THE INFLUENCE OF HISTORICAL AND CONTEMPORARY LANDSCAPE STRUCTURE ON PLANT BIODIVERSITY: EFFECTS ON SPECIES AND GENETICS

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A thesis submitted in partial fulfilment of the requirements of the University of Brighton for the degree of Doctor of Philosophy

March 2017

Abstract

Biodiversity describes diversity at different levels of biological organisation, including: habitat diversity; species diversity; and genetic diversity. Understanding the processes that contribute to maintaining biodiversity is a primary concern for both ecology and evolutionary biology. To this end, research into the factors influencing the different levels of biodiversity independently are widespread. However, little is understood about the relationship between the different levels. This study investigates the patterns of habitat, species, and genetic diversity in fragmented internationally important calcareous grasslands, and analyses the spatial and temporal factors influencing them. Finally, the relationship between these levels of biodiversity is examined. Within the South Downs National Park study area, substantial change to habitat diversity and landscape structure was measured between the 1930s and 2012. The transition of semi-natural habitat to agricultural land was the predominate change. Loss of habitat between the 1930s and 2012 was found to influence both species richness and species evenness of vegetation in twelve calcareous grassland study sites. By contrast, none of the variables examined explained the variation in species composition between sites. Further analysis, at the genetic level, for two target species showed that the amount of habitat loss was important in explaining the genetic variation in *Cirsium acaule*, and soil nutrients were important in explaining the variation of *Ranunculus bulbosus*. In contrast to the predictions of the species genetic diversity correlation theory, no relationship was established between species and genetic diversity. Similarly, no relationships were found between habitat diversity and diversity at the species or genetic level. Although there were similarities in the factors influencing different levels of biodiversity, habitat diversity, species diversity, and genetic diversity appear to be responding independently to the processes acting on them. As such efforts to conserve biodiversity should consider the influence of conservation strategies on biodiversity holistically, and not focus on a single measure.

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Glossary

Alpha diversity	Species diversity within a local species pool.
Beta diversity	The differentiation in species diversity among samples or sites.
Community	A group of populations of multiple species that are interacting within a habitat.
Habitat	An ecological or environmental area that is occupied by a population or community.
Habitat fragmentation	The division of contiguous habitat into smaller and more isolated patches
Land-cover	Classification of the land in relation to the vegetation or habitats present.
Landscape	An interactive area of land, similar in character throughout.
Landscape change	The change in the spatial structure of a landscape over time.
Landscape composition	The make-up of LULC classes within the landscape.
Landscape configuration	The spatial arrangement of LULC classes within the landscape.
Landscape ecology	The science of how the landscape interacts with ecological processes.
Landscape genetics	The study of the interaction between landscape spatial structure with gene flow and genetic spatial structure.
Land-use	Classification of how the land is used anthropogenically.
Land-use/Land-cover (LULC)	An amalgamation of the two terms used to describe the composition of the landscape.
Metapopulation	A set of multiple local populations that interact with one another.
(Habitat) Patch	Non-linear land areas of homogenous habitat that are distinct from their surroundings.
Population	A group of individuals from the same species

	within the same geographical area.
Species diversity	The amount and evenness of different species within a community of study
Spatial structure	The composition and configuration of landscape elements

Abbreviations

AIC	Akaike's information criterion
BAP	Biodiversity Action Plan
CAI	Core-area index
CCA	Canonical correspondence analysis
DEM	Digital elevation mode
F_{IS}	Measure of departure from the Hardy-Weinberg equilibrium within populations
F_{ST}	Measure of departure from the Hardy-Weinberg equilibrium between populations
GLM	Generalised linear model
HBIC	Hampshire Biodiversity Information Centre
H_e	Expected heterozygosity
H_o	Observed heterozygosity
IFM	Incidence function models
IUCN	International Union for Conservation of Nature and Natural Resources
K	Potassium
LULC	Land-use/land-cover
LUs	Livestock units
LUSB	Land Utilisation Survey of Britain
N	Nitrogen
NNR	National Nature Reserve
NSRI	National Soil Resources Institute
NVC	National Vegetation Classification
P	Phosphorus
PCA	Principal component analysis
RDA	Redundancy analysis
SBRC	Sussex Biodiversity Record Centre
SDNP	South Downs National Park
SGDC	Species-genetic diversity correlation
SHDI	Shannon diversity index
SLOSS	Single large or several small
SNCI	Site of Nature Conservation Importance
SSSI	Site of Special Scientific Interest

Acknowledgements

Thanks to Dr Niall Burnside for seeing through my flaws to the ability shadowed behind them, and having the confidence in me to take this PhD on. Your continued faith in me has been a constant surprise and a great inspiration!

Thanks to Dr Andy Overall, whose talents and generosity with time has enabled someone who had no prior training in genetics to conduct a whole research chapter focusing on it.

Thanks to Dr Matt Brolly and Dr Roger Smith for reading and providing valuable comments on draft versions.

Thanks to Neil Young, Kate Bush, and Miles Davis! I can’t imagine doing any of the lab and office work without you!

Finally thank you to my family. Thank you Jessica Tink, without whom I wouldn’t have done any of this. Thank you for your help at every stage in the past four and a bit years this has taken to complete. Thank you Oliver Tink for all the joy that you bring. Thank you Jonah Tink for all the smiles while I have been finishing this work off. You have all given this meaning to help me complete it.

I dedicate this work to my mother. I know you would have been proud of your little boy’s work.

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed

Dated

1. Introduction

1.1 Overview

Biodiversity describes diversity at different levels of biological organisation, including: habitat diversity; species diversity; and genetic diversity (Norse et al., 1986, Solbrig, 1991, Harper and Hawksworth, 1994, Heywood and Baste, 1995, Krebs, 1999). Maintaining biodiversity at each of these levels is one of the major challenges of international biodiversity conservation (Krebs et al., 1999).

Recent research has analysed the effects of historical landscape factors on biodiversity, although inconsistent findings have led to the failure to establish relationships. Furthermore, analysis of the relationship between spatial characteristics of the landscape and biodiversity is complicated by the extinction debt phenomenon, whereby after landscape change biodiversity may take time to come into equilibrium with the new landscape structure. As such, the underlying drivers of biodiversity are not clearly understood. Furthermore, increased understanding of how changing one component of biodiversity affects other components of biodiversity is necessary for viable long-term conservation.

Research is beginning to adopt a more holistic approach to the study of the processes maintaining biodiversity. Until recently the different components of biodiversity have typically been considered as exclusive entities. In particular species diversity and genetic diversity were associated with the domains of community ecology and population genetics respectively, with Clarke and Young (2000) commenting that the fields of ecology and genetics have traditionally worked as rivals with little cooperation. They argue that this is surprising considering the interaction between demographic and genetic processes in the course of extinction. Whilst several early papers (Lande, 1988, Caughley, 1994, Oostermeijer et al., 2003) outline the differences between demography and genetics, they also highlight that both factors and their interactions are important in the extinction process. Many researchers suggest that the integration of ecology and genetics is essential in achieving effective conservation management (Nunney and Campbell, 1993, Mills and Smouse, 1994, Schemske et al., 1994, Soule and Mills, 1998, Clarke and Young, 2000). Moreover, Picó and Van Groenendael (2007) comment that:

> *“To study the implications of fragmentation for species persistence, a multidisciplinary approach is required including the geography, ecology, and genetics of species analysed at large spatiotemporal scales and the development of measures at that scale necessary to recover biodiversity.”*

Vellend (2003) put forward the species-genetic diversity correlation (SGDC) hypothesis, which predicts that species and genetic diversity (within a single species) should be positively correlated across habitat patches or islands. The hypothesis is grounded in the principles of MacArthur and Wilson’s (1967) theory of island biogeography and Wright’s (1940) island model of population genetics. Vellend (2003) notes that whilst the mathematics of these two theories are different, they share near identical underlying logic: whilst the theory of island biogeography posits that species diversity is regulated by extinction and colonisation, the island model of population genetics posits that genetic diversity is regulated by rates of colonisation and local extinction. Compared to large populations, small populations experience higher rates of local extinction and genetic drift. In more isolated populations migration, and thus colonisation and gene flow, are lower than in less isolated populations.

A central consideration of this research was to combine the analysis of biodiversity at three levels, and to explore the nature of the relationship between them. More specifically, the research sought to evaluate the impact of landscape change on different levels of biodiversity within fragmented calcareous grasslands, in order to direct conservation and restoration efforts towards maintaining biodiversity.

The research focuses on calcareous grasslands on the South Downs National Park in South East England. At a European level semi-natural grasslands are recognised as key habitats for maintaining biodiversity (Poschlod and Bonn, 1998, Pykala, 2000). Agricultural intensification has led to the decline of such semi-natural grasslands and the flora and fauna they support (Hillier et al., 1990, Thomas, 1995).

1.2 Definition of landscape ecological terms

Various landscape ecological terms are used throughout this work. The following paragraph will define how they are used here and how they relate to one another.

An individual is used to describe one organism, and species to describe the type of organism. A group of individuals belonging to the same species in a particular geographical area is referred to as a population. Species are made up of populations, with a metapopulation defining a collection of local populations that interact with each other. A community is the aggregate of all the populations within a geographical area. The populations within a community are made up of individuals of particular species that interact with each other. Within a community each individual organism may represent a different species or a different genetic variant of a species. The dynamics of the population, and thus the species diversity and genetic diversity within, are determined by the birth, death, and movement of individuals. The ecological or environmental area occupied by a population or community is referred to as a habitat. A habitat is also used to refer to a collection of multiple habitat patches throughout a landscape. Habitat patch refers to non-linear land areas of homogenous habitat that are distinct from their surroundings. In Figure 1.1 each of the individual habitat polygons are habitat patches. To highlight this habitat patch a and habitat patch b have been singled out as distinct habitat patches. Groups of habitat patches of the same type collectively make-up a habitat, such as the five patches that make up habitat a, or the two patches that make-up habitat b (Figure 1.1). Landscape is used to describe an area that is spatially heterogeneous in at least one area of interest (Turner et al., 2002). The landscape in Figure 1.1 is comprised of a mosaic of four interacting habitat types.

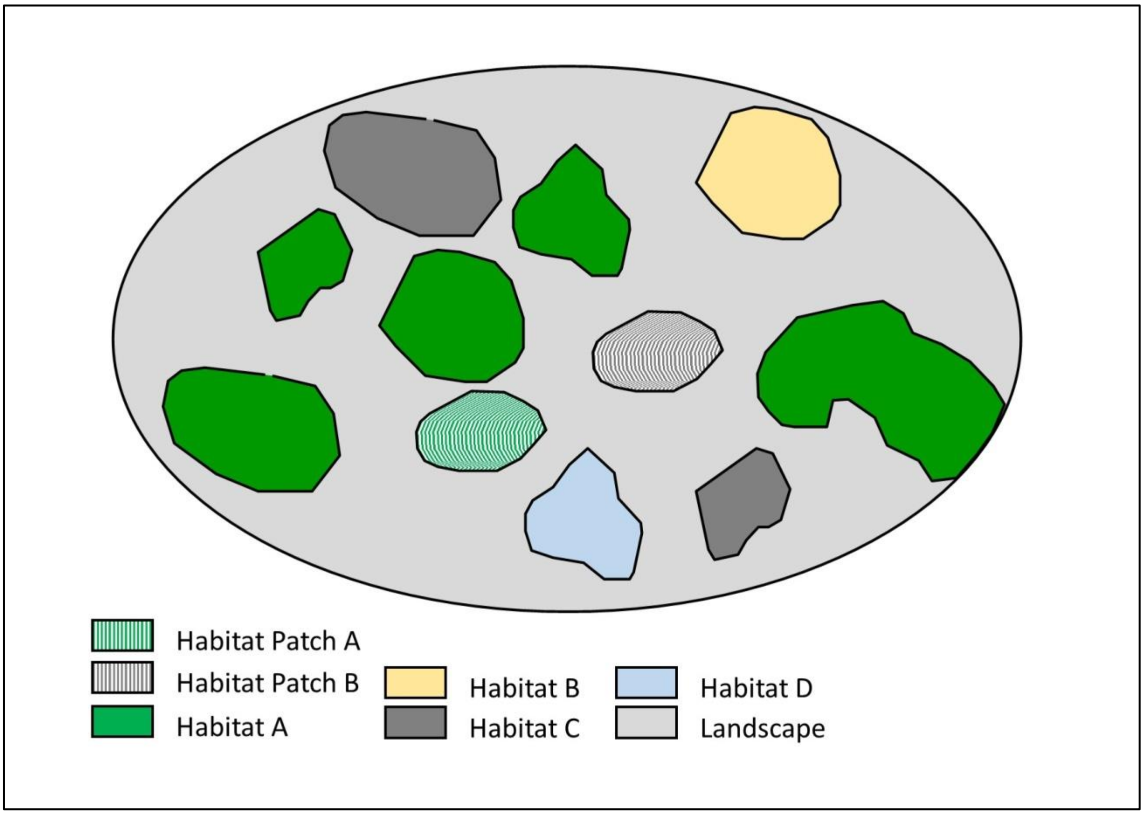


Figure 1.1 The distinctions between habitat patch, habitat, and landscape.

1.3 Research aim

Aims:

To investigate the patterns of habitat, species, and genetic diversity in fragmented calcareous grasslands, and to determine the spatial and temporal drivers of these three levels of biodiversity. This information can be used to direct conservation and restoration efforts towards maintaining biodiversity at all levels.

Objectives:

1. To develop data integration methods to model historical landscape structure, and analyse landscape change in the South Downs National Park.
2. To analyse the influence of historical, spatial, management, and abiotic variability on species diversity between the 1930s and 2012 in the South Downs National Park.
3. To analyse the influence of historical, spatial, management, and abiotic variability on genetic diversity between the 1930s and 2012 in the South Downs National Park.

4. To analyse the relationship between contemporary habitat diversity, species diversity, and genetic diversity in the South Downs National Park.

1.4 Research questions

The research worked to the following research questions:

i. What is the extent of change in habitat diversity and landscape structure within the SDNP between the 1930s and 2012?
ii. What patterns of fragmentation can be identified in twelve calcareous grassland study sites between the 1930s and 2012?
iii. To what extent do contemporary and historical landscape and environmental characteristics influence species diversity and composition?
iv. Is there evidence of an extinction debt in species diversity or composition?
v. What relationships exist between species diversity and species composition?
vi. What is the structure of gene flow across twelve calcareous grassland study sites for two plant species synonymous with calcareous grassland sites in South East England?
vii. To what extent do contemporary and historical landscape and environmental characteristics influence the genetic diversity of the two study species?
viii. What relationships exist between habitat, species, and genetic diversity, and is there evidence of a species genetic diversity correlation?

1.5 Thesis structure

1.5.1 Chapter 2. Literature review

This chapter serves to provide a context to the thesis by reviewing the literature relevant to the key research themes: biodiversity; landscape change; and the integration of habitat, species, and genetic diversity. This will include discussion and appraisal of existing and developing frameworks in relation to the study objectives

1.5.2 Chapter 3. Methodology: Study sites, species selection, and analysis methods

This chapter introduces the South Downs National Park (SDNP) study area, the calcareous grassland study habitat, the study sites, and the study species. The methods used to measure the components of biodiversity (habitat diversity, species diversity, genetic diversity) and the site variability (abiotic conditions, spatial structure, historical landscape structure, and management) are then presented. Finally, the methods used to analyse the relationship between the three components of biodiversity are presented.

1.5.3 Chapter 4. Habitat diversity and structure: contemporary and historical landscape patterns

The first research chapter, focusing on habitat diversity and historical landscape modelling. Initially, to provide a temporal element to the research, a model of the 1930s landscape was produced and validated. Secondly, this historical model was then used to analyse habitat diversity and structure in the study area between the 1930s and 2012. Both historical and contemporary habitat diversity and a range of spatial characteristics of the landscape are measured and discussed.

1.5.4 Chapter 5. The impact of abiotic, spatial, historical, and management variability on the composition and diversity of plant species

The second research chapter, focusing on species diversity. Initially the results of botanical surveys at twelve study sites are presented. The species records are then interrogated using multivariate methods (Redundancy Analysis) to investigate the effect and relative importance of a range of abiotic, spatial, historical, and management variables. The results are then discussed in relation to ecological theory and current research themes.

1.5.5 Chapter 6. The impact of historical and contemporary landscape structure on the genetic diversity of plant species

The third research chapter, focusing on genetic diversity. The results of laboratory analysis of genetic variation of two study species are presented. The data is then interrogated using multivariate methods (Redundancy Analysis) to investigate the effect and relative importance of a range of abiotic, spatial, historical, and management variables on genetic variability. The results they are then discussed in the context of population genetic theory and current research themes.

1.5.6 Chapter 7: Synthesis study: The relationship between habitat, species, and genetic diversity

In this chapter the results of the three research chapters are brought together for a synthesis study analysing relationships between the different levels of biodiversity. The broader implications of these relationships are then discussed.

1.5.7 Chapter 8: Discussion

This chapter begins with a discussion of the key findings of the research, with comment on their implications and applications. Each of the three research chapters will be discussed in turn, followed by a discussion of the broader implications of the results as a whole. The findings will be discussed in relation to the research questions.

1.5.8 Conclusion

The completion of the thesis aims stated in Chapter 1 are assessed. The limitations of the research are outlined, followed by recommendations for further research.

1.6 Nomenclature

Plants: Latin names follow Stace (2010).

Units: The International System of Units is used, with GIS distances in kilometres.

2. Literature review

2.1 Biodiversity

Biodiversity is the variety of life at all levels of biological organisation (Gaston and Spicer, 2004), defined by Wilson (2001) as:

> "*The variety of organisms considered at all levels, from genetic variants belonging to the same species through arrays of species to arrays of genera, families, and still higher taxonomic levels; includes the variety of ecosystems, which comprise both the communities of organisms within particular habitats and the physical conditions under which they live*."

As the above quote illustrates, biodiversity refers to diversity at three broad levels: communities, species, and genes (Norse et al., 1986, Solbrig, 1991, Harper and Hawksworth, 1994, Heywood and Baste, 1995, Krebs, 1999). The diversity of communities encompasses the diversity of biogeographic realms, biomes, provinces, ecoregions, ecosystems, habitats, and populations (Heywood and Baste, 1995). Diversity at the species level encompasses the number and evenness of species within a community of interest. Genetic diversity encompasses the variation in genetic structure between individuals within a population and between populations. The different levels are thought to be related and to act in synergy (Allendorf et al., 2012).

As a multidimensional concept, it is not appropriate to define biodiversity using a single measure (Magurran, 2004). However, Gaston (1996) comments that many studies imply that their findings concern biodiversity, typically by using the terms species diversity and biodiversity interchangeably (Hubbell, 2001, Magurran, 2004). Moreover, discussions of biodiversity loss typically focus on species extinctions, and not on local population declines (Ehrlich and Daily, 1993, Ehrlich, 1994), or declines of genetic and habitat diversity (Gaston, 1996). Similarly, measures of biodiversity are commonly used in conservation strategies. In such instances, one component of biodiversity is typically used as a surrogate for other, or all, levels of biodiversity. Most typically this is species diversity, occasionally genetic diversity, but rarely habitat diversity or a combination of the different components. Indeed it is at the species level where most research on biodiversity is focused, and this is often treated as the most fundamental level of biodiversity (Gaston and Spicer, 2004). Although species richness and diversity are often strongly positively correlated with other measures of biodiversity (Williams and Humphries, 1996), they should not be used as a surrogate for biodiversity (Purvis and Hector, 2000, Wilsey et al., 2005). In this vein Purvis and Hector (2000) comment that preserving species richness does not equate to preserving biodiversity.

2.1.1 Habitat diversity

Diversity at the habitat level (sensu Heywood and Baste, 1995) is perhaps the least studied component of biodiversity. This may in part be a factor of the difficulties in defining terms and classifying the different structures that communities encompass. For example, many different habitat classification systems exist, often with different sub-categories for further division. Moreover, there are often difficulties in deciding exactly where one community ends and another begins. Although habitat diversity is one of the better understood components of community diversity, a single term for the concept has yet to be established (Tews et al., 2004) (Figure 2.1). Henceforth habitat diversity will be used to describe the variety of habitats within a landscape.

The importance of habitat diversity is highlighted in the premise that ecological processes are influenced by spatial patterns in the landscape (Turner, 1989, Gustafson, 1998, Kupfer, 2012). As such, ecological processes are influenced by the diversity of habitats in a landscape. Contemporary habitat diversity is essentially a product of anthropogenic activity, specifically resulting from large scale landscape change. The transition of many different natural habitats to agricultural land commonly results in a lower habitat diversity. Landscapes are now typically dominated by agricultural land, with natural and semi-natural habitat persisting as small remnant patches. Few habitats exist that are not shaped by anthropogenic activity.

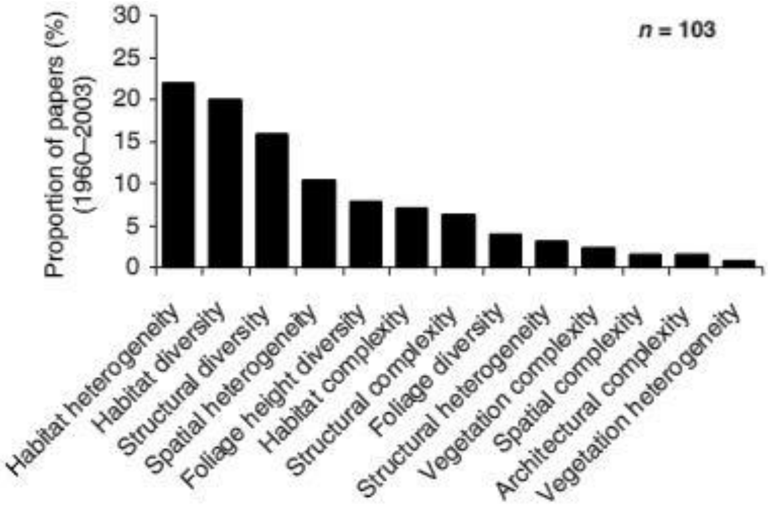


Figure 2.1. The frequency of different terminology used in publications to describe habitat diversity. From Tews et al. (2004).

2.1.2 Species diversity

Universally, across all taxonomic groups and environments, species are not equally common (Magurran, 2004). Some will be abundant, others moderately common, and some will be rare. Species diversity concerns both the number of species (species richness) and the amount of variability in the abundance of these species (species evenness) within a community of interest. It is the most commonly studied aspect of biodiversity, and is often used as a surrogate for biodiversity (Gaston and Spicer, 2004, Magurran, 2004). The study of species diversity is essentially the comparison of the diversity of one community with that of another.

Several authors comment that exactly what species diversity means is ambiguous (Hurlbert, 1971, Peet, 1974, Lande, 1996, Magurran, 2004, Tuomisto, 2010, Tuomisto, 2011). Central to these difficulties is that species diversity is composed of two distinct concepts: species richness, and species evenness. Species richness measures the number of species, while species evenness measures how similar species are in their abundance. As a result, the definition of species diversity is dependent upon the method of measurement. To this end, many species diversity indices exist, all placing subtly different emphasis on species richness and species evenness. As such the quantification of species diversity is largely a product of the weighting that is applied to these two different components. Thus, the judgement of whether one site is more diverse than another is largely dependent upon the choice of diversity measure used, an inevitable consequence of attempting to classify a multidimensional concept using a single value (Patil and Taillie, 1979). Multiple authors report differences in the ordering of sites by species diversity when comparing the values of two of the most popular measures (Hurlbert, 1971, Tothmeresz, 1995, Nagendra, 2002): the Shannon index (Shannon and Weaver, 1949) and the Simpson index (Simpson, 1949).

Whittaker (1960) introduced the concepts of alpha (α), beta (β) and gamma (γ) diversity. Alpha diversity describes the local species diversity, such as the diversity of a single sample or site. Beta diversity describes the differences in species composition between different samples or sites. Finally, gamma diversity concerns the species diversity of a regional species pool, such as the entire landscape of interest. Alpha and gamma diversity can be referred to as inventory diversity (Jurasinski et al., 2009), in that they differ only in scale. Beta diversity differs by concerning with the differences in species composition between different populations. As such, within conservation ecology, beta diversity can be used to analyse patterns of heterogeneity across an environment. For example, with reference to habitat fragmentation, beta diversity has been applied to research into the single large or several small (SLOSS) debate Wiersman and Urban (2005).

According to the theory of island biogeography (MacArthur and Wilson, 1967), species diversity is regulated by extinction and colonisation. Species diversity declines as species are lost from a community by extinction. By contrast the colonisation of a community by a new species increases species diversity. Species extinctions can be limited by the introduction of new individuals of an existing species to a population.

2.1.3 Genetic diversity

Genetic diversity describes the amount of variability in a population at the level of genetic markers. Markers are defined as sequence variants (e.g. single nucleotide polymorphisms) or repetitive sequences (e.g. microsatellites). Different variants are referred to as alleles, and combinations of alleles are genotypes. Genetic diversity is generated by two processes; mutation and migration (Frankham et al., 2009). Mutation causes changes to the nucleotides in a DNA sequence, and is how all genetic variation originates. Immigrants can augment the genetic diversity of one population by introducing the mutations of a different population. Once generated, genetic diversity is influenced by mutation, drift, migration, and selection (Hartl and Clark, 2007). Genetic drift describes the process where change in genetic composition is determined mainly by random processes instead of by natural selection.

At its basic level, evolution by natural selection is the change in genetic composition of populations in response to changes in their environment. Regions of the genome that do not influence the phenotype are neutral to the influence of natural selection and hence change is stochastic and largely influenced by genetic drift. This is only possible where there is genetic diversity (McNeely et al., 1990). Heterozygosity, the proportions of homozygous and heterozygous loci, is the most commonly used measure of genetic diversity (Frankham et al., 2009). A locus is homozygous where the genotype is comprised of two copies of the same allele, and heterozygous where it is comprised of two different alleles. Observed heterozygosity (H_o) is the proportion of heterozygotes in the total sample and expected heterozygosity (H_e) is the heterozygosity that would be expected under the Hardy-Weinberg equilibrium.

Loss of genetic diversity reduces a population’s ability to adapt to change, increasing the chances of extinction. In randomly mating populations that are experiencing no gene flow, mutation, genetic drift or natural selection, the genotype frequency is expected to follow Hardy-Weinberg expectations. For example, a locus with two alleles, *A* and *a*, at frequencies *p* and *q*, respectively, are expected to have *AA*, *Aa* and *aa* genotypes in the proportions p^2, $2pq$ and q^2, respectively. Departures from these genotype frequencies are referred to as departures from Hardy-Weinberg equilibrium. The most likely reasons for departures from these genotype frequencies result from inbreeding and subpopulations experiencing barriers to gene flow. Inbreeding is the production of offspring from related individuals, and leads to reduced reproduction and survival, known as inbreeding depression. Departures from the Hardy-Weinberg equilibrium can be measured using F-statistics (Wright, 1931, Wright, 1951), a suite of inbreeding coefficients used to measure genetic differences between populations based on the amount of heterozygosity in populations. In populations experiencing inbreeding (i.e., a departure from random mating), the population experiences an excess of homozygous genotypes (e.g., *AA* and *aa* genotypes) at the expense of heterozygous genotypes (e.g., *Aa*). The magnitude of this departure is measured by the statistic F_{IS}. Similarly, when subpopulations are not freely exchanging genes, the allele frequencies (e.g., *p* and *q*) can vary between subpopulations and again, there can be an excess of homozygosity within subpopulations relative to the total population's average allele frequencies. This departure is quantified by F_{ST}.

2.2 Biodiversity loss and landscape change: habitat fragmentation

Biodiversity loss is caused by a range of environmental and anthropogenic factors, of which landscape change is paramount (Lande, 1998, Fahrig, 2003, Pimm, 2008). Landscape change can occur as a result of fluctuations in abiotic conditions, biotic interactions, or natural disturbance and succession. However, in recent times, worldwide landscape change is thought to have been accelerated by anthropogenic factors (Vitousek et al., 1997). Under anthropogenic pressures the pattern of landscape change is often non-random, with the most productive land commonly being the most extensively modified, and with large remnant habitat patches likely to be located in less productive areas (Virkkala et al., 1994, Lindenmayer and Fischer, 2006). As a consequence, remnant vegetation is often restricted to land that is unsuitable for other uses, a phenomenon termed the worthless land hypothesis (Runte, 1977, Hall, 1988). Scott et al. (2001) report that nature reserves in the United States of America are predominantly situated at high elevations and on less productive soils, with the lower elevations and most productive soils extensively comprised of urban and agricultural land. Similarly, Burnside et al. (2002) show that remnant patches of calcareous grasslands on the West Sussex Downs in southern England are largely restricted to steep slopes. These slopes have acted as barriers to mechanised access and remained uncultivated, whilst the surrounding land has been converted. Such remnant vegetation may not be a representative sample of the habitat within its true ecological niche (Huggett and Cheesman, 2002), with the species best suited to the localised environment of the remnant land most likely to thrive. As such, localised environmental differences between habitat patches are expected to result in differences in the vegetation they support.

2.2.1 Habitat fragmentation

Contemporary land-use/land-cover (LULC) change typically occurs with the conversion of natural and semi-natural habitats into arable and urban land, leading to landscapes that are more homogenous in character with reduced habitat diversity (Farina, 2006). Such transformations lead to changes in the spatial characteristics of remnant habitat patches and can impact the ecological processes within these systems (Forman, 1995). Indeed, many natural and semi-natural habitats are now only found as small remnant patches. A loss of habitat extent is typically accompanied by increased fragmentation and isolation. Habitat fragmentation has three main consequences: remnant populations are reduced in size; the distance between populations increase; and the configuration of the habitat patch and the landscape is altered. These structural modifications affect ecological processes at multiple scales (Zschokke et al., 2000). Indeed, habitat fragmentation has been cited as being amongst the most important causes of global species extinction and biodiversity loss (Wilcox and Murphy, 1985, Pimm and Raven, 2000, Sala et al., 2000, Davies et al., 2001, Henle et al., 2004, Millennium Ecosystem Assessment, 2005, Dauber et al., 2006, Farina, 2006) as the following quotes emphasise:

> “*fragmentation is one of the most severe world-wide processes depressing biodiversity*” Farina (2006, p.128).
>
> (habitat fragmentation is) “*the most serious threat to biological diversity*” Wilcox and Murphy (1985) (p.884).

Such is the effect of habitat fragmentation on biodiversity, that a wealth of research into landscape change and habitat fragmentation has taken place since the 1970s (Diamond, 1975, Haila, 2002, Fahrig, 2003). This led to the establishment of habitat fragmentation as a central issue in landscape ecology at the turn of the century (Collinge, 1996, Collinge, 1998), with Wiens (1996) (p. 53) commenting that:

> “habitat fragmentation is widely regarded as a – if not the – central issue in conservation biology”.

It remains unclear whether isolated habitat fragments can sustain viable grassland plant populations (Ouborg, 1993, Honnay et al., 1999, Bruun, 2000, Krauss et al., 2004, Lindborg and Eriksson, 2004b). The theory of island biogeography (MacArthur and Wilson, 1967) proposes that small isolated patches have higher rates of extinction than populations in larger connected patches. Habitat fragmentation threatens plant health by lowering population size, and also by acting as a barrier to migration between patches. However, although habitat fragmentation can lead to species extinction (Young et al., 1996, Young and Clarke, 2000), many plants are well adapted to small and isolated habitats and can thus survive in habitat fragments (Lienert, 2004). The relationship between habitat fragmentation and individual plant species is complex, and is related to individual plant functional properties. Early successional species can typically fare well in habitat fragments. Adapting to the needs of intensive agriculture, results in grassland specialists being replaced by grassland generalists that are able to thrive in this altered environment (McKinney and Lockwood, 1999). Whilst short-lived species may quickly become extinct (Fischer and Stocklin, 1997, Matthies et al., 2004), others may recover if the seed-bank remains (Stocklin and Fischer, 1999).

Similarly, habitat fragmentation presents a threat to the genetic diversity of populations (Fahrig, 2003, Keller and Largiader, 2003, Jump and Penuelas, 2006). Small and fragmented populations are more susceptible to genetic drift and have a reduced probability of gene flow (Allendorf et al., 2012), which over time leads to a reduced genetic diversity and increased genetic differentiation between populations (Templeton et al., 1990, Frankham, 1996, Young et al., 1996, Aguilar et al., 2008). As such large and genetically diverse populations are expected to have more chance of long-term survival compared to small and genetically similar populations, as the ability of species and populations to adapt to landscape change is thought to be influenced by the levels of genetic diversity that are available for natural selection (Huenneke, 1991, Moritz, 2002, Frankham et al., 2009).

Two components of habitat fragmentation, isolation and connectivity, are commonly used synonymously (Tischendorf and Fahrig, 2001, Moilanen and Nieminen, 2002). However, they describe two different processes. Isolation measures the contrast between a habitat patch and its neighbours. Isolation is complete where all the surrounding patches have no similarity, decreasing as the quantity and similarity of surrounding patches increases. Patch connectivity, on the other hand, is concerned with the interactions between distinct populations (Moilanen and Nieminen, 2002). Patch connectivity describes the potential for immigration and colonisation of individuals and species from distinct populations. Moreover, scale is an important attribute of connectivity, with landscape connectivity and patch connectivity being different concepts (Tischendorf and Fahrig, 2001). Landscape connectivity refers to connectivity as a property of the entire landscape and is the realm of landscape ecology. Patch connectivity defines connectivity as an attribute of a patch and is the realm of metapopulation ecology.

A variety of methods are available for measuring patch connectivity. These range from simple nearest neighbour calculations to more complex formulae incorporating patch area, distances, and dispersal. Moilanen and Nieminen (2002) categorise three types of connectivity measures: nearest neighbour; buffer measures; and incidence function models (IFMs). The simplicity of calculating nearest neighbour distances has led to their extensive use in ecology (Prugh, 2009). However, the over simplistic nature of these measures led Moilanen and Nieminen (2002) to challenge whether it is a satisfactory measurement of connectivity. Buffer measures apply a buffer around a habitat patch, and analyse the amount of habitat within the buffer. Tischendorf and Fahrig (2000) suggest that connectivity measures should include measures of actual immigration rates. Moilanen and Nieminen (2002) however comment that in practise such data is typically not readily available. They propose that landscape metrics can be used as a surrogate for immigration. This is the principle behind IFMs, originally proposed by Hanski (1994). IFMs take into account the distance to all possible source populations, using a negative exponential dispersal kernel. Thus the closer a source population is to the focal patch, the larger it’s weighting.

Nearest neighbour and buffer measures can be termed structural connectivity measures, in that they measure the connectivity of landscape structures. By contrast IFM measures can be termed measures of functional connectivity, in that they attempt to measure the response of organisms to the structure of the landscape elements. Prugh (2009) found no difference between IFM and nearest neighbour measures in the prediction of patch occupancy and colonisation probabilities amongst twenty-four invertebrate, reptile, and amphibian metapopulations. Conversely, by testing the effectiveness of several connectivity measures in their ability to predict colonisation in two butterfly species, Moilanen and Nieminen (2002) found that nearest neighbour measures were inferior to buffer and IFM measures, leading them to propose a modified version of Hanski's (1994) orignal formula. However, Kindlmann and Burel (2008) argue that caution should be used with connectivity metrics, commenting that the view that components of a landscape are associated with a certain connectivity value should be abandoned. They argue that the same landscape may have different levels of connectivity for different species, or even for the same species temporally.

2.2.2 Habitat loss and fragmentation in Great Britain

Semi-natural habitats in Great Britain experienced dramatic declines in extent between the 1930s and the present day (Hooftman and Bullock, 2012). In particular, in recent decades the extent and ecological quality of semi-natural grasslands has been in decline throughout Britain, with long-term studies reporting losses greater than 80% (Fuller, 1987, Hooftman and Bullock, 2012) (Table 2.1). This has been driven by three principal causes: changes to the intensity (or the cessation) of land management; intensification of agricultural techniques; the development of land for urban or industrial use (JNCC, 2005, Haines-Young et al., 2006). Such change has resulted in habitats becoming increasingly fragmented, with remnant populations reduced in extent and the connectivity between them reduced (Green, 1990, Burnside et al., 2003, Hooftman and Bullock, 2012).

On the West Sussex Downs in south-east England, remaining patches of unimproved grassland are restricted to a narrow range of environments, namely on soils low in nutrients and with a high pH, steep slopes (10°-31°), northerly aspects, and land managed within environmental farming initiatives and schemes (Burnside et al., 2002, Critchley et al., 2002, Burnside et al., 2003, Haines-Young et al., 2003). Unimproved grassland patches remaining within the South Downs National Park are generally larger and more connected on the north facing escarpment. South facing patches are predominantly found on shallow sloping agricultural land and are generally more scattered, fragmented, and smaller. It has been repeatedly suggested that the resistance to change of these areas is likely due to the steepness of the land providing difficulties for mechanised access to these areas leaving them uncultivated (Darby, 1976, Buse, 1992, Burnside et al., 2003).

As a result of the intermittent nature of LULC surveys, limited data availability, and difficulties in integrating classifications from different surveys, the majority of previous studies of loss in extent of semi-natural grassland have focused on change over periods of less than thirty years (Table 2.1). More recently, methods of integrating data from the Land Utilisation Survey of Britain have been developed (Swetnam, 2007b, Hooftman and Bullock, 2012) allowing for LULC change analysis back to the 1930s.

Table 2.1 Reported losses of semi-natural grassland in Britain.

Habitat definition	Study region	Period of loss	Survey/data used in estimation	Source of information	Percentage change
Rough grassland	England and Wales	1933 - 1963	First and Second Land Utilisation Surveys	Coleman (1977)	-18.00
Unimproved lowland grassland	Lowland England and Wales	1930 - 1984	Various grassland surveys	Fuller (1987)	-91.67
Chalk grassland	England and Wales	1930 - 1998	First Land Utilisation Survey; Countryside Survey 2000	Swetnam (2007b)	-42.74
Calcareous grassland	Dorset	1930s - 2000	First Land Utilisation Survey; Land Cover Map 2000	Hooftman and Bullock (2012)	-82.98
Permanent grassland	England	1939 - 1959	Ministry of Agriculture Fisheries and Food statistics	Idle (1975)	-32.11
Calcareous grassland	England	1966 - 1988	Blackwood and Tubbs (1970) data, and county surveys	Keymer and Leach (1990)	-15.83
Unimproved grassland	West Sussex Downs	1971 - 1991	Aerial photographs	Burnside et al. (2003)	-58.22
Unimproved grassland	South Downs National Park (West Sussex)	1971 - 2001	Aerial photographs and Phase 1 habitat survey data	Mukupa (Unpublished)	-63.44
Calcareous grassland	Lowlands of England and Wales	1984 - 1998	Countryside Surveys 1984-2000	Haines-Young et al. (2003)	-3.00
Calcareous grassland	Great Britain	1990 - 1998	Countryside Surveys 1990 & 2000	Howard et al. (2003)	-19.75

2.2.3 Limitations of previous research into biodiversity loss and landscape change

The focus of much of the published landscape change research has been theoretical work to model the patterns of landscape change, with relatively little advancement in understanding of the consequential effects on ecological processes (Turner et al., 2001). Research into habitat fragmentation has also suffered from inconsistent use of terminology (Fahrig, 2003). Habitat fragmentation has been used interchangeably to describe habitat loss, the subdivision of habitats, increased habitat isolation, and reduced habitat connectivity (Lindenmayer and Fischer, 2006). With the quantification of both species diversity and habitat fragmentation being largely dependent upon the methods used to measure them, it should come as little surprise that the relationship has proved hard to define. Furthermore, much research into the effects of fragmentation on species has focused on vertebrates (Haila, 2002), with vegetation often being viewed simply as habitat for animals (Manning et al., 2004).

Fahrig (2003) comments that a strong negative correlation exists between habitat loss and biodiversity, whilst the correlation between habitat fragmentation and biodiversity is much weaker. Fahrig (2003) goes on to suggest that correlations of metrics such as patch isolation and patch size with biodiversity, are essentially indirect products of habitat loss as opposed to products of habitat fragmentation per se.

Although landscape change and fragmentation are major threats to biodiversity, other factors are also influential, and multivariate studies are beginning to highlight the importance and interactive effects of multiple factors (Barbaro et al., 2004). In particular, the biodiversity of plant species is a product of a complex interplay of variability in abiotic, spatial, historical, and management conditions.

2.3 The extinction debt

Although habitat loss and fragmentation are the leading causes of species loss (Wilcox and Murphy, 1985, Pimm and Raven, 2000, Sala et al., 2000, Davies et al., 2001, Henle et al., 2004, Millennium Ecosystem Assessment, 2005, Dauber et al., 2006, Farina, 2006), species response to changes in the landscape may not be immediate and often requires a substantial time delay following the initial impact. This extinction debt (Tilman et al., 1994) has been observed in a range of ecosystems and taxa (Kuussaari et al., 2009, Hylander and Ehrlen, 2013). Following landscape change, the conditions for a species to persist may no longer be met, for some remnant species. Whilst the changes may allow some species to thrive, and some to persist under sub-optimal conditions, others may not be able to survive in the long-term. Of the species that will not be able to survive in the long-term (i.e. have an extinction debt), many may still be able to persist for a number of generations before extinction.

As such, when only the contemporary landscape is used to assess biodiversity, failure to find a relationship between the contemporary landscape and biodiversity may be misinterpreted. Any conclusion that increasing habitat extent and improving connectivity between habitat patches are not important for conservation, are not valid when only the contemporary landscape has been considered. If biodiversity is a legacy of historical conditions, then the development of conservation programs must also consider the historical landscape. With increased knowledge of the extinction debt, conservation can work towards conserving species that are in extinction debt, by changing the conditions. As such it is essential for conservation to focus not only on the effects of landscape change on biodiversity, but to also consider temporal scales of biodiversity response to historical and ongoing landscape change (Eriksson and Ehrlen, 2001, Hanski and Ovaskainen, 2002, Lindborg and Eriksson, 2004b).

The nature of the relationship between the historical landscape and biodiversity in grassland habitats is not yet well understood. Some authors offer support for the extinction debt theory by reporting relationships between the historical landscape and contemporary species patterns (Partel and Zobel, 1999, Cousins and Eriksson, 2002, Lindborg and Eriksson, 2004b, Helm et al., 2006, Gustavsson et al., 2007, Krauss et al., 2010, Reitalu et al., 2012), whilst others have failed to find relationships (Bruun, 2000, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007). Lindborg and Eriksson (2004b) comment that contemporary species diversity within their semi-natural grassland study sites in

Sweden was largely a legacy of the landscape 50-100 years previous. They argue that contemporary species diversity had been formed under conditions no longer present. The time to extinction is affected by the strength of the environmental perturbation, the turnover rate of the species, and the availability of stable large patches (Ovaskainen and Hanski, 2002, Ovaskainen and Hanski, 2004). As such, long-lived plant species and species living just below the extinction threshold (i.e. where conditions are only slightly outside their requirements) in particular may persist for decades after landscape change before becoming extinct (Brook et al., 2003, Vellend et al., 2006, Kuussaari et al., 2009). As long as a species that is predicted to become extinct continues to persist, it is possible to conserve it through appropriate habitat restoration and landscape management (Hanski, 2000, Kuussaari et al., 2009).

2.4 Biodiversity frameworks

2.4.1 The Theory of Island Biogeography

MacArthur and Wilson’s (1967) Theory of Island Biogeography has been one of the most influential paradigms within landscape ecology and in particular within research of the interaction between habitat fragmentation and species diversity. The Theory of Island Biogeography was developed to explain the relationship between species richness and area that was observed by the authors within bird and ant populations on tropical islands. The fundamental principle of the Theory of Island Biogeography is that a positive relationship exists between the area of an island and species richness. As such it complements the basic principles of the species-area model, which states that species richness is a function of area (Boecklen & Gotelli, 1984; Rosenzweig, 1995; Rosenzweig 2004; Whittaker & Fernández-Palacios, 2007). Moreover, the Theory of Island Biogeography proposes that species diversity is regulated by extinction and colonisation. As such any factor that can affect extinction and colonisation can affect species diversity.

Although the theory was developed to describe patterns on islands, MacArthur and Wilson (1967) propose that the concepts are applicable in the wider context to “formerly continuous natural habitats now being broken up by the encroachment of civilisation” (p. 4). However, whilst the Theory of Island Biogeography provides a useful framework for studying the effects of fragmentation on species richness (Powledge, 2003), the theories grounding in true islands means that it has limitations when applied to habitat fragments (Laurance, 2008; Smith, 2010). Studies that apply the principles to habitat islands commonly make the assumption that habitat patches are isolated and surrounded by non-habitat (Lomolino, 2000). This is contrary to the growing consensus that remnant habitat patches can be influenced by the landscape matrix (Cook et al., 2002) and that studies at the landscape scale should consider connectivity, corridors, and metapopulation structure (Farina, 2006). Building on this, Forman (1995) put forward a revised explanation incorporating the additional characteristics of the landscape, which included habitat diversity, disturbance, area of patch interior, age, matrix heterogeneity, and isolation. Further revisions and analysis have been proposed by Lomolino (2000), Whittaker et al. (2008) and Santos et al. (2016).

2.4.2 Metapopulation Ecology

The 1980s saw a paradigm shift away from the Theory of Island Biogeography towards the metapopulation concept (Hanski 1989; Merriam 1991); Hanski and Simberloff, 1997; Hanski, 1999). The term metapopulation was first coined by Levins (1969), to describe a set of multiple local populations that interact with one another (Gilpin and Hanski, 1991; Hanski and Gilpin, 1991). Far from being isolated, metapopulations are connected by process such as gene flow and migration (Thrall et al., 2000).

Consistent with the Theory of Island Biogeography, the Levins Metapopulation Model (Levins, 1969) considers immigration and extinction as central processes in the stability of populations within patchy habitats. The concept of the Levins Metapopulation Model is that populations are spatially structured into groups of interacting local populations. A key notion of the Levins Metapopulation Model is its incorporation of a spatial aspect considering the position of individuals and populations in space. This contrasts with classical models of population biology that all individuals are equally likely to interact with one another. Under the principles of the Levins Metapopulation Model, increased fragmentation commonly results in local populations being in non-equilibrium of immigration and extinction (Olivieri et al., 1990). With populations ultimately being temporary, it is essential for conservation to preserve interactions and colonisation between populations (Thrall et al., 2000).

Source-sink metapopulations were described by Pulliam (1988; 1996) to outline that different habitat patches will be of varying quality and thus will differ in their capacities to support populations. Populations can either be defined as those where dispersal exceeds immigration (source populations) or those where immigration exceeds dispersal (sink populations). One of the most significant ecological implications of the theory is that through dispersal from a source population, a sink population can persist in regions that are characteristically outside their ecological niche (Pulliam, 1996).

2.4.3 Competitive exclusion

The Intermediate Disturbance Hypothesis (Grime, 1973, Connell, 1978) outlines that different habitats have different levels of disturbance from factors such as herbivores, pathogens, trampling, and environmental diseases, and availability of resources such as water, minerals, and light. Low levels of disturbance lead to decreased diversity through increased competitive exclusion leading to domination by species with a competitive strategy (K-selected). High levels of disturbance can disrupt stable ecosystems and reduce species diversity by making a habitat unsuitable, in turn leading to greater species movements. This condition is favourable for r-selected species, which are able to colonise open areas quickly when competition is low. By contrast an intermediate level of disturbance allows for more competitive K-selected species and less competitive r-selected species to occupy the same habitat, with a resultant optimal level of species diversity.

The Intermediate Disturbance Hypothesis is particularly relevant to semi-natural grasslands, such as calcareous grasslands, where high species diversity is thought to be a product of grazing acting as a regular disturbance (Price, 2003). Without disturbance, competitive exclusion would favour perennial grasses at the expense of many forb species therefore leading to a reduction in plant species diversity (Tilman, 1984, Collins and Gibson, 1995).

2.5 Factors affecting species diversity and composition

The following subsections review factors that have been cited as affecting species diversity.

2.5.1 Spatial structure and landscape change: Habitat loss and fragmentation

The dispersal of species across a landscape is influenced not only by species autecology, but is also a function of landscape dynamics (Tischendorf and Fahring, 2000). The importance of the spatial structure of habitats on species richness and diversity has been a major area of ecological research since the proposal of the theory of island biogeography (MacArthur and Wilson, 1967). The positive species-area relationship (also referred to as the species-area curve) has been demonstrated across a range of habitats worldwide (Rosenzweig, 1995, Lomolino, 2000). Despite ubiquitous reports supporting the principle of the species-area curve, research testing the principle continues, primarily because the relationship has not been fully explained.

Calcareous grasslands are an example of a habitat where the nature of the relationship between habitat area and species richness is unclear. Whilst some studies have found a positive species-area relationship (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012), other studies have found no relationship (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a). The Krauss et al. (2004) study tested the species-area relationship in both habitat specialists and habitat generalists. No differences were found between specialists and generalists, with both increasing as habitat area increased. The findings contrasted the predictions of Krauss et al. (2004) and also those of the theory of island biogeography (MacArthur and Wilson, 1967), which predicted that habitat specialists will be more greatly affected by habitat area than habitat generalists. It was concluded that this may have been due to the delayed extinction of some long-lived habitat specialist species under the process of the extinction debt.

One explanation for the inconsistent findings may be that an extinction debt may be dependent upon the extent of landscape change. In a review of 61 grassland fragmentation publications, Cousins (2009) notes that there is a theoretical threshold in relation to the amount of grassland remaining in a landscape. Specifically Cousins (2009) noted that in studies where less than 10% of the historical grassland remains in a landscape then the species richness is determined by present landscape patterns, whilst where greater than 10% remains species richness reflects historical landscape patterns. Few studies have directly tested the relationship between species diversity and habitat loss, although exceptions to this have noted a positive relationship (Cousins et al., 2003, Helm et al., 2006).

One area of debate surrounds the question of whether a single large or several small (SLOSS) patches are ecologically preferable. Hanski (1999) suggests that there is no universal answer to the SLOSS debate, and that the species composition within patches is key. If multiple small patches contain the same set of species, then it is likely that their combined number of species will be fewer than in a single large patch. However if each of the small patches has considerably different species compositions, then they may support higher species numbers than a single large patch.

As with habitat area, the exact nature of the relationship between connectivity and species diversity has proved difficult to determine. Some studies have found positive relationships (Bruun, 2000, Adriaens et al., 2006, Raatikainen et al., 2009), whilst others have found none (Krauss et al., 2004, Lindborg and Eriksson, 2004b, Helm et al., 2006). These differences may stem from the differences between the studies in the methods used for measuring connectivity and in quantifying species diversity. Connectivity influences species composition as different plant species respond differently to connectivity according to their functional properties (e.g. habitat generalist or specialist) (Dupré and Ehrlén, 2002) and life history (Bruun, 2000). Bruun (2000), found that long lived species of dry grasslands were not affected by connectivity, whilst for short lived species a positive correlation was found. Reitalu et al. (2010) comment that there are weaknesses in the measurement of connectivity, suggesting that livestock movements through the landscape should be incorporated. In order to overcome this they designed a study to include distance to the nearest village, grazing intensity, and past and present connectivity as exploratory variables. They found that grazing intensity, distance to an historical village, and present-day connectivity all influenced species diversity. However, these variables do not necessarily measure the livestock movement patterns that the authors indicated as important. Inclusion of information on livestock movement may allow for investigation of a further mechanism by which habitat fragmentation can influence species diversity, through its effect on species dispersal.

2.5.2 Historical landscape structure

Given the principles of the extinction debt, it is important to include a temporal scale to species diversity research (Eriksson and Ehrlen, 2001, Hanski and Ovaskainen, 2002, Lindborg and Eriksson, 2004b). As such the investigation of the relationship between temporal variables and species diversity has become a popular research theme. Simply stating or investigating relationships between species and contemporary landscapes does not necessarily unpack the whole story and limits investigation to a series of restricted variables. Several studies have found support for the extinction debt theory, by discovering relationships between contemporary species diversity in grasslands and aspects of the historical landscape. Relationships have been found between species diversity/richness and historical area (Helm et al., 2006), historical connectivity (Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins and Eriksson, 2008), site age and historical management (Partel and Zobel, 1999, Cousins and Eriksson, 2002, Gustavsson et al., 2007), and the historical amount of grassland in the area surrounding the patch (Reitalu et al., 2012). However, other studies contradict the theory, as they have found no relationship between species diversity in grasslands and the historical land-use of site (Bruun et al., 2001), historical area (Adriaens et al., 2006), and historical connectivity (Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007).

A study by Gibson and Brown (1991) found no difference in the number of species between ancient and disturbed calcareous grasslands. They conclude that small-scale species richness does not take a long time to develop and is therefore not dependent on the history of the site. Gibson and Brown (1991) postulated that small scale species richness was dependent on site specific variables and management. Differences between ancient and ex-arable calcareous grasslands were however apparent in the stages of succession evident by the species composition, which can take from decades to centuries to stabilise (Gibson and Brown, 1991).

2.5.3 Abiotic variability

2.5.3.1 Topography

Local and regional variation in topographic conditions can result in distinct differences in community structure (Rodwell, 1990, Rodwell, 1992). Topography influences the ecological conditions and as a result species structure, primarily through its influence on the amount of sunlight a site receives (Perring, 1959, Geiger, 1965, Oke, 1987). In the northern hemisphere, north facing land receives less direct sunlight and can be cooler and damper than adjacent south facing land, with Rorison et al. (1986) reporting an annual mean temperature difference of 2.5-3°C between adjacent calcareous grassland slopes . As a result they support a different range of flora (Tansley and Adamson, 1925, Tansley and Adamson, 1926, Perring, 1959, Burnside et al., 2002, Bennie et al., 2006). Rose (1995) comments that the flora of the south facing slopes of the South Downs is richer, particularly in orchids, with the north facing slopes having more lichens and bryophytes. Burnside et al. (2002) found that grasslands sites on shallow slopes were predominantly comprised of mesotrophic species, with a transition to calcareous communities as gradient increased. In addition to differences in species composition, south facing slopes have a higher species richness compared to north facing slopes (Perring, 1959, Hutchings, 1983) (Figure 2.2). The soils on steep slopes tend to form shallow and unstable rendzina soils compared to soils of flatter land (Balme, 1953, Trudgill, 1976, Bennie et al., 2006). Bennie et al. (2006) conclude that compared to shallow slopes, steeper slopes are more resistant to change due to phosphorous limitation in shallower rendzina soils. Keddy (2005) proposes that the more variation in conditions within a habitat the greater the number of species that will be found within. This a pattern summarised by the centrifugal model (Keddy, 1990, Wisheu and Keddy, 1992), which describes distributional patterns of species and vegetation along gradients that are caused by differences in environmental constraints.

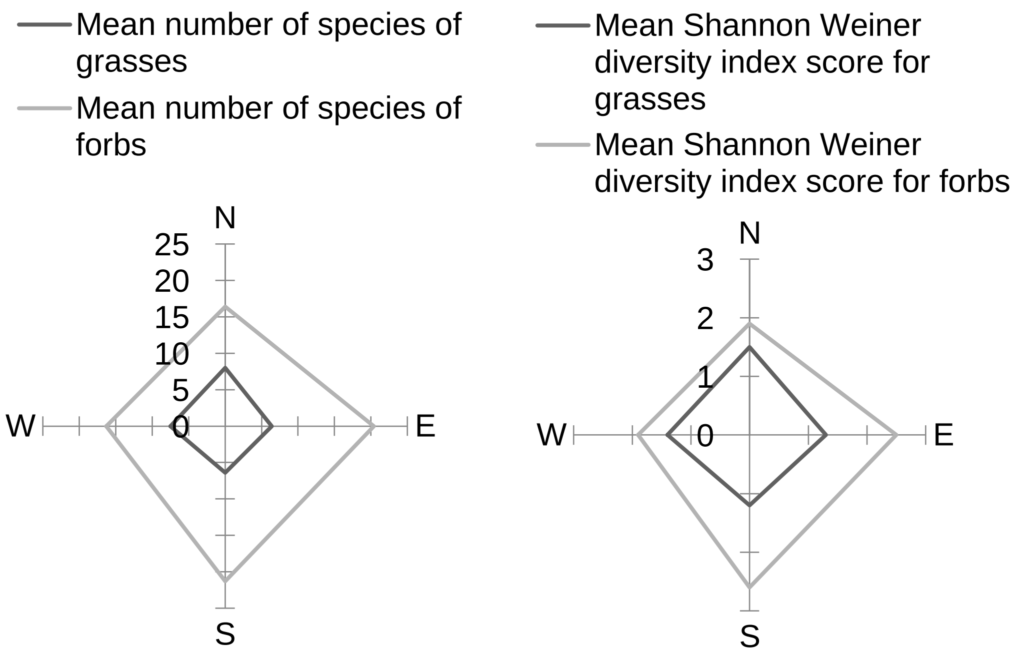


Figure 2.2 The relationship between aspect and species richness/diversity. The plots show the species richness and diversity of grasses and forbs plotted against compass points, and highlight higher values in the south and east compared to the north and west. Adapted from Hutchings (1983).

