Astragalus alpinus L.	VU	D2
Calamagrostis scotica Druce	VU	D1+2
Calamagrostis stricta (Timm) Koeler	VU	A2c
Chenopodium bonus-henricus L.	VU	A2c
Chenopodium glaucum L.	VU	A2c
Chenopodium murale L.	VU	A2c
Hordeum marinum Huds.	VU	A2c
Koeleria vallesiana (Honck.) Gaudin	VU	D2
Lathyrus aphaca L.	VU	A2c
Medicago minima (L.) L.	VU	A2c
Poa flexuosa Sm.	VU	D1
Poa glauca Vahl	VU	A2c
Pyrus cordata Desv.	VU	D1
Trifolium bocconei Savi	VU	D2
Trifolium incarnatum L. subsp. *molinerii* (Hornem.) Syme	VU	D2
Trifolium strictum L.	VU	D2
Vicia bithynica (L.) L.	VU	A2c
Vicia parviflora Cav.	VU	A2c
Calamagrostis purpurea (Trin.) Trin. subsp. *phragmitoides* (Hartm.) Tzvelev.	DD	
Festuca rubra L. subsp. *scotica* S. Cunn. Ex Al-Bermani	DD	

VU = Vulnerable; EN = Endangered; CR = Critically Endangered; EW = Extinct in the Wild; DD = Data Deficient; A2c = reduction in population size based on trend in Area of Occupancy or Extent of Occurrence; C2a(i) = Limited population size with continuing decline; D1 = Very restricted population of less than 1000 mature individuals; D2 = Very restricted population based on Area of Occurrence or number of locations (Data source: Cheffings and Farrell, 2005).

4.4.2 In situ *gap analysis*

A total of 803,755 occurrence records were obtained from the BSBI distribution database (BSBI, 2013), representing 213 of the 223 CWR taxa in the UK inventory. Ten taxa had no confirmed, fully georeferenced records dated from 1970 onwards at a precision of 2km by 2km or better; these taxa are listed in Table 4.3. The majority of records (752,821) belong to native or archaeophyte taxa and a further 49,804 records represent the neophyte taxa. The taxon with the highest number of records is cock's foot *Dactylis glomerata* L. with 35,592.

Table 4.3 List of CWR with no occurrence records reaching the appropriate quality standards for inclusion in gap analysis.

CWR	
Allium ampeloprasum var. *bulbiferum*	*Lathyrus japonicus* subsp. *maritimus*
Calamagrostis purpurea subsp. *phragmitoides*	*Medicago littoralis*
Festuca arenaria subsp. *arenaria*	*Pastinaca sativa* subsp. *sylvestris*
Festuca arenaria subsp. *oraria*	*Prunus incisa*
Festuca rubra subsp. *scotica*	*Trisetum flavescens* subsp. *purpurascens*

Taxon richness analysis showed that the areas of the UK richer in priority food, forage and fodder CWR are the south and the east, particularly the counties of Cornwall, Somerset, Dorset, Hampshire, Kent and the region of East Anglia, all with grid squares containing greater than 100 priority CWR (Fig. 4.2a). There are also grid squares containing over 100 CWR around Aberystwyth in Wales and in West Yorkshire in England. A single grid square in Scotland (around Edinburgh) contains greater than 75 priority CWR. Analysis of observation richness reveals that the locations with the highest numbers of records are Bedfordshire with over 15,500 records within two grid squares and Somerset with 9,000–10,500 records within one grid square in the county (Fig. 4.2b).

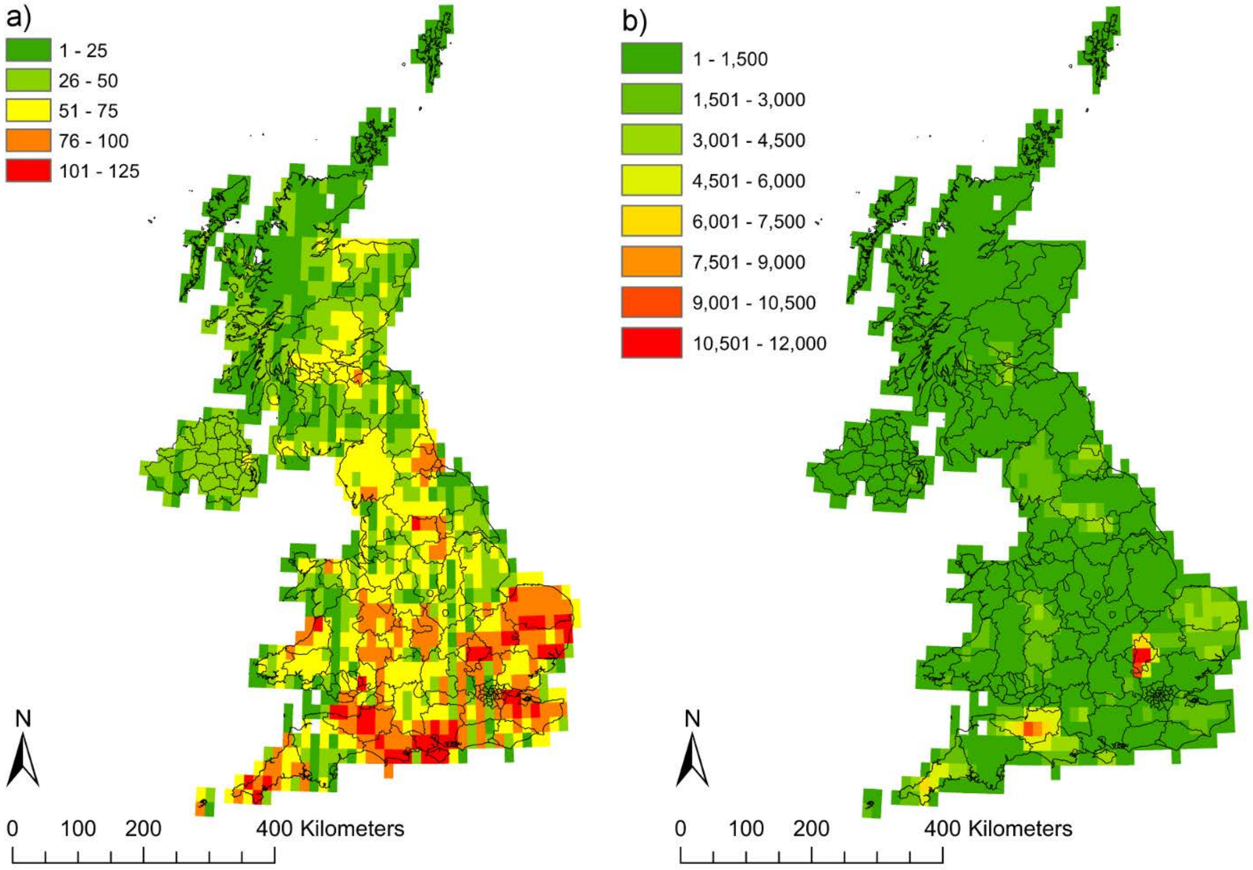

Figure 4.2 Richness analysis of the 213 CWR with occurrence records from the UK inventory. a) Taxon richness and b) Observation richness. Analysis uses a grid square size of 0.2 degrees.

A total of 27 grid squares across the UK were identified as an appropriate complementary network for the *in situ* conservation of priority food and forage/fodder CWR (Fig. 4.3). The network incorporates 211 out of the 223 priority CWR in the UK inventory (taxa with insufficient records could not be included). The top priority grid square containing the most priority CWR is in Purbeck, Dorset on the south coast of the UK with 124 taxa. The next priority site, in Cambridgeshire, had 17 CWR taxa that were not present in the previous site (with 107 CWR taxa in total). The next eight sites (making up the top ten) in order of highest priority to lowest are as follows with the number of additional taxa present in each square indicated and the total number of CWR present shown in brackets: Ceredigion in Wales 11(106), Bedfordshire in England 9(114), Aberdeenshire in Scotland 8(24), The Lizard Peninsula in England 7(104), Norfolk in England 4(103), Loch Lomond in Scotland 4(66), Somerset in England 3(104) and East London in England 3(106). Sites 14 to 27 each add just one additional taxon (i.e. a taxon not found in any of the preceding grid squares) to the network of grid squares (Fig. 4.4). Despite having no clear taxon hotspots, eight of the complementarity squares are within Scotland; a further two are within Wales. Three grid squares are in the north of England with the rest located in the south and east of the country. Out of the 211 taxa represented in the grid square network, 61.61% are found within five or more of the grid squares. Red clover *Trifolium pratense* L. is the only CWR found in all 27 grid squares.

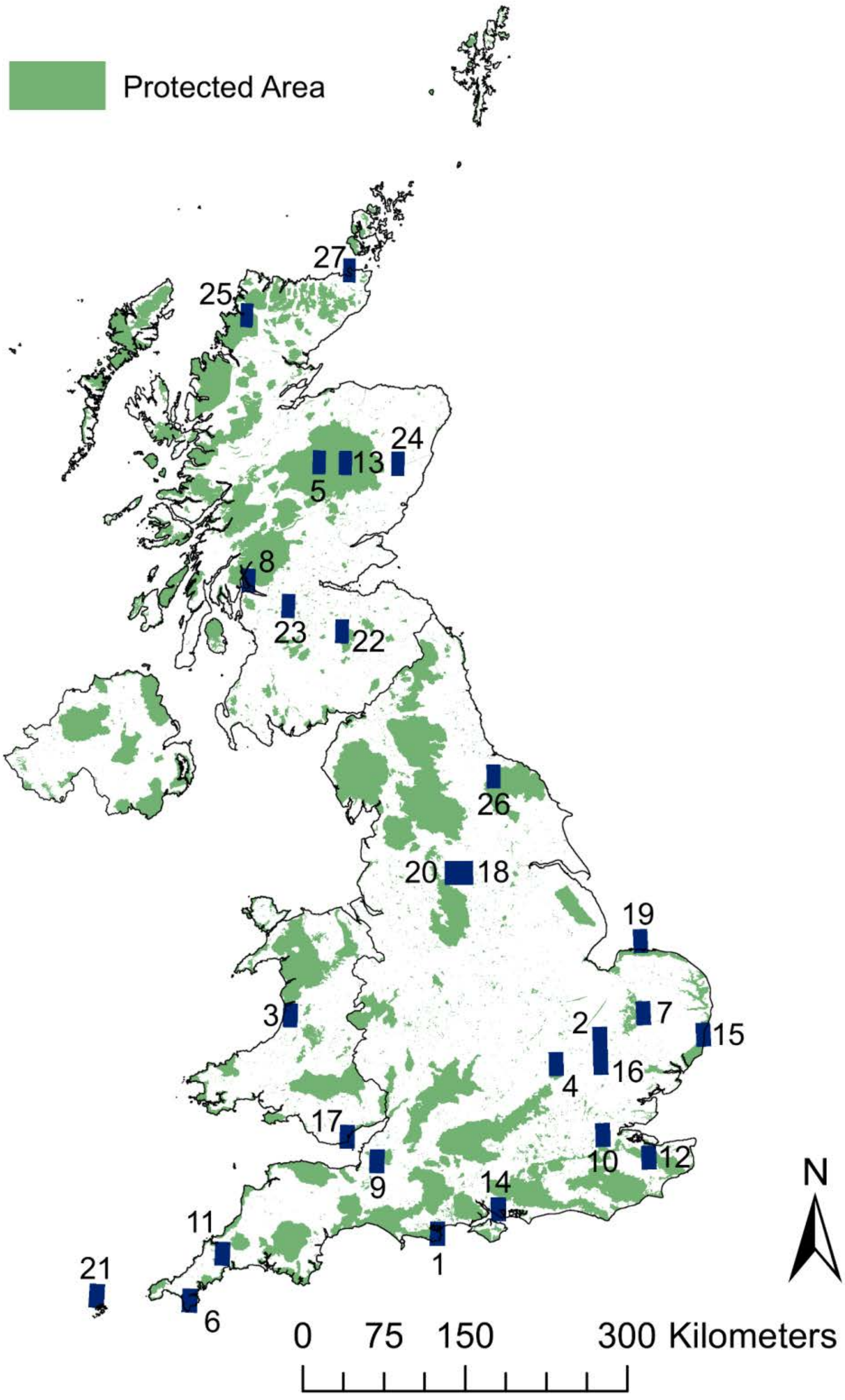

Figure 4.3 Network of 27 grid squares covering 211 of UK priority CWR identified using complementarity analysis. Grid squares identified may be suitable sites for establishing active *in situ* conservation.

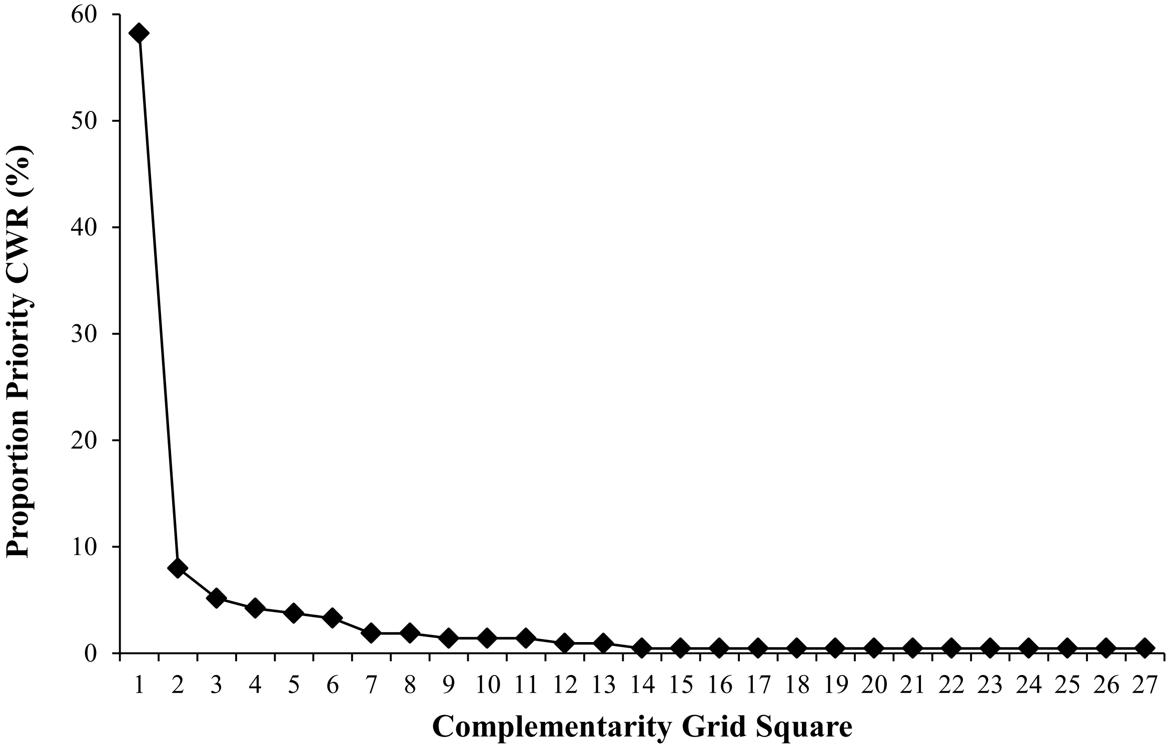

Figure 4.4 Percentage of additional CWR in each of the 27 complementarity grid squares (i.e. CWR not present in any of the preceding grid squares).

All 27 complementarity squares overlap with at least three protected areas and all include at least one SSSI. In terms of CWR occurrences, 31.05% of records are within protected areas in the UK. Only four of the 213 CWR with occurrence data have no records within protected areas, these are: wild black gooseberry *Ribes divaricatum* Douglas, buffalo currant *Ribes odoratum* H.L. Wendl., starry clover *Trifolium stellatum* L. and Hungarian vetch *Vicia pannonica* Crantz., all of which are neophyte taxa. Furthermore, 192 (90.14%) CWR have five or more occurrence records within protected areas, which indicates a reasonable representation of genetic diversity (Brown and Schoen, 1992; Neel and Cummings, 2003). However, the appropriate number of populations conserved will vary depending upon the breeding system of the species (Iriondo *et al.*, 2008b).

4.4.3 Ex situ *gap analysis*

A total of 1,034 accessions were found to be stored in genebanks for UK priority CWR. These records represent 146 CWR of the 223 CWR in the UK inventory. Just 16% of the 223 priority taxa have five or more accessions stored in genebanks, though two of these taxa have greater than 50 stored accessions and these are perennial rye grass *Lolium perenne* L. (338 accessions) and white clover *Trifolium repens* L. (65 accessions). Both are important UK forage species that are wild and cultivated in the UK, and new cultivars are occasionally selections of wild material. All of these 1,034 accessions are stored either in the Millennium Seed Bank, Warwick Genetic Resources Unit or the Institute of Biological, Environmental and Rural Sciences (IBERS) at Aberystwyth University in Wales. Of the 77 taxa without any stored accessions, the majority (53) are introduced taxa (archaeophytes and neophytes). In terms of GRS percentages, only three CWR reach the threshold of 30% showing their accessions are a good representation of the *in situ* distribution of these species, these are Plymouth pear *Pyrus cordata* Desv., Scottish small-reed *C. scotica* and starry clover *T. stellatum*.

The number of accessions and GRS values for each CWR were combined to assign a priority level for *ex situ* collecting to each CWR (Supplementary Table 4.3). Only one taxon was assigned the lowest priority level (6), being well represented in *ex situ* collections, and this was Plymouth pear *P. cordata*. The majority of taxa were assigned level three due to their having fewer than five accessions and a GRS below 30%. Seven taxa were assigned to priority level two, having stored accessions but no associated geo-reference data to determine their origin. Finally the 77 taxa with no accessions were assigned the top priority level of one, being most in need of collection of accessions (Supplementary Table 4.4).

4.5 Discussion

The importance of establishing conservation planning for CWR at the national level is increasingly being recognised (Maxted *et al*., 2015a) and a number of European countries including Spain, Finland and Italy have undertaken research to improve CWR conservation within their respective countries (Rubio Teso *et al*., 2013; Fitzgerald, 2013; Panella *et al*., 2014). Maxted *et al*. (2015a) are calling for the introduction of *in situ* conservation of CWR with complementary *ex situ* back up at the national level with integration into European conservation planning for CWR. Due to the likelihood that national priorities will not always overlap with wider regional priorities there is a need for conservation at both national and regional levels with integration between the two approaches. In terms of conservation planning, the UK presents an interesting case due to its division into the administrations of England, Wales, Scotland and Northern Ireland, each with its own conservation agency. The devolved nature of the UK administration adds a 'sub-national' level to the national/European model of CWR conservation. However, wider national (whole UK) priorities may differ from sub-national priorities and so an integrated approach is practical in this case. Support for this bottom-up approach is reflected in the collective agreement of NE, NRW and SNH on a single method for prioritising and assessing UK CWR conservation that would address national priorities, augmenting the efforts already undertaken at sub-national level (Fielder *et al*., 2015; submitted a; submitted b).

CWR are a part of 'biodiversity', defined by the CBD (1992) as 'the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems'. In this sense, the conservation agency for each administration with responsibility for biodiversity conservation also has responsibility for its CWR. However, at both UK and devolved administration levels, no active conservation measures aimed at the preservation of CWR and their genetic diversity currently exist, despite European and global commitments to secure these resources (CBD, 1992; 2010b; Planta Europa, 2008; European Union, 2011). Here, and separately in England, Wales and Scotland, the initial steps towards active conservation have been achieved through identification of CWR with potential for contributing to food security, assessment of their current level of protection and the outlining of recommendations for their integration into existing conservation planning. The main difference in the approaches taken at sub-national and national levels was seen in the application of criteria for CWR prioritisation rather than in the *in situ* or *ex situ* gap analysis methods.

The biggest difference in the prioritisation process between devolved administrations and the UK was the inclusion of neophytes in the UK CWR inventory. The decision to include introduced taxa in CWR priority lists is one with divided opinion in the literature. The Portuguese CWR inventory, one of the earlier national inventories, includes introduced taxa based on the argument that plant breeders require beneficial traits for crop improvement regardless of the origin of the material (Magos Brehm *et al*., 2008). It is also possible that the likelihood of fixation of new mutations under exposure to novel environmental conditions, may lead to the development of beneficial traits in introduced taxa. In contrast, the inventories from Spain, Finland, Italy and Cyprus all exclude introduced taxa with arguments largely surrounding the issue that it is easier to garner support from national governments for conservation of CWR that are part of the native flora, that introduced species are likely to have less diversity due to initial establishment of only a few individuals and that the conservation of any introduced CWR which are also invasive (i.e. causing economic or environmental harm (CBD, 1992)) should be avoided (Rubio Teso *et al*., 2013; Fitzgerald, 2013; Panella *et al*., 2014; Phillips *et al*., 2014). In the devolved administrations, neophyte CWR were excluded from the inventories primarily due a need to prioritise in light of limited resources. However, at the whole UK level neophytes have been included in the national inventory with the aim to establish conservation for nationally valuable neophytes, which would otherwise be missed at the devolved level. However, conservation of any invasive species should use *ex situ* approaches rather than *in situ*. There is no single correct approach to this issue, or the selection of prioritisation criteria in a broader sense, but rather decisions should be made with input from stakeholders to maximise their support and therefore the likelihood of establishing active conservation of CWR.

Approximately 27.5% of the land area of the UK is designated within protected areas (Defra, 2013). This is much higher than the 12.2% of global land area falling within protected areas (Chape *et al*., 2005) and a relatively high incidence of CWR within UK protected areas is apparent (over 90% of priority taxa represented in five or more protected areas). Despite representation in protected areas, these are only examples of potential passive conservation with no direct management or monitoring of CWR populations to ensure their persistence. It should be noted that passive conservation of CWR is the incidental occurrence of a CWR within a protected area, with no specific management in place to protect CWR populations, but where the existing management of the site is not detrimental to CWR populations (Maxted *et al*., 1997b). Incidence within protected areas may not always result in passive protection i.e. where priority habitat or species management conflicts with the needs of a CWR population. The implementation of active management for CWR will always be preferable and may be more easily established in sites already offering some level of passive protection. Complementarity grid squares propose a potential network of sites where multiple CWR can be actively conserved, aiding the planning and targeting of management at specific sites. Assessment of the genetic diversity of CWR within protected areas overlapping with these complementarity squares is necessary to assess their suitability as CWR genetic reserves (Iriondo *et al*., 2012). Though many CWR occur within protected areas, just over two thirds of the occurrences of priority CWR still fall outside of these designations. For comprehensive CWR conservation across the UK, an approach that targets *in situ* conservation both within and outside of protected areas is likely to be most effective. Many complementarity sites identified in England are also identified in the UK level analysis, particularly the sites in Purbeck, Cambridgeshire, on The Lizard Peninsula and in Somerset. There are fewer sites in Wales and Scotland that are also identified in the UK, though sites around Cardiff in Wales and Loch Lomond and the Cairngorms National Park in Scotland are present in both administration and UK analyses. The Gower Peninsula, the top priority complementarity site in Wales (Fielder *et al*., submitted a), is not identified in the UK gap analysis. The importance of the Gower Peninsula site for CWR conservation in Wales is clear and reinforces the need for a bottom-up approach to UK CWR conservation with the priorities of each administration addressed as well as those of national importance. Furthermore, the number of complementarity sites in common between the UK and devolved administrations suggests that CWR conservation can easily be incorporated into these sites without the need to establish a completely novel and independent UK network of CWR conservation sites.

Ex situ collections for priority CWR are more complete at the UK level than in each of the devolved administrations. This is likely to be because collection efforts by the Millennium Seed Bank, Kew have focused on acquiring at least one accession from each native plant within the UK, with 94% of native, orthodox taxa (whose seed can be dried and frozen for long-term storage and remain viable (Hong *et al*., 1998)) now collected and stored (Stephanie

Miles, pers. comm.). These efforts are targeted at the UK level and have not included the collection of introduced taxa. With respect to CWR it will be important that collections include archaeophyte and neophyte taxa as well as native taxa as they may also contain novel, adaptive and useful traits for use in crop development (Magos Brehm *et al*., 2008). It is not known which adaptive traits will be of use in the future so the collection of a range of accessions per CWR taxon representing its ecogeographic breadth is also advisable to ensure as much genetic variation within taxa is conserved as is practical (Crossa and Vencovsky, 2011).

4.5.1 Recommendations for enhancing CWR conservation in the UK

Recommendations have been outlined for the establishment of CWR conservation in England, Wales and Scotland (Fielder *et al*., 2015; submitted a; submitted b). However, as yet there is no scheme in place to translate this into implementation of active CWR management and monitoring; this should be a priority for each devolved administration. Once this foundation is in place, the recommendations below would take steps towards integration of sub-national and national conservation planning for CWR:

- Conservation of UK priority CWR should be incorporated into existing protected areas where possible. Priority CWR would benefit from being recognised and formally listed as ‘features of special interest/importance’ in each specific protected area and should be included in management plans to achieve long-term and active conservation. A minimum of five populations actively conserved per taxon (provided this many populations occur) is likely to be achievable, though discretion should be used in all cases. For example, a higher number of conserved populations may be appropriate for inbreeding taxa as more variation is found among populations of inbreeding taxa than those that are outbreeding (Brown and Marshall, 1995; Hamrick and Godt, 1989 and 1996; Hoban and Schlarbaum, 2014). As a first step, the conservation of UK priority CWR in protected areas recognised as having CWR of priority within a devolved administration would be appropriate e.g. the NNR on The Lizard in Cornwall. Nationally important CWR not represented in sub-national inventories should be identified and incorporated into conservation planning within the appropriate devolved administrations. This would result in a single *in situ* network of CWR conservation with sites of either sub-national importance or national importance or those recognised at both of these levels.

- *In situ* conservation planning should adhere to accepted minimum standards for the establishment of genetic reserves (Iriondo *et al*., 2012). This involves the selection of appropriate sites based on sound demographic surveying and genetic analysis, commitments to long-term conservation, the presence of CWR populations large enough to be viable in the long-term, recognition of the genetic reserve by appropriate authorities and formulation of clear conservation objectives and management and monitoring plans.

- Continue to survey the *in situ* distributions of priority CWR and store these records in an accessible and long-term database e.g. the BSBI distribution database (BSBI, 2013). It is critically important that up-to-date and specific recording to the sub-specific level becomes standard practice across the whole UK, as this will directly influence the reliability of the dataset for subsequent analyses. More recent and high quality occurrence records for the taxa listed in Table 4.3 are particularly encouraged.

- Gaps in *ex situ* collections of UK CWR should be filled. The collection of accessions should initially focus on taxa with no accessions (priority 1), including introduced taxa (Supplementary Table 4.4). Ultimately it should aim to collect a minimum of five accessions that represent the ecogeographic breadth of the taxon for all priority CWR, unless the taxon is a UK endemic in which case it should be increased as appropriate. Accessions should be representative of the taxon's full distribution (Hoban and Schlarbaum, 2014). Collection would ideally be informed by genetic diversity analysis and accessions collected would back up *in situ* populations associated with long-term active conservation. Duplication of collection efforts for the UK and in the devolved administrations should be avoided but equally insuring the collection of UK priority CWR that are not in the sub-national inventories is advised.

- Safety backups of accessions should be stored in separate genebanks to protect against sudden loss of material in any one location.

- The relationship between accessions held in long-term storage and population genetic changes over time should be explored and the appropriate intervals for updating *ex situ* collections determined per species to capture the genotypic evolution of *in situ* populations over time.

- Genebanks should undertake greater promotion of the conserved CWR material available to researchers, pre-breeders and plant breeders for use in crop improvement, encouraging their use in the development of new crop varieties and for use by any other appropriate parties/organisations and purposes (e.g. for long-term conservation, education and scientific research).

- Regular review of the inventory of priority CWR for the UK at a suggested interval of ten years is recommended, concurrent with reviews of the inventories for the devolved administrations. Future iterations of the inventory should be expanded to include other use categories of CWR. Reviews should first consider international policy but should also address national priorities.
- There should be a push to gain support for CWR conservation from the European Union through the Rural Development Programmes in each Member State delivered through the Common Agricultural Policy. It is important that there is wider recognition of the value of CWR and their role as a key component of ecosystem services.
- Assessment of climate change vulnerability of UK CWR is advisable. Such an assessment would provide valuable predictions as to the likely impact of climate change on CWR abundance and distributions over time and can be used to inform management approaches.
- Although this paper has focused on CWR for food security, it should be noted that all other categories of CWR (e.g. with uses in the medicinal, forestry, ornamental industries), wild harvested plants, landraces and heritage varieties are also lacking in conservation planning and further efforts will be required to achieve full conservation of all UK plant genetic resources.

4.6 Conclusion

The UK is in a unique situation as it has one of the most comprehensively studied floras in the world. Despite this, active conservation of its CWR is currently lacking. To account for the devolved approach to conservation in the UK, CWR inventories have been produced and gap analyses carried out separately for England, Scotland and Wales. It is hoped that the bottom-up approach undertaken will encourage appropriate organisations to incorporate CWR into conservation planning. It is equally important that CWR are assessed in terms of their national value and that conservation planning also takes this into account. The current study identifies nationally valuable CWR, key sites for *in situ* conservation and gaps in *ex situ* collections. Through the integration of these sub-national and national approaches, comprehensive, active and long-term management and monitoring of UK CWR could be achieved with complementary back-up of accessions in genebanks preserving the UK's national genetic resources, facilitating scientific research and education as well as providing a point of access for plant breeders to utilise the material to develop crop varieties with improved resilience to a changing climate. The methods used here can be easily applied to inform conservation planning for CWR in any country. The approach also demonstrates how conservation planning can be integrated within countries that have a level of devolution to sub-national administrations.

CHAPTER 5.

Distribution of crop wild relatives of conservation priority in the UK landscape

The work presented in this chapter has been accepted

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Conceived and designed the study: SJ, **HF**, JJH, NM, SS

Data collation and preparation: SJ, **HF**

Performed analysis: SJ

Interpreted results: SJ, **HF**

Wrote the paper: SJ, **HF***

Critically reviewed the paper: SJ, **HF**, JJH, NM, SS

*This chapter was written in close collaboration with Dr. Susan Jarvis, and much of the text was written by her. The work would not stand alone without her contribution, which is why her contribution has been included here. Full details of our relative contributions are given in the declaration on pages xix–xx at the beginning of the thesis.

5.1 Abstract

Many crop species suffer from a lack of genetic diversity, which reduces the ability of cultivars to withstand new pests or environmental stresses. The wild relatives of crop plants are an important source of genetic variation and can be used to introduce new traits into existing crops. Identification and conservation of crop wild relatives (CWR) is an important step to safeguard future food security. Recent efforts have identified geographical hotspots of CWR diversity in several countries but, as yet, there have been no surveys to identify the habitats and landscape features within these areas that might be most suitable for conservation efforts. Here, we use a UK-wide vegetation survey covering a range of both habitats and landscape features (e.g. fields, hedgerows, waterways and roadsides) to identify the habitats and features with the highest diversity of CWR identified as priority taxa for conservation. Priority CWR were most abundant in grassland habitats, although this was most striking in CWR related to forage and fodder crops. CWR related to food crops were most common in cropped and weedy areas, fertile grassland and lowland woodland. Within habitats, CWR diversity was significantly associated with linear features including hedgerows, roadsides, field boundaries and field margins. Our findings indicate that CWR of conservation interest are often associated with disturbed habitats and landscape features that are not considered as priorities under site-based conservation measures. We suggest that efforts to maintain linear features in hotspots of CWR diversity would be most effective at conserving the UK's CWR resource.

5.2 Introduction

Crop wild relatives (CWR) are wild plant species that are close relations of domesticated plants (Harlan and de Wet, 1971; Maxted *et al.*, 2006). Concerns about the lack of genetic diversity in many crop species have led to an increased interest in utilising the wider pool of genetic variation present in closely related plants to improve food security in the face of threats such as pests and disease. Food security is likely to be increasingly at risk in the longer term as climate change is predicted to reduce crop yields by an estimated 2% per decade, with the forecast set to worsen beyond 2050 (Porter *et al.*, 2014).

CWR host genetic diversity that could be used to tackle these issues through the introduction of traits such as pest and disease resistance, stress tolerance and increased yield to improve crops. The introduction of traits from wild relatives to cultivated plants has already led to a vast literature describing new varieties with improved characteristics (Maxted and Kell, 2009). For example, sugar beet (*Beta vulgaris* subsp. *vulgaris*) varieties with resistance to *Cercospora* leaf spot and Rhizomania have been developed with traits introduced from the wild relative, sea beet (*Beta vulgaris* subsp. *maritima*) (Munerati, 1932; Lewellen *et al.*, 1987; Biancardi *et al.*, 2002; Grimmer *et al.*, 2007). More recently sea beet accessions with abiotic stress tolerant traits have been identified and are being considered for use in breeding programmes (Stevanato *et al.*, 2013). Other examples of wild relative use in crop improvement include the transfer of mustard aphid resistance from wild *Brassica fruticulosa* Cirillo into cultivated *B. rapa* L. (Chandra *et al.*, 2004), the transfer of powdery mildew resistance from wild *B. carinata* A. Braun into cultivated *B. oleracea* L. (Tonguç and Griffiths, 2004) and the transfer of potato leafroll virus resistance from the wild Mexican species *Solanum verrucosum* Schtdl. to cultivated potato (Carrasco *et al.*, 2000).

Until recently little was known about the global distribution and abundance of CWR. The creation of the Harlan and de Wet Inventory of CWR in 2013 (Vincent *et al.*, 2013) marked a significant advance in our understanding of the number of global priority CWR taxa (1,667 CWR taxa with potential or proven use for crop improvement related to 173 crops of global importance) and their distributions. The inventory identified several global hotspots including a high concentration of CWR taxa around the Fertile Crescent, an area noted as both a historical centre of crop domestication and parts of which are subject to ongoing conflict. Thus efforts to establish active and systematic *in situ* and *ex situ* CWR conservation have been driven by the realisation that a lack of genetic diversity in crop plants is becoming a significant problem and that many CWR occur in areas where conservation is difficult to achieve.

Although the United Kingdom does not have the CWR diversity of the Fertile Crescent it does host a wide range of CWR taxa, many of which are related to crops of economic value such as the food crops brassicas, barley and sugar beet as well as forage and fodder crops including grasses, clovers and vetches. Current *in situ* CWR conservation in the UK is focused on a few very rare and threatened species. For example, *Pyrus cordata* is listed as Endangered in the English Red Data Book and has its own species recovery programme (Jackson, 1995; Stroh *et al.*, 2014) while *Asparagus prostratus,* which is Endangered according to the Vascular plant Red Data List for Great Britain, has undergone hand pollination and re-introduction to increase population sizes in the most vulnerable of sites (Cheffings and Farrell, 2005; King and Edwards, 2007; King *et al.*, 2007). There are currently no habitat based conservation measures targeted towards active CWR conservation, though approximately 35% of priority English CWR do gain some level of passive protection through presence within protected areas (Fielder *et al.*, 2015). Active conservation measures are, however, being developed on The Lizard Peninsula in Cornwall, based on genetic analyses of multiple CWR taxa, with a view to establishing this location as the first UK CWR genetic reserve (Fielder *et al.*, submitted d). Though ideally conservation strategies targeting CWR populations would consider genetic diversity (Frankel *et al.*, 1995; Magos Brehm *et al.*, 2012), such analyses are not always possible due to high costs. A more pragmatic approach is to support habitat based conservation measures as a tool for establishing targeted and active management of CWR populations in key locations.

Designation of sites or habitats of conservation interest for CWR has been hampered by the absence of an inventory of priority CWR and by a lack of knowledge regarding the habitats with the highest concentrations of CWR (Maxted, 2003). There is evidence to suggest that some CWR, such as wild oat (*Avena fatua*) and wall barley (*Hordeum murinum*), are more often associated with disturbed early-successional communities rather than perennial-dominated mid to late-successional communities (Grime, 1977; Maxted and Kell, 2009; Hopkins and Maxted, 2011). These disturbed habitats tend to have high levels of anthropogenic influence and as such, are not normally considered for conservation designation (JNCC, 2013). The abundance of CWR may also vary within habitats. Linear features such as road verges, field boundaries and streamsides are often relatively highly disturbed but act as important refuges for species not favoured in the modern intensively managed countryside (Smart *et al.*, 2002, 2006a) and could be important for CWR diversity. Whilst the margins of arable fields have attracted interest and agri-environment support as a refuge for rare arable weeds, food plants for lowland farmland birds and nectar plants for pollinating insects (Marshall and Moonen, 2002), their role in supporting CWR has never been examined and, as yet, there has been no formal analysis of the preferences of UK CWR for different habitats and landscape locations.

Recent research effort has produced inventories listing CWR of priority conservation interest for the UK as a whole and also in separate inventories for England, Wales and Scotland (Fielder *et al.* 2015; submitted a; submitted b; submitted c). This has led to more complete geographic analyses of hotspots of CWR abundance within each country. Here, we seek to extend this work by utilising the UK CWR inventory to assess the habitats and landscape features with the highest diversity of UK CWR. Identification of habitats and features with the highest number of CWR will inform conservation efforts for this valuable resource in the UK.

In the current study, the following hypotheses were addressed:

1. CWR are more likely to be located in disturbed habitats and areas with high anthropogenic influences than more stable communities, due to their often weedy growth habits (Jain, 1975; Maxted *et al.*, 1997c).
2. Combining the fact that CWR would be expected to be adapted to agricultural disturbance but not preferred by intensive cultivation we would expect CWR to be more likely to be found in linear features, particularly arable field margins.
3. If forage and fodder species are primarily grasses then it would be expected that either high or low productivity grasslands would be their preferred habitats. Since high productivity grasslands are more species poor we would expect lower productivity grasslands to be richer.
4. CWR that are more closely related to their associated crop are predicted to show more similar habitat preferences and so be more likely to be associated with agricultural habitat and landscape features.

5.3 Methods

5.3.1 Data collection

Data on the distribution of CWR in the landscape of Great Britain were taken from vegetation surveys conducted as part of the Countryside Survey (CS) of 2007 (Carey *et al.*, 2008). The detailed survey methodology of the Countryside Survey makes it an ideal resource for identifying habitat and landscape distribution patterns. The dataset consists of 11,685 quadrats from a stratified random sample of 589 1km squares across Great Britain (i.e. UK excluding Northern Ireland). Priority CWR in each quadrat were identified according to the UK and regional priority inventories (Fielder *et al.*, 2015; submitted a; submitted b; submitted c). The priority inventories contain CWR taxa that are considered to be most in need of conservation and differ between the regions due to differences in regional conservation priorities and species pools; for example, only the Welsh inventory considers CWR related to forestry crops (Supplementary Table 5.1). The UK priority list is not simply a product of the corresponding regional inventories as different conservation priorities were defined at each scale and criteria used to assign priority to CWR in each region were selected by the relevant stakeholders in each case. The UK priority CWR were then based on a new selection of criteria and were agreed between all regional stakeholders. Criteria used in the UK were: the use of the related crop, the native status of the CWR, the degree of relatedness of the CWR to the crop, the economic value of the related crop, the threat assessment of the CWR and the presence of any additional designations (Fielder *et al.*, submitted c). Only native or archaeophyte priority CWR were included in this analysis because non-native taxa may show different habitat preferences or, having recently arrived, may not yet have fully occupied preferred niche space. For each quadrat the proportion of taxa defined as priority CWR was calculated, thus accounting for variation in total species richness between quadrats due to quadrat size. Nomenclature for CWR follows Stace (2010).

5.3.2 Landscape drivers

Habitat types were defined using the aggregate classes from the Countryside Vegetation System (Bunce *et al.*, 1999). Eight classes (crops and weeds, tall grass and herbs, fertile grassland, infertile grassland, lowland woodland, upland woodland, moorland grass mosaic, heath and bog) were identified. The habitat classes are defined by separation along two axes representing fertility and disturbance e.g. crops and weeds are both highly fertile and highly disturbed whereas lowland woodland can be relatively fertile but has low disturbance. Within habitats, some CS quadrats were targeted on linear landscape features such as hedgerows, field margins, the banks of watercourses and road verges (Table 5.1). Fields, unenclosed land and other areas of habitat were sampled by other randomly located quadrat types (U and X). Quadrats designed to sample unusual or interesting habitats (Y quadrats) were excluded from the analysis as these were not sampled randomly. Other variables that might influence the proportion of CWR were also considered including the proportion of arable and urban areas in the surrounding 1km square (Table 5.2), which reflect potential associations of CWR with agricultural activities or human activity. Road area in the surrounding 1km was similarly included to reflect the potential influence of transport routes and road verges on CWR occurrence. The proportional cover of woody and annual plants and bare ground were chosen to reflect the successional stage and disturbance regime associated with the vegetation in each quadrat; CWR are often ruderal plants and might be expected to occur where the cover of annuals and bare ground is higher (Maxted and Kell, 2009). Finally, the Easting and Northing of the sites were included to account for any geographic variation in CWR abundance not explained by the other variables.

Table 5.1 Location of Countryside Survey quadrats in the landscape and quadrat characteristics (adapted from Carey *et al.*, 2008).

Quadrat type	**Associated landscape feature**	**Quadrat size**	**Number of quadrats per 1km survey square**
A	Arable fields	100m^2	5
B	Adjacent to field boundaries (hedges, walls or fences)	10m^2	5
H	Hedgerows	10m^2	2
M	Arable field margins	4m^2	Up to 15
R/V	Next to roads	11m^2	5
S/W	Next to watercourses	12m^2	5
U	Unenclosed land	4m^2	Up to 10
X	Randomly located	200m^2	5

Table 5.2 Explanatory variables included in models of landscape drivers of CWR occurrence.

Variable	**Description**	**Range**
Habitat type	Aggregate vegetation class (Bunce *et al.*, 1999)	N/A – see text
Quadrat type	Landscape feature in which quadrat occurred	N/A – see Table 5.1
Proportion arable area	Proportion of 1km square covered by arable land parcels	0–0.95
Proportion urban area	Proportion of 1km square covered by urban land parcels	0–0.91
Road area	Total area covered by roads in 1km square	0–20
Proportion woody cover	Proportion of total cover due to woody plants in quadrat	0–1
Proportion annual cover	Proportion of total cover due to annual plants in quadrat	0–1
Proportion bare ground	Proportion of bare ground in quadrat	0–1
Easting[1]	Longitudinal position of 1km square	072243–650966
Northing[1]	Latitudinal position of 1km square	037121–1217913

[1]British National Grid coordinate system

5.3.3 Statistical analysis

The relationships between the landscape drivers and CWR diversity were assessed with binomial mixed models with a logit link. The proportion of priority CWR taxa per quadrat was the response variable in all models and all terms in Table 5.2 were included as fixed terms. Continuous variables were standardised and centred prior to analysis. Collinearity between continuous predictors was tested for using variance inflation factors (VIFs) (Zuur *et al.*, 2009) and found to be low (all VIFs below 2) so all predictors were retained. Interaction terms between fixed effects were not included due to the high number of factor levels assessed. Due to the design of the survey the quadrats are nested within 1km squares and therefore the 1km square was included as a random intercept term to account for any unexplained between-square variation in the proportion of CWR.

A total of twelve models were constructed with the same fixed and random effect structures but varying response variables. Firstly, to assess the variables related to the overall distribution of CWR, a model was constructed with the proportion of all CWR from the UK inventory as the response variable. Four additional models were constructed to assess whether different types of CWR from the UK inventory were affected by different drivers. In this study, CWR were divided into those related to food crops and those related to forage and fodder crops. Forage/fodder CWR were defined as those related to crops used for fodder or forage for animal feed. Food CWR included plants related to crops used as vegetables, oil/fat producers, nuts, pseudocereals, pulses, fruits, flavourings, cereals, seeds or sugar (USDA, ARS, National Genetic Resources Program, 2015). Due to the different species present in each group (e.g. forage/fodder CWR comprise mostly grasses and forbs while food CWR are more variable including tree and shrub taxa) different patterns of occurrence in the landscape might be expected. The proportions of food or forage/fodder CWR from the UK inventory in each quadrat were calculated as response variables. Several CWR taxa are related to both food and forage/fodder crops and were included in both models.

CWR can also be grouped based on the degree of relatedness to their domesticated relatives. Relatedness can be defined by the ability to successfully cross taxa (the Gene Pool Concept, Harlan and de Wet, 1971) or, if crossing information is not available, by phylogenetic distance (the Taxon Group Concept, Maxted *et al*., 2006). CWR with the ability to cross easily with their related crop, or that are in the same species, are classed as Taxon Group (TG) or Gene Pool (GP) 1b (where TG or GP 1a are the cultivated varieties of a species). Taxa that are in the same genus as the crop, or that have limited ability to cross are placed in TG 2 to 4 or GP 2 and 3. Some CWR are related to multiple crop taxa and in such cases the most closely related species was used to define the GP or TG. The proportions of CWR taxa from the UK inventory in GP and TG 1 or 2 to 4 in each quadrat were used as response variables in the next two models.

There was some evidence that forage/fodder CWR in the dataset were more likely to be in TG or GP 1b, so four more models were constructed to assess whether differences in drivers associated with relatedness were maintained within each usage group. In each case the response variable was the proportion of taxa each usage/relatedness combination from the UK priority inventory.

Finally, to investigate regional differences in the drivers of CWR occurrence, three models were specified using the proportion of CWR derived from the English, Scottish and Welsh priority inventories respectively. For regional analyses only quadrats from that region were included in the model.

Parameters for all twelve models were estimated using Markov chain Monte Carlo sampling implemented in the R package MCMCglmm (Hadfield, 2010). Uninformative inverse Wishart prior distributions were used for the fixed effects. An uninformative parameter expanded (Cauchy) prior was used for the random effect to help convergence and estimation of the random effect variance. The prior on residual variation was fixed at 1. Models were run for 30,000 iterations with a 5,000 iteration burn in period and a thinning interval of 10 iterations. Three separate models were run to assess chain convergence visually and using the Gelman-Rubin statistic (Gelman *et al*., 2004). All analysis was conducted in R v.3.0.3 (R Core Team, 2014).

5.4 Results

Of the 211 native and archaeophyte taxa present across the four (UK and national) priority inventories of CWR, 129 were recorded in the 2007 Countryside Survey (Supplementary Table 5.1). The most commonly recorded species were *Holcus lanatus,* a fodder CWR, and *Rubus fruticosus* agg. (bramble), closely related to cultivated blackberry and raspberry.

Gelman-Rubin statistics for all chains in all models were between 1 and 1.01 after 30,000 iterations and were considered to be sufficiently converged. In each model, parameter estimates for the habitat and landscape feature levels are differences (contrasts) from the proportion of CWR expected in crops and weeds (the reference habitat type) and randomly located quadrats (the reference feature type).

The proportion of priority CWR across all UK quadrats showed significant differences in associations with habitats and landscape features (Fig. 5.1). Model coefficients for the model with the proportion of all CWR from the UK Inventory as the response are shown in Fig. 5.2a and Table 5.3. CWR were more likely to occur in grasslands than highly disturbed habitats (the baseline level of crops and weeds) but less likely to occur in heath and bog. Woodland areas and moorland grass mosaics had similar numbers of CWR taxa to cropped and weedy habitats. Priority CWR were more common in several landscape features compared to randomly selected quadrats. Arable field margins, hedgerows, field boundaries (including fences and walls) and road verges were all preferred localities for CWR taxa. By contrast, quadrats situated next to waterways had fewer priority CWR than randomly located quadrats. Of the covariates included, negative associations were seen with the cover of woody and annual plants and bare ground in the quadrat. Overall, priority CWR were more frequent in the south and east of the UK.

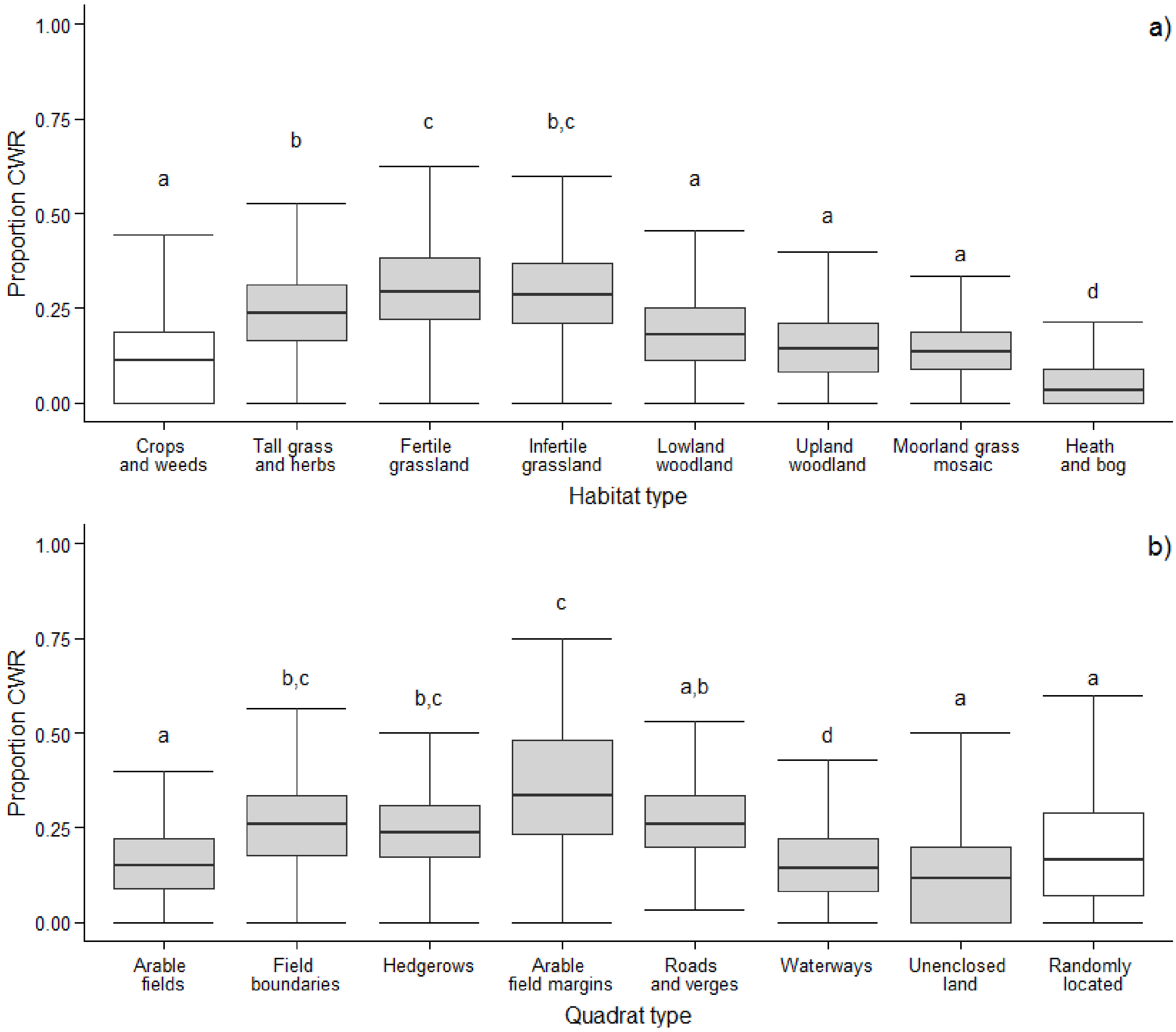

Figure 5.1 Boxplots of the proportion of CWR from the UK priority CWR inventory by a) habitat and b) quadrat type. Lines represent the median values, boxes are 25 and 75% percentiles and whiskers represent the most extreme data point within 1.5 times the interquartile range. The unshaded boxes indicate the factors used as reference levels in the models. Letters represent differences between groups defined by non-overlapping 95% credible intervals (see Table 5.3 for coefficients).

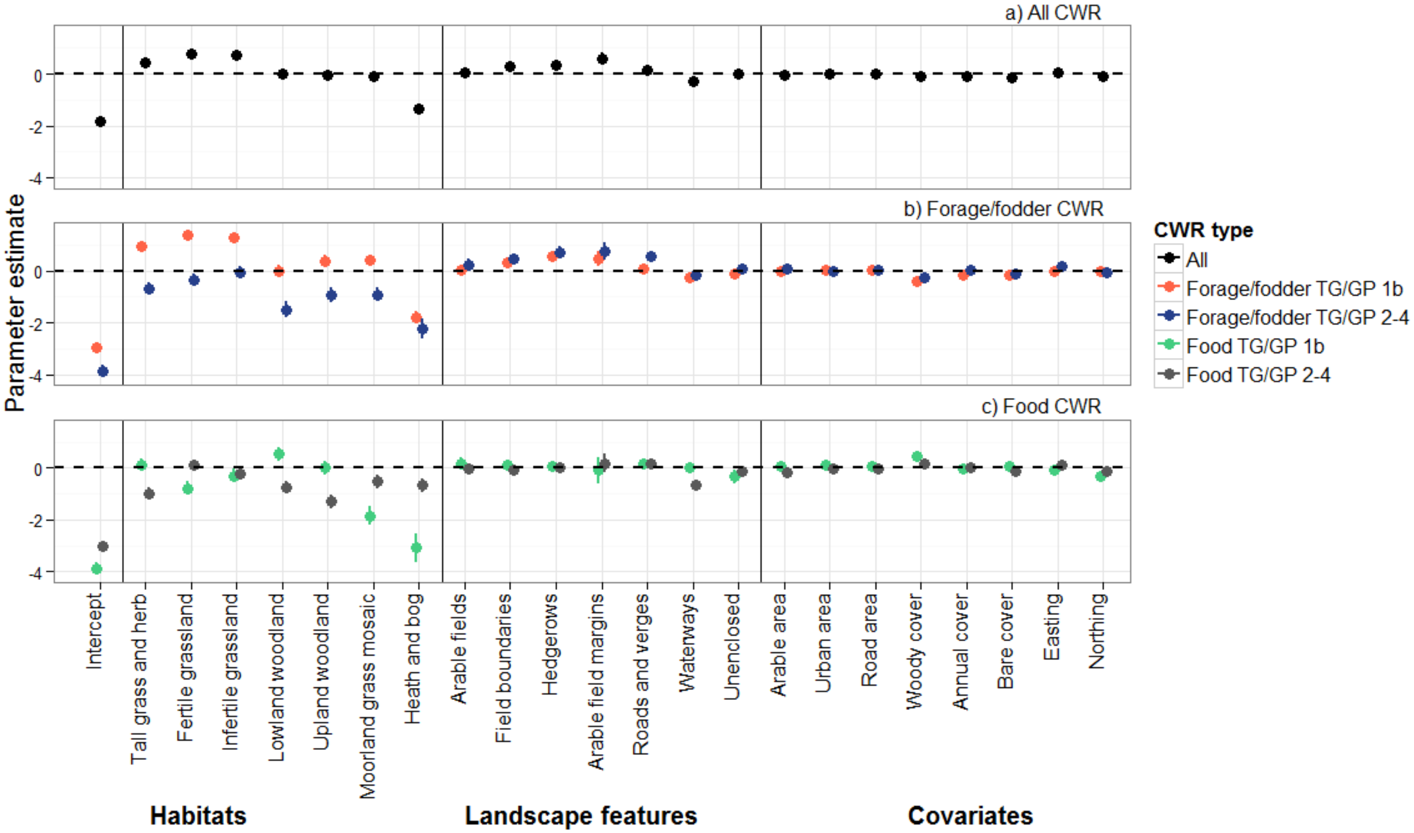

Figure 5.2 Parameter estimates for modelled effects of variables on the proportion of CWR in a quadrat. Panel a) shows parameter estimates for all CWR types from the UK Inventory, panels b) and c) separate CWR by both usage (fodder/forage vs food) and relatedness (TG/GP1b vs TG/GP2–4). Points are the mean of the posterior distribution and lines indicate the 95% credible interval.

Table 5.3 Parameter estimates and 95% credible intervals (in brackets) for each parameter in models of CWR occurrence either ungrouped (All CWR) or grouped by usage or relatedness. Parameters where 95% intervals do not overlap zero are highlighted in bold. Factors show contrasts from the reference (baseline) levels: crops and weeds (Habitats) and randomly located quadrats (Landscape features).

Parameter	**All CWR**	**Food CWR**	**Fodder CWR**	**TG/GP 1 CWR**	**TG/GP 2-4 CWR**
Intercept	**-1.84** (-1.99, -1.68)	**-2.5** (-2.69, -2.32)	**-2.33** (-2.49, -2.17)	**-2.81** (-2.99, -2.63)	**-3.09** (-3.29, -2.89)
Habitats					
Tall grass and herb	**0.42** (0.26, 0.57)	**-0.44** (-0.62, -0.26)	**0.49** (0.33, 0.65)	**1.15** (0.98, 1.33)	**-0.8** (-1, -0.61)
Fertile grassland	**0.76** (0.61, 0.92)	-0.06 (-0.25, 0.13)	**0.94** (0.78, 1.1)	**1.45** (1.27, 1.63)	**-0.59** (-0.79, -0.38)
Infertile grassland	**0.71** (0.55, 0.87)	**-0.25** (-0.45, -0.05)	**0.89** (0.72, 1.06)	**1.34** (1.16, 1.53)	**-0.35** (-0.56, -0.14)
Lowland woodland	0.02 (-0.15, 0.20)	-0.02 (-0.23, 0.19)	**-0.5** (-0.69, -0.32)	**0.8** (0.6, 1)	**-0.94** (-1.17, -0.71)
Upland woodland	-0.04 (-0.23, 0.14)	**-0.69** (-0.9, -0.46)	-0.05 (-0.24, 0.14)	**0.62** (0.42, 0.82)	**-0.78** (-1.03, -0.53)
Moorland grass mosaic	-0.08 (-0.26, 0.09)	**-0.75** (-0.97, -0.53)	-0.05 (-0.23, 0.13)	**0.4** (0.21, 0.6)	**-0.4** (-0.65, -0.16)
Heath and bog	**-1.36** (-1.56, -1.17)	**-0.96** (-1.19, -0.72)	**-2.15** (-2.37, -1.94)	**-1.79** (-2.01, -1.56)	**-0.36** (-0.62, -0.11)
Landscape features					
Arable fields	0.06 (-0.09, 0.20)	0.05 (-0.12, 0.22)	0.06 (-0.09, 0.21)	-0.02 (-0.18, 0.14)	**0.32** (0.13, 0.5)
Field boundaries	**0.28** (0.2, 0.36)	-0.03 (-0.13, 0.07)	**0.35** (0.27, 0.44)	**0.27** (0.19, 0.36)	**0.3** (0.18, 0.41)
Hedgerows	**0.35** (0.23, 0.48)	-0.07 (-0.21, 0.08)	**0.62** (0.49, 0.75)	**0.37** (0.24, 0.5)	**0.44** (0.26, 0.61)
Arable field margins	**0.56** (0.32, 0.81)	0.11 (-0.23, 0.43)	**0.6** (0.35, 0.84)	**0.48** (0.23, 0.74)	**0.57** (0.22, 0.91)
Roads and verges	**0.16** (0.09, 0.23)	**0.12** (0.03, 0.22)	**0.16** (0.09, 0.24)	**0.09** (0.01, 0.16)	**0.46** (0.35, 0.57)
Waterways	**-0.26** (-0.34, -0.19)	**-0.4** (-0.49, -0.3)	**-0.27** (-0.35, -0.19)	**-0.2** (-0.27, -0.12)	**-0.4** (-0.51, -0.29)
Unenclosed	-0.02 (-0.11, 0.08)	-0.11 (-0.23, 0.02)	-0.1 (-0.21, 0)	**-0.11** (-0.22, -0.01)	**0.14** (0.01, 0.27)
Covariates					
Arable area	-0.03 (-0.06, 0.01)	**-0.1** (-0.15, -0.05)	0 (-0.04, 0.04)	-0.02 (-0.06, 0.01)	0 (-0.07, 0.06)
Urban area	0.01 (-0.02, 0.04)	0.02 (-0.02, 0.06)	0.01 (-0.01, 0.04)	0.03 (0, 0.05)	-0.04 (-0.09, 0.01)
Road area	0.01 (-0.02, 0.03)	0 (-0.04, 0.04)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	-0.03 (-0.08, 0.02)
Woody cover	**-0.08** (-0.13, -0.02)	**0.35** (0.28, 0.41)	**-0.42** (-0.48, -0.36)	**-0.21** (-0.27, -0.15)	**0.19** (0.11, 0.26)
Annual cover	**-0.10** (-0.13, -0.07)	0.02 (-0.02, 0.05)	**-0.1** (-0.13, -0.07)	**-0.15** (-0.18, -0.11)	0.03 (-0.01, 0.07)

Parameter	All CWR	Food CWR	Fodder CWR	TG/GP 1 CWR	TG/GP 2-4 CWR
Bare cover	**-0.12** (-0.15, -0.09)	**-0.04** (-0.08, -0.01)	**-0.16** (-0.19, -0.12)	**-0.13** (-0.16, -0.1)	**-0.05** (-0.1, -0.01)
Easting	**0.07** (0.03, 0.10)	**0.07** (0.02, 0.11)	0.03 (0, 0.06)	-0.02 (-0.05, 0.01)	**0.24** (0.18, 0.3)
Northing	**-0.08** (-0.11, -0.05)	**-0.2** (-0.24, -0.16)	0 (-0.03, 0.03)	-0.03 (-0.06, 0)	**-0.18** (-0.24, -0.13)

CWR related to food and forage/fodder plants had different patterns of occurrence in the landscape (Table 5.3). Forage/fodder CWR were most common in grasslands whilst food CWR occurred most often in disturbed areas, fertile grassland and lowland woodland. Forage/fodder CWR dominated the preference for particular landscape features observed for all CWR, with food CWR occurring at similar frequencies in most landscape features, being slightly more frequent in roadsides and less frequent next to waterways. Food CWR were more likely to occur in quadrats with high cover of woody plants and quadrats in the south and east of the UK whilst forage/fodder CWR had a strongly negative association with cover of woody plants.

Overall, CWR in taxon or gene pool groups 2–4 were found most often in the baseline crops and weeds habitat type while more closely related CWR were more likely to be found in grassland or woodland habitats (Table 5.3). CWR differing in relatedness did not differ greatly in associations with landscape features, although there was a larger association with roads and verges for TG/GP 2–4 taxa and unenclosed land had proportionally more TG/GP 2–4 and fewer TG/GP 1b taxa. Because the usage of the related crop and relatedness were found to be confounded (forage/fodder CWR were more likely to be closely related to their cultivated relative; mean gene pool/taxon group for forage/fodder CWR = 2.11, food CWR = 2.67, $t_{1,97} = 2.13$, $P = 0.04$), distribution patterns of CWR in different relatedness groups were also assessed within each usage group (Supplementary Table 5.2, Table 5.4).

Table 5.4 Parameter estimates and 95% credible intervals for each parameter in each model of CWR occurrence grouped by relatedness within each usage group as shown in Fig. 5.2b,c. Parameters where 95% intervals do not overlap zero are highlighted in bold. Factors show contrasts from the reference (baseline) levels: crops and weeds (Habitats) and randomly located quadrats (Landscape features).

	Forage/fodder CWR		**Food CWR**	
Parameter	**TG/GP 1b**	**TG/GP 2-4**	**TG/GP 1b**	**TG/GP 2-4**
Intercept	**-2.94** (-3.12,-2.77)	**-3.88** (-4.1,-3.66)	**-3.86** (-4.12,-3.62)	**-3.04** (-3.25,-2.82)
Habitats				
Tall grass and herb	**0.97** (0.79,1.14)	**-0.66** (-0.88,-0.45)	0.12 (-0.11,0.36)	**-0.98** (-1.19,-0.76)
Fertile grassland	**1.4** (1.22,1.58)	**-0.35** (-0.58,-0.13)	**-0.81** (-1.07,-0.55)	0.12 (-0.08,0.34)
Infertile grassland	**1.28** (1.1,1.47)	-0.05 (-0.28,0.18)	**-0.34** (-0.6,-0.08)	**-0.24** (-0.46,-0.01)
Lowland woodland	0.01 (-0.2,0.21)	**-1.49** (-1.77,-1.21)	**0.51** (0.26,0.78)	**-0.74** (-0.98,-0.48)
Upland woodland	**0.4** (0.19,0.61)	**-0.93** (-1.22,-0.64)	-0.02 (-0.3,0.26)	**-1.3** (-1.58,-1.02)
Moorland grass mosaic	**0.4** (0.2,0.6)	**-0.92** (-1.19,-0.65)	**-1.89** (-2.23,-1.56)	**-0.53** (-0.78,-0.27)
Heath and bog	**-1.8** (-2.03,-1.56)	**-2.19** (-2.54,-1.84)	**-3** (-3.51,-2.5)	**-0.67** (-0.93,-0.39)
Landscape features				
Arable fields	0.01 (-0.15,0.17)	**0.25** (0.04,0.45)	0.17 (-0.05,0.4)	-0.03 (-0.24,0.18)
Field boundaries	**0.3** (0.22,0.39)	**0.45** (0.32,0.59)	0.12 (-0.02,0.26)	-0.06 (-0.17,0.06)
Hedgerows	**0.56** (0.42,0.7)	**0.71** (0.49,0.94)	0.05 (-0.13,0.23)	0.03 (-0.16,0.22)
Arable field margins	**0.47** (0.22,0.73)	**0.74** (0.39,1.1)	-0.07 (-0.61,0.42)	0.17 (-0.2,0.54)
Roads and verges	0.07 (-0.01,0.14)	**0.57** (0.46,0.69)	**0.17** (0.03,0.3)	**0.15** (0.05,0.25)
Waterways	**-0.28** (-0.35,-0.19)	**-0.16** (-0.29,-0.02)	0 (-0.14,0.14)	**-0.67** (-0.78,-0.55)
Unenclosed	**-0.12** (-0.23,-0.02)	0.1 (-0.09,0.28)	**-0.35** (-0.62,-0.09)	-0.1 (-0.24,0.03)
Covariates				
Arable area	-0.01 (-0.05,0.03)	0.06 (0,0.13)	0.05 (-0.01,0.12)	**-0.18** (-0.24,-0.11)
Urban area	0.02 (-0.01,0.05)	0 (-0.06,0.05)	**0.1** (0.05,0.14)	-0.04 (-0.09,0.01)
Road area	0.01 (-0.01,0.04)	0.02 (-0.03,0.07)	0.05 (0,0.1)	-0.04 (-0.09,0.01)
Woody cover	**-0.42** (-0.48,-0.35)	**-0.24** (-0.34,-0.13)	**0.44** (0.35,0.52)	**0.15** (0.07,0.23)

	Forage/fodder CWR		Food CWR	
Parameter	**TG/GP 1b**	**TG/GP 2-4**	**TG/GP 1b**	**TG/GP 2-4**
Annual cover	**-0.14** (-0.17,-0.11)	0.04 (-0.01,0.08)	-0.02 (-0.06,0.03)	0.04 (0,0.08)
Bare cover	**-0.15** (-0.18,-0.12)	**-0.11** (-0.16,-0.06)	0.05 (0,0.1)	**-0.11** (-0.16,-0.06)
Easting	0 (-0.04,0.03)	**0.18** (0.11,0.24)	**-0.08** (-0.15,-0.02)	**0.13** (0.07,0.18)
Northing	0.01 (-0.02,0.04)	-0.05 (-0.1,0.01)	**-0.33** (-0.39,-0.27)	**-0.13** (-0.18,-0.08)

Differentiating CWR based on both characteristics showed that closely related (TG/GP 1b) forage/fodder CWR dominated the overall CWR response, being much more common in all types of grasslands than the other groups (Fig. 5.2b, Table 5.4). Less closely related forage/fodder CWR (TG/GP 2–4), dominated by *Festuca rubra* agg., had similar habitat preferences to food CWR, being most frequent in disturbed and infertile grassland habitats, but shared landscape preferences with the closely related forage/fodder CWR, showing high frequencies in linear landscape features. Variation in habitat preferences were seen within food CWR with taxon/gene pool group 1b CWR being most common in lowland woodland and least common in heaths and bogs (Fig. 5.2c, Table 5.4). Less closely related food CWR were most common in disturbed habitats and fertile grassland. Both groups of food CWR showed a preference for roads and verges.

Although there were some differences in patterns between the different regions of the UK (Supplementary Figure 5.1), the uncertainty surrounding parameter estimates was much higher for the Welsh and Scottish CWR due to the smaller sample sizes and therefore although there was some variation in posterior means there was significant overlap in posterior distributions for most parameters.

5.5 Discussion

Despite the growing importance of conserving the diversity of CWR as a means of securing future agricultural production the analysis presented here is the first systematic analysis of the ecological preferences of priority CWR for a country, made possible through national interest in CWR conservation and the extensive dataset developed through the Countryside Survey in Great Britain. Understanding the habitats and landscape features with the greatest diversity of

CWR is important for informing *in situ* conservation strategies for crop genetic diversity to facilitate targeted management and monitoring in the most appropriate locations.

The first hypothesis that CWR would be most diverse in disturbed habitats with high anthropogenic influences was only partly supported by our data. Overall CWR were most common in fertile and infertile grasslands, the former of which is likely to be subject to human influence and disturbance through fertilisation and grazing. However, the habitat with the highest disturbance (the baseline crops and weeds habitat) had similar frequencies of CWR to woodlands and moorlands suggesting no overall preference for disturbed habitat types. The high proportion of CWR in grasslands was driven by the high proportion of forage and fodder CWR in the dataset, supporting the third hypothesis that forage and fodder CWR would be most likely to be found in grasslands. In particular, this pattern was driven by the more closely related forage and fodder CWR (those in taxon/gene pool group 1b). This group is dominated by several of the most common grass species in the UK including *Holcus lanatus* and *Lolium perenne*, which are commonly found in both fertile and infertile grasslands; fertile grasslands are likely to be managed to promote their occurrence. These taxa, and the dominant forage and fodder CWR in taxon/gene pool groups 2–4, also drive the overall association of CWR with linear landscape features, being more likely to be found in field boundaries, field margins, hedgerows and alongside roads than in randomly located quadrats. In particular, those forage and fodder CWR which are less tolerant of grazing e.g. *Arrhenatherum elatius* (Dostálek and Frantík, 2012) were commonly found in linear features (Supplementary Table 5.3). The second hypothesis that CWR would be more frequent in linear features than the surrounding habitat is therefore supported, and it appears that arable field margins have particularly high proportions of CWR related to forage and fodder crops.

The importance of linear features as refuge features for plant species in Britain has been previously linked to the relatively lower impact of heightened macronutrient availability and extremes of disturbance, which is frequently either intense or absent, that typify intensively farmed landscapes in the temperate zone (Smart *et al.*, 2002, 2006a). These twin pressures non-randomly filter species based on their traits. Thus ‘winners’ tend to be tall woody species or fast growing forbs and grasses while ‘losers’ are more likely to be shorter, slower-growing forbs (Tamis *et al.*, 2005; Smart *et al.*, 2006b; Walker *et al.*, 2009). Where linear features are subject to intermittent or frequent removal of biomass and lack of direct fertilizer application, conditions mimic lower intensity agriculture. Thus the many CWR species that are favoured by modern management regimes can coexist alongside CWR species less adapted to intensive farming regimes, resulting in higher proportions of CWR on linear features. Low occurrence of CWR on watersides compared to randomly located plots was a feature of both food and forage and fodder CWR. This suggests that few CWR are tolerant of either high soil moisture or of the late to mid-successional conditions increasingly prevalent on lowland stream and riversides in Britain (Carey *et al.*, 2008).

Food CWR were positively associated with road verges and negatively associated with waterways, although the effects were relatively small. Food CWR may show fewer strong patterns of occurrence with landscape features due to both the lower proportions recorded and the wider range of functional types within the group, which includes grasses, herbaceous plants and trees. The cultivated relatives of food CWR are generally grown in either arable fields or orchards. These habitats are likely to be classed as crops and weeds, although some orchards more closely resemble lowland woodland. Food CWR did have high abundance in both the baseline crops and weeds habitat type and in lowland woodland, suggesting that the distribution of CWR mirrored the habitats in which their cultivated relatives were grown, however fertile grasslands also had high numbers of food CWR. When food CWR were separated by relatedness it was apparent that the association with lowland woodland was driven by more closely related food CWR, while less closely related CWR were more common in fertile grassland and crops and weeds. Common closely related food CWR included the fruit and nut relatives *Rubus fruticosus* agg. and *Corylus avellana*, both of which are late successional woody taxa common in lowland wooded areas. The most common of the more distantly related food CWR (in taxon/gene pool groups 2–4) was *Trifolium repens*. Although *Trifolium* species are grown primarily as forage/fodder crops in the UK and are therefore strongly associated with fertile grasslands, they can also be used as food crops and therefore their relatives are classed as both food and forage and fodder CWR. Therefore, for food CWR, there was limited support for the fourth hypothesis that more closely related CWR would be more likely to share habitat preferences with their related crops. There was more support for this hypothesis in forage and fodder crops as closely related forage and fodder CWR were much more likely to be associated with fertile grasslands than less closely related forage and fodder CWR.

Overall, the proportion of CWR found was slightly higher in the south and in the east of the UK, a pattern also evident in the analysis of the UK geographic distribution of CWR taxa (Fielder *et al.*, submitted c). Many archaeophyte CWR, non-native taxa introduced before AD 1500, are likely to have their biogeographic origins in Mediterranean, eastern European or Asian biomes (Vincent *et al.*, 2013). This is may have led to the patterns observed in the UK with CWR being slightly less prevalent in the climatically harsher north of the country, though further analysis would be required to fully investigate this. There were few regional differences in patterns of CWR occurrence despite the variation in both available species pools and conservation priorities between regions. There was an indication that woody cover had a more negative effect in England and westerly areas were more impoverished in Scotland but for most parameters credible intervals overlapped, partly a reflection of the smaller amount of data used in each model.

Most crops were originally domesticated from wild and weedy plant species (Maxted and Kell, 2009). Many of the patterns of occurrence in CWR found in the current study can be explained in this context. Weedy or ruderal plants are usually stress intolerant and found in early successional habitats with high levels of persistent disturbance and potentially high productivity (Grime, 1977). As a consequence, CWR are unlikely to occur in stressful or late successional environments and are more likely to be typical of disturbed and productive habitats. Tree species generally used to cultivate fruits and nuts, are an exception to this rule. The wild relatives of these taxa are more commonly associated with stress tolerance and are found in mid- to late successional environments (Grime, 1977), although the pressures to obtain maximum yield means that species with highly stress-resistant traits were less likely to be domesticated.

The main caveat to our analysis is that it was not possible to assess the habitat and landscape of the rarest CWR as many of these taxa were not recorded in the Countryside Survey. Of those priority CWR taxa listed in the Great Britain Threatened Plants list (Cheffings and Farrell, 2005) only seven were recorded in the 2007 Countryside Survey with a total of only 19 records. Our results, therefore, cannot necessarily be generalised to these rarer taxa and should instead be considered to reflect the habitat preferences of the more common CWR species. Several of the common taxa, while listed in the priority inventory on the basis of their relatedness to and economic importance of cultivated relatives, are unlikely to ever be considered for conservation, as they are highly prevalent in the UK countryside. To assess the conservation needs of the rarer taxa, targeted surveys are required in combination with the broad scale analysis conducted here. In addition, the Countryside Survey does not effectively sample coastal areas, partly because the total area of coastal habitat in the UK is small. Many CWR of conservation importance have a coastal distribution (Preston *et al.*, 2002) and therefore the ability of the Countryside Survey to record these species was limited by the small number of coastal locations surveyed.

Geographical analyses have already been carried out within the UK (Fielder *et al.*, 2015; submitted a; submitted b; submitted c) to identify both hotspots of CWR diversity and minimum areas which are sufficient to contain all priority CWR. Within these outlined areas we can identify protected areas and establish conservation measures within them. The analysis conducted here means that we can also prioritise specific habitats and features within hotspots (whether within or outside of protected areas) allowing much more specific targeting of *in situ* CWR conservation. In particular, the results of this analysis suggest maintaining linear features such as field margins and road verges would be particularly useful. The continuation of prescriptions to maintain arable field margins in agri-environment schemes is therefore predicted to be important for CWR conservation. It will also be important to identify the distribution of genetic variation within CWR taxa, something that is not addressed here but which has been studied in detail on The Lizard Peninsula in Cornwall (Fielder *et al.*, submitted d). Genetic diversity of key CWR found on The Lizard and across the rest of the southwest of the UK was found to be geographically structured which suggests that UK conservation efforts for CWR will need to be widespread.