2.5.3.2 Soils

At the abiotic level the species composition of calcareous grasslands is a product of the environmental niche they occupy. Calcareous grasslands have characteristically thin, well drained and nutrient poor soils, and only species that can tolerate these conditions are able to thrive. Moreover, variability in soil nutrient availability can influence both species composition and diversity. The amount of available nitrogen (N) can have a major influence (Mountford et al., 1993, Willems et al., 1993, Smith, 1994). Tilman (1984) found that during early succession, of the nutrients they manipulated, nitrogen was the most limiting. Tilman (1984) found that the species composition of an early successional area can be influenced by the availability of soil nutrients, and thus the availability of limiting nutrients such as nitrogen may explain an element of spatial heterogeneity of vegetation. Nitrogen levels had a significant influence on the abundance of dominant plant species in the years immediately after a disturbance. Phosphorus (P) is a limiting factor (Willems et al., 1993, Janssens et al., 1998), and potassium (K) has the potential to affect the production of biomass (Van der Woude et al., 1994). However, Reitalu et al. (2012) found no relationship between soil properties and species richness of semi-natural grasslands in the Baltic Island of Öland (Sweden).

2.5.4 Management variability

Within managed semi-natural habitats, species diversity is influenced by the type and schedule of local management. On semi-natural grasslands, management, typically through grazing, mowing, or trampling, acts as a regular disturbance preventing succession and the build-up of nutrients (Critchley et al., 2004). In turn, this stops competitive species dominating and prevents the exclusion of grassland specialists (Price, 2003). New growth is regularly removed, and thus plants that are tolerant to grazing thrive. The outcome of these processes is that semi-natural grasslands have one of the highest species diversities of all habitats in Western Europe (Fischer and Stocklin, 1997), commonly supporting 25-30 species per 25 x 25cm quadrat (Hutchings, 1983, Mitchley and Grubb, 1986).

Some species benefit more from grazing and others from mowing (Jantunen, 2003). In the calcareous grassland of their study sites in Switzerland, Schlapfer et al. (1998) found species richness to be higher in grazed sites compared to mown ones. However, they comment that change from grazing to mowing only has a small influence on community structure, with 90% of species occurring in both grazed and mown sites. Similarly, grazing intensity can influence species composition, with some species benefitting from low intensity grazing and others from high intensity grazing (Hulme et al., 1999, Marriott et al., 2009). Low grazing intensity can help to conserve species diversity (Klimek et al., 2007), whilst high grazing intensity (Marriott et al., 2009) and abandonment of grazing (Luoto et al., 2003, Cousins and Eriksson, 2008) can both also result in reduced species diversity. Thus, consistent with the intermediate disturbance hypothesis (Grime, 1973, Connell, 1978), intermediate levels of grazing intensity result in the highest levels of species diversity (De Bello et al., 2006, Cousins and Eriksson, 2008).

Moreover, grazing intensity is a major factor influencing species composition (Barbaro et al., 2004, Sebastia et al., 2008). Sebastia et al. (2008) found that the type of grazer was important for species composition, with cattle increasing vegetation heterogeneity, whilst grazing by sheep favoured specific species of high conservation value. Similarly Pakeman (2004) reports that increased grazing intensity can be accompanied by an increase in species with: ruderal and competitive strategies; annual life strategies; seasonal regeneration by seed; flowering and seed dispersal early in the season; a higher requirement for light, and a low minimum height.

2.6 Factors affecting genetic diversity

Wright's (1940) island model of population genetics states that genetic diversity is regulated by rates of colonisation and local extinction. As such any factor that affects colonisation and local extinctions has the potential to affect genetic diversity. The following subsections examine the factors that have been related to variation in genetic diversity.

2.6.1 Isolation by distance

The theory of isolation by distance (Wright, 1943) predicts that, due to decreased likelihood for dispersal to more distant populations, a positive correlation exists between genetic distance and geographical distance. Therefore increased isolation of patches is commonly accompanied with a consequential increase in the genetic distance between individuals in discontinuous patches. Conversely, where distances between populations are small enough to allow sufficient gene flow, genetic differentiation is expected to be prevented. The results of recent studies in semi-natural grasslands have been mixed, with some finding evidence of isolation by distance (Jacquemyn et al., 2004, Honnay et al., 2007), whilst others have found none (Odat et al., 2004, Honnay et al., 2006, Rosengren et al., 2013, Dostalek et al., 2014).

2.6.2 Spatial structure and landscape change: Habitat loss and fragmentation

The term landscape genetics was coined in a seminal paper by Manel et al. (2003) to describe the study of the interaction between the landscape and genetic processes such as gene flow, genetic drift and selection. Manel et al.

(2003) proposed landscape genetics as the merging of landscape ecology and population genetics, commenting that the discipline combines “the tools of molecular genetics with the principles of ecological biogeography and landscape ecology”. The principle concept of landscape genetics is to identify genetic discontinuities and to correlate them to landscape features (Manel et al., 2003). The discipline emerged from the increasing use of landscape variables to explain spatial patterns in genetics (Manel et al., 2003), and provides a framework for explaining dispersal, migration, and gene flow based on spatial patterns of a landscape.

The magnitude of genetic drift, one of the processes that drives populations away from the Hardy-Weinberg equilibrium, is inversely related to effective population size. As such the stochastic effects of genetic drift will be greatest in smaller populations (Lynch et al., 1995, Young et al., 1996). In addition, increased selfing and mating amongst closely related individuals in small populations may lead to inbreeding and reduced numbers of heterozygotes (Schaal and Leverich, 1996, Young et al., 1996, Allendorf et al., 2012). This can lead to reduced fitness (Keller and Waller, 2002, Reed and Frankham, 2003). Although a positive relationship between population size and genetic variation is most common (Oostermeijer et al., 1994, Fischer and Matthies, 1998, Kery et al., 2000, Luijten et al., 2000, Vergeer et al., 2003, Brys et al., 2004, Hooftman et al., 2004, Hensen and Wesche, 2006, Leimu et al., 2006, Prentice et al., 2006, Baessler et al., 2010), the absence of this relationship is not uncommon (Jacquemyn et al., 2004, Leimu and Mutikainen, 2005, Honnay et al., 2007, Munzbergova et al., 2013). Meta-analysis by (Honnay and Jacquemyn, 2007) found that across 57 studies Mean gene diversity (He), percent polymorphic loci, and allelic richness were positively correlated with population size, but no relationship was found between population size and inbreeding coefficient (F_{IS}). Similarly, meta-analysis by Leimu et al. (2006) investigated the relationship between plant population size, fitness, and genetic variation. They found significantly positive relationships between population size and fitness (from 45 studies), and between population size and genetic variation (from 48 studies). One of the main determinants of population size is habitat size, and therefore it is not surprising that positive relationships between habitat patch area and plant genetic variability are often reported (Lienert et al., 2002a, Hooftman et al., 2004, Van Rossum and Triest, 2006, Jacquemyn et al., 2010, Dostalek et al., 2014).

Research into the effects of fragmentation and isolation of habitats upon genetic structure has developed from classic biogeography and metapopulation theories (MacArthur and Wilson, 1967, Levins, 1969). Fragmentation typically reduces population size and the connectivity between populations, thereby reducing or obstructing gene flow between populations, and leading to increased inbreeding and genetic drift (Soule and Wilcox, 1980, Schonewald-Cox et al., 1983, Gilpin and Soule, 1986, Lande and Barrowclough, 1987, Young et al., 1996). As a result of these processes, habitat fragmentation has decreased population genetic diversity within populations and increased genetic differentiation between populations (Leimu et al., 2006). Aguilar et al. (2008) carried out a meta-analysis of 101 publications, concluding that habitat fragmentation decreases the genetic diversity of plant populations. Expected heterozygosity, allelic richness, % polymorphic loci, and outcrossing rates were all negatively related to fragmentation, but no relationship was found with F_{IS}.

Although habitat fragmentation is often accompanied by reduced population size, the way it affects genetic drift and gene flow is not clearly understood. This is largely because its impact comes from the interaction of multiple factors. Studies that have focused on the relationship between habitat fragmentation and genetics have found that commonness does not prevent loss of genetic diversity in a species (Lienert et al., 2002a, Hooftman et al., 2004, Honnay and Jacquemyn, 2007), highlighting that common species are also susceptible to the negative effects of fragmentation. The effects of habitat fragmentation are expected to be more severe in recently fragmented populations (Huenneke, 1991, Gitzendanner and Soltis, 2000). Studies of the effect of fragmentation on plants has largely focused on population demographics (Aguilar et al., 2008), with an increased interest in the genetic consequences occurring in recent years (Lowe et al., 2005, Ouborg et al., 2006, Honnay and Jacquemyn, 2007). There is a general consensus that the effect of habitat fragmentation on population genetics are more complex than can be explained by simple theoretical models (Ewers and Didham, 2006, Feeley and Terborgh, 2008, Bacles and Jump, 2011). The response of a species to habitat fragmentation is dependent on multiple factors, not least its autecology and evolutionary history (Nazareno and Jump, 2012).

Several studies have found relationships between genetic diversity and contemporary connectivity (Lienert et al., 2002b, Lopez-Pujol et al., 2003, Hooftman et al., 2004, Van Rossum and Triest, 2006, Honnay et al., 2007, Fer and Hroudova, 2009). In particular Honnay et al. (2007) compared the influence of population size and connectivity on genetic diversity, and found a positive relationship between genetic diversity and connectivity, suggesting that gene flow was more important than populations size in determining genetic diversity. In contrast in a study by Jacquemyn et al. (2010) connectivity did not affect genetic diversity. Jacquemyn et al. (2010) did find a positive relationship between loss of extent of calcareous grassland patches and recent population bottlenecks in *Cirsium acaule*, suggesting that low genetic diversity in small populations may be due to loss of habitat extent.

However, to date most studies concerning the relationship between fragmentation and genetic diversity use population size, or a combination of population size and isolation as a surrogate for fragmentation. However, fragmentation is multi-faceted, and its influence on genetic diversity may stem from the interaction of population size, isolation, and matrix characteristics (Ezard and Travis, 2006). Ouborg et al. (2006) argue that population size and isolation should be viewed as separate parameters. Although habitat fragmentation at the landscape scale is expected to lead to reduced population size and increased isolation, this is not the only possible outcome (Fahrig, 2003). Different parameters of fragmentation act independently of one another, and as a result should not be used interchangeably (Ouborg et al., 2006). Moreover, small sample sizes and the effects of uncontrolled variables often confound fragmentation research. Nazareno and Jump (2012) comment that, particularly where hyper-variable microsatellite markers are used, small sample sizes can bias the conclusions drawn from studies.

2.6.3 Historical landscape structure

The discipline of phylogeography (Avise et al., 1987) explores the relationship between past habitat connectivity and contemporary genetic patterns at large spatial scales. These relationships exist as a considerable time period is required to reach genetic equilibrium (Koch and Kiefer, 2006, Soltis et al., 2006), with studies indicating historical landscape structure can be seen in genetic characteristics longer than it can in species characteristics (e.g. species diversity). However, much less is known about the relationship between historical landscape connectivity and genetic structure at smaller spatial and temporal scales (Munzbergova et al., 2013).

Few studies have explored the influence of historical landscape characteristics on contemporary genetic diversity. Exceptions include Jacquemyn et al. (2004) (studying *Primula elatior* in Belgium) and Prentice et al. (2006) (studying *Briza media* in semi-natural grasslands in Sweden) who both found positive relationships between site age and genetic diversity, and Munzbergova et al. (2013) who found a positive relationship between historical site connectivity and the genetic diversity of *Succisa pratensis* in semi-natural grasslands in Sweden. Munzbergova et al. (2013) comment that the past landscape structure (measured back to 1850) could be evidenced from contemporary genetic diversity patterns, despite extensive changes in the landscape.

2.6.4 Abiotic variability

Although they have repeatedly been shown to influence species diversity, few studies have examined the relationship between abiotic variables and genetic variation. However there is some evidence to suggest that topography is not related to genetic diversity (Ohsawa et al., 2008), that positive relationships exist between genetic diversity and phosphorous levels (de Vere et al., 2009), and that soil fertility can influence genetic variation (Huff et al., 1998).

2.6.5 Management variability

Several aspects of habitat management have been shown to affect genetic diversity. Last et al. (2014) found that intensive management was related to decreased genetic diversity for *Dactylis glomerata* in grasslands in Switzerland. Grazing patterns and movement of animals can affect genetic diversity through their influence on dispersal. Rico et al. (2014) found evidence of isolation by distance in *Dianthus carthusianorum* in ungrazed calcareous grasslands, but genetic distance in grazed patches within the same herding system was related to distance along the shepherding route. Rico et al. (2014) conclude that the population genetic diversity can be explained by patch connectivity by shepherding. Similarly, Honnay et al. (2006) argue that through increasing levels of seed exchange between grassland fragments, grazing may act to mitigate the genetic consequences of habitat fragmentation.

2.7 Integrating habitat, species and genetic diversity

The different levels of biodiversity often act synergistically (Allendorf et al., 2012), with ecological communities structured by processes operating at multiple scales (Ricklefs, 2004, Harrison and Cornell, 2008) and changes in ecosystem structure impacting upon processes controlling community species and genetic diversity. However they have typically been considered as individual entities and research into the relationships between the different levels has been scarce. Even one of the relationships considered as more established, that of a positive relationship between habitat diversity and species diversity, is in fact supported by little empirical evidence (Lundholm, 2009). Similarly research into the relationship between species diversity and genetic diversity is still in its infancy, with the nature of the relationship not yet well understood. Furthermore assessment of the relationship between habitat and genetic diversity, and between all three levels of biodiversity has largely been absent. This is particularly surprising considering that several authors have stressed the view that the integration of ecology and genetics is essential in achieving effective conservation management (Real, 1994, Soule and Mills, 1998, Clarke and Young, 2000, Vellend, 2003).

To knowledge, only one publication examines the relationships of biodiversity at the three levels. The paper by Gugerli et al. (2008) however, only presents a synopsis of the IntraBioDiv project, with no results of the analysis of the relationship between different levels of biodiversity. Furthermore, in their measurement of habitat diversity Gugerli et al. (2008) classify habitats based on climatic variables, as opposed to the use of a standardised habitat classification system.

Research into the relationship between landscape scale habitat diversity and species diversity has been more prevalent. The habitat heterogeneity hypothesis proposes that by providing niche opportunities for a variety of species, habitat diversity is important in maintaining species diversity (Whittaker, 1975, Shmida and Wilson, 1985, Ricklefs, 1987, Rosenzweig, 1995). The concept is based on niche theory, which states that each species is adapted for a specific set of abiotic and biotic environments (Hutchinson, 1957). The species within a habitat patch can be influenced by the effects of the surrounding habitat on the species pool and dispersal (Partel et al., 1996, Zobel, 1997, Norderhaug et al., 2000, Soderstrom et al., 2001, Akatov et al., 2005). Moreover, plant species may be able to survive in suboptimal conditions in a variety of neighbouring habitat types, and therefore a higher diversity of habitats provides more potential sources of new species. All habitats, natural or otherwise, are potential sources of new species and can contribute to patch level diversity, a phenomenon termed spatial mass effects (Shmida and Whittaker, 1981, Shmida and Ellner, 1984, Shmida and Wilson, 1985). In particular, spatial mass effects may result in habitat patches having increased diversity of habitat generalists, which are more able to become established under suboptimal conditions.

Some authors have argued that habitat diversity is a fundamental factor in the species diversity of plant communities (Tilman and Pacala, 1993, Chase and Leibold, 2003), and several authors have found evidence of a positive relationship between the habitat diversity and the diversity of plant species (Skov, 1997, Sotherton and Self, 2000, Pausas et al., 2003, Dufour et al., 2006, Poggio et al., 2010). Support for the relationship between habitat diversity and species diversity in semi-natural grasslands have been found by Bruun (2000), Reitalu et al. (2012), and Sutcliffe et al. (2015). The study by Bruun (2000) defined habitat diversity by classifying habitats based on soil pH values and potential solar radiation, rather than using habitat classifications. Reitalu et al. (2012) report that the richness of habitat specialist species was positively related to habitat diversity within 300m buffer areas. Finally, the Sutcliffe et al. (2015) study used habitat classifications as a measure of habitat diversity and reported a positive relationship between species diversity of plants and habitat diversity within a 2km buffer area. Other studies have looked at the relationship between species diversity and aspects of habitat diversity. For example Janisova et al. (2014) report that alpha diversity, the diversity of the local species pool, of plants in semi-natural grassland patches in Slovakia increased with increasing diversity and cover of natural and semi-natural habitats in the surrounding area. Conversely alpha diversity decreased with increased diversity or cover of non-natural habitats. In discussing their results, Janisova et al. (2014) comment that the species pool effect from connectivity to other grassland patches is stronger than the spatial mass effects. However, empirical evidence of the relationship between habitat diversity and species diversity is scarce, with a review of literature by Lundholm (2009) finding only 41 observational and 11 experimental studies testing the relationship across a range of taxa, with both negative and positive results reported. Whilst increased habitat diversity may lead to an increase in the number of habitat patches and niche opportunities, it can also lead to decreased patch size and increased isolation (Fahrig, 2003, Dufour et al., 2006), which can negatively influence species diversity. As such, more recently it has been proposed that intermediate levels of habitat diversity produce the highest species diversity (Fahrig et al., 2011, Redon et al., 2014).

The relationship between habitat diversity and genetic diversity has not previously been studied directly. However studies finding relationship between landscape composition and genetic variation offer some indication of the relationship between habitat and genetic diversity. A paper by the IntraBioDiv project (Manel et al., 2012) presents analysis of the relationship between genetic diversity and habitat diversity (measured by climatic variables). Across 13 alpine plant species they found that allele frequencies were most affected by precipitation and temperature, with topographical characteristics (solar radiation, slope, topographic exposure, soil humidity, aspect, and altitude) having little effect. Prentice et al. (2006) report a relationship between genetic variation and the structure of the surrounding habitat. Specifically genetic diversity within populations of *Briza media* was positively related to the amount of grassland in the landscape surrounding calcareous grassland fragments on the Baltic Island of Öland. However, no relationships were found between the genetic variation of *B. media* and the amount of arable land or forest in the surrounding area, with genetic diversity highest where the surrounding landscape is composed of grasslands. Prentice et al. (2006) conclude that the relationship is a product of increased connectivity, and therefore gene flow, with other grassland patches. Therefore there is a research need to simultaneously test the variables of habitat diversity and grassland connectivity to determine the relative influence of both these factors on genetic diversity.

2.7.1 The species-genetic diversity correlation

Several authors have commented on the parallel processes underlying theories of diversity at the species and genetic levels (Harper, 1977, Antonovics, 1978, Huston, 1994, Amarasekare, 2000, Hubbell, 2001). However it is only more recently that relationships between species and genetic diversity have gained renewed interest, largely driven by the special feature on community genetics in the journal *Ecology* in 2003 (Agrawal, 2003) and a seminal paper by Vellend (2003) which saw the birth of the species-genetic diversity correlation (SGDC) hypothesis.

On the similarities in the processes and concepts underlying both species and genetic diversity Antonovics (1976, p.238) commented that the:

> "*forces maintaining species diversity and genetic diversity are similar. An understanding of community structure will come from considering how these kinds of diversity interact*".

The similarities Antonovics (1976) refers to can be illustrated by the parallels in the logic underpinning the Theory of Island Biogeography (MacArthur and Wilson, 1967) and the island model of population genetics (Wright, 1940). Vellend (2003) notes that whilst the mathematics of these two theories is different, they share near identical underlying reasoning: whilst the Theory of Island Biogeography posits that species diversity is regulated by extinction and colonisation, the island model of population genetics posits that genetic diversity is regulated by rates of colonisation and local extinction. Both theories propose that smaller and more isolated populations will be less diverse. Small populations experience higher rates of local extinction and genetic drift, compared to large populations, whilst in more isolated populations migration, and thus colonisation and gene flow, are lower than in less isolated populations. These basic principles are generally well accepted, with research continuing to test and support the concepts. Moreover species and genetic diversity are influenced in similar ways by contemporary processes that influence population size, such as landscape, and habitat fragmentation.

However, the interaction between species and genetic diversity, and particularly the application of the relationship to the understanding of community structure, is less well understood. Despite the recognition of the similarities in the forces maintaining species and genetic diversity, research has tended to focus on either species diversity or genetic diversity, but rarely both together. Indeed, Clarke and Young (2000) comment that the fields of ecology and genetics have traditionally worked as rivals with little co-operation.

More recently the relationship has gained increased research attention, particularly following the introduction of SGDC hypothesis (Vellend, 2003), which posits that species and genetic diversity (within a single species) should be positively correlated across habitat patches or islands. The SGDC is based upon the theory that parallel ecological and evolutionary processes act on species and genetic diversity to produce parallel effects (Vellend, 2003, Vellend, 2004, Vellend and Geber, 2005). Specifically Vellend and Geber (2005) suggest that similar neutral (mutation, migration, drift) and adaptive (selection) processes control both types of diversity (Figure 2.3). The finite number of species in a population implies that both species and genes are susceptible to random fluctuations in abundance (i.e. drift). The effects of drift may be counteracted by the immigration of new species or novel alleles. The relationship between selection and diversity is more complex. At the basic level selection favours certain individuals over others, and these individuals may be different species or have different alleles.

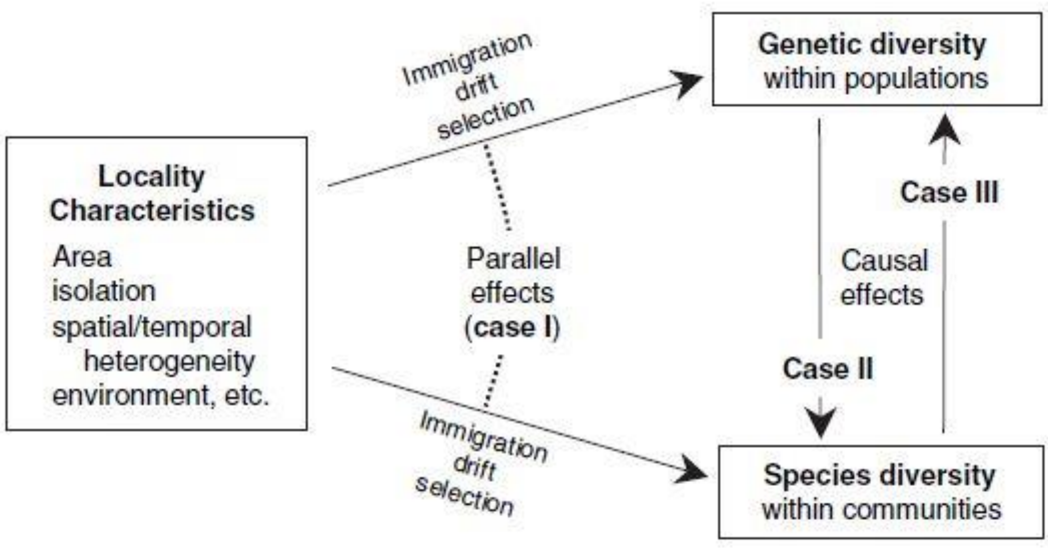


Figure 2.3 The parallel effects of locality characteristics on species and genetic diversity. Reproduced from Vellend and Geber 2005.

Underpinning the SGDC is the idea that diversity at one level may be dependent upon diversity at another. Vellend and Geber (2005) identify three principal ways in which species diversity and genetic diversity may influence one another. Firstly, if local characteristics affect the two diversity levels in similar ways, then a positive correlation would be expected. Secondly species diversity may affect genetic diversity as a result of its influence on selection. Finally, genetic diversity may affect species diversity. This can result either from genetic variation determining a population's performance and viability or by the genetic variation in a dominant species determining the biotic environment of a community. Indeed the results of a study by Lankau and Strauss (2007) indicate that mutual feedbacks exist between species and genetic diversity. Thus conservation efforts aimed at maintaining diversity at one level, should necessarily focus on maintaining diversity at the other level (Lankau and Strauss, 2007). Although understanding of the role of genetic diversity in maintaining species diversity in plant communities is limited, the concept dates back to the 1970s (Antonovics, 1976, Turkington and Harper, 1979). Whitlock et al. (2007) found support for the SGDC by showing that under constant environmental conditions more genetically diverse communities retained more species diversity and developed more similarities in species composition compared to less genetically diverse communities. Further support for the impact of genetic diversity in influencing the diversity and structure of communities comes from recent meta-analysis (Hughes et al., 2008, Whitham et al., 2012, Whitlock, 2014). As such, genetic diversity can moderate the response of communities to anthropogenic landscape change through responses to selection and adaptation (de Mazancourt et al., 2008, Norberg et al., 2012) and several authors argue that maintaining genetic diversity can be used as a management technique to benefit species richness (Crutsinger et al., 2006, Cook-Patton et al., 2011, McArt et al., 2012).

To initially test the SGDC hypothesis Vellend (2003) compared species and genetic data from fourteen islands, finding positive correlations in five of the data-sets. Partial correlation analysis identified that island area was the dominant variable with parallel effects, but also that species and genetic diversity are influenced by processes that extend beyond the theories of MacArthur and Wilson (1967) and Wright (1940). Although Vellend (2003) comments that with these findings the SGDC joins other theories as a basis for understanding biodiversity, the results were inconclusive and the study suffered from weaknesses in its methods. Vellend (2003) details that the fourteen data-sets, compiled from literature searches, are corresponding. However, although the data did correspond spatially there were substantial temporal differences. In the most extreme instance species data from 1960 was compared with genetic data from 1996.

Moreover further studies have reported mixed findings, with some providing support for a positive SGDC (Vellend, 2004, Vellend, 2005, Wehenkel et al., 2006, He et al., 2008, Odat et al., 2010, Wei and Jiang, 2012), some finding no correlation (Silvertown et al., 2009, Fady and Conord, 2010, Taberlet et al., 2012, Avolio and Smith, 2013), and some finding a negative correlation (Puşcaş et al., 2008). Finally, Odat et al. (2004) found mixed results, with within population genetic diversity (H_e) not related to species richness or evenness, and genetic difference between populations related to species evenness but not to species richness. The overall pattern has been summarised by Whitlock (2014) in meta-analysis looking more broadly at a range of studies that analysed the relationship between genetic diversity and ecological structure and functioning. In the 33 studies that considered neutral genetic diversity, no relationships were established between genetic diversity and species diversity, richness, or evenness.

Vellend (2005) offers an explanation for the inconsistency of SGDC studies, suggesting that if a community has a fixed total size and number of individuals that it can support, an increase in species richness equates to a decrease in the population size of the original species. Such decreases in population sizes would be expected to decrease genetic diversity through increased susceptibility to genetic drift, resulting in a negative relationship between species and genetic diversity (Vellend, 2005). A different explanation has been offered by Rosengren et al. (2013), who found a negative relationship between within population genetic diversity in the moss *Homalothecium lutescens* and vascular plant species richness. Rosengren et al. (2013) suggest that this relationship may be a product of the relationship between bryophytes and vascular plants, with the genetic diversity of bryophytes negatively affected by increased competition and reduced opportunities to become established where vascular plant species richness is high.

Helm et al. (2009) comment on the necessity of SGDC studies to not only test correlation between species and genetic diversity, but to also look at factors influencing them. SGDCs are most likely to occur where neutral processes, such as area and isolation, are the main drivers of diversity (Antonovics, 1976, Etienne and Olff, 2004, Vellend, 2005). Recent studies have been designed to test for factors having a parallel effect on species diversity and genetic diversity (Odat et al., 2004, Vellend, 2004, Cleary et al., 2006, Struebig et al., 2011, Avolio and Smith, 2013, Lamy et al., 2013). However, not all of these studies found the same factors to affect both species and genetic diversity. Exceptions were the studies by Lamy et al. (2013) which found that connectivity contributed to SGDCs, Vellend (2004) which found land-use history to be important, and Struebig et al. (2011) which found habitat size to be important. These three studies highlight the need to analyse the contribution of habitat characteristics that represent the three major processes driving diversity (drift, extinction, and immigration).

There has been limited study of the role of historical habitat connectivity to contemporary genetic diversity. Given the importance of this variable in plant populations due to the principle of extinction debt (Tilman, 1994), this is an area of research need. Munzbergova et al. (2013), provide an exception, and comment that their finding of a relationship between historical connectivity and genetic diversity corresponds to a previous study on species diversity in the same study area (Herben et al., 2006).

SGDC studies have been criticised for sampling too few individuals and/or too few populations (Nazareno and Jump, 2012). Another weakness has been the limited ecological characterisation of habitat patches (Lamy et al., 2013). The latter criticism means that although a relationship between species and genetic diversity has been tested, little information has been assembled regarding the underlying causes of the relationship.

2.8 Emerging themes and knowledge gaps

Many factors have been tested for their influence on the species and genetic diversity of vegetation in semi-natural grasslands. However, the nature of the relationships between some such factors and biodiversity is not clear, with different studies reporting positive, negative, or no relationships. Moreover, the results of research to date have failed to establish which factors influence biodiversity, and the relative importance of these different factors. This lack of understanding may be due to the complex and interactive influence of a range of different abiotic, spatial, historical, and management factors. As such, an opportunity exists to use multivariate methods to test the influence of multiple variables on species diversity and composition, and genetic diversity, in order to gain a greater understanding of the factors that are influential. This will also allow for the analysis of the relative importance of the factors influencing species and genetic diversity.

The extinction debt theory adds complexity to the study of the relationship between landscape spatial factors and species diversity. If a habitat patch is under an extinction debt, the theory predicts that the species within the patch may more closely reflect the historical structure of the site than the contemporary structure. Again, this highlights the importance of considering multiple factors simultaneously, when testing for factors that influence biodiversity. Furthermore, the majority of research to date has considered species diversity, without consideration of species composition. As such, it is not well understood if the factors influencing species diversity are the same as those influencing species composition.

The relationship between different levels of biodiversity is also not well understood. Although general theory has predicted relationships, particularly between habitat and species diversity, and between species and genetic diversity, evidence of these relationships is scant and has not produced consistent findings. Moreover, the relationships between habitat diversity and genetic diversity and between habitat, species, and genetic diversity have so far been largely ignored. Increased knowledge of the nature of the relationships between different levels of biodiversity will advance understanding of the multifaceted relationships that exist in grassland systems, and help to direct conservation efforts to maintain biodiversity at all levels.

2.9 Conclusions and relevance to the research questions

Over the past century landscapes worldwide have witnessed widespread change in diversity and structure. Specifically semi-natural habitats, such as calcareous grasslands, have become increasingly fragmented and more isolated from similar habitats types. To set the context for the thesis, the first two research questions analyse the extent of such changes on calcareous grasslands within the SDNP study area:

i. What is the extent of change in habitat diversity and landscape structure within the SDNP between the 1930s and 2012?
ii. What patterns of fragmentation can be identified in twelve calcareous grassland study sites between the 1930s and 2012?

What is less clear is how these changes influence species and genetic diversity. Moreover, many spatial, historical, abiotic, and management variables have been cited as influencing these two levels of biodiversity. The third research question addresses this gap in knowledge in relation to species diversity and composition:

iii. To what extent do contemporary and historical landscape and environmental characteristics influence species diversity and composition?

The extinction debt theory proposes that a time-lag exists between landscape change and a subsequent extinction in species. The fourth research question analyses the evidence of an extinction debt in the study sites:

iv. Is there evidence of an extinction debt in species diversity or composition?

The literature review identified a gap in knowledge in the relationship between species diversity and composition. Research typically studies one or other of these aspects, but rarely both simultaneously. Research question five was designed to address this gap, by assessing the relationship between species diversity and composition and to analyse the influence of the landscape of both of these concepts:

v. What relationships exist between species diversity and species composition?

As with species diversity, there is also a gap in knowledge as to how variation in spatial, historical, abiotic and management factors influence genetic diversity. Research question six and seven addressed this:

vi. What is the structure of gene flow across twelve calcareous grassland study sites for two plant species synonymous with calcareous grassland sites in South East England?
vii. To what extent do contemporary and historical landscape and environmental characteristics influence the genetic diversity of the two study species?

Finally, although theory suggests that a positive relationship exists between different levels of diversity, research to support this is scarce. The eighth research question analyses the relationship between habitat, species, and genetic diversity in the study area:

viii. What relationships exist between habitat, species, and genetic diversity, and is there evidence of a species genetic diversity correlation?

3. Methodology: Study sites, species selection, and analysis methods

3.1 Preamble

This Chapter will introduce the South Downs National Park (SDNP) research area, commenting on its characteristic features. Calcareous grasslands will then be discussed, with reference to their conservation importance. The twelve calcareous grassland study sites used throughout the thesis will then be presented. Following this, the two study species used in the genetic analysis will be introduced. The chapter then moves on to discuss the methodological approaches to measurements used throughout the research chapters: modelling historical landscape, measuring habitat scale spatial structure, measuring site variability, and measuring biodiversity.

3.2 Research design

This research used a mixed method approach to investigate landscape patterns and their effects on biodiversity. The study was carried out in three parts, each focusing on a different level of biodiversity: habitat diversity; species diversity; and genetic diversity. Each of these levels is represented in a separate research chapter, before a final synthesis of the three components and the relationship between them is discussed.

At each level of biodiversity, the research focused on twelve calcareous grassland sites within the SDNP. At each site data on biodiversity (habitat diversity, species diversity, and genetic diversity) was measured and analysed in relation to localised characteristics (abiotic, spatial, historical, and management). For analysis at the genetic diversity level two species with different dispersal strategies (*Cirsium acaule* and *Ranunculus bulbosus*) were selected.

3.3 Study area: The South Downs National Park

The SDNP is located in the counties of Hampshire, West Sussex, and East Sussex in the South East of England (Figure 3.1). The SDNP covers an area of over 16,500 hectares, measuring 114km from east to west. Previously an Area of Outstanding Natural Beauty, National Park status was designated in 2010. In a global context, the National Parks of the UK do not meet the criteria for the International Union for Conservation of Nature and Natural Resources (IUCN) category II: National Parks, as they are places where people work and live. Instead they are within category V: Protected Landscape. UK National Park designation is intended to preserve semi-natural land and cultural heritage. Most of the land is privately owned arable land, which is moderated with conservation (South Downs National Park Authority, 2012).

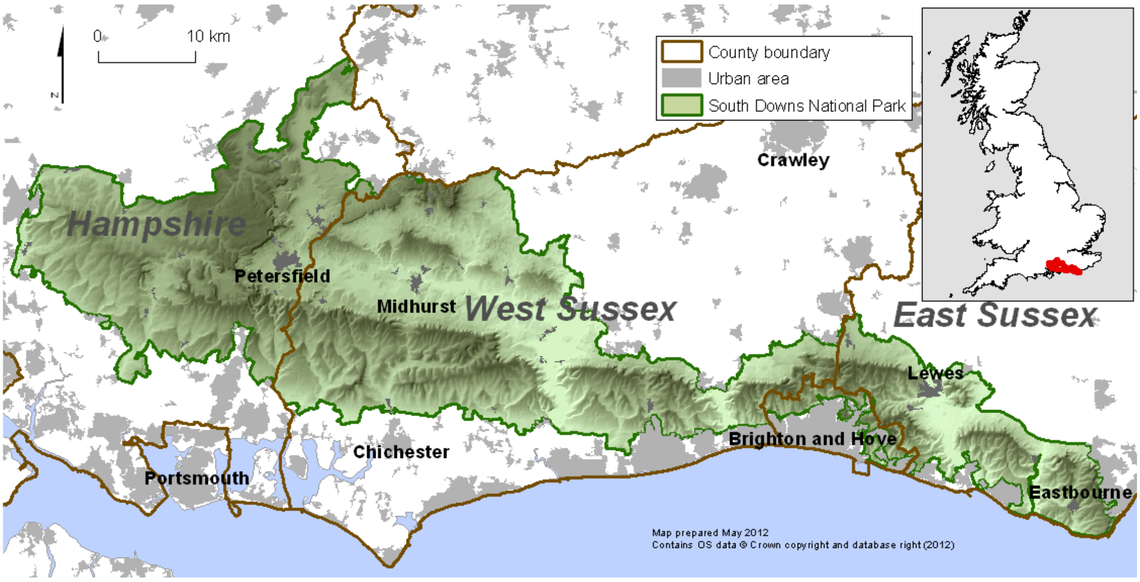
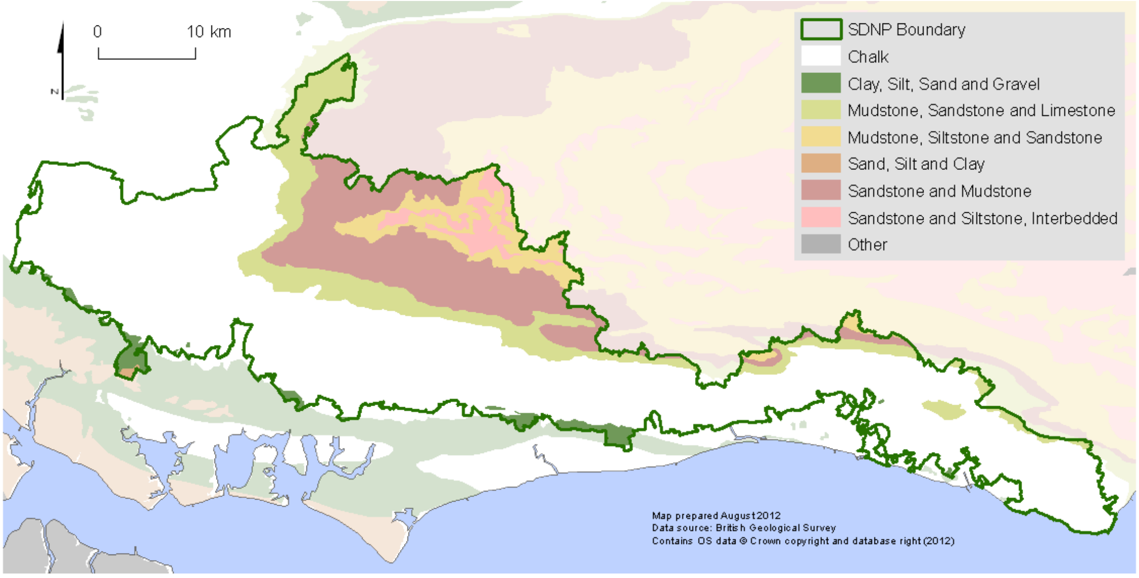


Figure 3.1 The location of the South Downs National Park in the South East of Great Britain. Original in colour.

Running through the heart of the SDNP is an underlying chalk bedrock (Figure 3.2). The chalk dates back to the Cretaceous period (65-100 million years ago), when marine deposits were laid down by the warm seas that covered the land (Avery, 1990, Mortimore, 1997, Brandon, 1998, South Downs National Park Authority, 2012). The chalk rests on earlier marine deposits of Greensand and Gault, as well as the Wealden sandstone and clay terrestrial deposits. Geological processes acted to push up and fold the Earth’s crust forming a dome of chalk. This dome has eroded over millions of years, with only the hills of the Downs remaining. Soft, fine-grained, and permeable to water, the distinctive properties of chalk have shaped the landscape of the SDNP (Brandon, 1998). Soils originating from chalk are characteristically thin, well drained and nutrient poor. Only plant species that are tolerant to such conditions thrive, and as a result grasslands on chalk support specialist species.

Moreover, plant growth is slow on such soils. The dip slopes and valley bottoms support deeper soils, whilst deposits of wind-blown soils on top of the chalk give rise to chalk heath habitats. The Wealden greensand soils are sandy, acidic, and nutrient poor, associated with wooded heath and commons.

Much of the distinctive landscape character of the SDNP are a product of the underlying chalk bedrock, which supports a diverse range of nationally and internationally important, natural and semi-natural ecosystems. Such characteristics provide are ideal for the study of the relationship between the landscape and biodiversity. Moreover, natural and semi-natural habitats with the SDNP now largely remain as small isolated fragments, providing an opportunity to study the effects of landscape change and habitat fragmentation on biodiversity.



Map prepared August 2012
Data source: British Geological Survey
Contains OS data © Crown copyright and database right (2012)

Figure 3.2. Bedrock geology within the SDNP. Original in colour.

3.4 Calcareous grassland

Most calcareous grasslands in Britain are situated on soils derived from underlying rock rich in calcium carbonate (Keymer and Leach, 1990). They are located on shallow, lime-rich soils, typically on limestone rock (including chalk) with pH values between 7 and 8.4 (Price, 2003). The underlying rocks are usually porous, and the soils freely-draining and well aerated. Soils lack nitrogen, potassium, and phosphorous, but are high in calcium carbonate ($CaCO_3$).

In addition to suitable soils, appropriate management is necessary as calcareous grasslands are principally biotic plagioclimax communities. They are not the naturally occurring climax communities of their environments, but the result of land management processes that arrests natural succession to scrub and subsequently woodland (Green, 1990). The major management process on grasslands in Britain is grazing, which acts as a continuous disturbance preventing succession and the build-up of nutrients (Critchley et al., 2003). In turn, this stops competitive species dominating and prevents the exclusion of grassland specialists (Price, 2003). New growth is continuously removed, and thus plants that are grazing tolerant thrive. Many species are adapted to grazing by an intercalary meristem, a system where leaves continuously grow from the base so that as they are defoliated they are able to produce new tissue (Fitter and Peat, 1994). Others have mechanical or chemical defences (such as wild thyme which is unpalatable to most herbivores (Crofts and Grayson, 1999).

Calcareous grasslands are of conservation importance because they support a diverse range of species, including rare and endangered ones (Willems, 2001), and are a recognised Biodiversity Action Plan (BAP) priority habitat within the UK. They are habitats with one of the highest species diversities in Europe (Willems, 1990), commonly supporting 25-30 species per 25x25cm quadrat (Hutchings, 1983, Mitchley and Grubb, 1986).

The National Vegetation Classification (NVC) (Rodwell, 1992) identifies fourteen calcareous grassland community types in Britain. The distribution of the fourteen calcareous grasslands broadly corresponds to the distribution of pervious calcareous bedrock in Britain, but beyond this pattern the lithology of the bedrock exerts relatively little influence on the range and composition of flora (Rodwell, 1990). Lowland calcareous grasslands are represented in the first nine groups (CG1-CG9) (Table 3.1), which are detailed by Rodwell (1992). In the SDNP six calcareous grassland community types are present (CG1-CG6), with CG2 the most abundant on the Sussex Downs (Figure 3.3).

Table 3.1 NVC calcareous grassland community types in Britain

CG1	*Festuca ovina - Carlina vulgaris* grassland
CG2	*Festuca ovina - Avenula pratensis* grassland
CG3	*Bromus erectus* grassland
CG4	*Brachypodium pinnatum* grassland
CG5	*Bromus erectus - Brachypodium pinnatum* grassland
CG6	*Avenula pubescens* grassland
CG7	*Festuca ovina – Thymus - Hieracium pilosella* grassland
CG8	*Sesleria albicans - Scabiosa columbaria* grassland
CG9	*Sesleria albicans - Galium sterneri* grassland
CG10	*Festuca ovina - Agrostis capillaris - Thymus praecox* grassland
CG11	*Festuca ovina - Agrostis capillaris - Alchemilla alpina* grass-heath
CG12	*Festuca ovina - Alchemilla alpina - Silene acaulis* dwarf-herb community
CG13	*Dryas octopetala - Carex flacca* heath
CG14	*Dryas octopetala - Silene acaulis* ledge community

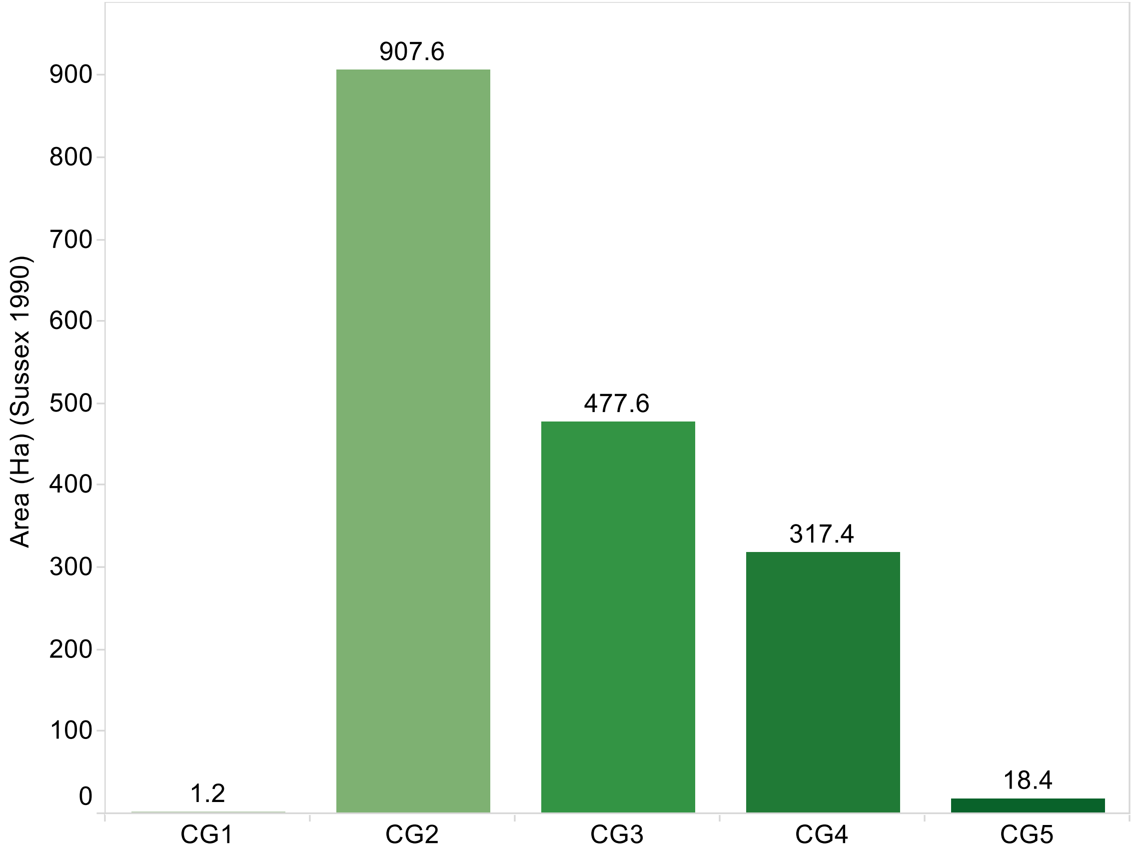


Figure 3.3 Lowland calcareous grassland NVC community types within the SDNP in Sussex. Adapted from Steven, 1992 & Steven and Muggeridge, 1992. See Table 3.1 for definitions.

There is an abundance of calcicole species in calcareous grasslands in the south and east, essentially related to the presence of excessively drained, base-rich and oligotrophic rendziniform soils (Rodwell, 1990). There is a high abundance of mesophytic *Arrhenatherum* herb species, such as *Galium verum*, *Plantago lanceolata*, *Trifolium repens*, *Lotus corniculatus*, and in more less heavily grazed patches *Arrenatherum elatius* and *Avenula pratensis* (Rodwell, 1990). The distinguishing feature of these grasslands however is the presence of *Mesobromin* plant species such as *Avenula pratensis*, *Briza media*, *Koeleria macrantha*, *Carex flacca*, *Sanguisorba minor*, *Helianthemum nummularium*, *Scabiosa columbaria*, *Leontodon hispidus* and in more lightly grazed areas , *Bromus erectus*, and *Brachypodium pinnatum*. In particular the warmer calcareous grassland in the south-east contain calcicole species with continental European distributions, such as *Cirsium acaule*, *Hippocrepis comosa*, and *Asperula cynanchica*, and nationally rare species such as *Polygala calcarea*, *Senecio integrifolius*, *Thesium humifusum*, *Euphrasia pseudokerneri*, *Orchis ustulata*, and *Herminium monorchis*.

As a result of their high species diversity, calcareous grasslands are ideal habitats to study patterns of species and genetic diversity. The high species diversity means that the influence of the study variables is likely to be more exaggerated than within a habitat with a lower species diversity. Localised variables contribute to differences in vegetation between sites. As such the results of this study could be used to guide research into other habitat types. Moreover calcareous grasslands have experienced a dramatic decline in extent over the past century, with most remnant patches remaining as small and isolated fragments (Fuller, 1987, Hooftman and Bullock, 2012), making them an ideal habitats for investigating the effects of landscape change and habitat fragmentation. In particular the calcareous grasslands within the SDNP are ideal for research purposes as they are habitats that have been subject to substantial transition and fragmentation in recent times (Burnside et al., 2003).

3.5 Study sites

Site selection/exclusion was based upon four central landscape criteria: site management, site spatial metrics, site history and site access availability. A total of twelve sites were selected to ensure an adequate amount of variation was included and ensure a range of landscape criteria were included. The twelve diverse sites were selected as a sample of the calcareous grasslands across the SDNP (Figure 3.4). Each site represented a habitat patch. Specifically each site was a contiguous area of calcareous grassland with a single owner and management approach. The selection sought to generate study sites with a range of abiotic, spatial, historical, and management conditions. Sites ranged in size from 6 hectares at Steep Down up to 153 hectares at Beachy Head. In addition to varying spatial characteristics, the conditions at each site were influenced by differences in aspect and slope. In terms of management, some sites were both grazed and mown, whilst others were only grazed, and at the time of the study Cissbury Ring was only mown (Table 3.2). Furthermore there was variation in grazer between sites, with some grazed by sheep, some by cattle, and some by multiple grazers (Table 3.2). Such variation facilitated the comparison between sites and across conditions. Detailed descriptions of each site are provided in the following subsections.

Table 3.2 Details on the mowing schedules and grazing stock for the twelve study sites

	Mows per year	Mowing schedule	Present grazing stocking level	Winter grazing
Arundel Park	1	Part mowing for hay in July	7 sheep for 12 months	Y
Beachy Head	3	Generally 3 times a year	10-15 per compartment	Y
Butser Hill	1	Mowing only on amenity areas in autumn.	220 ewes; 200 lambs for 5 months; 20-30 cattle for 3 months	N
Castle Hill Complex	-	-	30 cattle for 4 months; 100 sheep for 3 months; 18 ponies for 4 months	Y
Cissbury Ring	1	Grazing stopped in 2009. Mown once a year in summer.	-	N
Cradle Hill	1	Late winter/early spring	25 cows for 2 months over winter	Y
Devil's Dyke		-	14 cows for 12 months	Y
Harting Down West	1	Varies	350-400 sheep in winter across all of the site (not just west), and 15 cows all year	Y
Levin Down	1	Varies	130 sheep for 5 months; 15 cattle for 4 months	N
Malling Down	-	-	300 sheep for 12 months; 10-15 cattle for 4 months (winter)	Y
Southwick Hill	-	-	20 cattle for eight months (not winter)	N
Steep Down	-	-	20 sheep for 3 months; 20 cattle for three months	Y

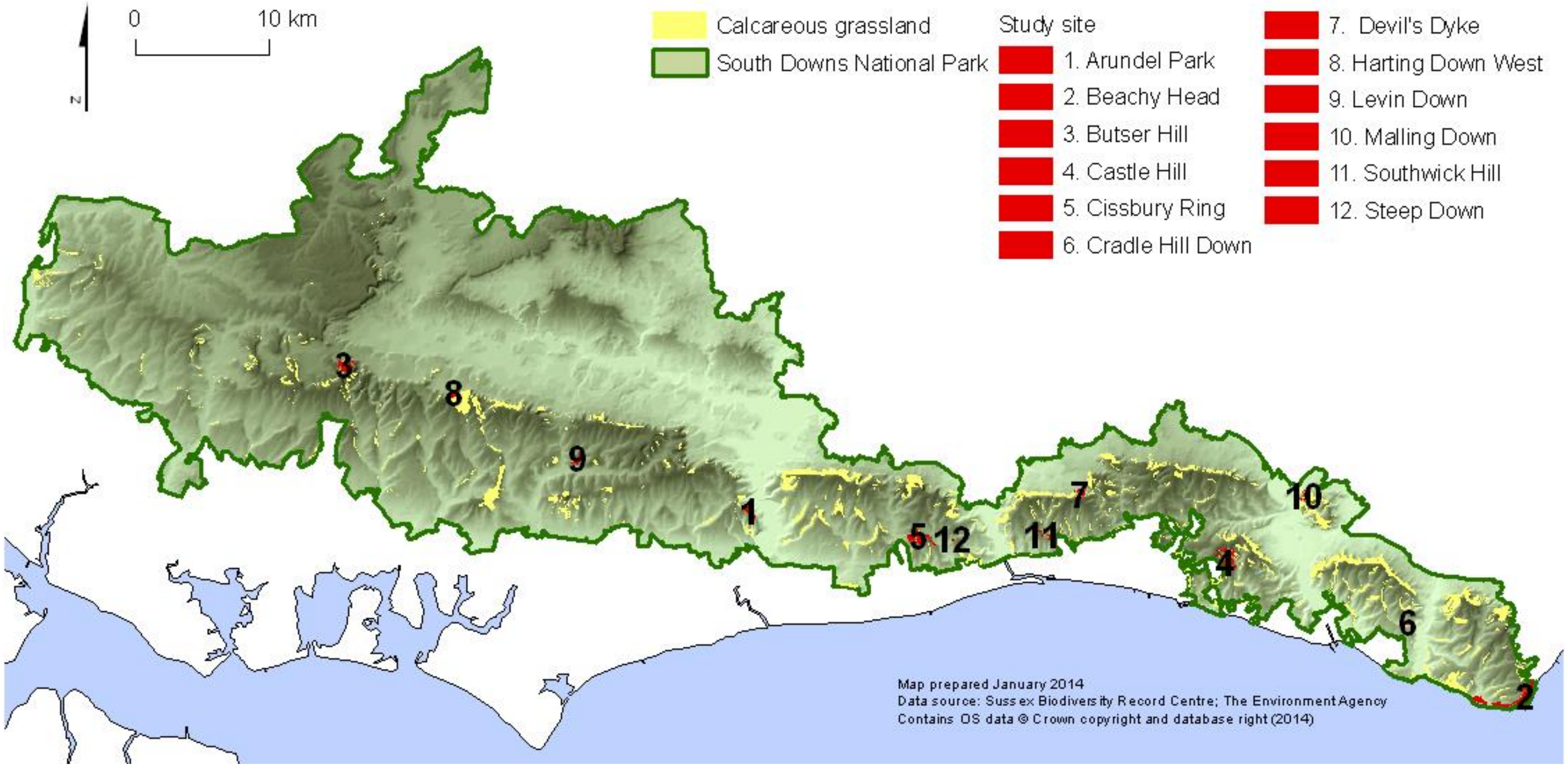


Figure 3.4 The location of the twelve study sites. Original in colour.

3.5.1 Arundel Park

Arundel Park (Figure 3.5) is a 26ha site that is one of five fragments of calcareous grassland patches within the Arundel Park Site of Special Scientific Interest (SSSI). The calcareous grassland fragments are interspersed with improved grassland and woodland. The site is part of the Arundel Estate managed by the Office of the Duke of Norfolk. The site is bordered to the east by the River Arun, which potentially acts as a barrier with sites on the opposite bank. The site is managed by both mowing and grazing. Annual mowing takes place once a year in July. Grazing occurs year-round by sheep, with a stocking density of 0.5 sheep per hectare, equating to 0.06 livestock units (LUs) per hectare. The site is NVC community type CG2, with patches of dense scrub and woodland (Steven, 1992). A previous survey by Steven (1992) identified high cover of *Sanguisorba minor*, *Helianthemum nummularium*, and *Leontodon hispidus*. *Bromus erectus* was uncommon and *Bracypodium pinnatum* absent. Steven (1992) recorded 74 species at the site.