5.6 Conclusion

This study presents the first analysis of the habitat and landscape feature preferences of multiple CWR taxa. This is possible within the UK due to the abundance of vascular plant survey data and the recent identification of CWR of highest priority for conservation. The results emphasise the importance of diverse approaches to conservation of CWR. Traditional conservation within protected areas alone may not be sufficient to conserve the full range of genetic diversity in CWR and should be complemented by conservation outside of protected areas, particularly in sites associated with linear features. Conservation outside of protected areas however, will present novel challenges for establishing long-term monitoring and active management and will require involvement and commitments from landowners. Nevertheless, if CWR (and the genetic diversity within) are to be comprehensively conserved across the country in order to meet the UK's commitments to the Convention on Biological Diversity (CBD, 1992; 2010a; 2010b) and the International Treaty for Plant Genetic Resources for Food and Agriculture (FAO, 2001), these challenges must be addressed. Since agri-environment schemes are likely to be a significant pan-European mechanism for delivering CBD commitments, it would be both desirable and relatively straightforward to evaluate whether existing UK scheme options will indirectly foster favourable conditions for CWR in their preferred habitats and locations.

CHAPTER 6.

A genetic assessment of crop wild relatives on The Lizard Peninsula, Cornwall and its suitability to become the first UK genetic reserve

The work presented in this chapter has been submitted to the

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Conceived and designed the study: **HF**, JJH, BFL, NM

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Wrote the paper: **HF**

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6.1 Abstract

Crop wild relatives (CWR) are a largely untapped resource with vast potential for use in crop improvement, contributing to meeting ever-increasing food production demands in the face of crop pests, diseases and climate change. Little systematic or active conservation exists in the UK for these valuable taxa despite increasing acknowledgement of their significant economic value in global, European and national policy. A genetic diversity study using AFLPs was performed on eight CWR on The Lizard, Cornwall to investigate the site's suitability for establishment of the first UK CWR genetic reserve. Results revealed that the CWR on The Lizard hold as much genetic diversity as populations from other southwest UK sites despite representing a much smaller geographic area. Further, this diversity was largely distinct from southwest populations, indicating The Lizard as a suitable location for *in situ* conservation of UK CWR diversity, while also revealing that The Lizard is not, on its own, sufficient to conserve the genetic diversity of all CWR in the southwest, highlighting the need for a network of complementary UK genetic reserves. We present the first systematic genetic analysis of multiple CWR, with evidence to support establishment of a national multi-CWR genetic reserve with active and long-term management informed by genetic diversity data. It represents a model that can now be applied globally.

6.2 Introduction

The world's human population is predicted to reach 10.1 billion within the next ninety years (United Nations, 2011). To accommodate this ever-increasing population, food production needs to increase by 70% (World Bank, 2008; Godfray *et al*., 2010). At the same time, climate change is predicted to reduce crop yield by 2% per decade with more severe yield declines forecast beyond 2050 (Porter *et al*., 2014). This dramatically increases the call for higher yielding, but also more resilient, crops to supply rapidly increasing demand; hence the utilisation of plant genetic resources such as crop wild relatives (CWR) for crop improvement will become ever more important (Hopkins and Maxted, 2011).

CWR are wild plants, which due to their close genetic and taxonomic relationship to a crop offer a largely untapped pool of genetic diversity, holding the potential to improve crop varieties and their resilience through plant breeding (Maxted *et al*., 2006). Factors driving biodiversity loss at national and global levels (habitat destruction, changes to management practice, and, in the longer term, climate change) also threaten CWR (Maxted and Kell, 2009).

Despite increasing threat to CWR and their importance in crop improvement they have rarely been considered a conservation priority and are poorly conserved both *in situ* (Maxted, 2003) and *ex situ* (Dias *et al*., 2012) as a result. CWR conservation is, however, becoming a higher priority in global, regional and national policy. The Convention on Biological Diversity (CBD), which includes the Global Strategy for Plant Conservation and Strategic Plan for Biodiversity (CBD, 1992; 2010a; 2010b), and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2001) emphasise the importance of systematic conservation strategies, particularly *in situ* conservation of genetic diversity complemented by *ex situ* back up. The Harlan and de Wet CWR inventory (Vincent *et al*., 2013) takes the first systematic step towards achieving this goal.

In Europe, the European Strategy for Plant Conservation (ESPC) (Planta Europa, 2008) has identified the need to establish reserves that hold genetic conservation of CWR as high priority. Little systematic, active conservation exists within the UK, but CWR conservation is now a national priority, being identified as a priority action in the *Biodiversity 2020* strategy for England (Defra, 2011).

CWR conservation efforts should include information on genetic diversity to maximise intra-specific diversity and the potentially useful gene variants conserved (Schoen and Brown, 1993), a key concept behind the establishment of CWR genetic reserves. In Europe some genetic studies have identified potential sites for genetic reserve establishment such as for *Brassica* wild relatives in Italy (Branca *et al*., 2012) and *Beta patula* Aiton in Madeira (Pinheiro de Carvalho *et al*., 2012) but few target multiple CWR or meet the standards outlined in Iriondo *et al*. (2012). Currently no CWR genetic reserves exist in the UK.

In a recent study of CWR distributions across England it was found that The Lizard is a hotspot of CWR taxon diversity, with only Purbeck, Dorset containing a higher number of CWR (Fielder *et al*., 2015). Furthermore, The Lizard is a site that potentially harbours high levels of genetic diversity due to its varied ecogeography and underlying geology; The Lizard has the largest outcrop of serpentine rock in England (approximately 50km^2), which gives rise to ultra-basic soils rich in magnesium and low in calcium, uncommon in Britain (Andrews, 1998). There also exists complex microclimatic variation related to topography and maritime exposure.

The Lizard is already a key site in England for conservation. The peninsula in its entirety is designated as an Area of Outstanding Natural Beauty (AONB), the site is a Special Area of Conservation (SAC) for a variety of habitats including dry Atlantic coastal heaths and vegetative cliffs of the Atlantic coast (The Lizard, 2013). It also hosts eight Sites of Special Scientific Interest (SSSI) protected under the Wildlife and Countryside Act, 1981 (The Lizard, 2013). Approximately two thousand hectares of The Lizard are designated as a National Nature Reserve (NNR) managed by Natural England and other areas are owned and managed by the National Trust and Cornwall Wildlife Trust (The Lizard, 2013).

The extent of conservation management already in place and the potential for the ecogeographic characteristics of the area to give rise to interesting genetic variation, make The Lizard a suitable candidate for a CWR genetic reserve. The objective of our study was to undergo a direct assessment of genetic diversity in multiple CWR on The Lizard to investigate its suitability as an appropriate location for the first UK CWR genetic reserve and to produce a baseline of genetic data for future monitoring.

To achieve this, the following two questions were addressed:

1. What is the level of genetic diversity on The Lizard both within and among populations for eight CWR?
2. How different is the genetic diversity on The Lizard for these CWR compared with that elsewhere in the southwest of the UK?

Finally, an assessment of the evidence is made against the standards proposed for genetic reserves (Iriondo *et al*., 2012) to determine whether The Lizard could qualify as a CWR genetic reserve (Supplementary Table 6.1).

6.3 Materials and methods

6.3.1 Study species

Eight CWR occurring on The Lizard were selected (*Allium schoenoprasum* L., *Allium ursinum* L., *Asparagus prostratus* Dumort., *Beta vulgaris* L. subsp. *maritima* (L.) Arcang., *Daucus carota* L. subsp. *gummifer* (Syme) Hook. f., *Raphanus raphanistrum* L. subsp. *maritimus* (Sm.) Thell., *Trifolium occidentale* Coombe and *Trifolium repens* L.). These were selected due to i) potential economic value (each is related to a crop of high economic importance in the UK and used as either human food or animal forage/fodder), ii) their being contrasting taxa covering both inbreeders/outbreeders as well as widespread/restricted range taxa (Table 6.1) and iii) being present throughout the study site with known locations (Hopkins, 1983; Osborne, 2010).

6.3.2 Data collection

During spring 2012 and 2013 sampling was carried out for the eight CWR. Leaf material and associated demographic data were collected from up to seven sites per taxon on The Lizard (Fig. 6.1) and up to a further seven sites from southwest UK within Cornwall, Devon, Dorset, Somerset, Gloucestershire and Glamorgan (Supplementary Table 6.2). Sites represented the geographical ranges of these taxa in the southwest of the UK (see Table 6.1 for description of UK ranges). Sampling was carried out within the restrictions of available time and resources. For each taxon it was ensured that all sites were a minimum of 1km apart to limit the likelihood of gene flow between sites (although two sites of *A. prostratus* were approximately 720m apart due to its limited distribution and accessibility of sites).

Table 6.1 Summary of the life histories of each of the eight study CWR species sampled on The Lizard Peninsula.

Taxon (Common name)	Related crop (Crop use)	Life History		
		Life Form	**Geographic Range (within UK)**	**Breeding System**
Allium schoenoprasum (Wild chives)	Chives (Human food)	Perennial Herbaceous	Local, in SW and N England and S Wales	Mixed – animal
Allium ursinum (Ramsons)	Garlic (Human food)	Perennial Herbaceous	Frequent, locally abundant over most of British Isles	Mixed – animal
Asparagus prostratus (Wild asparagus)	Asparagus (Human food)	Perennial Herbaceous	Very local in SW England and S Wales	Outcrossing – animal
Beta vulgaris subsp. *maritima* (Sea beet)	Sugar beet (Human food)	Perennial Herbaceous	Frequent, on coasts of British Isles except N & C Scotland	Outcrossing – wind
Daucus carota subsp. *gummifer* (Sea carrot)	Carrot (Human food)	Biennial Herbaceous	Frequent, S and W Britain	Outcrossing – animal
Raphanus raphanistrum subsp. *maritimus* (Sea radish)	Radish (Human food)	Biennial to Perennial Herbaceous	Frequent, coast of British Isles, N to Outer Hebrides but absent from most of E Britain and N Scotland	Outcrossing – animal
Trifolium occidentale (Western clover)	White clover (Animal forage and fodder)	Perennial Herbaceous	Local, SW England, Glamorgan, Anglesey	Mixed – animal
Trifolium repens (White clover)	White clover (Animal forage and fodder)	Perennial Herbaceous	Abundant throughout British Isles	Outcrossing – animal

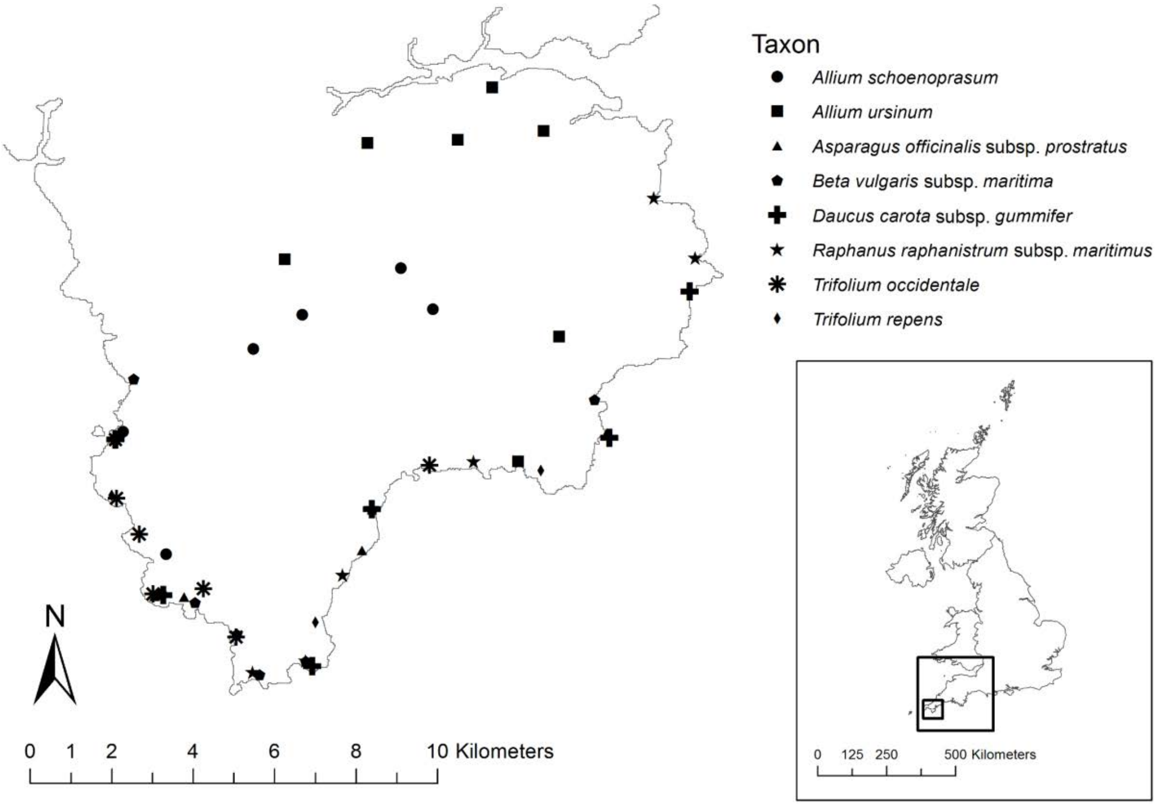

Figure 6.1 The Lizard Peninsula, showing the 52 collection sites for eight CWR. Inset: southwest survey area (large box) and The Lizard survey area (small box).

For each taxon, 20 leaf samples (100mg fresh weight per sample) were collected from different plants across the full range of each site. Samples were immediately stored in airtight plastic bags containing drying indicator silica gel (Chase and Hills, 1991). A total of 1,860 samples were collected and analysed using amplified fragment length polymorphism (AFLP) molecular markers.

6.3.3 AFLP method

Molecular work was undertaken at the Institute of Biology, Ecology and Rural Science (IBERS), University of Aberystwyth, Wales. Genomic DNA was extracted using the DNeasy

96 Plant Kit (Qiagen, 2012). The AFLP method was completed according to IBERS' standard protocol (Skøt *et al*., 2005), based on Vos *et al*. (1995).

6.3.4 Genotyping

AFLP profiles generated from the ABI 3730x1 capillary sequencer were visualised and the peaks automatically scored as present (1) or absent (0) in GENEMAPPER 4.0 (Applied Biosystems). Peak scorings were then verified manually, allowing correction of misclassified peaks (Bonin *et al*., 2004). Sample profiles that could not be scored unambiguously were excluded from subsequent analysis. The number of sample profiles analysed is shown in Table 6.2.

6.3.5 Error rate

Steps taken to reduce technical and human errors included the automation of laboratory work, the aid of highly skilled laboratory staff at IBERS and the inclusion of a pre-selective amplification step in the protocol to reduce mismatched amplification products (Vos *et al*., 1995). Genotyping errors were reduced in line with Bonin *et al*. (2004) and Bonin *et al*. (2007) including the analysis of replicates of between five and ten percent of samples.

For each taxon, error rates were calculated per locus (ratio of the total number of mismatches between samples and replicates to the number of replicated individuals) and for the whole dataset (the number of mismatches per sample divided by the number of peaks per sample, expressed as a percentage) (Bonin *et al*., 2004; Pompanon *et al*., 2005). Error rates per locus were below 0.1 (in line with Bonin *et al*., 2004 recommendations), except for *A. prostratus*, which was 0.1667. Loci contributing high levels of error to the dataset were removed. The total number of loci analysed per taxon is shown in Table 6.2.

Table 6.2 Numbers of Lizard and southwest UK sites surveyed, samples collected, AFLP profiles scored for presence/absence of peaks, primer pairs used, total numbers of marker loci identified and number of polymorphic loci included in analysis.

CWR	**Lizard sites**	**Southwest sites**	**Samples collected**	**AFLP profiles scored**	**Primer pair 1 (*Eco*RI/*Mse*I)**	**Primer pair 2 (*Eco*RI/*Mse*I)**	**Total loci**	**Polymorphic loci**
Allium schoenoprasum	7	1	160	159	ACG/CTG	AGC/CGC	220	79
Allium ursinum	7	7	280	267	ACG/CTG	AGC/CGC	205	50
Asparagus prostratus	3	0	60	59	AGC/CGC	ATG/CAG	126	54
Beta vulgaris subsp. *maritima*	7	6	260	259	ATC/CAC	ATC/CAG	281	127
Daucus carota subsp. *gummifer*	7	7	280	278	ACT/CTT	ATC/CAC	268	105
Raphanus raphanistrum subsp. *maritimus*	7	6	260	257	ATC/CAC	ATG/CAG	312	133
Trifolium occidentale	7	7	280	279	ATC/CAC	ATG/CAG	355	46
Trifolium repens	7	7	280	272	AGC/CGC	ATC/CAC	365	80

6.3.6 Statistical analysis

For each taxon, the two primer pair binary matrices generated by GENEMAPPER 4.0 were combined and run through AFLP-SURV 1.0 (Vekemans *et al*., 2002) to determine genetic diversity and population structure (Lynch & Milligan, 1994). AFLP markers are dominant, hence allele frequencies were calculated using AFLP-SURV'S Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999). Caution should be taken when interpreting results from polyploid taxa as this method of estimating allele frequencies assumes a maximum of two alleles per locus. Where this assumption is not met, it can lead to over-estimation of allele frequencies and under-estimation of genetic variation within populations (Lynch & Milligan, 1994). In this study both *A. prostratus* and *T. repens* are tetraploid and do not meet this assumption. Despite this, Hargreaves *et al*. (2010) argue that because *T. repens* is an allotetraploid showing disomic inheritance (Caradus, 1995; Weidema, 1996), any such effects should not prevent the analysis of AFLP data using AFLP-SURV. The remaining six taxa in the study are diploid and therefore meet this assumption (Stace, 2010).

Another underlying assumption of AFLP-SURV is that populations do not deviate from Hardy-Weinberg genotypic proportions (Wright's inbreeding coefficient (F_{IS}) = 0). This assumption was made for all taxa with the exception of *A. ursinum* for which an F_{IS} of 0.5 was used due to evidence of both sexual and vegetative reproduction as well as self-compatibility (Oborny *et al*., 2011; Herden *et al*., 2012). *A posteriori* AMOVA analyses support this assumption. To test the significance of differentiation among populations (F_{ST}), 1,000 permutations were performed. Using AFLP-SURV the following descriptive statistics were calculated: proportion of polymorphic loci at the five percent confidence level expressed as a percentage (PLP), expected heterozygosity (H_E), mean expected heterozygosity within populations (H_W) and Wright's fixation index (F_{ST}) (Lynch & Milligan, 1994).

Principal coordinate analysis (PCoA) was performed in GENALEX 6.501 (Peakall and Smouse, 2006; Peakall and Smouse, 2012). PCoA plots based on pairwise genetic distance were generated to illustrate the patterns of variation between individuals, PCoA plots based on Nei's genetic distances revealed distances between whole populations.

GENALEX 6.501 was also used to perform an analysis of molecular variance (AMOVA) for each taxon (Excoffier *et al*., 1992). AMOVA determines the partitioning of genetic variation within and among populations of a particular taxon (Excoffier *et al*., 1992; Michalakis and Excoffier, 1996), allowing conclusions to be drawn regarding the breeding system of a taxon. The significance of AMOVA results was calculated using 999 random permutations of the dataset.

Finally, a Mantel test for matrix correspondence was undertaken (Mantel, 1967) using 999 permutations to determine whether there was a relationship between geographic and genetic distances for each taxon. This test computes a correlation coefficient by comparison of two distance matrices, one showing the logarithmic transformation of geographic distances between populations based on coordinate data and one showing pair-wise F_{ST} between populations transformed to $F_{ST}/(1-F_{ST})$ according to Rousset (1997).

6.4 Results

6.4.1 What is the level of genetic diversity on The Lizard both within and among populations for eight CWR?

Levels of genetic diversity within populations on The Lizard show clear differences between taxa (Table 6.3). The highest genetic diversity is in the obligate outcrosser *A. prostratus* (H_W = 0.323, S.E. = 0.008) whilst the taxon with the lowest is *T. occidentale* (H_W = 0.063, S.E. =

0.005), which is self-compatible and also undergoes vegetative spread (Coombe, 1961; British Flora, 2014) although AMOVA suggests it is primarily outbreeding with 72% of variation partitioned within populations (PhiPT = 0.278; $P = 0.001$).

Table 6.3 Mean within-population expected heterozygosity (H_W) and standard error (S.E) for eight CWR on The Lizard and southwest UK.

	Lizard		**Southwest**	
Taxon	**H_W**	**S.E.(H_W)**	**H_W**	**S.E.(H_W)**
Allium schoenoprasum	0.191	0.008	0.165	0.022
Allium ursinum	0.086	0.005	0.128	0.020
Asparagus prostratus	0.323	0.008	N/A	N/A
Beta vulgaris subsp. *maritima*	0.146	0.009	0.162	0.007
Daucus carota subsp. *gummifer*	0.101	0.002	0.098	0.006
Raphanus raphanistrum subsp. *maritimus*	0.086	0.006	0.115	0.022
Trifolium occidentale	0.063	0.005	0.061	0.011
Trifolium repens	0.134	0.007	0.132	0.007

Differentiation among populations, calculated as Wright's F-statistic (F_{ST}) for each taxon, also shows clear differences between taxa. Population differentiation is considerably higher for *A. ursinum* ($F_{ST} = 0.505$, S.E. = 0.197) than any other taxon, whereas *D. carota* subsp. *gummifer* and *T. repens* show low levels of differentiation among populations ($F_{ST} = 0.025$, S.E. = 0.184; $F_{ST} = 0.024$, S.E. = 0.233 respectively). The remaining taxa show intermediate levels of differentiation among populations. AMOVA results support these findings with most genetic variation observed in *A. ursinum* partitioned among populations (60%) rather than within (40%) (PhiPT = 0.597; $P = 0.001$), typical of an inbreeding taxon. In contrast, for all other taxa, most genetic variation was partitioned within populations, confirming these as outbreeding taxa.

PCoA analyses uncovered patterns and geographic partitioning of genetic diversity found on The Lizard (Figs. 6.2 and 6.3). For *A. schoenoprasum*, both individual sample and population PCoA analyses separate coastal populations Asch06 and Asch05 on the west coast of The

Lizard from the four inland populations (Asch01, 02, 03 and 07), located in protected heathland Goonhilly Downs, and one other coastal population Asch04 (Figs. 6.2a and 6.3a).

A. ursinum shows two populations, A.urs01 and A.urs02 on the Helford Estuary, are genetically distinct from the remaining five *A. ursinum* populations sampled on The Lizard (Figs. 6.2b and 6.3b). The three *A. prostratus* populations sampled are distinct from each other (Figs. 6.2c and 6.3c). *B. vulgaris* subsp. *maritima* individuals and populations from The Lizard show no clear distinction with the exception of Beta02 (Figs. 6.2d and 6.3d). *R. raphanistrum* subsp. *maritimus* reveals some structure in genetic diversity across The Lizard with populations Raph03 and Raph07 clustering separately from populations further south (Raph01, 02 and 06) and those further north (Raph04 and 05) (Fig. 6.2f). The clustering of Raph04 and 05 is also observed in Fig. 6.3f, separated by coordinate two (18.43% variation).

No clear pattern in genetic diversity is apparent among populations of *D. carota* subsp. *gummifer*, *T. occidentale* or *T. repens* (Figs. 6.2e,g,h and 6.3e,g,h). However, a pattern is revealed within populations of *T. repens* where individuals cluster into two separate groups (Fig. 6.2h); further study is necessary to fully understand this pattern.

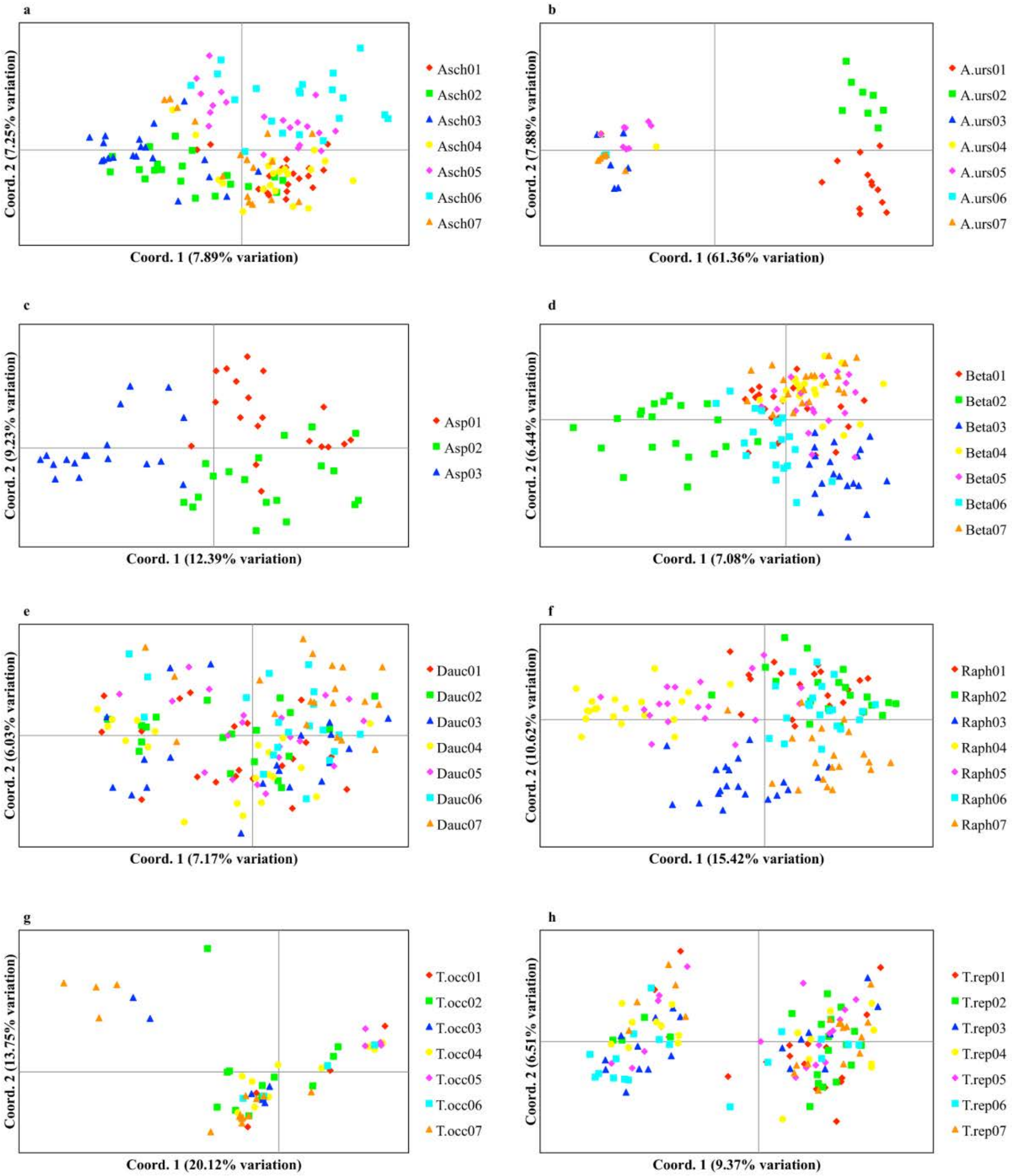

Figure 6.2 PCoA plots of all sampled individuals from The Lizard of the eight CWR studied. a) *Allium schoenoprasum*; b) *Allium ursinum*; c) *Asparagus prostratus*; d) *Beta vulgaris* subsp. *maritima*; e) *Daucus carota* subsp. *gummifer*; f) *Raphanus raphanistrum* subsp. *maritimus*; g) *Trifolium occidentale*; h) *Trifolium repens*.

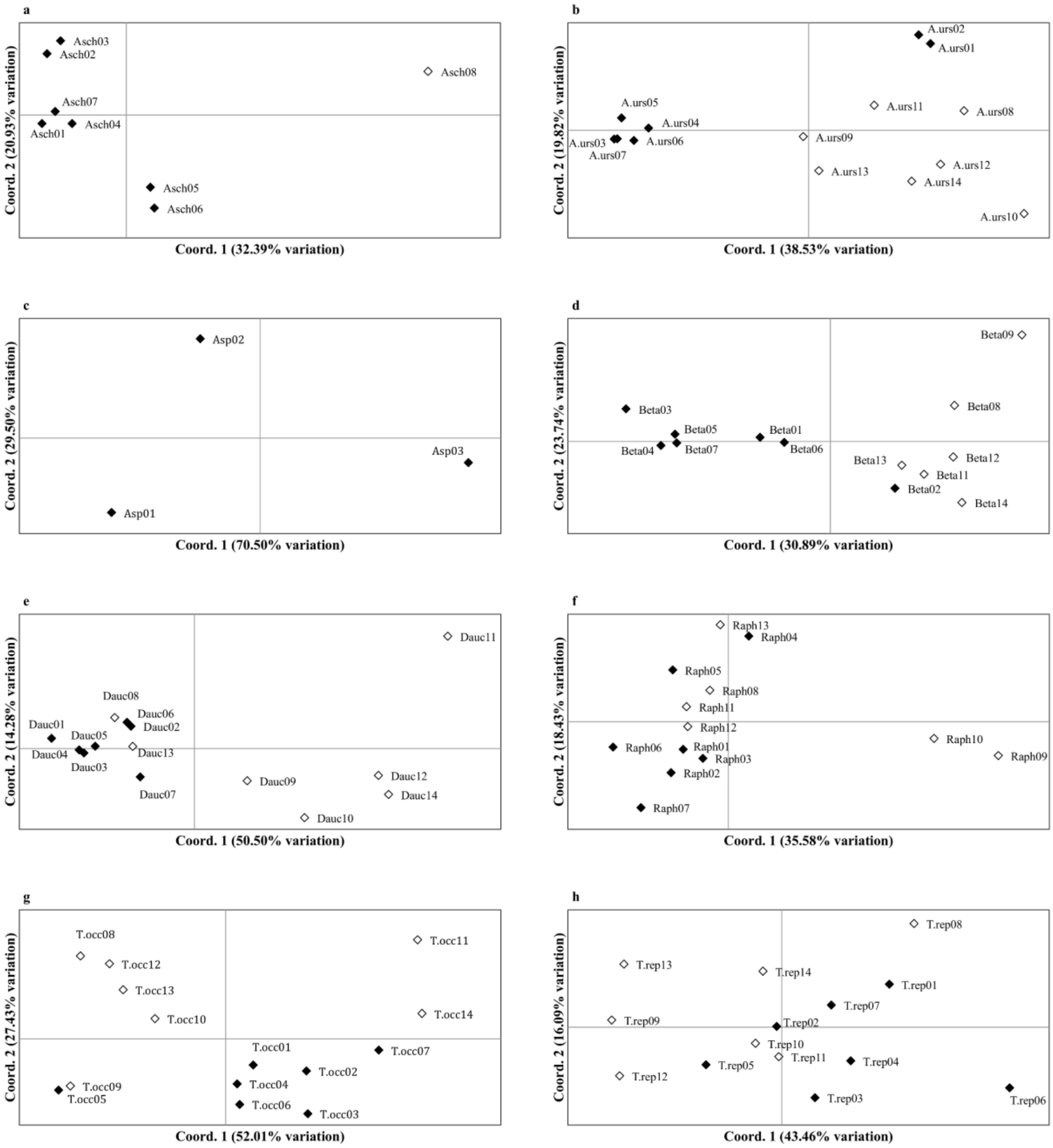

Figure 6.3 PCoA plots of all sampled populations of the eight CWR studied. a) *Allium schoenoprasum*; b) *Allium ursinum*; c) *Asparagus prostratus*; d) *Beta vulgaris* subsp. *maritima*; e) *Daucus carota* subsp. *gummifer*; f) *Raphanus raphanistrum* subsp. *maritimus*; g) *Trifolium occidentale*; h) *Trifolium repens*. Populations with black data points are collected from The Lizard, populations with unfilled data points are those collected from outside The Lizard within the southwest of the UK.

6.4.2 How different is the genetic diversity on The Lizard for these CWR compared with that elsewhere in the southwest of the UK?

As with populations from The Lizard, the level of genetic diversity within populations in southwest UK differs between taxa (Table 6.3). H_W ranges from 0.061 (S.E. = 0.011) in *T. occidentale* to 0.165 (S.E. = 0.022) in *A. schoenoprasum*. No significant differences were found between H_W values for The Lizard and the southwest for any of the taxa.

PCoA analysis of populations indicates that for most taxa The Lizard populations are genetically distinct from those in the southwest (Fig. 6.3). The only taxon for which this is not the case is *R. raphanistrum* subsp. *maritimus*.

Mantel tests for each taxon provided further support for the distinction observed between CWR at Lizard and southwest sites. Significant isolation by distance was found for *A. schoenoprasum* (*P* = 0.003), *D. carota* subsp. *gummifer* (*P* = 0.001), *T. occidentale* (*P* = 0.043) and *T. repens* (*P* = 0.043) (Fig. 6.4).

See summary of full results in Table 6.4, including management recommendations for The Lizard CWR populations.

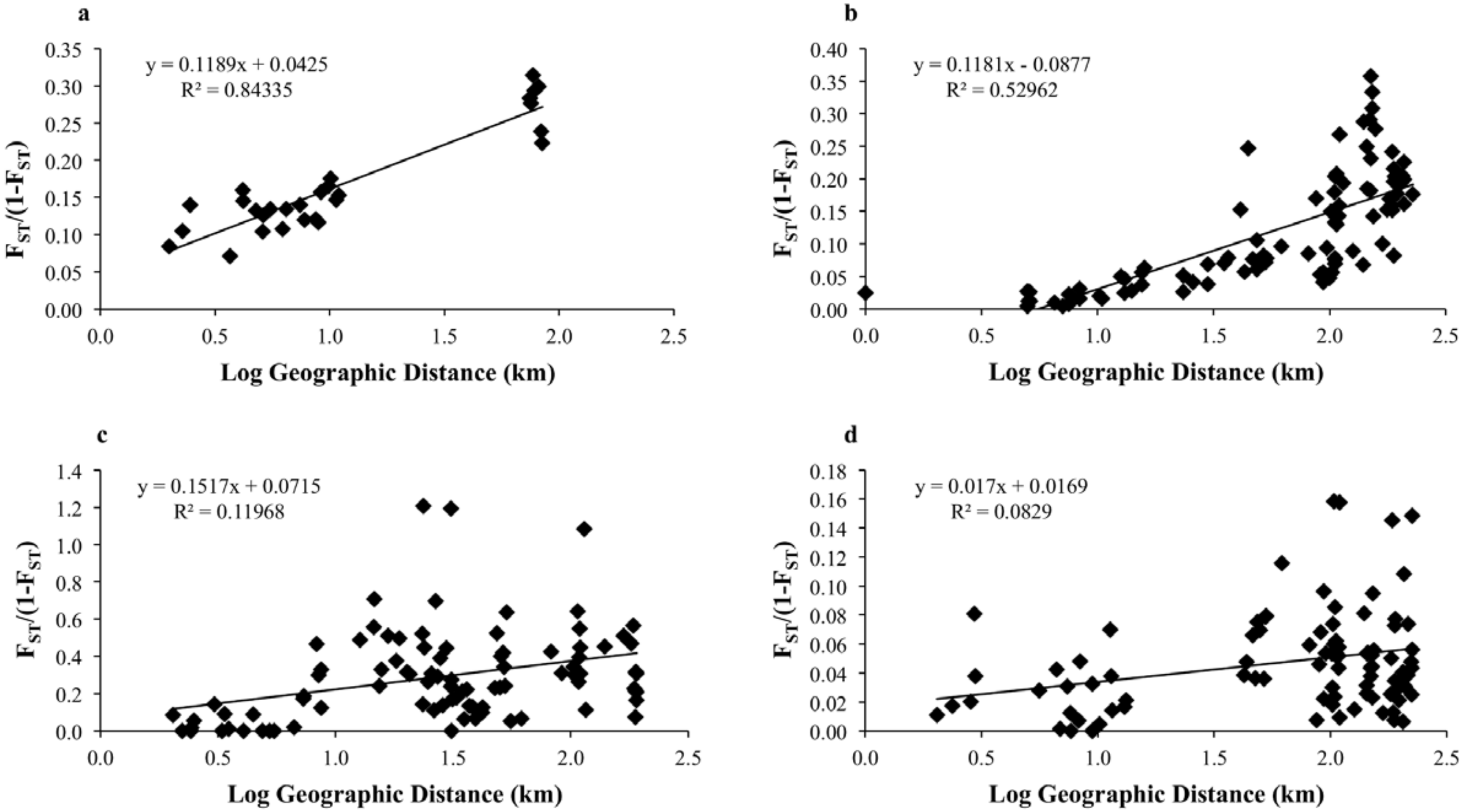

Figure 6.4 Mantel tests, performed with 999 permuations, showing significant relationships between genetic and geographic distance for (a) *Allium schoenoprasum*; (b) *Daucus carota* subsp. *gummifer*; (c) *Trifolium occidentale*; (d) *Trifolium repens*.

Table 6.4 Summary of genetic data and associated management implications for The Lizard populations for all eight study taxa. Within population variation is based on AMOVA test results across all southwest and Lizard populations; the Mantel test is also based on all southwest and Lizard populations and significant correlation coefficients are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$) or *** ($P \leq 0.001$).

Taxon (Common name)	**Genetic Statistics**				**Management Implications for Lizard Populations**
	H_W	**F_{ST}**	**Within pop. var. (%)**	**Mantel correlation coefficient (r)**	
Allium schoenoprasum (Wild chives)	0.191	0.116	78	0.918**	• Quite high diversity on Lizard • Average differentiation among populations • Strongly outbreeding • Designate a few larger areas to conserve range of genetic diversity • Due to isolation by distance, ensure conserved sites represent range of geographical locations • Continue current management (pony/cattle grazing and controlled burning of heath) • Monitor regularly (once every 5 years initially and then once every 15 years) to ensure population sizes are maintained
Allium ursinum (Ramsons)	0.086	0.505	40	0.153	• Quite low diversity on Lizard • Very high differentiation among populations • Clonal and sexual reproduction occurs • Designate several small areas to conserve range of genetic diversity • Little management intervention occurs in these woodland habitats, some path maintenance and tree safety management • No change in management currently necessary • Monitor regularly (once every 5 years initially and then once

					every 15 years) to ensure population sizes are maintained
Asparagus prostratus (Wild asparagus)	0.323	0.099	83	-0.910	• Very high genetic diversity • Average differentiation among populations • Lizard contains the majority of the UK populations of this taxon • All known populations on The Lizard are actively monitored and managed • Continuation of routine monitoring and management is recommended • Management consists primarily of cattle grazing in NNR sites, with some short periods (*ca*. 3 weeks per year) of pony grazing around Terrick Colt, a site managed by the National Trust) • There are some concerns regarding overgrazing of some *Asparagus* sites and as such, regular monitoring of population sizes is highly encouraged • In other areas of The Lizard it is known that the hottentot fig, an invasive species, threatens some populations. Regular clearance of this species from known *Asparagus* sites already occurs and should continue
Beta vulgaris subsp. *maritima* (Sea beet)	0.146	0.080	81	0.280	• Quite high diversity on Lizard • Average differentiation among populations • Designate a few large populations to conserve the range of genetic diversity • Populations tend to be large • Regular monitoring of populations is recommended (once every 5 years initially and then once every 15 years) to

					ensure maintenance of population sizes • Little active management appears to be necessary for this taxon but current management at each site should be maintained e.g. clearance of hottentot fig at Lizard Point • Sea beet population at Coverack has highest genetic diversity, agreement should be made between land owner and conservation organisations to enable monitoring of population size over time
Daucus carota subsp. *gummifer* (Sea carrot)	0.101	0.025	83	0.728***	• Average diversity on Lizard • Very little differentiation among populations • Designate a small number of large populations to conserve the range of genetic diversity • Due to isolation by distance, ensure conserved sites represent extremes of range on The Lizard • Populations are large and continuous but regular monitoring of populations is recommended (once every 3 years initially and then once every 8 years) to ensure maintenance of population sizes • Continue current management (sites generally grazed by cattle or ponies and occasional scrub clearance carried out around areas with rocky outcrops)
Raphanus raphanistrum subsp. *maritimus* (Sea radish)	0.086	0.221	68	0.204	• Quite low diversity on Lizard • High differentiation among populations • Designate a few large sites to conserve range of genetic diversity • Little management intervention appears necessary as only annual coast path maintenance/hedge strimming occurs at the

					majority of sites • Highest genetic diversity and largest population is at Pistil Meadow which has not been managed for *ca*. 4 years • Population monitoring at regular intervals (once every 3 years initially and then once every 8 years) is recommended to ensure current low levels of management are appropriate for maintenance of population sizes
Trifolium occidentale (Western clover)	0.063	0.085	72	0.346*	• Low diversity on Lizard • Little differentiation among populations • Vegetative spread occurs • Designate a minimum of two large areas to conserve range of genetic diversity • Due to isolation by distance, ensure conserved sites represent geographic extremes • Continue current management (primarily cattle grazing in either Nov to Feb or May to Oct with periodic resting of land) • Monitoring of population sizes across its range every 3 years is recommended initially to prevent loss of genetic diversity and to ensure overgrazing does not occur (once populations are known to be stable, monitor once every 15 years) • If management practices are changed at any time, the effect on population size should be very carefully monitored

Trifolium repens (White clover)	0.134	0.024	93	0.288*	• Average diversity on Lizard • Very little differentiation among populations • Taxon largely ubiquitous • No management necessary at present • Should be occasionally monitored (once every 15 years) to ensure no decrease in population sizes

6.5 Discussion

Estimates of genetic diversity and differentiation reflect the life histories of each taxon. This closely follows patterns observed by Hamrick and Godt (1989; 1996) where life history traits, including life form, breeding system and geographic range, influenced plant genetic diversity. The influence of life history traits on genetic diversity can inform conservation management plans. For example, the inbreeder *A. ursinum* has heavy seeds which spread just 2.5m or less over a 20-year period (Ernst, 1979), greatly limiting dispersal and therefore gene flow between populations. In light of this, *A. ursinum* will require several sites to be conserved across its range due to high differentiation among populations. In contrast, to conserve the full range of genetic diversity in widespread, outcrossing plants such as *D. carota* subsp. *gummifer* and *T. repens*, fewer sites need to be conserved due to high levels of gene flow resulting in little differentiation between populations.

Taking *A. schoenoprasum* as an example, when planning conservation management for this species on The Lizard it will be important that representative populations from both coastal and inland sites are targeted for active conservation monitoring and management, due to the population structure observed in PCoA analyses. In practical terms, as populations of all eight taxa appear to be thriving on The Lizard, it is recommended that current site management regimes are maintained but that formal routine monitoring of population sizes, and ideally genetic diversity, is introduced. The patterns of genetic diversity revealed in this study are a baseline for monitoring (Marfil *et al*., 2015), and will enable identification of negative trends and appropriate adjustment of management plans in response. Such plans can target conservation efforts in a time and resource efficient manner whilst ensuring the full range of genetic diversity in target taxa is protected in the long-term (see Table 6.4 for management implications for The Lizard).

H_W results indicate that a similar level of genetic diversity exists between populations on The Lizard and those from the southwest, despite sites on The Lizard representing a much smaller geographic area and having populations which are much closer in proximity to one another than those sampled across the southwest. This suggests that genetic diversity found on The Lizard for these CWR is close to the maximum diversity found across the southwest. Moreover, the genetic diversity found on The Lizard is largely distinct from diversity found elsewhere in the southwest. For taxa where distinction between different sites is found, site selection for conservation will depend on geographic separation between sites to ensure the maximum range of diversity within the taxon can be conserved. In contrast, where no obvious distinction is found, site selection can be made independently of geographic distance between sites.

Four of the eight CWR studied displayed a statistically significant correlation between geographic and genetic distance. For taxa showing isolation by distance it suggests that when selecting appropriate sites for conservation at any level (i.e. populations within a single genetic reserve, genetic reserves within a network across a country or even globally), genetic diversity conserved will be maximised if sites are selected from extremes of their range. This indicates the need for a complementary network of UK genetic reserves, to effectively conserve the full range of CWR diversity.

To further evaluate the levels of distinction in genetic diversity between The Lizard and other locations, the results of other studies in the literature can be considered. However for some taxa, few, if any, studies of genetic diversity in wild populations exist, and fewer still within the UK.

High levels of heterozygosity and polymorphism, based on isozymes, were previously found in a population of *A. prostratus* in Port Eynon, South Wales (Kay and John, 1995). This corroborates the findings of this study where both H_E and PLP values in the three Lizard populations are also high (Asp01: $H_E = 0.316$, PLP = 92.6%; Asp02: $H_E = 0.339$, PLP = 87%; Asp03: $H_E = 0.314$, PLP = 75.9%). These high levels of diversity are likely to decline due to the deleterious effects of small population sizes (Kay and John, 1995) highlighting the importance of conservation of this taxon across The Lizard and the rest of its narrow UK range.

B. vulgaris subsp. *maritima* has been the focus of a number of genetic diversity studies. Cureton *et al*. (2006) and Richards *et al*. (2014) studied genetic diversity within and among wild populations of sea beet around Poole harbour, Dorset and along the Atlantic coast of France respectively using microsatellites. Their results show a much higher H_E than the results obtained for The Lizard and southwest UK populations. However, microsatellites are hyper-variable, and there is clear evidence that comparisons of genetic diversity parameters based on different molecular markers should be made with caution (Levsen *et al*., 2008). Consequently, the lower level of genetic diversity in *B. vulgaris* subsp. *maritima* in the current study by no means implies that the taxon is unimportant when considering conservation planning within the UK.

The most reliable comparison of genetic diversity between studies can be made for *T. repens* (Hargreaves *et al*., 2010). The results presented by Hargreaves *et al*. (2010) show a mean H_E of 0.108 across all populations sampled within England. This value is significantly lower than that from The Lizard ($H_E = 0.134$, $P \leq 0.01$) suggesting that Lizard populations are more genetically diverse. Populations in both studies display high homogeneity with little differentiation between populations. This comparison suggests that Lizard populations with their higher levels of genetic diversity than the rest of the UK, would be an appropriate target for conservation of white clover genetic diversity. The Lizard could also be considered as complementary to the island of St. Kilda in Scotland, a site recommended by Hargreaves *et al*. (2010) for white clover conservation due to its unique genetic diversity in comparison with the rest of the UK.

Establishment of complementary CWR genetic reserves using the method presented here targets genome-wide genetic diversity within and among populations, aiming to conserve the maximum possible range of genetic diversity found within CWR, allowing for the likelihood of adaptive traits being conserved. This approach differs from the traditional approach to conservation, which targets species or habitat conservation and aims only to maintain species or habitat conservation *per se*. Genetic reserve based conservation aims to maximise the range of genetic diversity conserved, providing the foundation for long-term species or habitat survival (Dulloo *et al*., 2008). Consequently, even in locations already under conservation management, additional measures for CWR should be established, as existing protections do not consider genetic diversity and may even be detrimental to CWR populations if they conflict with the requirements of other protected species.

AFLPs are commonly used to guide conservation planning (Watson-Jones *et al*., 2006; Hargreaves *et al*., 2010; Greene *et al*., 2014; Marfil *et al*., 2015). It should be recognised that the genome-wide genetic diversity revealed may or may not correlate with adaptive genetic diversity, with evidence both supporting (Schoen and Brown 1993; Richardson *et al*., 2009; Johnson *et al*., 2011) and disputing (Reed and Frankham, 2001; McKay and Latta, 2002) this relationship, but the strongest argument in favour being that there are many examples of AFLP markers being associated/linked with adaptive trait Quantitative Trait Loci. Though the presence of agriculturally valuable traits cannot be guaranteed to be present on The Lizard, in light of the high concentration of CWR, the diverse ecogeography and the existing commitments to protection of biodiversity in this location alongside the results of this study, it is recommended that additional measures are put in place across The Lizard to conserve CWR.

6.6 Conclusion

This study presents the first systematic genetic analysis of multiple CWR within a potential genetic reserve, which will meet the required set of standards set out by Iriondo *et al*. (2012) (Supplementary Table 6.1).

A high level of genetic diversity within CWR on The Lizard is revealed, which is also largely distinct from other UK sites. This, together with the high number of CWR present within this area (93 taxa), provides strong support for establishing this site as the first UK CWR genetic reserve with active conservation monitoring and management of all CWR present complemented by *ex situ* back up of populations. Doing so would directly respond to ITPGRFA, target 9 of the GSPC, Aichi target 13 of the CBD Strategic Plan, action 10 of the Biodiversity Strategy 2020, target 9 of the ESPC and one of Defra's Biodiversity 2020 strategy priority actions (FAO, 2001; 2010a; 2010b; European Union, 2011; Planta Europa, 2008; Defra, 2011). In addition, the varied habitats, microclimates and topography of the peninsula mean that taxa may be better able to redistribute themselves within the area as climate changes.

Recommendations for conservation management of each of the studied CWR largely consist of maintaining current management regimes but now with regular monitoring of population sizes and possibly genetic diversity at defined intervals should population sizes drop significantly. Complementary *ex situ* collecting of conserved *in situ* populations is also recommended. In this way it will be possible to detect changes in population size or health at an early stage, providing an opportunity to review site management to ensure maintenance of CWR and genetic diversity in the long-term.

In a time when future food security looks ever more precarious in the face of climate change it is essential that plant genetic resources across the world are identified and systematically conserved. There should be a focus upon improving *in situ* conservation efforts with complementary *ex situ* back up to facilitate their continued evolution through ongoing adaptation to changing environments (Ford-Lloyd *et al*., 2014) and the long-term availability and accessibility of genetically diverse material for crop improvement. The establishment of national networks of CWR genetic reserves, the selection of which is based on genetic diversity data and which meet the required standards, would be a significant step towards achieving this fundamental goal.

CHAPTER 7.

Next-generation sequencing: a powerful tool for targeting *ex situ* collection of crop wild relatives

7.1 Introduction

CWR are poorly represented in *ex situ* collections (Dias *et al*., 2012; Maxted *et al*., 2008c). It was recently calculated that approximately 50% of all *ex situ* accessions worldwide represent only ten crop species, indicating a high degree of bias towards the collection of cultivated germplasm from crops of high economic importance rather than their more genetically diverse wild relatives (Kilian and Graner, 2012). Other estimates suggest that 94% of CWR species within Europe are not stored *ex situ* at all, emphasising that vast gaps exist in current collections (Maxted and Kell, 2009). Worryingly, it was reported that international collection efforts have been in decline since 1995, though conversely national collection efforts were reported to be increasing along with an awareness of the specific importance of collecting CWR germplasm (FAO, 2010).

In order to contribute to improved future food security it is crucial that complementary *in situ* and *ex situ* conservation approaches for CWR are established systematically (Maxted *et al*., 1997b; Greene, 2014). Genetically diverse CWR populations will need to be conserved *in situ* to maintain evolutionary processes that will not only enable their long-term survival but will also prevent the loss, through genetic erosion, of potentially useful adaptations that can be used in crop improvement. However it is equally important that genetically diverse CWR material is stored *ex situ* as an insurance against the loss of *in situ* populations, as well as ensuring these potentially useful adaptations and traits can be characterised and made available to plant breeders (Maxted *et al*., 1997b). Moreover, it is likely that *ex situ* conservation will become increasingly important if the predicted changes in climate progress at a faster rate than plant populations are able to adapt (FAO, 2010). Therefore, it is necessary to fill the gaps in the *ex situ* conservation of CWR through further collection and storage of material. Any additional collection in the field though should not be random but should be specifically targeted to ensure the maximum range of genetic diversity can be collected and maintained within genebanks, for both conservation and use.

To achieve this, collection gaps must be identified. Castañeda-Álvarez *et al*. (2015) highlight that for this to be possible, collecting missions for CWR should be informed by molecular genetic analyses, through calculation of measures of genetic diversity and distance within and among accessions already conserved within genebanks. Camadro (2012) supports this by affirming that the structure of population genetic diversity of CWR must be analysed to be able to collect representative samples for conservation and use. This was demonstrated by Shan *et al*. (2005) who uncovered the patterns of genetic variation in global genebank collections of chickpea wild relatives in the genus *Cicer* using AFLPs. *Cicer* accessions originating from southeastern Turkey and Palestine were found to have the highest levels of genetic diversity. Shan *et al*. (2005) conclude that, based on their results, further collection should take place in the sites with the highest genetic diversity and surrounding areas. Equally though, they suggest collection should also focus on sites that may have lower genetic diversity but which are located in areas with a distinct ecogeography. A similar study was conducted by Ferguson *et al*. (1998) on lentil wild relatives using RAPD markers to investigate genetic diversity and distance between populations to find areas of high diversity and areas of unique diversity. By applying molecular genetic analyses such as these to other CWR collections it is likely to enhance the genetic diversity captured in genebanks. Moreover, the use of modern sequencing techniques to discover single nucleotide polymorphism (SNP) diversity will enable faster, more direct, high-throughput analyses of genomic variation across genebank accessions, and where appropriate within adaptive genes rather than non-transcribed regions of the genome.

SNPs are simply defined as single changes in the base sequence of DNA. For a base position to be confirmed as an SNP it is generally accepted that the least common allele should occur with a frequency equal to or greater than 1% (Vignal *et al*., 2002). SNPs are generally bi-allelic due to both their relatively low mutation rate, making the probability of multiple changes at any one locus unlikely, and a bias towards transition mutations (purine to purine or pyrimidine to pyrimidine) rather than transversion mutations (purine to pyrimidine and vice versa) (Vignal *et al*., 2002). This can be seen as a disadvantage when compared to the polymorphic nature of markers such as microsatellites, however the fact that SNPs are universal markers, the most abundant source of genetic variation in the genome and also suited to next-generation sequencing (NGS) technologies (Ren *et al*., 2013), this small drawback is more than overcome making them highly suited to genetic diversity studies.

First generation sequencing technology, automated Sanger sequencing (Metzker, 2010), has been employed very successfully over the years, including for the completion of the first human genome sequence (IHGC, 2004). However it has now been surpassed by other newer technologies. NGS has become a widely used technique due to its ability to process millions of sequence reads in parallel, a feat which was beyond previous Sanger sequencers (Mardis, 2008). Not only can it generate this huge volume of data in a much shorter space of time but also it is much less expensive than previous sequencing techniques (Mardis, 2008; Metzker, 2010). As the costs of NGS continue to rapidly decrease (Wetterstrand, 2015), so the potential for more routine use in conservation planning is increasing.

Many NGS sequencing technologies are commercially available. The leading sequencing platforms include those from Roche (e.g. 454 GS FLX Titanium), Illumina/Solexa (e.g. HiSeq) and Life Technologies/Applied Biosystems (e.g. SOLiD). All of these platforms rely on different sequencing chemistries (pyrosequencing, reversible termination and sequencing by ligation respectively) and therefore differ in equipment and reagent costs, read lengths, run times and amount of data generated per run (Moorthie *et al*., 2011). The Roche 454 platform is known to generate the longest reads out of the three most common platforms, with average read lengths of 330bp reported by Metzker (2010) and up to 700bp according to Liu *et al*. (2012). These long read lengths have the benefit of improving the mapping of repetitive sequences (Metzker, 2010). The SOLiD platform typically gives the shortest read lengths of 85bp whereas Illumina platforms yield read lengths of 100bp (Liu *et al*., 2012). Roche platforms also benefit from having the shortest run times of less than 24 hours whereas Illumina runs on the HiSeq 2000 platform take four days on a single-end run and nine days on a paired-end run (Metzker, 2010). The more recent Illumina HiSeq 2500 platform yields higher amounts of data but takes 11 days to complete a paired-end run (Illumina, 2015a). The SOLiD platform runs take even longer at seven days (single-end run) to 14 days (paired-end run) (Liu *et al*., 2012). Roche platforms are also particularly suited to studies using targeted enrichment methods (Metzker, 2010). Targeted enrichment allows the sequencing of specific regions of a genome, avoiding the costs of whole genome sequencing and allowing more detailed analysis of the regions of interest (Moorthie *et al*., 2011). However, Illumina is currently the most widely used sequencing platform due to it yielding the highest data output (up to 1TB) compared to other platforms (Illumina, 2015a). The low costs of Illumina sequencing reagents (HiSeq 2000: $0.07 per million bases) compared to other platforms, particularly Roche (which has very high reagent costs of $10 per million bases for the GS FLX Titanium platform), and the ability of Illumina sequencers to carry out both de novo sequencing and re-sequencing make this platform a cost-efficient and versatile choice (Liu *et al*., 2012).

NGS technologies are increasingly being discussed in terms of their potential use in plant breeding for crop improvement. Currently the emphasis seems to be on the use of NGS in screening current germplasm (both crop and PGR accessions) stored in genebanks to identify beneficial traits, either through mining for identification of individual alleles or through mapping of quantitative trait loci (Kilian and Graner, 2012; Pérez-de-Castro *et al*., 2012). This approach of characterising stored germplasm using high throughput technology will be invaluable in increasing the volume and quality of information about genebank collections that is available to breeders. This in turn will allow targeted development of new crop varieties with increased yields and resilience to biotic and abiotic stresses.

In contrast, the objective of the current study was to demonstrate the use of NGS in ascertaining whether or not the existing germplasm is likely to be representative of the range of CWR genetic diversity found in natural populations, with a particular focus on adaptive genetic diversity. Where collections are representative, there is no need for further collection. Where gaps are identified in germplasm collections the genetic diversity information revealed by NGS can be used to target further collecting of germplasm. In this way, the range of diversity and therefore the range of potentially beneficial traits stored in genebanks can be optimised.

Over the last 15 years *Medicago truncatula* Gaertn. (Fabaceae family) has become a well-established model legume species (Branca *et al*., 2011; Ben *et al*., 2012). It has many qualities that make it an excellent model; its genome has been sequenced, it is diploid (2n = 16), self-fertile, has a small genome (500–550 million base pairs) and a short generation time (Young *et al*., 2009; Young *et al*., 2011; Zhou *et al*., 2011). It is also a CWR of the economically valuable forage crop, alfalfa (*M. sativa* L. subsp. *sativa*). *M. truncatula* has not yet been classified within the Gene Pool concept of *M. sativa* subsp. *sativa* (Vincent *et al*., 2013), suggesting that crossing experiments have yet to be carried out. Despite this, it is already recognised as having potential for conferring disease resistance in alfalfa and can itself be used as a forage crop (USDA, ARS, National Genetic Resources Program, 2015). For the reasons outlined above, *M. truncatula* was selected for use in this study to demonstrate the use of NGS in targeting collection of germplasm.

To achieve the study's objective, an initial approach was selected which used Roche 454 sequencing platform along with Roche Nimblegen's Sequence Capture technology (Roche NimbleGen, 2011), a method of targeted enrichment, to isolate and sequence genomic DNA from *M. truncatula* genes involved in stress response pathways. This approach would enable the assessment of SNP variation in adaptive genes linked to environmental stress, particularly relevant to the field of conservation genetics and CWR conservation in light of climate change and other threats to species genetic diversity. A total of 70 *M. truncatula* genes involved in stress response pathways (e.g. water, salt, temperature and oxidative stress) were selected for the study (Supplementary Table 7.1), primarily through searches of nucleotide sequence similarity between genes of known function in the sequenced genome of *Arabidopsis thaliana* (L.) Heynh. using NCBI's Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990; Lamesch *et al.*, 2011). Other genes were identified from a study of SNP diversity in drought stress associated genes in *Trifolium pratense* L. and a study of brown plant hopper resistance in rice *Oryza sativa* L. (Hargreaves, 2011; Ab-Ghaffar, 2012). Using the Sequence Capture method, a further aim was to investigate whether it would be possible to capture these same 70 genes from accessions of other, closely related, legume species and successfully sequence them. These species were: *Trifolium repens* L., *T. pratense* L., *Vicia johannis* Tamamsch, *V. narbonensis* L., *Lens culinaris* Medik. and *Lathyrus annuus* L. If it had been possible to isolate sequences from related species and map them in relation to the *M. truncatula* reference genome, this could overcome limitations relating to the lack of sequenced wild plant reference genomes, and particularly CWR reference genomes, which are restricting the use of NGS in plant conservation studies (Michael and VanBuren, 2015).

Unfortunately, due to a range of financial and resource limitations outlined in more detail in section 8.3 the approach using Roche 454 sequencing, Sequence Capture and legume species related to *M. truncatula* ultimately was not pursued and an alternative method of whole genome re-sequencing using the Illumina HiSeq sequencing platform was carried out as outlined in full in section 7.2 below. To meet the study objective using the Illumina sequencing platform, the project required the following four stages. Ultimately however, time limitations due to the issues experienced using the initial Roche sequencing method meant that only the first stage was achieved.

1. Carry out whole genome re-sequencing of 13 *M. truncatula* accessions using the Illumina HiSeq 2500.
2. Identify SNP variation within genes relating to stress response pathways (Supplementary Table 7.1).
3. Determine the levels of genetic diversity and patterns of genetic distance within and among accessions, based on SNP variation identified in stress response genes.
4. Make recommendations as to where gaps currently exist in *M. truncatula* genebank collections and where further collecting is likely to improve the genetic diversity conserved. This can be achieved by identifying accessions with high levels or distinctive genetic diversity and comparing these results to the ecogeographic regions from which they originate. This will help inform where further collecting would be most likely to yield additional and complementary genetic diversity.

7.2 Materials and methods

7.2.1 Study species

Twenty seed accessions of *M. truncatula* were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. Four seeds per accession were planted in a greenhouse at the University of Birmingham and 53% germinated. Leaf material from each plant was removed and snap frozen in liquid nitrogen for long-term storage at minus 20°C. At least two plants grew successfully for thirteen of the accessions and these were selected for the study (Table 7.1). Fig. 7.1 illustrates the countries within the native range of *M. truncatula* with collection sites of the 11 accessions of wild origin indicated (the two wild accessions lacking coordinate data are mapped to the centre of their country of origin).

Table 7.1 Accessions and number of plants sequenced with associated passport data.

Accession ID	Country of origin	Longitude	Latitude	Type	Number of plants
53087	Turkey	27.1833	39	Wild	4
53787	Czech Republic	NULL	NULL	Wild	4
54082	Malta	NULL	NULL	Cultivated	3
54084	Australia	NULL	NULL	Cultivated	3
53156	Morocco	-3.25	34.9667	Wild	2
53247	Libya	13.6333	32.4333	Wild	2
53366	Jordan	35.6	31.6167	Wild	2
53954	Lebanon	36.0167	33.8667	Wild	2
54081	Hungary	NULL	NULL	Wild	2
55085	Syria	36.9069	34.8722	Wild	2
57157	Italy	13.75	37.2167	Wild	2
58156	Portugal	-8.38333	37.7333	Wild	2
132528	Azerbaijan	49.7281	40.2889	Wild	2

Longitude and latitude displayed in decimal degrees; 'NULL' indicates no coordinates are available.

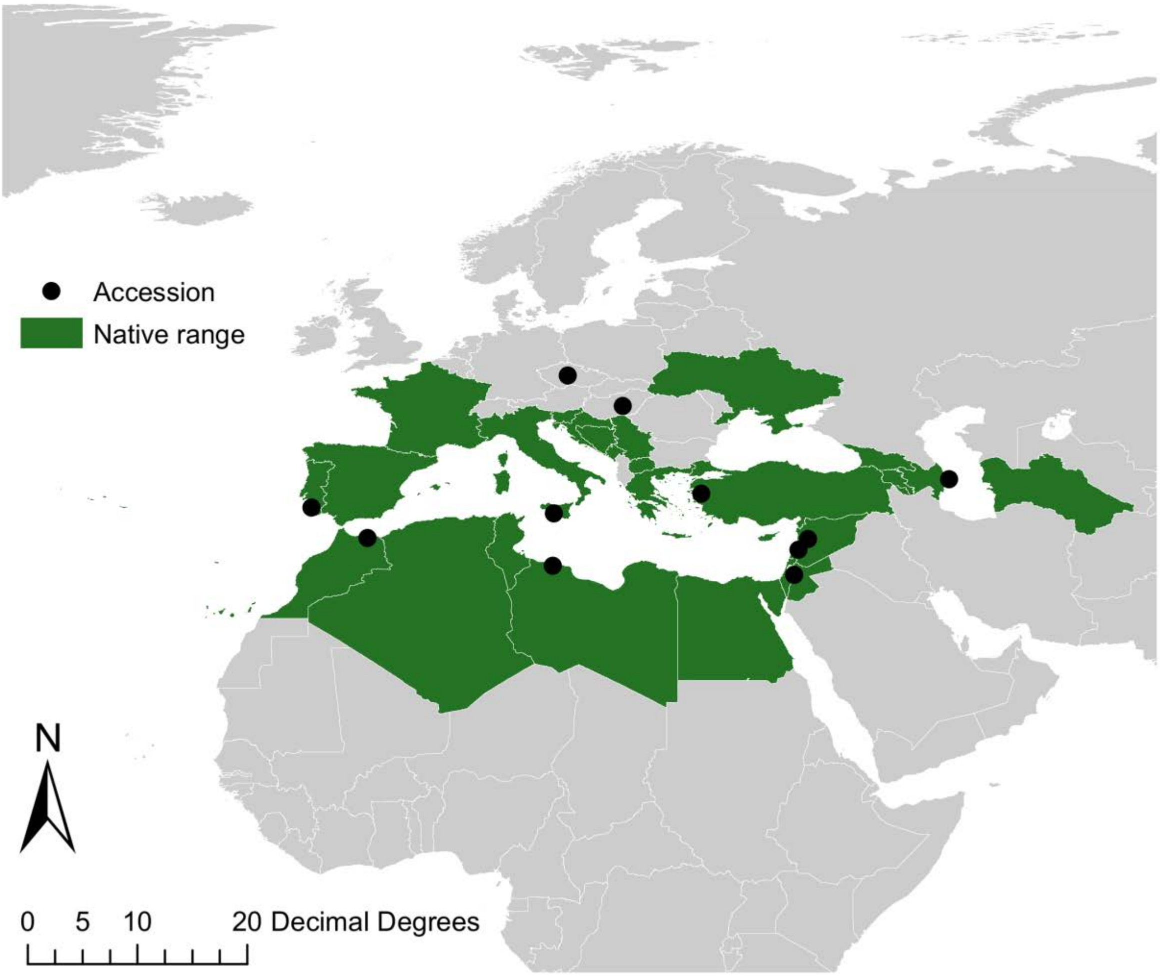

Figure 7.1 The native range of *M. truncatula* according to USDA, ARS, National Genetic Resources Program (2015) and the collection locations of all 11 wild accessions.

7.2.2 DNA extraction

DNA extractions from the leaf material of all 32 *M. truncatula* plants (hereafter referred to as samples) were carried out using the QIAGEN DNeasy® Plant Mini Kit (Qiagen, 2012). The standard protocol was performed on 100mg of each sample with two minor adjustments for optimisation. Firstly, the RNase incubation time was extended from 10 minutes to 30 minutes and an extra dry spin was included prior to elution of the DNA, to remove contaminants. All samples were then placed in a SpeedVac vacuum concentrator for approximately one hour until the volume of each sample was reduced by half (from 200µl to 100µl). Samples were then quantified using a NanoDrop spectrophotometer to determine the amount of DNA yielded and its purity. Purity readings (260/280 ratio) fell within the range of 1.50 and 2.00 indicating the samples were sufficiently free from residual contaminants such as phenol (Nanodrop, 2012). The protocol yielded between 408.96ng to 4967.42ng of DNA, sufficient to continue to the library preparation step. Nanodrop readings were found to be more reliable at higher DNA concentrations.

7.2.3 Genomic DNA library preparation

Thirty-two libraries (one per sample) were produced according to the standard low sample protocol of the Illumina TruSeq® DNA PCR-Free method (Illumina, 2013). The protocol involves fragmentation of genomic DNA using Covaris shearing, end repair, DNA fragment size selection (550 base pair insert size), adenylation of 3' ends of the DNA insert and ligation of adaptors to enable hybridisation to a flow cell during sequencing. Adaptors each included a unique index sequence to facilitate multiplexing of sample libraries. Two multiplexed pools, each containing 16 sample libraries, were produced (Table 7.2). Library preparation was carried out at the Center for Genomics and Bioinformatics, Indiana University, Indiana, USA.

Table 7.2 Unique Illumina PCR-Free DNA index sequences ligated to each sample in the two library pools.

Pool	Sample name	Index set	Illumina PCR-Free DNA index	Illumina PCR-Free DNA index sequence
1	53087 1	A	2	CGATGT
1	53087 2	A	4	TGACCA
1	53087 3	A	5	ACAGTG
1	53087 4	A	6	GCCAAT
1	53787 1	A	7	CAGATC
1	53787 2	A	12	CTTGTA
1	53787 3	A	13	AGTCAA
1	53787 4	A	14	AGTTCC
1	54082 1	A	15	ATGTCA
1	54082 2	A	16	CCGTCC
1	54082 3	A	18	GTCCGC
1	54084 1	A	19	GTGAAA
1	54084 2	B	22	CGTACG
1	54084 3	B	23	GAGTGG
1	53156 1	B	25	ACTGAT
1	53156 2	B	27	ATTCCT
2	53247 3	B	1	ATCACG
2	53247 2	B	3	TTAGGC
2	53366 1	B	8	ACTTGA
2	53366 2	B	9	GATCAG
2	53954 1	B	10	TAGCTT
2	53954 2	B	11	GGCTAC
2	54081 1	B	20	GTGGCC
2	54081 2	B	21	GTTTCG
2	55085 1	B	22	CGTACG
2	55085 2	B	23	GAGTGG
2	57157 1	B	25	ACTGAT
2	57157 2	B	27	ATTCCT
2	58156 1	A	2	CGATGT
2	58156 2	A	4	TGACCA
2	132528 1	A	5	ACAGTG
2	132528 2	A	6	GCCAAT

Library pool quantification using qPCR was then carried out at the University of Birmingham according to the KAPA Biosystems standard protocol for Illumina sequencing platforms (KAPA, 2011). Accurate quantification of libraries is essential as overestimation of library concentrations can result in low cluster density on the sequencing flow cell, and underestimation can result in over clustering. In each case, sub-optimal sequencing results are obtained (KAPA, 2011). The concentrations of library pools 1 and 2 were 1.865nM and 1.8nM respectively.

7.2.4 Cluster generation

Denaturation of DNA in library pool 1 was carried out by taking 12.5µl of pool 1, adding 12.5µl of 0.1M NaOH and leaving at room temperature for five minutes. A volume of 25µl of Tris-HCl + 0.1% Tween 20 was then added as a buffer, resulting in a concentration of 450pM. A volume of 1075µl of hybridisation buffer (HT1) was then added to dilute the denatured library pool to a concentration of 20pM. A further 300µl of HT1 was then added to reach a final concentration of 12pM (a concentration selected to produce optimal cluster density). The same process was followed to denature and dilute library pool 2. A volume of 120µl of each 12pM pool was then loaded onto a HiSeq flow cell. Four flow cell lanes were loaded with pool 1 and a further four lanes were loaded with pool 2. Oligonucleotides complementary to the adaptor sequences ligated to the sample DNA are present on the flow cell, allowing isothermal bridge amplification of DNA fragments using the automated Illumina cBot (Illumina, 2012). This produces clonal clusters of single molecule DNA templates, which are attached to the flow cell with sequencing primers hybridised to the free ends of the templates, ready for sequencing.

7.2.5 Whole genome re-sequencing

After successful cluster generation, the HiSeq flow cell was then transferred from the cBot to the Illumina HiSeq 2500 sequencer. A TruSeq® v3 run was carried out according to the Illumina protocol (Illumina, 2014). The process uses 'Sequencing by Synthesis', where DNA is synthesised from the sequencing primers, which were annealed to the DNA templates in the cBot. Each incorporated base emits a fluorescent signal that is detected by the HiSeq 2500 and the specific basecall at each position is determined (Illumina, 2015b). A total of 209 cycles were carried out to produce pair end reads (100 cycles for read 1, nine cycles for index reads and 100 cycles for read 2). Paired end reads enable more accurate alignments to a reference genome (Illumina, 2015b). A depth of coverage of approximately 15x was achieved per sample.

7.2.6 Mapping reads to reference genome

The 100bp paired end sequence reads generated as outputs from the Truseq® run were demultiplexed using index reads. Reads were then mapped to the *M. truncatula* reference genome v4.0 (Tang *et al*., 2014) using the Burrows-Wheeler alignment tool (BWA, 2013). The BWA-MEM (maximal exact matches) algorithm was used in paired end mode and is suitable for aligning Illumina reads from 70bp to 1Mbp in length (BWA, 2013). Alignments were then sorted and indexed using SAMtools v1.1 (Li *et al*., 2009).

7.3 Results and discussion

Using the methods described above, whole genome re-sequencing was successfully carried out to produce 32 sequenced genomes (13 accessions) of *M. truncatula*. The data are in the required format to enable downstream analyses, including SNP identification in genes linked to stress responses (Supplementary Table 7.1). Population genetic analyses based on SNP variation in stress associated genes will achieve the objective set out in section 7.1, to demonstrate the application of NGS in targeting the collection of CWR germplasm to fill gaps in genebank collections. As sequencing costs continue to drop this method could be adopted by genebanks to ensure the range of natural adaptive genetic diversity in wild CWR populations is conserved *ex situ*. Investment in sequencing genebank accessions would mean that these data are also available to be used in the characterisation of germplasm and could be used as a baseline for monitoring genetic diversity through the process of seed regeneration (van Treuren and van Hintum, 2014). All of these are valuable applications that would help towards ensuring the long-term availability of genetically diverse germplasm for utilisation by plant breeders.

The cost of sequencing has reduced substantially since the $3 billion spent to sequence the human genome (approximately $1 per base pair), with the advent of NGS responsible for a particularly steep drop in cost from 2007 onwards (IHGC, 2004; Wetterstrand, 2015). In the current study the cost of re-sequencing a single genome of *M. truncatula*, inclusive of DNA extraction and library preparation costs, was approximately $615, equating to less than $1.25 per million base pairs. This demonstrates how much more affordable this technology is becoming and its potential to be used more widely within the field of PGR conservation and use. Ultimately, this method was significantly cheaper than it would have been using the

Sequence Capture methodology to sequence only the regions of interest. Moorthie *et al.* (2011) also recognise that overall, the sequencing of whole genomes is a more cost-effective approach than targeted enrichment methods, though additional costs of data analysis must also be considered when planning NGS research (Sboner *et al.*, 2011).

The first plant genome to be sequenced was that of *A. thaliana* in 2000 (The Arabidopsis Genome Initiative, 2000). Since then more than one hundred plant genomes have been sequenced, 63% of which are crop plants and the remaining 37% of which consist of model and non-model plants and crop wild relatives (Michael and VanBuren, 2015). There is a need for more plant genomes, particularly CWR, to be sequenced to facilitate detailed genetic analyses of crops gene pools to target germplasm collection appropriately to fill gaps in the genetic diversity conserved (van Treuren *et al.*, 2012). McCouch (2013) also suggests that all plant samples available in genebanks under the ITPGRFA should be sequenced in order to 'mine biodiversity for food security' to produce new, more resilient crop varieties. However, in contrast to the small diploid genomes of plants such as *A. thaliana* and *M. truncatula*, the challenges presented by large genomes, occurrence of polyploidy, extensive repetitive sequences and structural variation can make resolving complex plant genomes highly problematic due to the short reads generated using NGS (Michael and VanBuren, 2015). Third generation sequencing, which is now becoming commercially available, has the ability to produce longer sequence reads, with higher throughput, at faster speeds with higher accuracy than NGS platforms (Thudi *et al.*, 2012). For example, Pacific Biosciences are using SMRT technology that enables the sequencing of one DNA strand by a single DNA polymerase (Thudi *et al.*, 2012). This process can sequence 1–3 nucleotides every second and produces reads of up to 10,000 base pairs in length (McCarthy, 2010). Technologies such as this will take significant steps towards the sequencing of the most complex plant genomes, making this data available for use in CWR conservation and crop improvement.

Ultimately, it is not possible to collect all of the genetic diversity present in wild populations and to store this *ex situ*, due to limited resources (Hoban and Schlarbaum, 2014). Equally, it is not possible to know how representative germplasm collections truly are of the genetic diversity found in the wild (van Treuren *et al*., 2012). Despite this, genetic diversity data can be used to target the collection of populations with high or unique genetic diversity. Furthermore, to combine this information with ecogeographic data could further improve the representativeness of CWR collections and Ecogeographic Land Characterisation (ELC) maps are a tool that could facilitate this. Maps such as these categorise the landscape of a geographic area based on select ecogeographic variables that shape the distribution of a species (Parra-Quijano *et al*., 2008). Knowledge of ELC categories and the extent of genetic diversity of accessions from within and among categories would enable genebank managers to make more informed decisions regarding where further collection would be most likely to yield high or unique genetic diversity. The recent release of CAPFITOGEN tools make the production of ELC maps relatively simple (Parra-Quijano *et al*., 2014), allowing this method to be used effectively alongside genetic diversity data to significantly improve the completeness of CWR genebank collections.