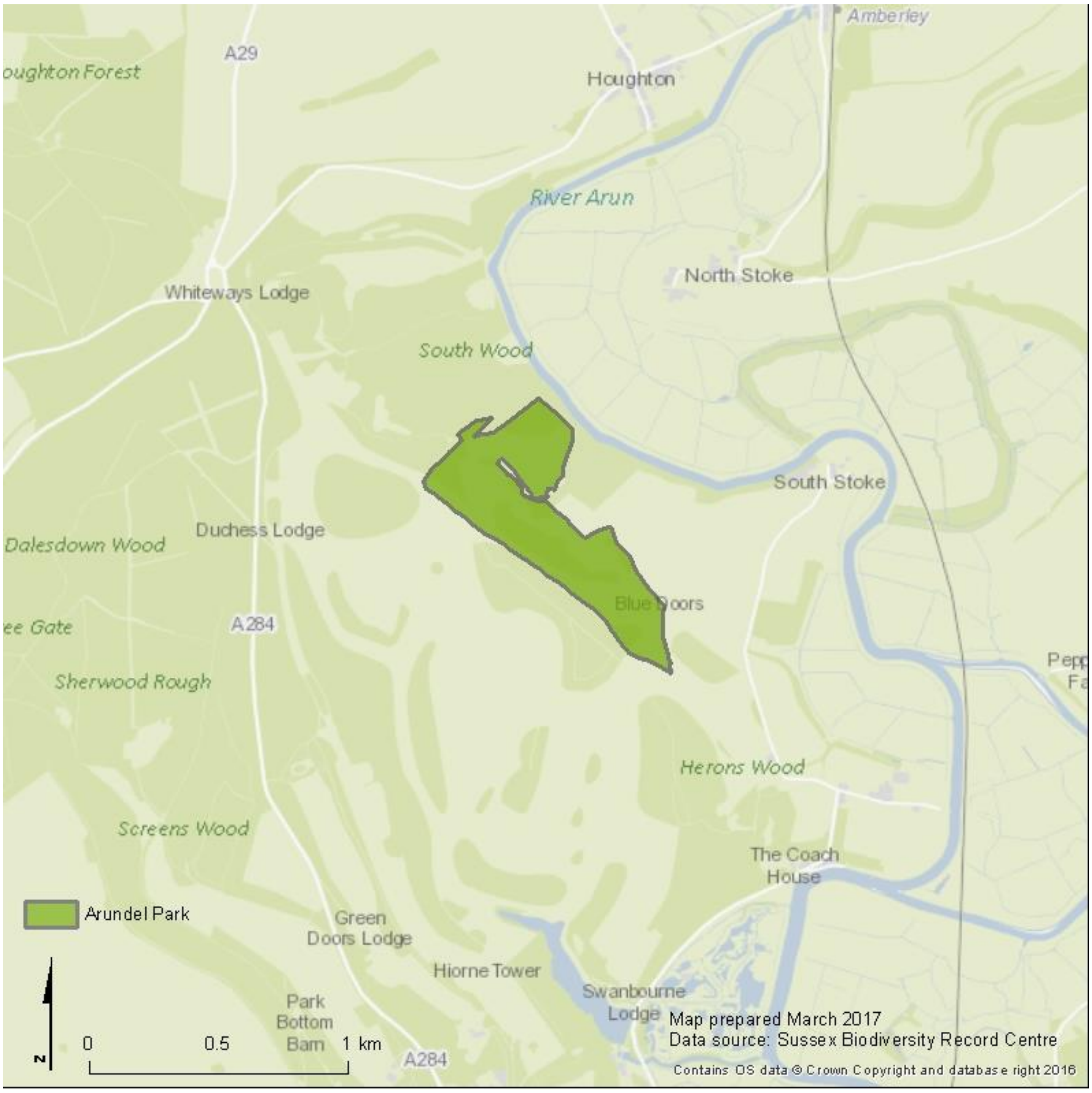


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Figure 3.5 The location of Arundel Park study site. Original in colour.

3.5.2 Beachy Head

Beachy Head (Figure 3.6) is the largest of the study sites at 152.5ha. The Beachy Head site is an extensive area of contiguous calcareous grassland stretching along the top of the coastal cliffs from Birling Gap to Eastbourne. It forms part of the Seaford to Beachy Head SSSI, and is owned by Eastbourne Borough Council. The site is bounded by the coast to the south and east, with neighbouring grassland sites to the north and west. The grassland at Beachy Head is maintained by mowing, grazing, and trampling. Mowing generally occurs three times a year. Cattle graze the site for four months of the year, with fifteen individuals rotating between different compartments. Stocking levels are 0.03 LUs per hectare. This site is comprised of NVC calcareous grassland types

CG2, CG3, and CG4, as well as mesotrophic grassland, and dense scrub (Henderson, 1979, Steven and Muggeridge, 1992). The site has varying species richness, with some areas of high species richness and some scrub. 104 species were recorded at the site by Steven and Muggeridge (1992).

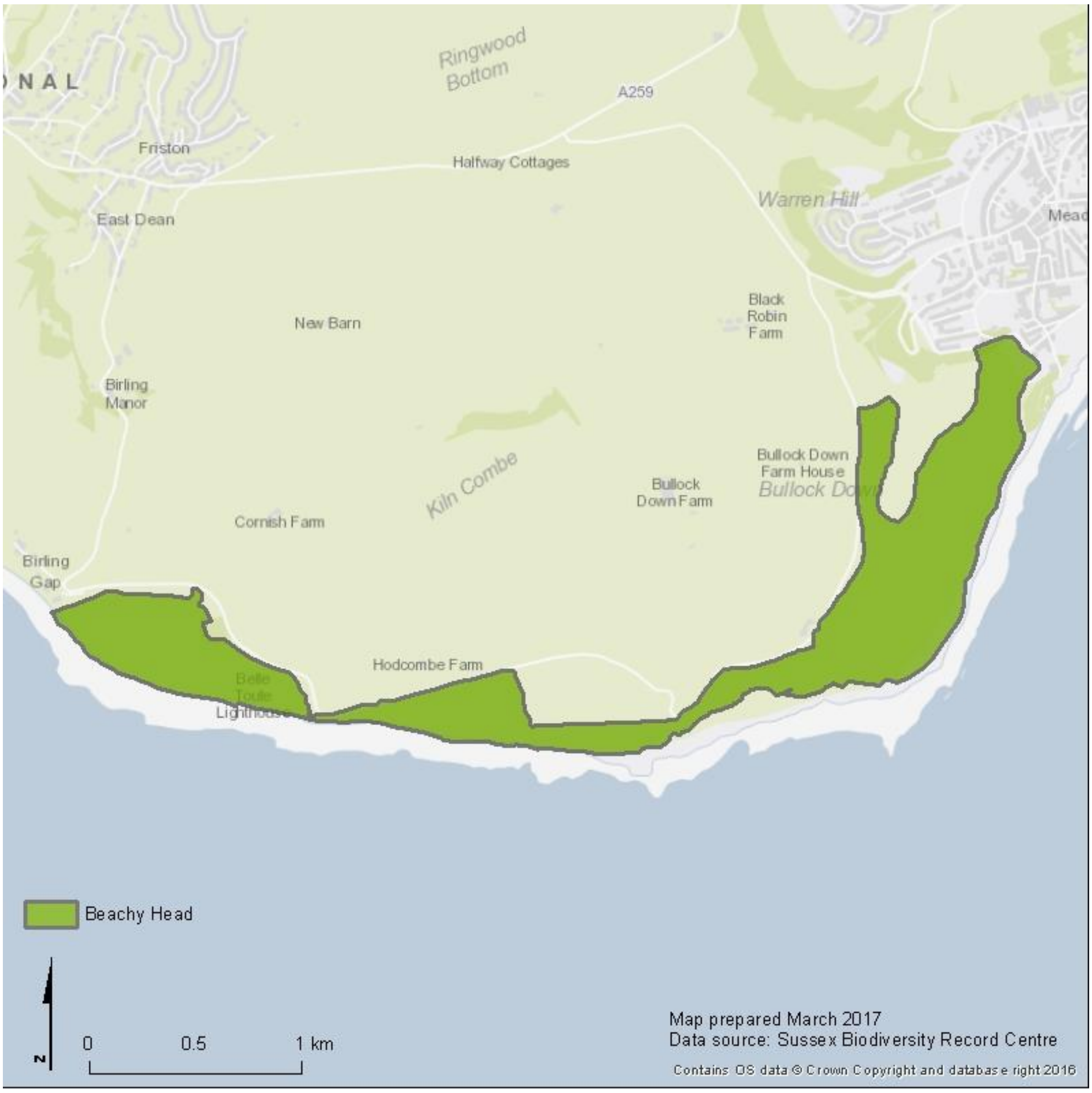

Figure 3.6 The location of Beachy Head study site. Original in colour.

3.5.3 Butser Hill

The Butser Hill site (Figure 3.7) measures 61.5ha. Part of the Queen Elizabeth Country Park and Butser Hill NNR (National Nature Reserve), the site is managed by Natural England as Butser Hill SSSI. Butser Hill contains the highest point in the SDNP, and is the second largest calcareous grassland site in Hampshire. Management is by a combination of mowing and grazing by cattle, sheep, and rabbits. Approximately 30 cattle graze the site for three months of the year (during summer). Additionally 220 sheep and 200 lambs graze for five months between spring and autumn. Combined stocking levels are 0.33 LUs per hectare. Mowing occurs annually on the amenity areas in autumn. The site is comprised of NVC calcareous grassland types CG2, CG3, CG6, as well as mesotrophic grassland, dense scrub, and areas of woodland (Pardon, 1990). Pardon (1990) comments that the site has a reasonable diversity of calcareous grassland communities, although the diversity is not outstanding considering its large area. In 1987 a total of 93 species were recorded (south area of site only) (Pardon, 1990).

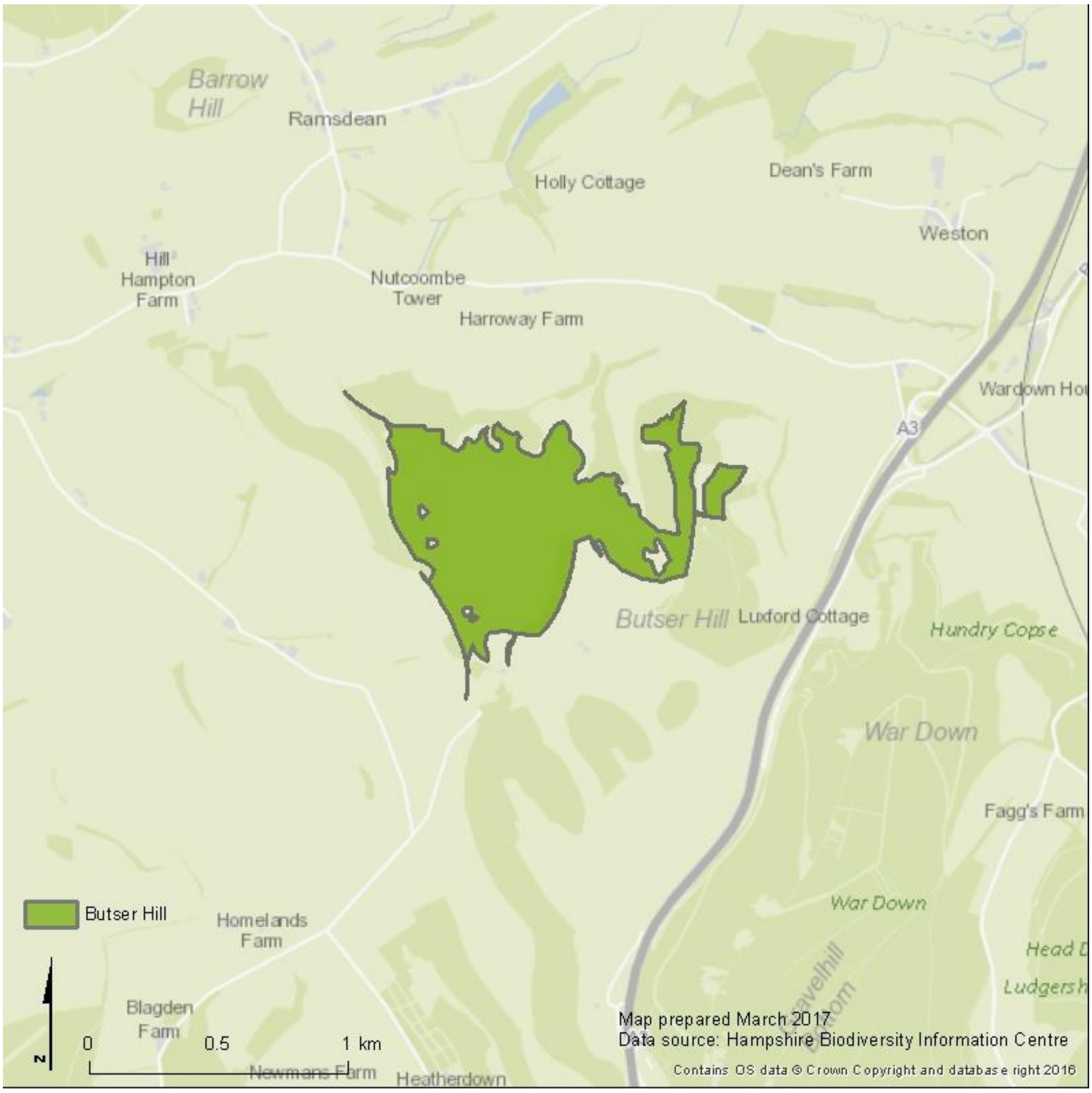

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Figure 3.7 The location of Butser Hill study site. Original in colour.

3.5.4 Castle Hill Complex

Castle Hill Complex (Figure 3.8) is a 92ha site, owned and managed by Natural England as Castle Hill SSSI and part of the Castle Hill NNR. The site is one of the most species-rich calcareous grasslands in England (Rose, 1995), with a number of rare and uncommon species (Steven and Muggeridge, 1992). For the past 30 years the site has been grazed by cattle in autumn and sheep in winter, with occasional additional winter grazing by Exmoor ponies aimed at restricting the abundance of *Brachypodium* species. Stocking levels are 30 cattle for 4 months a year, 100 sheep for 3 months, and 18 ponies for 4 months. The valley bottoms of the site have been harvested 3 times in the past 30 years. NVC communities CG2, CG3, CG4, CG5 are present along with some mesotrophic grassland communities (Steven and Muggeridge, 1992). Steven and Muggeridge (1992) comment that the site is of varying quality, with some species rich areas, and some species poor areas that have previously been improved. Species richness of 67 was recorded in a section of the site by Steven and Muggeridge (1992).

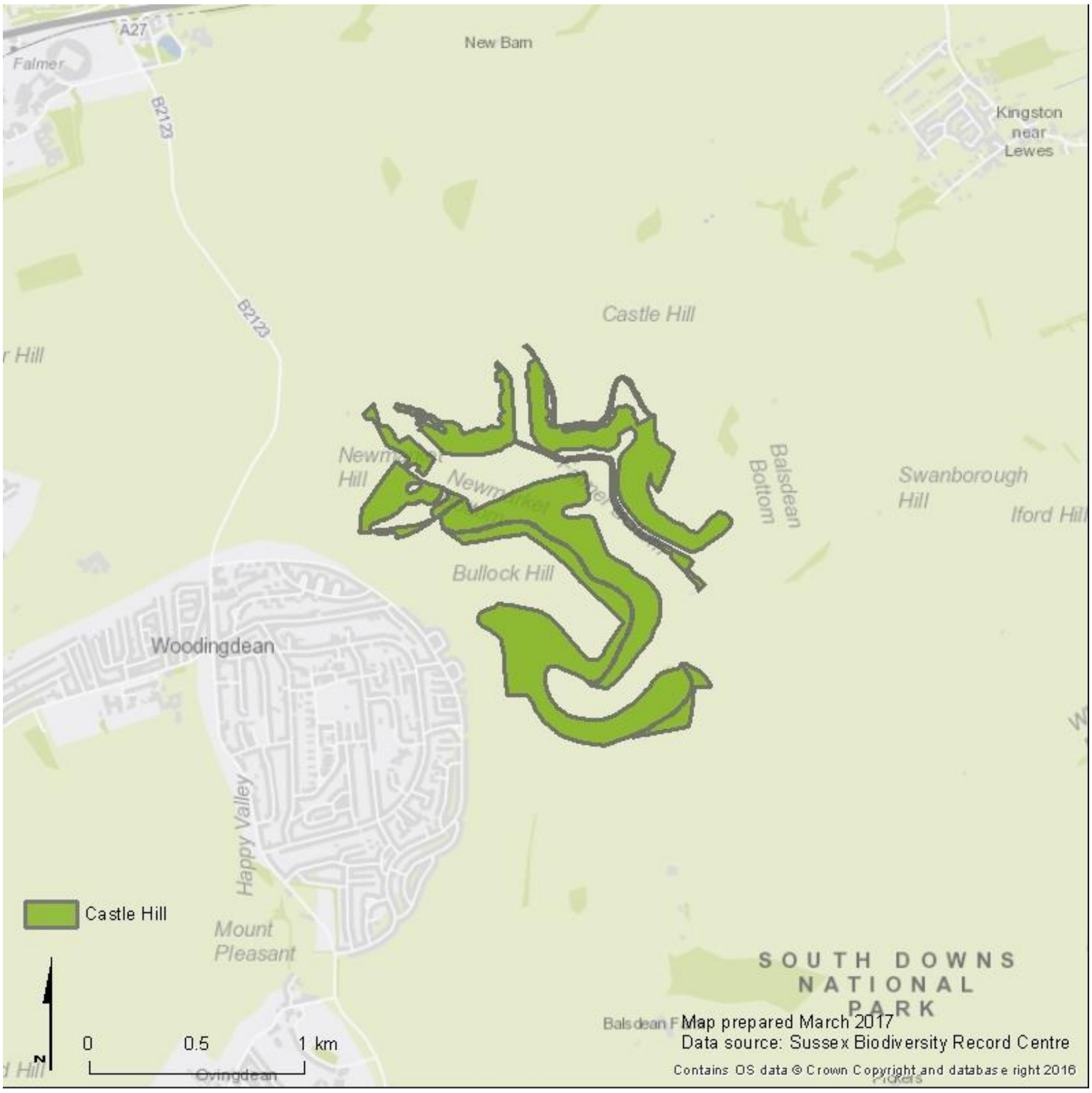

Figure 3.8 The location of Castle Hill Complex study site. Original in colour.

3.5.5 Cissbury Ring

Cissbury Ring (Figure 3.9) is a 38ha site detached from the main escarpment of the South Downs. The Cissbury Ring site is a hill fort owned by the National Trust, and is part of Cissbury Ring SSSI. Most of the site is gently sloping with aspects in all directions. At the time of this study the site was managed by cutting and collection. The re-establishment of grazing is planned in the coming years, having been suspended in approximately 2009 due to breeding birds. Prior to this time there was occasional grazing by cattle. The site is comprised of NVC community types CG1, CG2, CG3, CG4, with some mesotrophic grassland and calcifugous grasslands communities. Steven (1992) comments that although parts show signs of past improvement, generally the site is unimproved and species rich. Steven (1992) recorded 74 species at the site.

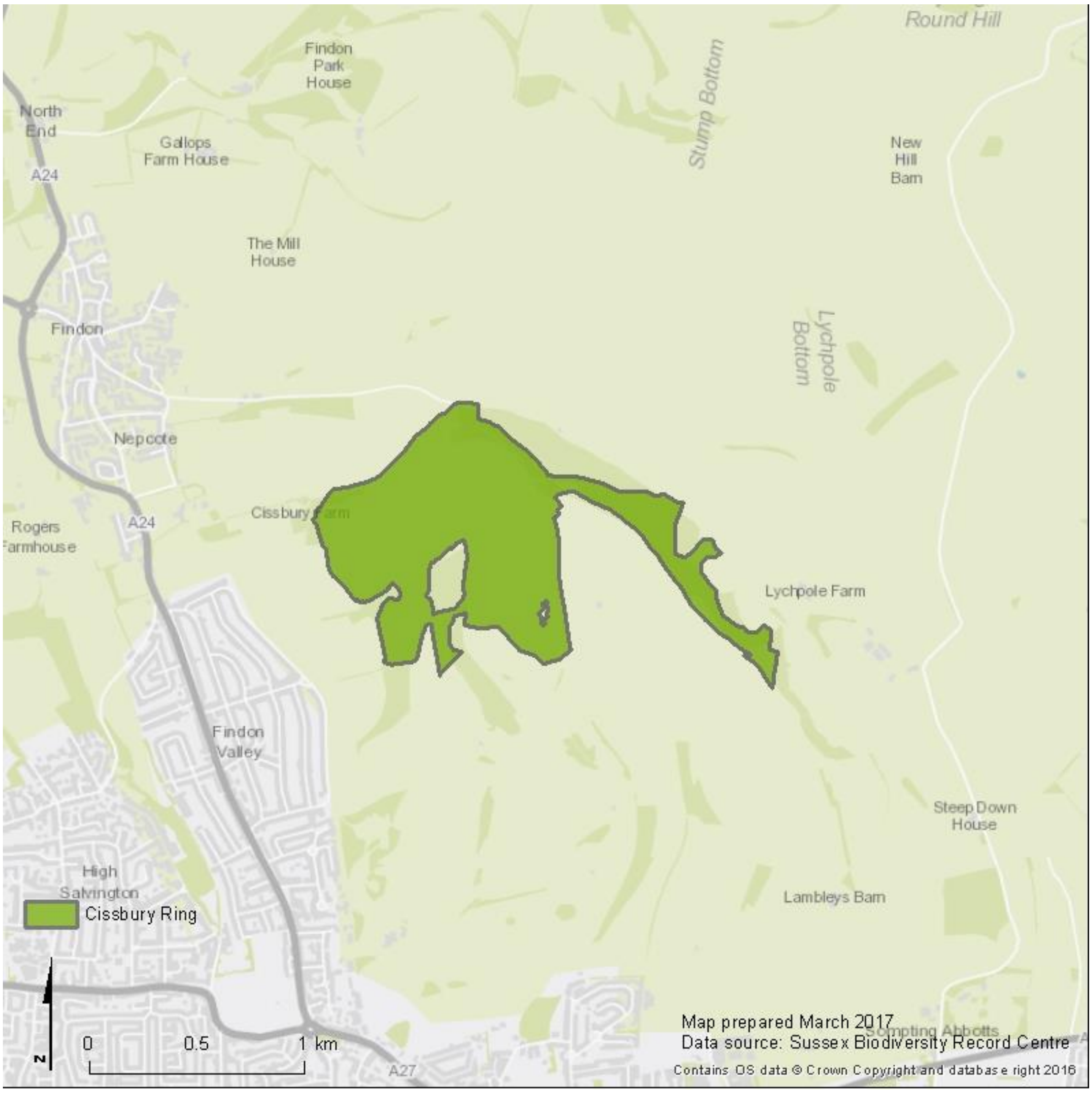

Figure 3.9 The location of Cissbury Ring study site. Original in colour.

3.5.6 Cradle Hill Down

At 38ha, the Cradle Hill Down site (Figure 3.10) is an important inland stretch of grassland that provides a link between the grasslands of the main escarpment of the South Downs and the coastal grasslands. Cradle Hill Down is owned by the National Trust, and part of the Seaford to Beachy Head SSSI. At an average of 40m above sea level, the site has the lowest elevation of the twelve study sites. Cradle Hill Down is a north facing site. The site is managed by mowing once a year in late winter or early spring and grazing by cattle. 25 cattle graze the site for two months of the year in winter, and this management has been consistent for the past 20 years. The site contains large areas of scrub, but with patches of species rich grassland on the lower part of the slope, with NVC communities CG2, CG3 and mesotrophic grasslands present. Steven and Muggeridge (1992) recorded 43 species at the site.

Figure 3.10 The location of Cradle Hill Down study site. Original in colour.

3.5.7 Devil's Dyke

The Devil's Dyke site (Figure 3.11) covers an area of 35ha, and is comprised of opposite north and south facing slopes. Located at the eastern extreme of the Fulking Escarpment, Devil's Dyke is owned by the National Trust and is part of Beeding Hill to Newtimber Hill SSSI. Since 2000 the site has been grazed year-round by 14 cattle. Prior to this sheep grazed the site, along with cattle grazing every other year. NVC community types present at the site are CG2, CG3, CG4, and mesotrophic grassland communities. The opposing slopes of the site are different in character, with the north facing slope having very shallow soil and the south facing slope fairly deep soil (Steven, 1992). Species richness of 78 was recorded by Steven (1992).

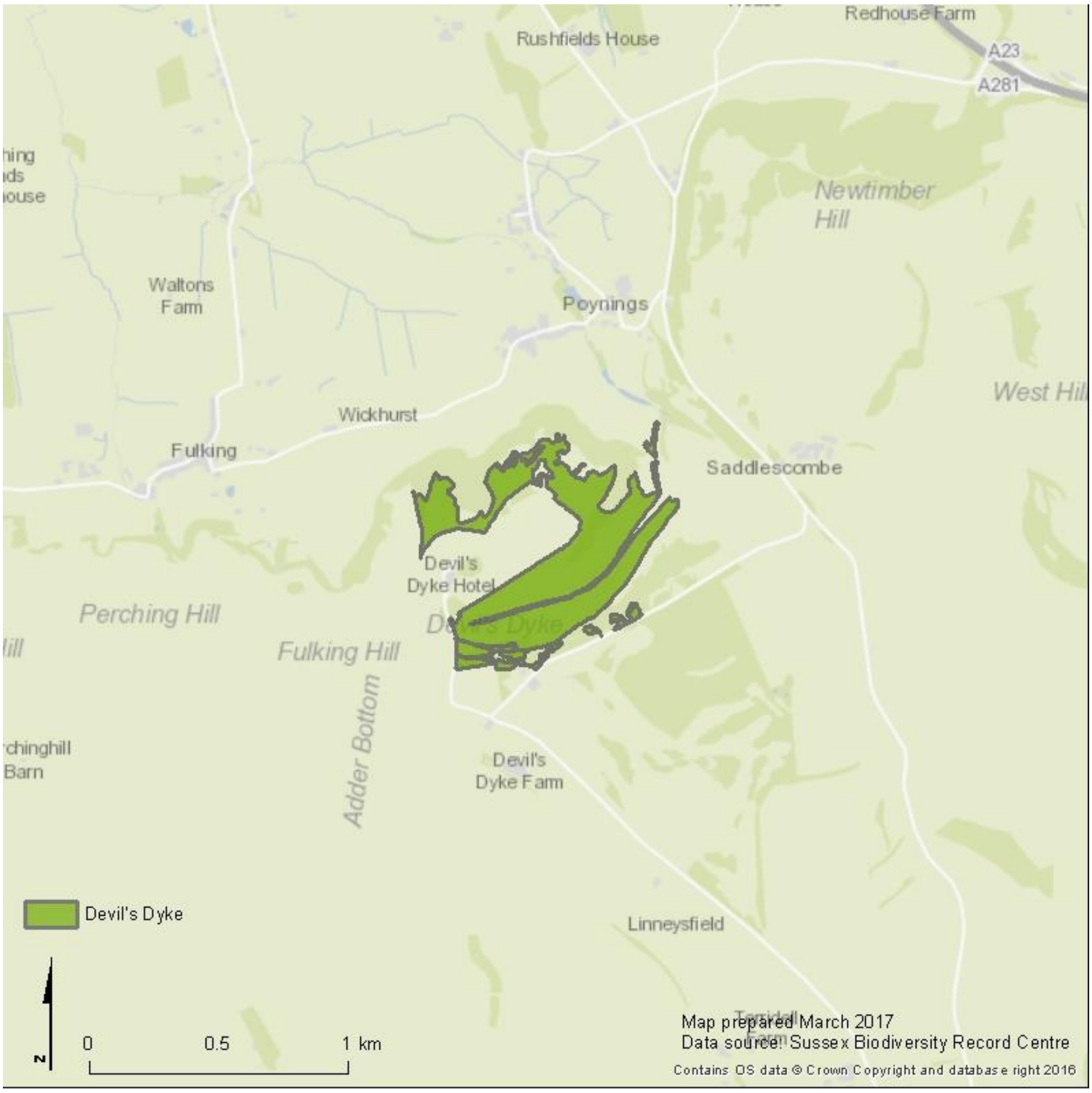

Figure 3.11 The location of Devil's Dyke study site. Original in colour.

3.5.8 Harting Down West

Part of the Harting Downs SSSI, the Harting Down West site (Figure 3.12) was converted from agricultural land in the 1970s (Steven, 1992, Rose, 1995) and measures 33.4ha. Owned by the National Trust, the site is part of the main escarpment of the Downs and is predominantly north facing. The site is mowed annually in spring. Winter grazing has been carried out by cattle and sheep since 1989. Historically grazing may have been more intense with commoner's rights allowing grazing intensity at up to four times the present levels. NVC community types CG2, CG3, CG4, and mesotrophic grasslands are found at the site. The grassland is generally low in species richness with woodland occupying the borders of the grassland. Steven (1992) comments that *Brachypodium pinnatum* is absent from the site with *Bromus erectus* dominating. Species richness of 58 was recorded at the site by Steven (1992).

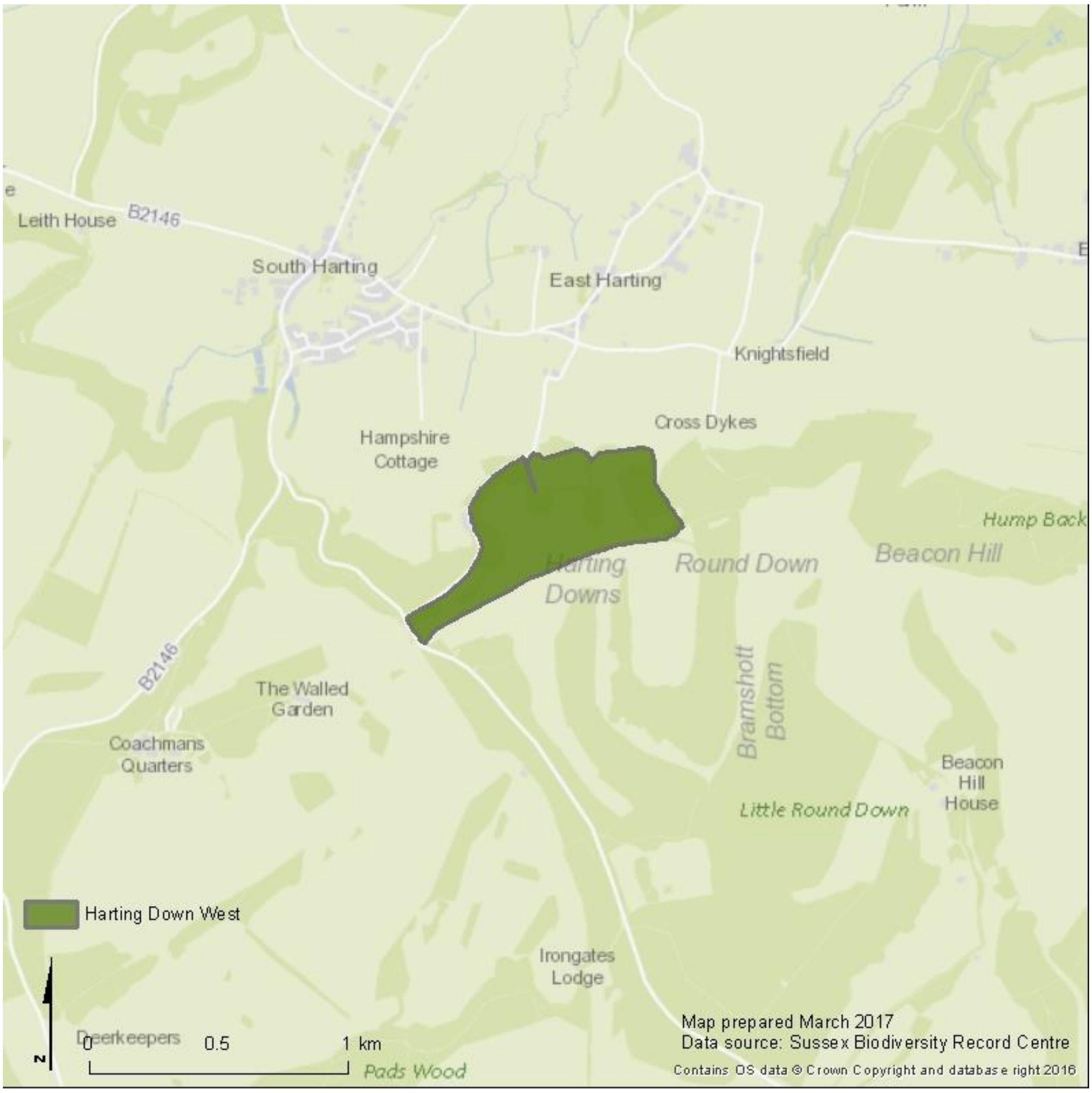

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Figure 3.12 The location of Harting Down West study site. Original in colour.

3.5.9 Levin Down

A designated SSSI, Levin Down (Figure 3.13) is owned and managed as a nature reserve by the Sussex Wildlife Trust. The site measures 33ha and is south and south-east facing. The site is mowed annually on a rotational basis.

Since 1981 the site has been grazed by cattle and sheep, with intensities varying between different compartments of the site. The site was predominantly open grassland until the mid-20th century, before grazing began to decline. A large part of Levin Down was ploughed during the war, but the South and East facing slopes were left. Parts of the site are comprised of scattered and dense scrub, with NVC communities CG2, CG3, and mesotrophic grasslands present. Steven (1992) comment that *Bracypodium pinnatum* is absent, with large populations of *Helianthemum nummularium* and *Campanula glomerata*. 61 species were recorded at the site by (Steven, 1992).

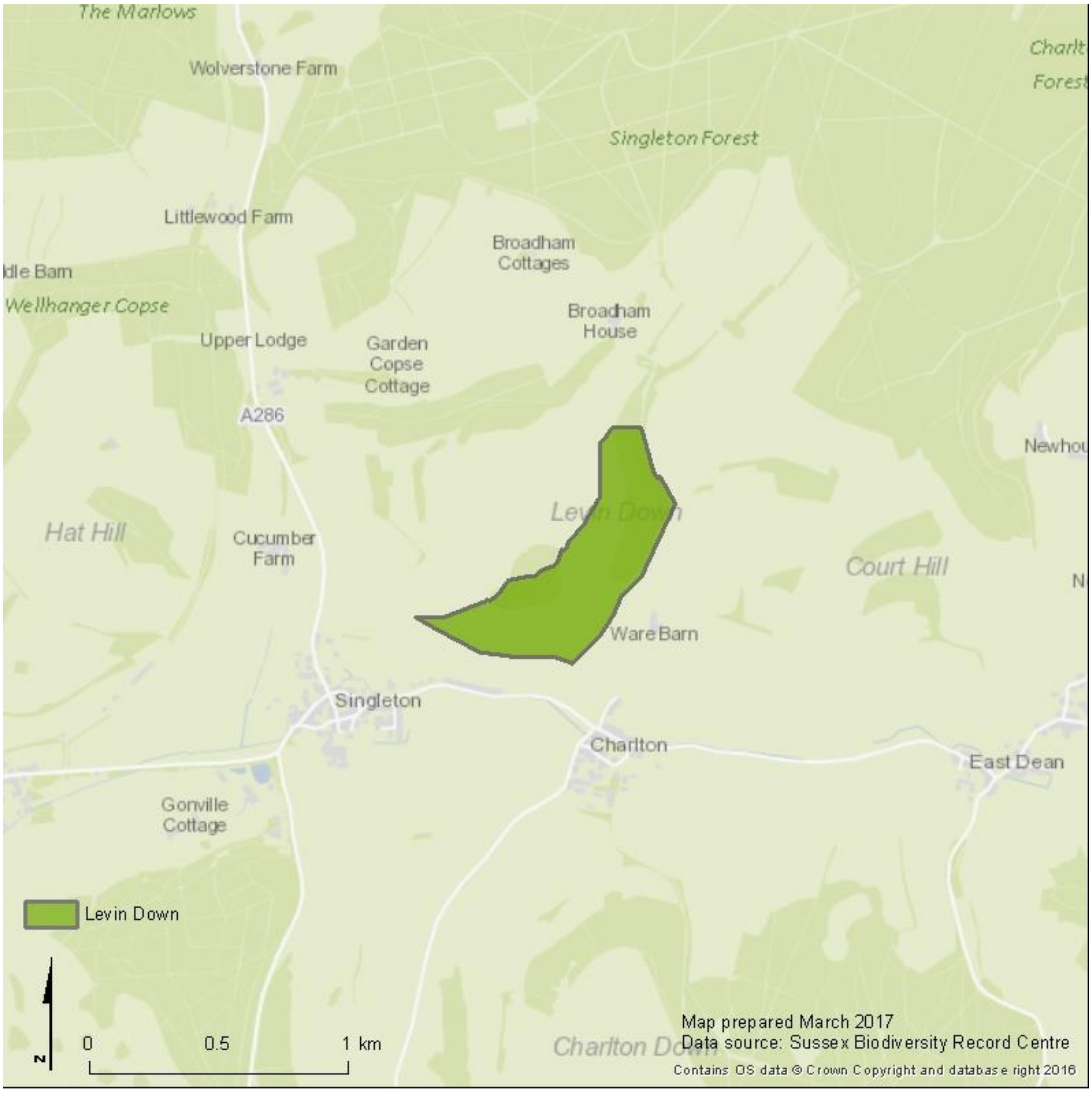

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Figure 3.13 The location of Levin Down study site. Original in colour.

3.5.10 Malling Down

In addition to Levin Down, Malling Down (Figure 3.14) is the second of the sites managed by the Sussex Wildlife Trust, and is part of the western end of the Lewes Downs SSSI. Malling Down to is part of the largest remaining unploughed area in East Sussex (Rose, 1995). The site is 28.3ha. The site has been grazed by sheep and cattle since 1983. Stocking levels are 300 sheep and 10-15 cattle over winter. The site is comprised on CG2 and mesotrophic grassland communities. The site is generally species rich, with Steven and Muggeridge (1992) recording 86 species, although patches are species poor.

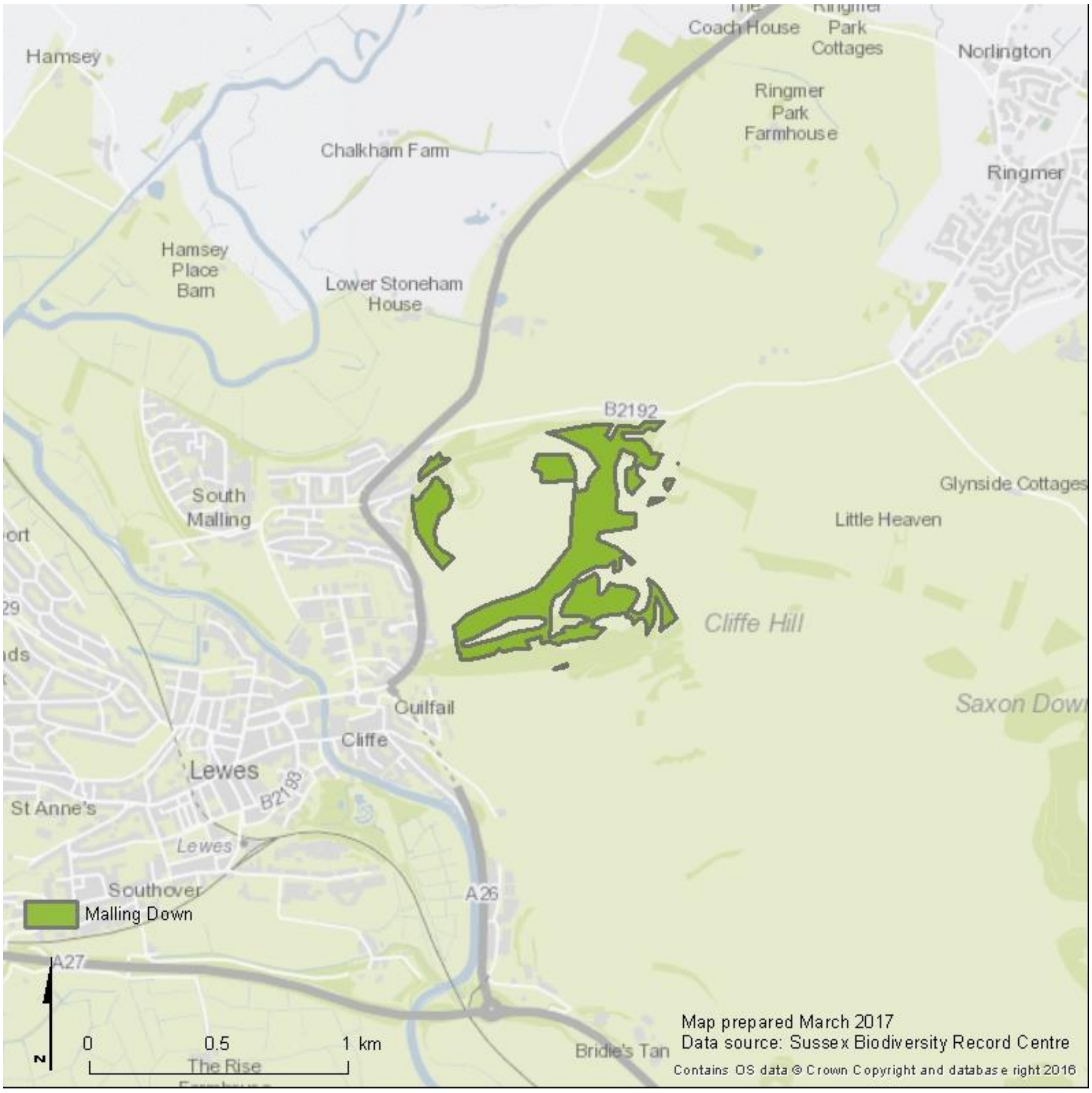

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Figure 3.14 The location of Malling Down study site. Original in colour.

3.5.11 Southwick Hill

Mostly owned by the National Trust and part private, Southwick Hill (Figure 3.15) is a designated Site of Nature Conservation Importance (SNCI). The site is located on the dip slope and is thus predominantly of a northerly aspect with gentle slopes. Southwick Hill measures 30.1ha. The site is generally heavily grazed. For the past 20 year 20 cattle have grazed the site year-round. NVC community types CG2, CG3, CG5, and mesotrophic grassland and calcifugous grasslands and montane communities are present. A large proportion of the site is comprised of dense scrub. Species richness of 57 was recorded by Steven (1992).

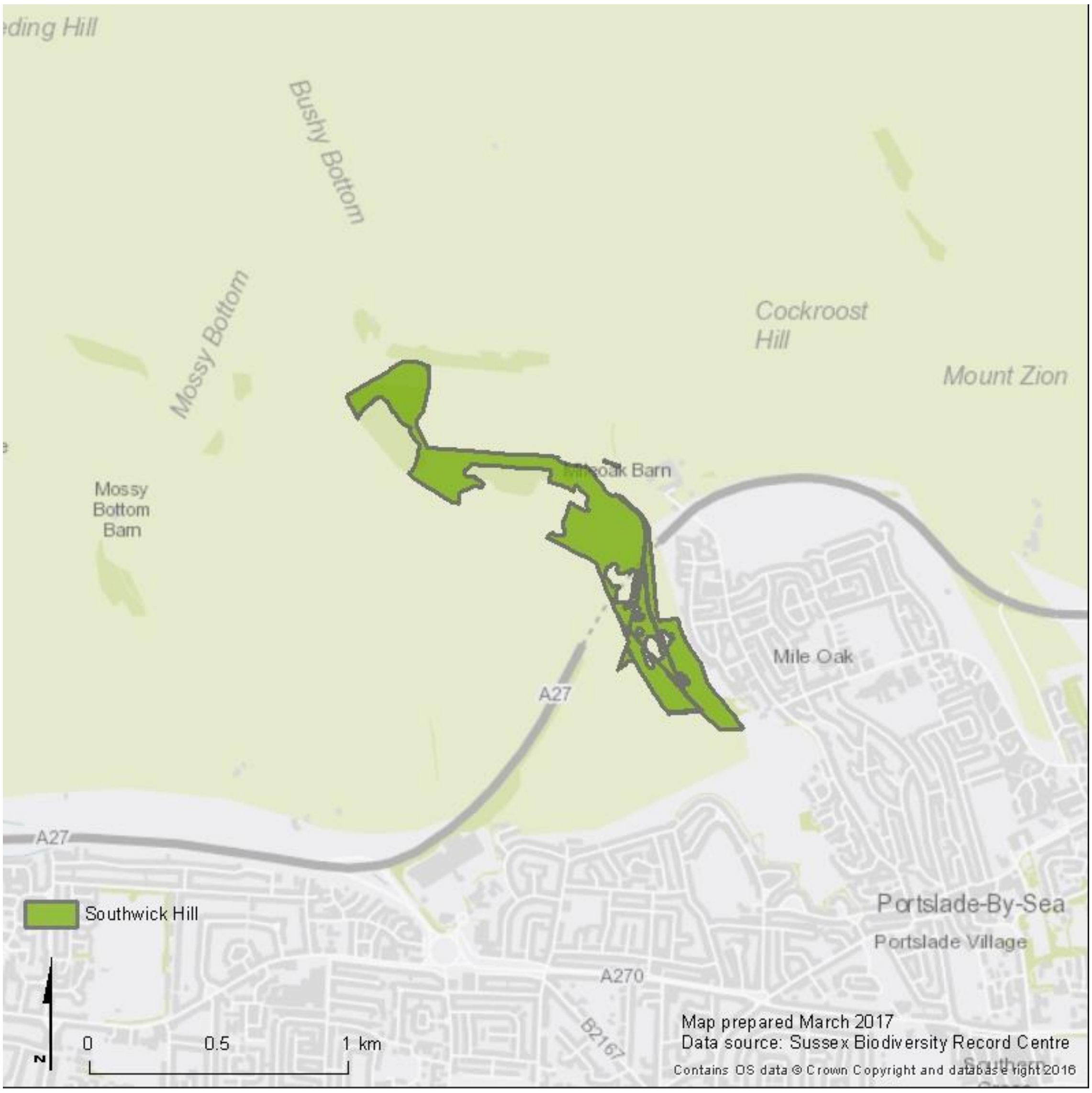


Figure 3.15 The location of Southwick Hill study site. Original in colour.

3.5.12 Steep Down

Steep Down (Figure 3.16) is part of the Sompting Estate and is a designated SNCI. A small site at 6ha surrounded by a matrix of agricultural land. The site is predominantly north-west facing and is south of the main stretch of the South Downs. The site was grazed up until the 1970s when grazing ceased, with occasional grazing resuming in the 1980s and 1990s. Since 2007 the site has been grazed by both sheep and cattle at variable rates. Due to its steepness the site is not mowed. The site has a NVC CG2, CG3, and mesotrophic grassland community types with patches of dense scrub, particularly at the base of the slope. Steven (1992) recorded 19 species, commenting that *Brachypodium pinnatum* was dominant in parts of the site.

Figure 3.16 The location of Steep Down study site. Original in colour.

3.6 Study species

Two species of plants were selected for genetic analysis on the basis of autecology and suitability for genetic analysis. This facilitated the analysis of genetic diversity at the study sites.

3.6.1 *Cirsium acaule* Dwarf Thistle

Cirsium acaule is a perennial herb of the *Asteraceae* family. *C. acaule* reaches its geographical limit within the British Isles, where it is found north to Yorkshire and west to the Welsh borderlands (Figure 3.17). *C. acaule* grows on shallow soils, particularly on the chalk escarpments. Characteristically most abundant on rendzina soils with a high pH, it is restricted to calcareous soils and as such can be considered a calcicole. *C. acaule* is largely restricted to calcareous grasslands communities although it is also present in low abundances in mesotrophic grassland communities (MC5 and MC11) (Fitter and Peat, 1994). *C. acaule* is typically denser on southern facing slopes, often being absent from north facing slopes at the northern limit of its distribution (Pigott, 1968). *C. acaule* is most plentiful where the grass is short (2-15cm) (Pigott, 1968), and although it can persist in longer grass the establishment of seedlings is prevented. Ellenberg indicator values for *C. acaule* are: light = 9; moisture = 4; reaction = 8, nitrogen = 3; salt tolerance = 0 (Hill et al., 1999).

C. acaule has a horizontal, branched sympodial rhizome, at the apex of which a compact rosette forms. The purple rosette flowers from June to September. *C. acaule* is gynodioecious, having some individuals with female flowers and other individuals with hermaphrodite flowers. The leaves of *C. acaule* are eaten by sheep and rabbits, but the rosette is rarely destroyed and is avoided by cattle (Pigott, 1968). The main agent of dispersal for *C. acaule* is wind, and as such long-distance dispersal is possible (Pigott, 1968, Fitter and Peat, 1994). *C. acaule* is a long-lived species, with clonal patches estimated to be 60-80 years old (Pigott, 1968). Microsatellite genetic markers have been developed for *C. acaule* by Jump et al. (2002) who isolated nine loci.

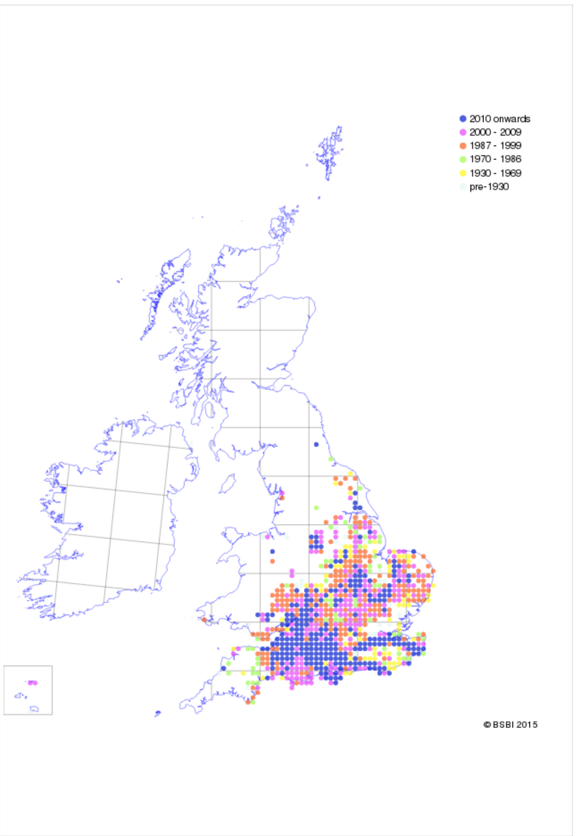


Figure 3.17. The distribution of *C. acaule* in Britain and Ireland. Reproduced from the Online Atlas of the British and Irish Flora, Biological Records Centre.

3.6.2 *Ranunculus bulbosus* Bulbous buttercup

A perennial herb of the *Ranunculaceae* family, *Ranunculus bulbosus* is widespread through-out Britain (Figure 3.18). Primarily a lowland species, it is typically present within grazed dry calcareous and mesotrophic grassland communities. It is in greatest abundance under sunny conditions and thus it is more common on south facing slopes. The abundance of *R. bulbosus* is a function of site age, grazing intensity, and drainage, and it is not able to persist in competition with taller species. Although *R. bulbosus* is often abundant in soils with a high pH, it is not considered a calcicole as it is present on acidic soils that are well drained. Ellenberg values for *R. bulbosus* are: light = 7; moisture = 4; reaction = 7; nitrogen = 4; salt tolerance = 0 (Hill et al., 1999).

R. bulbosus plants have 1-10 stems that arise from an annually renewed corm, with 1-5 bright yellow flowers per stem. Flowering occurs April to June with dispersal of seeds a month later. *R. bulbosus* is a diploid (2n = 16). *R. bulbosus* is visited by a range of generalist pollinators, most typically *Diptera, Coleoptera* and *Halictidae*, and more rarely *Apidae* (Harper, 1957, Steinbach and Gottsberger, 1994). The flower of *R. bulbosus* is typically avoided by grazers, and dispersal is predominantly barochorous, although long-distance dispersal can occur through grazing animals and birds which feed on the seeds and bulbs (Harper, 1957, Sarukhan, 1974). Although traditionally reported as being self-incompatible (Coles, 1973, Lundqvist, 1990), Matter et al. (2013) found selfing rates of 45.7% in an experimental population in Switzerland. Eight species-specific microsatellite markers have been developed by Matter et al. (2012). These markers are highly polymorphic, with allele numbers ranging from seven to sixty-three.

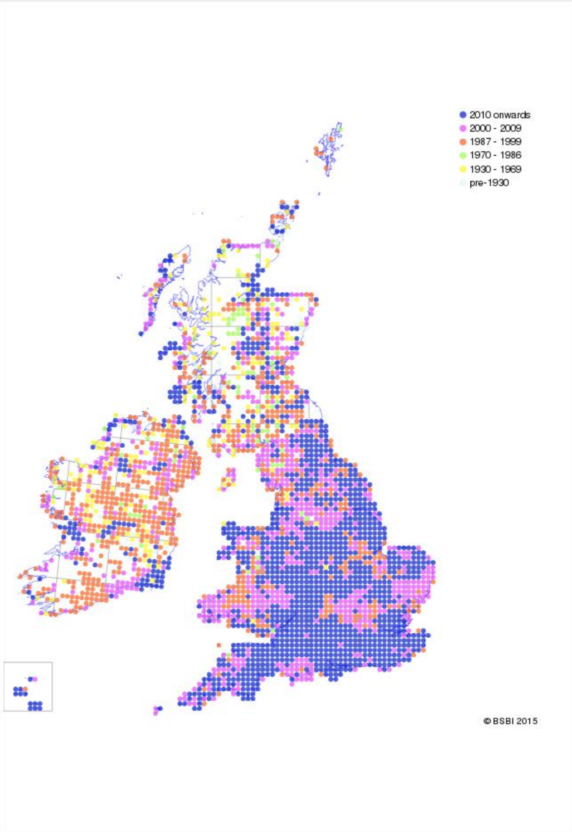


Figure 3.18. The distribution of *R. bulbosus* in Britain and Ireland. Reproduced from the Online Atlas of the British and Irish Flora, Biological Records Centre.

3.7 Measuring habitat scale spatial structure

The spatial structure of habitats within the landscape was analysed using landscape composition and habitat configuration metrics. All LULC types were included in the habitat scale analysis. As measures of landscape composition, the total area of the different LULC types and their percentage of the total area of the SDNP were calculated in ArcMap 10.1 (ESRI, 2012). To measure landscape configuration the following metrics were calculated in FRAGSTATS (McGarigal and Marks, 1995): Number of patches, Mean patch area, Core Area Index (CAI), and Average Nearest Neighbour.

The number of patches refers to the total number of patches of each LULC type within the landscape, and mean patch area is mean area of these patches. Habitat scale CAI measures represent the percentage of a land that is comprised of core area. This metric is expressed as:

Equation 3.1 Habitat scale Core Area Index

$$CAI = \frac{\sum_{j=1}^{n} \frac{{A_{ij}}^{c}}{A_{ij}}(100)}{n_i}$$

> Where $\sum_{j=1}^{m}$ is the sum across all patches of the corresponding patch type, ${A_{ij}}^{c}$ = core area (m^2) of patch ij based on an edge depth (here the edge depth was set to 10m, equal to the resolution of the cells), A_i = area (m^2) of patch ij, and n$_i$ is the total number of patches in the landscape.

Average Nearest Neighbour represents the mean Euclidean distance between a LULC patch and the nearest patch of the same LULC type. This is calculated for each LULC in the landscape and the mean value found.

Equation 3.2 Habitat scale Average Nearest Neighbour

$$Average\ Nearest\ Neighbour = \frac{\sum_{j=1}^{n} h_{ij}}{n_i}$$

> Where $\sum_{j=1}^{n}$ is the sum across all patches of the corresponding patch type, n$_i$ is the number of patches of the same type.h$_{ij}$ is the distance in metres from patch ij to the nearest neighbouring patch of the same LULC type.

3.8 Measuring site variability

Whilst the characteristics of the landscape exert some influence on species patterns, Reitalu et al. (2012) argue that the characteristics of the local habitat explain more of the variation in species richness of semi-natural grassland patches than do the characteristics of the landscape. Variability in topography, soils, spatial structure, historical spatial structure, and management was assessed across the twelve study sites. The purpose was to compile a database of site characteristics to be used to analyse relationships between site variability and biodiversity.

The extent of the historical sites was determined by using the 'Select By Location' tool within ArcMap 10.1 (ESRI, 2012). The tool parameters were set to select patches from the historical calcareous grassland model that overlapped with the extent of the contemporary sites.

3.8.1 Topographic variability

Area, elevation, slope, and aspect were all calculated in ArcMap 10.1 (ESRI, 2012). Elevation, slope, and aspect were all derived from a digital elevation model (DEM). For this purpose the OS Terrain 50 OpenData dataset was used, which has a resolution of 50m. Elevation was calculated as the mean elevation of the site. A slope file was created and data from the attribute table was used to calculate the mean slope. Similarly an aspect file was created and aspect values (in degrees) were converted into eight cardinal directions. To classify sites the mode for the cardinal values was used. Although slope data can provide some information on the amount of sunlight received, calculations of solar radiation provide more meaningful data. As such mean solar radiation in kilowatt hours per metre squared (kW h/m2) was calculated for each site. Whilst radiation varied across a site, a mean value was used to classify the potential solar radiation across the site, similar to the method used by Bruun (2000).

3.8.2 Soil variability

At each site, three 20g soil samples were taken for laboratory analysis. pH values were measured using a calibrated Mettler Toledo pH meter with an Inlab

413 SG electrode. Nitrogen (N), Phosphorus (P), Potassium (K), values were tested using the HI-3895 soil test kit by Hanna Instruments. 106.7 ml of distilled water was added to each 20g soil sample (ratio 1.5 water: 8 soil), and left to stand for twenty-four hours. The solutions (2.5 ml for N and P; 0.5 ml for K) were then transferred to test tubes, and mixed with the appropriate reagents. N and P levels were then recorded using a colorimetric method, and K using a turbidimetric method.

3.8.3 Spatial variability

Spatial variability was included to analyse the influence of spatial structure, habitat loss, and habitat fragmentation, on species and genetic structure. McGarigal et al. (2005) suggest that there are five main spatial components to habitat loss and fragmentation: habitat extent, habitat subdivision, patch geometry, habitat isolation, and habitat connectedness. To this end appropriate metrics for each of these components were identified, with an additional measure of percentage change in patch size from the 1930s to 2012.

Patch extent was calculated in ArcMap 10.1 (ESRI, 2012). Patch subdivision concerns the breaking up of a contiguous habitat into separate patches. Patch density within 1km of each site was used as a measure of subdivision. The number of patches within a 1km buffer of each site was calculated in ArcMap 10.1 (ESRI, 2012), and converted in to a density value:

Equation 3.3 Patch subdivision

$$Subdivision = \frac{n}{Ai}$$

> Where *n* = the number of patches within 1km of site i, and *Ai* = the total area in hectares of site *i.*

Patch geometry considers the spatial character of habitat patches, of which there are numerous aspects. One aspect, core area, is particularly relevant to habitat fragmentation. Large contiguous habitats will generally have a large core area. As habitats break into multiple patches they simultaneously increase their edge perimeter, in turn decreasing the size of the core area. Core area can be defined as the total area of the patch, once the edge area has been eliminated. To compare core area statistics between sites with different areas, a core area index (CAI) can be calculated, representing the percentage of a site that is comprised of core area. This metric is expressed as:

Equation 3.4 Patch level Core Area Index

$$\boldsymbol{CAI = \frac{{A_{ij}}^{c}}{A_{ij}}\,(100)}$$

Where ${A_{ij}}^{c}$ = core area (m^2) of patch ij based on an edge depth (here the edge depth was set to 10m, equal to the resolution of the cells); A_{ij} = area (m^2) of patch *ij*.

To measure connectivity the modified version of the incidence function model (IFM) formula proposed by Moilanen and Nieminen (2002) was selected. IFMs attempt to use landscape metrics as a surrogate for immigration rates. Specifically, IFMs take into account the distance to all source populations, with a negative exponential dispersal kernel. The formula proposed by Moilanen and Nieminen (2002) is expressed as:

Equation 3.5 Patch connectivity

$$S_i = A_i^c \sum_{j \neq i} exp\,(-\propto d_{ij}) A_j^b$$

Where Si is the connectivity of patch i. The sum can be taken over all patches where *j≠i;* A_i is the area of patch *i*; *b* and *c* are constant parameters scaling the effect of emigration and immigration as a function of patch area (set to 0.3 after Moilanen and Nieminen (2002)); d_{ij} is the distance between patches *i* and *j;* $\propto$ is a constant setting the migrant survival rate over the distance d_{ij} (set to 0.3 to correspond to a migration distance of 3.3km after Helm et al. (2006)); A_j is the area of patch *j*.

Percentage change was measured as the percentage of the remnant site area compared to the 1930s site area, and relates to the loss of area aspect of fragmentation. It is expressed as:

Equation 3.6 Patch area percentage change

$$Percentage\ change_i = \left(\frac{2012\ A_i}{1930s\ A_i}\right) \times 100$$

Where *Ai* = area of patch *i*

3.8.4 Historical variability

Historical site spatial structure was measured using patch area, CAI, and connectivity using the same method as for the contemporary sites (section 3.8.3).

3.8.5 Management variability

Management variables measures were gathered through communication with the land owners and site managers for each site, with the responses consolidated through subsequent inspection of sites during the field visits. Although this opened the data to inconsistencies, no standardised records of these variables exist. It was considered that the effect of inconsistencies in the responses would not significantly influence the results, and the variables requested were selected as unambiguous ones that would be consistently interpreted. The following information was gathered: type of management (mowing, grazing, mowing and grazing); grazer (sheep, cattle, other); number of grazers; mowing schedule. The number of grazers was used to calculate grazing densities in Livestock Units (LUs) per hectare. The number of grazers were converted into LUs using the livestock values given by Nix (2008) (Table 3.3).

Table 3.3 Livestock Unit values

	Livestock value
Cattle	1.00
Lowland sheep	0.11
Lambs	0.04
Ponies	0.8

For each site LUs were calculated as follows:

Equation 3.7 Livestock Units

$$\boldsymbol{LUs = \frac{(Livestock\ value \times months\ of\ year\ grazing)}{12}}$$

To convert LUs into density values, allowing for comparisons between sites, the LUs for each site was divided by the site area (ha).

3.9 Measuring biodiversity

3.9.1 Habitat diversity

The most commonly utilised measures of habitat diversity evaluate two separate aspects of diversity: richness and evenness. Habitat richness refers to the number of habitats in a landscape, and habitat evenness refers to the relative percentage of each of these habitats. Two such indices that have been particularly popular are the Shannon and Simpson indices (Forman, 1995), borrowed from community ecology where they have been used to quantify species diversity. The Shannon index of diversity (Shannon and Weaver, 1949) (Equation 3.8) emphasises the richness component of diversity and rare habitats, whereas the Simpson index (Simpson, 1949) emphasises the evenness component and the dominant habitat (Haines-Young and Chopping, 1996, Riitters et al., 2000). As such Nagendra (2002) recommends use of the Shannon index where the measurement of rare habitat types is of interest, and the Simpson index where the dominant habitat is of interest.

Equation 3.8 The Shannon index of diversity (SHDI)

$$\boldsymbol{SHDI = -\sum_{i=1}^{N} p_i \times \ln p_i}$$

where N = the number of land cover types, p_i = the proportional abundance of the *ith* habitat type.

3.9.2 Species richness and diversity

Measuring species diversity is not a straight forward procedure, with numerous methods existing for its calculation. Typically species diversity is measured as the number of species (species richness), the difference between species within a population (species evenness), or a combination of both (species diversity). Furthermore, there are numerous ways to measure each of these concepts. The term species diversity is characterised by confusion of specific terminology and difficulties in interpreting results (Krebs, 1999). The difficulties in measuring and quantifying species diversity is that it involves quantifying two independent concepts: species richness (the number of species), and species evenness. The difference between species diversity measures are typically in the weighting given to these concepts. For example the Simpson’s Index is weighted towards the most dominant species in the sample, whereas the Shannon-Weiner index is weighted towards richness and evenness within a sample (Magurran, 2004). Patil and Taillie (1979) comment that this is an inevitable consequence of attempting to classify a multidimensional concept as a single value. Multiple authors report differing orders of site diversity when comparing the values of different measures of species diversity, such as the Shannon-Weiner index with Simpson’s index (Hurlbert, 1971, Tothmeresz, 1995, Nagendra, 2002). To this end Southwood and Henderson (2000) comment that diversity ordering is essential before comparing communities with a single nonparametric measure. The principle of such a technique is that the different parameters used in different diversity measures result in different classifications of diversity. Therefore, by calculating and plotting diversity using a range of parameters, it is possible to detect which sites are ranked consistently. Several authors outline appropriate methods (Renyi, 1961, Hill, 1973, Tothmeresz, 1995). The method proposed by Renyi (1961) is expressed as:

Equation 3.9 Diversity ordering

$$\mathrm{H}\alpha\ (\mathrm{p1}, \mathrm{p2}, \ldots, \mathrm{pn}) = \left(log \sum_{i=1}^{S} p_{i}^{\alpha} \right) / (1-\alpha)$$

Where α = the entropy of order (α≥0, α≠1), *S* = species richness, and *p* = the proportional abundance of the *i*th species.

Where diversity ordering shows that sites are not ranked consistently the use of species diversity measures is not appropriate and instead species richness measures should be used. At the most basic level, species richness can be described as the total number of species in a community. However this is difficult to achieve in the field, as increased sample effort will almost always lead to an increase in the number of species recorded. This point is well highlighted by Connor and Simberloff (1978), who showed that “the number of botanical collecting trips to each of the Galápagos Islands is a better predictor of species numbers than are area, elevation, or isolation”. As such, when gathering species data by sampling, using the number of observed species will produce a negative bias in measuring species richness.

Statistical species richness estimators provide a means to overcome the shortcoming of using the number of observed species. An overwhelming number of measures are available (Colwell and Coddington, 1994), with little consensus of the most appropriate technique. Smith and Belle (1984) recommend use of the one such estimator, the Jackknife, for small samples and another, the Bootstrap, for larger samples. Unfortunately, no recommendation is made as to what may be a suitable point of division in the classification of small and large samples size, although Krebs (1999) recommends that 100 quadrats is a reasonable point. In a comparative test of the measures Colwell and Coddington (1994) report the Chao 2 and second-order Jackknife techniques as remarkably accurate with small samples. Walther and Martin (2001) found the Chao and the Jackknife estimators to be the least biased and more precise, with Palmer (1990) reporting that better estimates were obtained from Jackknife compared to Bootstrapping methods.

Equation 3.10 Second-order Jackknife

$$\boldsymbol{J_2 = S_{obs} + \left[\frac{Q_1(2m-3)}{m} - \frac{Q_2(m-2)^2}{m(m-1)}\right]}$$

where S_{obs} = the number of species observed at the site; Q_1 = the number of species that occur in one quadrat only (unique species); Q_2 = the number of species that occur in precisely two quadrats; and m = the total number of quadrats sampled.

Species evenness was measured using the Simpsons measure of evenness, which is calculated as:

Equation 3.11 Simpsons measure of species evenness

$$E_{1/D} = \frac{1/D}{S}$$

Where D = Simpson’s index of diversity, and S = the number of species in the sample.

Two further measures addressed the within site spatial variation in species richness. Namely, within site alpha diversity and beta diversity were measured after Partel et al. (2001). Specifically mean site alpha diversity was calculated as the mean species richness across all quadrats on a site-by-site basis. Within site beta diversity examined the level of similarity in species richness values between all quadrats on a site-by-site basis. As such, within site beta diversity values reflected the evenness of the distribution of species composition across a site. Within site beta diversity was calculated using 1-Sorensen’s similarity index (Equation 3.12). The Sorensen’s similarity index was selected as the most appropriate similarity/dissimilarity index due to the increased weighting of the number of joint occurrences in its calculation making this index preferable for sample surveys where the true number of species may not have been recorded (Krebs, 1999).

Equation 3.12 Sorensen's similarity index formula

$$S = 1 - \frac{2A}{2A + B + C}$$

> Where A= the number of species present in both quadrats, B = the number of species present in quadrat B, and C = the number of species present in quadrat C.

These two measurements add complexity to the species richness measure, by analysing the variation in species richness across each site.

3.9.3 Species composition

Whilst species richness and diversity provide important information about the ecological characteristics of an area of study, species composition offers different information and its analysis can therefore provide a deeper understanding than species richness and diversity alone. A weakness with using measures of species richness/diversity to quantify diversity can be illustrated using an example of a site which has two closely related species and comparing it to a site with two distantly related species. Species richness/diversity measures will calculate the sites to be equally diverse, which does not seem intuitive. Diversity is not comprised only of the number of species, but also the identity of those species (Jennings et al., 2008). Unlike species richness and diversity, species composition cannot be represented by a single value.

Moreover, a common criticism of species diversity indices is that they lose so much of the original species information by condensing it into a single number (Krebs, 1999). It was this obstacle that led to the development of dominance-diversity curves (Whittaker, 1965). Dominance-diversity curves plot proportional abundance of species in a sample on a log scale against species rank to produce a curve that can describe the evenness and relative dominance of species in a sample. Moreover dominance-diversity curves can be used to identify the most dominant, and the rare species within a sample. Dominance-diversity curves can also be used to complement species richness values, which do not incorporate dominance calculations. An impression of the ecological condition of a site can be gleaned from the abundance of particular categories of species. Of particular relevance to calcareous grassland condition is information on the abundance of forb, grass, calcicole, ancient site indicator, positive indicator, and agricultural/negative indicator species. Identification of core and rare species and their relative abundance will also be of value.

Throughout this research species composition was measured in two ways. At each site the abundance of every recorded species was complied. This information formed the matrix of dependent variables in the redundancy analysis carried out in Chapter 5. Redundancy analysis allows for the analysis of relationships between a matrix of dependent variables, in this case species composition, with a matrix of independent variables, such as landscape variables.

The second way in which species composition was analysed was to measure the frequencies of dominant, characteristic, and core species at each site. Age of site indicator species are after Gibson and Brown (1991) and Karlik and Poschlod (2009). Calcicole species were identified after Ellenberg indicator values (Ellenberg et al., 1991), with strong calcicoles identified as species with F (Moisture) ≤4, R (pH) ≥7, N (Nitrogen) ≤ 4. Ellenberg indicator values for the species recorded were taken from Hill et al. (1999). Positive and agricultural/negative site indicators are after JNCC (2004). Species were classified as core (≥75% frequency), intermediate (>25%<75%), or rare (≤25%) based upon their frequencies across the twelve study sites. A full list of these species is provided in the appendix (Table A.9.2).

3.9.4 Genetic diversity

3.9.4.1 Isolation by distance

Gene flow can occur primarily between populations with a close proximity, resulting in genetic diversity increasing with distance between sites, known as isolation by distance (Wright, 1943). However under certain conditions gene flow can be independent of geographical distance and may be driven by other processes. In order to measure for this effect, isolation by distance was calculated using the method detailed by (Nei, 1972) (Equation 3.13).

Equation 3.13. Isolation by distance (Nei, 1972)

$$D = -\ln\frac{J_{XY}}{\sqrt{J_{XX}J_{YY}}}$$

> where $J_{XY} = \sum_{i=1}^{m}\sum_{j=1}^{r} x_{ij}y_{ij}/r$, $J_{XX} = \sum_{i=1}^{m}\sum_{j=1}^{r} x_{ij}^{2}y_{ij}/r$, x_{ij} is the frequency of the ith allele at the jth locus in population X, and y_{ij} is the frequency of the ith allele at the jth locus in population Y.

3.9.4.2 Genetic variability

Genetic variation was measured using expected heterozygosity (H_e) and observed heterozygosity (H_o). The Hardy-Weinberg H_e was calculated as the probability that two randomly selected alleles at a given locus within a population were different. Mean H_e was then calculated across all loci. H_o was estimated as the share of heterozygous genotypes in the overall pool of genotypes. Additionally inbreeding was measured using F-statistics (Wright, 1931). F_{IS} was used to measure the amount of inbreeding of individuals within sub-populations (Equation 3.14). F_{ST} was used to measure the amount of inbreeding within sub-populations in relation to the total population (Equation 3.15).

Equation 3.14. F_{IS}

$$\boldsymbol{F_{IS} = 1 - \frac{H_o}{H_e}}$$

> Where H_o = the observed heterozygosity, and H_e = the expected heterozygosity (e.g. the Hardy-Weinberg expectation).

Equation 3.15. F_{ST}

$$F_{ST} = \frac{var(p)}{p(1-p)}$$

Where var(p) = the variance in allele frequency *p* measured across all sub-populations; p(1-p) = maximum variance possible.

4. Habitat diversity and structure: contemporary and historical landscape patterns

4.1 Preamble

Firstly, this chapter focuses on modelling the historical landscape of the SDNP, with an approach for constructing comparable land-use/land-cover maps using data from different temporal periods described and validated. The modelled landscapes will then be used within the second focus of the chapter: the analysis of the habitat diversity and structure. Specifically there will be analysis of the historical and contemporary habitat diversity and structure, and temporal change of the SDNP at landscape and habitat scales. This habitat data will be carried forward into the analysis of species and genetic diversity.

4.2 Introduction

4.2.1 Landscape change in Britain

Research into landscape change in Britain has found that semi-natural habitat types were subject to substantial declines in extent during the 20th century (Fuller, 1987, Burnside et al., 2003, Hooftman and Bullock, 2012) (Table 2.1). A recent study by Hooftman and Bullock (2012) analysed change in calcareous grassland extent in Dorset over nearly seventy years, reporting a decline of greater than 80%. Similarly, other studies detecting change over periods of more than fifty years report declines of 43% of chalk grassland in England and Wales (Swetnam, 2007b), and 92% of unimproved lowland grassland in England and Wales (Fuller, 1987). In particular semi-natural habitat classes have most typically been converted to agricultural land. Burnside et al. (2003) found that between 1971 and 1981 grassland in the West Sussex Downs had a transition probability of 0.48 to remain as grassland and 0.40 to be converted to arable land, whilst for the period 1981 to 1991 it was more likely to have been converted to arable (transition probability 0.39) than to have remained as grassland (probability 0.36). Similarly Hooftman and Bullock (2012) found that between the 1930s and 2000, transition probabilities from managed calcareous grassland to arable land was 0.49, and to remain as calcareous grassland 0.03. Much of this change is thought to have occurred in the period immediately after World War II, when under the plough-up policies grassland was converted to arable land in the drive for self-sufficiency. Government subsidies under the

1947 Agriculture Act encouraged the ploughing up, fencing, and draining of vast areas of unspoilt grassland (Duffey et al., 1974, Ratcliffe, 1977, Hindmarch and Pienkowski, 2000, Leonard, 2007).

As a result of these changes the affected habitats have become smaller in extent, more isolated, and increasingly fragmented (Burnside et al., 2003, Hooftman and Bullock, 2012). Hooftman and Bullock (2012) found that between the 1930s and 2000, the mean size of managed calcareous grassland patches had decreased from 40.4 hectares to 2.5 hectares. Interestingly the reverse was found by Burnside et al (2003), who report an increase of mean patch size of unimproved grasslands on the West Sussex Downs from 5.8 ha in 1971 to 8.7 ha in 1981, perhaps as a result of the number of patches decreasing from 815 patches in 1971 to 227 patches in 1991. Between the 1930s and 2000 the mean distance of managed calcareous grassland patches to a large (>5ha) patch of the same habitat increased from 82m to 465m (Hooftman and Bullock, 2012). Similarly, Burnside et al. (2003) report an increased nearest neighbour distance from 187m in 1971 to 341m in 1991.

4.2.2 The historical landscape and landscape change

Research into important ecological processes, such as loss of biodiversity, often requires the analysis of change over time (Johnston, 1998). Indeed, the relationship between the spatial structure of landscape elements and biodiversity has been a major area of ecological research since the proposals within the island model of population genetics (Wright, 1940) and the Theory of Island Biogeography (MacArthur and Wilson, 1967), that smaller and more isolated islands have lower genetic diversity and species diversity than larger and more connected islands. Based on the principles outlined in these theories, the relationship between contemporary landscape structure and contemporary biodiversity continues to be a popular research theme. However, the failure to find relationships between the contemporary landscape and biodiversity has led to recent research investigating other factors to explain biodiversity. In particular the relationship between the historical landscape and biodiversity has received increasing research attention (Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins and Eriksson, 2008).

Such research has typically focused on the species level of biodiversity, with contemporary species patterns shown to be influenced by historical area (Helm et al., 2006), historical connectivity (Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins and Eriksson, 2008), site age and historical management (Partel and Zobel, 1999, Cousins and Eriksson, 2002, Gustavsson et al., 2007), and the historical amount of grassland in the area surrounding the patch (Reitalu et al., 2012). However, other studies have found no relationship between species diversity in grasslands and the historical land-use of a site (Bruun et al., 2001), historical area (Adriaens et al., 2006), and historical connectivity (Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007). Similarly, at the genetic level, genetic diversity can be influenced by historical site connectivity (Munzbergova et al., 2013) and site age (Jacquemyn et al., 2004, Prentice et al., 2006).

In addition to the consideration of the static historical and contemporary landscapes, temporal change in landscape structure also impacts upon biodiversity. Landscape change and its associated causes and consequences are key research topics in landscape ecology around the world (Wu and Hobbs, 2002, Hobbs and Wu, 2007). Indeed temporal change in landscape structure, and in particular the loss, fragmentation, and isolation of habitats, are amongst the most important causes of global species extinction and biodiversity loss (Wilcox and Murphy, 1985, Pimm and Raven, 2000, Sala et al., 2000, Henle et al., 2004, Dauber et al., 2006, Farina, 2006).

4.2.2.1 Modelling the historical landscape and landscape change

As detailed in the previous section, the inclusion of historical landscape factors is an important component of research into biodiversity. Landscape pattern analysis (LPA) is used to assess changes in landscape configuration over time (Turner et al., 2001, Farina, 2006), and has been used to successfully document the fragmentation of a range of landscape types (Herold et al., 2002, Turner et al., 2003, Deng et al., 2009, Hooftman and Bullock, 2012). Landscape pattern metrics can be calculated for different time periods, and to analyse temporal change (Dunn et al., 1991). Long-term LPA has been limited as a result of the intermittent nature of historical records, limited data availability, and inconsistencies between data from different surveys. The lack of consistent data over time is paramount, with (Johnston, 1998) p. 120) commenting that data used in landscape change analysis needs to be mapped "*perfectly and consistently*". If these stipulations are not met, factors unrelated to landscape change, such as differences in the scale, extent, classification, or generalisation of the data, may erroneously be detected as landscape change. There are two dimensions to data inconsistencies: the form of the data (thematic differences); and the scale of the data (spatial differences) (Moody and Woodcock, 1994, Wickham and Ritters, 1995, O'Neill et al., 1996).

Thematic differences are rooted in the method and purpose of original data collection. There has been no long-standing standardised method for classifying LULC survey data in Britain, an issue that the Phase 1 Habitat guidelines (JNCC, 2010) attempt to address. As a consequence each LULC survey tends to use a unique classification system, making comparisons between two different surveys challenging. Moreover, thematic differences can stem from the format of the data, which can be in qualitative form as historical maps, survey data, and aerial photography, and in quantitative form as remotely sensed imagery (Lwin et al., 2012). This lack of a consistent method for recording LULC is a major limitation in our ability to monitor and understand change in LULC over time. The Land Cover Maps, produced by the Centre for Ecology & Hydrology, serve as a useful example of the inherent nature of thematic inconsistencies. The Land Cover Maps project mapped land-cover for the whole of Britain in 1990, 2000, and 2007. However, even though they were produced by the same organisation, using data collected by satellite, the maps are not readily comparable as different classification systems were used. Thematic integration methods can minimise the problems of data inconsistencies by reclassifying different data-sets into a common classification system (Jansen and Gregorio, 2002). Turner et al. (2001) comment that the choice of classification is critical and should be consistent with the aim of the analysis. Moreover, it is important to balance the desire to examine data as far back as possible historically, against the resolution of the analysis (Petit and Lambin, 2002).

In addition to being thematically equivalent, for the purpose of comparison LULC data also needs to correspond spatially (Congalton and Green, 2009). Spatial differences are grounded in the generalisations made to produce the data. Data generalisation, the process where features are "*reduced and/or modified in terms of their size, shape and numbers*" (Balodis, 1988) (p.71) is "*an inherent characteristic of all geographical data. All maps, whether digital or analogue, are generalised representations of reality*" (Joao, 1998) (p.1). Therefore problems are inherent when making comparisons with data published at different scales or resolutions. In comparing the differences between identical features mapped at scales of 1:10,000 and 1:50,000, Joao (1998) found that the measurement of a road length altered by between +6% and -8.5% compared to the original data, highlighting the significance of spatial inconsistencies. In the same study features were displaced by up to 34.6 metres. Similarly, Moody and Woodcock (1994) discovered that changing the grain size of their LULC data from 30m to 1km affected several landscape metrics including patch size, patch density, and landscape diversity. Increasing grain size potentially results in rarer LULC patches being under-represented and small LULC patches being lost. Where data-sets have different scales, spatial integration methods can be used to identify the most suitable spatial scale to transform the different data-sets.

Once both thematic and spatial data integration has been completed, the output data-sets will have the same classification system and spatial scale. It is then possible to carry out change detection analysis. The process of landscape change detection has been enhanced by the advancement of GIS. LULC data for multi-temporal periods can be stored and analysed within a GIS database, allowing for the comparison of LULC change across the entire population of interest.

It is perhaps a result of these difficulties and challenges in measuring long-term landscape change, that the majority of European temporal landscape change studies have focused on periods of less than thirty years (Keymer and Leach, 1990, Burnside et al., 2003, Haines-Young et al., 2003, Howard et al., 2003).

4.2.3 Habitat diversity and landscape structure

4.2.3.1 Habitat diversity

Habitat diversity, the variety of habitats within a landscape of interest, is an important component of biodiversity. However research typically uses species diversity as a proxy for biodiversity, and subsequently research into habitat diversity is surprisingly scarce. Moreover, the majority of research into habitat diversity has focused on its relationship with species diversity, rather than analysing it as an individual concept. Research into the relationship between landscape structure and habitat diversity has been absent, and in particular little is known about how historical landscape structure and temporal landscape change influence contemporary habitat diversity. This is particularly surprising given the recent research emphasis on the relationship between biodiversity and the historical landscape (Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins and Eriksson, 2008).

Measuring both the contemporary and the historical habitat diversity will allow for analysis of the change in habitat diversity over time, and allow for subsequent analysis of the relationship between habitat diversity and diversity at the species and genetic level.

4.2.3.2 Landscape structure

There are two components to landscape structure: landscape composition and landscape configuration. Landscape composition refers to the make-up of LULC classes within a landscape. Changes in landscape composition results from changes in the variety and abundance of LULC types, and concerns the conversion of land from one LULC class into another. Landscape configuration refers to the spatial arrangement of LULC classes within a landscape. Change in landscape configuration results from changes in the spatial character, arrangement, position, and orientation of landscape elements (McGarigal, 2015). It occurs at patch level (e.g. the shape of an individual habitat patch), class level (e.g. the connectivity of habitat patches), and landscape level (e.g. the arrangement of LULC classes in relation to one another throughout the landscape). While landscape composition and configuration are independent processes they often work in tandem, with the effects of landscape composition change often exacerbated by simultaneous changes to landscape configuration.

Whilst information about patterns in the landscape may be the focus of landscape configuration measures, this information is not what drives most research that uses them. The aim of spatial analysis is to analyse the ecological processes that the landscape metrics drive and to detect ecological properties of the landscape. However, several authors argue that at present our ability to measure landscape configuration outweighs our understanding of its effects on ecological processes (Dramstad et al., 1998, Gustafson, 1998, Turner et al., 2001, Li and Wu, 2004, Kupfer, 2012). Thus the choice of metrics used in analysis should be directed by the aims of the analysis (Turner et al., 2001).

4.2.4 Historical habitat surveys in Britain

The Doomsday Book of 1086 could be considered as the first attempt at a comprehensive survey of landscape composition in Great Britain. However, the first broad scale surveys were the Land Utilisation Survey of Britain (LUSB) in the 1930s (Stamp 1947), and the Second Land Use Survey in the 1960s (Coleman, 1961). The LUSB was directed by L. Dudley Stamp, surveying the whole of the country to classify every parcel of land designated on Ordnance Survey 1:10560 scale maps from the 1890s. The surveys were completed by an estimated 250,000 children from 10,000 schools, identifying nine main types of land utilisation (Stamp, 1934). One of the major difficulties in measuring landscape change using the LUSB stems from its classification of grasslands. In the LUSB, grasslands were classified as either ‘heath and moorland’ or ‘meadow and permanent grass’, which contrasts the more contemporary classification of acidic grasslands, calcareous grasslands, mesotrophic grasslands, and heathlands. To this end, methods of modelling data from the LUSB into contemporary classifications have been developed (Swetnam, 2007b, Hooftman and Bullock, 2012). However, neither of these studies carried out a full validation of all LULC types using independent LULC data. Swetnam (2007b) validated the method using a basic quality assurance (detailed in (Swetnam, 2007a), whereas Hooftman and Bullock (2012) compared their modelled 1930s landscape to vegetation surveys from the same period.

More recently Phase 1 habitat surveys have been carried out at county scale. The Phase 1 classification system was developed by the Nature Conservancy Council through the 1970s, 80s, and 90s, as a standardised system to record semi-natural vegetation and other wildlife habitats as part of large-scale surveys. Phase 1 surveys continue to be carried out at county level, with the technique also used to classify habitats in desk based aerial photography projects (West Sussex County Council, 1996).

4.2.5 Study aims

The two major aims of this chapter are (1) to produce an independently validated model of the historical landscape of the SDNP, and (2) to assess contemporary, historical, and temporal change in habitat diversity and landscape structure. This will facilitate the study of the relationship between historical landscape structure and both species and genetic diversity (Chapters 5 & 6), and will enable the discussion of the relationship between habitat diversity with other levels of biodiversity (Chapter 7).

4.3 Methods

4.3.1 Data

LULC data from contemporary and historical periods were used. LULC data for 2012 was provided by Hampshire Biodiversity Information Centre (HBIC) and Sussex Biodiversity Record Centre (SBRC). For the historical LULC, data from the LUSB was used to model the 1930s landscape. Digitised versions of the original LUSB paper maps were provided by the Environment Agency. For the thematic integration of LULC data (see section 4.3.2), soil data was provided by the National Soil Resources Institute (NSRI) in the form of the NATMAP Soilscapes dataset.

4.3.2 Historical landscape modelling

Given the limitations of temporal landscape change analysis (see Chapter 2), pre-processing of LULC data is essential (Petit and Lambin, 2002). Data integration techniques have been developed to minimise differences between data. However, no standardised approach has been established, with the optimal method dependent upon the aim of the research, and the quality and quantity of the data (Gennaretti et al., 2011).

4.3.2.1 Thematic integration

The first stage in the modelling was thematic integration. The aim of this step was to re-organise the two data-sets so that they contained equivalent and corresponding LULC classifications. Particular attention was focused on one of the areas of biggest discrepancy between the data-sets, the classification of grasslands. The LUSB classified grassland areas into two broad groups: heath and moorland; or meadow and permanent grass. Within the HBIC and SBRC data-sets, four specific types of grassland are detailed: acid grassland, calcareous grassland, mesotrophic grassland, and heathland. Thus the first step of the modelling was to translate the LUSB grassland classes into the four classes of the HBIC and SBRC data (Figure 4.1). To achieve this the modelling methods of Swetnam (2007b) and Hooftman and Bullock (2012) were adapted. The grassland classes of the LUSB were integrated with NSRI Soilscapes data to translate them into the four contemporary classes (Table 4.1). For the remaining LULC classes, thematic integration was carried out in line with the methods outlined by Petit and Lambin (2001) and Petit and Lambin (2002). The revised LUSB data was used as the template layer for which the HBIC and SBRC habitat data-sets were integrated into.

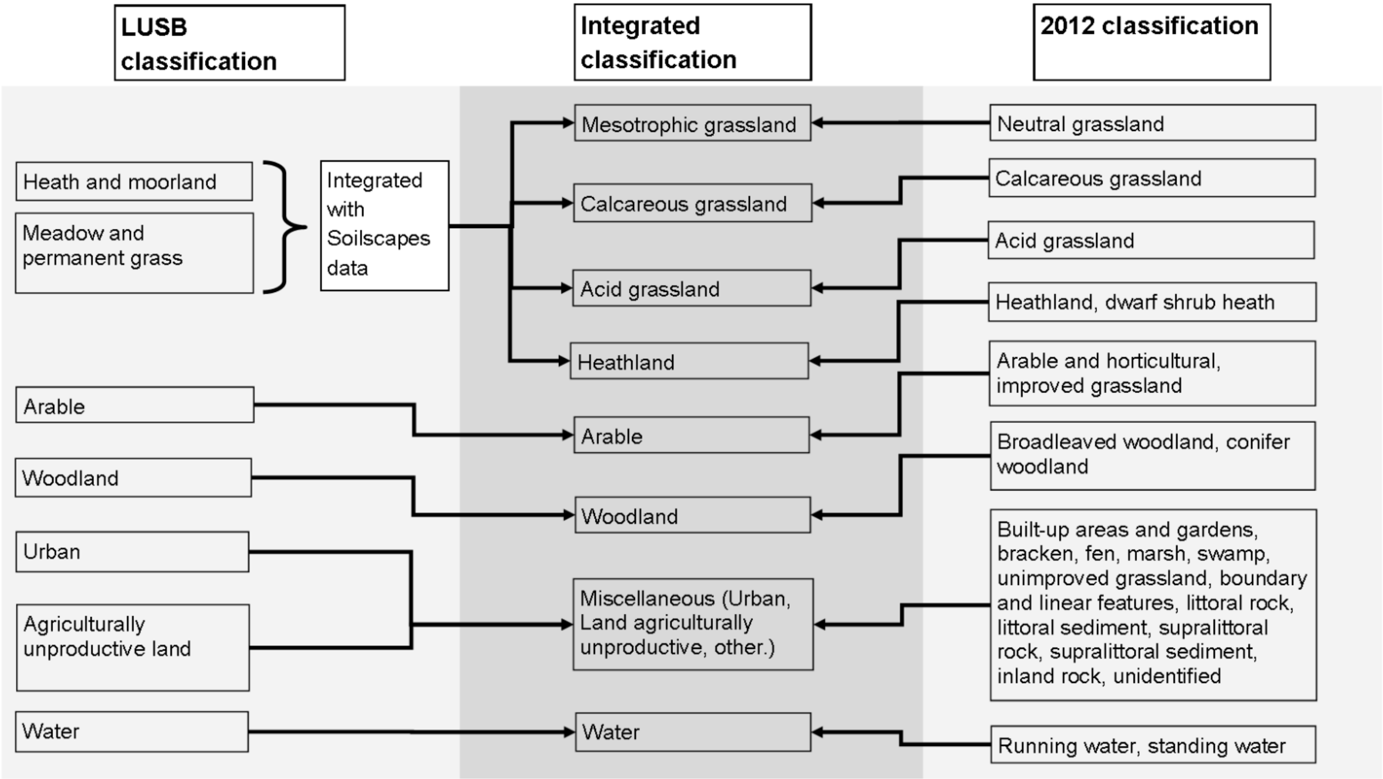


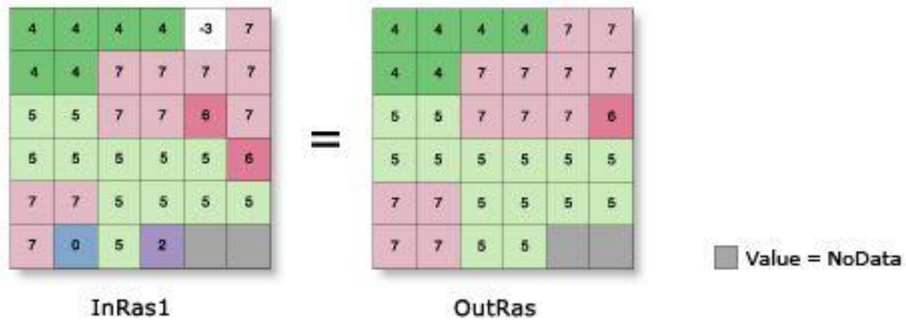
Figure 4.1. Thematic integration of the habitat data. Following this step data from both the 1930s (left section) and 2012 (right section) were classified into eight identical classes (middle section).

Table 4.1 Criteria used to translate broad grassland categories into contemporary grassland classifications. Soilscapes data was combined with LUSB classifications to determine the contemporary LULC type listed in the table. Adapted from Hooftman and Bullock (2012).

LUSB category / Soilscapes category	Heath and moorland	Meadow and permanent grass
Fen peat soils	Other	Other
Freely draining lime-rich loamy soils	Calcareous	Calcareous
Freely draining slightly acid but base-rich soils	Mesotrophic	Mesotrophic
Freely draining slightly acid loamy soils	Mesotrophic	Mesotrophic
Freely draining slightly acid sandy soils	Heathland	Acid
Freely draining very acid sandy and loamy soils	Heathland	Acid
Loamy and clayey floodplain soils with naturally high groundwater	Mesotrophic	Mesotrophic
Loamy and clayey soils of coastal flats with naturally high groundwater	Mesotrophic	Mesotrophic
Loamy soils with naturally high groundwater	Acid	Acid
Naturally wet very acid sandy and loamy soils	Heathland	Acid
Sand dune soils	Other	Other
Shallow lime-rich soils over chalk or limestone	Calcareous	Calcareous
Slightly acid loamy and clayey soils with impeded drainage	Mesotrophic	Mesotrophic
Slowly permeable seasonally wet acid loamy and clayey soils	Heathland	Acid
Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils	Mesotrophic	Mesotrophic
Sea	Water	Water
Water	Water	Water

4.3.2.2 Spatial integration

Spatial integration was carried out to ensure that data from both sources were set to the same resolutions, thereby reducing the effects of discrepancies between different data-sets. Spatial integration was carried out using the technique developed by Petit and Lambin (2001) and Petit and Lambin (2002). Based on the principles of Riitters et al. (1995), a template data-set is identified, commonly the data with the coarsest resolution. Other data-sets are then generalised to the resolution of the template data, and an incremental range of larger resolutions. Next, five landscape metrics (Landscape shape index, Shannon's diversity index, the mean patch fractal dimension, the total core area index, and the total edge contrast index) are used to identify the resolution at which the landscape structure is most similar between the template data and the generalised data. Using this method the data-sets were generalised to a resolution equal to that of the finest data-set, in this case the 10m resolution of the LUSB data, and then progressively aggregated to 50m resolutions (later referred to as generalised maps) using the majority filter tool within the Spatial Analyst toolbar of ArcMap 10.1 (ESRI, 2012). Majority filtering is an iterative technique that replaces cells based on the majority of contiguous neighbouring cells (Figure 4.2).



4	4	4	4	-3	7
4	4	7	7	7	7
5	5	7	7	8	7
5	5	5	5	5	6
7	7	5	5	5	5
7	0	5	2		

InRas1

=

4	4	4	4	7	7
4	4	7	7	7	7
5	5	7	7	7	6
5	5	5	5	5	5
7	7	5	5	5	5
7	7	5	5		

OutRas

Figure 4.2 The majority filter method for generalisation. In this figure majority filter is applied to the input raster using the four orthogonal neighbouring cells. Where the majority of these neighbouring cells is the same, the value of the cell is re-classified. Reproduced from ESRI (2012a).

The spatial structure of the generalised maps was measured using five metrics (landscape shape index, Shannon's diversity index, mean patch fractal dimension, total core area index, and total edge contrast index). These metrics have been identified as non-redundant metrics in representing landscape structure (Riitters et al., 1995, Petit and Lambin, 2002). The Euclidean normalised distance between the target map and each generalised map was then calculated in turn. The resolution producing the minimum mean Euclidean normalised distance between metrics (Equation 4.1) was selected as the optimal resolution.

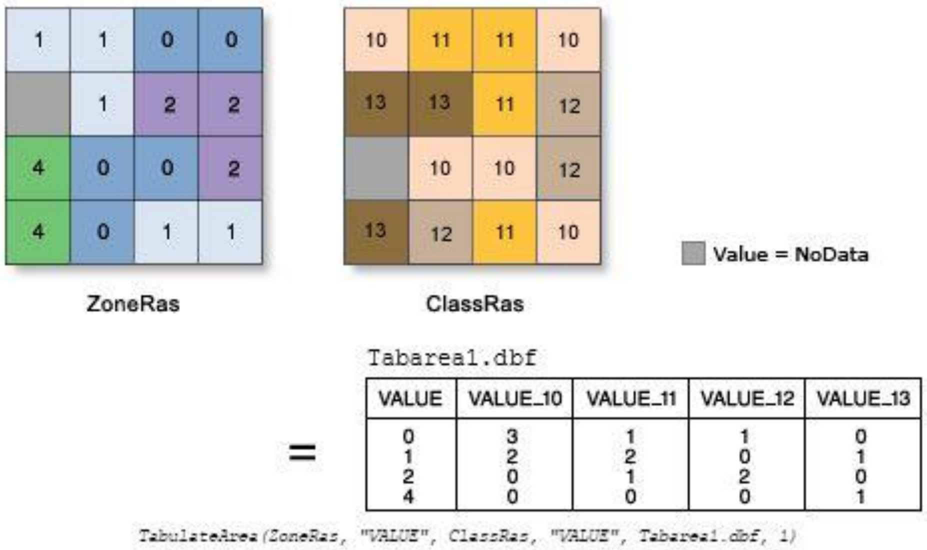
Equation 4.1. The Euclidean normalised distance between metrics

$$d_{st} = \sqrt{\left(\frac{a_Y - a_Z}{s_a}\right)^2 + \left(\frac{b_Y - b_Z}{s_b}\right)^2 + \left(\frac{c_Y - c_Z}{s_c}\right)^2 + \left(\frac{d_Y - d_Z}{s_d}\right)^2 + \left(\frac{e_Y - e_Z}{s_e}\right)^2}$$

Where a = Mean shape index, b = Shannon's diversity index, c = Mean patch fractal dimension, d = Core area index, e = total edge contrast index, Y = target map, Z = generalised map, and s = standard deviation.

4.3.2.3 Model validation

To validate the grassland modelling, the four grassland classes of the HBIC and SBRC habitat data-sets were first transformed into the original two LUSB grassland classes (heath and moorland, meadow and permanent grass). The modelling method was then carried out on the transformed data, producing a new version of HBIC and SBRC data-set. Two versions of the HBIC and SBRC data-sets were then available: one with the original LULC classifications, and one with the modelled classifications. The Tabulate Area function within the Spatial Analyst toolbox of ArcMap 10.1 (ESRI, 2012) was used to compare the original classifications with the modelled ones. Tabulate Area seeks to calculate cross-tabulated areas between two data-sets (Figure 4.3). As such it is possible to detect the transition of classifications from the original data to the modelled data.



1	1	0	0
▨	1	2	2
4	0	0	2
4	0	1	1

ZoneRas

10	11	11	10
13	13	11	12
▨	10	10	12
13	12	11	10

ClassRas

▨ Value = NoData

=

Tabarea1.dbf

VALUE	VALUE_10	VALUE_11	VALUE_12	VALUE_13
0	3	1	1	0
1	2	2	0	1
2	0	1	2	0
4	0	0	0	1

```
TabulateArea(ZoneRas, "VALUE", ClassRas, "VALUE", Tabarea1.dbf, 1)
```

Figure 4.3 The tabulate area method. Cross-tabulated areas are calculated between two data-sets to produce an output table. In this figure of the cells with a value of 0 in the ZoneRas data, have values of 10 (3 cells), 11 (1 cell), or 12 (1 cell) in the ClassRas data. Reproduced from ESRI (2012b)

To validate the modelling at a broader level, Kappa coefficients were calculated to measure the agreement between the original and modelled data. The Kappa coefficient (Equation 4.2) measures inter-rater agreement, which takes agreement by chance into consideration.

Equation 4.2. The Kappa coefficient

$$k = \frac{\mathrm{Pr}(a) - \mathrm{Pr}(e)}{1 - \mathrm{Pr}(e)}$$

Where Pr(a) = the observed agreement, and Pr(e) = the chance agreement.

4.3.3 Habitat diversity and landscape structure

4.3.3.1 Habitat diversity

Historical and contemporary habitat diversity was measured using the Shannon index of diversity (SHDI, Equation 3.8) (Shannon and Weaver, 1949). The SHDI was selected as it is recommended for landscape management within an ecological framework (Nagendra, 2002) and has been used in a similar study by Krauss et al. (2004). SHDI was calculated at two scales, within 1km and 5km radii. These scales were selected to incorporate the dispersal range of the study species and are similar to those used by Krauss et al. (2004). Additionally, habitat diversity was measured at the patch scale. For each study site habitat diversity was measured at two scales: a 1km buffer; and a 5km buffer. This analysis was carried out for both the 1930s and the 2012 extent of the sites.

4.3.3.2 Landscape structure

Historical and contemporary landscape composition and configuration were measured using a range of metrics (Table 4.2). Full definitions of these metrics are provided in Chapter 3.

Table 4.2 Metrics used to measure landscape composition and configuration

Landscape composition	Landscape configuration
Area (Ha) (AREA)	Number of patches (NP)
Percentage of landscape (%LAND)	Mean patch area (MPA)
	Core area index (CAI)
	Average nearest neighbour (m) (ANN)

To assess the nature of LULC change between the 1930s and 2012, transition probabilities were calculated using the tabulate area function within ArcMap 10.1 (ESRI, 2012). The transition probabilities measured the area of one LULC type that made the transition to another LULC type between the two periods of study. Each LULC type was interrogated to establish the area of land that made the transition from one LULC type to another. These area values were then used to calculate transition probabilities.

4.3.3.3 Patch level structure

In order to carry out analysis at the habitat scale, twelve calcareous grassland study sites were identified. The study sites were carefully selected from the breadth of the South Downs National Park, to include a range of abiotic, spatial, and management conditions (see Chapter 3). For each of the twelve study sites a range of metrics were calculated to measure site fragmentation between the 1930s and 2012. McGarigal et al. (2005) comment that there are five main spatial components to habitat loss and fragmentation: habitat extent, habitat subdivision, patch geometry, habitat isolation, and habitat connectedness. To this end appropriate metrics for each of these components were identified, with an additional measure of percentage change in patch size from the 1930s to 2012 (Table 4.3). Full definitions of these metrics are provided in Chapter 3

Table 4.3 Fragmentation metrics and abbreviations

Metric	Abbreviation
Patch extent (Ha) (1930s & 2012)	PE
Patch extent percentage change (1930-2012)	Habitat loss (%)
Subdivision (2012)	Sub
Core area index (1930s & 2012)	CAI
Connectivity (1930s & 2012)	Conn

4.4 Results

4.4.1 Historical landscape modelling

4.4.1.1 Thematic integration

The thematic integration resulted in eight comparable LULC types for each dataset (Figure 4.1). Of the original seven 1930s classifications, three remained unchanged (arable, woodland, and water). The separate 'urban' and 'agriculturally unproductive land' classifications were grouped into one new classification: miscellaneous. The two classifications 'heath and moorland' and 'meadow and permanent pasture' were integrated with the Soilscapes data to produce the four classifications: acid grassland, calcareous grassland, mesotrophic grassland, and heathland. The 2012 data originally contained twenty-four classifications, which were integrated into the eight study classifications. The major reclassification of the 2012 data was a suite of thirteen classes that were transferred into the miscellaneous classification (Figure 4.1).

4.4.1.2 Spatial integration

A resolution of 10 metres was found to be the optimal level of generalisation, as this was the resolution where the Euclidean normalised distance between metrics was the smallest (Table 4.4). Beyond this resolution the Euclidean normalised distance between metrics increased, meaning that the structure of the landscapes became less similar. Therefore, in relation to the variables of Equation 4.1, a 10 metre resolution was used for subsequent analysis.

Table 4.4 Distance in the five-dimensional space of the landscape metrics between the generalised maps and the target maps.

Resolution (metres)	Euclidean normalised distance between metrics
10	3.51
15	3.73
20	4.38
25	4.43
50	4.82

4.4.1.3 Model validation

An overall agreement of 83% was found between the original and the modelled grassland classifications. This translated to a Kappa coefficient of >72%, representing a substantial agreement (Landis and Koch, 1977). The modelling of calcareous grasslands was particularly successful, with 94% being correctly modelled (Table 4.5). The remaining 6% was modelled as mesotrophic grassland, with no calcareous grassland being modelled as acid grassland or heathland, and <0.01as other LULC types (Table 4.5). Similarly 99% of heathland was modelled as heathland, with the remaining 1% classified as a combination of mesotrophic grassland, calcareous grassland, and other LULC types (Table 4.5). Mesotrophic grasslands were correctly modelled for 68% of cells, with 16% modelled as calcareous grassland, 9% as other LULC types, 4% as heathland, and 2% as acid grassland (Table 4.5). The modelling was least successful for acid grasslands, where only 2% were correctly classified (Table 4.5). Acid grassland was typically classified as mesotrophic grassland (66%) or heathland (31%), with <1% classified as calcareous grassland or other LULC types (Table 4.5). However this result may be a factor of the scarcity of acid grasslands in 2012, when it represented only 0.2% of the region.

Table 4.5 Agreement between original and modelled land-use/land-cover classifications. Figures in rows represent what each modelled habitat is in actual habitat. Figures in columns represent what each actual habitat has been modelled as.

Modelled LULC / Actual LULC	Mesotrophic grassland	Calcareous grassland	Acid grassland	Heathland	Other
Mesotrophic grassland	**0.68**	0.16	0.02	0.05	0.09
Calcareous grassland	0.06	**0.94**	0.00	0.00	<0.01
Acid grassland	0.66	<0.01	**0.02**	0.31	<0.01
Heathland	<0.01	<0.01	0.00	**0.99**	<0.01

4.4.2 Habitat diversity and landscape structure

4.4.2.1 Habitat Diversity

Across the SDNP, the diversity of habitats as measured using the SHDI in the landscape decreased from 1.72 in the 1930s to 1.14 in 2012. At the patch level, within 1km buffers mean SHDI for all sites was significantly greater in the 1930s (t=2.69, p=0.02) (Table 4.6), with all twelve study sites having higher values in the 1930s (Table 4.6). Similarly at the 5km buffer scale, the mean SHDI for all sites was significantly different in the 1930s than 2012 (t=4.53, p<0.001), although one site (Beachy Head) had a higher value in 2012 (Table 4.6).

Table 4.6 Habitat diversity at study sites in both the 1930s and 2012

	SHDI 1930s 1km	SHDI 2012 1km	SHDI 1930s 5km	SHDI 2012 5km
Arundel Park	1.56	1.39	1.55	1.23
Beachy Head	1.23	1.23	1.30	1.33
Butser Hill	1.37	1.22	1.51	1.03
Castle Hill Complex	1.24	0.77	1.28	1.01
Cissbury Ring	1.17	0.76	1.22	0.93
Cradle Hill	1.29	1.21	1.37	1.22
Devil's Dyke	1.17	1.12	1.24	1.11
Harting Down West	1.38	1.19	1.69	0.98
Levin Down	1.32	0.85	1.50	1.01
Malling Down	1.49	1.47	1.41	1.33
Southwick Hill	1.17	0.91	1.24	1.06
Steep Down	1.17	0.53	1.22	0.88
Mean	1.30	1.05	1.38	1.09

4.4.2.2 Landscape structure

The modelled 1930s data presented a landscape with relative evenness between four main LULC types: calcareous grasslands, arable land, mesotrophic grasslands, and woodland (Table 4.7, Figure 4.4, Figure 4.5). The most dominant LULC type was calcareous grassland (29%, 47,200Ha). Together with arable land (22%, 36,200Ha), mesotrophic grasslands (20%, 33.500Ha), and woodland (18%, 34,600Ha), the four dominant habitats comprised 89% of the total area. By 2012 this had changed to a landscape dominated by arable land (61%, 100,700Ha) (Table 4.7, Figure 4.4, Figure 4.6). The amount of woodland increased to 21% of the area (34,675Ha). All grassland types became substantially reduced in extent, now comprising less than 6% of the total area, compared to 55% in the 1930s. In particular calcareous grasslands lost 88% of their 1930s extent.

For calcareous grasslands and heathland, the substantial decrease in extent did not coincide with a decrease in the number of habitat patches (Table 4.7). Calcareous grasslands lost nearly 88% of their 1930s extent, but the number of patches only fell by 15% (from 1036 to 882). Despite a loss of 81% of habitat area, the number of heathland patches increased by 62% (from 105 to 275). This and the substantial decrease in mean patch area of these habitats indicates that these habitats now remain as small fragment patches. Moreover all grassland types had a reduced core area index value, again indicating a reduced fragment size. An increased average nearest neighbour distance for acid grasslands, calcareous grasslands, and mesotrophic grasslands suggests connectivity to other patches of the same grassland type has decreased substantially.