7.4 Conclusion

A lack of technical and bioinformatics expertise was a major limitation in this project. Despite this, sequence data for 32 whole genomes of *M. truncatula* from the ICARDA genebank in Syria have been successfully produced. This is a substantial dataset that can now be used in a population genetics study using SNP variation in genes linked to stress responses to reveal the extent and pattern of genetic diversity already maintained in this collection. Together with the use of ELC maps, the genetic diversity data generated can be used to find gaps in this *ex situ* collection to help target further collecting missions. NGS, and now third generation sequencing, are capable of producing huge volumes of data making them powerful tools for use in population genetics studies to inform conservation planning. The sequencing method used in this project and the intended subsequent analysis can be applied to any CWR with a sequenced genome. The cost of sequencing will only continue to decrease, making this technique evermore accessible to genebanks. It will significantly improve the completeness of *ex situ* collections and ultimately the range of genetic diversity available to plant breeders to produce novel, resilient crop varieties that are more likely to withstand environmental change.

CHAPTER 8.

General Discussion

8.1 Overview

The UK is a contracting party to the Convention on Biological Diversity (CBD, 1992) and the ITPGRFA (FAO, 2001), with a responsibility to achieve targets set out by these, as well as the GSPC and Strategic Plan for Biodiversity 2011–2020, to actively conserve PGR including CWR and their genetic diversity through complementary *in situ* and *ex situ* measures (CBD, 2010a; 2010b). Further policy instruments, the EU Biodiversity Strategy 2020 (European Union, 2011) and ESPC (Planta Europa, 2008), reiterate these targets in a European context. Each of the devolved administrations of the UK have their own biodiversity strategy (Defra, 2011; Welsh Assembly Government, 2006; Scottish Government, 2013b; DOENI, 2002), all of which aim to preserve biodiversity and protect our natural capital, including agricultural genetic diversity and CWR. Despite UK and devolved administration commitments and responsibilities to respond to these policy instruments it has not been possible until now to implement any CWR conservation measures due to a lack of scientific knowledge to inform conservation decision-making. The research presented in this thesis fills this knowledge gap by identifying priority CWR for food security across the UK, revealing gaps in their current conservation both *in situ* and *ex situ* and making recommendations to relevant stakeholders to facilitate the establishment of systematic CWR conservation for the UK and devolved administrations. The methodologies developed and used in this thesis were selected to cover the fundamental concept that to achieve effective, active and long-term CWR conservation there is a need for rigorous conservation planning. Any approach taken must incorporate complementary *in situ* (both within and outside of protected areas) and *ex situ* conservation planning, informed by the range and pattern of genetic diversity found within target taxa (Maxted *et al*., 1997b; Greene *et al*., 2014). Chapters 2–4 and Appendix 1 (Fielder *et al*., 2015; submitted a; submitted b; submitted c) took a broad, holistic approach considering appropriate conservation actions required for all priority food, forage and fodder CWR across the administrations of England, Wales and Scotland and also nationally across the whole UK. Chapter 5 (Jarvis *et al*., in press) addressed how *in situ* CWR conservation can be further targeted in appropriate locations by demonstrating the habitat preferences of CWR across the UK, these results can be incorporated into national and administration level conservation planning. Though all of these chapters highlight the need for conservation of the genetic diversity within CWR and make recommendations with this in mind, Chapters 6 (Fielder *et al*., submitted d) and 7 both aimed to directly investigate the range of genetic diversity within CWR. The former focused on the application of genetic diversity analyses in justifying the establishment of *in situ* genetic reserves and the latter focused on its *ex situ* application in assessing the genetic diversity stored in genebanks to enable better decision making and targeted collection. On completion of this research there remains a requirement for the implementation of the recommendations made to meet global, European and national targets. However, the recommendations have been developed through consultation with the conservation organisations for each of the devolved administrations of the UK and this has resulted in policy discussions that are leading to implementation. Fundamentally, implementation must result in the systematic, long-term conservation of the range of genetic diversity in UK CWR but also must link this with the use of this genetically diverse material in crop improvement.

8.2 From research to practice

In the field of conservation research and particularly research concerning food security, it is crucial that there is a bridge between academic studies and the relevant stakeholders ultimately responsible for creating change in practice. Meilleur and Hodgkin (2004) suggest that links between recommendations for conservation of CWR and the establishment of their protection and management are weak. Without strengthening this bridge between research and policy, conservation decision making and management practices, active conservation and use, CWR are likely to remain poorly conserved and at risk of genetic erosion due to factors including climate change, habitat destruction and land management. This in turn will limit the availability of genetically diverse material to use in crop improvement initiatives and ultimately will compromise our future food security.

The main results obtained through gap analyses in Chapters 2–4 and Appendix 1 (Fielder *et al*., 2015; submitted a; submitted b; submitted c) are presented in Table 8.1. They highlight key areas where research has revealed a need for on-the-ground conservation action. For example, it would be expedient to focus on the 11–15% of threatened CWR in each administration and the UK to ensure the persistence of key population sizes and their existing range of genetic diversity. Furthermore, complementarity results demonstrate that a network of as few as 15 sites (or potential CWR genetic reserves) is required to conserve the majority of English priority CWR, nine and 11 sites for common and rare priority CWR respectively in Wales, 22 sites to conserve priority CWR in Scotland and up to 27 sites would be required for priority CWR in the UK inventory. These networks would be sufficient to contain at least five distinct *in situ* populations for 38% of priority CWR in Wales and up to 61% in the UK as a whole. Many of the sites recommended in the devolved administration overlap with those of national UK priority including Purbeck, The Lizard Peninsula, sites in Cambridgeshire, Somerset in England, around Cardiff in Wales and in the Cairngorms National Park in Scotland. Considering this, efforts to establish networks of CWR genetic reserves in each administration should integrate with UK-wide efforts to produce one *in situ* network of sites, which are either of sub-national importance, national importance or both. Prior to this project no protected areas in the UK met the minimum standards necessary for CWR genetic conservation (Iriondo *et al*., 2012). There was a need, where priority CWR populations occur within existing protected areas, for management plans to be adjusted to incorporate CWR and long-term monitoring to be established, this is now happening on The Lizard and is also underway on the Isle of Purbeck.

Equally though, to achieve the recommended minimum of five conserved populations for all CWR (Brown and Briggs, 1991; Dulloo *et al*., 2008), active management of CWR in habitats outside of protected areas will also need to be established. Rare CWR are unlikely to be present in greater than five complementarity sites overall due to their restricted ranges so conservation representing their full distributions should be the focus in these cases. Results from Chapter 5 (Jarvis *et al*., in press), concerning the habitat preferences of priority CWR in the UK, can be combined with the broad results from each administration and the UK to inform CWR management and monitoring by target *in situ* conservation more effectively in the habitats where CWR are most likely to be found. Finally, *ex situ* gap analyses were able to highlight where further collection of CWR accessions is required. Results indicate that CWR in Scotland are severely under-collected but also that only 16% of priority CWR in England and the UK reach the advised minimum of five or more genebank accessions and as few as 7% and 2% reach this threshold in Wales and Scotland respectively. Some of the gaps in *ex situ* collections can be attributed to the collection priorities of the MSB, Royal Botanic Gardens, Kew. Their focus has been to collect all UK native, orthodox (able to be dried and stored without damage), seed-bearing species and they now hold close to 94% of these species (S. Miles pers. comm.). However, this does not include archaeophyte or neophyte taxa, which in the case of CWR are also valuable for the potential genetic diversity contained within them.

Table 8.1 Summary of key results for the UK and devolved administrations from Chapters 2–4 and Appendix 1 (Fielder *et al.*, 2015; submitted a; submitted b; submitted c).

	England	**Wales**	**Scotland**	**United Kingdom**
Priority CWR	148	122	120	223
Percent priority CWR threatened	14	15	11	12
Suggested complementarity sites	15	9 (C) 11 (R)	22	27
Percent CWR in five or more complementarity sites	53	38 (C) 0 (R)	42	61
Number of *ex situ* conserved accessions for priority CWR	687	238	40	1034
Percent CWR with five or more accessions	16	7	2	16
Percent CWR with no accessions	44	76	91	35

(C) = Common CWR; (R) = Rare CWR.

The key recommendations made in the preceding chapters, relevant to stakeholders and policy makers, that need to be addressed for CWR conservation in the UK include:

1. The establishment of an integrated *in situ* network of CWR genetic reserves of sub-national, national or dual importance, that incorporate the full range of the genetic diversity of priority CWR. Recommended sites include Purbeck and The Lizard Peninsula in England, The Gower Peninsula in Wales and a site overlapping with the Loch Lomond and Trossachs National Park in Scotland. The added value of carrying out separate gap analyses for common and rare CWR in Wales could also be explored in England, Scotland and the whole UK to further validate the sites selected;
2. The protection of CWR populations outside of protected areas, particularly in habitats shown to be associated with higher incidence of CWR (e.g. field boundaries, hedgerows and road verges). This could be achieved through incorporation of CWR

conservation into the Common Agricultural Policy's Rural Development programme via government Countryside Stewardship schemes;

3. The establishment of The Lizard as the first CWR genetic reserve in the UK as a priority, closely followed by the Isle of Purbeck, which has the highest UK concentration of CWR taxonomic diversity and;
4. The collection of genetically diverse CWR material to fill *ex situ* gaps in genebanks, including archaeophyte and neophyte taxa.

The research carried out in Chapters 6 (Fielder *et al.*, submitted d) and 7 both present methodologies that can easily be replicated to assess the genetic diversity within and among populations *in situ* or accessions *ex situ*. Gaining an understanding of the range and patterns of genetic diversity in the wild and within existing genebank collections is crucial so that CWR with high or distinctive genetic diversity can be maintained *in situ*, enabling ongoing adaptation to changing environmental conditions (Ford-Lloyd *et al.*, 2014), and *ex situ*, enabling the establishment of a complete back up of diverse material in genebanks available for germplasm screening. Using the model developed and described in this thesis, genetic diversity data can be integrated into conservation planning. This again highlights the need for communication and collaboration between research and practice. Bridging this gap will maximise the genetic diversity conserved across all CWR and will increase the likelihood that agriculturally valuable traits will be preserved and made available for use in crop improvement.

Across the UK and within the devolved administrations of England, Wales and Scotland, representatives of each conservation agency (NE, NRW and SNH) were consulted throughout the undertaking of the research presented in this thesis. Their input was invaluable in ensuring the results and ultimately the recommendations made in the UK, in each administration and in the case study of The Lizard as a potential CWR genetic reserve, were relevant and attainable. Their involvement also provides a promising future, that the impetus from this work will be taken forward and that the conservation of CWR in the UK will be systematic and comprehensive. This is best demonstrated in a summary of the actions that are already underway, or have been proposed, by representatives from each administration's conservation agency.

Within England the following actions have been completed or are underway:

- The results of The Lizard genetic analysis have been disseminated to National Nature Reserve managers from The Lizard and the southwest of the UK.
- Though Defra does not currently wish to formally create any new national protected area designations (e.g. statutory CWR genetic reserves), options are currently being explored to incorporate CWR management into existing protected areas as 'informal genetic reserves', a proxy for formal recognition. For example, CWR could be listed as special interest features within SSSI designations or incorporated into NNR management plans, which are reviewed every five years; the latter is likely to be the quickest and simplest option.
- This has already been achieved on The Lizard where CWR have already been included in the NNR management plan. The Lizard NNR managers are close to meeting the minimum genetic reserve standards proposed by Iriondo *et al.* (2012). To achieve the minimum standards, The Lizard still requires an agreed framework for the reporting of ongoing monitoring (for The Lizard and England, the lead authority should logically be NE, and NE should report to Defra for all national and

international reporting purposes) and funding to enable start-up activities such as seed collection of priority populations to be stored at the MSB, Kew (Supplementary Table 6.1). If CWR are listed as special interest features in existing protected areas then ongoing costs should become part of national priority biodiversity conservation activities.

- Options to enable conservation outside of protected areas are also being explored, including CWR conservation in the Rural Development Programme for England through agri-environmental schemes such as the new Countryside Stewardship.
- The results of The Lizard genetic analysis were also disseminated to local stakeholders in Purbeck, Dorset; another potential site for a CWR genetic reserve to complement The Lizard. Stakeholders included representatives from Natural England, The National Trust, Dorset County Council and The Farming and Wildlife Advisory Group for the southwest and all were interested in taking CWR conservation forward.
- Spatial data depicting all 15 complementarity site boundaries across England were requested by NE and subsequent analysis has been carried out to map the overlap of existing statutory protected areas with all complementarity sites to identify the most appropriate designations to incorporate CWR management and monitoring.

Less progress has been made so far in other administrations but some initial steps forward have been taken. Within Wales, spatial data depicting all common and rare complementarity sites were requested by NRW to pass on to the relevant staff for each corresponding statutory site in Wales. Options to disseminate the work carried out in Wales to the Welsh Government are being explored. In Scotland, SNH have suggested that this research should now be disseminated to the Scottish Government and a wider audience within SNH to provide the momentum to move CWR conservation further up the agenda.

8.3 Limitations

Despite the key involvement of conservation organisations in this research, a lack of funding and resources to fully support the implementation of the recommendations made throughout this thesis is a significant limitation. It will be necessary to gain recognition and support at the governmental level to secure a nationwide and long-term commitment to improving CWR conservation across the UK. This process has begun as a result of this project and will continue to be a priority action for the UK Plant Genetic Resources Group – the scientific committee with responsibility for advising government on PGR issues.

The results achieved in any gap analysis study will be highly dependent on the pool of taxa chosen for the analysis and the availability of CWR occurrence record data. This is reflected in the results of the conservation planning work carried out for the UK by Maxted *et al.* (2007) in comparison with the results presented in Chapter 4 (Fielder *et al.*, submitted c). For example, the 2007 complementarity analysis demonstrated that 17 sites would be sufficient to contain two thirds of UK priority CWR, whereas the current study requires 27 to contain close to 100% of the current CWR inventory and only two sites to reach two thirds. In fact, to conserve 100% of 2007 priority CWR for the UK, as many as 69 sites would have been required. The key differences between the method used in 2007 and that used in Chapter 4 are the differing species lists due to different prioritisation methods and the use of lower resolution occurrence data (10km by 10km CWR data points) in the former; a minimum of 2km by 2km CWR data points were used in Chapter 4. In addition, the current study involved devolved administration conservation organisations. Maxted *et al.* (2007) warn that without the inclusion of CWR in the remit of conservation planning, any analysis will only have been an academic exercise. Though the results of the two studies differ substantially, the involvement of conservation agencies in deciding the methodology in the current study has ensured the results are supported and this has already meant that this is more than just an academic exercise.

In addition, though the work carried out to enhance CWR conservation across the UK was supported by NE (England), NRW (Wales) and SNH (Scotland) it did not involve representatives from the Department of the Environment in Northern Ireland. This was because no staff were available to advise on the methodology and prioritisation criteria. This meant that a separate Northern Ireland CWR conservation project could not be undertaken, however CWR within Northern Ireland were included in the full UK analysis. Though no specific sites from Northern Ireland were identified as important in a national UK context for *in situ* conservation of CWR, it is likely that there are CWR of specific value to that administration that would benefit from stakeholder involvement to support the implementation of measures to safeguard CWR genetic diversity.

In the genetic work carried out using AFLP markers on The Lizard a total of eight CWR taxa were sampled of the 93 CWR recorded on the peninsula. Due to time and financial limitations it was not possible to sample more taxa than this without reducing the number of samples per population below 20. Bonin *et al*. (2007) highlight that though some AFLP studies suggest 30 individuals per population is appropriate to give reliable results (Krauss, 2000), most studies fall below this optimum, likely due to resource limitations. As a result, it would not have been appropriate to reduce the number of individual samples collected per population. Therefore, the taxa selected included a range of different life histories to ensure the results were a representative sample of wider CWR genetic diversity on The Lizard. Equally, the same resource limitations prevented the sampling of more populations across the southwest or even extending the study to cover the whole UK. However, the results were sufficient to identify patterns of genetic diversity in all eight CWR and were able to demonstrate the value of CWR on The Lizard site in the context of the southwest of the UK.

The limitation which undoubtedly has had the greatest impact on the work presented here concerns the issues experienced in the use of novel NGS sequencing techniques for improved targeting of CWR collection. While the use of targeted resequencing techniques, such as Roche's Sequence Capture technology introduced in Chapter 7, provide a means of sequencing genomic regions of interest at greater depth of coverage and lower overall costs compared to whole genome resequencing (Metzker, 2010), this technique ultimately proved unsuccessful. Although the costs associated with sequencing projects are plummeting, a limitation remains in the requirement for sufficient expertise to successfully carry out technical laboratory work. The continued failure to produce results through targeted resequencing led to rising project costs as reagents had to be replaced to troubleshoot and repeat protocols. Subsequently, whole genome resequencing became the more financially viable and time efficient option, and has produced a dataset ready for analysis. Further limiting factors in the use of NGS in this project to produce the desired results included, the requirement of expertise in bioinformatics analysis, the provision of hardware capable of processing large datasets and ultimately a lack of time to overcome these obstacles.

8.4 Wider implications – Europe and beyond

All *in situ* conservation efforts ultimately must be implemented at the national level (Maxted *et al*., 2013b) and the research completed here seeks to secure CWR at this scale. The UK is a contracting party to the CBD, it's GSPC and Strategic Plan, as well as the ITPGRFA (CBD, 1992; 2010a; 2010b; FAO, 2001) and so has a strong commitment to delivering biodiversity conservation that includes the protection of CWR and their genetic diversity.

Across Europe, a concept has been presented that integrates the *in situ* conservation of CWR genetic diversity at the national level with the European/regional level (Maxted *et al*., 2013b). The concept proposes a bottom-up approach where CWR are secured at the national level and nationally important populations of European value are identified for inclusion in a European *in situ* conservation network. This works in tandem with a top-down approach whereby populations of European value, not necessarily matching national priorities, are identified and their protection is also established (Maxted *et al*., 2013b). The process undertaken in the UK takes the bottom-up approach and therefore will be easily integrated with a European top-down approach when this is established. Maxted *et al*. (2015b) also emphasise the need for a global level to be added to this concept and they highlight that a consultation process and technical workshop entitled 'Toward establishment of a global network for *in situ* conservation and on-farm management of PGRFA' (FAO, 2014) was held in 2012 where this was discussed and encouraged. The point being that to conserve CWR diversity and maximise its potential use there is a need to fully integrate CWR conservation at national, regional and global levels.

Though the UK is not located in areas of the world with the richest and most genetically diverse CWR (Vavilov, 1926), it is still home to a range of valuable CWR including relatives of brassica, sugar beet, barley and many forage and fodder crops. It is also an example of one of best-studied floras in world (Preston, 2002). The detailed study of UK CWR and their conservation presented in this thesis is a useful case study of how GIS and genetic diversity methodologies can be integrated and used in CWR conservation planning along with the crucial involvement of stakeholders. It is anticipated that the basic approaches taken here can be effectively implemented within any country across the world.

8.5 Future research

1. Following on from establishing conservation of CWR taxonomic and genetic diversity across the UK, an assessment of climate change vulnerability of CWR will be essential to ensure their appropriate conservation management for long-term persistence. Such analysis may involve: a. taxon specific ecological niche modelling (Phillips *et al.*, 2006) to define the current fundamental and realised niches (Hutchinson, 1957) of target CWR, b. a comparison of a taxon's current niche with future climate scenario models to predict the longevity of potential genetic reserve sites (e.g. The Lizard Peninsula), the likely shift and extent of suitable niche environments available as the climate changes (Hijmans and Graham, 2006) and the probability that suitable conditions will remain within the boundaries of sites managed for CWR, and c. consideration of the taxon's life history and range of genetic diversity to understand its capacity to adapt to novel environmental conditions or to migrate to environments matching the conditions within its current niche (Pearson, 2006). Vitt *et al.* (2010) highlight that plant species with short generation times, long-distance seed dispersal mechanisms and high genetic variability are more likely to adapt or migrate successfully as the climate changes. In contrast, species with long generation times, short-distance dispersal mechanisms and limited genetic diversity will have a much higher risk of extinction, especially in increasingly fragmented landscapes (Hannah, 2008; Loss *et al.*, 2011). CWR highly vulnerable to climate change in the UK, identified through ecological niche modelling, should be carefully managed. They

should clearly be sampled and representative diversity held *ex situ* but also in extreme cases where local extinction is likely, assisted migration may be considered appropriate (Vitt *et al*., 2010). However, this conservation technique is controversial, generating much debate (see Hewitt *et al*., 2011 for detailed review) and would require careful monitoring of translocated populations and implementation on a species by species basis (Loss *et al*., 2011).

2. Ecogeographical land characterisation (ELC) maps are a recently developed but valuable tool for revealing patterns in ecogeographic variables (climatic, edaphic and bioclimatic) that shape the distributions of plant species across a landscape (Parra-Quijano *et al*., 2008). Their use has already been demonstrated in improving the ecogeographic (and by proxy, genetic) diversity in genebank collections and the selection of appropriate locations for CWR genetic reserves (Parra-Quijano *et al*., 2012b,c). Most suited to studies of individual species or groups of closely related species, due to the need to select ecogeographic variables that are likely to determine their patterns of adaptation (Parra-Quijano *et al*., 2012b), ELC maps were not suited to the approaches taken within the UK as part of the research presented in this thesis. This was due to the analysis including multiple, distantly related taxa and the need for fine resolution coastal environmental data overlapping with the coastal distributions of many UK CWR (Preston, 2002), which is unavailable in the existing ELC mapping tools (Parra-Quijano *et al*., 2014). However, ELC maps could provide a further insight into the ecogeographic representativeness of the proposed genetic reserve networks for selected individual CWR of particular value or those which are most threatened, to further validate the reserve locations. As discussed in Chapter 7, ELC maps could also be valuable when used in conjunction with genetic diversity data to improve the

targeting of collection of genebank accessions. The recent release of CAPFITOGEN tools make the development of ELC maps relatively simple and accessible to all users (Parra-Quijano *et al*., 2014), overcoming limitations relating to lack of appropriate expertise.

3. The innate use of a CWR is within its genetic diversity. As costs of sequencing continue to fall it would be foolish not to take advantage of this technology to increase our understanding of genetic diversity to aid their conservation and the discovery of useful traits. McCouch (2013) suggests that all non-duplicate plant samples available in genebanks under the ITPGRFA should be sequenced so this information can be combined with passport and phenotypic data in an internationally accessible database to improve conservation and utilisation of CWR. The 3000 rice genomes project (CAAS, IRRI and BGI (2014) has successfully sequenced 3000 rice accessions (landraces), demonstrating that this approach is highly possible and can be applied to CWR genebank collections with relative ease.

4. To complement genetic diversity studies of CWR it would also be advisable to further investigate the extent to which phenotypic plasticity and stress-induced recombination can facilitate survival of populations under changing environmental conditions. Species or individuals able to vary phenotypic traits may have the capacity to buffer the effects of environmental change, increasing their chance of survival, without the need to undergo genetic adaptation (Gratani, 2014). Phenotypic plasticity may however only be a short-term solution and genetic adaptation may ultimately be required for long-term species persistence (Richter *et al*., 2012). Chromosomal recombination has also been demonstrated to increase under stressful environmental conditions, this is particularly apparent in studies by Lebel *et al*. (1993) and Zhong

and Priest (2011), therefore increasing the capacity for genetic adaptation under changing environmental conditions. These are expanding areas of research that deserve further consideration in relation to species survival, adaptability and conservation planning (Forsman, 2014).

5. Further research concerning how CWR can be actively conserved outside of protected areas is necessary. Incorporation of CWR into governmental schemes such as rural development programmes alongside long-term monitoring of CWR populations would enable the evaluation of the efficacy of this approach.

8.6 Conclusion

The United Kingdom may not hold the same range of genetic diversity in CWR as centres of diversity such as the Fertile Crescent in the Middle East (Vavilov, 1926; Vincent *et al*., 2013), however it is still home to a range of economically valuable taxa that are under-conserved *in situ* and under-represented in genebanks. Additionally, the UK has made international commitments to secure its plant genetic resources through the CBD GSPC and Strategic Plan and the ITPGRFA (CBD, 1992; 2010a; 2010b; FAO, 2001).

The research presented in this thesis highlights how CWR conservation can be enhanced throughout the UK using a combination of GIS and genetic diversity methodologies to improve both *in situ* conservation (in genetic reserves, outside of protected areas and in appropriate habitats) and *ex situ* conservation (through more targeted collection of accessions) of CWR. The involvement of relevant stakeholders has proven a key success of this work and emphasises the value of communication between researchers, conservation organisations, governmental departments, germplasm users, etc., which crucially has started to bridge the gap between research and practice; a fundamental principle required to achieve effective conservation and use of CWR.

The approaches taken here are highly repeatable and so could be replicated within other countries in Europe and beyond to further enhance CWR conservation. Equally, it is important that a bottom-up approach is adopted where national CWR conservation efforts such as this, feed into European efforts that ultimately feed into global CWR conservation programmes. Full integration at all levels is essential to secure these genetic resources and the genetic diversity contained within them in all parts of their range.

Only through a comprehensive, integrated and long-term approach to CWR conservation, and their use, can we take positive steps towards feeding an increasing population under evermore challenging climatic conditions, through the development of novel, more resilient crop varieties. Charles Godfray provides a timely warning that ‘if we fail on food, we fail on everything’ (Godfray, 2011). This emphasises the need for a significant change in the attitudes of society as a whole in understanding our environment, and the interactions and impact we have upon it. Nevertheless, in recent years we have seen the release of instrumental policy documents, increasing national, European and global commitments to improved CWR conservation, the rising profile of the value of CWR and the increasing availability and accessibility of tools and methodologies to facilitate CWR conservation and their use. Together, all of these advancements suggest that while we should not be complacent, we can be optimistic about the future and our food security.

References

Ab-Ghaffar, M.B. (2012) *Transcriptomics analysis of phloem-feeding insect resistance in rice germplasm*. Ph.D. University of Birmingham.

Adugna, A., Snow, A.A., Sweeney, P.M., Bekele, E. and Mutegi, E. (2013) Population genetic structure of *in situ* wild Sorghum bicolor in its Ethiopian center of origin based on SSR markers. *Genetic Resources and Crop Evolution* 60: 1313–1328.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. (2004) Emerging infectious diseases in plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* 19: 535–544.

Andrews, J.R. (1998) The Lizard Complex. In: Selwood, E.B., Durrance, E.M. and Bristow, C.M. (eds.), *The Geology of Cornwall*. University of Exeter Press, Exeter. Pp. 21–30.

Ben, C., Toueni, M., Montanari, S., Tardin, M-C., Fervel, M., Negahi, A., Saint-Pierre, L., Mathieu, G., Gras, M-C., Noël, D., Prospéri, J-M., Pilet-Nayel, M-L., Baranger, A., Huguet, T., Julier, B., Rickauer, M. and Gentzbittel, L. (2012) Natural diversity in the model legume *Medicago truncatula* allows identifying distinct genetic mechanisms conferring partial resistance to *Verticillium* wilt. *Journal of Experimental Botany* 64(1): 317–332.

Berlingeri, C. and Crespo, M.B. (2012) Inventory of related wild species of priority crops in Venezuela. *Genetic Resources and Crop Evolution* 59: 655–681. doi: 10.1007/s10722-011-9709-2

Biancardi, E., Lewellen, R.T., de Biaggi, M., Erichsen, A.W. and Stevanato, P. (2002) The origin of rhizomania resistance in sugar beet. *Euphytica* 127: 383–397.

Bijlsma, R., Bundgaard, J. and Boerema, A.C. (2000) Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *Journal of Evolutionary Biology* 13: 502–514.

Bijlsma, R. and Loeschcke, V. (2012) Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications* 5: 117–129.

Bilz, M., Kell, S.P., Maxted, N. and Lansdown, R.V. (2011) *European Red List of Vascular Plants*. Luxembourg: Publications Office of the European Union.

Blows, M.W. and Hoffmann, A.A. (2005) A reassessment of genetic limits to evolutionary change. *Ecology* 86: 1371–1384.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C. and Taberlet, P. (2004) How to track and assess genotyping errors in population genetic studies. *Molecular Ecology* 13: 3261–3273.

Bonin, A., Ehrich, D. and Manel, S. (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16: 3737–3758.

Boon, C. and Outen, A.R. (2011) *Flora of Bedfordshire*. Bedfordshire Natural History Society, UK.

Braithwaite, M.E., Ellis, R.W. and Preston, C.D. (2006) *Change in the British Flora 1987–2004*. Botanical Society of the British Isles, London.

Branca, A., Paape, T.D., Zhou, P., Briskine, R., Farmer, A.D., Mudge, J., Bharti, A.K., Woodward, J.E., May, G.D., Gentzbittel, L., Ben, C., Denny, R., Sadowsky, M.J., Ronfort, J., Bataillon, T., Young, N.D. and Tiffin, P. (2011) Whole-genome nucleotide diversity, recombination and linkage disequilibrium in the model legume *Medicago truncatula*. *PNAS* 108(42): E864–E870.

Branca, F., Argento, S. and Tribulato, A. (2012) Assessing genetic reserves in Sicily (Italy): The *Brassica* wild relatives case study. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford, UK. Pp. 52–58.

British Flora (2014) *Online atlas of the British and Irish flora*. [online] Available at: http://www.brc.ac.uk/plantatlas/

Brown, A.H.D. and Briggs, J.D. (1991) Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. In: Falk, D.A. and Holsinger, K.E. (eds.), *Genetics and conservation of rare plants*. Oxford University Press, New York. Pp. 99–199.

Brown, A.H.D. and Schoen, D.J. (1992) Plant population genetic structure and biological conservation. In: Sandlund, O.T., Hindar, K., and Brown, A.H.D. (eds.), *Conservation of Biodiversity for Sustainable Development*. Oxford University Press, London.

Brown, A.H.D. and Marshall, D.R. (1995) A basic sampling strategy: theory and practice. In: Guarino, L., Ramantha Rao, V. and Reid, R. (eds.), *Collecting plant genetic diversity: Technical guidelines*. CABI Publishing, Wallingford. Pp. 75–92.

BSBI (2013) *BSBI Distribution Database.* Botanical Society of Britain and Ireland. [online] Available at: http://bsbidb.org.uk

Bunce, R.G.H., Barr, C.J., Gillespie, M.K., Howard, D.C., Scott, W.A., Smart, S.M., van de Poll, H.M. and Watkins, J.W. (1999) *Vegetation of the British Countryside - The Countryside Vegetation System*. Department of the Environment, Transport and the Regions, London.

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K.E., Carr, G.M., Chanson, J., Chenery, A.M., Csirke, J., Davidson, N.C., Dentener, F., Foster, M., Galli, A., Galloway, J.N., Genovesi, P., Gregory, R.D., Hockings, M., Kapos, V., Lamarque, J-F., Leverington, F., Loh, J., McGeoch. M.A., McRae, L., Minasyan, A., Hernández Morcillo, M., Oldfield, T.E.E., Pauly, D., Quader, S., Revenga, C., Sauer, J.R., Skolnik, B., Spear, D., Stanwell-Smith, D., Stuart, S.N., Symes, A., Tierney, M., Tyrrell, T.D., Vié, J-C. and Watson, R. (2010) Global biodiversity: Indicators of recent declines. *Science* 328: 1164–1168.

BWA (2013) *Burrows-Wheeler Alignment Tool – Manual references pages*. [online] Available at: http://bio-bwa.sourceforge.net/bwa.shtml

CAAS, IRRI and BGI (2014) The 3,000 rice genomes project. *Giga Science* 3: 7.

Camadro, E.L. (2012) Relevance of the genetic structure of natural populations, and sampling and classification approaches for conservation and use of wild crop relatives: potato as an example. *Botany* 90: 1065–1072.

Caradus, J.R. (1995) White clover *Trifolium repens* L. In: Smartt, J. and Simmonds, N.W. (eds.), *Evolution of crop plants*. Longman Group, UK. Pp. 306–312.

Carey, P.D., Wallis, S., Chamberlain, P.M., Cooper, A., Emmett, B.A., Maskell, L.C., McCann, T., Murphy, J., Norton, L.R., Reynolds, B., Scott, W.A., Simpson, I.C., Smart, S.M. and Ullyett, J.M. (2008) *Countryside Survey: UK results from 2007*. NERC/Centre for Ecology and Hydrology.

Carrasco, A., de Galarreta, J.R., Rico, A. and Ritter, E. (2000) Transfer of PLRV resistance from *Solanum verrucosum* Schlechdt to potato (*S. tuberosum* L.) by protoplast electrofusion. *Potato research* 43: 31–42.

Castañeda-Álvarez, N.P., de Haan, S., Juárez, H., Khoury, C.K., Achicanoy, A., Sosa, C.C., Bernau, V., Salas, A., Heider, B., Simon, R., Maxted, N. and Spooner, D.M. (2015) *Ex Situ* Conservation Priorities for the Wild Relatives of Potato (*Solanum* L. Section *Petiola*). *PLOS One* 10(4): e0122599. doi:10.1371/journal.pone.0122599

CBD (1992) *Convention on Biological Diversity*. Secretariat of the Convention on Biological Diversity. Montreal, Canada.

CBD (2002) *Strategic Plan for the Convention on Biological Diversity 2002–2010*. Secretariat of the Convention on Biological Diversity. Montreal, Canada.

CBD (2010a) *Global Strategy for Plant Conservation*. Secretariat of the Convention on Biological Diversity. Montreal, Canada.

CBD (2010b) *Strategic Plan for Biodiversity 2011–2020*. Secretariat of the Convention on Biological Diversity. Montreal, Canada.

CCRA (2012) *UK Climate Change Risk Assessment: Government Report*. Defra, HM Government. [online] Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69487/pb13698-climate-risk-assessment.pdf

Chandra, A., Gupta, M.L., Banga, S.S. and Banga, S.K. (2004) Production of an interspecific hybrid between *Brassica fruticulosa* and *B. rapa*. *Plant breeding* 123: 497–498.

Chape, S., Harrison, J., Spalding, M. and Lysenko, I. (2005) Measuring the extent and effectiveness of protected areas as an indicator for meeting global biodiversity targets. *Philosophical transactions of the royal society B* 360: 443–455.

Charlesworth, D. and Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

Chase, M.W. and Hills, H.H. (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

Cheffings, C.M. and Farrell, L. (eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. (2005) *The Vascular Plant Red Data List for Great Britain*. Species Status 7: 1–116. Joint Nature Conservation Committee, Peterborough.

Chuda, A. and Adamus, A. (2009) Aspects of interspecific hybridization within edible Alliaceae. *Acta Physiologiae Plantarum* 31: 223–227.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A.J. and Wehner, M. (2013) Long-term Climate Change: Projections, Commitments and Irreversibility. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K. Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Coombe, D.E. (1961) *Trifolium occidentale*, a new species related to *T. repens* L. *Watsonia* 5: 68–87.

Critchlow-Watton, N., Dobbie, K.E., Bell, R., Campbell, S.D.G., Hinze, D., Motion, A., Robertson, K., Russell, M., Simpson, J., Thomson, D. and Towers, W. (eds.) (2014) *Scotland's State of the Environment Report. Scotland's Environment Web*. [online] Available at: http://www.environment.scotland.gov.uk/media/92572/state-of-environment-report-2014.pdf

Crossa, J. and Vencovsky, R. (2011) Basic sampling strategies: theory and practice. In: Guarino, L., Ramanatha Rao, V. and Goldberg, E. (eds.), *Collecting Plant Genetic Diversity: Technical Guidelines – 2011 Update*. Bioversity International, Rome, Italy. Ch. 5.

Cureton, A.N., Newbury, H.J., Raybould, A.F. and Ford-Lloyd, B.V. (2006) Genetic structure and gene flow in wild beet populations: the potential influence of habitat on transgene spread and risk assessment. *Journal of Applied Ecology* 43: 1203–1212.

Davies, P.A., Lulsdorf, M.M. and Ahmad, M. (2007) Wild relatives and biotechnological approaches. In: Yadav, S.S., McNeil, D.L. and Stevenson, P.C. (eds.), *Lentil: An Ancient Crop for Modern Times*. Springer, The Netherlands. Pp. 225–240.

Defra (2010) *Agriculture in the UK 2010*. Department for Environment, Food and Rural Affairs.

Defra (2011) *Biodiversity 2020: A strategy for England's wildlife and ecosystem services*. Department for Environment, Food and Rural Affairs. London, UK.

Defra (2013) *UK Biodiversity Indicators in your pocket 2013*. Department for Environment, Food and Rural Affairs. London, UK. [online] Available at: http://jncc.defra.gov.uk/page-4229

Defra and JNCC (on behalf of the Four Countries' Biodiversity Group) (2012) *UK Post-2010 Biodiversity Framework*. JNCC, Peterborough. [online] Available at: http://jncc.defra.gov.uk/pdf/UK_Post2010_Bio-Fwork.pdf

Dias, S., Dulloo, M.E. and Arnaud, E. (2012) The role of EURISCO in promoting use of agricultural biodiversity. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 270–277.

Dines, T. (2008) *A Vascular Plant Red Data List for Wales*. Plantlife International, Salisbury. [online] Available at: http://www.bsbi.org.uk/Welsh_Red_List.pdf

DOENI (2002) *Northern Ireland Biodiversity Strategy*. Department of the Environment for Northern Ireland. [online] Available at: http://www.doeni.gov.uk/niea//nibs2002.pdf

DOENI (2011) *Conserving Biodiversity: Biodiversity Lists*. Department of the Environmental Northern Ireland. [online] Available at: http://www.doeni.gov.uk/niea/biodiversity/sap_uk.htm

DOENI (2012) *Digital Datasets*. Department of the Environment for Northern Ireland. [online] Available at: http://www.doeni.gov.uk/niea/other-index/digital-intro.htm

Dostálek, J. and Frantík, T. (2012) The impact of different grazing periods in dry grasslands on the expansive grass *Arrhenatherum elatius* L. and on woody species. *Environmental Management* 49: 855–861.

Dulloo, M.E., Labokas, J., Iriondo, J.M., Maxted, N., Lane, A., Laguna, E., Jarvis, A. and Kell, S.P. (2008) Genetic Reserve Location and Design. In: Iriondo, J.M., Maxted, N. and Dulloo, E. (eds.), *Conserving Plant Genetic Diversity in Protected Areas*. CABI Publishing, Wallingford. Pp. 23–64.

Ehlenfeldt, M.K. and Ballington, J.R. (2012) *Vaccinium* species of section *Hemimyrtillis*: their value to cultivated blueberry and approaches to utilization. *Botany* 90: 347–353.

Ernst, W.H.O. (1979) Population biology of *Allium ursinum* in northern Germany. *Journal of Ecology* 67: 347–362.

Esquinas-Alcázar, J. (2005) Protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nature Reviews Genetics* 6: 946–953.