Table 4.7 Spatial statistics for the 1930s and 2012 land-use/land-cover types across the SDNP

	Area (Ha) (% of total)		**Number of patches**		**Mean patch area**		**Percentage change in number of patches**	**Core area index**		**Average nearest neighbour (m)**	
	1930s	**2012**	**1930s**	**2012**	**1930s**	**2012**		**1930s**	**2012**	**1930s**	**2012**
Woodland	29,121 (17.8%)	34,674 (21%)	2727	12116	10.8	2.9	-73.1%	88.2	80.9	66.3	28.8
Arable	36,231 (21.8%)	100,686 (60.9%)	1892	3631	19	27.7	45.8%	91.1	91.3	94.7	21.3
Mesotrophic grassland	33,507 (20.3%)	2,932 (1.8%)	1766	1005	19	2.9	-84.7%	89.6	75	52.3	174.6
Calcareous grassland	47,190 (28.6%)	5,700 (3.7%)	1036	882	45.6	6.9	-84.9%	93.2	84.8	29.8	132.9
Acid grassland	6,012 (3.6%)	383 (0.2%)	631	183	9.5	2.1	-77.9%	86	81.9	62.7	614.2
Heathland	3,381 (2%)	647 (0.4%)	105	275	31.9	2.4	-92.5%	92.3	79	183.8	87.2
Miscellaneous	7,852 (4.7%)	19,259 (11.4%)	3132	42045	2.5	0.4	-84%	74.5	70.6	101.4	24.2
Water	1,970 (1.2%)	983 (0.6%)	734	18393	2.6	0.1	-96.2%	62.9	24.2	99.3	59.6

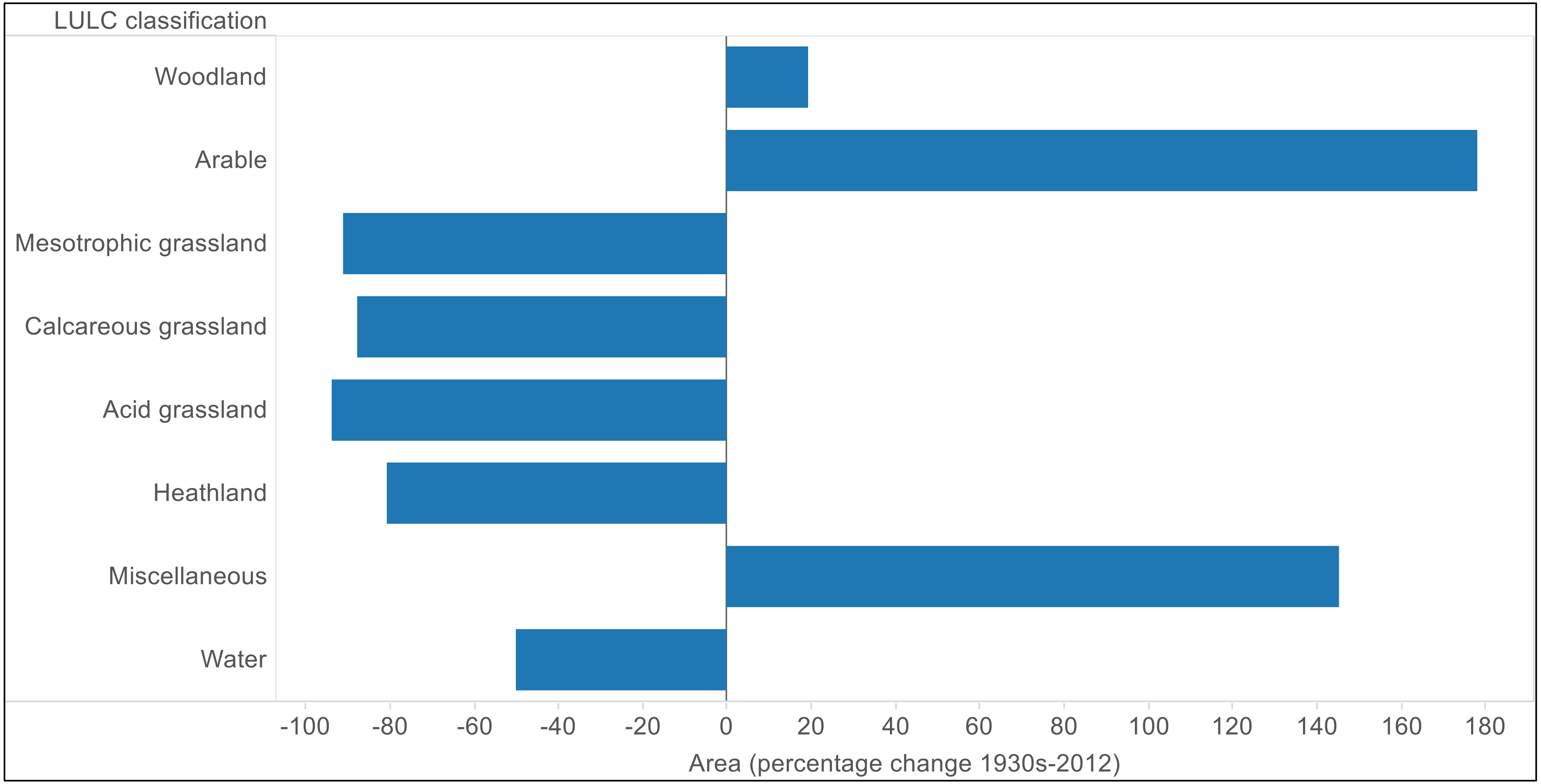


Figure 4.4 Percentage change in the area of land-use/land-cover types from the 1930s to 2012.

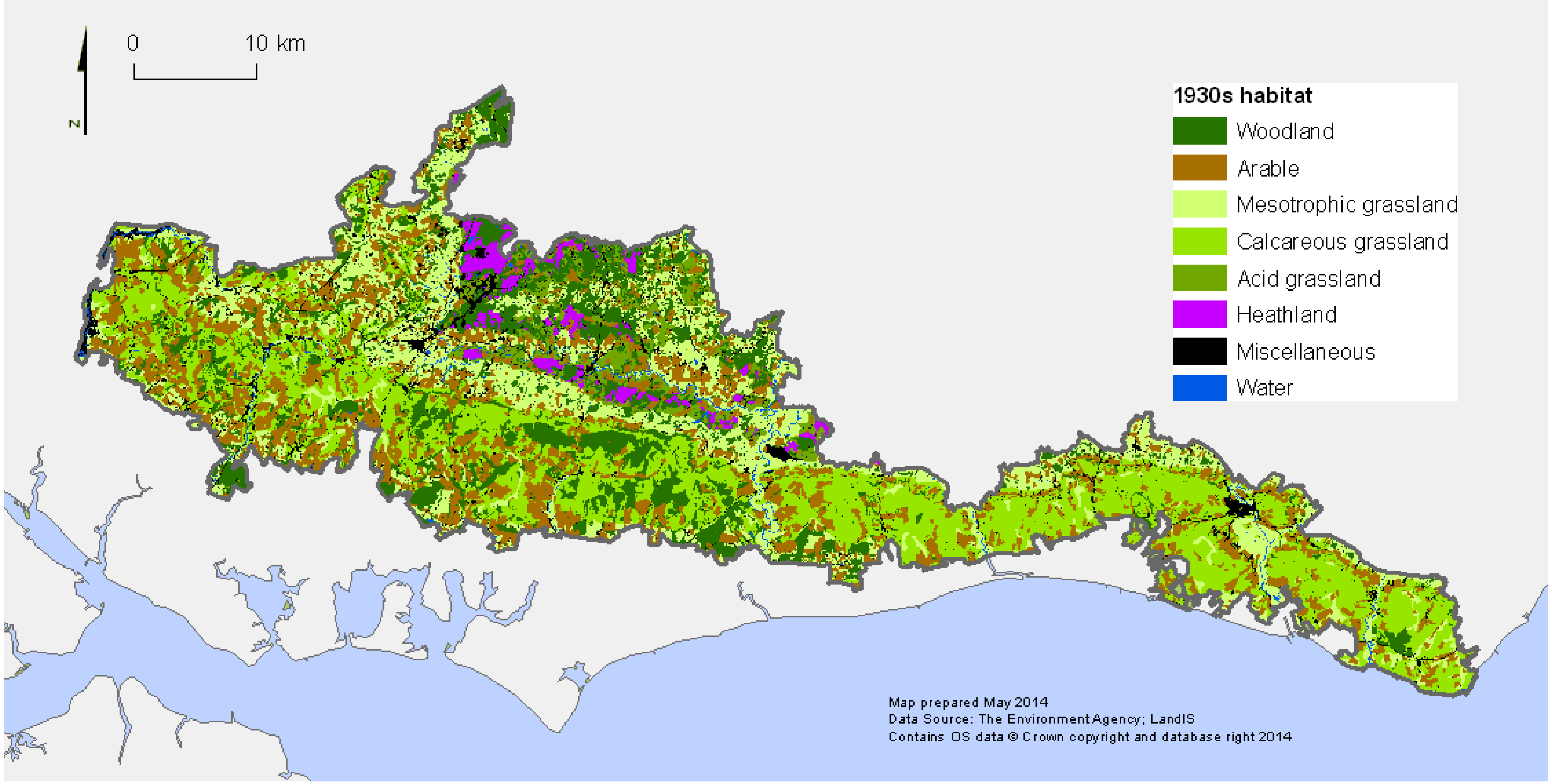
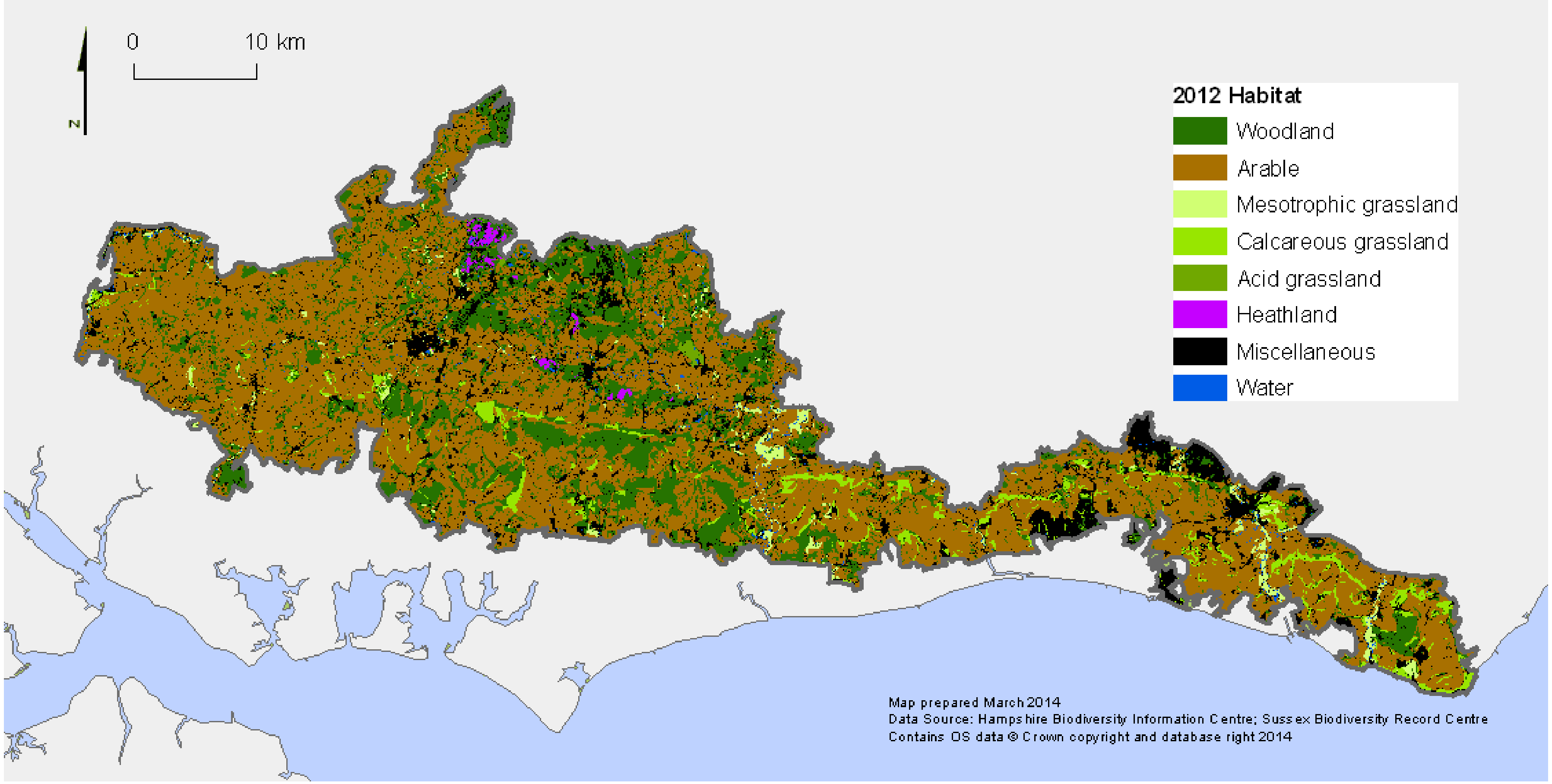


Figure 4.5 The modelled habitat of the SDNP in the 1930s. Original in colour.



Map prepared March 2014
Data Source: Hampshire Biodiversity Information Centre; Sussex Biodiversity Record Centre
Contains OS data © Crown copyright and database right 2014

Figure 4.6 The habitat of the SDNP in 2012. Original in colour.

Transition probabilities revealed that only woodland (transition probability 73%) and arable land (transition probability 87%) were more likely to have remained as the same LULC type than to have been converted (Table 4.8). The miscellaneous LULC types were equally likely to have remained miscellaneous or to have been converted (transition probability 50%). However, all the four grassland types were substantially more likely to have been converted than to have remained as the same LULC type. 1930s calcareous grassland was nearly seven times more likely to be arable land in 2012 than to have remained as calcareous grassland (transition probability 69%). Mesotrophic (transition probability 72%) and acid grasslands (transition probability 66%) were also largely converted to arable land, with heathland most commonly converted to woodland (transition probability 56%). The low transition probabilities from one grassland class to another between the 1930s and 2012, provides further validation of the modelling process. The largest such transition saw 2% of the modelled 1930s acid grasslands classified as mesotrophic grassland in 2012. The transition probability for water was surprisingly low (20%), however this is largely due to differences in resolution between the 1930s and the 2012 data. Water classifications were disproportionately affected by this issue due to its relatively small extent, and in particular small patch sizes. See section 4.2.2.1 for more discussion of the influence of scale.

Table 4.8 Transition probabilities across the SDNP (1930s-2012). Values in rows represent the transition probability values of the 1930s classifications in 2012. Values in columns represent what each 2012 classification was in the 1930s.

2012 1930s	Woodland	Arable	Mesotrophic grassland	Calcareous grassland	Acid grassland	Heathland	Miscellaneous	Water
Woodland	**0.73**	0.18	0.00	0.01	0.00	0.01	0.06	0.00
Arable	0.04	**0.87**	0.01	0.01	0.00	0.00	0.07	0.00
Mesotrophic grassland	0.08	0.72	**0.05**	0.01	0.01	0.00	0.13	0.01
Calcareous grassland	0.11	0.69	0.01	**0.10**	0.00	0.00	0.09	0.00
Acid grassland	0.15	0.66	0.02	0.00	**0.00**	0.00	0.16	0.01
Heathland	0.56	0.12	0.00	0.00	0.03	**0.13**	0.15	0.00
Miscellaneous	0.12	0.33	0.03	0.01	0.00	0.00	**0.50**	0.01
Water	0.12	0.31	0.12	0.00	0.00	0.01	0.24	**0.20**

4.4.2.3 Patch scale analysis

Between the 1930s and 2012 there was a decline in extent of all twelve study sites, with only two sites (Arundel Park and Beachy Head) maintaining more than 10% of their 1930s extent (Table 4.9, Figure 4.7). The site with the biggest loss in extent was Harting Down West, which was reduced by 3281 hectares to its 2012 extent of 33.4 hectares. The site with the smallest loss in extent was Arundel Park. This site was reduced in extent by 28 hectares, which due to its small size in the 1930s represents a 45% loss. With the exception of Arundel Park, all sites had reduced core area index values. Similarly all but one site (Beachy Head) had reduced connectivity values in 2012 compared to the 1930s. The subdivision values further highlight the fragmentation of the sites, with higher values representing increased division of sites into multiple smaller patches. Across all sites there were significant decreases in area (W=218, p=0.0001), CAI (W=215.5, p=0.0002), and connectivity (t=2.91. p=0.013) (Table 4.9).

Table 4.9 Fragmentation and isolation statistics of study sites in both the 1930s and 2012

	Area 1930 (Ha)	Area 2012 (Ha)	Habitat loss (%)	CAI 1930s	CAI 2012	Connectivity 1930	Connectivity 2012	Subdivision 2012
Arundel Park	62	34.2	-44.8%	84.9	90.3	6.7	4.4	1.1
Beachy Head	621	152.8	-75.4%	93.7	91.6	3.6	5.9	0.5
Butser Hill	2483	63.6	-97.4%	92	89.1	35.8	9.8	1.6
Castle Hill Complex	2344	91.8	-96.1%	95	78.8	22.7	14	1.4
Cissbury Ring	2969	107	-96.4%	94.9	92	7.43	7.3	0.3
Cradle Hill	2848	8.2	-99.7%	95.2	78.3	23.3	8.7	1
Devil's Dyke	3085	35.3	-98.9%	94.7	76.1	11.2	11	1.1
Harting Down West	3315	33.4	-99.0%	93.1	91.9	32.8	9	0.3
Levin Down	1743	32.7	-98.1%	93.3	91.7	20.0	5.3	0.6
Malling Down	463	28.3	-93.9%	93.5	71	21.1	5.6	0.6
Southwick Hill	3085	30.1	-99.0%	94.7	74.6	11.2	8.1	1.1
Steep Down	2969	6	-99.8%	94.9	80.8	7.4	3.5	0.7
Median*/Mean**	2666*	33.8*	-91.4%	94.2*	85*	17**	7.72**	0.86

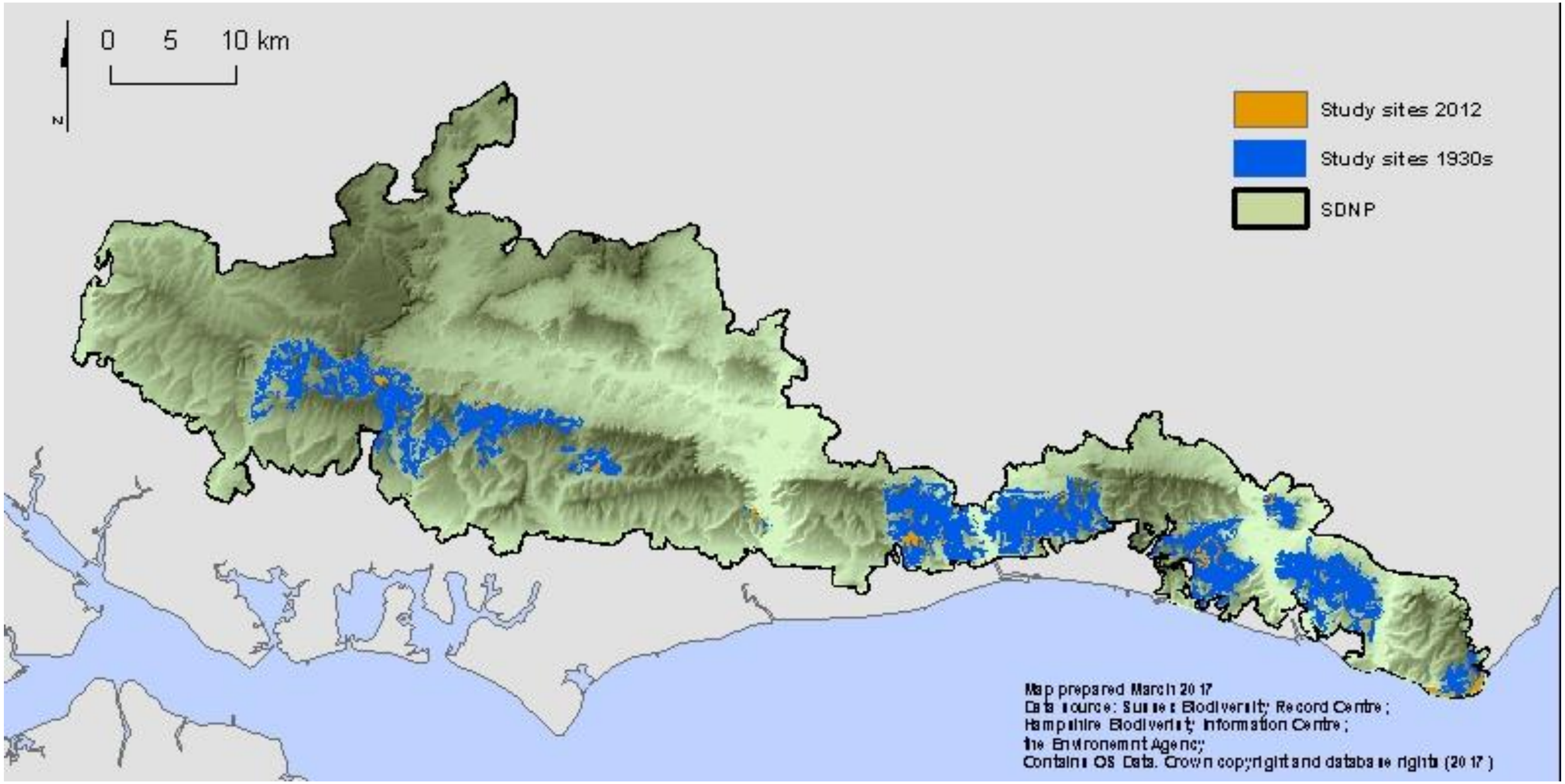


Figure 4.7 The extent of the study sites in the 1930s and 2012. Original in colour.

4.5 Discussion

4.5.1 Historical landscape modelling

Historical LULC data is typically either not available or not directly comparable to contemporary LULC data. These factors have acted as major limitations in our ability to monitor and understand landscape change. To overcome these limitations and to analyse landscape change in the SDNP, a method for modelling the historical landscape was formed based on previous work by Swetnam (2007b) and Hooftman and Bullock (2012). Validation of the method showed an overall accuracy of habitat modelling of 83%, with calcareous grassland modelling being particularly successful (94% modelling accuracy). This accuracy level is similar to those reported for the Land Cover Map 2000 (Fuller et al., 2002b) and of CORINE maps (Feranec et al., 2007). However, the different characteristics of the source data for the 1930s and 2012 LULC may explain some of the loss of accuracy. Furthermore, spatial differences between the data-sets could result from distortion during the scanning of the original 1930s Land Utilisation Survey maps (Swetnam, 2007b), and as georeferencing was carried out for the whole country in one phase (Fuller et al., 2002a).

The validation of the modelling shows the potential for using the method to generate comparable LULC data across different temporal periods. Such data extends the possibilities for assessing and monitoring landscape change. Not only does it allow for the comparison of contemporary LULC data that use different classification systems, but it also allows for long-term landscape change analysis through the comparison historical LULC data with contemporary LULC data. Whilst this technique can be used to measure landscape change, losses may be underestimated as extant habitat may be degraded in terms of an altered species composition and diversity (Hooftman and Bullock, 2012). Indeed, this effect has been noted in studies that have re-surveyed remnant habitat patches (Bennie et al., 2006, Keith et al., 2009, Newton et al., 2012).

This chapter describes an approach for building detailed and comparable habitat maps using different data sources, building on the methods of Swetnam (2007b) and Hooftman and Bullock (2012). One of the main achievements of this approach was to enable the construction of comparable maps from data 80 years apart. Validation of the modelling provided evidence of the robust nature of the method, offering confidence in the reclassification and simplification of LULC data within this methodology. Validation was carried out by using the modelling method to re-classify LULC data from 2012 and examine the agreement between the original and the modelled data. An overall agreement of 83% between the original and the modelled data was obtained. The process was particularly accurate in modelling calcareous grasslands, where a 94% agreement was found.

Previous studies, from which this method was adapted, have not validated their models in such a way. Swetnam (2007b) first developed a method for modelling the 1930s Land Utilisation Survey data. However the research focused on the nature of change over multiple spatial and temporal scales, and as such the modelling of the 1930s LULC was not validated other than by a basic quality assurance process (detailed in (Swetnam, 2007a). Hooftman and Bullock (2012) validated their modelled 1930s LULCs by comparing them with an independent dataset of vegetation surveys. These vegetation surveys were from the same area as their study and also from the 1930s. However, one of the main challenges in modelling historical landscapes in Britain is in the classification of grassland types, a product of the temporal changes to grassland classifications and the quantity of different systems for classifying grasslands. The independent surveys that Hooftman and Bullock (2012) used to validate their model aggregated all grassland types, and thus there was no validation of the method’s ability to model grassland types. By providing a validation of grassland habitats, the modelling validation results presented here highlight the validity of the method for modelling historical grassland types.

4.5.2 Habitat diversity and landscape structure

Between the 1930s and 2012 the landscape composition of the SDNP has been shown to have changed substantially. In the 1930s the landscape was composed of a matrix of arable, woodland, calcareous grassland, and mesotrophic grasslands, which by 2012 had been transformed into a landscape dominated by arable land, with woodland also prominent. Central to this pattern was the loss of semi-natural grasslands between the 1930s and 2012. Transition analysis revealed that by 2012 semi-natural habitats were more likely to have been converted to arable land than to have remained as they were in the 1930s. This pattern of landscape change is comparable with the findings of several previous studies (Fuller, 1987, Burnside et al., 2003, Swetnam, 2007b, Hooftman and Bullock, 2012).

Once analysis moved onto a site based scale, further differences and changes became evident within individual sites. Habitat diversity surrounding the twelve study sites was significantly higher in the 1930s than 2012, at both 1km and 5km scales. Indeed, over both scales, only Beachy Head at the 5km scale had higher habitat diversity in 2012. Contemporary habitat diversity varied between sites, being particularly low at Steep Down. This site is an isolated patch of calcareous grassland in a matrix dominated by arable land. The highest habitat diversity was found at Beachy Head and Malling Down. In contrast to Steep Down, these two sites in particular were surrounded by a matrix of different habitat types. Whilst previous studies have documented contemporary habitat diversity surrounding grassland patches (Janisova et al., 2014, Sutcliffe et al., 2015), to current knowledge no previous studies have done this on a temporal scale. As such the findings here provide an important insight into the decline in habitat diversity surrounding calcareous grasslands within the SDNP. This finding is particularly important as it provides opportunities for the study of the relationship between change in habitat diversity and contemporary species and genetic diversity.

4.5.3 Limitations/spatial statistics

Recently there has been an emphasis on the spatial aspect of ecological patterns and processes (Turner et al., 2001), leading Kupfer (2012) to comment that the quantification of landscape configuration has become a fundamental pursuit within landscape ecology. To this end there has been the development of numerous spatial statistics that aim to quantify particular aspects of landscape structure. The release of the FRAGSTATS program in 1995 (McGarigal and Marks, 1995) has allowed for uncomplicated calculation of an array of landscape metrics, with the program having been cited thousands of times (Kupfer, 2012). Kupfer (2012) is critical of this phenomenon, arguing that relationships between landscape metrics and ecological processes are often presumed rather than established. Moreover, Riitters et al. (1995) comment that many landscape metrics are correlated to one-another. Therefore, whilst the landscape metrics used were carefully selected to measure specific patterns, they are not direct measures of ecological processes.

It is also important to recognise that measuring temporal change, using static data has its limitations. There are three dimensions to geographical data: location, attributes, and time (Berry, 1964, Bullock et al., 1974, Haggett et al., 1977, Sinton, 1978, Langran, 1992). Building on this theme Sinton (1978) developed a framework that proposes that each of these three components is fixed, controlled, or measured. Most mapped data presents a static view, and thus the time attribute is fixed. This creates a problem for measuring temporal change using sequential mapped data. Each series of data represents a snapshot at a given time, and the detection of change is investigated between these snapshots. What cannot be detected is what was present at any point between the snapshots. Therefore where change is detected, it can only be said that there was change at some point between the two snapshots. Information on the time, progression, or dynamics of change cannot be assumed. Equally, where no change is detected from one snapshot to the next, it cannot be ascertained that there was no change in the intervening period.

4.6 Conclusion

A validated method for modelling the historical landscape allowed for the analysis of LULC over an eighty year period. This analysis revealed a substantial loss in semi-natural habitat, mainly due to a transition of semi-natural habitats to arable land. The landscape changed from one with a greater diversity of habitats, to one dominated by a duopoly of arable land and woodland. Semi-natural grassland habitats were shown to have become smaller and less connected. This landscape scale pattern was mirrored at site level, with a significant decline in extent, core area index, connectivity, and adjacent habitat diversity of the twelve study sites. The validation of the modelling method allows for further analysis of the patterns of historical landscape change, and can be applied to a series of LULC data-sets in order to analyse the timings of landscape change between multiple periods. The analysis and results allow for the investigation of the relationship between changing site spatial structure and components of biodiversity, the focus of the chapters 5 & 6.

5. The impact of abiotic, spatial, historical, and management variability on the composition and diversity of plant species

5.1 Preamble

Having considered habitat diversity in Chapter 4, this chapter moves the focus of analysis on to the next level of biodiversity, that of species diversity. Relationships between species diversity and a range of abiotic, spatial, historical, and management factors will be analysed using multivariate methods. The historical factors will be drawn from the historical modelling detailed in Chapter 4.

5.2 Introduction

Across a range of habitats, species richness and composition are influenced by variability in abiotic conditions (Benton, 2009), spatial factors (Fahrig, 2003), historical factors (Matlack, 1994, Singleton et al., 2001), and management factors (Schaffers, 2002, Pakeman and Marriott, 2010). Despite extensive research on semi-natural grasslands, the relationship between species patterns and landscape variability remains unclear. Although the influence on species patterns of many factors have been tested, few relationships have been established, with studies producing inconsistent results. One possible reason for these inconsistencies is that studies have tended to focus on only one aspect of variability, highlighting that the diversity and composition of species in semi-natural grasslands cannot be explained effectively by studying factors in isolation, but instead require consideration of multiple interacting factors. Given that plant communities are structured by ecological processes operating at multiple scales (Ricklefs, 2004, Harrison and Cornell, 2008), a greater understanding of how different factors interact to influence species patterns is necessary for effective conservation of calcareous grasslands and the species they contain.

5.2.1 Variability in species diversity

A range of abiotic, spatial, historical, and management factors have been tested for their influence on the species diversity of semi-natural grassland vegetation. However, despite the quantity of research, a lack of consistency in the findings of different studies means that few relationships have been established. Indeed, within calcareous grasslands the nature of one of the most fundamental notions of landscape ecology, the species-area relationship, is not clear. Some authors report positive correlations (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012), with others finding no relationship (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a).

Multivariate techniques have been employed to study the relationship between species diversity and multiple variables. However, these studies have generally restricted analysis to related variables, such as groups of spatial variables (Krauss et al., 2004, Kiviniemi, 2008). Few studies have tested explanatory variables from a range of different groups, with exceptions provided by Raatikainen et al. (2009) and Reitalu et al. (2012). Raatikainen et al. (2009) tested the importance of habitat area, connectivity, and management on species richness in semi-natural grasslands using generalised linear models (GLMs) and variation partitioning. The variation partitioning revealed that spatial variables explained 8% of the variability in species richness, local variables 5%, and management variables 3%, with the combined effects explaining 35%. Reitalu et al. (2012) used GLMs to test for the effects of contemporary spatial variables, contemporary and historical landscape variables, contemporary management variables, and soil variables, on species richness in semi-natural grasslands in Sweden. They found that site species richness of grassland specialists was influenced by habitat heterogeneity, area, continuity, grazing intensity, and the amount of grassland surrounding the site in 1800.

5.2.2 Variability in species composition

Comparatively less is known about the effect of site variability on species composition than on species diversity. To this end a small number of studies have employed ordination techniques such as canonical correspondence analysis (CCA) and redundancy analysis (RDA), which allow for the analysis of relationships between a matrix of dependent variables, such as species composition, with a matrix of independent variables, such as landscape variables. Barbaro et al. (2004) used CCA to test the effects of lithology, soil moisture, and management on species composition in calcareous grasslands.

They found that lithology was the most important of these variables, explaining 12% of the variability, with the combined effects of lithology and management explaining 24% of the variability. Similarly Alard et al. (2005) used partial CCA to examine the effects of a range of historical (historical land-use, historical tree cover) and environmental (soil nutrients, slope, elevation, exposition, biotic, landscape, management) variables, on species composition in calcareous grasslands. It was found that 67% of the variability in species composition could be explained by the combined effects of historical and environmental variables.

5.2.3 Integrating the study of species diversity and species composition

A limited number of studies have considered the effects of explanatory variables on both species diversity and composition. Sebastia et al. (2008) used CCA to test the effects of abiotic and management variables on species composition of Pyrenees grassland sites. They found that abiotic and grazing variables explained 40% of the variability in species composition. Similarly, using regression analysis, Sebastia et al. (2008) found that bedrock, exposure, stoniness, and grazing intensity influenced species richness, with no variables influencing species evenness. Using variation partitioning and RDA Klimek et al. (2007) analysed the relative contribution of groups of variables to variability in species richness and species composition. They found management variables to be most significant in influencing variability in species richness, with this group accounting for 12% of the variation. In particular there was a negative relationship between the application of nitrogen based fertiliser and species richness, and a positive relationship between grazing intensity and species richness. Environmental variability explained the largest amount of variability (8%) of species composition, with elevation, slope, solar radiation, and soil quality significantly contributing to this effect.

The findings of these studies suggest that species diversity and species composition are influenced by different variables. Moreover, Gibson and Brown (1991), comment that species richness is most influenced by contemporary pressures, and species composition by long-term and historical processes. This stresses the need for research to incorporate the analysis of both species richness and species composition, not only because they measure different aspects of the ecological condition of site, but also because they respond differently to changing environments. This requirement is further highlighted by Partel et al. (2001), who examined the relationship between species richness and composition. Partel et al. (2001) report that patterns exist between the two measures, commenting that in order to study the reasons for variability in species richness it is necessary to also study species composition. A further study by Reitalu et al. (2009) recommends that studies of plant diversity in semi-natural grasslands considers both species richness and evenness. Reitalu et al. (2009) found that species richness and evenness respond differently to habitat fragmentation, with species evenness more sensitive to site isolation.

5.2.4 Extinction debt

The extinction debt theory (Tilman et al., 1994) proposes that there may be a period after landscape change where the diversity of species in a habitat is not in sync with the modified habitat configuration. Instead, the species composition was formed under the historical conditions. Whilst some species may become locally extinct immediately, others may persist for several generations before they can no longer survive in the modified habitat. As such, these species are in an extinction debt, as without conservation efforts they will become extinct. There have been several approaches to testing for evidence of an extinction debt, but most typically researchers have analysed the relationship between contemporary species richness with past and present habitat characteristics (Kuussaari et al., 2009). Where species diversity is better described by the historical than by the contemporary landscape, the existence of an extinction debt is assumed. Within grassland habitats some researchers have found relationships between the historical landscape and species diversity, offering evidence of an extinction debt (Bruun et al., 2001, Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins et al., 2007, Krauss et al., 2010). By contrast, in finding the contemporary landscape to be more related to contemporary species diversity than the historical landscape, others have failed to find evidence of an extinction debt (Adriaens et al., 2006, Oster et al., 2007, Cousins and Eriksson, 2008). Where an unpaid extinction debt is detected, appropriate conservation measures can counteract future biodiversity loss. Moreover, the prospect of an extinction debt necessitates biodiversity studies to consider both the contemporary and the historical landscape. Conclusions about relationships between biodiversity and the landscape drawn from studies that only consider the contemporary landscape may not detect the nature of the relationship if biodiversity is related to an historical landscape structure.

To date most research into the extinction debt has focused on species richness. Research into the extinction debt in species composition, and to other levels of biodiversity, has been largely absent. An exception is provided by Alard et al. (2005) who found that the historical management was important in influencing species composition in calcareous grasslands.

For an extinction debt to be evidenced, species diversity would be more closely matched to historical factors than contemporary factors. Conversely, if there was no extinction debt present then species diversity would be more closely matched to contemporary factors than historical factors.

5.2.5 Study rationale

By considering the influence of multiple factors on both species richness and composition, this study will provide novel information on the interactions between site variability and species, which can be used to inform and develop effective conservation initiatives. As biotic plagioclimax communities with high species diversity at fine scales and many species at their environmental limit, calcareous grasslands in Britain provide an ideal setting to study the relationship between such variability and species patterns. Furthermore, with sites that have varied abiotic conditions, spatial structure, history, and management, the South Downs National Park offers an ideal context for the study. To date, no multivariate studies have been undertaken on calcareous grasslands in the UK. Yet extensive change to spatial and management characteristics over the past century mean these threatened habitats present a unique opportunity to examine the effects of these multiple landscape factors on species richness and composition. Knowledge of the nature of the relationship between site variability and species is essential for the development of appropriate management plans and ultimately the successful conservation of habitats and the species they support.

5.2.6 Study aim

The aim of this study is to examine the effects of landscape variability on species diversity and composition in calcareous grasslands. This aim will help to further understanding of the effects of landscape scale changes on the species diversity and composition within internationally important calcareous grassland systems.

5.3 Methods

5.3.1 Vegetation surveys: species data collection

Twelve calcareous grassland study sites in the South Downs National Park were selected as detailed in Chapter 4. At each site fieldwork was carried out over fourteen weeks between June and September 2013. During this fieldwork period there was a daily average of 7.6 hours of sunshine, and average daily rainfall was 1.03mm. At each site eighteen 1m^2 quadrats were used to sample vegetation, equalling 216 quadrats in total. The use of 1m^2 quadrats is consistent with previous surveys on calcareous grasslands (Gibson and Brown, 1991, Partel et al., 2001, Jantunen, 2003, Lindborg and Eriksson, 2004b, Butaye et al., 2005, Cousins et al., 2007, Cousins and Eriksson, 2008). To determine the number of quadrats required for a representative sample, sample effort curves were produced (Section 5.4.1.1) for both species diversity and species composition, at an initial study site known to be species rich (Devil's Dyke, 78 species recorded by Steven (1992)). The number of quadrats was kept the same across all sites to ensure consistency. As the sites varied in extent, this resulted in smaller distances between quadrats at the smaller sites compared to the larger sites. It was decided that the sample effort analysis validated this approach and ensured that a representative sample of the whole site was generated from the surveys. Quadrats were positioned using a stratified random approach, and within each quadrat a list of the names and abundance (percentage cover) of all species of grasses, forbs, and sedges was recorded. In addition the abundance of bare ground, litter, mosses, and fungi was recorded.

5.3.2 Species diversity measures

Diversity ordering (Renyi, 1961) was carried out to assess the suitability of the data for species diversity analysis. Species richness was calculated using second-order Jackknife estimates (Burnham and Overton, 1978) (Equation 3.10). The second-order Jackknife is a nonparametric estimator, suitable for improving observed species richness counts from small samples. It is a nonparametric estimator in that it is not based on the parameter of a species abundance model that has been fitted to the data. The method considers the number of species found in only one quadrat and the number found in precisely two quadrats. By taking this measure to ensure that the experimental unit (i.e. species richness) represented the totality of the unit (i.e. the study site) pseudoreplication (sensu Hurlbert (1984)) was avoided.

Species evenness was calculated using Simpson's measure of evenness (Equation 5.1). Alpha diversity was measured as the mean number of species per 1m^2 quadrat at each site. Beta diversity was taken as the mean similarity between quadrats within a site, measured as 1-Sorensen's similarity index (Equation 5.2). The Sorensen's similarity index was selected as the most appropriate similarity/dissimilarity index due to the increased weighting of the number of joint occurrences in its calculation making this index preferable for sample surveys where the true number of species may not have been recorded (Krebs, 1999).

Equation 5.1 Simpson's measure of species evenness

$$E_{1/D} = \frac{1/D}{S}$$

Where D = Simpson's species diversity value, and S = species richness.

Equation 5.2 Sorensen's similarity index formula

$$S = 1 - \frac{2A}{2A + B + C}$$

Where A= the number of species present in both quadrats, B = the number of species present in quadrat B, and C = the number of species present in quadrat C.

5.3.3 Species composition measures

The number of characteristic and core species at a site was analysed. The composition of characteristic species was determined by assessing the abundance of calcicole, forb, grassland, ancient site indicator, positive indicators, and agricultural/negative indicator species. Age of site indicator species are after Gibson and Brown (1991) and Karlik and Poschlod (2009). Calcicole species were identified after Ellenberg indicator values (Ellenberg et al., 1991), with strong calcicoles identified as species with F (Moisture) ≤4, R (pH) ≥7, N (Nitrogen) ≤ 4. Ellenberg indicator values for the species recorded were taken from Hill et al. (1999). Positive and agricultural/negative site indicators are after JNCC (2004). Species were classified as core (≥75% frequency), intermediate (>25%<75%), or rare (≤25%) based upon their frequencies across the twelve study sites. A full list of these species is provided in the appendix (Table A.9.2).

5.3.4 Site variability measures

To understand the influence of site variability on species richness and composition, a set of explanatory variables were selected (Table 5.1). These explanatory variables were specifically selected to measure variability between sites in abiotic conditions and soils, spatial structure, historical spatial structure, and management. The collection of data for site variability is explained in detail in Chapter 3. To reduce co-linearity of variables, correlated variables were identified and reduced. Where variables were correlated, those with the highest explanatory influence on species diversity were selected for inclusion. A correlation matrix based on Pearson's correlation coefficients was produced, and where significantly correlated variables were identified ($p<0.05$) only one was selected for further analysis.

Table 5.1 Site variables

Environmental/ management	Slope (°)*, elevation (m)*, radiation (kW h/m2), pH, N, P, K, management type*, grazer, livestock unit/ha (LSU)
Spatial/ Historical	Area (ha), core area index (CAI), lost area (ha)*, habitat loss (%), connectivity, subdivision, 1930s area (ha)*, 1930s core area index*, 1930s connectivity*, 1930s subdivision

*Variables marked were excluded from the final analysis due to co-linearity with other variables.

5.3.5 Data analysis

5.3.5.1 Variability in species diversity

To analyse the relationship between the measures of species diversity and the explanatory variables generalised linear models (GLMs) were used in the R package (Team., 2013). GLMs allow for generalising of linear regression by relating the linear model to the response variable using a link function. For the species richness a GLM with a logarithmic link function following a Poisson distribution was used. A Poisson distribution was used as the species richness values were count data, with no upper bound. For species evenness a GLM with a quasi-binomial distribution was used. A quasi binomial distribution was chosen as the beta diversity data is proportional. For alpha diversity a GLM with a Gaussian distribution was used, as the response was normally distributed. For beta diversity a GLM with a quasi-binomial distribution was used. A quasi binomial distribution was chosen as the beta diversity data is proportional.

5.3.5.2 Variability in species composition

To identify the factors influencing the variability in species richness and species composition RDA with forward selection was implemented using the VEGAN package (Oksanen et al., 2015) for R. RDA is an ordination method that allows for the simultaneous analysis of data from different tables. This was ideal for the present purposes, allowing simultaneous analysis of site variability and species compositions data. Detrended canonical correspondence analysis, with detrending by segments, revealed that the maximum gradient length for all components was less than 2. Thus a linear method of ordination, such as RDA, was appropriate (Lepš and Šmilauer, 2014). RDA was carried out independently for species composition (all species), and species composition (characteristic species). A forward selection process was used, with significance tested by ANOVA like permutation tests (1000 permutations). Variables where $p>0.05$ were excluded from the model. Species composition values (% cover) were log transformed prior to analysis. This is standard practise for species composition data, which is often asymmetric as a result of exponential species growth in favourable conditions (Legendre and Legendre, 1998).

5.4 Results

5.4.1 Survey results

5.4.1.1 Sample effort curves

Sample effort curves were produced for one of the study sites known to have high species richness (Devil’s Dyke, 78 species recorded by Steven (1992)). The sample effort curves for species diversity revealed that eighteen quadrats were sufficient for the running mean number of species and the cumulative species diversity to flatten to within 5% variation from the mean (Figure 5.1). Additionally, at eighteen quadrats 95% of the species of the total number of species were recorded (Figure 5.1). Similarly, at eighteen quadrats the running mean percentage cover of the seven most abundant species flattened to within 5% variation of their mean abundance (Figure 5.2). As such, eighteen quadrats were sampled at the other eleven sites. On completion of the surveys, sample effort curves were then produced for these sites to verify if the sampling was sufficient for all sites. Again it was found that by the 18th quadrat the curves for both the cumulative number of species (Figure 5.3) and the running mean number of species (Figure 5.4) had flattened.

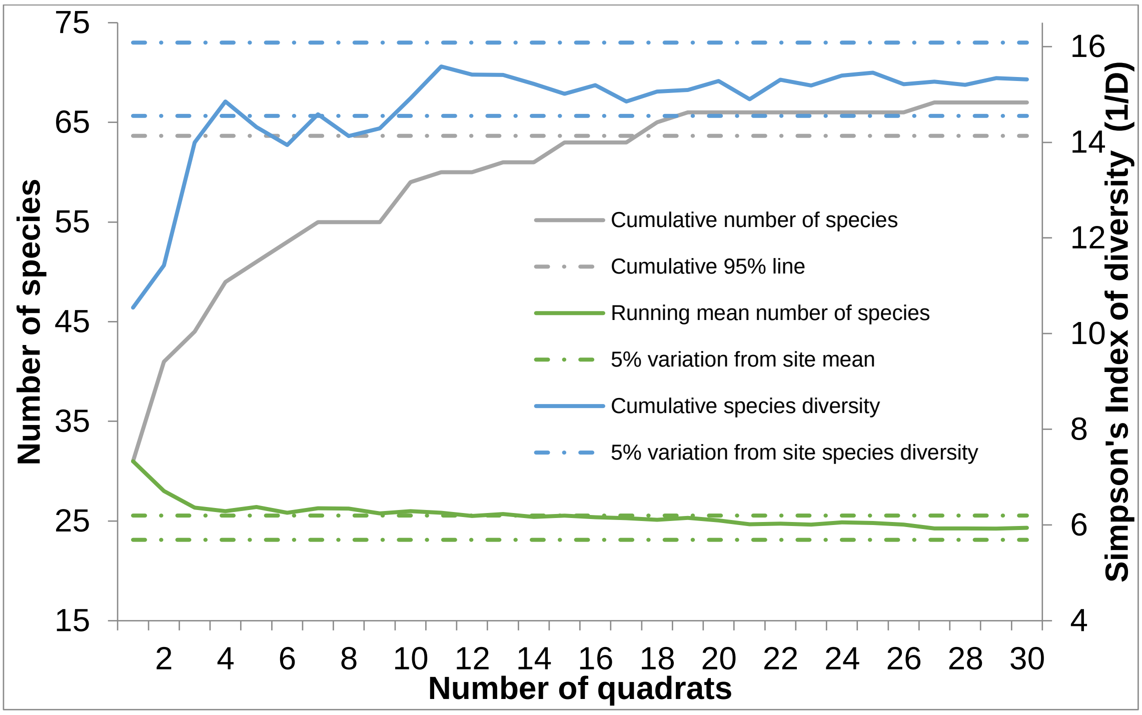


Figure 5.1 Sample effort curves for species diversity at Devil’s Dyke.

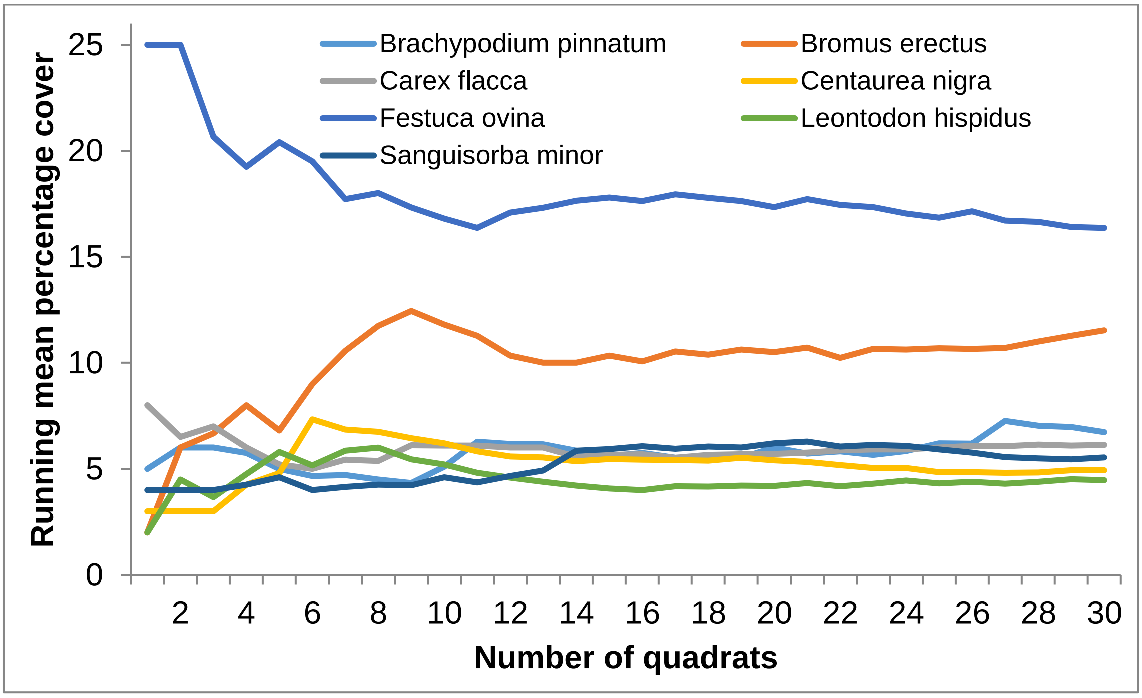


Figure 5.2 Sample effort curves for the percentage cover of the seven most abundant species at Devil’s Dyke.

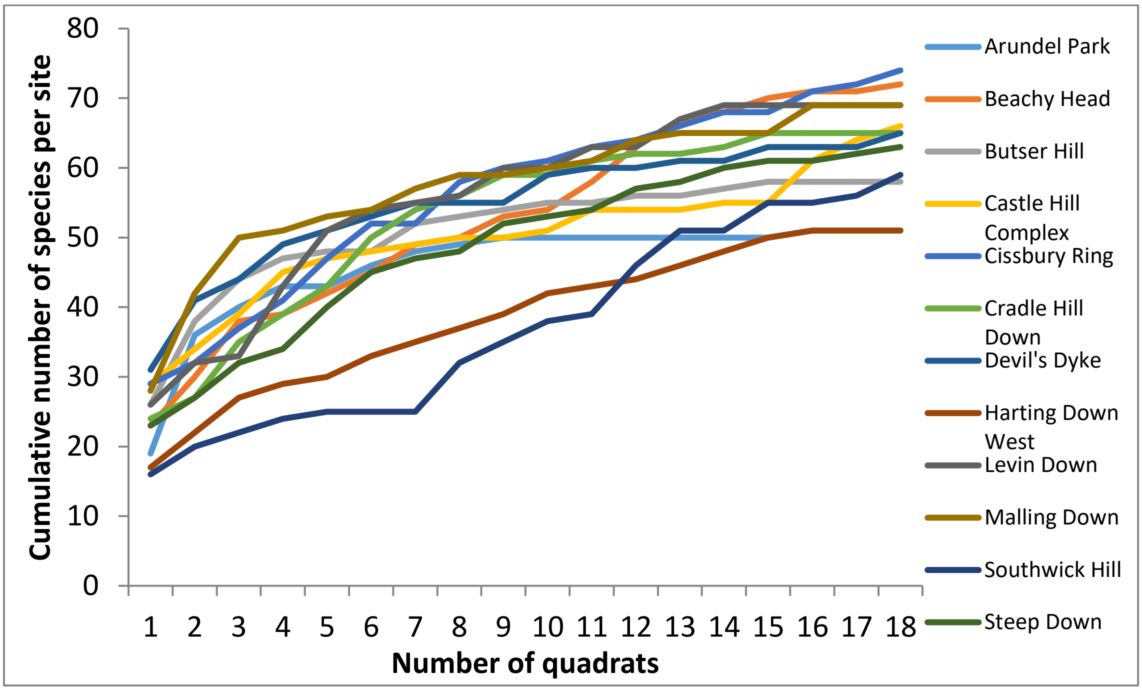


Figure 5.3 Sample effort curves (cumulative number of species) for all sites.

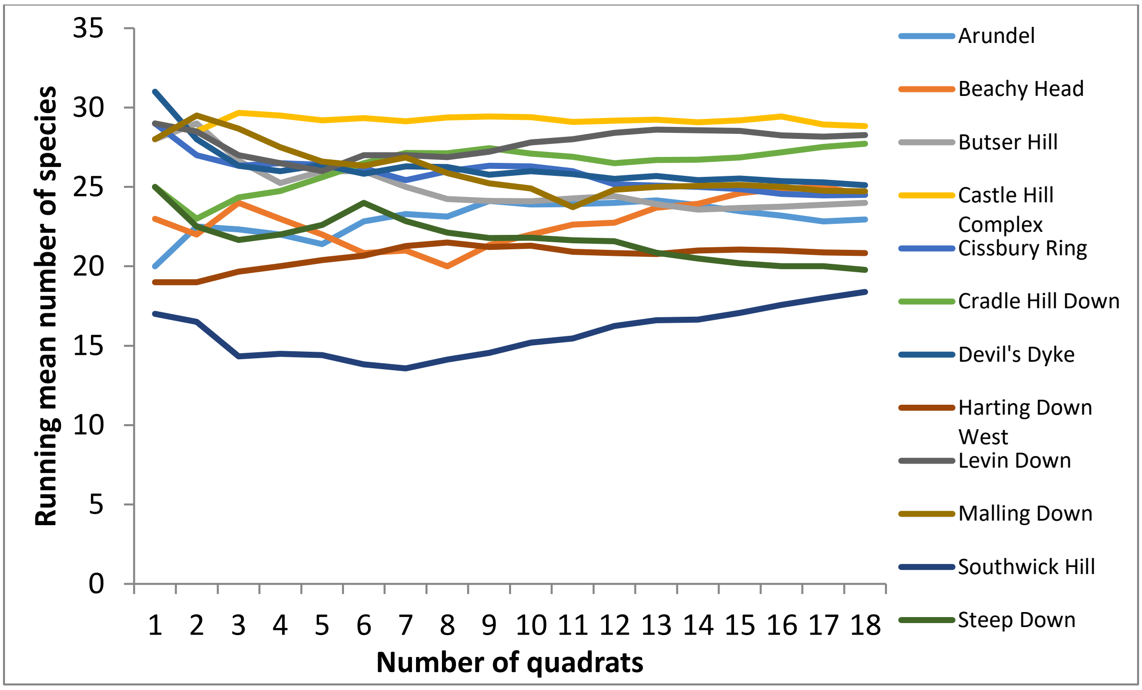


Figure 5.4 Sample effort curves (running mean number of species) for all sites.

5.4.1.2 Species diversity

Diversity ordering revealed that using a species diversity measure was not appropriate for the data (Figure 5.5). Therefore second-order Jackknife species richness estimates (hereafter referred to as species richness), and species evenness were used for the analysis. The second-order Jackknives increased the richness values by between six species (Arundel Park) and twenty-seven species (Cissbury Ring and Devil's Dyke) (Figure 5.6).

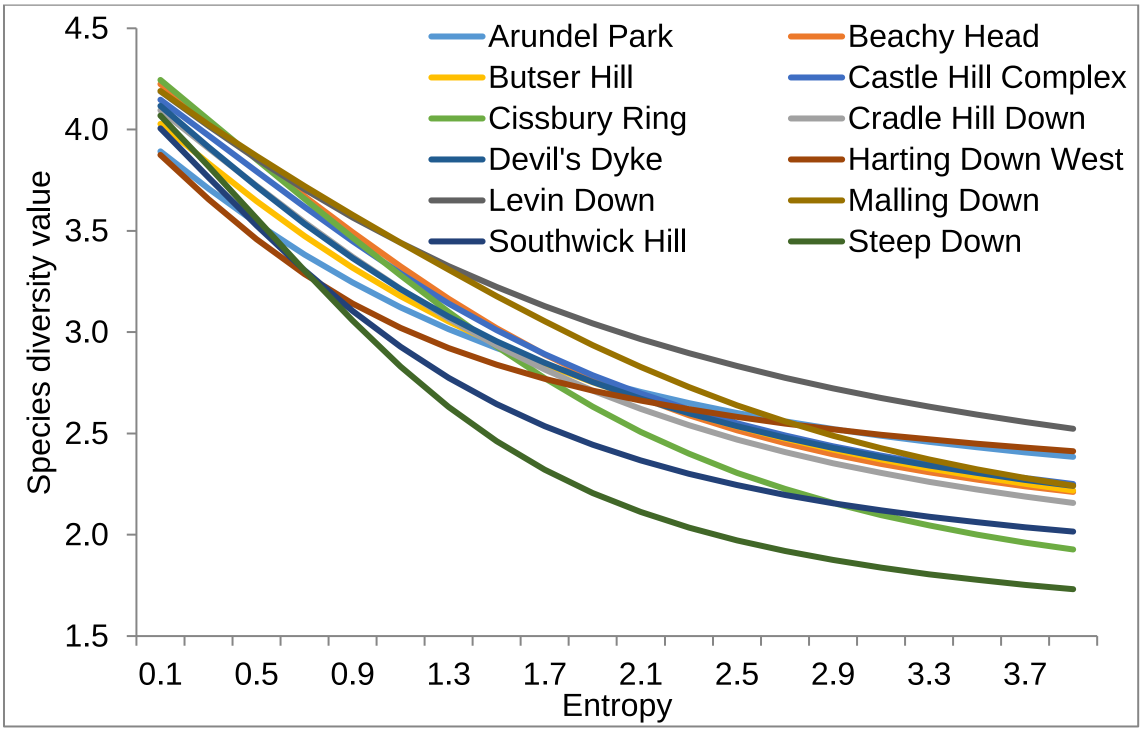


Figure 5.5 Diversity ordering for the twelve study sites. Species diversity values are plotted for each site, using increasing entropy values within the calculation. The figure shows using an entropy value of 0.1 results in Cissbury Ring being calculated as the most species diverse and Harting Down West as the least species diverse. However, using entropy values greater than 1.3 results in Levin Down being the most species diverse and Steep Down the least species diverse.

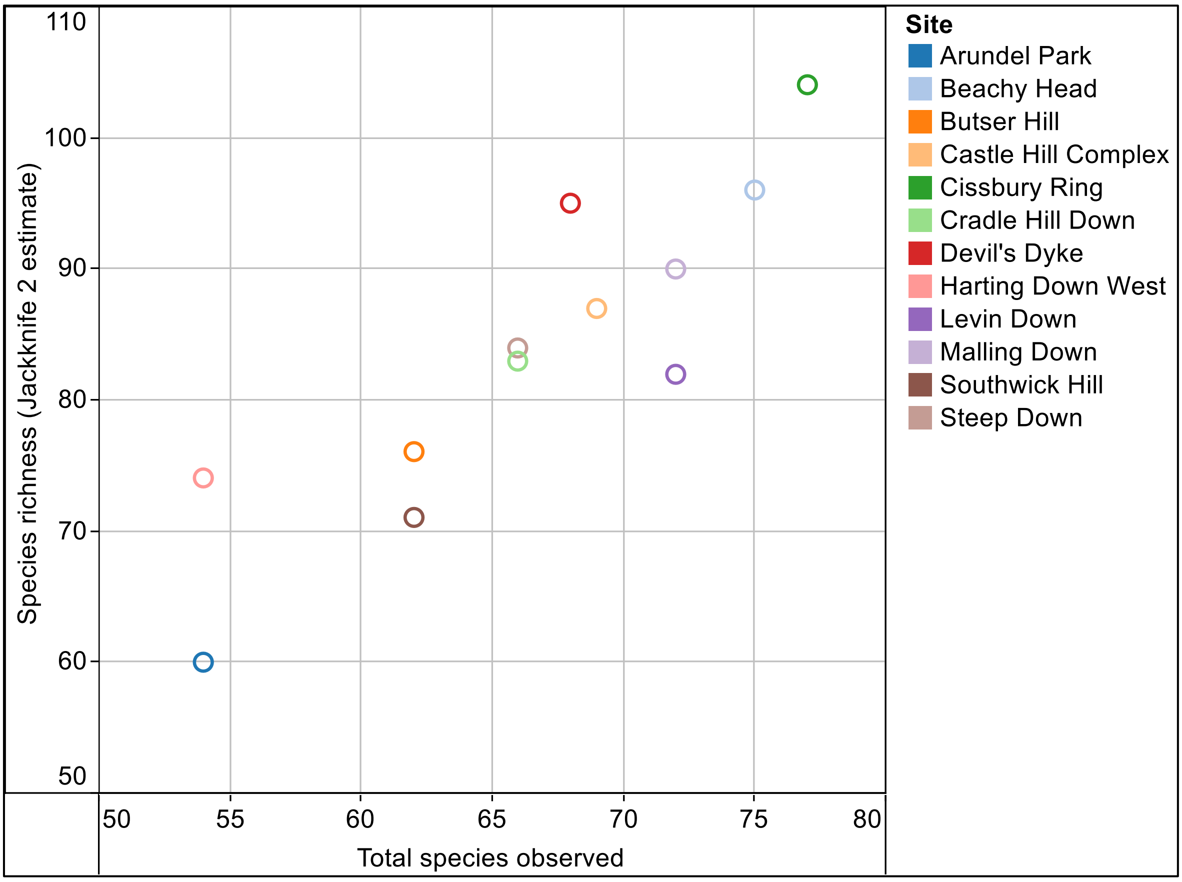


Figure 5.6 The relationship between observed species richness and second-order Jackknife estimates.

Species richness varied across the twelve sites (Table 5.2). The mean number of species per site was 84, ranging from 60 (Arundel Park) to 104 (Cissbury Ring) (Table 5.2). The total number of species estimated across all sites was 168. Similarly there was a range in species evenness from 0.13 (Steep Down) up to 0.29 (Arundel Park) (Table 5.2). There was also variability in mean alpha diversity with values ranging from 17.6 species (Southwick Hill) to nearly 30 species (Castle Hill Complex) (Table 5.2). The mean alpha diversity for all sites was 23.8 species per m^2. Mean Beta diversity was 0.42 ±0.06, and ranged from 0.33 (Castle Hill Complex) to 0.53 (Southwick Hill, and Steep Down) (Table 5.2). Four sites (Castle Hill Complex, Cradle Hill Down, Devil’s Dyke, and Levin Down) had no quadrats where less than 20 species were recorded (Figure 5.7). Conversely, two sites (Southwick Hill and Steep Down) both had quadrats with only 10 species present. The highest number of species in a single quadrat was found at Malling Down, where over half (37 out of 72) of the species at the site were present in a single quadrat.

Table 5.2 Species diversity values for the twelve study sites showing species richness (Second-order Jackknife estimate), species evenness (E_{1D}), mean alpha diversity, and mean beta diversity.

	Species richness	Species evenness	Mean within site alpha diversity (±s.d.)	Within site beta diversity
Arundel Park	60	0.29	22.6 (±4.1)	0.38
Beachy Head	96	0.20	24.1 (±6.3)	0.49
Butser Hill	76	0.24	22.6 (±3.7)	0.41
Castle Hill Complex	87	0.22	29.7 (±2.8)	0.33
Cissbury Ring	104	0.17	25.3 (±3.6)	0.44
Cradle Hill Down	83	0.22	27.7 (±3.4)	0.37
Devil's Dyke	95	0.22	25 (±2.6)	0.38
Harting Down West	74	0.27	19.6 (±2.1)	0.34
Levin Down	82	0.28	27.2 (±2.8)	0.41
Malling Down	90	0.25	25.6 (±5.2)	0.46
Southwick Hill	71	0.18	17.6 (±4.5)	0.53
Steep Down	84	0.13	18.5	0.53
Mean (all sites)			23.8 (±5.3)	0.42 (±0.06)

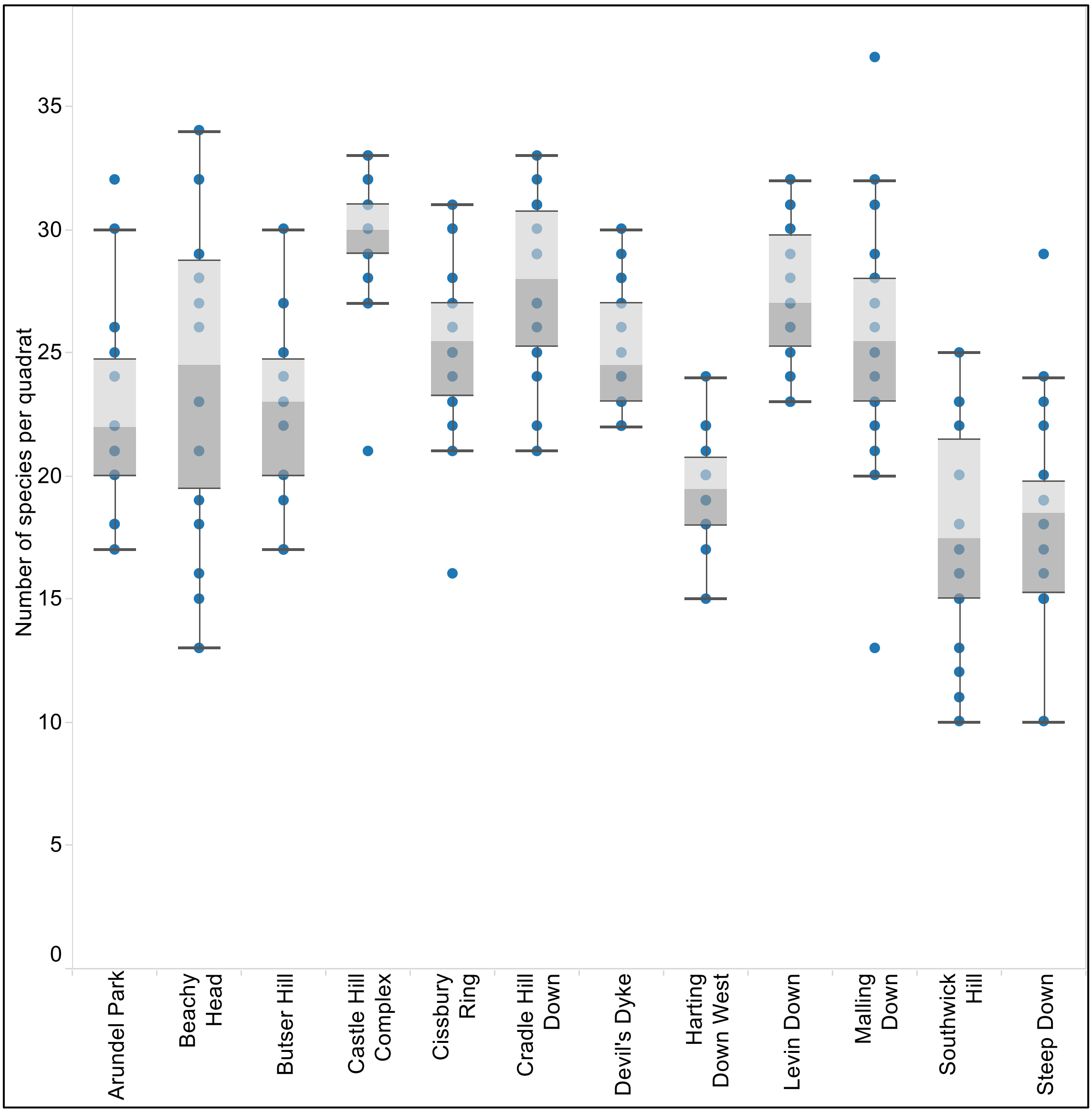


Figure 5.7 Boxplot of the number of species per quadrat at each site. Points represent the observed species richness of the eighteen quadrats at each site (fewer than eighteen points appear at a site where more than one quadrat have the same species richness). Median, upper quartile, and lower quartile range are presented.

5.4.1.3 Species composition

Species composition varied across the sites, with the mean similarity between sites being 0.73 (Sorensen similarity index), ranging from the least similar of 0.64 (Arundel Park and Southwick Hill) to the most similar of 0.83 (Arundel Park and Butser Hill) (Table 5.3). At all study sites grass species were the most abundant species (Figure 5.8). *Festuca ovina* was the most abundant species at nine sites, *Brachypodium pinnatum* at two, and *Bromus erectus* at one (Figure 5.8).

Table 5.3 Similarity in species composition between sites (Sorensen's similarity index) expressed as a decimal percentage.

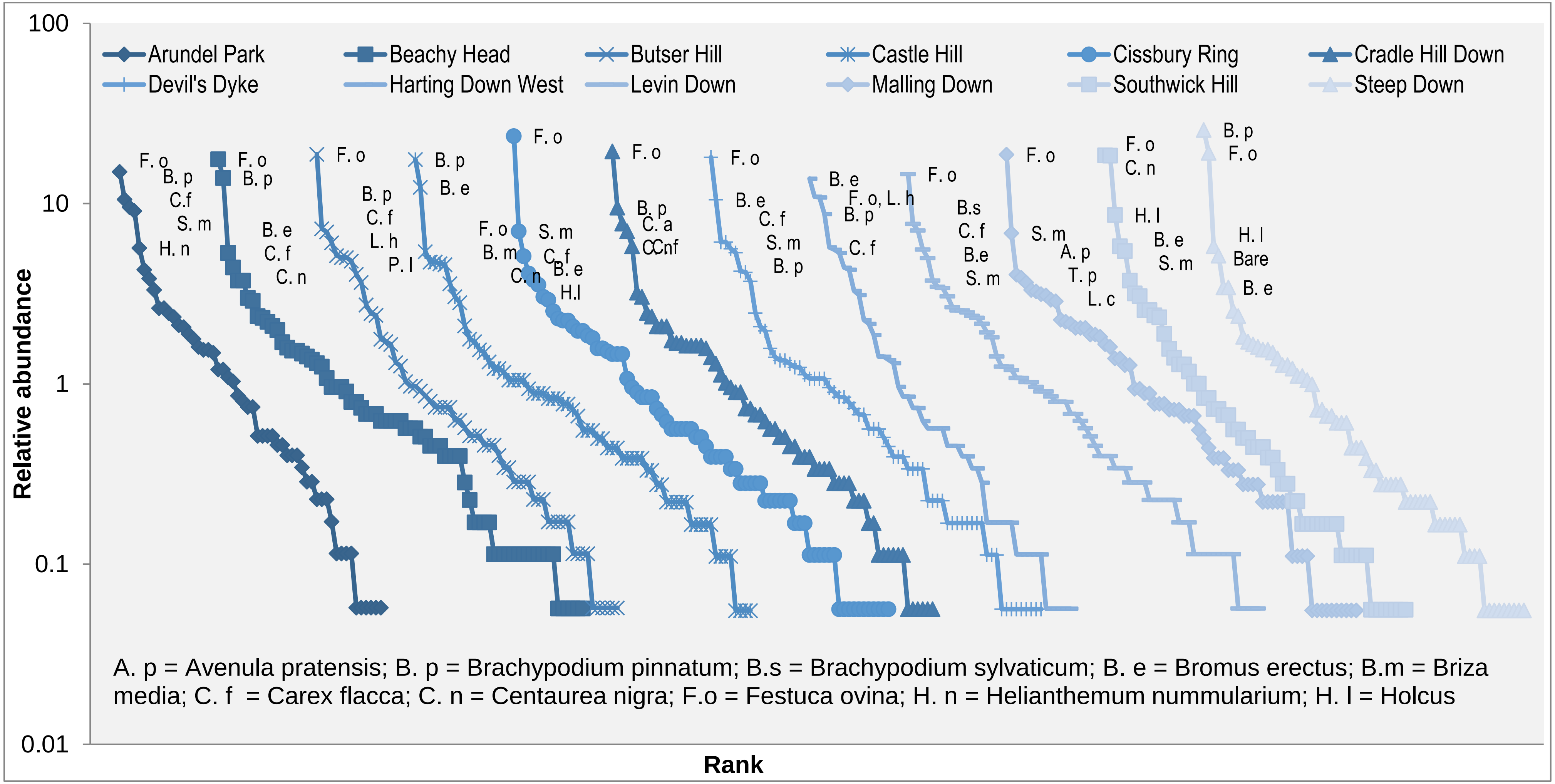
	Beachy Head	Butser Hill	Castle Hill Complex	Cissbury Ring	Cradle Hill Down	Devil's Dyke	Harting Down West	Levin Down	Malling Down	Southwick Hill	Steep Down
Arundel	0.73	0.83	0.70	0.72	0.75	0.75	0.80	0.73	0.70	0.64	0.72
Beachy Head		0.70	0.74	0.74	0.79	0.76	0.70	0.75	0.75	0.73	0.74
Butser Hill			0.72	0.76	0.73	0.74	0.79	0.79	0.70	0.65	0.67
Castle Hill Complex				0.74	0.77	0.80	0.70	0.77	0.74	0.73	0.70
Cissbury Ring					0.74	0.73	0.70	0.77	0.74	0.71	0.69
Cradle Hill Down						0.79	0.73	0.78	0.72	0.72	0.77
Devil's Dyke							0.74	0.76	0.76	0.71	0.76
Harting Down West								0.68	0.67	0.66	0.73
Levin Down									0.76	0.67	0.70
Malling Down										0.66	0.72
Southwick Hill											0.70

Forty-six species could be classified as core species (≥75% frequency across all sites) (See Appendix A1 for a full list of species). The range in the number of core species was small (6), with a minimum of 40 species (Harting Down West and Southwick Hill), and a maximum of 46 species (Beachy Head) (Table 5.4). The range in rare species was greater (10) with a minimum of 2 at Arundel Park and a maximum of 12 at Cissbury Ring. The number of forb species ranged from 38 at Arundel Park up to 55 species at Cissbury Ring (Table 5.4). Whilst the number of forb species was positively related to species richness, the number of grass species was not (Figure 5.9). The number of grasses ranged from 10 species at Devil’s Dyke and Steep Down to 16 at Cissbury Ring and Southwick Hill. In particular Devil’s Dyke had a high species richness (95) but a low number of grasses (10), whilst Southwick Hill had a low species richness (74) but a high number of grass species (16). The most calcicole species were found at Castle Hill Complex (31), and the least at Harting Down West (19). The high number of calcicoles at Castle Hill Complex was interesting as the site was sixth for its species richness. Indeed 45% of the recorded species at Castle Hill Complex were calcicole species. Conversely Steep Down was ranked sixth in terms of species richness, yet has the joint second lowest number of calcicole species. A natural break in the data highlights two types of site: sites high in calcicole species (≥25 species) (8 sites); and sites low in calcicole species (≤21 species) (4 sites).

These findings complement the species richness values by offering a more descriptive interpretation. For example, results suggest that the species richness of Southwick Hill may have been elevated by its diversity of grasses rather than its diversity of typical calcareous grassland species. By contrast the high number of core and calcicole species at Castle Hill Complex highlight that its ecological value is greater than would be interpreted from its species richness value alone.

Table 5.4 The number of core and characteristics species at each site

	Core species	Intermediate species	Rare species	Forbs	Grasses	Calcicole species	Ancient indicator species	Positive indicator species	Agricultural/negative indicator species
Arundel Park	41	9	2	38	11	21	6	15	5
Beachy Head	46	18	9	53	14	27	9	20	8
Butser Hill	41	15	4	43	12	25	7	23	7
Castle Hill Complex	43	17	7	52	14	31	8	18	4
Cissbury Ring	45	18	12	55	16	30	9	22	4
Cradle Hill	44	15	5	48	12	28	8	21	8
Devil's Dyke	43	18	5	52	10	26	7	20	4
Harting Down West	40	9	3	36	12	19	5	16	4
Levin Down	43	19	8	52	13	29	9	22	4
Malling Down	43	17	10	50	14	27	7	21	5
Southwick Hill	40	15	5	40	16	21	6	16	7
Steep Down	41	16	7	48	10	21	7	19	5



A. p = Avenula pratensis; B. p = Brachypodium pinnatum; B.s = Brachypodium sylvaticum; B. e = Bromus erectus; B.m = Briza media; C. f = Carex flacca; C. n = Centaurea nigra; F.o = Festuca ovina; H. n = Helianthemum nummularium; H. l = Holcus

Figure 5.8 Dominance/diversity curves for the twelve study sites. At each site the top five most abundant species are identified.

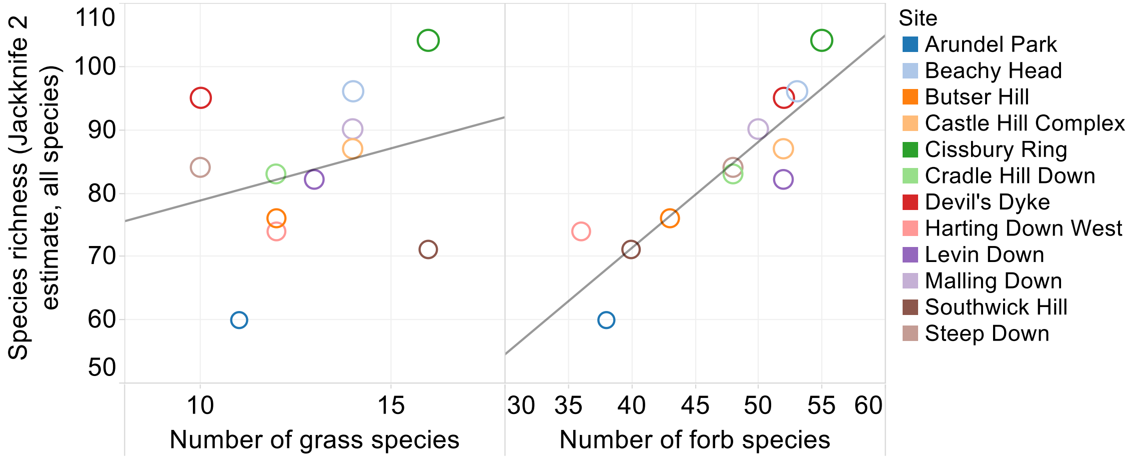


Figure 5.9 The relationship between species richness and the number of forb and grass species

5.4.2 Relationships between species, alpha, and beta diversity

The relationships between the four measures of species diversity (species richness, species evenness, alpha diversity, and beta diversity) was tested using a Spearman's Rho test, with p values adjusted with Bonferroni corrections for six tests. No relationships were found between the four measures (Table 5.5).

Table 5.5 Relationship between species richness, alpha diversity, and beta diversity using Spearman's Rho tests.

	Species evenness	Alpha diversity	Beta diversity
Species richness	-0.47	0.45	0.13
Species evenness		0.3	-0.65
Alpha diversity			-0.55

Bonferroni adjustment for six tests: p=0.05 becomes 0.009

5.4.3 Site variability

Area, connectivity, habitat loss (%), and LSU were not normally distributed and so were transformed (Log base 10, except habitat loss (%) which was transformed by log base 10 + 1) prior to analysis. 1930s area and 1930s CAI were skewed by outlying values at Arundel Park, and so were excluded from further analysis. Co-linearity was found between the following sets of variables: habitat loss (area) and habitat loss (%); 1930s connectivity and 1930s subdivision. Habitat loss (%) and 1930s subdivision were carried forward for further analysis, as they displayed a stronger linear relationship with species diversity. An overview of the site variability is provided in Table 5.6.

Table 5.6 Descriptive statistics for the explanatory variables. Greyed-out variables were not used in the final analysis, but are included here for completeness.

	Variable	Mean (±s.d.)	Minimum	Maximum
Environmental/management variables	Slope (°)	14.2 (±2.2)	9.4	17.5
	Elevation (m)	114.3 (±43.5)	40	199.7
	Radiation (kW h/m^2)	3.8 (±0.2)	3.4	4.1
	pH	6.8 (±0.4)	5.9	7.2
	N (Qualitative scale)	3.5 (±1.5)	1	6.3
	P (Qualitative scale)	2.4 (±0.3)	2	3
	K (Qualitative scale)	3.8 (±1.6)	1	6.2
	Management type	Qualitative		
	Grazer	Qualitative		
	LSU	0.4 (±0.4)	0	1.3
Spatial/historical variables	Area (ha)	52 (±42.1)	6	152.8
	Core area index (CAI)	83.9 (±7.6)	71	92
	Lost area (ha) (1930s - 2012)	2113.4 (±1121.2)	27.8	3281.4
	Habitat loss (%)	8.5 (±15.5)	0.2	55.2
	Connectivity	7.7 (±2.9)	3.5	14
	Subdivision	0.9 (±0.4)	0.29	1.64
	1930s area (ha)	2165.3 (±1110.1)	62	3314.8
	1930s core area index	93.3 (±2.7)	84.9	95.2
	1930s connectivity	17 (±10.1)	3.8	35.8
	1930s subdivision	32.8 (±15.4)	6	59

5.4.4 Variability in species diversity

5.4.4.1 Site variability and species richness

For the landscape variables a model including area, habitat loss (%), and 1930s subdivision was found to explain the most variation (lowest Akaike's information criterion (AIC) value=93.81). Using these variables in the GLM all three were found to be significant in explaining the variation in species richness (Table 5.7). For the environmental and management variables a model including pH, radiation, and grazer was found to explain the most variation (lowest AIC= 89.2). Using these variables in the GLM highlighted significant influence of radiation and grazing by sheep (Table 5.7).

Table 5.7 The importance of the explanatory variables in explaining the variability in species richness, using a GLM. * p<0.05; ** p<0.01, *** p<0.001

		Estimate	Standard error	P
Landscape variables	(Intercept)	4.37	0.16	$<2e^{-16}$***
	1930s subdivision	$0.5e^{-02}$	$0.25e^{-02}$	0.048*
	Area	0.24	0.10	0.016*
	Habitat loss (%)	-0.24	0.10	0.013*
Management variables	(Intercept)	1.17	1.15	0.31
	pH	0.18	0.10	0.07
	Radiation	0.53	0.22	0.013*
	Grazer Multiple	$0.44e^{-02}$	0.07	0.95
	Grazer None	0.25	0.13	0.054
	Grazer Sheep	-0.22	0.10	0.03*

5.4.4.2 Site variability and species evenness

For the landscape variables a model including habitat loss (%), subdivision, and 1930s subdivision was found to explain the most variation (lowest AIC=14.08). Using these variables in the GLM, habitat loss (%) and 1930s subdivision were found to be significant in explaining the variation in species evenness. For the environmental and management variables a model including radiation, K, and P was found to explain the most variation (lowest AIC= 14.06). However, using these variables in the GLM no significant relationships were found.

Table 5.8 The importance of the explanatory variables in explaining the variability in species evenness, using a GLM. * p<0.05; ** p<0.01, *** p<0.001

		Estimate	Standard error	P
Landscape variables	(Intercept)	-1.98	0.24	3.57E-05***
	Habitat loss (%)	0.51	0.16	0.013*
	Subdivision	-0.01	0.01	0.4
	1930s subdivision	0.02	0.005	0.015*

5.4.4.3 Site variability and alpha diversity

For the landscape variables a model including connectivity, habitat loss (%), and subdivision was found to explain the most variation (lowest AIC=71.38). Using these variables in the GLM, no variables were found to be significant in explaining the variation in alpha diversity between sites. For the environmental and management variables a model including radiation, pH, and N was found to explain the most variation (lowest AIC= 66.53). However, using these variables in the GLM no significant relationships were found.

5.4.4.4 Site variability and beta diversity

For the landscape variables a model including area, CAI, and connectivity was found to explain the most variation power (lowest AIC=21.9). Using these variables in the GLM connectivity was found to be significant in explaining the variation in beta diversity. For the environmental and management variables a model including radiation, and pH was found to explain the most variation (lowest AIC= 21.74). However, using these variables in the GLM no significant relationships were found.

Table 5.9 The importance of the explanatory variables in explaining the variability in beta diversity, using a GLM. * p<0.05; ** p<0.01, *** p<0.001.

		Estimate	Standard error	P
Landscape variables	(Intercept)	6.75	3.63	0.1
	Area	0.24	0.20	0.27
	CAI	-3.56	1.93	0.10
	Connectivity	-0.08	0.03	0.02*

5.4.5 Variability in species composition

None of the variables were significant in explaining the variation in species composition (Table 5.10, Table 5.11, Figure 5.10, Figure 5.11). However, when analysing the composition of forb species in isolation, contemporary CAI values were found to be important (Table 5.12, Figure 5.12). In particular the abundancies of *Viola hirta*, *Euphrasia officinalis*, *Viola riviniana*, and *Pimpinella saxifrage* were positively related to CAI, and the abundancies of *Centaurea nigra*, *Cirsium arvense*, *Senecio jacobaea*, and *Phyteuma orbiculare* were negatively related to CAI values (Table 5.12, Figure 5.12). In an attempt to understand the complexities of this relationship analysis was carried out to investigate the relationship between plant traits (Ellenberg indicator values, mean abundance, core species rating) and its contribution to the RDA axis. However, no relationships were found.

Table 5.10 Results of the RDA between species composition and landscape variables

	AIC	F value	Pr(>F)
Connectivity	29.40	1.42	0.10
CAI	29.54	1.28	0.16
Subdivision.30s	29.89	0.96	0.51
Habitat loss (%)	29.89	0.96	0.55
Subdivision	30.09	0.78	0.79
Area	30.19	0.69	0.89

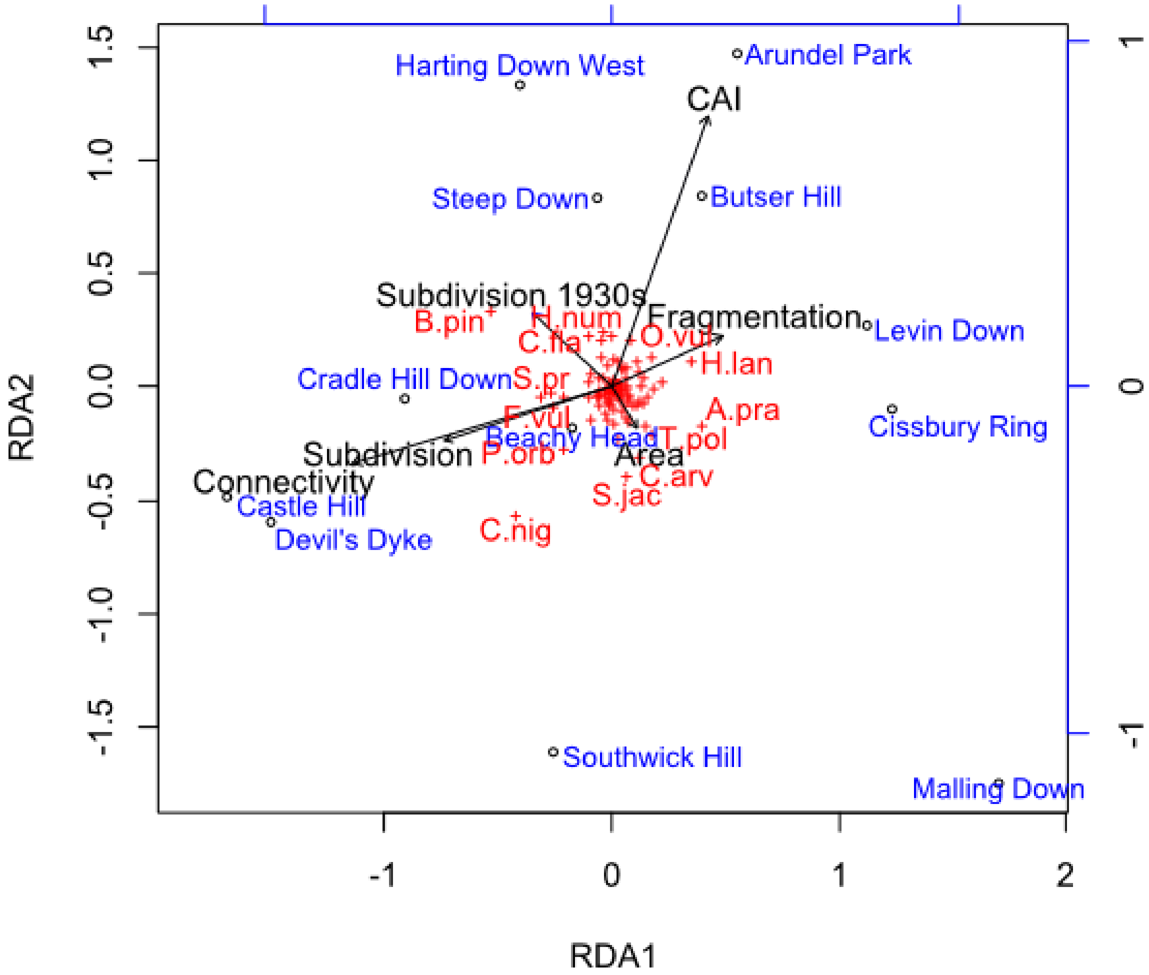


Figure 5.10 Ordination tri-plot showing the relationship between sites, species, and landscape variables.

Table 5.11 Results of the RDA between species composition and environmental/management variables

	AIC	F	Pr(>F)
Radiation	29.44	1.38	0.11
K	29.56	1.26	0.18
Grazer	30.69	1.15	0.22
N	29.77	1.07	0.34
pH	29.78	1.06	0.36
P	29.86	0.98	0.47
LSU	30.08	0.79	0.84

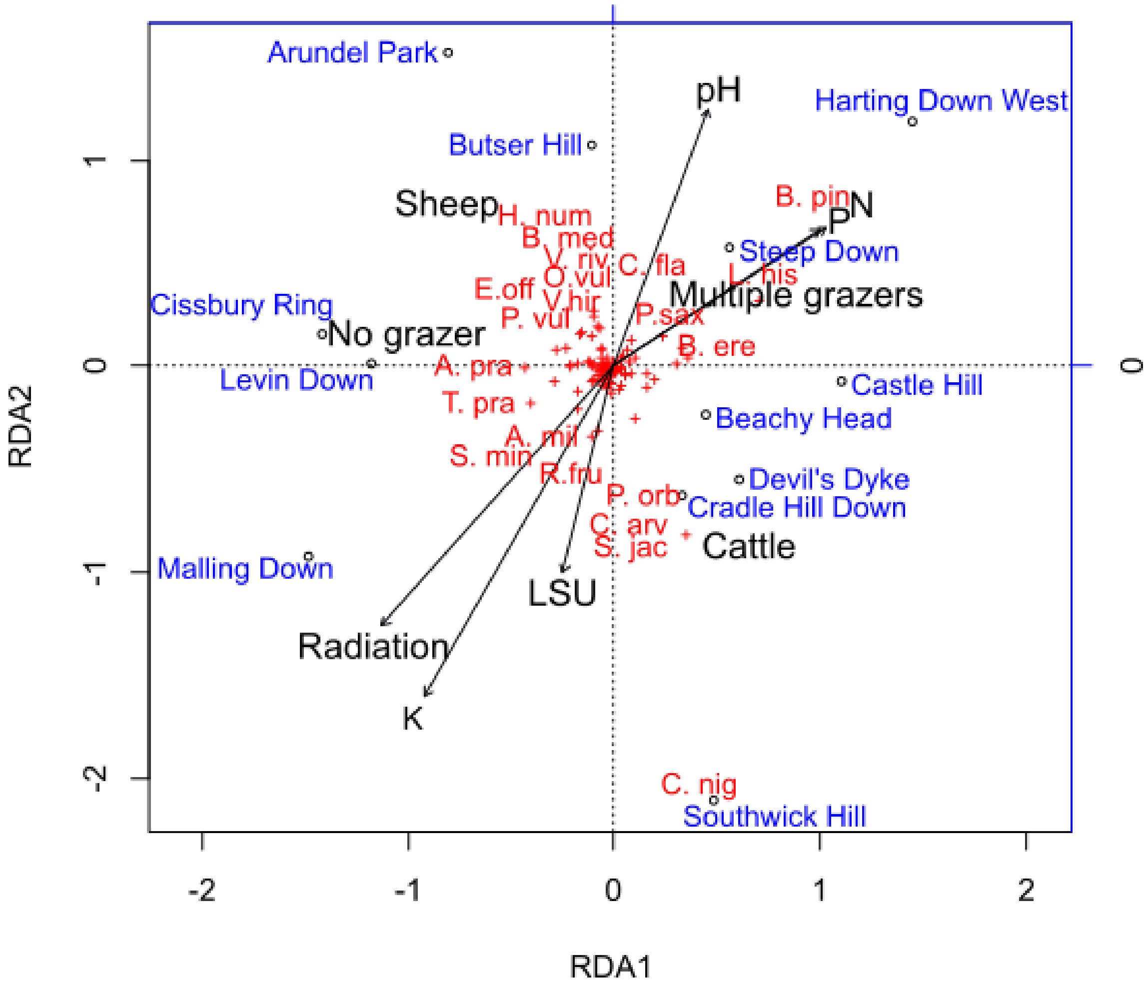


Figure 5.11 Ordination tri-plot showing the relationship between sites, species, and environmental/management variables.

Table 5.12 Results of the RDA between forb species composition and CAI. *p=<0.05

	AIC	F value	Variance explained	Pr(>F)
CAI	23.548	1.84	15.52%	0.015*

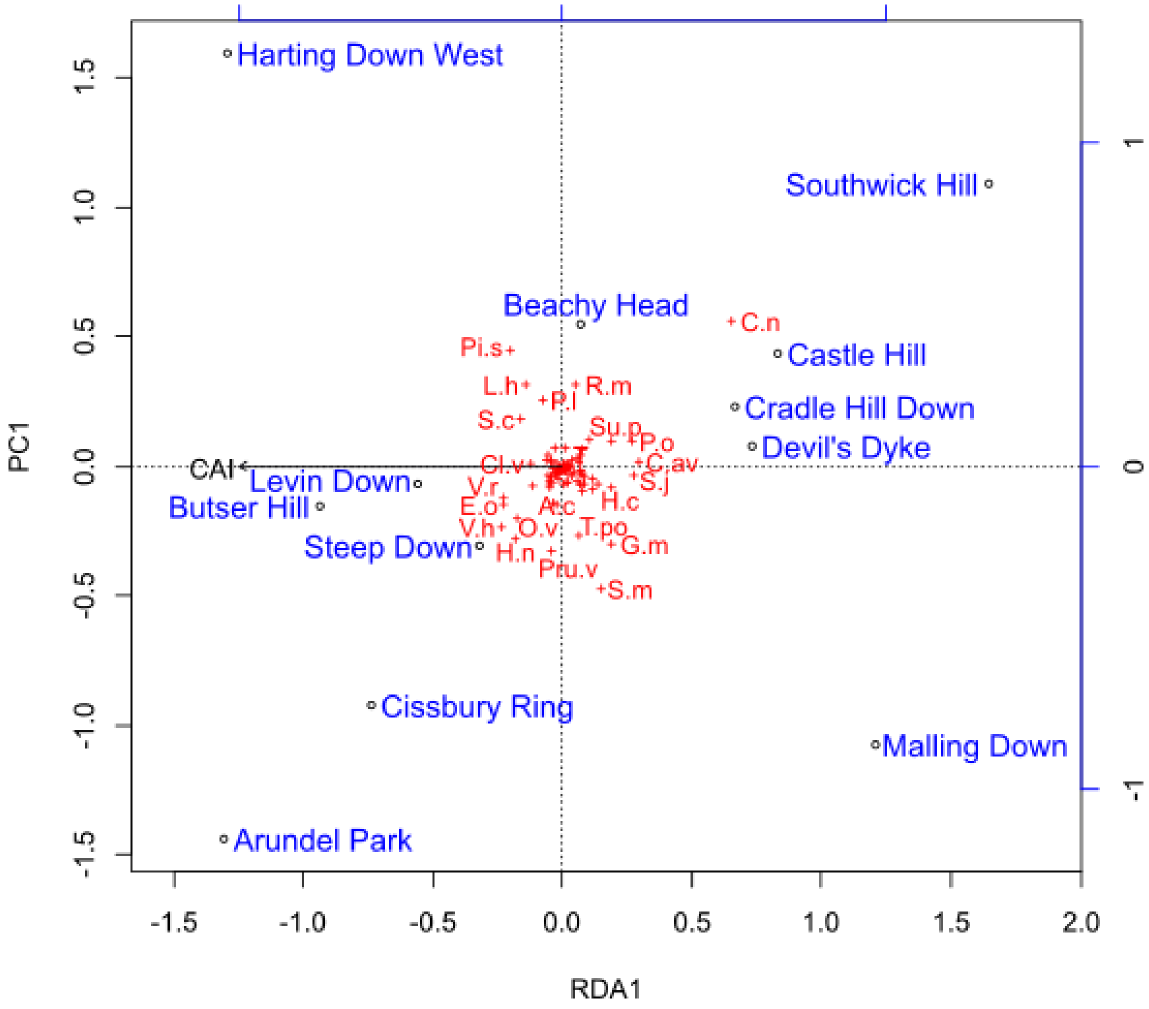


Figure 5.12 Ordination tri-plot showing the relationship between sites, forb species, and CAI.

5.5 Discussion

5.5.1 Species diversity and composition

Diversity ordering showed that the use of species diversity measures was not appropriate for the survey data. Using different entropy values to calculate species diversity resulted in different ordering of the sites. As such species richness, species evenness, alpha diversity, and beta diversity were used. Specifically, using different entropy values in the calculation of species diversity produced different outcomes in terms of the diversity levels of the sites. There was great variety between sites in measures of species richness (from 60-104), species evenness (0.13-0.29), alpha diversity (17.6-29.7), and beta diversity (0.33-0.53). To gain a better understanding of the characteristics of the species contributing to species richness, relationships with forbs and grasses independently found that whilst the number of forb species was positively related to species richness, the number of grass species was not. The lack of a relationship between the number of grasses and species richness was largely due to a low variance in the number of grasses between sites (variance 4.2). By comparison there was a greater variation in the number of forbs between sites (variance 41.1). As such, each site contained most of the total pool of grass species, with only a small number of rarer grasses not present at all sites. By contrast, the composition of forbs varied more greatly from one site to another.

No relationship was found between alpha and beta diversity in calcareous grassland sites across the South Downs. This showed that species richness at a site was not a product of all quadrats containing a high proportion of the species pool of the site. Similarly species richness at a site was not the sum of quadrats with dissimilar species compositions. As such, at sites on the South Downs high species richness can be seen as a product of both consistently high species diversity and of the sum of patches of low species richness with different species composition. Interestingly, this finding contrasts that of a study on calcareous grasslands in Estonia by Partel et al. (2001), who report a negative relationship between alpha and beta diversity, and showed that each quadrat in their study contained most of the species pool with little variability in species composition between quadrats.

5.5.2 Variability in species diversity

The study sought to assess the influence and effect of each site’s landscape structure on species diversity. After excluding correlated variables, six spatial and historical factors (area, core area index, habitat loss (%), connectivity, subdivision, and1930s subdivision), and seven environmental/management factors (radiation, pH, N, P, K, grazer, livestock units) were selected for use in the analysis.

Based on accepted landscape ecological theories (MacArthur and Wilson, 1967, Hanski, 1999), and habitat specific empirical evidence (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Cousins and Eriksson, 2008, Raatikainen et al., 2009), contemporary site area and connectivity would be expected to explain part of the variation in species richness and composition. The results of other studies question these results (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Krauss et al., 2004, Lindborg and Eriksson, 2004a, Lindborg and Eriksson, 2004b, Helm et al., 2006). In line with these expectations, area was found to have a significant influence in explaining species richness, with sites with a larger area having higher species richness. This finding concurs with several previous studies that have established positive species-area relationships in semi-natural grasslands (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012). However, the results contrast those of other studies that have failed to find evidence of the species-area relationship in semi-natural grasslands (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a). These findings indicate that the importance of area on species richness is landscape specific. On the South Downs and numerous other study areas (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012), area is an important factor determining species richness, whilst in other study areas species richness has been shown to be dependent upon other factors and has no relationship with area (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a).

Similarly, in agreement with some studies (Krauss et al., 2004, Lindborg and Eriksson, 2004b, Helm et al., 2006), but contrary to others (Adriaens et al., 2006, Cousins and Eriksson, 2008, Raatikainen et al., 2009, Reitalu et al., 2012), the results of GLMs found no relationship between species diversity and contemporary connectivity. As such, the relationship between species diversity and area and connectivity remains unclear. The mixed nature of the results of studies exploring these relationships suggests that although there may be some effect of area and connectivity on species richness, their impact may be dependent on a range of other variables. Moreover, the contrasting findings may reflect the unique and often slow response of different plant species to fragmentation (Eriksson, 1996, Eriksson and Ehrlen, 2001).

The metrics habitat loss (%), subdivision, and connectivity were included to assess fragmentation of both the contemporary and historical structure of the study sites. Of these measures 1930s subdivision and habitat loss were found to be important in the variability of both species richness and species evenness. The importance of both contemporary and historical measures of fragmentation in influencing both species richness and species evenness highlights the importance of fragmentation on species diversity. The effect of habitat loss (%) was negative with species richness and evenness, and the effect of 1930s subdivision was positive, both suggesting that increased fragmentation is linked to decreased species diversity. This concurs with the results of previous studies in semi-natural grasslands (Cousins et al., 2007, Krauss et al., 2010), which have related increased fragmentation to decreased species diversity. The relationship between historical fragmentation and species diversity is a particularly interesting and novel finding. Although several previous studies have investigated the relationship between historical connectivity and contemporary species diversity (Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins et al., 2007, Cousins and Eriksson, 2008, Reitalu et al., 2009, Reitalu et al., 2012), to knowledge this is the first study to use broader measures of historical fragmentation, making this a novel finding.

Fahrig (2003) suggests that biodiversity loss does not result from habitat fragmentation directly, but is a product of the associated habitat loss. In finding habitat loss (%) and 1930s subdivision to be important in the variation in species diversity between sites, this study challenges the comments of Fahrig (2003). The current study showed that whilst habitat loss is important, other components of fragmentation also have an effect on species diversity.

Reitalu et al. (2009) comments that in semi-natural grasslands, species richness is particularly sensitive to habitat fragmentation, whilst species evenness is not. Similarly Vellend (2004) found that species richness of forest herbs was negatively related to habitat isolation, whereas there was no relationship with species evenness. The results of this study are contrary to the comments by Reitalu et al. (2009), with both species richness and evenness being influenced by components of habitat fragmentation. These differences may result from the lack of a widely accepted method for measuring fragmentation. Reitalu et al. (2009) measured fragmentation as the percentage of different land-cover types surrounding grassland patches. By contrast the present study used three separate components of fragmentation (habitat loss, connectivity, and subdivision) of both the contemporary and historical landscape.

Of the historical variables tested, only 1930s subdivision was shown to be important in explaining the variation in species diversity. By contrast, previous studies have found historical patch area (Helm et al., 2006, Krauss et al., 2010) and historical connectivity (Lindborg and Eriksson, 2004b, Helm et al., 2006, Reitalu et al., 2012) to be important in explaining variability in species diversity. The results of the present study may reflect the specific history of the South Downs National Park. As grasslands on the South Downs were historically large and contiguous, area and connectivity may not have been a limiting factor in the 1930s. Historical area and connectivity to other sites was greater than contemporary values, and may have been ample for the flow of species between sites.

In addition to the influence of the spatial variables, two environmental/management variables (solar radiation and grazing by sheep) were important in explaining species richness. RDA revealed that the relationship between species richness and solar radiation was positive and supports previous studies which have found relationships between species richness/composition and aspect/slope (Tansley and Adamson, 1925, Tansley and Adamson, 1926, Perring, 1959, Hutchings, 1983, Rose, 1995, Burnside et al., 2002, Bennie et al., 2006, Raatikainen et al., 2009). These studies attribute the effects on species richness and composition to either slope or aspect. By including solar radiation as a variable, the results here support the comments of several authors (Perring, 1959, Geiger, 1965, Oke, 1987) that the influence of topography on species patterns is primarily a result of its influence on the amount of sunlight a site receives.

Whilst previous studies have found that grazing intensity (Hulme et al., 1999, De Bello et al., 2006, Klimek et al., 2007, Cousins and Eriksson, 2008, Marriott et al., 2009) and the type of management (grazing or mowing) (Schlapfer et al., 1998, Jantunen, 2003) influence species richness/composition, few previous studies have found a relationship between the type of grazer and species patterns. One exception is provided by Sebastia et al. (2008), who found that the type of grazer influenced species composition. In particular Sebastia et al. (2008) found that grazing by cattle increased vegetation heterogeneity, whilst grazing by sheep favoured specific species of high conservation value. The Sebastia et al. (2008) study looked at the influence of abiotic factors and management on species composition. A multivariate study by Klimek et al. (2007) looked at a range of contemporary environmental factors and management, and found grazing intensity explained some of the variation in species richness. The results of this study contribute to the findings of both Sebastia et al. (2008) and Klimek et al. (2007) by showing that the type of grazer can contribute to the variation in species richness in multivariate analysis.

Klimek et al. (2007) found management variables to be most significant in influencing variability in species richness, with this group of variables accounting for 12% of the variation. In particular there was a negative relationship between the application of nitrogen based fertiliser and species richness, and a positive relationship between grazing intensity and species richness. Although previous studies have reported associations between soil nutrients and grassland vegetation (Mountford et al., 1993, Willems et al., 1993, Smith, 1994, Van der Woude et al., 1994, Janssens et al., 1998) the only previous multivariate study on semi-natural grasslands to analyse soil nutrient variability was by Klimek et al. (2007), who found that Nitrogen fertilisation was negatively related to species richness in managed grasslands.

There was a contrast in the results between the different measures of species diversity. Five factors were important in the variability of species richness, two factors in the variability of species evenness, and no factors for the variability of alpha or beta diversity. This complements the findings of several previous studies that have found that different components of species diversity have different relationships with explanatory variables (Partel et al., 2001, Klimek et al., 2007, Sebastia et al., 2008, Reitalu et al., 2009). This further evidences the importance of the selection of measure used to quantify species diversity.

5.5.3 Variability in species composition

No relationships were found between site variability and species composition when considering all species. This contrasts with the findings of previous studies, which have linked species composition to a range of landscape, historical, environmental, and management factors (Barbaro et al., 2004, Alard et al., 2005, Klimek et al., 2007, Marini et al., 2007, Karlik and Poschlod, 2009). Alard et al. (2005) found that 67% of the variability in species composition in their calcareous grassland study sites in France, could be explained by the combined effects of historical (historical land-use, historical tree cover) and environmental (soil nutrients, slope, elevation, exposition, biotic, landscape, management) variables.

In contrast to the results for species diversity, there was no relationship between species composition and solar radiation. This also contrasts with the results of Klimek et al. (2007) who found that environmental variability explained 8% of the variability in species composition, with elevation, slope, solar radiation, and soil quality significantly contributing to this effect. Moreover the lack of a relationship between species composition and solar radiation contrasts with several previous studies that have found that sites with different aspects support a different range of flora (Tansley and Adamson, 1925, Tansley and Adamson, 1926, Perring, 1959, Burnside et al., 2002, Bennie et al., 2006). Rose (1995) comments that the flora of the south facing slopes of the South Downs are richer, particularly in orchids, with the north facing slopes having more lichens and bryophytes. Therefore, as the present study focused on grasses, forbs, and sedges, the effect of solar radiation on lichens and bryophytes was not measured. As such the results highlight that in the study area solar radiation is not important in explaining the variability of grass, forb, and sedge species.

No relationships were found between species composition and management, in contrast to previous studies that have found differences in composition between grazed and mowed sites (Schlapfer et al., 1998, Jantunen, 2003), that grazing intensity influences species composition (Barbaro et al., 2004, Pakeman, 2004), and that the type of grazer influences species composition (Sebastia et al., 2008). With the exception of the studies by Barbaro et al. (2004) and Sebastia et al. (2008), the other studies mentioned above have looked at the influence of management on species independently, not allowing for the impacts of other factors. Although the Barbaro et al. (2004) studies looked at multiple factors, they only tested the impact of contemporary environmental factors and management, not including any measure of the historical landscape. Similarly the study by Sebastia et al. (2008) looked at abiotic and management factors, not including spatial or historical factors. Therefore the findings of the present study may indicate that although management factors may bear some influence on vegetation, they may not be as important as other factors in determining species composition.

In contrast to the lack of a relationship between the explanatory variables and species composition, when testing the relationship between the explanatory variables and the composition of forb species alone, relationships were identified. CAI values were found to be significant in explaining 15.5% of the variation in the composition of forb species. In particular the abundance of *Viola hirta*, *Euphrasia officinalis*, *Viola riviniana*, and *Pimpinella saxifrage* were positively related to CAI, and the abundances of *Centaurea nigra*, *Cirsium arvense*, *Senecio jacobaea*, and *Phyteuma orbiculare* were negatively related to CAI values. However, no relationships between position on the RDA axis and the abundance or frequency of the forb species was found. That the relationship is between forbs and core area index, as opposed to the area, is interesting and suggests that the proportion of core area within the habitats is important rather than the total extent. From a conservation point of view this shows the importance not only on maintaining and restoring the overall extent of habitats, but also in providing sufficient core areas for species to thrive. The finding of a relationship with forbs, but not all species, supports the notion that habitat specialists are expected to be more sensitive to habitat change (Helm et al., 2006, Kuussaari et al., 2009).

Previous research has shown nitrogen (N) to be important in species composition (Mountford et al., 1993, Willems et al., 1993, Smith, 1994, Klimek et al., 2007), and phosphorus (P) to be a limiting factor (Willems et al., 1993, Janssens et al., 1998). Alard et al. (2005) analysed multiple environmental and management variables, finding higher phosphorous pentoxide levels to be associated with species such as *B. erectus*, *Arrhenatherum elatius*, *Seseli libanotis*, and *Genista tinctoria* (Klimek et al., 2007). Tilman et al. (1999) found that *Taraxacum officinale* had a higher requirement for phosphorous (K) and its biomass was more limited by K than the five grass species present in their study: *Agrostis tenuis, Alopecurus pratensis, Dactylis glomerata, Festuca rubra, and Poa pratensis*. As such, increased availability of K may allow particular forb species to outcompete grass species. Conversely where levels of K are low, some forbs may be limited and outcompeted by grasses that are less dependent on K levels. This suggests that K influences the composition of forb species, a relationship which to date has received relatively little research attention compared to relationships with N and P. However the present study did not replicate these findings, failing to find a relationship between N, P, or K and species composition.

5.6 Conclusion

The results of this study show that the variation in species richness between calcareous grassland sites can be explained by area, habitat loss, 1930s subdivision, solar radiation levels, and grazing by sheep. Similarly species evenness can be explained by habitat loss and 1930s subdivision. No factors were significant in explaining the variation in species composition between sites when considering all species, although the composition of forb species was influenced by contemporary CAI values.

The relationship between species richness and both historical and contemporary factors suggests that the calcareous grassland study sites may be in a state of transition, where they are beginning to reflect the contemporary structure of the site, but are still retaining some of the characteristics of their historical structure.

The implications of the study are that maintenance and conservation measures for calcareous grasslands should be directed by targeted outcomes. Where the conservation of high species richness is desired then both contemporary and historical factors of the site should be considered. For the protection of target species, contemporary landscape variables may be more important. Moreover, the study provides evidence of the presence of an extinction debt in calcareous grasslands in the UK. This finding offers essential information for UK policies to act to conserve these habitats before an extinction debt is realised.

6. The impact of historical and contemporary landscape structure on the genetic diversity of plant species

6.1 Preamble

Having considered habitat and species diversity in the previous two chapters, this chapter focuses on genetic diversity. Relationships between the genetic diversity of two study species and a range of abiotic, spatial, historical, and management factors will be analysed using multivariate methods. Differences in the results between the two species are discussed, along with discussion of the implications of the overall findings for conservation management.

6.2 Introduction

6.2.1 The role of genetics in the extinction debt theory

The ability of species and populations to adapt to landscape change is thought to be influenced by the level of genetic variation that is available for natural selection (Frankham et al., 2009). Genetic variation, both within and between populations, is influenced by gene flow, natural selection, adaptation, and stochastic processes such as genetic drift. Landscape change, and in particular reduced habitat connectivity and population size, can increase a population’s susceptibility to genetic drift and reduce gene flow, leading to genetic erosion and increased differentiation between populations (Young et al., 1996).

The extinction debt theory (Tilman et al., 1994) proposes that whilst some species may be able to initially persist after landscape change, if they are not suited to the new landscape characteristics they will eventually become extinct after a long enough time-lag. A delayed response to landscape change results in the species present within a population being out of sync with the characteristics of the landscape, and bearing more resemblance to the historical landscape. Typically relationships between landscape variables and species richness have been the focus of research into the extinction debt. However, by focusing on diversity at the species level, the extinction debt can only be observed once species have become extinct. If analysis at the species level offers no evidence of an extinction debt in a landscape, it cannot be known if this is due to the true lack of an extinction debt in the landscape, or if the extinction debt is yet to be paid. As such measures of species diversity may lead to the underestimation of threats to biodiversity. However, data on genetic variation can provide information on the actual condition of populations, and their relationship with landscape change. Whilst the effects of landscape change on species diversity may be delayed due to extinction debt, the effects on genetic variation may be more acute (Helm et al., 2009). As such it is important to consider the genetic scale in order to gain insight into the extent of an extinction debt in fragmented landscapes (Honnay et al., 2006). However, attempts to detect an extinction debt by studying at the genetic scale are yet to receive considered research attention, although research into the relationship between genetic variation and the historical landscape is a growing research theme (Jacquemyn et al., 2004; Prentice et al., 2006; Munzbergove et al., 2013).

6.2.2 Genetic variation and landscape variables

Gene flow between populations can occur primarily between populations in close proximity, leading to spatial autocorrelation of genetic variation. Under these circumstances, as geographical distance between sites increases so does genetic distance, a phenomenon referred to as isolation by distance (Wright, 1943). The effect of isolation by distance in grasslands is not well understood, with some authors finding a significant isolation by distance effect (Jacquemyn et al., 2004, Honnay et al., 2007), and others finding none (Odat et al., 2004, Honnay et al., 2006, Rosengren et al., 2013, Dostalek et al., 2014).

The lack of a consistent relationship between genetic and geographical distance, suggests that within grassland habitats gene flow may be independent of geographical distance and may instead be driven by other processes. Moreover, a lack of isolation by distance may, to some extent, be a product of the historical characteristics of the site. In explaining their failure to find an isolation by distance effect, Rosengren et al. (2013) note that the populations of their study were connected 180 years prior to the analysis and therefore may share a common gene pool. Although the subsequent fragmentation of these populations would be expected to be accompanied by genetic drift, slow population turnovers may lead to an extinction debt effect whereby change in genetic variation has yet to be realised.