ESRI (2011) *ArcGIS Desktop Release 10*. Environmental Systems Research Institute, Redlands CA.

European Union (2011) *The EU biodiversity strategy to 2020*. European Commission. [online] Available at: http://ec.europa.eu/environment/nature/info/pubs/docs/brochures/2020%20Biod%20brochure%20final%20lowres.pdf

Eurostat (2013) *European Commission*. [online] Available at: http://epp.eurostat.ec.europa.eu

Evans, N., Baierl, A., Semenov, M.A., Gladders, P. and Fitt, B.D.L. (2008) Range and severity of a plant disease increased by global warming. *Journal of the Royal Society Interface* 5: 525–531.

Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. *Genetics* 131: 479–491.

FAO (1997) *The state of the world's plant genetic resources for food and agriculture*. Food and Agriculture Organization of the United Nations, Rome.

FAO (2001) *International Treaty on Plant Genetic Resources for Food and Agriculture*. Food and Agriculture Organisation of the United Nations.

FAO (2009) *Country report on the state of plant genetic resources for food and agriculture: United Kingdom*. Department for Environment, Food and Rural Affairs, London.

FAO (2010) *The second report on the state of the world's plant genetic resources for food and agriculture*. Commission on genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome.

FAO (2011) *Second global plan of action for plant genetic resources for food and agriculture*. Commission on genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome.

FAO (2014) *Concept note on global networking on* in situ *conservation and on-farm management of plant genetic resources for food and agriculture*. Information document to the 7th Session of the Intergovernmental Working Group on Plant Genetic Resources for Food and Agriculture (CGRFA/WG-PGR-7/14/Inf.3), Commission for Genetic Resources for Food and Agriculture, Rome, Italy. [online] Available at: http://www.fao.org/3/a-ml477e.pdf

FAO (2015) *Coping with climate change – the roles of genetic resources for food and agriculture*. Commission on genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome.

FAOSTAT (2013) *Agricultural Crop Production Data*. [online] Available at: http://faostat3.fao.org/home/E

Ferguson, M.E., Ford-Lloyd, B.V., Robertson, L.D., Maxted, N. and Newbury, H.J. (1998) Mapping the geographical distribution of genetic variation in the genus *Lens* for the enhanced conservation of plant genetic diversity. *Molecular Ecology* 7: 1743–1755.

Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J., Ford-Lloyd, B. and Maxted, N. (2015) Enhancing the conservation of crop wild relatives in England. *PLOS One* 10: e0130804. doi: 10.1371/journal.pone.e0130804

Fielder, H., Burrows, C., Woodman, J., Ford-Lloyd, B. and Maxted, N. (submitted a) Enhancing the conservation of crop wild relatives in Wales. *New Journal of Botany*.

Fielder, H., Smith, C., Ford-Lloyd, B. and Maxted, N. (submitted b) Enhancing the conservation of crop wild relatives in Scotland. *Journal for Nature Conservation*.

Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J, Burrows, C., Woodman, J., Smith, C., Ford-Lloyd, B. and Maxted, N. (submitted c) Enhancing the conservation of crop wild relatives in the United Kingdom. *Botanical Journal of the Linnean Society*.

Fielder, H., Brotherton, P., Hopkins, J., Hosking, J., Ford-Lloyd, B. and Maxted, N. (submitted d) A genetic assessment of the crop wild relatives of the Lizard Peninsula, Cornwall and its suitability to become the first UK genetic reserve. *Journal of Applied Ecology*.

Fitzgerald, H. (2013) *The national crop wild relative strategy report for Finland*. MTT, Report 121. 95 pp. [online] Available at: http://jukuri.mtt.fi/bitstream/handle/10024/481549/mttraportti121.pdf

Flowers, T.J. and Flowers, S.A. (2005) Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management* 78: 15–24.

Fondevilla, S., Torres, A.M., Moreno, M.T. and Rubiales, D. (2007) Identification of a new gene for resistance to powdery mildew in *Pisum fulvum*, a wild relative of pea. *Breeding Science* 57: 181–184.

Ford-Lloyd, B.V., Kell, S.P. and Maxted, N. (2008). Establishing conservation priorities for crop wild relatives. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 110–119.

Ford-Lloyd, B.V., Schmidt, M., Armstrong, S.J., Barazani, O., Engels, J., Hadas, R., Hammer, K., Kell, S.P., Kang, D.M., Khoshbakht, K., Li, Y., Long, C., Lu, B-R., Ma, K., Nguyen, V.T., Qiu, L., Ge, S., Wei, W., Zhang, Z. and Maxted, N. (2011) Crop Wild Relatives—Undervalued, Underutilized and under Threat? *BioScience* 61: 559–565.

Ford-Lloyd, B., Engels, J.M.M. and Jackson, M. (2014) Genetic resources and conservation challenges under the threat of climate change. In: Jackson, M., Ford-Lloyd, B. and Parry, M. (eds.), *Plant Genetic Resources and Climate Change*. CABI Publishing, Wallingford. Pp. 16–37.

Forestry Commission (2014) *Chalara dieback of ash* (Chalara fraxinea). Forestry Commission. [online] Available at: http://www.forestry.gov.uk/chalara#spread

Forsman, A. (2014) Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* 1–9. doi: 10.1038/hdy.2014.92

Frankel, O.H. and Soulé, M.E. (1981) *Conservation and Evolution*. Cambridge University Press, New York.

Frankel, O.H., Brown, A.H.D. and Burdon, J.J. (eds.) (1995) *The conservation of plant biodiversity*. Cambridge University Press, Cambridge, UK.

Franks, J.R. (1999) *In situ* conservation of plant genetic resources for food and agriculture: a UK perspective. *Land Use Policy* 16: 81–91.

Frese, L., Nachtigall, M., Enders, M. and Pinheiro de Carvalho, M.Â.A. (2012) Beta patula Aiton: genetic diversity analysis. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 45–51.

Garg, R., Verma, M., Agrawal, S., Shankar, R., Majee, M. and Jain, M. (2014) Deep transcriptome sequencing of wild halophyte rice, *Porteresia coarctata*, provides novel insights into the salinity and submergence tolerance factors. *DNA Research* 21: 69–84.

Gelman, A., Carlin, J.B., Stern, H.S. and Rubin, D.B. (2004) *Bayesian data analysis*. Chapman and Hall/CRC, Boca Raton, FL.

Ghamkhar, K., Snowball, R. and Bennett, S.J. (2007) Ecogeographical studies identify diversity and potential gaps in the largest germplasm collection of bladder clover (*Trifolium spumosum* L.). *Australian Journal of Agricultural Research* 58: 728–738.

Gilpin, M.E. and Soulé, M.E. (1986) Minimum viable populations: The processes of species extinctions. In: Soulé, M.E. (ed.), *Conservation biology: The science of scarcity and diversity*. Sinauer Associates, Sunderland Mass. Pp. 13–34.

Godfray, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C. (2010) Food security: The challenge of feeding 9 billion people. *Science* 237: 812–818.

Godfray, H.C.J. (2011) Food for thought. *PNAS* 108(50): 19845:19846.

González-Orozco, C.E., Brown, A.H.D., Knerr, N., Miller, J.T. and Doyle, J.J. (2012) Hotspots of diversity of wild Australian soybean relatives and their conservation *in situ*. *Conservation Genetics* 13: 1269–1281.

Goodman, R.M., Hauptli, H., Crossway, A. and Knauf, V.C. (1987) Gene transfer in crop improvement. *Science* 236: 48–54.

Government Office for Science (2011) *Foresight. The Future of Food and Farming*. Final Project Report. The Government Office for Science, London. [online] Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/288329/11-546-future-of-food-and-farming-report.pdf

Grando, S., von Bothmer, R. and Ceccarelli, S. (2001) Genetic diversity of barley: use of locally adapted germplasm to enhance yield and yield stability of barley in dry areas. In: Cooper, H.D., Spillane, C. and Hodgink, T. (eds.), *Broadening the Genetic Base of Crop Production*. CABI/FAO/IPRI, New York, USA/Rome, Italy/Rome, Italy. Pp. 351–372.

Gratani, L. (2014) Plant phenotypic plasticity in response to environmental factors. *Advances in Botany* 2014(4): 1–17. doi: 10.1155/2014/208747

Green, N., Campbell, G., Tulloch, R. and Scholten, M. (2009) Scottish Landrace Protection Scheme. In: Veteläinen, M., Negri, V. and Maxted, N. (eds.), *European landraces: on-farm conservation, management and use*. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy. Pp. 233–243.

Greene, S.L., Kisha, T.J., Yu, L. and Parra-Quijano, M. (2014) Conserving plants in gene banks and nature: investigating complementarity with *Trifolium thompsonii* Morton. *PLOS One* 9: e105145.

Gregory, P.J. and Marshall, B. (2012) Attribution of climate change: a methodology to estimate the potential contribution to increases in potato yield in Scotland since 1960. *Global Change Biology* 18: 1372–1388.

Grenier, C., Bramel-Cox, P.J. and Hamon, P. (2001) Core collection of sorghum: I. Stratification based on eco-geographical data. *Crop Science* 41: 234–240.

Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* 111: 1169–1194.

Grimmer, M.K., Trybush, S., Hanley, S., Francis, S.A., Karp, A. and Asher, M.J.C. (2007) An anchored linkage map for sugar beet based on AFLP, SNP and RAPD markers and QTL mapping of a new source of resistance to Beet necrotic yellow vein virus. *Theoretical and Applied Genetics* 114: 1151–1160.

Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* 33: 1–22.

Hajjar, R. and Hodgkin, T. (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156: 1–13.

Hamrick, J.L. and Godt, M.J.W. (1989) Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L. and Weir, B.S. (eds.), *Plant population genetics, breeding and genetic resources*. Sinauer Associates inc., Sunderland, Massachusetts. Pp 43–63.

Hamrick, J.L. and Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions: Biological Sciences* 351: 1291–1298.

Hannah, L. (2008) Protected areas and climate change. *Annals of the New York Academy of Sciences* 1134: 201–212.

Hargreaves, S., Maxted, N., Hirano, R., Abberton, M., Skøt, L. and Ford-Lloyd, B.V. (2010) Islands as refugia of *Trifolium repens* genetic diversity. *Conservation Genetics* 11: 1317–1326.

Hargreaves, S. (2011) *Genetic diversity studies of* Trifolium *species from the extremes of the UK*. PhD thesis, University of Birmingham.

Harlan, J.R. and de Wet, J.M.J. (1971) Toward a rational classification of cultivated plants. *Taxon* 20: 509–517.

Hawkes, J.G., Maxted, N. and Ford-Lloyd, B.V. (2000) *The* ex situ *conservation of plant genetic resources*. Springer, Netherlands.

Henry, R.J. (2014) Genomic strategies for germplasm characterization and the development of climate resilient crops. *Frontiers in Plant Science* 5: 1–4.

Herden, T., Neuffer, B. and Friesen, N. (2012) *Allium ursinum* L. in Germany – surprisingly low genetic variability. *Feddes Repertorium* 123: 81–95.

Hewitt, N., Klenk, N., Smith, A.L., Bazely, D.R., Yan, N., Wood, S., MacLellan, J.I., Lipsig-Mumme, C. and Henriques, I. (2011) Taking stock of the assisted migration debate. *Biological Conservation* 144: 2560–2572.

Heywood, V.H. and Zohary, D. (1995) A catalogue of the wild relatives of cultivated plants native to Europe. *Flora Mediterranea* 5: 375–415.

Heywood, V.H. and Dulloo, M.E. (2006) In Situ *Conservation of Wild Plant Species – a Critical Global Review of Good Practices*. IPGRI Technical Bulletin No. 11. IPGRI, Rome.

Hijmans, R.J. and Spooner, D.M. (2001) Geographic distribution of wild potato species. *American Journal of Botany* 88(11): 2101–2112.

Hijmans, R.J. and Graham, C.H. (2006) The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Change Biology* 12: 2272–2281.

Hijmans, R.J., Guarino, G. and Mathur, P. (2012) *DIVA-GIS Version 7.5*. Manual [online] Available at: http://www.diva-gis.org/documentation

HLPE (2012) *Food security and climate change*. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome. [online] Available at: www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/HLPE_Reports/HLPE-Report-3-Food_security_and_climate_change-June_2012.pdf

Hoban, S. and 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.

Hoffman, D.L., Chong, J., Jackson, E.W. and Obert, D.E. (2006) Characterization and mapping of a crown rust resistance gene complex (*Pc58*) in TAM O-301. *Crop science* 46: 2630–2635.

Höglund, J. (2009) *Evolutionary conservation genetics*. Oxford University Press, New York.

Hong, T.D., Ellis, R.H. and Linington, S. (1998) *Compendium of information on seed storage behaviour*. The Royal Botanic Gardens.

Hopkins, J.J. (1983) *Studies of the Historical Ecology, Vegetation and Flora of the Lizard District, Cornwall, with Particular Reference to Heathland.* PhD thesis, Bristol University.

Hopkins, J. and Maxted, N. (2011) *Crop wild relatives: plant conservation for food security*. Natural England, Peterborough, UK.

Hunter, D. and Heywood, V. (eds.) (2011) *Crop Wild relatives. A Manual of* In Situ *Conservation*. Earthscan, London, Washington DC.

Hutchinson, G.E. (1957) Concluding remarks. In: *Cold Spring Harbor Symposia on Quantitative Biology* 22: 415–427.

Idohou, R., Ephrem Assogbadjo, A., Fandohan, B., Nounagnon Gouwakinnou, G., Glele Kakai, R.L., Sinsin, B. and Maxted, N. (2012) National inventory and prioritization of crop wild relatives: case study for Benin. *Genetic Resources and Crop Evolution* 60(4): 1337–1352. doi: 10.1007/s10722-012-9923-6

IHGC International Human Genome Consortium (2004) Finishing the euchromatic sequence of the human genome. *Nature* 431: 931–945.

Illumina (2012) *cBot User Guide*. Part# 15006165 Rev. K. Illumina. [online] Available at: http://supportres.illumina.com/documents/myillumina/45afc37e-10de-4ad5-9bc3-ee4ed3431bce/cbot_ug_15006165_k_public.pdf

Illumina (2013) *TruSeq® DNA PCR-Free Sample Preparation Guide*. Part# 15036187 Rev. B. Illumina. [online] Available at: http://supportres.illumina.com/documents/documentation/chemistry_documentation/samplepreps_truseq/truseqdnapcrfree/truseq-dna-pcr-free-sample-prep-guide-15036187-b.pdf

Illumina (2014) *HiSeq® 2500 System User Guide*. Part# 15035786 Rev. D. Illumina. [online] Available at: http://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/hiseq2500/hiseq-2500-user-guide-15035786-d.pdf

Illumina (2015a) *HiSeq 2500 Specifications*. Illumina [online] Available at: http://www.illumina.com/systems/hiseq_2500_1500/performance_specifications.html

Illumina (2015b) *An introduction to Next-Generation Sequencing Technology*. Publication Number: 770-2012-008. Illumina. [online] Available at: http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Iriondo, J.M., Dulloo, E. and Maxted, N. (eds.) (2008a) *Conserving Plant Genetic Diversity in Protected Areas: Population Management of Crop Wild Relatives*. CABI Publishing, Wallingford.

Iriondo, J.M., Ford-Lloyd, B., De Hond, L., Kell, S.P., Lefèvre, F., Korpelainen, H. and Lane, A. (2008b) Plant population monitoring methodologies for the *in situ* genetic conservation of CWR. In: Iriondo, J.M., Maxted, N. and Dulloo, M.E. (eds.), *Conserving Plant Genetic Diversity in Protected Areas*. CABI Publishing, Wallingford. Pp. 88–123.

Iriondo, J.M., Maxted, N., Kell, S.P., Ford-Lloyd, B.V., Lara-Romano, C., Labokas, J. and Magos Brehm, J. (2012) Quality standards for genetic reserve conservation of crop wild relatives. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and

Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 72–77.

Iriondo, J.M., Fielder, H., Fitzgerald, H., Kell, S.P., Labokas, J., Magos-Brehm, J., Negri, V., Phillips, J., Rubio-Teso, M.L., Sensen, S., Taylor, N. and Maxted, N. (2015) National Strategies for the Conservation of CWR. In: Maxted, N., Dulloo, M.E. and Ford-Lloyd, B.V. (eds.), *Enhancing Crop Genepool Use: Capturing wild relative and landrace diversity for crop improvement*. CABI Publishing, Wallingford. Pp. XX-XX.

IRRI (2013) *Wild parent spawns super salt-tolerant rice*. International Rice Research Institute [online] Available at: http://irri.org/news/media-releases/wild-parent-spawns-super-salt-tolerant-rice

IUCN (2012) *The IUCN Red List of Threatened Species*. Version 2012.1. [online] Available at: http://www.iucnredlist.org

Jackson, A. (1995) The Plymouth pear – the recovery programme for one of Britain's rarest trees. *British Wildlife* 6: 273–278.

Jackson, M.T. and Ford-Lloyd, B.V. (1990) Plant genetic resources–A perspective. In: Jackson, M.T., Ford-Lloyd, B.V. and Parry, M.L. (eds.), *Climatic Change and Plant Genetic Resources*. Belhaven Press, London. Pp. 1–17.

Jaggard, K.W., Qi, A. and Semenov, M.A. (2007) The impact of climate change on sugarbeet yield in the UK: 1976–2004. *Journal of Agricultural Science* 145: 367–375.

Jain, H.K. (1988) Plant genetic resources and policy. *Trends in Biotechnology* 6(3): 73–77.

Jain, S.K. (1975) Genetic reserves. In: Frankel, O.H. and Hawkes, J.G. (eds), *Crop genetic resources for today and tomorrow*. Cambridge University Press, Cambridge, UK. Pp. 379–396.

Jarvis, A., Williams, K., Williams, D., Guarino, L., Caballero, P.J. and Mottram, G. (2005) Use of GIS for optimizing a collecting mission for a rare wild pepper (*Capsicum flexuosum* Sendtn.) in Paraguay. *Genetic Resources and Crop Evolution* 52: 671–682.

Jarvis, A., Upadhyaya, H., Gowda, C.L.L., Aggarwal, P.K., Fujisaka, S. and Anderson, B. (2008) *Climate change and its effect on conservation and use of plant genetic resources for food and agriculture and associated biodiversity for food security*. Food and Agriculture Organisation. [online] Available at: http://www.fao.org/docrep/013/i1500e/i1500e16.pdf

Jarvis, S., Fielder, H., Hopkins, J.J., Maxted, N. and Smart, S. (in press) Distribution of crop wild relatives of conservation priority in the UK landscape. *Biological Conservation*.

JNCC (2011) *Conservation designations for UK taxa*. Joint Nature Conservation Committee, Peterborough. [online] Available at: http://jncc.defra.gov.uk/page-3408

JNCC (2013) *Guidelines for the selection of biological SSSIs. Part 1: Rationale, Operational Approach and Criteria for Site Selection*. Joint Nature Conservation Committee.

JNCC (2014) *Designations associated with status based lists*. Joint Nature Conservation Committee. [online] Available at: http://jncc.defra.gov.uk/page-3425

Johnson, R.C., Kisha, T.J., Pecetti, L., Romani, M. and Richter, P. (2011) Characterization of *Poa supina* from the Italian Alps with AFLP markers and correlation with climatic variables. *Crop Science* 51: 1627–1636.

Jones, P.G., Beebe, S.E., Tohme, J. and Galwey, N.W. (1997) The use of geographical information systems in biodiversity exploration and conservation. *Biodiversity and Conservation* 6: 947–958.

KAPA (2011) *KAPA Library Quantification Kits for Illumina Sequencing Platforms – Technical Data Sheet v4.11*. Boston, USA and Cape Town, South Africa: KAPA Biosystems.

Kay, Q.O.N. and John, R. (1995) *The conservation of scarce and declining species in lowland Wales: population genetics, demographic ecology and recommendations for future conservation in 32 species of lowland grassland and related habitats: CCW Science Report No. 110*. Countryside Council for Wales, Bangor.

Kell, S.P., Knüpffer, H., Jury, S.L., Maxted, N. and Ford-Lloyd, B.V. (2005) *Catalogue of Crop Wild Relatives for Europe and the Mediterranean*. Available online via the PGR Forum Crop Wild Relative Information System (CWRIS – http://www.pgrforum.org/cwris/cwris.asp) and on CD-ROM. © University of Birmingham, UK.

Kell, S.P., Knupffer, H., Jury, S.L., Ford-Lloyd, B.V. and Maxted, N. (2008) Crops and wild relatives of the Euro-Mediterranean region: making and using a conservation catalogue. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 69–109.

Kell, S.P., Maxted, N. and Bilz, M. (2012) European crop wild relative threat assessment: knowledge gained and lessons learnt. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 218–242.

Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W. and Maxted, N. (2015) China's crop wild relatives: Diversity for agriculture and food security. *Agriculture, Ecosystems and Environment* doi:10.1016/j.agee.2015.02.012

Khoury, C.K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A. and Struik, P.C. (2013) An inventory of crop wild relatives of the United States. *Crop Science* 53: 1–13. doi: 10.2135/cropsci2012.10.0585

Khoury, C.K., Bjorkman, A.D., Dempewolf, H., Ramírez-Villegas, J., Guarino, L., Jarvis, A., Rieseberg, L.H. and Struik, P.C. (2014) Increasing homogeneity in global food supplies and the implications for food security. *PNAS* 111: 4001–4006.

Khoury, C.K., Heider, B., Castañeda-Álvarez, N.P., Achicanoy, H.A., Sosa, C.C., Miller, R.E., Scotland, R.W., Wood, J.R.I., Rossel, G., Eserman, L.A., Jarret, R.L., Yencho, G.C., Bernau, V., Juarez, H., Sotelo, S., de Hann, S. and Struik, P.C. (2015) Distributions, *ex situ* conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato

[*Ipomoea batatas* (L.) Lam., *I.* series *Batatas*]. *Frontiers in Plant Science* 6(251). doi: 10.3389/fpls.2015.00251

Kilian, B. and Graner, A. (2012) NGS technologies for analyzing germplasm diversity in genebanks. *Briefings in Functional Genomics* 2(1): 38–50.

King, H. and Edwards, B. (2007) Hand pollination of a single female wild asparagus *Asparagus prostratus* plant near Ferrybridge in Dorset, using pollen taken from plants in Cornwall, southwest England. *Conservation Evidence* 4: 73–76.

King, H., Rich, T.C. and Cordrey, L. (2007) Comparing hand pollination success with natural pollination of wild asparagus *Asparagus prostratus*, at colonies on The Gower, Pembrokeshire, west Wales, and in Cornwall, south-west England. *Conservation Evidence* 4: 69–72.

Krauss, S.L. (2000) Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology* 9: 1241–1245.

Lakew, B., Eglinton, J., Henry, R.J., Baum, M., Grando, S. and Ceccarelli, S. (2011) The potential contribution of wild barley (*Hordeum vulgare* ssp. *spontaneum*) germplasm to drought tolerance of cultivated barley (*H. vulgare* ssp. *vulgare*). *Field Crops Research* 120: 161–168.

Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D.L., Garcia-Hernandez, M., Karthikeyan, A.S., Lee, C.H., Nelson, W.D., Ploetz, L., Singh, S., Wensel, A. and Huala, E. (2011) The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Research* 2011: 1–9. doi:10.1093/nar/gkr1090

Landucci, F., Panella, L., Lucarini, D., Gigante, D., Donnini, D., Kell, S., Maxted, N., Venanzoni, R. and Negri, V. (2014) A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop Science* 54: 1628–1644.

Lebel, E.G., Masson, J., Bogucki, A. and Paszkowski, J. (1993) Stress-induced intrachromosomal recombination in plant somatic cells. *PNAS USA* 90: 422–426.

Levsen, N.D., Crawford, D.J., Archibald, J.K., Santos-Geurra, A. and Mort, M.E. (2008) Nei's to Bayes': comparing computational methods and genetic markers to estimate patterns of genetic variation in *Tolpis* (Asteraceae). *American Journal of Botany* 95: 1466–1474.

Lewellen, R.T., Skoyen, L.O. and Erichsen, A.W. (1987) *Breeding sugarbeet for resistance to rhizomania: Evaluation of host-plant reactions and selection for and inheritance of resistance*. Proceedings of the 50th Congress of the IIRB (International Institute for Beet Research), Brussels. Pp. 139–156.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* 25: 2078–2079.

Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L. and Law, M. (2012) Comparison of Next-Generation Sequencing Systems. *Journal of Biomedicine and Biotechnology* 2012: 251364. doi:10.1155/2012/251364

Loreau, M., Oteng-Yeboah, A., Arroyo, M.T.K., Babin, D., Barbault, R., Donoghue, M., Gadgil, M., Häuser, C., Heip, C., Larigauderie, A., Ma, K., Mace, G., Mooney, H.A., Perrings, C., Raven, P., Sarukhan, J., Schei, P., Scholes, R.J. and Watson, R.T. (2006) Diversity without representation. *Nature* 442: 245–246.

Loss, S.R., Terwilliger, L.A. and Peterson, A.C. (2011) Assisted colonization: Integrating conservation strategies in the face of climate change. *Biological Conservation* 144: 92–100.

Lozano, F.D., Rebelo, A.G. and Bittman, R. (2012) How plant inventories improve future monitoring. *Biodiversity and Conservation* 21: 1937–1951.

Lynch, M. and Milligan, B.G. (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3: 91–99.

Mace, G.M., Norris, K. and Fitter, A.H. (2012) Biodiversity and ecosystem services: a multilayered relationship. *Trends in Ecology and Evolution* 27(1): 19–26.

MacNair, M.R. (1997) The evolution of plants in metal-contaminated environments. In: Bijlsma, R. and Loeschcke, V. (eds.), *Environmental Stress, Adaptation, and Evolution*. Birkhauser Verlag, Basel. Pp. 3–24.

MAFF (1995) *United Kingdom Country Report for FAO*. Ministry of Agriculture, Fisheries and Food, London.

Magos Brehm, J., Maxted, N., Ford-Lloyd, B.V. and Martins-Loução, M.A. (2008) National inventories of crop wild relatives and wild harvested plants: case study for Portugal. *Genetic Resources and Crop Evolution* 55: 779–796.

Magos Brehm, J., Maxted, N., Martins-Loução, M.A. and Ford-Lloyd, B.V. (2010) New approaches for establishing conservation priorities for socio-economically important plant species. *Biodiversity and Conservation* 19(9): 2715–2740.

Magos Brehm, J., Ford-Lloyd, B.V., Maxted, N. and Loução, M.A. (2012) Using neutral genetic diversity to prioritise crop wild relative populations: a Portuguese endemic case study for *Dianthus cintranus* Boiss. & Reut. subsp. *barbatus* R. Fern. & Franco. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford, UK. Pp. 193–210.

Mallikarjuna, N., Senapathy, S., Jadhav, D.R., Saxena, K., Sharma, H.C., Uphadhyaya, H.D., Rathore, A. and Varshney, R. (2011) Progress in the utilization of *Cajanus platycarpus* (Benth.) Maesen in pigeonpea improvement. *Plant Breeding* 130: 507–514.

Mammadov, J., Aggarwal, R., Buyyarapu, R. and Kumpatla, S. (2012) SNP markers and their impact on plant breeding. *International Journal of Plant Genomics* 2012: 1–11.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Journal of Cancer Research* 27: 209–220.

Marais, G.F., McCallum, B. and Marais, A.S. (2008) Wheat leaf rust resistance gene *Lr59* derived from *Aegilops peregrina*. *Plant Breeding* 127: 340–345.

Mardis, E.R. (2008) The impact of next-generation sequencing technology on genetics. *Trends in Genetics* 24(3): 133–141.

Marfil, C.F., Hidalgo, V. and Masuelli, R.W. (2015) *In situ* conservation of wild potato germplasm in Argentina: examples and possibilities. *Global Ecology and Conservation* **3**: 461–476.

Margules, C.R. (1989) Introduction to some Australian developments in conservation evaluation. *Biological Conservation* 50: 1–11.

Marshall, E.J.P. and Moonen, A.C. (2002) Field margins in northern Europe: their functions and interactions with agriculture. *Agriculture, Ecosystems & Environment* 89: 5–21.

Maxted, N., Hawkes, J.G., Guarino, L. and Sawkins, M. (1997a) Towards the selection of taxa for plant genetic conservation. *Genetic Resources and Crop Evolution* 44: 337–348.

Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G. (1997b) Complementary conservation strategies. In: Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G. (eds.), *Plant Genetic Conservation: The* In Situ *Approach*. Chapman & Hall, London. Pp. 20–55.

Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G. (1997c) *Plant genetic conservation: the* in situ *approach*. Chapman & Hall: London.

Maxted, N. (2003) Conserving the genetic resources of crop wild relatives in European Protected Areas. *Biological Conservation* 113: 411–417.

Maxted, N., Mabuza-Diamini, P., Moss, H., Padulosi, S., Jarvis, A. and Guarino, L. (2004) *Systematic and ecogeographic studies on crop genepools 11: An ecogeographic study African* Vigna. International Plant Genetic Resources Institute, Rome, Italy. 414 pp.

Maxted, N., Ford-Lloyd, B.V., Jury, S., Kell, S. and Scholten, M. (2006) Towards a definition of a crop wild relative. *Biodiversity and Conservation* 15: 2673–2685.

Maxted, N., Scholten, M., Codd, R. and Ford-Lloyd, B. (2007) Creation and use of a national inventory of crop wild relatives. *Biological Conservation* 140: 142–159.

Maxted, N. and Kell, S.P. (2008) Linking *in situ* and *ex situ* conservation with use of crop wild relatives. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 450–467.

Maxted, N., Dulloo, E., Ford-Lloyd, B.V., Iriondo, J.M. and Jarvis, A. (2008a) Gap analysis: a tool for complementary genetic conservation assessment. *Diversity and Distributions* 14: 1018–1030.

Maxted, N., Iriondo, J.M., Dulloo, M.E. and Lane, A. (2008b) Introduction: The integration of PGR conservation with protected area management. In: Iriondo, J.M., Maxted, N. and Dulloo, M.E. (eds.), *Conserving plant genetic diversity in protected areas*. CABI Publishing, Wallingford. Pp. 1–22.

Maxted, N., Kell, S.P. and Ford-Lloyd, B.V. (2008c) Crop wild relative conservation and use: establishing the context. In: Maxted, N., Dulloo, E., Ford-Lloyd, B., Kell, S.P. and Iriondo, J.M. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 3–30.

Maxted, N. and Kell, S. (2009) *Establishment of a global network for the* in situ *conservation of crop wild relatives: status and needs*. Commission on Genetic Resources for Food and Agriculture, Food and Agriculture Organization of the United Nations, Rome, Italy. 211 pp.

Maxted, N., Kell, S.P., Ford-Lloyd, B.V., Dulloo, M.E. and Toledo, A. (2012) Toward the systematic conservation of global crop wild relative diversity. *Crop Sciences* 52: 774–785.

Maxted, N., Magos Brehm, J. and Kell, S. (2013a) *Resource book for the preparation of national conservation plans for crop wild relatives and landraces*. University of Birmingham, UK. [online] Available at: http://www.fao.org/fileadmin/templates/agphome/documents/PGR/PubPGR/ResourceBook/TEXT_ALL_2511.pdf

Maxted, N., Avagyan, A., Frese, L., Iriondo, J.M., Magos Brehm, J., Singer, A. and Kell, S.P. (2013b) *Preserving diversity: a concept for* in situ *conservation of crop wild relatives in Europe*. *In Situ* and On-farm Conservation Network, European Cooperative Programme for Plant Genetic Resources. Rome, Italy.

Maxted, N. (2015) Comment in: Goldenberg, S. The doomsday vault: the seeds that could save a post-apocalyptic world. *The Guardian*. [online] Available at: http://www.theguardian.com/science/2015/may/20/the-doomsday-vault-seeds-save-post-apocalyptic-world

Maxted, N., Avagyan, A., Frese, L., Iriondo, J.M., Magos Brehm, J., Singer, A. and Kell, S.P. (2015a) *ECPGR Concept for* in situ *conservation of crop wild relatives in Europe*. Wild Species Conservation in Genetic Reserves Working Group, European Cooperative Programme for Plant Genetic Resources, Rome, Italy.

Maxted, N., Amri, A., Castañeda Álvarez, N., Dias, S., Dulloo, M.E., Fielder, H., Ford-Lloyd, B.V., Iriondo, J.M., Magos Brehm, J., Nilsen, L-B., Thorman, I., Vincent, H. and Kell, S.P. (2015b) Joining up the dots: a systematic perspective of crop wild relative conservation and use. In: Maxted, N., Dulloo, M.E. and Ford-Lloyd, B.V. (eds.), *Enhancing Crop Genepool Use: Capturing wild relative and landrace diversity for crop improvement*. CABI Publishing, Wallingford. Pp. XX-XX.

McCarthy, A. (2010) Third generation DNA sequencing: Pacific Biosciences' single molecule real time technology. *Chemistry and Biology Innovations* 17: 675–676.

McCouch, S. (2013) Feeding the future. *Nature* 499: 23–24.

McKay, J.K. and Latta, R.G. (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution* 17: 285–291.

MEA (2004) *Ecosystems and Human Well–being: Biodiversity Synthesis Report*. Millennium Ecosystem Assessment. [online] Available at: http://www.unep.org/maweb/en/Index.aspx

Meilleur, B.A. and Hodgkin, T. (2004) *In situ* conservation of crop wild relatives. *Biodiversity and Conservation* 13: 663–684.

Met Office (2015) *UK Climate*. Met Office. [online] Available at: http://www.metoffice.gov.uk/public/weather/climate

Metzker, M.L. (2010) Sequencing technologies – the next generation. *Nature Reviews Genetics* 11: 31–46.

Michael, T.P. and VanBuren, R. (2015) Progress, challenges and the future of crop genomes. *Current Opinion in Plant Biology* 24: 71–81.

Michalakis, Y. and Excoffier, L. (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142: 1061–1064.

Moorthie, S., Mattocks, C.J. and Wright, C.F. (2011) Review of massively parallel DNA sequencing technologies. *The HUGO Journal* 5: 1–12.

Munerati, O. (1932) Sull'incrocio delle barbabietola coltivata con la bete selvaggia della costa adriatica. *Industria saccarifera italiana* 25: 303–304.

Nanodrop (2012) *Interpretation of nucleic acid 260/280 ratios*. T123-Technical Bulletin. Delaware, USA: Thermo Scientific. [online] Available at: http://www.nanodrop.com/Library/T123-NanoDrop-Lite-Interpretation-of-Nucleic-Acid-260-280-Ratios.pdf

National Botanic Garden of Wales (2014) *Welsh Rare Plants Project*. National Botanic Garden of Wales. [online] Available at: http://www.gardenofwales.org.uk/science/welsh-rare-plants-project/

Natural England (2014) *Publications, maps and data*. Natural England. [online] Available at: http://webarchive.nationalarchives.gov.uk/20140605110407/http://www.naturalengland.org.uk/publications/data/default.aspx

Neel, M.C. and Cummings. M.P. (2003) Genetic consequences of ecological reserve design guidelines: An empirical investigation. *Conservation Genetics* 4: 427–439.

NERC (2013) *Section 41 Species – Priority Actions Needed. Natural Environment and Rural Communities Act 2006*. [online] Available at: http://publications.naturalengland.org.uk/publication/4958719460769792

NIWT (2002) *National Inventory of Woodland and Trees – Wales*. Forestry Commission: Edinburgh. [online] Available at: http://www.forestry.gov.uk/website/forestry.nsf/byunique/hcou-54pg9v

NRW (2012) *Maps and data*. Natural Resources Wales. [online] Available at: www.ccgc.gov.uk

NRW (2014) *Forestry*. Natural Resources Wales. [online] Available at: http://naturalresourceswales.gov.uk/forestry/?lang=en

Oborny, B., Botta-Dukát, Z., Rudolf, K. and Morschhauser, T. (2011) Population ecology of *Allium ursinum*, a space-monopolizing clonal plant. *Acta Botanica Hungarica* 53: 371–388.

ONS (2014) *Statistical Bulletin: Annual Mid-year Population Estimates, 2013*. Office for National Statistics. [online] Available at: http://www.ons.gov.uk/ons/dcp171778_367167.pdf

Osborne, J. (2010) *Exploring the potential of the Lizard, Cornwall, as a genetic reserve for the long-term conservation of crop wild relatives*. MRes thesis, University of Birmingham.

Panella, L., Landucci, S., Torricelli, R., Gigante, D., Donnini, D., Venanzoni, R. and Negri, V. (2014) *The national crop wild relative strategy for Italy: first steps to be taken*. PGR Secure. [online] Available at: http://www.pgrsecure.bham.ac.uk/sites/default/files/documents/public/National_CWR_Conservation_Strategy_Italy.pdf

Parra-Quijano, M., Draper, D. and Iriondo, J.M. (2003) Assessing *in situ* conservation of *Lupinus* spp. in Spain through GIS. *Crop Wild Relative* 1: 8–9.

Parra-Quijano, M., Draper, D. and Iriondo, J.M. (2007) GIS-based evaluation of the *in situ* conservation of a crop wild relative: the case of Spanish lupins. *Bocconea* 21: 105–116.

Parra-Quijano, M., Draper, D., Torres, E. and Iriondo, J.M. (2008) Ecogeographical representativeness in crop wild relative *ex situ* collections. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 249–273.

Parra-Quijano, M., Iriondo, J.M. and Torres, E. (2012a) Applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. *Spanish Journal of Agricultural Research* 10(2): 419–429.

Parra-Quijano, M., Iriondo, J.M. and Torres, E. (2012b) Ecogeographic land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genetic Resources and Crop Evolution* 59(2): 205–217.

Parra-Quijano, M., Iriondo, J.M., Frese, L. and Torres, E. (2012c) Spatial and ecogeographic approaches for selecting genetic reserves in Europe. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford, UK. CABI Publishing, Wallingford. Pp. 20–28.

Parra-Quijano, M., Torres Lamas, E., Iriondo, J.M. and López, F. (2014) *Capfitogen Tools: Programme to strengthen national plant genetic resource capacities in Latin America*. Version 1.2, FAO.

Peakall, R. and Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Peakall, R. and Smouse, P.E. (2012) GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537–2539.

Pearson, R.G. (2006) Climate change and the migration capacity of species. *Trends in Ecology and Evolution* 21: 111–113.

Pérez-de-Castro, A.M., Vilanova, S., Cañizares, J., Pascual, L., Blanca, J.M., Díez, M.J., Prohens, J. and Picó, B. (2012) Application of genomics tools in plant breeding. *Current Genomics* 13: 179–195.