When gene flow is independent of geographical distance, a population's genetic variation may be influenced by other landscape factors. This effect has been particularly well documented in relation to the loss, fragmentation, and isolation of habitats. Heterozygosity, the state of having two different alleles of the same gene, is lost from a population at a faster rate as the size of a population decreases. Specifically, over a generation, heterozygosity is lost at a rate of $\frac{1}{N}$, where N is the sample size (Gillespie, 1998). This together with a reduced probability of gene flow means that small and fragmented populations will become less genetically diverse over time (Ellstrand and Elam, 1993, Young et al., 1996, Allendorf et al., 2012). Specifically, habitat fragmentation can lead to decreased genetic variation within populations through inbreeding, and increased genetic differentiation between populations, known as population subdivision (Leimu et al., 2006).

There has been recent interest in the effect of contemporary landscape structure and environmental variables on plant genetic variation and inbreeding. Positive relationships have been reported between genetic variation (measured by expected heterozygosity (H_e)) and patch size (Hooftman et al., 2004, Prentice et al., 2006, Baessler et al., 2010), the proportion of similar habitat in the surrounding area (Prentice et al., 2006, Baessler et al., 2010), and habitat diversity in the surrounding area (Baessler et al., 2010). Negative relationships have been found between plant genetic variation and patch isolation (Honnay et al., 2007), population size (Baessler et al., 2010), and the combined effects of patch size and patch isolation (Lienert et al., 2002b). Others have failed to find relationships between the contemporary landscape and genetic variation (Honnay et al., 2006). In addition to these observed relationships, most of these studies tested other variables and found no relationships. For example, although a positive relationship between population size and plant genetic variation is most typical (Hooftman et al., 2004, Prentice et al., 2006, Baessler et al., 2010), the absence of this relationship is not uncommon (Jacquemyn et al., 2004, Leimu and Mutikainen, 2005, Honnay et al., 2007, Munzbergova et al., 2013). However, meta-analysis studies show that a positive relationship exists between genetic variation and population size. Meta-analysis by Honnay and Jacquemyn (2007) found that across 57 studies plant genetic variation (H_e, percent polymorphic loci, and allelic richness) was positively correlated with population size. Similarly, meta-analysis of 48 studies by Leimu et al. (2006) investigated the relationship between plant population size and genetic variation finding significantly positive relationships between population size and genetic variation.

Research into the relationship between genetic variation and historical variables has flourished since the turn of this century. Although an array of different historical variables have been examined, there has been a strong focus on habitat age, with fewer studies exploring spatial aspects of the historical landscape. Positive relationships have been shown between genetic variation and habitat patch age, with higher genetic variation in older sites (Cronberg, 2002, Jacquemyn et al., 2004, Prentice et al., 2006, Munzbergova et al., 2013, Rosengren et al., 2013), particularly where plant populations have not reached an equilibrium with the modified conditions of the contemporary landscape (Bolliger et al., 2014). Rosengren et al. (2013) used stepwise multiple linear regression to study the genetic variation of the moss *Homalothecium lutescens* in relation to measures of connectivity, management, species richness, and the historical landscape. It was found that allelic richness was positively related to the area of old grassland in the surrounding area, and within population genetic variation (Nei's H_S) (Nei, 1973) was higher in older grasslands patches, but negatively related to vascular plant species richness. Where spatial aspects of the historical landscape have been tested, inconsistent results have been found. Some studies have found no relationship between genetic variation and historical landscape configuration (historical patch size, historical patch connectivity) (Honnay et al., 2006, Helm et al., 2009). By contrast others have found positive relationships between genetic variation and historical patch connectivity (Munzbergova et al., 2013).

Helm et al. (2009) found that genetic variation in quaking-grass *Briza media* was better described by the current landscape than the historical one, and argue that this shows the fast rate of response of genetic variation to landscape change in this species. Honnay et al. (2006) similarly found that genetic variation of common kidneyvetch *Anthyllis vulneraria* was related to contemporary but not historical factors. By contrast, Munzbergova et al. (2013) found that genetic variation of devil's-bit scabious *Succisa pratensis* was related to both the contemporary and the historical landscape. Munzbergova et al. (2013) used bi-directional step-wise linear regression to study the genetic variation of *Succisa pratensis* in relation to a range of contemporary landscape, and historical landscape factors. It was found that current genetic variation (measured by Nei's H_S) was positively related to patch suitability, patch size, patch age, and historical patch connectivity, but negatively related to the number of flowering plants. Nei's inbreeding coefficient (Nei, 1973) for the current population was positively related to patch connectivity in 1850, but negatively related to patch connectivity in 1900 and 1945, and to the number of flowering plants.

The relationship between inbreeding and landscape factors is less well studied than the relationship between genetic variation and landscape factors. Exceptions have noted a positive relationship F_{ST} and patch size (Hooftman et al., 2004), and a positive relationship between genetic variation within populations (F_{IS}) and the combined effects of patch size and patch isolation (Lienert et al., 2002b). Meta-analysis of 57 studies by Honnay and Jacquemyn (2007) found no relationship between population size and F_{IS}. Therefore, within grassland habitats, not only is the relationship between the landscape and genetic variation unclear, but there is also little understanding of whether the relationships between genetic variation and landscape factors result from inbreeding or genetic differentiation.

A small number of studies have also alluded to the potential that F_{ST} and F_{IS} may respond differently to historical and contemporary characteristics. Studying butterflies, Keyghobadi et al. (2005) found that F_{ST} was most highly correlated to contemporary forest cover, whilst F_{IS} was most highly correlated with historical forest cover. Similarly Rosengren et al. (2013) found that genetic variation in the moss *Homalothecium lutescens* was higher in older grassland patches, however no effect was noted in F_{ST}, which is to be expected (Jakobsson et al., 2013). In a further study, Jacquemyn et al. (2004) found that within population genetic diversity (F_{IS}) was higher in populations in older forests. Jacquemyn et al. (2004) also found that genetic differentiation between populations (F_{ST}) in older patches was significantly greater than in younger patches, in contrast to both Keyghobadi et al. (2005) and Rosengren et al. (2013). Whilst genetic variation may respond more quickly to landscape change, it can take many generations for genetic drift to significantly impact upon genetic structure (Young et al., 1996, Tomimatsu and Ohara, 2003).

6.2.3 Genetic variation and species traits

The relationship between plant traits and plant community response to landscape change is not well understood (Lindborg, 2007, Aggemyr and Cousins, 2012). Species with dispersal methods that more easily allow for long distance dispersal, such as those dispersed by wind, would be expected to be less sensitive to habitat fragmentation than species with short-distance dispersal strategies. Although it would be predicted that plant species with traits allowing them to colonise and/or avoid extirpation in small and isolated remnant habitat patches would be less susceptible to the negative impacts of habitat fragmentation, empirical results concerning the advantages and disadvantages of different dispersal methods in fragmented landscapes have been inconsistent. Some studies have found that dispersal by animals enables species to survive habitat loss (Marini et al., 2012), and others that a positive relationship between the presence of species dispersed by animals and patch size (Dupré and Ehrlén, 2002). Others have found no relationship between dispersal method and patch connectivity or area (Lindborg, 2007), or that colonisation and extirpation are not related to dispersal mechanisms (McCune and Vellend, 2015). Fewer studies have considered the relationship between dispersal traits and genetic variability. One exception, provided by Hooftman et al. (2004), investigated genetic variation of two species with different dispersal strategies: the insect pollinated *Succisa pratensis* and the wind pollinated *Carex davalliana*. No differences in genetic variation were observed between the two species. As such there is a need to determine the traits of plant species that make them less vulnerable to landscape change (McCune and Vellend, 2015). Recent research has found that the dispersal traits of grassland species are explained by historical factors, with the wind and animal dispersal potential significantly related to grassland age and the amount of grassland in the historical landscape (Purschke et al., 2012). The diversity of dispersal traits is related to historical landscape factors and long-term management (Purschke et al., 2014).

6.2.4 Study rationale

This study uses multi-variate methods to assess the genetic variation of two lowland calcareous grassland plant species with different dispersal methods, in relation to landscape and environmental factors. This will provide novel information in the interactions between site variability and genetic variation, which can be used to inform and develop effective conservation initiatives. With sites that have varied abiotic conditions, spatial structure, history, and management, the South Downs National Park offers an ideal context for the study. Extensive change to spatial and management characteristics over the past century mean calcareous grassland habitats present a unique opportunity to examine the effects of these multiple landscape factors on genetic variation. Knowledge of the nature of the relationship between site variability and genetic variation is essential for the development of appropriate management plans and ultimately the successful conservation of habitats and the species they support.

6.2.5 Study aim

The aim of this study is to examine the effects of landscape variability on genetic diversity within and between calcareous grassland populations.

6.3 Methods

6.3.1 Selection of study species

Two species with a high frequency across calcareous grasslands in the study area (Tansley and Adamson, 1926, Mitchley and Grubb, 1986, Steven, 1992, Steven and Muggeridge, 1992, Holm, 2011) were chosen: *Cirsium acaule*, and *Ranunculus bulbosus*. The selection of species at high frequencies was paramount as they offered replicate samples compared to more localised rare species. High frequency species presented the opportunity to determine a reliable picture of the regions historical landscape. Common and widespread plants are typically considered to be more genetically variable compared to rare species (Karron, 1987, Hamrick and Godt, 1989, Ellstrand and Elam, 1993, Spielman et al., 2004), although this can be counteracted by hybridisation, recent speciation, multiple origins, or recent population bottlenecks (Lewis and Crawford, 1995, Purdy and Bayer, 1995, Friar et al., 1996, Smith and Pham, 1996). As such, common species can be more susceptible to relative loss of genetic variation as a result of habitat fragmentation (Aguilar et al., 2008). A list of suitable species was identified from the core species sampled in the study area (Appendix Table A.9.1). Two species with contemporary frequencies of 100%, differing life strategies, and with sufficient microsatellite markers already developed to facilitate genetic analysis were selected.

6.3.2 Study sites

In order to ensure compatibility with the research on species diversity (Chapter 5), the same twelve study sites were used (detailed in Chapter 3). At each site ten individuals of each species were sampled, consistent with the number of individuals sampled in a similar study by Munzbergova et al. (2013). Moreover, Munzbergova et al. (2013) validated the use of this sample size using a rarefaction technique. For each individual four loci were analysed, thus for each site a total of forty pairs of alleles per species were analysed.

6.3.3 Site variability measures

To ensure consistency with the research on species diversity, the same factors were used to measure site variability as detailed in Chapter 5 (Table 6.1)

Table 6.1 The site variables that were used in the analysis

Environmental/ management	Slope (°), elevation (m), radiation (kW h/m2), pH, N, P, K, management type, grazer, livestock unit/ha (LSU)
Spatial/ historical	Area (ha), core area index (CAI), lost area (ha), habitat loss (%), connectivity, subdivision, 1930s area (ha), 1930s core area index, 1930s connectivity, 1930s subdivision

6.3.4 DNA extraction and microsatellite analysis

At each of the twelve study sites, leaf tissue (circa 20mg) was collected from ten individuals and immediately dried in silica. Samples were collected randomly across the full extent of the site in the summer (June-September) of 2013. DNA was extracted from the leaf tissue using a DNeasy Plant Kit (Qiagen).

For genetic analysis of *C. acaule*, four microsatellite markers developed by Jump et al. (2002) were used, and for *R. bulbosus* four microsatellite markers developed by Matter et al. (2012) were used. Microsatellite DNA markers are highly polymorphic repeats of a short sequences of nucleotides. Microsatellite markers are commonly used to identify genetic variation and similarity at the individual level (e.g., forensic DNA fingerprints employ microsatellites (Gill et al., 1985)) and have the advantage over other methods of measuring DNA variation due to their high variability. Where microsatellites have been identified, analysis can be carried out on DNA samples from individuals within a population to calculate the number of repeats that are present in each individual. Microsatellites have higher mutation rates leading to higher levels of variation, and therefore offer more potential to detect change in genetic variation both within and between populations. Meta analyses by Aguilar et al. (2008) found no effect on the measurement of genetic parameters depending on the use of different genetic markers (allozymes vs. DNA based). For both species, four microsatellites were amplified (Table 6.2).

The samples were amplified by polymerase chain reaction (PCR) using 4µl of DNA (5 – 50ng/sample) and 21µl of a PCR master mix (final concentration: 1xPCR buffer, 0.2mM of each dNTP, 1.5mM $MgCl_2$, 0.5µM primer and 1unit of Platinum *Taq* (Invitrogen)) specific to each primer (detailed in Jump et al. (2002), Matter et al. (2012)). PCR is a technique that amplifies specific sequences of DNA, creating millions of copies from small samples. The copies can then be used for analysis of genetic variation. Genotyping of the PCR product was carried out by Source Bioscience, UK.

Table 6.2. Primer names and the 5’ Applied Biosystems primer dyes (6-Fam/Hex)

Species	Primer	Dye	Citation
Cirsium acaule	CACA01	FAM	Jump et al. (2002)
	CACA04	FAM	
	CACA05	FAM	
	CACA24	HEX	
Ranunculus bulbosus	RB204	FAM	Matter et al. (2012)
	RB206	HEX	
	RB302	FAM	
	RB306	HEX	

6.3.5 Data analysis

6.3.5.1 Genetic variation and inbreeding

Genetic variation is most typically measured by heterozygosity. Expected heterozygosity (H_e) describes the proportion of heterozygotes that would be expected under the Hardy-Weinberg equilibrium, and observed heterozygosity (H_o) describes the actual observed heterozygosity measured within a sample. The Hardy-Weinberg equilibrium is the genotype frequencies that would be expected in a randomly mating population that is experiencing no gene flow, mutation, genetic drift, or natural selection. Departure from the Hardy-Weinberg equilibrium is typically measured using F-statistics, a suite of inbreeding coefficients. F_{IS} measures the genetic variation within individuals within a population. It is reflective of inbreeding within individuals, as when inbreeding increases, heterozygosity within individuals and hence populations decreases. F_{ST} is a measure of population differentiation, and concerns the genetic variation between subpopulations within the total populations.

The following measures of genetic variability/differentiation were calculated in the GDA program (Lewis and Zaykin, 2001):

Expected heterozygosity (H_e)
Observed heterozygosity (H_o)
Inbreeding co-efficient (F_{IS})
Population differentiation (F_{ST})

To test for the significance of departure from random mating, bootstrapping of F_{IS} was carried out at the 95% level, providing confidence intervals.

6.3.5.2 Genetic effects of site variability

Isolation by distance was measured using a matrix of genetic and geographic distances, with genetic distances calculated after Nei (1972). Mantel tests (Mantel, 1967) with 9999 permutations were used to analyse the matrix in the ADE4 package (Dray and Dufour, 2007) for R. To analyse the relationship between the measures of genetic variation/structure and the explanatory variables, generalised linear models (GLMs) were used. A GLM with a quasi-binomial distribution was used.

6.4 Results

6.4.1 DNA extraction and microsatellite analysis

For *C. acaule* four microsatellite markers were successfully genotyped (Table 6.3). Initially CACA16 was tested, but did not successfully PCR so CACA24 was used instead. For *R. bulbosus* three microsatellite markers were successfully genotyped, with the fourth marker (RB302) being unsuccessful (Table 6.3). Overall genotyping was more successful for *C. acaule*, with an average of 92% of samples successfully genotyped across four microsatellites. For *R. bulbosus* an average of 66% of samples were successfully genotyped across three microsatellites.

Table 6.3 Number of samples successfully genotyped

Microsatellite (species)	Number of samples successfully genotyped (out of 120 samples)
CACA01 (*C. acaule*)	103
CACA04 (*C. acaule*)	109
CACA05 (*C. acaule*)	117
CACA24 (*C. acaule*)	114
RB204 (*R. bulbosus*)	76
RB206 (*R. bulbosus*)	82
RB302 (*R. bulbosus*)	0
RB306 (*R. bulbosus*)	79

6.4.2 Genetic variation and inbreeding

For both species genetic variation (H_e) was high at each locus (Table 6.4) and at each site (Table 6.5). In both species there was variation in H_e between sites. In *C. acaule* H_e ranged from 0.53 at Cradle Hill Down to 0.71 at Southwick Hill (Table 6.5). In *R. bulbosus* the range in H_e was between 0.37 at Beachy Head and 0.77 at Malling Down (Table 6.5). For both species all microsatellites had a positive F_{IS} (Table 6.4), evidencing inbreeding. On a site by site basis six sites had F_{IS} values significantly greater than 0 (Table 6.4) for *C. acaule*, evidencing that this species was inbred at these sites. For *R. bulbosus*, six sites had F_{IS} values significantly greater than 0, and two sites had values significantly less than 0. The sites with values significantly less than 0 (Harting Down West and Levin Down) are evidencing outbreeding, suggesting high levels of gene flow from outside populations. There were no relationships between the two species in H_e, H_o, or F_{IS} between sites (Table 6.6). Overall F_{ST} values for both species were significantly greater than 0 (Table 6.4), highlighting barriers to gene flow between populations.

Table 6.4 Expected heterozygosity (H_e), observed heterozygosity (H_o), F_{IS}, and F_{ST} for each loci

Locus	H_e	H_o	F_{IS}	F_{ST}
CACA01 (*C. acaule*)	0.65	0.55	0.15	0.13
CACA04 (*C. acaule*)	0.77	0.47	0.39	0.1
CACA05 (*C. acaule*)	0.55	0.44	0.20	0.04
CACA24 (*C. acaule*)	0.83	0.68	0.18	0.02
All *C. acaule*	**0.7**	**0.53**	**0.18***	**0.07***
RB204 (*R. bulbosus*)	0.77	0.51	0.33	0.12
RB206 (*R. bulbosus*)	0.73	0.35	0.52	0.12
RB306 (*R. bulbosus*)	0.71	0.51	0.29	0.001
All *R. bulbosus*	**0.74**	**0.46**	**0.33***	**0.08***

* denotes significance at $p \leq 0.05$, using bootstrapping of F_{IS}.

Table 6.5 Observed heterozygosity (H_o) and F_{IS} by site with upper and lower confidence intervals

	C. acaule			*R. bulbosus*		
	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}
Arundel Park	0.61	0.66	-0.09	0.74	0.67	0.11
Beachy Head	0.64	0.54	0.16*	0.37	0.25	0.34*
Butser Hill	0.62	0.48	0.25	0.77	0.33	0.60*
Castle Hill Complex	0.67	0.58	0.15	0.65	0.55	0.18
Cissbury Ring	0.66	0.49	0.27*	0.66	0.32	0.53*
Cradle Hill Down	0.53	0.56	-0.06	0.48	0.13	0.74*
Devil's Dyke	0.69	0.37	0.49*	0.70	0.49	0.31
Harting Down West	0.66	0.49	0.27*	0.40	0.51	-0.32*
Levin Down	0.67	0.54	0.19*	0.63	0.79	-0.28*
Malling Down	0.69	0.67	0.04	0.75	0.66	0.12
Southwick Hill	0.71	0.63	0.12	0.73	0.21	0.74*
Steep Down	0.68	0.38	0.45*	0.73	0.42	0.44*
All sites	**0.65**	**0.53**	**0.19***	**0.63**	**0.44**	**0.32***

* denotes significance at $p \leq 0.05$, using bootstrapping of F_{IS}.

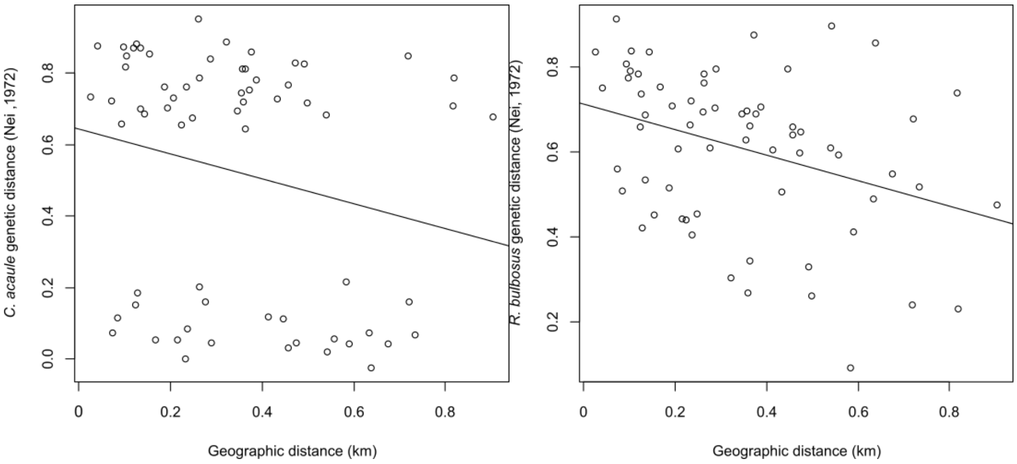
Table 6.6 The relationship between measures of genetic variability and inbreeding between *C. acaule* and *R. bulbosus*. Values represent Spearman's rho correlation coefficients.

	R. bulbosus			
		H_e	H_o	F_{IS}
C. acaule	H_e	0.24		
	H_o		0.2	
	F_{IS}			-0.05

6.4.3 Genetic effects of site variability

6.4.3.1 Isolation by distance

Isolation by distance was assessed using a Mantel test to compare the geographic distance and genetic distance of the twelve sites. The analysis showed that, although there was a general negative trend, there was no significant relationship between geographic and genetic distance for either *C. acaule* (Mantel's r =-0.22, p>0.9) (Figure 6.1(a)) or *R. bulbosus* (Mantel's r =-0.35, p>0.9) (Figure 6.1 (b)).



(a) **(b)**

Figure 6.1 The relationship between geographic distance and genetic distance of *C. acaule* (a), and the relationship between geographic distance and genetic distance of *R. bulbosus* (b).

6.4.3.2 Variability and H_e of *C. acaule*

For the landscape variables a model including connectivity, area, and CAI was found to explain the most variation (lowest AIC = 18.29). However, using these variables in the GLM no variables were significant in explaining the variation in H_e of *C. acaule*. For the environmental variables a model including pH, P, and K was found to explain the most variation (lowest AIC = 18.3). However, using these variables in the GLM no variables were significant in explaining the variation in H_e of *C. acaule*.

6.4.3.3 Variability and H_o of *C. acaule*

For the landscape variables a model including 1930s subdivision, CAI and habitat loss (%) was found to explain the most variation (lowest AIC = 22.84). Using these variables in the GLM habitat loss (%) was shown to be significant in explaining the variation in H_o of *C. acaule* (Table 6.7, Figure 6.2). For the environmental variables a model including pH, P, and K was found to explain the most variation (lowest AIC = 23.9). However, using these variables in the GLM no variables were significant in explaining the variation in H_o of *C. acaule*.

Table 6.7 Importance of the explanatory variables in explaining the variability in Hₒ of *C. acaule*

	Estimate	Std. Error	Pr(>|t|)
(Intercept)	8.003921	4.754214	0.1308
Subdivision.30s	0.010007	0.007452	0.2161
CAI	-4.506103	2.531781	0.113
Habitat loss (%)	0.734152	0.257192	0.0213*

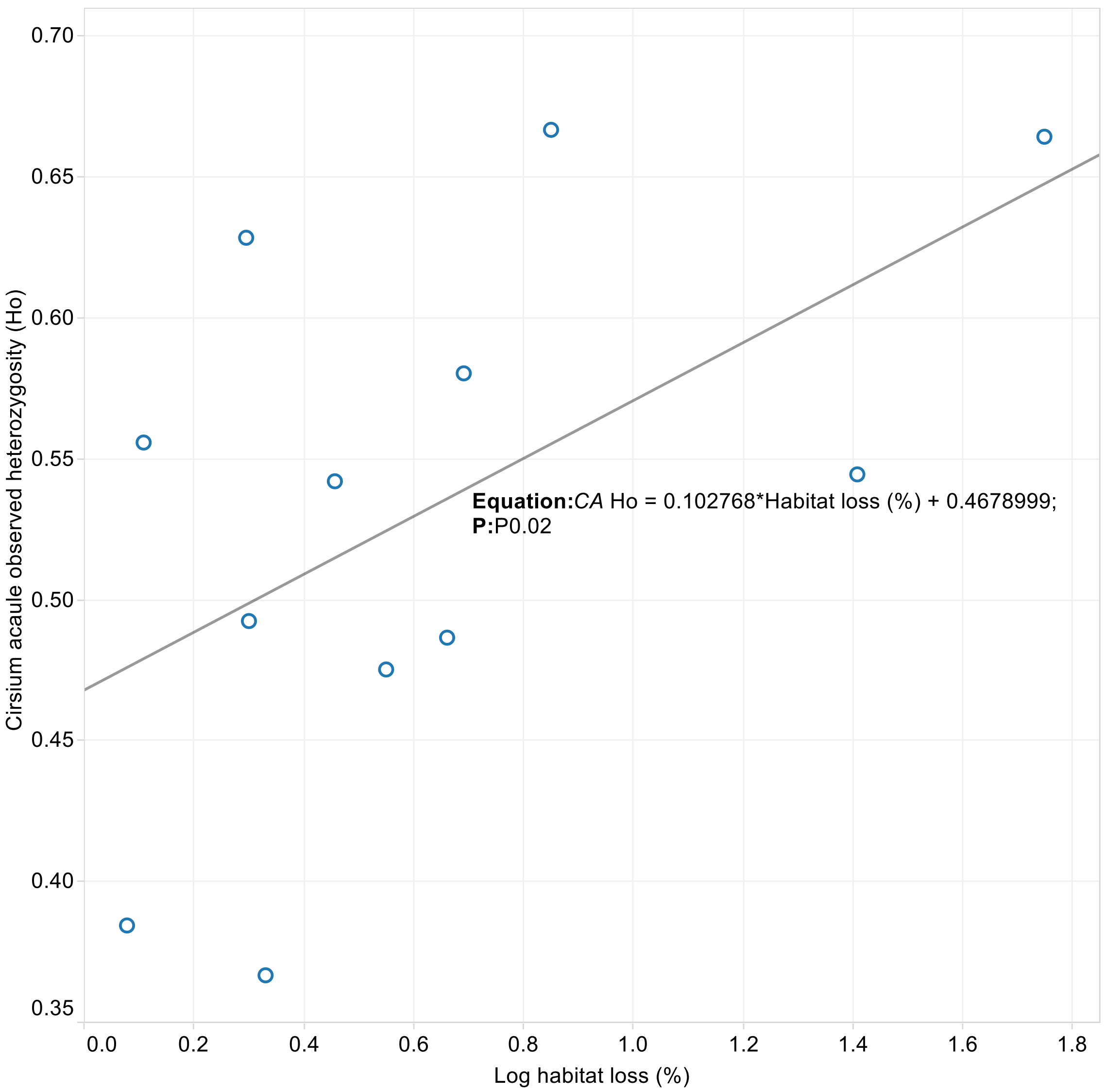


Figure 6.2 The relationship between observed heterozygosity of *C. acaule* and habitat loss (%).

6.4.3.4 Variability and F_{IS} of C. acaule

For the landscape variables a model including CAI, connectivity, habitat loss (%), and 1930s subdivision was found to explain the most variation (lowest AIC = -6.26). Using these variables in the GLM habitat loss (%) was shown to be significant in explaining the variation in F_{IS} of *C. acaule* (Table 6.8, Figure 6.3). For the environmental variables a model including pH, N, K, grazer, and LSU was found to explain the most variation (lowest AIC = -3.32). However, using these variables in the GLM, no variables were significant in explaining the variation in F_{IS} of *C. acaule*.

Table 6.8 Importance of the explanatory variables in explaining the variability in F_{IS} of *C. acaule*

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-3.21737	2.31691	-1.389	0.2075
Connectivity	0.021295	0.018179	1.171	0.2798
CAI	1.937981	1.22092	1.587	0.1565
1930s subdivision	-0.00827	0.004058	-2.038	0.081
Habitat loss (%)	-0.34314	0.119396	-2.874	0.0239*

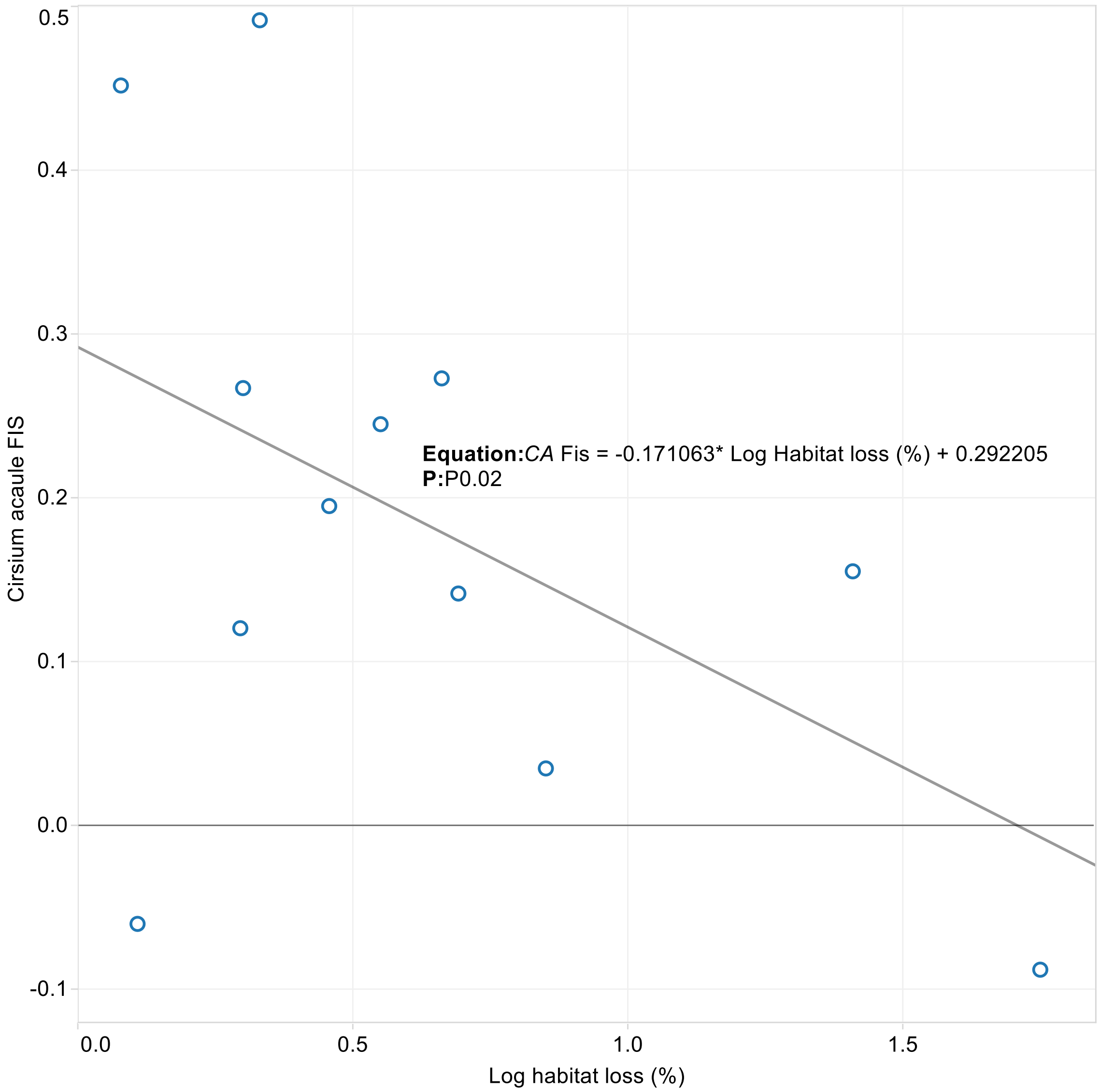


Figure 6.3 The relationship between F_{IS} of *C. acaule* and habitat loss (%).

6.4.3.5 Variability and H_e of *R. bulbosus*

For the landscape variables a model including connectivity, subdivision, and CAI was found to explain the most variation (lowest AIC = 21.12). However, using these variables in the GLM no variables were significant in explaining the variation in H_e of *R. bulbosus*. For the environmental variables a model including radiation, N, and K was found to explain the most variation (lowest AIC = 18.85). Using these variables in the GLM, N and K were shown to be significant in explaining the variation in H_e of *R. bulbosus* (Table 6.9).

Table 6.9 Importance of the explanatory variables in explaining the variability in H_e of *R. bulbosus*

	Estimate	Std. Error	Pr(>|t|)
(Intercept)	5.89414	2.84224	0.07181
Radiation	-1.3804	0.75272	0.10402
K	0.22078	0.07612	0.01987*
N	-0.27218	0.07689	0.00762**

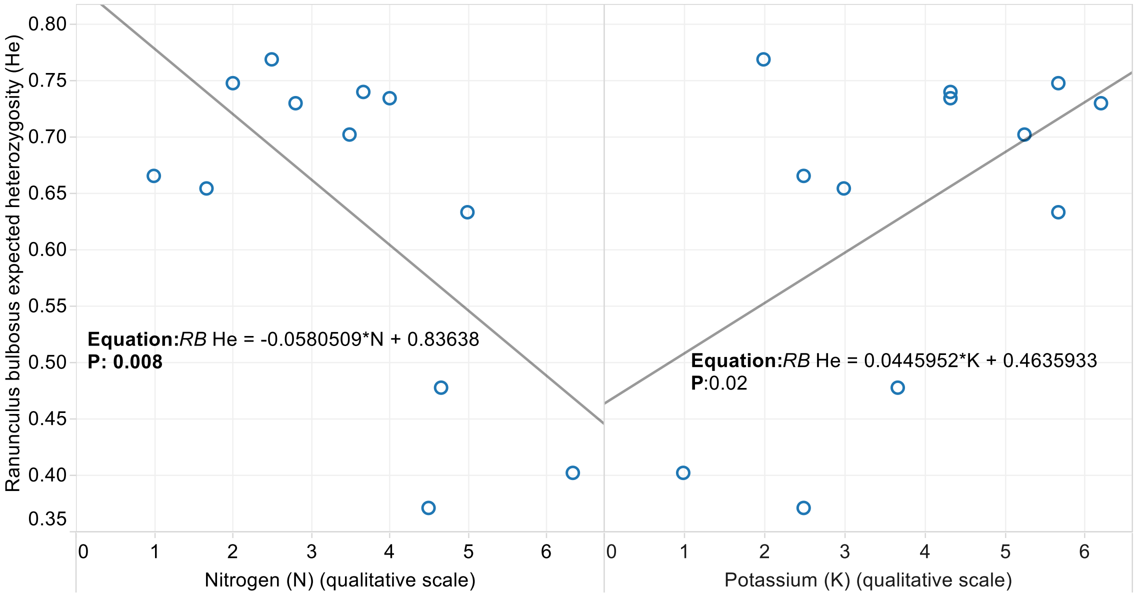


Figure 6.4 The relationship between H_e of *R. bulbosus* and soil nitrogen and potassium.

6.4.3.6 Variability and H_o of R. bulbosus

For the landscape variables a model including 1930s subdivision, current subdivision, and habitat loss (%) was found to explain the most variation (lowest AIC = 22.28). However, using these variables in the GLM no variables were significant in explaining the variation in H_o of *R. bulbosus*. For the environmental variables a model including pH, P, and K was found to explain the most variation (lowest AIC = 23.81). However, using these variables in the GLM no variables were significant in explaining the variation in H_o of *R. bulbosus*.

6.4.3.7 Variability and F_{IS} of *R. bulbosus*

For the landscape variables a model including 1930s subdivision, current subdivision, and habitat loss (%) was found to explain the most variation (lowest

AIC = 12.51). However, using these variables in the GLM no variables were significant in explaining the variation in F_{IS} of *R. bulbosus.* For the environmental variables a model including pH, N, P, K, grazer, and LSU was found to explain the most variation (lowest AIC = -0.39). However, using these variables in the GLM no variables were significant in explaining the variation in F_{IS} of *R. bulbosus*.

6.5 Discussion

6.5.1 Genetic variation and inbreeding

Across the four loci analysed, H_e in *C. acaule* was 0.7. This value is higher than the 0.58 reported across the same loci by Jump et al. (2002), with the difference mainly stemming from CACA04 where H_e was 0.77 compared to the 0.42 reported by Jump et al. (2002). However, the results are within the range of 0.48-0.73 reported across sixteen populations by Jacquemyn et al. (2010). Across the four loci analysed H_o in *C. acaule* was 0.53. This was comparable with a value of 0.60 reported across the same loci by Jump et al. (2002), who also reported similar values on a locus by locus basis. There was significant excess of homozygosity (positive F_{IS}) of *C. acaule* at six sites, indicating inbreeding. At no sites was there indication of outbreeding by an excess of heterozygosity (negative F_{IS}). By contrast Jump (2002) found thirteen out of fourteen populations to be within Hardy Weinberg equilibrium (F_{IS} not significantly different from 0), with the fourteenth having excess heterozygosity (negative F_{IS}), indicative of outbreeding. Genetic differentiation between sites was indicated by a significantly positive F_{ST} value (0.07) across all sites.

Across all three loci H_e in *R. bulbosus* was 0.74, comparable with the value of 0.77 obtained across the same loci by Matter et al. (2013). Across all three loci H_o in *R. bulbosus* was 0.46, higher than the value of 0.32 obtained across the same loci by Matter et al. (2013). This difference mainly stemmed from RB204 where H_o was 0.51 compared to the 0.16 reported by Matter et al. (2013). There was significant excess of homozygosity (positive F_{IS}) of *R. bulbosus* at six sites, indicating inbreeding. In addition, an excess of heterozygosity (negative F_{IS}) was recorded at two sites, indicating outbreeding. Overall F_{IS} was 0.38, comparable with a value of 0.32 reported by Matter et al. (2013). Also in common with Matter et al. (2013) an excess of homozygosity (positive F_{IS}) was recorded at each marker, although the values reported here are lower, particularly at RB204 (0.33 compared to 0.78). An excess of homozygosity between sites was indicated by a significantly positive F_{ST} value (0.08). This result was similar to that reported by Matter et al. (2013) (0.07).

6.5.2 Genetic variation and landscape variables

Analysis revealed that genetic variation in *C. acaule* was best explained by habitat lost. As with the findings at the species diversity level, this suggests that landscape change is important in contemporary diversity at the genetic level. By contrast, levels of soil N and K were the most important variable in explaining of the genetic variation in *R. bulbosus*. These differences may arise from the different dispersal strategies of the two species. *C. acaule* is predominantly dispersed by wind (Pigott, 1968, Fitter and Peat, 1994), and as such has the potential for occasional long distance dispersal (Higgins and Richardson, 1999, Cain et al., 2000). *R. bulbosus*, however, is predominantly dispersed barochorously in the immediate vicinity of the parent flower (Harper, 1957, Sarukhan, 1974).

The findings of the genetic study highlight that the relationship between site variability and genetic variability is complex, and that genetic variation may result from interactions between particular traits of a species with the unique landscape and environmental conditions at each site. Overall the results show that at the genetic level different species respond differently to landscape characteristics, and so it is imperative that conservation attempts consider the synecology of species.

6.5.3 Interspecific differences: genetic variation and species traits

There was little consistency in genetic variation and inbreeding between *C acaule* and *R. bulbosus*. On a site by site basis there were no relationships between the two species for H_e, H_o, or F_{IS}. This shows that there were differences between the two species in their response to barriers to gene flow. The complexities of this relationship become more apparent following the GLM. The observed heterozygosity and F_{IS} of *C. acaule* was related to the extent of habitat loss, whereas expected heterozygosity of *R. bulbosus* was related to contemporary soil characteristics.

Differences between the species may stem from their different dispersal strategies. *C. acaule* is predominantly dispersed by wind (Pigott, 1968, Fitter and Peat, 1994), whereas *R. bulbosus* is predominantly dispersed barochorously (Harper, 1957, Sarukhan, 1974). Wind dispersal means that occasional long distance dispersal is likely (Higgins and Richardson, 1999, Cain et al., 2000) in *C. acaule*, whereas the barochorous dispersal of *R. bulbosus* is typically in the immediate vicinity of the parent flower. Matter et al. (2013) found that dispersal was less than 1m for 60% of all outcrossed mating events in *R. bulbosus* and greater than 100m in 15.9%, with an observed mean dispersal distance of 29.2m. This would suggest reduced barriers to gene flow from isolation in *C. acaule.* However, although there is no relationship between the two species, the results do not indicate lower genetic variation and increased inbreeding in *R. bulbosus* compared to *C. acaule*. Indeed at several sites *R. bulbosus* has more genetic variation than *C. acaule*, and *R. bulbosus* has significant levels of outbreeding at two sites. Although the mean dispersal distance of *R. bulbosus* reported by Matter et al. (2013) was small, they also found mating events up to 362 metres, suggesting that pollen flow may be sufficient to allow gene flow between fragmented grasslands. Whilst the different dispersal strategies of the two species do not appear to be limiting genetic variation, it may still be influential. The different methods of dispersal may be affected differently by different factors of the landscape and environment. For example isolation may interact differently with *C. acaule* than *R. bulbosus*, but these effects may be offset by similarly different responses to soil nutrients levels. In a study of two species with different dispersal methods (Hooftman et al., 2004) found no differences in genetic variation, supporting the findings of the present study that the relationship between site variability and genetic variability is a complex one. As such the genetic variation may reflect interactions of the species traits with the unique landscape and environmental conditions at each site.

For both species, the failure to find an effect of isolation by distance contradicts previous studies. Significant isolation by distance was reported in *C. acaule* by Jump et al. (2003) and in *R. bulbosus* by Hahn et al. (2012). Other studies in calcareous grasslands have also provided inconsistent results, with some reporting a significant isolation by distance (Honnay et al., 2007), and others finding none (Honnay et al., 2006). These contrasting findings highlight the possibility that the effects of isolation by distance may be species specific, and related to the traits of individual species. Moreover, the effects of isolation by distance may be offset by other landscape characteristics.

6.5.4 Genetic variation and the extinction debt

Interestingly, for both species none of the spatial characteristics of the contemporary landscape were important in determining genetic variation. Although there was variation in the contemporary spatial metrics that have been related to genetic variation (e.g. patch size, patch isolation, amount of similar habitat in the surrounding area), most previous studies have established relationships between contemporary spatial characteristics and genetic variation (Hooftman et al., 2004, Prentice et al., 2006, Honnay et al., 2007, Baessler et al., 2010, Munzbergova et al., 2013, Rosengren et al., 2013), although Honnay et al. (2006) similarly found no relationships. The failure to identify contemporary spatial characteristics as important may be a reflection of the scale of change in the landscape. Long-lived plant species often have a unique and slow response to landscape change. Therefore the genetic structure of species may not be in equilibrium with the contemporary landscape. The extinction debt theory proposes that instead, diversity may be more related to the historical landscape. However, no relationships between genetic diversity and the historical landscape were found either. The effects of landscape change to plant species can occur generations after the event. As such there may be a time-lag in which the genetic structure is still responding to landscape change. As such, in the study area the genetic patterns of the species may be in a state of transition, whereby they have responded to landscape change to an extent that they are no longer related to the 1930s landscape. However, they have not yet reached equilibrium with the contemporary landscape. Indeed the relationship between H_O in *C. acaule* and habitat change supports this notion.

Although the length of the time-lag of the extinction debt is species specific and is influenced by localised conditions, in species diversity it has been found at periods of forty years plus (Lindborg and Eriksson, 2004b, Krauss et al., 2010). Given that genetic variation is predicted to respond more quickly to landscape change than species diversity (Helm et al., 2009), the seventy year period between the measures of the historical and contemporary landscape in the present study may be a long enough period for an extinction debt to have been fulfilled. With modification of the landscape since the 1930s, the genetic patterns of the vegetation may be in equilibrium with a more recent pattern of landscape structure, or may be in a state of transition as it continues to adjust to the changing landscape. The genetic patterns of the species are yet to achieve equilibrium with the contemporary landscape and may thus be susceptible to loss of genetic variation once the adaptation to the contemporary landscape is fulfilled. This is the first study to fail to identify relationships between contemporary or historical factors, with previous work by (Helm et al., 2009) finding the genetic variation of *B. media* to be related to the contemporary landscape, but not the historical landscape.

6.6 Conclusion

In both *C. acaule* and *R. bulbosus*, genetic variation and inbreeding varied between sites, however the nature of this variation was not consistent between the two species. As such it can be implied that *C. acaule* and *R. bulbosus* respond differently to barriers to gene flow. Moreover, in addition to the contrast between *C. acaule* and *R. bulbosus*, the results also contrast those from other grassland species studied previously (Leimu et al., 2006, Honnay et al., 2007, Munzbergova et al., 2013). It therefore appears that the genetic response of grassland plant species to landscape change and landscape variability is species specific.

Overall the evidence of an extinction debt was inconclusive, but for both species the findings suggested that landscape change and contemporary factors were more important in influencing genetic structure than historical factors were. As such the genetic variability is in likely in a state of transition whereby it is responding to landscape change, yet still retaining some characteristics of the historical landscape.

7. Synthesis study: The relationship between habitat, species, and genetic diversity

7.1 Preamble

In the three preceding chapters the effects of site variability have been analysed for habitat, species, and genetic diversity. This chapter seeks to synthesise the findings of this analysis by summarising the relationship between habitat, species, and genetic diversity.

7.2 Introduction

7.2.1 Habitat-species-genetic diversity relationships

It has been commented that the different levels of biodiversity often act synergistically (Allendorf et al., 2012), yet research testing the relationships between them has been scarce. Research into the three-way relationship between habitat, species, and genetic diversity, is absent. Moreover the knowledge of the two-way relationships between these three levels of diversity is not well understood. There is little empirical support for the notion that habitat diversity and species diversity are related (Lundholm, 2009), and the relationship between habitat diversity and genetic diversity is largely untested. Similarly although recent research has tested the relationship between species and genetic diversity, results have been inconsistent.

Both the habitat heterogeneity hypothesis and the spatial mass effects theory hypothesise a positive relationship between habitat diversity and species diversity. The habitat heterogeneity hypothesis suggests that higher habitat diversity provides more niche opportunities for a variety of species (Hutchinson, 1957, Whittaker, 1975, Shmida and Wilson, 1985, Ricklefs, 1987, Rosenzweig, 1995). The spatial mass effects theory proposes that as different habitats contain different species, a high diversity of habitats equates to a greater potential source of new species (Shmida and Whittaker, 1981, Shmida and Ellner, 1984, Shmida and Wilson, 1985). However, despite some authors finding a positive relationship between habitat diversity and the diversity of plant species (Skov, 1997, Sotherton and Self, 2000, Bruun et al., 2001, Pausas et al., 2003, Dufour et al., 2006, Poggio et al., 2010, Janisova et al., 2014, Sutcliffe et al., 2015), empirical evidence of the relationship is scare, with a review of literature by Lundholm (2009) finding only 41 observational and 11 experimental studies testing the relationship across a range of taxa, with both negative and positive results reported.

7.2.2 The species genetic diversity correlation

The species-genetic diversity correlation (SGDC) (Vellend, 2003) argues that parallel processes act on species and genetic diversity to produce parallel effects. Specifically Vellend and Geber (2005) suggest that similar neutral (mutation, migration, drift) and adaptive (selection) processes control both types of diversity. As such, species and genetic diversity (within a single species) are predicted to be positively correlated across habitat patches or islands. Research into the SGDC in plant species can be summarised by meta-analysis across 33 studies by Whitlock (2014), which found no relationships between neutral genetic diversity and either species diversity, richness, or evenness. However, much of the research into the SGDC has focused on forest habitats (Vellend, 2004, Vellend, 2005, Wehenkel et al., 2006, Struebig et al., 2011, Wei and Jiang, 2012). Within grasslands Odat et al. (2010) report a positive SGDC in ribwort plantain *Plantago lanceolata*, whilst Odat et al. (2004) found no SGDC in the meadow buttercup *Ranunculus acris*. Odat et al. (2004) did find a relationship between genetic differentiation between populations and species diversity. In addition to testing for a correlation between species and genetic diversity, studies are also beginning to investigate the factors influencing the relationship. Factors found to be contribute to SGDCs include connectivity (Lamy et al., 2013), land-use history (Vellend, 2004), and habitat size (Struebig et al., 2011). Other studies found different factors to effect species diversity and genetic diversity (Odat et al., 2004, Cleary et al., 2006, Avolio and Smith, 2013).

7.2.3 Study rationale

These studies highlight the need to analyse the contribution of habitat characteristics that represent the three major processes driving diversity (drift, extinction, and immigration). There has been a recent research interest in the influence of historical factors on both species diversity (Partel and Zobel, 1999, Bruun et al., 2001, Cousins and Eriksson, 2002, Lindborg and Eriksson, 2004b, Adriaens et al., 2006, Helm et al., 2006, Cousins et al., 2007, Gustavsson et al., 2007, Oster et al., 2007, Cousins and Eriksson, 2008, Reitalu et al., 2012) and genetic diversity (Cronberg, 2002, Jacquemyn et al., 2004, Honnay et al., 2006, Prentice et al., 2006, Helm et al., 2009, Munzbergova et al., 2013, Rosengren et al., 2013). However, to date, the relationship between historical factors and the SGDC has not been well studied, although the finding that land-use history contributes to the SGDC (Vellend, 2004) suggests that historical factors may be important in the SGDC. Information on the relationships between different levels of biodiversity is important for biodiversity and conservation research, as it is important to understand how conservation efforts to increase one level of diversity are likely to affect another level of diversity. Moreover, for conservation management knowledge of the relationship between levels of biodiversity will aid decision making. For example knowledge of the relationship between habitat scale factors, such as habitat size and connectivity, and landscape scale factors, such as habitat heterogeneity, will allow for more informed decisions in conservation strategies.

7.3 Method

Principal component analysis (PCA) was carried out to identify patterns between the different levels of diversity. Where the loading plots suggested correlations between different measures of diversity, Pearson correlation tests were carried-out to test the significance of the relationships. Habitat diversity was measured at two spatial distances (1km radius and 5km radius) both historically (1930s) and contemporarily (2012). Species richness second-order Jackknife estimates were used for species diversity, and expected heterozygosity (H_e) of the two study species were used for genetic diversity.

In order to identify groupings between the different factors, clusters analysis was carried-out. A complete linkage method with a similarity level of 50 was used.

The SGDC was directly tested by running Pearson’s correlation tests on between species richness, and H_e of the two study species. P values were adjusted using the Bonferroni correction for multiple comparisons.

7.4 Results

PCA highlighted associations between the different components of biodiversity (Figure 7.1). Axis 1 accounted for 46% of the variation (eigenvalue 3.25), and was most highly correlated with historical habitat diversity (1930s SHDI 1km) (+0.5 correlation). Axis 2 accounted for 22.1% of the variation (eigenvalue 1.55) and was most highly correlated with H_e in *R. bulbosus* (-0.56 correlation). Based on the loading plot, the relationship between species richness and historical habitat diversity (SHDI 1930s 5km), and between H_e in *C. acaule* and contemporary habitat diversity (SHDI 2012 5km) were tested. A significantly negative relationship was found between species richness and historical habitat diversity (Pearson correlation coefficient = -0.6, p=0.038).

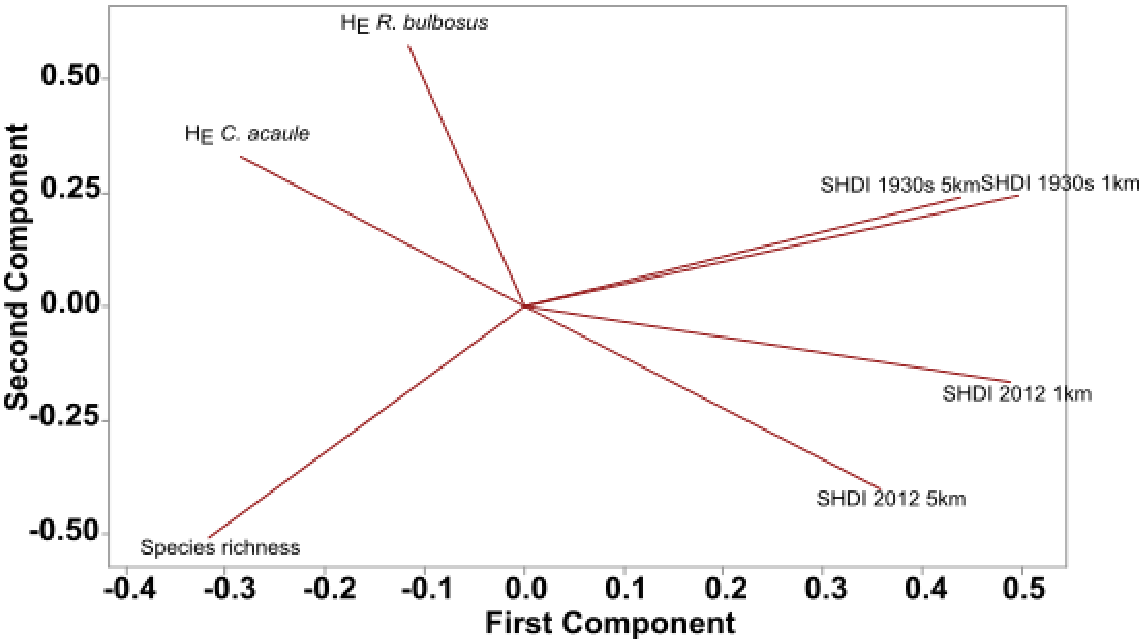


Figure 7.1 PCA loading plot of the different levels of diversity.

Cluster analysis grouped the factors into three clusters (Figure 7.2). Within these groups there was no overlap between different levels of biodiversity, with habitat diversity, species diversity, and genetic diversity being separated into three distinct groups.

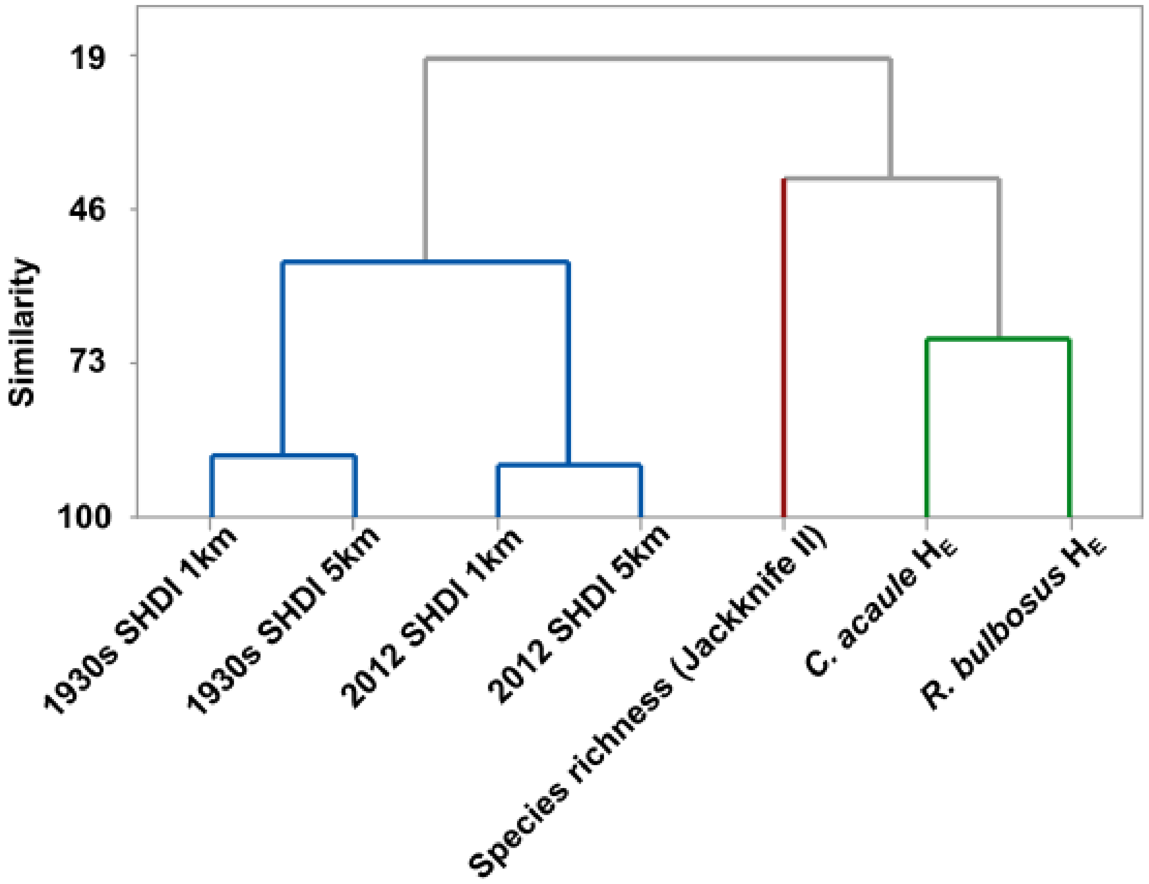


Figure 7.2 Dendogram of the cluster analysis of the different components of biodiversity.