Phillips, J., Kyratzis, A., Christoudoulou, C., Kell, S. and Maxted, N. (2014) Development of a national crop wild relative conservation strategy for Cyprus. *Genetic Resources and Crop Evolution* 61: 817–827.

Phillips, O.L. and Meilleur, B. (1998) Usefulness and economic potential of the rare plants of the United States: A status survey. *Society for Economic Botany* 52(1): 57–67.

Phillips, S.J., Anderson, R.P. and Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.

Pickering, R., Ruge-Wehling, B., Johnston, P.A., Schweizer, G., Ackermann, P. and Wehling, P. (2006) The transfer of a gene conferring resistance to scald (*Rhynchosporium secalis*) from *Hordeum bulbosum* into *Hordeum vulgare* chromosome 4HS. *Plant Breeding* 125: 576–579.

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T. and Cliff, B. (1997) Economic and environmental benefits of biodiversity. *BioScience* 47: 747–757.

Pinheiro de Carvalho, M.Â.A., Nóbrega, H., Freitas, G., Fontinha, S. and Frese, L. (2012) Towards the establishment of a genetic reserve for *Beta patula* Aiton. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 36–44.

Planta Europa (2008) *A Sustainable Future for Europe; the European Strategy for Plant Conservation 2008–2014*. Plantlife International (Salisbury, UK) & Council of Europe (Strasbourg, France).

Plantlife (2012) *Wild Plant Horizons: Taking forward the Global Strategy for Plant Conservation 2011–2020*. Plantlife International, Salisbury. [online] Available at: http://www.plantlife.org.uk/uploads/documents/GSPC_Template_v4.pdf

Plantlife (2014) *Plant Link Cymru (PLINC)*. Plantlife International. [online] Available at: http://www.plantlife.org.uk/wales/wild_plants/plinc/

Pompanon, F., Bonin, A., Bellemain, E. and Taberlet, P. (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics* 6: 847–859.

Porter, J.R., Xie, L., Challinor, A.J., Cochrane, K., Howden, S.M., Iqbal, M.M., Lobell, D.B. and Travasso, M.I. (2014) Food security and food production systems. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Edi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R. and White, L.L. (eds.), *Climate Change 2014: Impacts, Adaptation, and*

Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Prescott-Allen, R. and Prescott-Allen, C. (1986) *The first resource: Wild species in the North American economy*. Yale University, New Haven, CT.

Preston, C.D., Pearman, D.A. and Dines, T.D. (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 University Press, UK.

PwC (2013) Crop wild relatives: *A valuable resource for crop development*. Price Waterhouse Cooper. [online] Available at: http://pwc.blogs.com/files/pwc-seed-bank-analysis-for-msb-0713.pdf

Qiagen (2012) *DNeasy® Plant Handbook*. US: Qiagen.

Ramírez-Villegas, J., Khoury, C., Jarvis, A., Debouck, D.G. and Guarino, L. (2010) A gap analysis methodology for collecting crop gene pools: A case study with *Phaseolus* beans. *PLoS ONE* 5(10): 1–18.

R Core Team (2014) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rebelo, A.G. (1994) Iterative selection procedures: centres of endemism and optimal placement of reserves. In: Huntley, B.J. (ed.), *Botanical Diversity in Southern Africa*. National Botanical Institute, Pretoria. Pp. 231–257.

Redden, R. (2013) New approaches for crop genetic adaptation to the abiotic stresses predicted with climate change. *Agronomy* 3: 419–432.

Reed, D.H. and Frankham, R. (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* 55: 1095–1103.

Reed, D.H. and Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230–237.

Ren, J., Sun, D., Chen, L., You, F.M., Wang, J., Peng, Y., Nevo, E., Sun, D., Luo, M-C. and Peng, J. (2013) Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. *International Journal of Molecular Sciences* 14: 7061–7088.

Rich, T.C.G., Motley, G.S. and Kay, Q.O.N. (2005) Population sizes of three rare Welsh endemic *Sorbus* species (Rosaceae). *Watsonia* 25: 381–388.

Richards, C.M., Reeves, P.A., Fenwick, A.L. and Panella, L. (2014) Genetic structure and gene flow in *Beta vulgaris* subspecies *maritima* along the Atlantic coast of France. *Genetic Resources and Crop Evolution* 61: 651–662.

Richardson, B.A., Rehfeldt, G.E. and Kim. M. (2009) Congruent climate-related genecological responses from molecular markers and quantitative traits for western white pine (*Pinus monticola*). *International Journal of Plant Sciences* 170: 1120–1131.

Richter, S., Kipfer, T., Wohlgemuth, T., Guerrero, C.C., Ghazoul, J. and Moser, B. (2012) Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* 169: 269–279.

Roche NimbleGen (2011) *NimbleGen SeqCap EZ Library LR User's Guide Version 2.0*. Roche NimbleGen, USA.

Rodrigues, A.S.L., Andelman, S.J., Bakarr, M.I., Boitani, L., Brooks, T.M., Cowling, R.M., Fishpool, L.D.C., da Fonseca, G.A.B., Gaston, K.J., Hoffmann, M., Long, J.S., Marquet, P.A., Pilgrim, J.D., Pressey, R.L., Schipper, J., Sechrest, W., Stuart, S.N., Underhill, L.G., Waller, R.W., Watts, M.E.J. and Yan, X. (2004) Effectiveness of the global protected area network in representing species diversity. *Nature* 428: 640–643.

Rousset, F. (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.

Rubio Teso, M.L., Iriondo, J.M., Parra, M. and Torres, E. (2013) *National strategy for the conservation of crop wild relatives of Spain*. PGR Secure. [online] Available at: http://www.pgrsecure.bham.ac.uk/sites/default/files/documents/public/National_CWR_Conservation_Strategy_Spain.pdf

Sboner, A., Mu, X.J., Greenbaum, D., Auerbach, R.K. and Gerstein, M.B. (2011) The real cost of sequencing: higher than you think! *Genome Biology* 12: 125.

Scheldeman, X. and van Zonneveld, M. (2010) *Training Manual on Spatial Analysis of Plant Diversity and Distribution*. Bioversity International, Rome, Italy.

Schlenker, W. and Roberts, M.J. (2009) Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. *PNAS* 106: 15594–15598.

Schløtterer, C. (2004) The evolution of molecular markers – just a matter of fashion? *Nature Reviews Genetics* 5: 63–69.

Schoen, D.J. and Brown, A.H.D. (1993) Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. *PNAS USA* 90: 10623–10627.

Scholten, M., Maxted, N., Kell, S.P. and Ford-Lloyd, B.V. (2008) Creation of a National Crop Wild Relative Strategy: a Case Study for the United Kingdom. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.), *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 120–142.

Scottish Executive (2004) *Scotland's biodiversity: it's in your hands. A strategy for the conservation and enhancement of biodiversity in Scotland*. Scottish Executive, Edinburgh. [online] Available at: http://www.gov.scot/Resource/Doc/25954/0014583.pdf

Scottish Government (2013a) *Scottish Biodiversity List*. The Scottish Government. [online] Available at: http://www.gov.scot/Topics/Environment/Wildlife-Habitats/16118/Biodiversitylist/SBL

Scottish Government (2013b) *2020 challenge for Scotland's biodiversity. A strategy for the conservation and enhancement of biodiversity in Scotland*. Scottish Government, Edinburgh. [online] Available at: http://www.gov.scot/Resource/0042/00425276.pdf

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M.N. (2006) An overview of molecular marker methods for plants. *African Journal of Biotechnology* 5(25): 2540–2568.

Shan, F., Clarke, H.C., Plummer, J.A., Yan, G. and Siddique, K.H.M. (2005) Geographical patterns of genetic variation in the world collections of wild annual Cicer characterized by amplified fragment length polymorphisms. *Theoretical and Applied Genetics* 110(2): 381–391.

Skøt, L., Humphreys, M.O., Armstead, I., Heywood, S., Skøt, K.P., Sanderson, R., Thomas, I.D., Chorlton, K.H. and Ruaraidh Sackville Hamilton, N. (2005) An association mapping approach to identify flowering time genes in natural populations of *Lolium perenne* (L.). *Molecular Breeding* 15: 233–245.

Skøt, L., Humphreys, J., Humphreys, M.O., Thorogood, D., Gallagher, J., Sanderson, R., Armstead, I.P. and Thomas, I.D. (2007) Association of candidate genes with flowering time and water-soluble carbohydrate content in *Lolium perenne* (L.). *Genetics* 177: 535–547.

Smart, S.M., Bunce, R.G., Firbank, L.G. and Coward, P. (2002) Do field boundaries act as refugia for grassland plant species diversity in intensively managed agricultural landscapes in Britain? *Agriculture, Ecosystems & Environment* 91: 73–87.

Smart, S.M., Marrs, R.H., Le Duc, M.G., Thompson, K., Bunce, R.G.H., Firbank, L.G. and Rossall, M.J. (2006a) Spatial relationships between intensive land cover and residual plant species diversity in temperate farmed landscapes: Land-use and residual species diversity patterns. *Journal of Applied Ecology* 43: 1128–1137.

Smart, S.M., Thompson, K, Marrs, R.H., Le Duc, M.G., Maskell, L.C. and Firbank, L.G. (2006b) Biotic homogenization and changes in species diversity across human-modified ecosystems. *Proceedings of the Royal Society B: Biological Sciences* 273: 2659–2665.

SNH (2012) *Natural Spaces*. Scottish Natural Heritage. [online] Available at: http://gateway.snh.gov.uk/natural-spaces/index.jsp

SRDP (2015) *Scottish Rural Development Programme*. The Scottish Government. [online] Available at: https://www.ruralpayments.org/publicsite/futures/

Stace, C. (1997) *New Flora of the British Isles Second Edition*. Cambridge University Press, United Kingdom.

Stace, C.A., Ellis, R.G., Kent, D.H. and McCosh, D.J. (eds.) (2003) *Vice-County Census Catalogue of the Vascular Plants of Great Britain, the Isle of Man and the Channel Islands*. Botanical Society of the British Isles, London.

Stace, C. (2010) *New Flora of the British Isles Third Edition*. Cambridge University Press, United Kingdom.

Stevanato, P., Trebbi, D., Biancardi, E., Cacco, G., McGrath, J.M. and Saccomani, M. (2013) Evaluation of genetic diversity and root traits of sea beet accessions of the Adriatic Sea coast. *Euphytica* 189: 135–146.

Stocker, T.F., Qin, D., Plattner, G.-K., Alexander, L.V., Allen, S.K., Bindoff, N.L., Bréon, F.-M., Church, J.A., Cubasch, U., Emori, S., Forster, P., Friedlingstein, P., Gillett, N., Gregory, J.M., Hartmann, D.L., Jansen, E., Kirtman, B., Knutti, R., Krishna Kumar, K., Lemke, P., Marotzke, J., Masson-Delmotte, V., Meehl, G.A., Mokhov, I.I., Piao, S., Ramaswamy, V., Randall, D., Rhein, M., Rojas, M., Sabine, C., Shindell, D., Talley, L.D., Vaughan, D.G. and Xie, S.-P. (2013) Technical Summary. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* . Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Stroh, P.A., Leach, S.J., August, T.A., Walker, K.J., Pearman, D.A., Rumsey, F.J., Harrower, C.A., Fay, M.F., Martin, J.P., Pankhurst, T., Preston, C.D. and Taylor, I. (2014) *A Vascular Plant Red List for England*. Botanical Society of Britain and Ireland, Bristol, UK.

Tamis, W.L.M., van't Zelfde, M., van der Meijden, R., Groen, C.L.G. and Udo de Haes, H.A. (2005) Ecological interpretation of changes in the Dutch flora in the 20th century. *Biological Conservation* 125: 211–224.

Tang, H., Krishnakumar, V., Bidwell, S., Rosen, B., Chan, A., Zhou, S., Gentzbittel, L., Childs, K.L., Yandell, M., Gundlach, H., Mayer, K.F.X., Schwartz, D.C. and Town, C.D. (2014) An improved genome release (version Mt4.0) for the model legume *Medicago truncatula*. *BMC Genomics* 15: 312. doi:10.1186/1471-2164-15-312

Tanksley, S.D. and McCouch, S.R. (1997) Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. *Science* 277: 1063–1066.

Tatum, L.A. (1971) The Southern corn leaf blight epidemic. *Science* 171: 1113–1116.

Taylor, N.G., Kell, S., Holubec, V., Parra-Quijano, M., Chobot, K. and Maxted, N. (in prep) A crop wild relative conservation strategy for the Czech Republic.

Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science* 327: 818–822.

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.

The Lizard (2013) *the Lizard: Linking the Lizard Countryside Partnership*. [online] Available at: http://www.the-lizard.org/

Thudi, M., Lu, Y., Jackson, S.A., May, G.D. and Varshney, R.K. (2012) Current state-of-art of sequencing technologies for plant genomics research. *Briefings in Functional Genomics* 2: 3–11.

Tonguç, M. and Griffiths PD. 2004. Transfer of powdery mildew resistance from *Brassica carinata* to *Brassica oleracea* through embryo rescue. *Plant Breeding* 123: 587–589.

UKCP09 (2009) *Adapting to Climate Change: UK Climate Projections*. Department for Environment, Food and Rural Affairs (Defra), London. [online] Available: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69257/pb13274-uk-climate-projections-090617.pdf

UK NEA (2011) *The UK National Ecosystem Assessment: Synthesis of the Key Findings*. UNEP-WCMC, Cambridge.

UKNPI (2013) *United Kingdom National Plant Inventory*. IBERS, Aberystwyth University [online] Available at: https://www.google.com/fusiontables/DataSource?docid=1WYDRRhsRq2oAe6jhB4rV4c1eRc1yMzIhcCCUp7s#chartnew:id=8

United Nations (2011) *World Population Prospects*. UN Department of Economic and Social Affairs, Population Division. [online] Available at: http://esa.un.org/unpd/wpp/index.htm

United Nations (2013) *World Population Prospects: The 2012 Revision, Highlights and Advance tables*. Working paper No. ESA/P/WP.228. Department of Economic and Social Affairs, Population Division. United Nations, New York. [online] Available at: http://esa.un.org/wpp/Documentation/pdf/WPP2012_HIGHLIGHTS.pdf

USDA, ARS, National Genetic Resources Program (2015) *Germplasm Resources Information Network – (GRIN)* [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. Available at: http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?view=index&language=en

van de Wouw, M., Kik, C., van Hintum, T., van Treuren, R. and Visser, B. (2010) Genetic erosion in crops: concept, research results and challenges. *Plant Genetic Resources: Characterization and Utilization* 8(1): 1–15.

Van Treuren, R., Coquin, P. and Lohwasser, U. (2012) Genetic resources collections of leafy vegetables (lettuce, spinach, chicory, artichoke, asparagus, lamb's lettuce, rhubarb and rocket salad): Composition and gaps. *Genetic Resources and Crop Evolution* 59(6): 981–997.

Van Treuren, R. and van Hintum, T.J.L. (2014) Next-generation genebanking: plant genetic resources management and utilization in the sequencing era. *Plant Genetic Resources: Characterzation and Utilization* 12(3): 298–307.

Vaughan, D.A., Balázs, E. and Heslop-Harrison, J.S. (2007) From crop domestication to super-domestication. *Annals of Botany* 100: 893–901.

Vavilov, N.I. (1926) The centres of origin of cultivated plants. *Works of applied botany and plant breeding* 1: 17–75.

Vekemans, X., Beauwens, T., Lemaire, M. and Roldán-Ruiz, I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11: 139–151.

Vignal, A., Milan, D., SanCristobal, M. and Eggen, A. (2002) A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution* 34: 275–305.

Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N.P., Guarino, L., Eastwood, R., León, B. and Maxted, N. (2013) A prioritized crop wild relative inventory to help underpin global food security. *Biological Conservation* 167: 265–275.

Vitt, P., Havens, K., Kramer, A.T., Sollenberger, D. and Yates, E. (2010) Assisted migration of plants: Changes in latitudes, changes in attitudes. *Biological Conservation* 143: 18–27.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research* 23: 4407–4414.

Wales Biodiversity Partnership (2015) *Section 42 lists*. Wales Biodiversity Partnership. [online] Available at: http://www.biodiversitywales.org.uk/Section-42-Lists

Walker, K.J. (2003) One species lost every year? An evaluation of plant extinctions in selected vice-counties since 1900. *Watsonia* 26: 291–302.

Walker, K.J., Preston, C.D. and Boon, C.R. (2009) Fifty years of change in an area of intensive agriculture: plant trait responses to habitat modification and conservation, Bedfordshire, England. *Biodiversity and Conservation* 18: 3597–3613.

Watson-Jones, S.J., Maxted, N. and Ford-Lloyd, B.V. (2006) Population baseline data for monitoring genetic diversity loss for 2010: A case study for *Brassica* species in the UK. *Biological Conservation* 132: 490–499.

Weidema, I.R. (1996) Inheritance of the isozyme isocitrate dehydrogenase (IDH) in a natural population of polyploidy white clover (*Trifolium repens* L.). *Hereditas* 125: 19–24.

Welsh Assembly Government (2006) *Environment Strategy for Wales*. Welsh Assembly Government. Cardiff, UK. [online] Available at: http://wales.gov.uk/docs/desh/publications/060517environmentstrategyen.pdf

Welsh Assembly Government (2010) *Climate Change Strategy for Wales*. Welsh Assembly Government for Wales. Cardiff, UK. [online] Available at: http://wales.gov.uk/docs/desh/publications/101006ccstratfinalen.pdf

Welsh Assembly Government (2011) *Environmental Strategy Annual Report 2010–2011*. Welsh Assembly Government. Cardiff, UK. [online] Available at: http://wales.gov.uk/docs/desh/publications/111221environmentstrategyen.pdf

Welsh Government (2014) *The Wales Rural Development Programme 2014–2020 (RDP)*. Welsh Government. [online] Available at: http://wales.gov.uk/topics/environmentcountryside/farmingandcountryside/cap/ruraldevelopment/rural-development-plan-for-wales-2014-2020/?lang=en

Wetterstrand, K.A. (2015) *DNA Sequencing Costs*. Data from the NHGRI Genome Sequencing Program (GSP). [online] Available at: http://www.genome.gov/sequencingcosts/

Wissuwa, M., Kondo, K., Fukuda, T., Mori, A., Rose, M.T., Pariasca-Tanaka, J., Kretzschmar, T., Haefele, S.M. and Rose, T.J. (2014) Unmasking novel loci for internal phosphorus utilization efficiency in rice germplasm through genome-wide association analysis. *PLOS One* 10: e0124215

World Bank (2008) *World development report 2008: Agriculture for Development*. World Bank, Washington DC.

Young, N.D. and Udvardi, M. (2009) Translating *Medicago truncatula* genomics to crop legumes. *Current Opinion in Plant Biology* 12: 193–201.

Young, N.D., Debelle, F., Oldroyd, G.E., Geurts, R., Cannon, S.B., Udvardi, M.K., Benedito, V.A., Mayer, K.F., Gouzy, J., Schoof, H., Van de Peer, Y., Proost, S., Cook, D.R., Meyers, B.C., Spannagl, M., Cheung, F., De Mita, S., Krishnakumar, V., Gundlach, H., Zhou, S., Mudge, J., Bharti, A.K., Murray, J.D., Naoumkina, M.A., Rosen, B., Silverstein, K.A., Tang, H., Rombauts, S., Zhao, P.X., Zhou, P., Barbe, V., Bardou, P., Bechner, M., Bellec, A., Berger, A., Berges, H., Bidwell, S., Bisseling, T., Choisne, N., Couloux, A., Denny, R., Deshpande, S., Dai, X., Doyle, J.J., Dudez, A.M., Farmer, A.D., Fouteau, S., Franken, C., Gibelin, C., Gish, J., Goldstein, S., Gonzalez, A.J., Green, P.J., Hallab, A., Hartog, M., Hua, A., Humphray, S.J., Jeong, D.H., Jing, Y., Jocker, A., Kenton, S.M., Kim, D.J., Klee, K., Lai, H., Lang, C., Lin, S., Macmil, S.L., Magdelenat, G., Matthews, L., McCorrison, J., Monaghan, E.L., Mun, J.H., Najar, F.Z., Nicholson, C., Noirot, C., O'Bleness, M., Paule, C.R., Poulain, J., Prion, F., Qin, B., Qu, C., Retzel, E.F., Riddle, C., Sallet, E., Samain, S., Samson, N., Sanders, I., Saurat, O., Scarpelli, C., Schiex, T., Segurens, B., Severin, A.J., Sherrier, D.J., Shi, R., Sims, S., Singer, S.R., Sinharoy, S., Sterck, L., Viollet, A., Wang, B.B., Wang, K., Wang, M., Wang, X., Warfsmann, J., Weissenbach, J., White, D.D., White, J.D., Wiley, G.B., Wincker, P., Xing, Y., Yang, L., Yao, Z., Ying, F., Zhai, J., Zhou, L., Zuber, A., Denarie, J., Dixon, R.A., May, G.D., Schwartz, D.C., Rogers, J., Quetier, F., Town, C.D. and Roe, B.A. (2011) The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature* 480: 520–524.

Zeven, A.C. and Zhukovsky, P.M. (1975) *Dictionary of Cultivated Plants and Their Centres of Diversity. Excluding Ornamentals, Forest Trees and Lower Plants*. PUDOC, Wageningen, The Netherlands.

Zhang, L., Pickering, R.A. and Murray, B.G. (2001) *Hordeum vulgare* x *H. bulbosum* tetraploid hybrid provides useful agronomic introgression lines for breeders. *New Zealand Journal of Crop and Horticultural Science* 29: 239–246.

Zhivotovsky, L.A. (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* 8: 907–913.

Zhong, W. and Priest, N.K. (2011) Stress-induced recombination and the mechanism of evolvability. *Behavioral Ecology and Sociobiology* 65: 493–502. doi: 10.1007/s00265-010-1117-7

Zhou, C., Han, L., Pislariu, C., Nakashima, J., Fu, C., Jiang, Q., Quan, L., Blancaflor, E.B., Tang, Y., Bouton, J.H., Udvardi, M., Xia, G and Wang, Z. (2011) From Model to Crop: Functional Analysis of a STAY-GREEN Gene in the Model Legume *Medicago truncatula* and Effective Use of the Gene for Alfalfa Improvement. *Plant Physiology* 157: 1483–1496.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, Berlin.

Appendix 1.

Enhancing the conservation of crop wild relatives in Wales

The work presented in this appendix has been submitted to the

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Author contributions:

Conceived and designed the study: **HF**, CB, BFL, NM

Data collation and preparation: **HF**

Performed analysis: **HF**

Interpreted results: **HF**

Wrote the paper: **HF**

Critically reviewed the paper: **HF**, CB, JW, BFL, NM

A1.1 Abstract

To feed the world's human population in the face of threats such as climate change, pests and diseases and habitat destruction, crops will need to be bred which are more resilient and higher yielding. Crop wild relatives (CWR) offer a solution to this as they contain higher levels of genetic diversity and contain beneficial traits, which can be used in crop improvement. CWR however, are also threatened and are generally poorly conserved; this is also true within Wales. Here, appropriate conservation for CWR in Wales is outlined to ensure the active and long-term maintenance of populations *in situ* and accessions *ex situ*. To achieve this, an inventory of 122 priority Welsh CWR was developed, hotspots of CWR taxon diversity were identified and a network of complementary genetic reserves were proposed. The Gower Peninsula was identified as the most taxon rich area of Wales for both common and rare/scarce CWR. Extensive gaps in *ex situ* collections were also identified for which further collecting is necessary. Implementation of the recommendations made will provide systematic and long-term conservation of CWR in Wales and will ensure this resource is available for use in crop improvement.

A1.2 Introduction

The need to conserve crop wild relatives (CWR) and the genetic diversity within them is increasingly highlighted in global and European policy documents (Convention on Biological Diversity (CBD), Global Strategy for Plant Conservation (GSPC), Strategic Plan for Biodiversity (CBD, 1992; 2010a; 2010b), European Strategy for Plant Conservation (ESPC) (Planta Europa, 2008) and EU Biodiversity 2020 strategy (European Union, 2011)), this is due to their potential as a resource for tackling the issue of future food security.

It is predicted that the human population will reach approximately nine billion people by the year 2050 (United Nations, 2011). This will require food production to increase significantly on limited land whilst confronting the increasing threats from a changing climate (Government Office for Science, 2011). This is compounded by the fact that crops are often bred for uniformity and so have limited genetic diversity, which in turn limits their capacity for adaptation (Jain, 1988). Meeting this challenge will require the development of crops which produce higher yields and are also much more resilient to biotic and abiotic stresses.

CWR are wild plants related to socio-economic crops (Maxted *et al*., 2006). Having never been through the process of domestication they are likely to contain much higher levels of genetic diversity, and therefore novel traits, in comparison with crops themselves (Hopkins and Maxted, 2011). The use of these novel traits in plant breeding would take positive steps towards meeting the food security challenge. CWR however, are themselves threatened. Like all wild plants, they are likely to be susceptible to drivers of change, most notably: habitat degradation and loss, novel pests and diseases and climate change (Maxted and Kell, 2009). Until recently, little was being done to conserve CWR either *in situ* or *ex situ* (Maxted, 2003; Maxted *et al*., 2008c; Dias *et al*., 2012) but with recent policy developments their conservation is being placed higher and higher on international, regional and national agendas.

In response to policy, many conservation strategies have now been developed in a range of different countries worldwide (Finland: Fitzgerald, 2013; Spain: Rubio Teso *et al*., 2013; Cyprus: Phillips *et al*., 2014; Italy: Panella *et al*., 2014; Czech Republic: Taylor *et al*., in prep; for *Vigna* in Africa: Maxted *et al*., 2004; *Phaseolus* in the Americas: Ramírez-Villegas *et al*., 2010 and for wild soybean in Australia: Gonzalez-Orozco *et al*., 2012). These national strategies identify CWR taxa that are a priority for conservation (listed in a CWR inventory, Maxted *et al*., 2013a), and they then work to identify gaps in current CWR conservation using a complementary *in situ* and *ex situ* approach (Maxted *et al*., 2008a). This in turn enables the development of an overall strategy, which outlines recommendations for management of priority CWR for active and long-term conservation and use.

CWR conservation planning is now also being developed within the UK. Conservation is managed separately in each of the devolved administrations in the UK and as a result it was deemed most appropriate to carry out separate assessments of current CWR conservation for England (Fielder *et al*., 2015), Scotland (Fielder *et al*., submitted b) and Wales. This facilitates working directly with conservation organisations in each country to ensure there is support for the strategy and any recommendations made.

In Wales, conservation is based around the framework of the Environment Strategy for Wales (Welsh Assembly Government, 2006). The strategy sets out 39 outcomes, focussing on issues such as mitigation of climate change, sustainable use of resources and maintaining biodiversity, which it hopes to have achieved by 2026. Outcome 19 states that by 2026 ‘the loss of biodiversity [will have] been halted and we [will be able to] see a definite recovery in the number, range and genetic diversity of the species'. In the absence of outcomes specifically addressing agricultural genetic diversity, CWR conservation should fall under the umbrella of outcome 19, which highlights the need to halt loss of genetic diversity. However, in a recent review of the strategy's progress (Welsh Assembly Government, 2011) it was acknowledged that this outcome has not yet been achieved and it is apparent that CWR are not yet actively conserved in Wales.

Over the next few decades, the UK Climate Change Impact Programme (UKCIP) predicts that Wales is likely to experience increasing annual mean temperatures (increasing by 2–2.5˚C by 2050), hotter, dryer summers and more winter rainfall (UKCP09, 2009; Welsh Assembly Government, 2010). These changes in Wales are likely to lead to increased incidences of flooding as well as periods of drought in the summer, habitat and species losses and increased risks from new pests and diseases (UKCP09, 2009). Although the impacts of climate change are likely to be more severe in other regions of the world (Welsh Assembly Government, 2010), the impacts projected for Wales provide a strong argument for establishing active CWR conservation to secure this vital source of genetic variation for crop improvement to maintain and increase food production and its resilience.

There are a number of organisations working to achieve active plant conservation in Wales. Plant Link Cymru (PLINC) acts as a forum for these organisations to communicate and collaborate (Plantlife, 2014). One particular focus of PLINC is to regularly review progress towards achieving the GSPC targets (CBD, 2010a). Target nine of the GSPC states that '70 per cent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species [will be] conserved' by 2020. So far in Wales, however, genetic diversity conservation has been focused on landraces, and conservation of CWR genetic diversity has not yet been addressed in a systematic manner across the country (Plantlife, 2012).

The aim of the current study was to assess the current state of Welsh CWR conservation through the identification of priority CWR and gaps in their current conservation, both *in situ* and *ex situ*. Based on these results recommendations were then made, suggesting positive steps to be taken in Wales to establish systematic and active CWR conservation that will preserve their genetic diversity and ensure this resource is available in the long-term for use in crop improvement.

A1.3 Materials and methods

A1.3.1 CWR checklist and inventory

A checklist of the Welsh flora was extracted from the Vice County Census Catalogue (VCCC) (Stace *et al*., 2003) and a checklist of all UK CWR was extracted from the Crop Wild Relative Information System (CWRIS) (Kell *et al*., 2005). These two lists were matched against one another to produce a final checklist of all CWR occurring within Wales. The taxonomy of the checklist was then harmonised with the British Flora (Stace *et al*., 1997). The final list contains 1,251 CWR taxa (see Supplementary Table A1.1a).

To prioritise the checklist, five criteria were selected through a process of consultation with Natural Resources Wales (NRW). This ensured that the resulting CWR inventory would contain taxa relevant to current conservation priorities in Wales. The five criteria are listed below:

1. Use of the related crop – Using GRIN Taxonomy for Plants (USDA, ARS, National Genetic Resources Program, 2015), the uses of crops related to Welsh CWR were

identified. Wild relatives associated with crops grown for human food or animal forage and fodder were prioritised. Trees grown in the forestry industry were also identified using GRIN Taxonomy and their associated wild taxa were also prioritised.

2. Native status – Native and archaeophyte taxa in Wales were prioritised based on native status data from the VCCC (Stace *et al*., 2003).

3. Economic value of the related crop – The economic value of human food crops was determined using agricultural statistics, taken as a five year mean, extracted from FAOSTAT (2013) between 2007 and 2011, Eurostat (2013) between 2007 and 2011 and Defra (2010) between 2006 and 2010. These were crop production quantity data (million tonnes) at the global, European and UK levels, crop production value at producer price data (millions of euros) and crop production at market prices data (£ million) at the UK level respectively. The wild relatives of all crops listed in these statistics were prioritised. As was also found by Kell *et al*. (2012), comparable statistics for forage and fodder crops were not available. Economic values of forestry taxa were extracted from the National Inventory of Woodland and Trees – Wales (NIWT, 2002). Native tree species listed in NIWT (2002) were prioritised.

4. Degree of relatedness to the crop – Using the Gene Pool (GP) and Taxon Group (TG) concepts (Harlan and de Wet, 1971; Maxted *et al*., 2006). CWR in GP1b are easily crossable with their related crop, CWR in GP2 are crossable but with lower success rates and CWR in GP3 require advanced techniques to facilitate crosses between CWR and their associated crop. Where GP classifications for CWR are lacking the TG concept is used. CWR in TG1b are most closely related to their associated crop (within the same species), CWR in TG2 are in the same taxonomic section as the crop,

TG3 indicates the same subgenus and TG4 indicates the same genus. Data for food and forage/fodder crops were extracted from the Harlan and de Wet inventory of globally important CWR (Vincent *et al*., 2013). CWR within GP1b and 2 and Taxon Groups 1b, 2 and 3 of their related crops were prioritised. Taxa for which taxonomic classifications below genus level were unavailable were assigned to TG4. In addition, taxa in GP1a or TG1a were not prioritised as they are recognised as being cultivated taxa, which have no wild populations in Wales. Where CWR were associated with more than one crop gene pool, the highest GP/TG classification was used as the basis for prioritisation. Forestry taxa were not prioritised using this criterion as they are commercially grown rather than having been through a domestication process and so do not have wild relatives.

5. Threat Assessment – Data were extracted at the global, regional and national levels from the IUCN Red List of Threatened Species (IUCN, 2012), European Red List of Vascular Plants (Bilz *et al*., 2011) and the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005). Any taxa categorised as Critically Endangered, Endangered, Vulnerable or Near Threatened in any of the three assessments were prioritised. Data were also extracted from the Vascular Plant Red Data List for Wales (Dines, 2008) and all threatened taxa identified.

A1.3.2 In situ *gap analysis*

Occurrence data for all priority Welsh CWR were obtained from five separate sources: the BSBI distribution database (BSBI, 2013) and the four Welsh local record centres (South East Wales Biodiversity Records Centre (SEWBReC), West Wales Biodiversity Information Centre (WWBIC), Biodiversity Information Service for Powys and Brecon Beacons National

Park (BIS) and North Wales Environmental Information Service (Cofnod)). Data were then collated and checked to ensure all records were unique.

NRW were then consulted as to how this raw data should be filtered to develop a final dataset containing reliable occurrence records. Records without sufficient passport data to determine location were removed from the dataset (i.e. those lacking coordinates and a location description). Records with a precision equal to or lower than $2km^2$ (tetrad) were also removed from the dataset. Only records listed as having been ‘confirmed’ were selected for the analysis. Only records made from 1970 onwards were considered recent enough to be able to assume the record was still valid. Finally, it was suggested by NRW that two separate *in situ* gap analyses for Welsh CWR should be carried out, one for common CWR (occurring in greater than 100 hectads across Great Britain) and another for rare/scarce CWR (occurring in 100 or fewer hectads across Great Britain) based on categories used in the production of Rare Plant Registers (JNCC, 2014).

Two Geographical Information System (GIS) software packages, DIVA-GIS 7.5.0 (Hijmans *et al*., 2012) and ArcMap 10.0 (ESRI, 2011), were used to carry out the *in situ* gap analyses for common and rare/scarce Welsh CWR. The country boundary file for the UK was downloaded from DIVA-GIS (www.diva-gis.org) and the Welsh boundary extracted. Methods described in Scheldeman and van Zonneveld (2010) and Hijmans *et al*. (2012) were followed in order to carry out the four *in situ* techniques listed below:

- Taxon and observation richness – allowing identification of taxon hotspots and regions of recording bias. This was carried out twice, once for common CWR and once for rare/scarce CWR. Both richness measures were obtained using the ‘point to grid’ function in DIVA-GIS with a grid cell size of 0.05 degrees.

- Comparison of occurrence data with protected areas – a measure of *in situ* passive conservation of CWR was made through the use of spatial join tools in ArcMap 10.0. Boundary shape files for statutory protected areas were obtained from the Countryside Council for Wales website (www.ccgc.gov.uk).
- Complementarity analysis – two separate complementarity analyses were carried out for common and rare/scarce CWR with an aim to identify complementary sites across Wales, which could be appropriate for establishing CWR genetic reserves. This method follows that proposed by Rebelo (1994), using an iterative method of grid cell selection to select a network of sites containing as many different taxa in as few grid cells as possible. A grid cell size of 0.05 was used.
- Minimum five populations – results of the complementarity analyses were further explored to identify which taxa were represented in five or more grid cells. Active *in situ* conservation of at least five populations from a range of geographic locations is a minimum requirement as it increases the chances of a taxon's survival in the event of stochastic and/or anthropogenic change (Brown and Briggs, 1991; Dulloo *et al.*, 2008).

A1.3.3 Ex situ *gap analysis*

The UK National Plant Inventory (UKNPI, 2013) and the Millennium Seed Bank, Kew provided *ex situ* accession data relating to all Welsh priority CWR taxa. This was used as the basis of the *ex situ* gap analysis. Data from both sources were combined and filtered to ensure only accurately georeferenced records were included. The final dataset was used to carry out the following:

- The numbers of *ex situ* accessions per priority CWR originating from within Wales were identified. As a back up of the five populations recommended to be conserved *in situ*, a threshold of five accessions was also set. Priority CWR with five or more accessions are considered sufficiently conserved *ex situ*. Accessions below this threshold will require further collecting.

- Taxa with accessions collected from within Wales underwent an analysis of geographical representativeness, where geographic coverage of accessions was compared with geographical coverage of occurrence records. This produced a geographical representativeness score (GRS) for each taxon, using the 'circular area statistic' as described by (Ramírez-Villegas *et al*., 2010). Taxa for which GRS values were lower than the threshold of 30% were considered in need of further collecting (Ramírez-Villegas *et al*., 2010; Phillips *et al*., 2014).

- These two results were combined to classify each priority CWR into one of the levels in Table A1.1, each of which corresponds to the urgency for further collecting.

Table A1.1 *Ex situ* priority levels and recommended actions required to ensure gaps in *ex situ* collections are sufficiently filled (Source: Fielder *et al.*, submitted b).

Priority Level	**Criteria**	**Action required**
1	No accessions	Highest priority for collection; Ensure minimum of five accessions are collected representing the full range of the CWR
2	Has accessions but none are georeferenced or the location data is restricted	High priority for collection; Georeference existing accessions; Further collection of accessions which are representative of CWR range required
3	Fewer than five accessions and GRS lower than 30%	Increase accessions to a minimum of five; ensure representative of range
4	Fewer than five accessions but GRS greater than 30%	Increase accessions to a minimum of five; confirm that current stored accessions are representative of CWR range
5	Greater than or equal to five accessions but GRS lower than 30%	Improve geographic representativeness of accessions through further targeted collection
6	Greater than or equal to five accessions and GRS greater than 30%	Sufficiently represented in *ex situ* collections

A1.4 Results

A1.4.1 CWR inventory

The Welsh national inventory of priority CWR contains 122 taxa (105 species and 17 subspecies, see Table A1.2 for a summary of results and Supplementary Table A1.1b or the Plant Genetic Resources Diversity Gateway, http://pgrdiversity.bioversityinternational.org, for the full inventory). Of these 122 taxa, 24 (19.7%) are recorded as being rare or scarce due to their occurrence in fewer than 100 hectads across Great Britain. Approximately two thirds (80 taxa) of the inventory are related to crops used for the production of human food. The remaining third consists of CWR related to forage/fodder crops and forestry species (23 and 19 taxa respectively). Of the 20 families represented in the inventory, Poaceae, Brassicaceae and Rosaceae contain the most genera with 16, 6 and 6 respectively. The three genera with the highest taxon richness are *Trifolium* (clovers, 11 taxa), *Vicia* (vetches, 10 taxa) and *Chenopodium* (goosefoots, 8 taxa).

Table A1.2 Summary of inventory of 122 priority CWR in Wales.

Family	No. of genera	No. of species	No. of infra-specific taxa	Native status
Aceraceae	1	1		N
Apiaceae	3	1	4	N
Asteraceae	2	2		A
Betulaceae	1	1	2	N
Brassicaceae	6	6	4	A & N
Chenopodiaceae	2	8	1	A & N
Corylaceae	1	1		N
Ericaceae	1	3		N
Fabaceae	5	24	2	A & N
Fagaceae	2	3		N
Geraniaceae	1	1		N
Grossulariaceae	1	1		N
Liliaceae	2	7		A & N
Linaceae	1	2		N
Oleaceae	1	1		N
Plantaginaceae	1	1		N
Poaceae	16	22	1	A & N
Rosaceae	6	16	2	A & N
Salicaceae	1	1	1	N
Ulmaceae	1	3		N
Totals	**55**	**105**	**17**	

A = Archaeophyte, N = Native.

Thirty-four food crops with native or archaeophyte CWR in Wales are of economic value according to agricultural statistics. At the global scale, mean production quantities of crops (between 2007 and 2011) with wild relatives in Wales were identified (FAOSTAT, 2013). Sugar beet was shown to have the highest production quantity of 239.6 million tonnes. Other crops with high production quantities included barley, onions and apples (Fig. A1.1). Mean production values at producer prices at the European scale (Eurostat, 2013) illustrated that barley had the highest economic value (8,520 million Euros) with rapeseed, apples and sugar beet also showing high values (above 3,000 million Euros). Mean value of production statistics (at market prices) extracted from Defra (2010) support these results, indicating barley and rapeseed have the highest values in England. Economically valuable forestry species (listed in the NIWT, 2002) included oak, ash, birch and beech trees. The 76 taxa related to these 34 crops and trees of economic value were listed within the Welsh inventory of priority CWR.

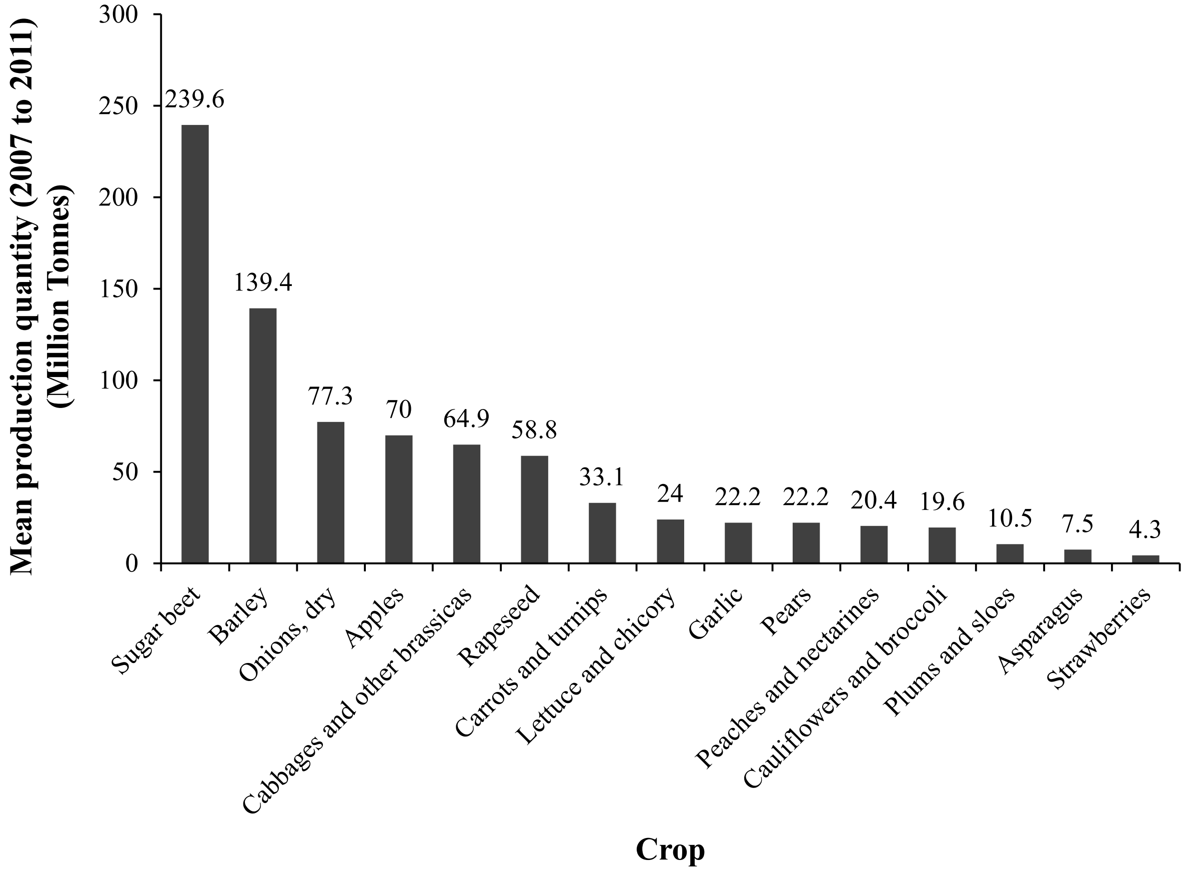

Figure A1.1 Global mean production quantity (million tonnes) between 2007 and 2011 for socio-economic crops with native or archaeophyte CWR occurring within Wales (Data source: FAOSTAT, 2013).

Only 25% of priority Welsh CWR were classified using the Gene Pool concept, with 18% falling within GP 1b, 3% in GP 2 and 4% in GP 3 (Fig. A1.2). This suggests only a quarter of CWR in Wales have undergone crossing experiments to determine the closeness of their genetic relationship to their associated crop/s. Fifty-nine percent of taxa were assigned a Taxon Group (classification of relatedness to a crop based on taxonomic hierarchy in the absence of genetic crossing experiments), with the majority falling within TG1b. The 19 forestry species do not have Gene Pool or Taxon Group classifications because cultivated trees tend to be selected ecotypes from wild populations (Hunter and Heywood, 2011) rather than wild species that have undergone generations of change through domestication.

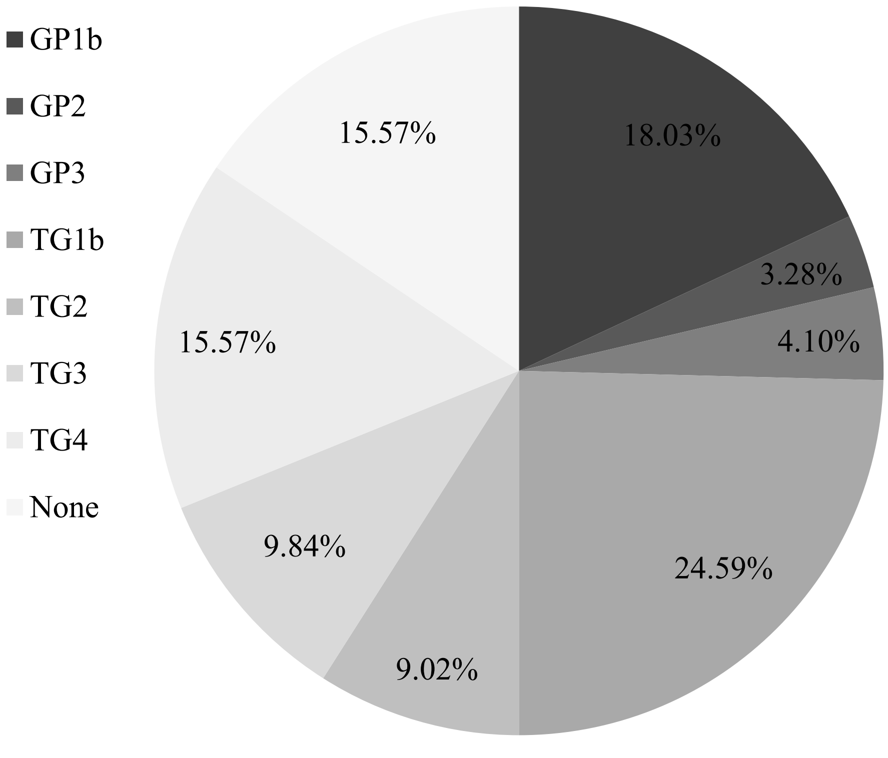

Figure A1.2 Percentage of taxa classified in Gene Pools 1b to 3 (GP1 to 3), Taxon Groups 1b to 4 (TG1b to 4) and taxa with no classification in the Welsh national inventory of priority CWR, showing the degree of relatedness of CWR to their associated crops.

Of the 122 priority CWR taxa in the Welsh inventory, 14.8% (18 taxa) are threatened according to the Vascular Plant Red Data List for Great Britain (Cheffings and Farrell, 2005) and the Vascular Red Data List for Wales (Dines, 2008). Taxa classified as Vulnerable, Endangered or Critically Endangered in either the Welsh or British red lists are listed in Table A1.3. Ley's whitebeam *Sorbus leyana* Wilmott is the only taxon to be listed as Critically Endangered in both the Welsh and British red lists. Other Critically Endangered taxa include wild asparagus *Asparagus prostratus* Dumort., true service-tree *Sorbus domestica* L., round-leaved whitebeam *Sorbus eminens* E.F. Warb. and upright clover *Trifolium strictum* L. A total of nine CWR taxa were found to have a higher threat classification in Wales than Britain as a whole and seven CWR taxa had a lower threat classification in Wales.

Table A1.3 Threatened taxa listed in the Welsh CWR inventory.

Taxon	**Great Britain Red List**	**Wales Red List**
Sorbus leyana Wilmott	CR	CR
Sorbus domestica L.	CR	EN
Asparagus prostratus Dumort.	EN	CR
Sorbus eminens E.F. Warb.	EN	CR
Trifolium strictum L.	VU	CR
Sorbus leptophylla E.F. Warb.	EN	EN
Vicia bithynica (L.) L.	VU	EN
Chenopodium murale L.	VU	EN
Allium oleraceum L.	VU	VU
Chenopodium glaucum L.	VU	VU
Sorbus minima (Ley) Hedl.	VU	VU
Bromus secalinus L.	VU	NT
Lathyrus palustris L.	NT	VU
Hordeum marinum Huds.	VU	LC
Poa glauca Vahl	VU	LC
Chenopodium bonus-henricus L.	VU	LC
Allium ampeloprasum L.	LC	VU
Sinapis arvensis L.	LC	VU

LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered, CR = Critically Endangered (Data Source: Cheffings and Farrell, 2005; Dines, 2008).

A1.4.2 In situ *gap analysis*

Two separate *in situ* gap analyses were carried out for priority CWR identified in the Welsh national inventory. The first contained the 98 taxa classified as common throughout Great Britain and the second contained the 24 taxa classified as rare or scarce. No records of high enough quality existed for the two subspecies eastern parsnip *Pastinaca sativa* L. subsp. *urens* (Req. ex Godr.) Čelak. or wild parsnip *P. sativa* subsp. *sylvestris* (Mill.) Rouy & E. G. Camus and so they were not able to be included in the analysis. Both subspecies were classified as rare/scarce, leaving a total of 22 taxa in the rare/scarce *in situ* gap analysis. A total of 191,664 occurrence records were included in the analysis (190,771 occurrence records for common CWR and 893 occurrence records for rare and scarce CWR).

Taxon richness analysis of the 98 common CWR reveals hotspots along the south coast of Wales (particularly the Gower peninsula, areas surrounding Cardiff and the lower Wye Valley), north Wales (the northern reaches of Denbighshire and Flintshire) and a number of small localities along the west coast in the county of Ceredigion between Aberystwyth and Cardigan (Fig. A1.3a). In comparison with the number of observations made across Wales, higher recording effort is apparent in two grid squares around the Cardiff area and in one grid square around Chepstow (in the Wye Valley) in comparison with the rest of the country (Fig. A1.3b). The presence of the National Museum Wales in Cardiff may account for the higher numbers of observations in those specific grid squares.

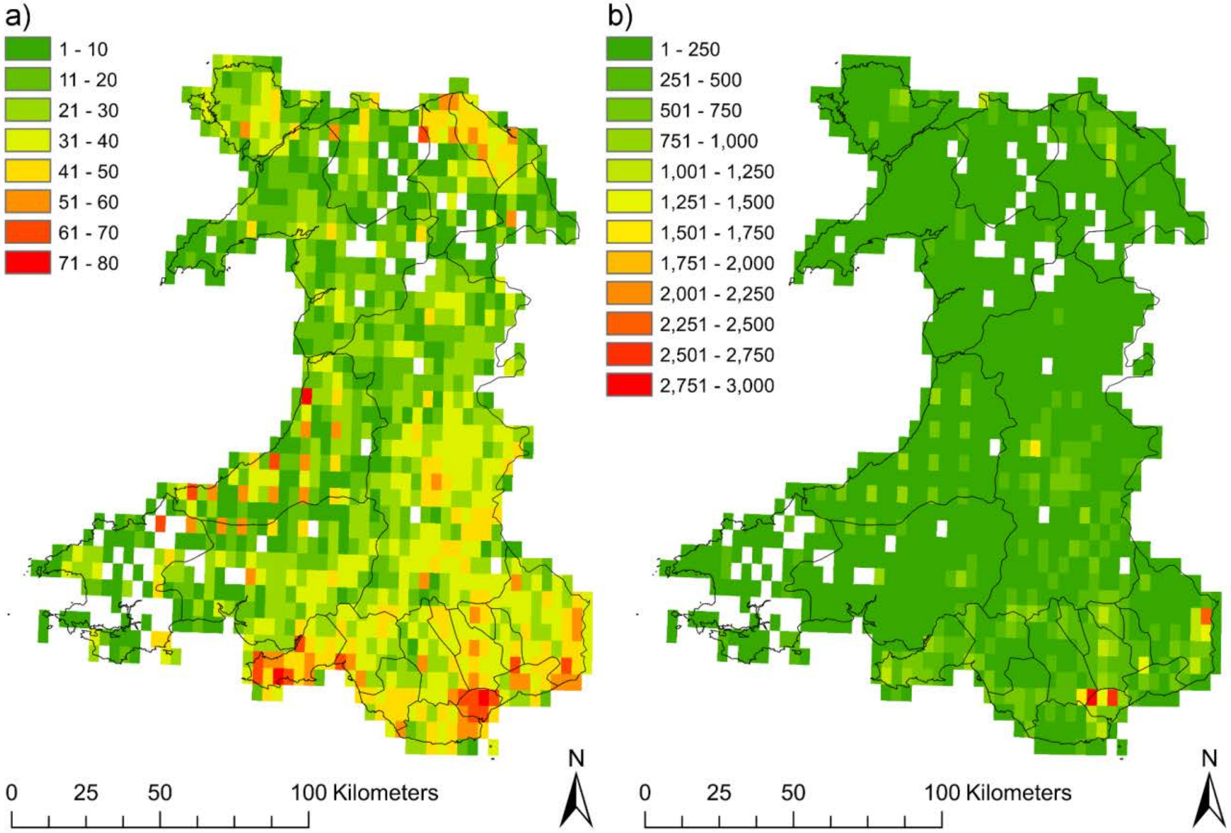

Figure A1.3 Richness analysis for the 98 common taxa with occurrence data points in the Welsh national CWR inventory using a grid square size of 0.05 degrees. a) Taxon richness and b) Observation richness.

Taxon richness analysis of the 22 rare and scarce CWR also reveals the Gower Peninsula as a key hotspot (Fig. A1.4a). In addition, a site in the south east of the county of Powys (Brecon Beacons) and coastal areas surrounding Newport in the south of Wales are also highlighted as hotspots, the former having a high number of *Sorbus* species. The grid square with the highest number of records is located in the Brecon Beacons, south Powys (Fig. A1.4b), which overlaps with the taxon hotspot in Fig. A1.4a. One grid square covering Flat Holm Island, part of the county of Cardiff, has the second highest number of occurrence records (Fig. A1.4b).

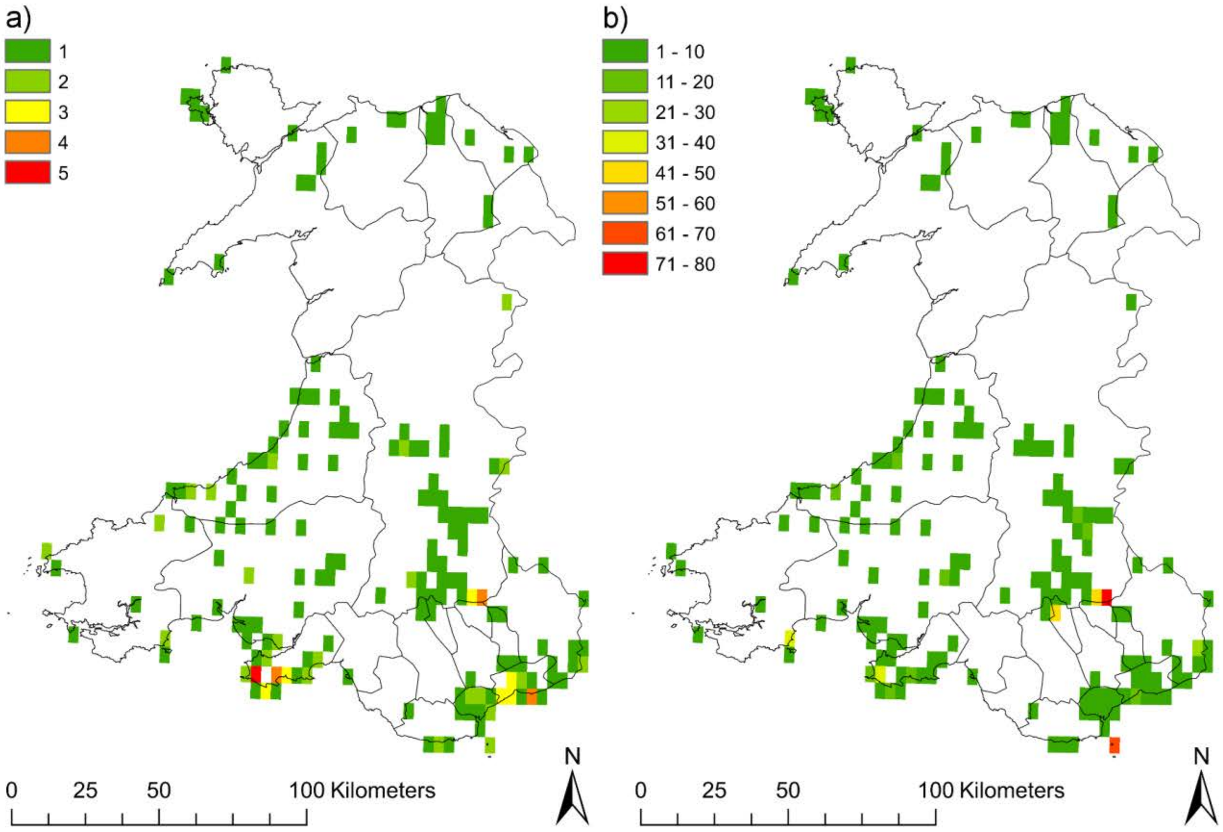

Figure A1.4 Richness analysis for the 22 rare taxa with occurrence data points in the Welsh national CWR inventory using a grid square size of 0.05 degrees. a) Taxon richness and b) Observation richness.

Analysis of the location of occurrence records reveals that over one third (37.65%) of all common, rare and scarce occurrence records fall within statutory Welsh protected areas. All common taxa, except for one, have at least two records within protected areas. Wall barley *Hordeum murinum* L. subsp. *murinum* is the exception with only three records, none of which fall within a protected area (one is located on the west coast, south of Aberystwyth and the remaining two are close to the Severn bridge in the south east of Wales). However, further records for this taxon recorded to species level exist but were not included in the analysis due to uncertainty over which subspecies they belonged to. It was found that all rare/scarce CWR have at least one occurrence record within protected areas. Interestingly, six rare/scarce taxa have no records outside of a protected area (wild asparagus *A. prostratus*, glaucous meadow-grass *Poa glauca* Vahl, white mustard *Sinapis alba* L. subsp. *alba*, thin-leaved whitebeam *S. leptophylla* E.F. Warb, Ley’s whitebeam *S. leyana*, lesser whitebeam *S. minima* (Ley) Hedl.).

The complementarity analysis carried out for common CWR showed that a total of nine grid squares would be sufficient to contain all 98 common taxa (Fig. A1.5a). The highest priority grid square is located in the county of Cardiff with 79 taxa being recorded at this site. However, there are a particularly high number of occurrence records taken in this location so it is possible that the results are biased to some degree toward this grid square (Fig. A1.3b), it was treated with discretion as a result and will require ground-truthing. Grid squares two, three and four collectively contain 87.76% of all common priority CWR and so are considered the highest priority. Grid square two is located on the Gower Peninsula with a total of 77 CWR taxa. A further 49 taxa are within grid square three, located in north Ceredigion, seven of which are not found in square two. A total of thirty-three taxa are then contained within grid square four in west Powys, two of which are not present in grid squares two and three.

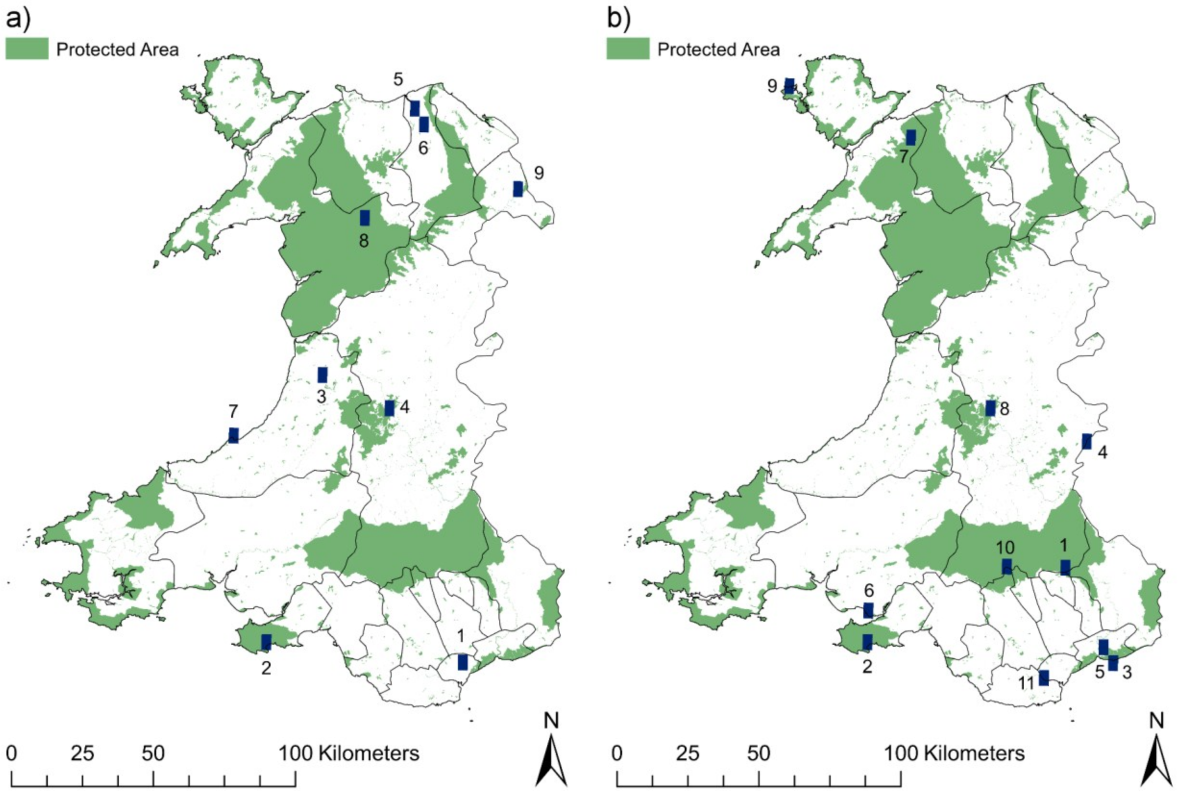

Figure A1.5 Complementarity analyses for a) 98 common Welsh priority CWR showing the locations of all nine priority grid squares/candidate sites for CWR genetic reserves and b) 22 rare and scarce Welsh priority CWR showing the locations of all 11 priority grid squares/candidate sites for CWR genetic reserves.

The complementarity analysis carried out for rare CWR taxa showed that a total of 11 grid squares would be sufficient to contain all 22 rare and scarce taxa included in the gap analysis (Fig. A1.5b). The top three priority sites each contain four taxa and there is no overlap between them (i.e. the four taxa recorded in square one are different to those in square two etc.). These three sites are located in southeast Powys, the Gower Peninsula and in coastal areas by Newport in south Wales respectively. Though the top priority site is potentially associated with recording bias (Fig. A1.4b), this is likely to be because all four taxa are *Sorbus* species, the majority of which are threatened (Table A1.3). Only two grid squares were common to both analyses. These were the sites on the Gower Peninsula and in west Powys. In addition, it was found that only 37.8% of common taxa were found in five or more grid squares. Of the rare taxa, only one was located in more than one grid square and this was sea clover *Trifolium squamosum* L. though it was still only in a total of two squares.

A1.4.3 Ex situ *gap analysis*

A total of 238 accessions collected from within Wales were found to exist in UK genebanks. Of the 122 priority Welsh CWR it was found that only 29 taxa had any stored accessions, leaving 93 taxa without any *ex situ* material collected from within Wales and therefore classified as priority level 1 (Supplementary Table A1.2). Furthermore, it was found that over half of these accessions (52.1%) were of perennial rye grass *Lolium perenne* L., with cock's foot *Dactylis glomerata* L. having the next highest number of accessions (17). A total of nine taxa had five or more separate stored accessions, only two of which are considered rare or scarce (wild asparagus *A. prostratus* and thin-leaved whitebeam *S. leptophylla*.) GRS results indicate that *ex situ* collections for only four taxa have a geographical representativeness value above the recommended threshold of 30% and all of these are classified as rare or scarce (round-leaved whitebeam *S. eminens*, thin-leaved whitebeam *S. leptophylla*, upright clover *T. strictum* and Bithynian vetch *Vicia bithynica* (L.) L.). Combining these results, only thin-leaved whitebeam *S. leptophylla* is classified as sufficiently represented in *ex situ* collections (priority level 6), seven CWR belong to priority level 5 with five or more accessions but a GRS below 30%. The remaining CWR have fewer than five accessions, except for wild asparagus *A. prostratus* that has five but is in priority level 2 due to the geographical location of those accessions being restricted. Overall, it can be seen that very few taxa have *ex situ* collections that are representative of their *in situ* distribution and the majority require further collection efforts. See Supplementary Table A1.2 for a full list of the number of accessions stored per priority Welsh taxon, GRS values and their priority levels for further collecting.

A1.5 Discussion

A1.5.1 Prioritisation to create a CWR inventory

The selection of appropriate criteria for prioritisation to produce an inventory is application specific. This is reiterated in the assessment of England CWR conservation where Fielder *et al*. (2015) stress the importance of involving relevant stakeholders in this development process to ensure national support for any conservation recommendations made as well as ensuring that international targets are also considered. The Welsh priority CWR inventory was developed with input from NRW to achieve this.

Though there are similarities between the Welsh inventory of priority CWR and the English equivalent (Fielder *et al*., 2015), including the consultation with conservation organisations and the use criteria such as economic value and degree of relatedness to a crop, there are also some key differences. The Welsh inventory has fewer priority CWR taxa than the English inventory (122 and 148 taxa respectively), despite the Welsh inventory not only including

CWR related to food and forage/fodder crops but also forestry taxa as well. This is likely to be due to Wales having a smaller total flora, which was reflected in the total number of CWR identified in Wales and listed in the CWR checklist (1,251 in Wales and 1,471 in England). The decision to include forestry taxa in the Welsh inventory was taken early on through discussion with NRW. It was considered necessary to include trees due to their increasing threat from pest and diseases such as the recent Chalara ash dieback outbreak (Forestry Commission, 2014) and also due to their economic importance for timber production (NRW, 2014). The English inventory does not include forestry taxa, however Fielder *et al*. (2015) highlight that future work should also consider conservation of other categories of CWR including forestry species, medicinal plants, plants with industrial application etc.

Red list threat assessments were used in the prioritisation process to create the Welsh CWR inventory (Cheffings and Farrell, 2005; Dine, 2008; Bilz *et al*., 2011; IUCN, 2012). IUCN red list data has been widely used as a criterion in many conservation strategies as it provides a thorough analysis of multiple aspects of threat e.g. population size and geographic range (Venezuela: Berlingeri and Crespo, 2012; Benin: Idohou *et al*., 2012; Finland: Fitzgerald, 2013; Spain: Rubio Teso *et al*., 2013; Italy: Panella *et al*., 2014). Red lists, however, do not yet consider how these threats impact the genetic diversity within species. In the case of CWR, threat to genetic diversity should be a major consideration as it is the diversity within species that is the key to developing improved crop varieties.

A1.5.2 In situ *gap analysis*

To carry out the *in situ* gap analysis of priority CWR, NRW advised that common CWR should be analysed separately from rare/scarce CWR. This was to ensure that the analysis was more transparent, creating a clearer picture of conservation priorities for each of these groups.

This is an idea that is supported by Hopkins and Maxted (2011) who suggest that not only should rare taxa be targeted for systematic conservation but that common taxa may prove to contain areas of rich genetic diversity due to their abundance and geographic partitioning. For common CWR, specific and targeted areas within a taxon's range should be a priority for conservation whereas for rare/scarce taxa it will be more appropriate to conserve their whole range. There is an added value in carrying out these two analyses separately rather than combining all species into the same gap analysis, this is because sites identified as important both for common and rare species can be further prioritised over sites that may only contain common species.

The inclusion of occurrence data from all four Welsh Local Record Centres (LRC) in addition to the data obtained from the BSBI distribution database (BSBI, 2013), greatly improved the robustness of the *in situ* analysis. Similar volumes of data were obtained from each LRC, ensuring that each region within Wales was well represented. Only two areas appeared to have fewer occurrence records and these were locations within Pembrokeshire in the southwest and Denbighshire in the northeast, however it is thought that this may not be due to a lack of records but a lack of records at the resolution of tetrad ($2km^2$) or better, or that county recorders hold more data but that not all of it is yet available through LRCs and the BSBI distribution database.

Taxon hotspot analyses revealed the south of Wales to be richer in CWR than the north. This is a pattern reflected in the English gap analysis and also in an analysis of CWR locations in the UK Countryside Survey (Fielder *et al.*, 2015; Jarvis *et al.*, in press). However, even in areas with high concentrations of CWR, few examples of their active conservation exist within Wales. The Welsh Rare Plants Project, run jointly between the National Museum Wales and the National Botanic Garden of Wales seeks to actively conserve approximately 20 rare plants, four of which are CWR (National Botanic Garden of Wales, 2014). As a result, some active conservation does exist for wild asparagus *A. prostratus*, thin-leaved whitebeam *S. leptophylla*, Ley's whitebeam *S. leyana* and lesser whitebeam *S. minima* (Kay and John, 1995, Rich *et al*., 2005; King *et al*., 2007) but there are no examples of systematic and multi-species approaches to CWR conservation across the country.

The complementarity analysis is the first stage in identifying appropriate broad-scale locations for implementing CWR conservation, with the aim of forming a network of complementary genetic reserves (Fitzgerald, 2013). In Wales, locations were identified for both common and rare/scarce taxa separately with only two grid cells showing overlap between the two analyses (Gower Peninsula and west Powys). With remote analyses such as the GIS methods used in the current study, it is very important to ground-truth the results to ensure the plants in question are in the locations indicated by the analysis (Fitzgerald, 2013). In the case of Wales, it is likely that more taxa will be present in each grid square than indicated in the analysis, particularly in grid cells containing rare taxa where it is likely that the common taxa will also be present.

Similarly to England, the majority of CWR occurrence records in Wales were located outside protected areas (Fielder *et al*., 2015). Though a network of genetic reserves across the country would make significant progress towards active and systematic conservation of CWR it would not, on its own, be sufficient to ensure the full range of priority CWR are suitably conserved. Many CWR are considered weedy taxa, which are often associated with disturbed, early-successional habitats (Maxted and Kell, 2009) and so would usually fall outside the boundaries of a typical protected area; these habitat preferences are further supported by Jarvis *et al*. (in press), who found that CWR across the UK are often associated with linear landscape features, including field margins and road verges. As such, active conservation and management of CWR outside of protected areas should be encouraged perhaps through provision of incentives for stakeholders and landowners.

A1.5.3 Ex situ *gap analysis*

CWR in Europe are poorly represented in genebanks (Dias *et al*., 2012). Analysis of *ex situ* accessions of priority CWR has revealed a similar picture in Wales. Less than a quarter of Welsh priority CWR have any stored accessions whereas in England, this figure stands at just over 50% (Fielder *et al*., 2015). It is likely that plant collection efforts have so far focussed on collecting one or two accessions from across the UK as a whole, rather than ensuring the conservation of the range of genetic diversity within each devolved administration. Now that these gaps have been identified in Wales it is possible for a more targeted and systematic approach to be taken in collecting accessions. This will also help towards reducing the bias towards accessions of perennial rye grass *L. perenne* in current collections.

GRS analysis revealed further gaps in *ex situ* collections. Many CWR are lacking accessions but very few of those with accessions have their full geographic range represented *ex situ*. Only four taxa had a GRS score above 30% (the recommended threshold for geographic representativeness (Ramírez-Villegas *et al*., 2010; Phillips *et al*., 2014). These four CWR are all listed as either Critically Endangered or Endangered in the Vascular Plant Red Data List for Wales (Dines, 2008). This finding is likely to be due to their highly restricted geographical ranges, which will mean that one accession would already represent a large proportion of their total distribution in the wild. Though this makes it difficult to make strong conclusions from the GRS analysis, it does give a broad picture of the representativeness of *ex situ* collections.

A more robust method for analysing the representativeness of *ex situ* collections would be to use genetic data. Though such techniques are more expensive and time consuming than the approach taken in Wales, they are becoming increasingly affordable and will in time become a viable option for analyses such as these. Until this becomes routinely affordable it is possible to consider ecogeography as a proxy for genetic diversity, using climatic, edaphic and geophysical variables to define unique 'adaptive scenarios' across a geographic region (Parra-Quijano *et al*., 2014). This approach is best suited to analysis of a specific taxon or group of related taxa (Parra-Quijano *et al*., 2014) and so was not appropriate for the current analysis of 122 CWR. Within more fine-scale analyses this would be a valuable technique.

A1.5.4 Recommendations for enhancing CWR conservation in Wales

- The Welsh inventory of priority CWR should be reviewed and updated at regular intervals (e.g. once every 10 years). This will allow conservation organisations to continue to tailor the list of priority CWR in the face of climate change and changing national and international priorities. The process of reviewing and updating the inventory should always include conservation organisations and stakeholders such as NRW to ensure active conservation of CWR is maintained.

- The data available for prioritisation should be continuously improved. For example, there is a need for economic value data for forage and fodder crops, more data on crop gene pool concepts for a broader range of taxa and a method to assess threats to genetic diversity.

- A network of complementary genetic reserves conserving the ranges of taxonomic and genetic diversity within CWR should be established. Steps should be taken to investigate the suitability of the Gower Peninsula as the first CWR genetic reserve in Wales, it having the highest number of both common and rare/scarce CWR. The remaining locations for both common and rare CWR should then also be investigated and ground-truthed. All CWR genetic reserves should be established in a manner that meets the required standards (Iriondo *et al*., 2012).

- Active conservation of CWR outside of protected areas should be encouraged. A dialogue between organisations (e.g. Welsh government, NRW) and landowners will be necessary, along with appropriate incentives. A link could be made between CWR conservation and the Wales Rural Development Programme 2014–2020 (Welsh Government, 2014).

- Genetic diversity studies should be carried out for priority CWR to determine the level and partitioning of genetic diversity in these taxa across Wales. In this way, a more informed selection of sites suitable for genetic reserves and for management outside of protected areas could be made. Where this is not possible, ecogeographic analyses could be carried out (Parra-Quijano *et al*., 2012b).

- A minimum of five populations per priority CWR should be conserved *in situ* (Brown and Briggs, 1991; Dulloo *et al*., 2008). For taxa whose range is too limited to achieve this, an effort to ensure conservation of the maximum range of genetic diversity is advised. This threshold should be adjusted appropriately according to the life histories of the CWR under consideration.

- Communication between the different conservation organisations (and departments within organisations) is vital for the coordination of conservation activities for CWR and genetic resources in general. This could be achieved through the PLINC Wales group (Plantlife, 2014).

- CWR occurrence records should be continuously updated. Current records maintained by the four LRC and the BSBI distribution database (BSBI, 2013) provide an extensive and detailed dataset. It is important that this is maintained and that new records are transferred from county recorders to LRCs and the BSBI distribution database. Records should be as precise as possible, using at least six figure grid references and recording to subspecies level where possible.

- Collection efforts should focus on achieving a minimum of five geographically distinct accessions per priority CWR, where possible (Brown and Briggs, 1991; Dulloo *et al.*, 2008). This should be adjusted appropriately according to geographic range and life histories of target CWR. Initial collection efforts should focus on the 93 CWR in priority level 1 with no accessions stored in genebanks (Supplementary Table A1.2).

- Genetic analyses of current collections should be undertaken to determine any overlap between stored accessions and to identify instances where further collecting would provide additional genetic diversity for the collection. Where genetic analyses are not a viable option, ecogeographic analyses should be undertaken as a proxy using methods described by Parra-Quijano *et al.* (2014).

- To ensure collections capture the genotypic evolution of *in situ* populations over time, the relationship between accessions held in long-term storage and population genetic changes over time should be explored.

- Duplicates of all accessions should be stored in other genebanks as a safety back up and existing accessions should be maintained according to genebank ‘best practices’.

- Agreements between landowners (within and outside of protected areas) and genebanks should be formulated to protect the rights of the former from whose land the accessions have been collected. Agreements should specify any restrictions on the use of the CWR material.

A1.6 Conclusion

The current study presents the first inventory of Welsh priority CWR and an assessment of their conservation in Wales. It has been developed with input and support from NRW to ensure that the results are relevant to current conservation priorities in Wales and that it responds to international policy. The results highlight the importance of establishing a network of *in situ* conservation in the form of both genetic reserves and conservation outside of protected areas with appropriate management. This should be complemented by a comprehensive *ex situ* back up of material which should be accessible to plant breeders for use in crop improvement. With this in mind, long-term active conservation and also improved use of CWR will be achievable, contributing to widespread efforts to improve future food security in line with European and global policy.