No relationships were found between species diversity and genetic diversity for either species (species diversity and *C. acaule* genetic diversity: Pearson's correlation coefficient = 0.2, p = 0.6; species diversity and *R. bulbosus* genetic diversity: Pearson correlation coefficient = 0.8, p = 0.6).

7.5 Discussion

7.5.1 Habitat-species-genetic diversity relationships

Levels of contemporary habitat diversity, species diversity, and genetic diversity are not related within the study area. As the first study to directly test the relationship between diversity at these three levels, this is a novel finding, and is of particular significance as it contradicts the predictions of theory. The habitat heterogeneity hypothesis predicts that habitat and species diversity will be correlated as a result of higher habitat diversity providing more niche opportunities for a variety of species (Hutchinson, 1957, Whittaker, 1975, Shmida and Wilson, 1985, Ricklefs, 1987, Rosenzweig, 1995). Similarly, the spatial mass effects theory also predicts that habitat and species diversity will be correlated, but as a result of a high diversity of habitats offering more potential sources of new species (Shmida and Whittaker, 1981, Shmida and Ellner, 1984, Shmida and Wilson, 1985). Indeed, several authors have found positive relationship between habitat diversity and species diversity in plants (Skov, 1997, Sotherton and Self, 2000, Bruun et al., 2001, Pausas et al., 2003, Dufour et al., 2006, Poggio et al., 2010, Janisova et al., 2014, Sutcliffe et al., 2015). However, as a review of literature by Lundholm (2009) shows, the nature of the relationship between habitat and species diversity is not yet well understood.

The inclusion of historical habitat diversity in the analysis, highlighted a negative relationship between historical habitat diversity and species richness. This is the first study to test this relationship, with the result particularly interesting in comparison to the failure to find a relationship between contemporary habitat diversity and species diversity. Together, these findings show that there are relationships between the historical landscape structure and contemporary species diversity. Where species diversity reflects the historical landscape structure, it can be as a result of the contemporary species structure being shaped by characteristics of the historical landscape. Following landscape change, some species may continue to persist although they are no longer suited to the modified landscape. After a long enough time-lag such species may become extinct, under the extinction debt process.

7.5.2 The species-genetic diversity correlation

Species and genetic diversity are hypothesised to be correlated as a result of parallel processes acting on them to produce parallel effects (Vellend, 2003). However, no evidence of a species genetic correlation was found for either *C. acaule* or *R. bulbosus*. These findings further evidence the complexity of the relationship between species and genetic diversity. Although some authors have found support for a positive SGDC (Vellend, 2004, Vellend, 2005, Wehenkel et al., 2006, He et al., 2008, Odat et al., 2010, Wei and Jiang, 2012), several others have failed to find a positive relationship (Puşcaş et al., 2008, Silvertown et al., 2009, Fady and Conord, 2010, Taberlet et al., 2012, Avolio and Smith, 2013). Indeed, the results are in line with meta-analysis by Whitlock (2014), which found no overall relationship between species diversity and neutral genetic diversity across 33 studies.

The lack of a relationship between species and genetic diversity may result from differences in the factors found to be important in influencing them. As such, the parallel processes theorised to be acting on species and genetic diversity are not having parallel effects. Species diversity is predominantly being influenced by area, habitat loss, 1930s subdivision, solar radiation levels, and grazing by sheep. By contrast genetic diversity was found to be independent of the study variables (*C. acaule*), or influenced by levels of nitrogen and potassium in the soil (*R. bulbosus*).

The results highlights that, despite the theory behind the SGDC, too little is still understood about the relationship between species and genetic diversity to be able to recommend management for high genetic diversity in order to enhance species diversity.

8. Discussion

8.1 Preamble

The aim of this research was to investigate the patterns of habitat, species, and genetic diversity in fragmented lowland calcareous grasslands, and to determine the spatial and temporal drivers of these three levels of biodiversity. In order to address this aim the study worked to answer eight research questions. In the following sections, the results of the study will be related to each research question in turn.

8.2 What is the extent of change in habitat diversity and landscape structure within the SDNP between the 1930s and 2012?

To analyse temporal change in landscape structure in the SDNP, the modelled historical LULC data was compared with the contemporary LULC data. This revealed a substantial level of landscape transformation between the 1930s and 2012. In the 1930s the landscape was allocated comparatively evenly to four main habitats: arable (22%), woodland (18%), calcareous grasslands (29%), and mesotrophic grasslands (20%). However by 2012 the SDNP was dominated by arable land (61%), with woodland also prominent (21%). By contrast semi-natural habitats (grassland and heathland) had substantially declined to total only 6% of the SDNP.

This transformation and loss is a major driver in the decline in habitat diversity between the 1930s and 2012. Between the 1930s and 2012 a decline in habitat diversity across the SDNP was found, with simultaneous declines in habitat diversity surrounding all the study sites. Although previous studies have documented the transition of natural and semi-natural habitat to arable land over the past century (Burnside et al., 2003, Swetnam, 2007b, Hooftman and Bullock, 2012), few previous studies have analysed temporal change in habitat diversity. Exceptions have, however, reported a decline of habitat diversity in agricultural landscapes (Bengtsson-Lindsjo et al., 1991, Ihse, 1995, Olsson et al., 2000). Therefore the results of the present study provide an indication of the extent of change in habitat diversity in Britain. Furthermore they document the transition from a diverse landscape of natural and semi-natural habitats in the

1930s to landscape dominated by arable land in 2012, within the SDNP study area.

Similarly, Burnside et al. (2002) documented substantially decreased landscape evenness, one of the components of landscape diversity, on the West Sussex Downs between 1971 and 1981, and further decreases between 1981 and 1991. The findings also support several previous studies that have reported a decline in habitat diversity in agricultural landscapes (Bengtsson-Lindsjo et al., 1991, Ihse, 1995, Olsson et al., 2000). Under the habitat heterogeneity hypothesis, niche theory, and the spatial mass effects theory, the consequences of this decline in habitat diversity would be a decline in niche opportunities and thus less opportunities to maintain species diversity (Hutchinson, 1957, Whittaker, 1975, Shmida and Wilson, 1985, Ricklefs, 1987, Tilman and Pacala, 1993, Rosenzweig, 1995, Chase and Leibold, 2003). However, the nature of the relationship between habitat diversity and species diversity is unclear (Lundholm, 2009).

Over the eighty-year period of the two data-sets an overall loss of 82% of semi-natural habitats in the SDNP is revealed. In particular, losses included: calcareous grasslands (88%); mesotrophic grasslands (91%); acid grasslands (94%); and heathland (81%). Using data from two periods, the present study makes no attempt to pinpoint the timings of the LULC change detected. Thus it is interesting to compare the results to those of Burnside et al. (2003), who reported a 58% decline in unimproved grassland habitat extent between 1971 and 1991. Based on the West Sussex Downs, the Burnside et al. (2003) study is contained within the region used in the present study, and highlights that substantial losses to grassland habitat continued between 1971 and 1991. Whilst the results of the present study measured landscape change over an eighty year period, the study by Burnside et al. (2003) measured change between 1971, 1981, and 1991. Based on the West Sussex section of the SDNP, Burnside et al. (2003) found that the loss of semi-natural grassland and the increase in arable land continued within this period. However, comparison of the 1991 data for West Sussex with the 2012 data for the entire SDNP, suggest that LULC may have remained relatively stable in this period. A comparison between these studies shows that arable land comprised 64.8% of the West Sussex Downs in 1991, compared to 60.9% for the whole SDNP in 2012, and semi-natural grassland comprised 4.2%, compared with 6.1% for the whole SDNP in 2012.

Moreover, the findings are similar to previous studies that measured the loss of grassland habitat in Britain more generally. In a study of LULC change in Dorset between the 1930s and 2000, Hooftman and Bullock (2012) reported a loss of 74% of semi-natural habitats, with declines of 83% of calcareous grassland, 97% of mesotrophic grasslands, 61% of acid grasslands, and 56% of heathlands. Similarly, in other studies over periods greater than fifty years, Fuller (1987) reported a 92% loss of unimproved grasslands, and Swetnam (2007b) reported declines in chalk grasslands of 43% . The majority of previous studies that measure the loss of grasslands in Britain have focused on ecologically short time intervals, with only three studies covering periods greater than fifty years (Fuller, 1987, Swetnam, 2007b, Hooftman and Bullock, 2012). However, the periods examined in those specific studies and this study, provide a valuable opportunity to examine the effects of historical landscape structure on ecological processes.

The major direction of LULC change was the conversion of grassland and heathland to arable land. All types of 1930s grassland and heathland had a higher probability of being converted to arable land by 2012 than to have remained as grassland. In particular 1930s calcareous grasslands had only a 10% chance of remaining as calcareous grasslands by 2012, but a 69% probability of being converted to arable land. Indeed arable land gained area from all of the other LULC types, including 18% of the 1930s woodland. These changes resulted in arable land increasing in extent by 172% from the 1930s to 2012. Woodland appeared to have retained its 1930s extent (73% of 1930s woodlands remained woodland in 2012). Furthermore the model suggests that the overall woodland extent increased by 19.1%. These findings are similar to those of Hooftman and Bullock (2012), who reported an increase of 25% over a similar period of time. In the present study woodland particularly gained extent from heathlands, with 56% of the 1930s heathlands being woodlands by 2012.

Transition to woodland was the second largest factor in the loss of calcareous grasslands (transition probability of 11%), followed by loss to miscellaneous (typically urbanisation) (transition probability of 9%). These findings are in agreement with those of Burnside et al. (2003), who reported a large transition of grasslands to arable lands (40% between 1971-81, and 39% between 1981-91), and Hooftman and Bullock (2012) who reported 47% of calcareous grassland making a transition to arable land between the 1930s and 2000. In contrast the present study and the study by Hooftman and Bullock (2012), Burnside et al. (2003) found substantial areas of grasslands were not converted in the period of their study (48% of 1971 grasslands remained in 1981, and 36% of 1981 grasslands remained in1991). These findings by Burnside et al. (2003) suggest that the rate of conversion may have steadied by this period. In agreement to the model results presented here, Hooftman and Bullock (2012) report that the main transition of heathland was to woodland. However in contrast to the present study, Hooftman and Bullock (2012) found considerable transition of heathland to arable, built-up, and improved grassland.

8.3 What patterns of fragmentation can be identified in twelve calcareous grassland study sites between the 1930s and 2012?

The focus of this thesis was on the effects of patch level variability on biodiversity. As such, twelve calcareous grassland study sites with different characteristics were carefully selected. In line with the substantial landscape change across the SDNP, at the patch level substantial change to both landscape composition and configuration was also observed. This allows for the analysis of the relationship between change in landscape structure and biodiversity.

Across the twelve sites selected for more detailed analysis, the fragmentation metrics revealed significant losses in area, core area index values, and connectivity. Indeed, all twelve of the study sites were subject to substantial loss in their extent between the 1930s and 2012, with ten of the sites being reduced by more than 90%. These substantial and similar levels of loss have been reported in previous work by Hooftman and Bullock (2012) who report a decrease in mean patch area of 82% in rough calcareous grasslands and 94%

in managed calcareous grasslands between the 1930s and 2000. Moreover, the significant reduction in connectivity mirrors the findings of Hooftman and Bullock (2012) who found that connectivity decreased between the 1930s and 2000 for calcareous grasslands, acid grasslands and heathlands. An expected consequence of reduced connectivity would be less opportunity for colonisations and gene flow between populations, thus limiting species and genetic diversity under the principles of the Theory of Island Biogeography and Wrights island model of population genetics. Moreover, previous work has linked habitat diversity to species diversity (Fahrig et al., 2011, Redon et al., 2014), and thus the decline in habitat diversity found may be replicated in other levels of biodiversity. However, the generalisation of this relationship remains under question (Lundholm, 2009).

Reductions in habitat area may lead to reductions in species richness in line with the species-area hypothesis (MacArthur and Wilson, 1967, Rosenzweig, 1995, Lomolino, 2000). However, the nature of the relationship in calcareous grasslands has been inconsistent, with some studies finding a positive relationship (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012), and other studies have found no relationship (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a). Reductions in habitat patch area are likely to have been accompanied by reductions in the population sizes of some species (Fahrig, 2003), which is, in turn, likely to have resulted in a reduction in genetic diversity (Leimu et al., 2006, Honnay and Jacquemyn, 2007). Similarly, the changes in landscape composition led to increased fragmentation and reduced connectivity between semi-natural grassland patches. The likely outcome of such change is the reduced movement of species between grassland patches (Soons et al., 2005, Soons and Ozinga, 2005). This in turn is predicted to result in reductions in species diversity (Ouborg, 1993, Honnay et al., 1999, Bruun, 2000, Krauss et al., 2004, Lindborg and Eriksson, 2004b) and genetic diversity (Young et al., 1996, Young and Clarke, 2000).

In addition to this loss in extent, there was evidence of fragmentation of semi-natural habitats with substantially reduced core areas, number of patches, and mean patch areas, with a simultaneous increase in the average nearest distance to another patch of the same grassland type. The decline in the number of patches for each grassland type was less dramatic than the loss in total extent. This phenomenon was most apparent in calcareous grasslands where the 88% decline in extent was coincidental with a decline in the number of patches by 15%, and suggested that remnant grasslands were small, fragmented patches. The structure of heathland was particularly interesting with an increase in the number of patches from 105 in the 1930s to 275 in 2012 (162% increase). This was largely a result of large patches being broken down into multiple smaller patches. Calcareous grassland patches reduced from a mean of 45.6ha in the 1930s to 6.9ha in 2012, observation that are similar to the changes reported by Hooftman and Bullock (2012) in managed calcareous grasslands in Dorset between the 1930s and 2000 (40.4ha to 2.5ha). Similarly changes in mean patch size for heathland (31.9ha to 2.4ha), mesotrophic grassland (19ha to 2.9ha), acid grasslands (9.5ha to 2.1ha) and woodland (10.8ha to 2.9ha), were all comparable with the changes reported by Hooftman and Bullock (2012) (heathland: 52.6ha to 6.3ha; rough mesotrophic grassland: 10.1ha to 2.4ha; acid grassland 9.1ha to 2.6ha; and woodland 7.5ha to 5.1ha).

The identification of a reduced number of patches and an increased average nearest neighbour for grasslands also concurs with the findings of Burnside et al. (2003). However, Burnside et al. (2003) reported an increased mean patch area in unimproved grasslands between 1971 and 1991, in contrast to the findings of this study. This may reflect differences that emerge due to the larger temporal period of this study compared to the shorter timescale within their study, and the difficulty in identifying specific site boundaries within the 1930s data. The 1930s site boundaries were derived within ArcMap 10.1 (ESRI, 2012) by selecting contiguous features from the 1930s calcareous grassland data that overlapped the contemporary extent of the site. In the 1930s there were large contiguous areas of calcareous grassland across the SDNP. As a result, the area classified as the 1930s sites were typically much vaster than that contemporary sites. Moreover, the 1930s site for two of the contemporary sites

(Cissbury Ring and Steep Down) covered the same area. It was decided that despite this shortcoming, the validation of the 1930s data meant that the extent and locations of the 1930s sites were valid.

Although previous studies have documented the transition of natural and semi-natural habitat to arable land over the past century (Burnside et al., 2003, Swetnam, 2007b, Hooftman and Bullock, 2012), only a limited number of previous studies have explicitly measured temporal change in habitat diversity (Bengtsson-Lindsjo et al., 1991, Ihse, 1995, Olsson et al., 2000). Therefore these results presented here provide an indication of the extent of change in habitat diversity in Britain, and document the transition from a diverse landscape of natural and semi-natural habitats in the 1930s to landscape dominated by arable land in 2012, within the SDNP study area.

8.4 To what extent do contemporary and historical landscape and environmental characteristics influence species diversity and composition?

Focusing on the twelve calcareous grassland study sites, analysis of the field surveys revealed the variability in species richness between sites. In relation to the historical, spatial, management, and abiotic characteristics of the sites, this variability in species richness was shown to be influenced by the contemporary area, historical subdivision, habitat loss (%), solar radiation, and grazing by sheep. Species evenness was found to be influenced by habitat loss (%) and historical subdivision.

The extent of habitat loss at sites was found to be important in explaining the variability of both species richness and species evenness, with negative relationships found for both of these measures of species diversity. This finding offers support for the notion that fragmentation of habitats has a negative influence on species diversity, and concurs with the results of previous studies in semi-natural grasslands (Cousins et al., 2007, Krauss et al., 2010).

The results of the present study show that on the South Downs, both contemporary and historical factors are important in explaining species richness. This finding is similar to previous studies (Bruun et al., 2001, Cousins et al., 2007), however, other studies have found only historical factors (Lindborg and Eriksson, 2004b, Helm et al., 2006, Krauss et al., 2010), or only contemporary factors influenced species diversity (Adriaens et al., 2006, Oster et al., 2007, Cousins and Eriksson, 2008).

In contrast to the findings for species diversity, no relationships were found between the explanatory variables and species composition. However, core area index values explained 15.5% of the variation in the composition of forb species. The finding of a relationship with forbs but not all species supports the notion that habitat specialists are expected to be more sensitive to habitat change and extinction debt (Helm et al., 2006, Kuussaari et al., 2009). Specifically, the results suggest that the composition of forb species is sensitive to changes in contemporary core area. Conversely, the composition of other species appears to be less predictable, and not related to any of the explanatory variables used in the study.

Livestock units were not found to be significant in influencing either species richness or composition. This finding may be explained by the Intermediate Disturbance Hypothesis (Grime, 1973, Connell, 1978), which outlines that intermediate levels of disturbance produce the highest levels of species richness. The nature of the testing of variables within this study meant that linear relationships between the variables were being tested.

The results add further evidence of the complexity of the relationship between species diversity/composition and site variability. Despite a recent interest in studying the influence of contemporary and historical variables, there has been little consistency in the factors found to effect species diversity and composition. To date a range of abiotic, spatial, historical, and management factors have been found to effect species diversity and composition in semi-natural grasslands. However, there has been no consistency between studies the factors found to influence species diversity, and moreover contradictory results have been found for some factors.

Implications of the findings are that conservation efforts focusing on species diversity should consider not only methods to make the contemporary landscape characteristics more favourable, but also need to consider the influence of the historical landscape on conservation efforts. Moreover, the results implicate the requirement to consider the effects of conservation efforts on both species diversity and species composition. Strategies to maintain or improve species diversity may have corresponding negative effects for the persistence of particular species. Conversely, when attempting to conserve particular species, the effect of appropriate conservation of such species should consider the potential impact on species diversity.

8.5 Is there evidence of an extinction debt in species diversity or composition?

For an extinction debt to be evidenced, species diversity would be more closely matched to historical factors than contemporary factors. Conversely, if there was no extinction debt present then species diversity would be more closely matched to contemporary factors than historical factors. Both contemporary (area, solar radiation, management) and historical (habitat loss (%) and 1930s subdivision) factors were important in explaining species diversity in the present study, a finding consistent with previous studies (Bruun et al., 2001, Cousins et al., 2007). By contrast, Helm et al. (2006), Lindborg and Eriksson (2004b), and Krauss et al. (2010) found that historical landscape structure, but not contemporary landscape structure, was related to species richness. The authors conclude that these findings provide evidence that the plant species patterns were formed under landscape conditions that no longer exist, and that the sites are in a period of extinction debt. Further contrasting findings have found that contemporary factors were more important than historical factors in explaining species richness (Adriaens et al., 2006, Oster et al., 2007, Cousins and Eriksson, 2008). These differences may be the result of different methodological approaches of the different studies.

The influence of both contemporary and historical factors on species diversity suggests that the study sites may be in a period of transition, where they are beginning to reflect the contemporary structure of the site, but are still retaining some of the characteristics of their historical structure. Moreover, the results illustrate the complex nature and interplay between both historical and contemporary landscape structure on species presence, abundance, and persistence across the landscape. Extinction debt occurs due to the lagged response of species to the loss and fragmentation of habitat patches (Tilman et al., 1994). Some species may not be optimally suited to the present configuration of the patch they inhabit and are therefore likely to become locally extinct without appropriate change to site structure. Indeed previous studies have found species to be under an extinction debt for periods of between forty and three-hundred-and-fifty years (Bruun et al., 2001, Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins et al., 2007, Krauss et al., 2010).

In contrast to the results for species diversity, the composition of forb species was found to be related to contemporary factors (CAI) but not historical factors. Alard et al. (2005) found that historical variables (colonisation by trees in 1952, 1973 and 1991, and past land-use in 1824, 1914 and 1991) explained 30% of the variability in species composition. These variables focus on the historical management of the site, in contrast to the historical variables in the present study that focus on spatial structure. As such the relationship with species composition detected by Alard et al. (2005) but not in the present study suggests that the historical management of a site may be more influential in species composition than historical spatial structure.

Kuussaari et al. (2009) suggest that the extinction debt is related to spatial and temporal scales, with the response being faster in smaller and isolated habitats. Moreover, following a review of studies of the extinction debt in grassland plants, Cousins (2009) comments that, generally, in landscapes with less than 10% of grassland remaining, plant species richness is more related to contemporary than historical landscapes. The overall figure of remaining calcareous grassland from the SDNP between the 1930s and 2012 is 12%, a figure on the borderline of the 10% cut-off proposed by Cousins (2009). This would suggest that the losses in extent of calcareous grassland may be at a tipping point. At present enough of the habitat remains for some of the historical characteristics to still influence species patterns. However the loss has been significant enough for the species patterns to lose some connection with historical factors and begin to reflect contemporary factors. Moreover there is the suggestion that any further loss of habitat may lead to a greater loss of the influence of historical factors on contemporary species diversity. Furthermore, the conclusions of Cousins (2009) suggest that any further loss in calcareous grassland extent in the study region may result in species already in an extinction debt becoming locally extinct.

In this study there was a period of approximately eighty years between the historical measures and the contemporary measures. Two studies covering shorter periods of time failed to find relationships between species richness and the historical landscape: Oster et al. (2007) found that connectivity in 1946-1954 did not influence contemporary species richness; and Cousins et al. (2007) found no relationship between species richness and connectivity 50 years before. However, several other authors have found relationships between species richness/diversity and the historical landscape over similar or greater periods of time: Lindborg and Eriksson (2004b) found that historical connectivity was related to species diversity at both 50 and 100 years before present; (Reitalu et al., 2012) found that the amount of grassland surrounding a site in 1800 influenced contemporary species richness of grassland specialists; (Gustavsson et al., 2007) found that the land-use of a grassland site in the 18th and 19th century influenced contemporary species richness; and (Helm et al., 2006) found that area and connectivity in the 1930s influenced species richness in 2001. By contrast, Adriaens et al. (2006) found no relationship between species richness and patch area or connectivity in 1775, 1850, 1875, 1905, 1968, or 1984. Overall, these findings suggest that the eighty year time period between the historical and contemporary data in the present study is sufficient to test the effects of the historical landscape on species patterns.

The links between the contemporary species patterns and the historical landscape highlight the importance of future conservation within the study area. Where contemporary biodiversity is a legacy of historical conditions, then the development of conservation programs must also consider the historical landscape. With increased knowledge of the extinction debt theory, conservation can work towards conserving species that are in extinction debt, by changing the conditions. As such it is essential for conservation to focus not only on the effects of landscape change on biodiversity, but to also consider temporal scales of biodiversity response to historical and ongoing landscape change (Eriksson and Ehrlen, 2001, Hanski and Ovaskainen, 2002, Lindborg and Eriksson, 2004b).

Overall, from the analysis of both species and genetic diversity, the existence of an extinction debt is unclear. Although both species diversity and genetic diversity were related to habitat loss and species diversity was related to 1930s subdivision, there was no overwhelming evidence that biodiversity more closely related to the historical landscape than the contemporary landscape. Indeed, both species diversity (area, radiation, and grazer) and the genetic diversity of *R. bulbosus* (soil nitrogen and potassium) were related to several contemporary characteristics. This may highlight that the study sites are in a period of transition whereby the still reflect aspects of the historical landscape, but are also in the process of adapting to landscape change. This may reflect differences between species in their response to landscape change.

8.6 What relationships exist between species diversity and species composition?

Species diversity was related to factors of both the contemporary and historical landscape, as well as to the local environmental conditions and management. By contrast the composition of all species was not related to any of the study variables, and the composition of forbs only to contemporary CAI values. This initially appears to contradict the suggestion of Gibson and Brown (1991) that species richness does not take a long time to develop, but species composition can take centuries to stabilise. On this principle, it may be expected that species richness would be related to the contemporary landscape as it more quickly adapts to landscape change. The slower process of development of species composition may be expected to relate to the historical landscape until it has had a long enough time-lag to come into equilibrium with the contemporary landscape. However, the results presented here suggest that site history remains important in determining contemporary species richness, but is not influential in determining species composition. Although species richness may be reflective of the historical landscape, the composition of these species is not. Whilst the present study failed to find a relationship between the historical landscape and species composition, relationships were found between contemporary variables and the composition of forb species. Intuitively it may be read that the relationship between species richness and historical variables suggests that species richness is responding slowly to changes in the landscape. Conversely, the lack of a relationship between species composition and historical variables may suggest that species composition has responded more quickly to landscape change. However, the relationship between the historical landscape and species richness may be because after landscape change species richness is more quickly able to return to historical levels. It may not be that the species richness of sites with a high level of historical subdivision have remained as a reflection of its historical configuration throughout the eighty year period of the study, but that sites with a historical high level of subdivision have been more quickly recolonised or colonised by new species following local species extinctions after initial habitat loss. Indeed the failure to find a similar relationship with species composition supports this notion, suggesting that whilst species richness may reflect the historical landscape, this species richness is comprised of a different set of species that are determined by contemporary variables.

Despite the relationship between species diversity and historical factors, there was no relationship between species diversity or composition and historical site connectivity, in contrast to the findings of previous research (Lindborg and Eriksson, 2004b, Helm et al., 2006, Reitalu et al., 2012). This may reflect that site connectivity was not a limiting factor for species in the 1930s. As stated previously, historical connectivity to other sites was greater than contemporary connectivity, and may have been ample for the flow of species between sites.

Previous studies that have found relationships between species richness and historical factors have concluded that this is evidence of an extinction debt (Lindborg and Eriksson, 2004b, Helm et al., 2006). Extinction debt describes the delayed extinction of species that are not in equilibrium with their environment.

An extinction debt would lead to the extinction of species that were not able to persist under modified conditions. As such it would be expected to impact on both species richness and species composition at similar rates. As a species becomes extinct species richness is lowered, and the species no longer contributes to species composition. However, the results suggest that species richness and species composition differ in their response to the study variables. Species richness was influenced by both historical and contemporary variables, whilst species composition was not influenced by either.

In contrast to the studies concerning species diversity, to date, few studies have tested the relationship between species composition and historical factors. One exception is provided by Alard et al. (2005), although this study tested historical management as opposed to the historical spatial structure measures of the present study. Therefore the results presented here provide an important first insight into the nature of the effect of historical spatial factors on species composition. Moreover, the study is the first to document that historical factors influence species diversity and species composition in different ways. The study highlights the importance of measuring both species richness and composition. Previous studies on semi-natural grasslands have made conclusions based upon species richness/diversity (Krauss et al., 2004, Lindborg and Eriksson, 2004b, Helm et al., 2006, Cousins et al., 2007, Reitalu et al., 2012) or species composition (Barbaro et al., 2004, Alard et al., 2005) in isolation, with very few studies incorporating aspects of both (Klimek et al., 2007). By analysing species richness and composition together, the results presented here are the first to describe the underlying and interactive nature of the relationship between landscape variability and species patterns.

8.7 What is the structure of gene flow across twelve calcareous grassland study sites for two plant species synonymous with calcareous grassland sites in South East England?

Population differentiation (F_{ST}) for both species was significantly greater than zero ($p<0.05$). This indicates an excess of homozygosity between sites due to differing allele frequencies, which typically results from barriers to gene flow. H_e was higher in *R. bulbosus* than *C. acaule*, with H_o higher in *C. acaule* than *R.*

bulbosus. There was little consistency in F_{IS} between the species, with only three sites having complimentary significant F_{IS} values for both species. By contrast, two sites had significant inbreeding of *C. acaule* and significant outbreeding of *R. bulbosus*. This shows that there were differences between the two species in their response to barriers to gene flow.

No differences in the level of genetic variation of the two study species were found. Similarly, in a study of two species with different dispersal methods, Hooftman et al. (2004) found no differences in genetic variation. Genetic variation was shown to be independent of the distance between sites, showing that factors other than distance act as barriers to gene flow.

There was no evidence of isolation by distance in *C. acaule*. Interestingly the populations appeared to be either genetically similar or genetically distant, with few relationships in-between (Figure 6.1(a)). This pattern was consistent irrespective of geographical distance, offering further evidence that the populations are panmitic. There appears to be gene flow between all populations, as evidenced by the samples with low genetic distance across all geographical distances. Further research may wish to investigate the nature of the pattern displayed in (Figure 6.1(a)), however for the purposes of this research it can be concluded that there is no relationship between geographical and genetic distance in *C. acaule*.

By contrast, Jump et al. (2003) found a significant correlation between genetic distance and geographical distance in five *C. acaule* populations in England. However some of the populations studied by Jump et al. (2003) were at the edge of their geographic range, where there is a decline in density of populations and in seed production (Jump and Woodward, 2003). As a consequence of declining population density and seed production, populations become increasingly isolated (Brown, 1984), which can lead to pronounced geographical structuring in genetic variation (Lesica and Allendorf, 1995). Furthermore the populations studied by Jump et al. (2003) were separated by distances of up to 300km, compared to a maximum distance of 90km in the present study. As a wind dispersed species, occasional long-distance dispersal may occur in *C. acaule* (Higgins and Richardson, 1999, Cain et al., 2000). However, shorter distances provide fewer barriers to gene flow, and as such the shorter distances between the sites in the present study may have reduced the impact of isolation by distance. By contrast the greater distances between sites in the Jump et al. (2003) study may explain the discrepancies between the results presented here. Moreover, because of an increased distance from their periphery, the SDNP populations are likely to be less affected by the declines in density and seed production experienced by some of the populations studied by Jump et al. (2003). This finding also highlights that in the SDNP factors other than distance between sites are responsible for the genetic variation between sites.

No effect of isolation by distance was found in *R. bulbosus*, in contrast to Hahn et al. (2012) studying nine populations in the Swiss Alps. The study by Hahn et al. (2012) was conducted at elevations of between 1200-1800 metres above sea level in a mountainous landscape. As such, distance may have acted as a greater barrier to gene flow than in the SDNP, and may explain the differences in the results of the studies. Therefore, whilst the excess homozygosity reported by Hahn et al. (2012) can in part be attributed to distance between sites acting as a barrier to gene flow, in the SDNP other factors influence the dispersal of *R. bulbosus*.

8.8 To what extent do contemporary and historical landscape and environmental characteristics influence the genetic diversity of the two study species?

Habitat loss (%) was important in explaining the variability of both H_o and F_{IS} in *C. acaule*. A positive relationship was found between habitat loss (%) and H_o, with a negative relationship between habitat loss (%) and F_{IS}. Both of these relationships are counterintuitive, as habitat loss would be expected to have a negative effect on genetic variation and lead to increased inbreeding as the population size decreases. Moreover, the findings contrast the comments of previous authors who have argued that inbreeding is greatest within more fragmented populations (Ellstrand and Elam, 1993, Young et al., 1996, Allendorf et al., 2012). These findings may result from traits of the species; specifically that *C. acaule* is a calcicole species specialised to grassland habitats. Although the markers employed are assumed to be neutral, the preponderance of positive heterozygosity-fitness correlations suggests that microsatellite markers can often be linked to genes under selection (Morgante et al., 2002). Specialism, by nature, is expected to result in a loss of genetic variation across large parts of the genome because it corresponds to a phenotype optimally adapted to a restricted niche (Li et al., 2014). Adaptation by the process of directional natural selection can decrease genetic variation in populations by selecting for or against a specific gene and its neighbourhood. Moreover, the restricted habitat requirement of specialist species can result in lower abundance, more limited gene flow, and smaller effective population sizes in comparison to generalist species (Habel et al., 2015). As a result specialist species can have reduced genetic variation and increased genetic differentiation (Crnokrak and Barrett, 2002, Louy et al., 2007, Habel et al., 2013), compared to higher genetic variation and lower genetic differentiation in generalist species (Louy et al., 2007, Dennis et al., 2011, Habel et al., 2013). A reduction in inter-species competition may then arise as habitat loss leads to species extinctions under the principles of the species-area relationship. This loss of competition can then lead to the prediction that by reducing the intensity of selection to specialise, populations become more genetically diverse as a result of genetic variants becoming selectively neutral. Alternatively, once interspecies competition has ceased, intraspecies competition can be mitigated by populations adapting (by disruptive selection) to alternative resources no longer used by competitors (Svanback and Bolnick, 2007). As such, habitat loss may be leading to a reduction in inter-species competition, allowing *C. acaule* to become less specialised, and as a result more genetically variable. Conversely, at sites that have experienced lower levels of habitat loss, *C. acaule* has continued to specialise and as a result has lower levels of genetic variation at these sites.

No variables were important in explaining the variability in H_e of *C. acaule*. Similarly, none of the environmental/management variables were found to be important in explaining any of the genetic variation/inbreeding measures for *C. acaule*. The failure to find a relationship between management and genetic variation is particularly interesting as it contrasts previous studies in other plant species (Honnay et al., 2006, Last et al., 2014, Rico et al., 2014). The patterns identified in the studies by (Honnay et al., 2006) and (Rico et al., 2014) refer specifically to grazing. The wind dispersal strategy of *C. acaule* may therefore explain the contrasting findings, as the species is less dependent on grazers for transportation of genes, and so is less influenced by management factors.

Although none of the explanatory variables were important in explaining H_e or F_{IS} of *R. bulbosus*, levels of soil nitrogen and potassium were found to be important in explaining the variability in the observed heterozygosity. Few previous studies have tested the relationship between genetic variation and abiotic factors. However the results for *R. bulbosus* are consistent with those of (de Vere et al., 2009) who report a positive relationship between phosphorous levels and genetic variation (allelic richness) in *Cirsium dissectum*. One possible explanation for this relationship is the possible increased flowering in soils with increased phosphorous (Jongejans et al., 2008). Increased flowering could lead to increased reproduction, and as a consequence increased genetic variation. Alternatively, phosphorous may promote reproduction through increasing the survival of seedlings (de Vere et al., 2009). A further explanation could be the effect of phosphorous as a limiting factor in species diversity (Willems et al., 1993, Janssens et al., 1998). Lower species diversity could lead to higher populations of the fewer species present at a site, and this higher population size would be expected to have higher genetic variation. However this explanation is not satisfactory on several counts. First, it contradicts the ideas of the species-genetic diversity correlation; second, no relationship was found between species diversity and phosphorous; and third, nitrogen is also a limiting factor in species diversity (Mountford et al., 1993, Willems et al., 1993, Smith, 1994, Klimek et al., 2007), yet in contrast to the finding for phosphorous a negative relationship existed between nitrogen and genetic variation of *R. bulbosus*. As such this relationship may be specific to a particular trait of *R. bulbosus*. Further investigation into the relationship between this species and soil nutrients is required to further explain the relationship.

No relationship was found between the genetic variability and historical factors, showing that the genetic variation of *R. bulbosus* may be responding quickly to landscape change, and is being driven by contemporary factors within the landscape. This finding supports the findings of previous studies that have found genetic variability to be related to contemporary but not historical factors (Honnay et al., 2006, Helm et al., 2009). However whilst the contemporary factors important for *R. bulbosus* were soil nutrients, Honnay et al. (2006) found contemporary management to be important in influencing the genetic variation of *Anthyllis vulneraria*, and Helm et al. (2009) found *Briza media* was related to contemporary habitat connectivity. As such, although the genetic variation of all three species shows relationships with contemporary factors, the nature of the relationship appears to be species specific. Moreover, other studies have by contrast found historical factors to influence genetic variation of plant species (Munzbergova et al., 2013).

8.9 What relationships exist between habitat, species, and genetic diversity, and is there evidence of a species genetic diversity correlation?

There was no relationship between contemporary habitat diversity, species diversity, and genetic diversity. This again shows the complexity of biodiversity within calcareous grasslands. The SGDC theory suggests that parallel processes act on species and genetic diversity to produce similar responses. However, Vellend and Geber (2005) comment that high competition pressure may restrict the alpha niche of a species and thus may stabilise selection. As such a negative relationship between species and genetic diversity would be witnessed. Vellend and Geber (2005) comment that there may be a trade-off between the effects of parallel processes and the effects of competition pressure. Indeed there may be a point at which these two polarising factors have an equal weight, resulting in no relationship between species and genetic diversity. The high species richness of calcareous grasslands may result in a sufficient level of competition pressure for such an effect, explaining the lack of a SGDC found here.

Alternatively, the failure to find a correlation between species and genetic diversity may be explained by the differences in the factors influencing these two levels of biodiversity. The SGDC theory proposes that parallel processes act on both species and genetic diversity to produce a parallel response. However, this study found different factors to be important in explaining species and genetic diversity. Moreover, for the two study species the factors explaining variability in genetic diversity were different. As such, it appears that species and genetic diversity respond in different ways to the parallel processes acting on them. Differences in how species and genetic diversity are affected by the characteristics of a site may result from differences in their response to landscape change. Although genetic diversity can take a long time to stabilise (Koch and Kiefer, 2006, Soltis et al., 2006), genetic diversity would be expected to respond more quickly to landscape change than species diversity (Helm et al., 2009), as the results of this study suggest.

Although both theory (the habitat heterogeneity hypothesis and the spatial mass effects theory) and empirical studies (Skov, 1997, Sotherton and Self, 2000, Bruun et al., 2001, Pausas et al., 2003, Dufour et al., 2006, Poggio et al., 2010, Janisova et al., 2014, Sutcliffe et al., 2015) suggest that habitat diversity and species diversity may be positively related, the nature of the relationship is not fully understood (Lundholm, 2009). The observed variation in habitat diversity across study sites provided not only the opportunity to test the relationship between habitat diversity and species diversity, but was extended to examine the three-way relationship between habitat, species, and genetic diversity.

9. Conclusion

9.1 Key findings

This study has shown that historical survey data can be used to model the historical landscape in a way that it is comparable to contemporary data. From this, substantial change in landscape composition, landscape configuration, habitat diversity, and the structure of lowland calcareous grassland habitat patches within the SDNP were found.

Species diversity and composition varied between the twelve lowland calcareous grassland study sites. GLMs showed that, for species richness this variability could be explained by contemporary site area, the level of subdivision in the 1930s, the percentage habitat loss, the amount of solar radiation, and grazing by sheep. Similarly species evenness could be explained by the level of subdivision in the 1930s and the percentage habitat loss. The factors influencing species composition were less clear. RDA showed that no factors were found to be influential in explaining the composition of all the recorded species, however, 15.5% of the variation in composition of forb species could be explained by core area index values.

For the two study species genetic variation was not a product of the distance between study sites. GLMs found that the genetic variation of *C. acaule* was positively influenced by the amount of habitat loss, whilst the genetic variation of *R. bulbosus* was influenced by levels of soil N and K.

Although similar factors influenced variability in the different levels of diversity, no relationships were established between habitat diversity, species diversity, and genetic diversity. This highlights the need for biodiversity conservation efforts to consider the factors influencing all levels of biodiversity, and not to focus on one measure.

9.2 Limitations

Although the historical landscape modelling method was validated, there will be come discrepancies between the model outputs and the actual historical landscape. Sources of error may be from distortion during the scanning of the original 1930s Land Utilisation Survey maps, and because georeferencing was carried out for the whole country in one phase. Furthermore in the detection of landscape change between the 1930s and 2012 the different characteristics of the source data may have resulted in discrepancies. The landscape change analysis was also restricted to two points in time: the 1930s and 2012. As such, no assumptions can be made that the change over time was linear.

A range of landscape metrics were used to analyse both the contemporary and the historical landscape. Although these metrics were carefully selected to measure particular aspects of the landscape, the relationship between these metrics and ecological processes requires caution. Kupfer (2012) argues that relationships between landscape metrics and ecological processes are often presumed rather than established. Similarly, connectivity between sites can be underestimated as plant populations can remain in the landscape, but not necessarily within the specific grassland habitats on which connectivity is being measured (Oster et al., 2007).

In the genetic analysis of *R. bulbosus*, the microsatellite marker RB302 did not successfully genotype. In addition, the other three markers for *R. bulbosus* had a lower success rate than the four markers used for *C. acaule*. As such the analysis of genetic variation for *R. bulbosus* was based on less data than for *C. acaule*.

9.3 Recommendations for further study

This study found a positive relationship between site area and species richness, which supports the species-area hypothesis, the Theory of Island Biogeography (MacArthur and Wilson, 1967), metapopulation theory (Hanski, 1999), and several other studies on calcareous grasslands (Bruun, 2000, Krauss et al., 2004, Adriaens et al., 2006, Cousins et al., 2007, Oster et al., 2007, Raatikainen et al., 2009, Reitalu et al., 2012). However, other studies have failed to find a relationship between area and species richness in calcareous grasslands (Eriksson et al., 1995, Partel and Zobel, 1999, Kiviniemi and Eriksson, 2002, Lindborg and Eriksson, 2004a). As such the nature of the relationship between species richness and area within calcareous grasslands is not clear. However, a wealth of studies that measure the relationship have been published, and so meta-analysis of these studies could offer a clearer picture of the relationship.

The results of this study provided limited information on the factors influencing species composition. However, the finding that the composition of forbs was influenced by core area index values suggests that the characteristics of the species may be important. Further research could therefore investigate the factors influencing species composition using different or more detailed categorisations of species.

Different factors were found to influence the genetic variation of the two study species. However, reasons for these differences were unclear, with no apparent relationship between the influential factors and the dispersal methods of the species. Further research on other calcareous grassland species is therefore recommended in order to gain a clearer understanding of the nature of the interaction between genetic variation, landscape factors, and dispersal methods.

This research found no evidence of a relationship between species diversity and genetic diversity. Whilst this is finding is congruent with some previous research, it contradicts the SGDC theory. As such there is an opportunity for future research to establish if the findings reported here are consistent for other species within fragmented calcareous grasslands, or if differences in the relationship exist for different plant species. Such further research on different species would allow for the detection of more general patterns and to analyse patterns for particular species traits. Here, two plants with different dispersal patterns were studied and produced similar results in terms of no positive SGDC. However, the relationship between plant traits and plant community response to landscape change is not well understood (Lindborg, 2007, Aggemyr and Cousins, 2012). Therefore future research could examine the effects of different plant traits on the SGDC.

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Appendices

Table A.9.1 Full list of species and their frequencies within study sites across the SDNP. The results from the present study (2013 column) are provided alongside data from previous studies within the SDNP. Core species for each period as in black, with rare and intermediate species appearing in grey. 1920s data sourced from Tansley and Adamson (1925), 1990s data sourced from Steven (1992) and Steven and Muggeridege (1992), 2013 data from present study.

	1920s	1990s	2013
Achillea millefolium	76	90	100
Agrimonia eupatoria	10	21	92
Agrostis stolonifera	54	77	100
Anthoxanthum odoratum	70	45	8
Asperula cynanchica	76	70	92
Avenula pratensis	95	92	75
Avenula pubescens	70	66	58
Bellis perennis	65	49	75
Brachypodium pinnatum	20	32	92
Briza media	95	83	100
Bromopsis erecta	62	86	100
Carex caryophyllea	52	77	92
Carex flacca	100	99	100
Carlina vulgaris	54	34	75
Centaurea nigra	67	89	83
Cirsium acaule	98	97	100
Crataegus monogyna	0	40	92
Dactylis glomerata	72	100	100
Euphrasia officinalis agg	60	53	83
Festuca ovina	95	100	100
Galium mollugo	7	71	92
Galium verum	65	76	100

Gentianella amarella	40	52	83
Helianthemum nummularium	15	32	75
Hieracium pilosella	76	78	92
Holcus lanatus	47	74	100
Koeleria macrantha	85	76	8
Leontodon hispidus	80	94	100
Linum catharticum	93	90	92
Lotus corniculatus	90	100	100
Medicargo lupulina	50	77	67
Phleum pratense bertolonii	32	51	83
Phyteuma orbiculare	70	63	58
Pimpinella saxifraga	85	91	83
Plantago lanceolata	100	100	100
Plantago media	60	73	75
Polygala calcarea	0	15	83
Polygala vulgaris	54	67	100
Prunella vulgaris	65	86	100
Ranunculus bulbosus	65	78	100
Rubus fruticosus agg.	0	24	75
Sanguisorba minor	95	95	100
Scabiosa columbria	90	89	92
Senecio jacobaea	37	76	92
Thymus praecox arcticus	98	88	92
Trifolium pratense	87	95	100
Trifolium repens	35	46	100
Trisetum flavescens	70	74	25
Veronica chamaedrys	45	23	100
Viola hirta	47	85	92
Viola riviniana	27	29	100

Table A.9.2 Complete list of calcicole, forb, grass, and indicator species recorded at the twelve study sites. Age of site indicator species are after Gibson and Brown (1991) and Karlik and Poschlod (2009). Calcicole species were identified after Ellenberg indicator values (Ellenberg et al., 1991), with strong calcicoles identified as species with F (Moisture) ≤4, R (pH) ≥7, N (Nitrogen) ≤ 4. Ellenberg indicator values for the species recorded were taken from Hill et al. (1999). Positive and agricultural/negative site indicators are after JNCC (2004).

Calcicole	Forbs	Grasses	Ancient site indicator	Positive indicator	Agricultural/negative indicator
Agrimonia eupatoria	*Achillea millefolium*	*Agrostis stolonifera*	*Asperula cynanchica*	*Agrimonia eupatoria*	*Bellis perennis*
Anacamptis pyramidalis	*Agrimonia eupatoria*	*Alopecurus pratensis*	*Bromus erectus*	*Anthyllis vulneraria*	*Cirsium arvense*
Anthyllis vulneraria	*Anacamptis pyramidalis*	*Anthoxanthum odoratum*	*Carex caryophyllea*	*Asperula cynanchica*	*Cirsium vulgare*
Asperula cynanchica	*Anthyllis vulneraria*	*Arrhenatherum elatius*	*Festuca ovina*	*Campanula glomerata*	*Cynosurus cristatus*
Avenula pratensis	*Arenaria serpyllifolia*	*Avena fatua*	*Filipendula vulgaris*	*Carex caryophyllea*	*Holcus lanatus*
Avenula pubescens	*Asperula cynanchica*	*Avenula pratensis*	*Galium verum*	*Carex flacca*	*Lolium perenne*
Brachypodium pinnatum	*Bellis perennis*	*Avenula pubescens*	*Helianthemum nummularium*	*Carlina vulgaris*	*Senecio jacobaea*

Calcicole	**Forbs**	**Grasses**	**Ancient site indicator**	**Positive indicator**	**Agricultural/negative indicator**
Bromopsis erecta	*Blackstonia perfoliata*	*Brachypodium pinnatum*	*Hippocrepis comosa*	*Centaurea nigra*	*Trifolium repens*
Campanula glomerata	*Calluna vulgaris*	*Brachypodium sylvaticum*	*Koeleria macrantha*	*Centaurea scabiosa*	*Trisetum flavescens*
Carex caryophyllea	*Campanula glomerata*	*Briza media*	*Veronica officinalis*	*Cirsium acaule*	
Carlina vulgaris	*Campanula rotundifolia*	*Bromus erectus*		*Clinopodium vulgare*	
Centaurea scabiosa	*Carlina vulgaris*	*Carex caryophyllea*		*Filipendula vulgaris*	
Cirsium acaule	*Centaurea nigra*	*Carex flacca*		*Galium verum*	
Clinopodium vulgare	*Centaurea scabiosa*	*Cynosurus cristatus*		*Geranium sp*	
Cotoneaster horizantalis	*Centaurium erythraea*	*Dactylis glomerata*		*Helianthemum nummularium*	
Daucus carota	*Cerastium fontanum*	*Danthonia decumbens*		*Hieracium pilosella*	
Filipendula vulgaris	*Cirsium acaule*	*Festuca ovina*		*Hippocrepis comosa*	
Galium mollugo	*Cirsium arvense*	*Holcus lanatus*		*Leontodon hispidus*	

Calcicole	**Forbs**	**Grasses**	**Ancient site indicator**	**Positive indicator**	**Agricultural/negative indicator**
Gentianella amarella	*Cirsium dissectum*	*Koeleria macrantha*		*Lotus corniculatus*	
Helianthemum nummularium	*Cirsium eriophorum*	*Lolium perenne*		*Polygala calcarea*	
Hieracium pilosella	*Cirsium vulgare*	*Luzula campestris*		*Polygala vulgaris*	
Hippocrepis comosa	*Clinopodium vulgare*	*Phleum pratense bertolonii*		*Potentilla erecta*	
Knautia arvensis	*Cornus sanguinea (g)*	*Trisetum flavescens*		*Primula veris*	
Koeleria macrantha	*Cruciata laevipes*	*Unknown grass B*		*Sanguisorba minor*	
Leontodon hispidus	*Dactylorhiza fushii*			*Scabiosa columbria*	
Leucanthemum vulgare	*Daucus carota*			*Thymus praecox arcticus*	
Medicargo lupulina	*Euphrasia officinalis agg*			*Thymus pulegioides*	
Ophrys fuciflora	*Filipendula vulgaris*				
Orchis ustulata	*Fragaria vesca*				
Origanum vulgare	*Galium mollugo*				
Phyteuma orbiculare	*Galium verum*				

Calcicole	**Forbs**	**Grasses**	**Ancient site indicator**	**Positive indicator**	**Agricultural/negative indicator**
Picris hieracoides	*Gentianella amarella*				
Pimpinella saxifraga	*Geranium sp*				
Plantago media	*Glechoma hederacea*				
Polygala calcarea	*Gymnadenia conopsea*				
Primula veris	*Helianthemum nummularium*				
Ranunculus bulbosus	*Hieracium pilosella*				
Salvia pratensis	*Hippocrepis comosa*				
Sanguisorba minor	*Hypericum hirsutum*				
Scabiosa columbria	*Hypericum perforatum*				
Thymus pulegioides	*Knautia arvensis*				
Trisetum flavescens	*Leontodon hispidus*				
Viola hirta	*Leucanthemum vulgare*				
	Linum catharticum				

Calcicole	Forbs	Grasses	Ancient site indicator	Positive indicator	Agricultural/negative indicator
	Lotus corniculatus				
	Medicargo lupulina				
	Ononis repens				
	Ophrys fuciflora				
	Orchis ustulata				
	Origanum vulgare				
	Pastinaca sativa				
	Phyteuma orbiculare				
	Picris hieracoides				
	Pimpinella saxifraga				
	Plantago lanceolata				
	Plantago media				
	Polygala calcarea				
	Polygala vulgaris				
	Potentilla anserina				
	Potentilla erecta				
	Potentilla reptans				

Calcicole	**Forbs**	**Grasses**	**Ancient site indicator**	**Positive indicator**	**Agricultural/negative indicator**
	Potentilla sterilis				
	Primula veris				
	Prunella vulgaris				
	Ranunculus acris				
	Ranunculus bulbosus				
	Rhianthus minor				
	Rumex acetosa				
	Rumex acetosella				
	Salvia pratensis				
	Sanguisorba minor				
	Scabiosa columbria				
	Senecio jacobaea				
	Stachys officinalis				
	Stellaria graminea				
	Succisa pratensis				
	Taraxacum spp				
	Teucrium scorodonia				

Calcicole	Forbs	Grasses	Ancient site indicator	Positive indicator	Agricultural/negative indicator
	Thymus praecox arcticus				
	Thymus pulegioides				
	Trifolium pratense				
	Trifolium repens				
	Ulex europaeus				
	Umbelliferae sp.				
	Unknown B				
	Unknown C				
	Unknown D				
	Unknown E				
	Unknown X				
	Veronica arvenis				
	Veronica chamaedrys				
	Veronica officinalis				
	Vicia cracca				
	Viola hirta				

Calcicole	Forbs	Grasses	Ancient site indicator	Positive indicator	Agricultural/negative indicator
	Viola riviniana				

Table A.9.3 Contribution to total variance (×10^2) in CAI values of species in the redundancy analysis (RDA) for forb species and CAI.

Species	RDA (×10^2)	Species	RDA (×10^2)	Species	RDA (×10^2)
Centaurea nigra	65.51	Stachys officinalis	2.79	Unknown B	-2.15
Cirsium arvense	29.27	Potentilla erecta	2.04	Gymnadenia conopsea	-2.17
Senecio jacobaea	27.74	Vicia cracca	1.92	Asperula cynanchica	-2.55
Phyteuma orbiculare	27.04	Picris hieracoides	1.90	Lotus corniculatus	-2.84
Galium mollugo	19.03	Cruciata laevipes	1.89	Rumex acetosa	-3.16
Succisa pratensis	18.59	Glechoma hederacea	1.87	Rumex acetosella	-3.24
Hippocrepis comosa	18.57	Plantago media	1.81	Hieracium pilosella	-3.34
Sanguisorba minor	15.24	Daucus carota	1.66	Veronica officinalis	-3.70
Ranunculus bulbosus	13.93	Potentilla anserina	1.56	Fragaria vesca	-4.01
Polygala vulgaris	11.84	Unknown C	1.04	Prunella vulgaris	-4.04
Linum catharticum	11.31	Arenaria serpyllifolia	1.01	Pastinaca sativa	-4.31
Filipendula vulgaris	10.47	Veronica arvenis	1.01	Ranunculus acris	-4.43
Cirsium acaule	8.62	Agrimonia eupatoria	0.99	Teucrium scorodonia	-4.47
Trifolium	8.51	Umbelliferae	0.70	Campanula	-4.48

pratense		sp.		glomerata	
Achillea millefolium	7.60	Cirsium eriophorum	0.68	Gentianella amarella	-4.65
Cerastium fontanum	7.59	Unknown D	0.58	Blackstonia perfoliata	-4.89
Veronica chamaedrys	7.32	Centaurium erythraea	0.49	Geranium sp	-5.12
Polygala calcarea	7.11	Orchis ustulata	0.40	Cirsium vulgare	-5.31
Trifolium repens	7.11	Unknown X	0.40	Galium verum	-5.74
Taraxacum spp	7.03	Hypericum hirsutum	0.36	Calluna vulgaris	-6.20
Knautia arvensis	6.97	Medicargo lupulina	0.20	Plantago lanceolata	-7.28
Carlina vulgaris	6.80	Unknown E	0.20	Campanula rotundifolia	-11.34
Hypericum perforatum	6.66	Potentilla sterilis	-0.49	Clinopodium vulgare	-12.20
Thymus praecox arcticus	6.58	Stellaria graminea	-0.61	Leontodon hispidus	-13.98
Anthyllis vulneraria	6.30	Salvia pratensis	-1.04	Scabiosa columbria	-16.21
Anacamptis pyramidalis	5.70	Ulex europaeus	-1.04	Origanum vulgare	-17.80
Primula veris	5.40	Cornus sanguinea (g)	-1.05	Helianthemum nummularium	-18.43
Rhianthus minor	4.92	Thymus pulegioides	-1.05	Pimpinella saxifraga	-20.12
Ononis repens	4.32	Leucanthemum vulgare	-1.32	Viola riviniana	-22.66
Ophrys Afuciflora	3.92	Centaurea scabiosa	-1.63	Euphrasia officinalis agg	-22.95

Bellis perennis	3.65	Potentilla reptans	-1.64	Viola hirta	-23.63
Dactylorhiza fushii	3.27	Cirsium dissectum	-1.65		