The biodiversity of the Wealden ghyll woodlands: species richness, abundance and distribution patterns in a rare and fragmented habitat

Andrew R. Flint

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

March 2014

The University of Brighton

- For my mum -

Dungeon-Ghyll Force

William Wordsworth

Along the river's stony marge
The sand-lark chants a joyous song;
The thrush is busy in the wood,
And carols loud and strong.
A thousand lambs are on the rocks,
All newly born! both earth and sky
Keep jubilee, and more than all,
Those boys with their green coronal;
They never hear the cry,
That plaintive cry! which up the hill
Comes from the depth of Dungeon-Ghyll.

Said Walter, leaping from the ground,
"Down to the stump of yon old yew
We'll for our whistles run a race."
--Away the shepherds flew;
They leapt--they ran--and when they came
Right opposite to Dungeon-Ghyll,
Seeing that he should lose the prize,
"Stop!" to his comrade Walter cries--
James stopped with no good will:
Said Walter then, exulting, "Here
You'll find a task for half a year.

"Cross, if you dare, where I shall cross--
Come on, and tread where I shall tread."
The other took him at his word,
And followed as he led.
It was a spot which you may see
If ever you to Langdale go;
Into a chasm a mighty block
Hath fallen, and made a bridge of rock:
The gulf is deep below;
And, in a basin black and small,
Receives a lofty waterfall.

Wordsworth's notes:

Title: "Ghyll," in the dialect of Cumberland and Westmoreland, is a short and, for the most part, a steep narrow valley, with a stream running through it.

"Force" is the word universally employed in these dialects for waterfall.

Abstract

The Wealden ghyll woodlands are associated with unique plant assemblages that include nationally rare bryophyte species with oceanic affiliations. The identification and monitoring of this type of 'priority' habitat, recognised as important in terms of regional and national biodiversity, is a central facet of the UK Biodiversity Action Plan (UKBAP). Despite the acknowledged importance of ghyll woodlands for non-vascular plant species, previous studies attempting to examine and characterise the ghyll woodlands have neglected to include these bryophyte communities. This research identifies and characterises the Wealden ghyll woodlands through an examination of the spatial and temporal distributions of bryophyte and flowering plant species. The research also seeks to provide baseline data against which biodiversity levels can monitored. In order to identify and contextualise the importance of ghyll woodland in terms of regional biodiversity, survey data was collected from other types of ancient woodland throughout the region for comparative analysis. The study involved the collection of species and environmental data from a total of 1440 random quadrats from 60 survey sites situated throughout the Weald, as well as the use of archive survey data collected during two 20 year periods (1951-1970 and 1976-1995). A number of statistical approaches including general linear modelling, ANOSIM, Mann-Whitney U and Spearman rank correlation analysis were used to identify the environmental correlates of spatial and temporal changes in species distributions. Spatial analysis indicated that ghyll woodland is restricted to the stream valleys themselves which were significantly richer in bryophyte and flowering plant species than the surrounding woodlands. NVC classifications assigned to the ghylls indicated the presence of 'oceanic' plant communities that are associated with damp, humid microclimatic conditions. A number of authors have explained the presence of oceanic bryophytes within the ghylls as being the result of a damp, humid microclimate present within the stream valleys. However, the study found no significant differences between climatic conditions within the ghyll valleys and those in the surrounding ancient woodlands. ANOSIM analysis indicated that community composition was influenced by site substrate, with clay and sandstone ghyll woodlands containing significantly different plant communities. Chi-squared analysis identified a temporal increase in the ratio of oceanic bryophytes and ancient woodland indicator flowering plant species during the study period. Analysis of Ellenberg indicator values indicated a move towards more shade-tolerant plant communities within the ghyll woodlands.

The patchily distributed ghyll woodlands were examined for signs of habitat fragmentation through genetic analysis of the bryophyte *Conocephalum conicum* (Great Scented Liverwort) using the random amplification polymorphic DNA technique (RAPD). Wright's fixation index (F_{ST}) and Nei's coefficient of gene variation (G_{ST}) both indicated a loss of genetic diversity characteristic of genetic isolation. A Mantel test based on Nei's genetic distance values indicated that the genetic isolation observed was not correlated with the geographical distance between populations.

The study indicated that temporal changes are occurring in the composition of ghyll woodland plant communities and that bryophyte populations are displaying symptoms of genetic isolation. The study illustrates the importance of some form of monitoring program if the biodiversity value of these sites is to be maintained.

Table of Contents

List of Figures

List of Tables

Appendices

Acknowledgements

This thesis is dedicated to Amanda, Tonika, Cali, Zed and Nellie. Amanda you have always been there to support me, giving up your time at university so that I could get finished and keeping me sane when the pressure was on. I know how many of the day to day chores you took on so that I could concentrate on my thesis and without your help this would never have been possible. Tonika, Cali, Zed and Nellie, you have gone without many things over the years so that Mum and Dad could carry out their studies and you've never complained. Studying with four children around could have been a nightmare, but instead you all went out of your way to help and made things so much easier. Thank you.

My sincere thanks also go out to a number of people who helped me during this study. Firstly, I would like to thank my supervisors, Dr Dawn Scott and Dr Niall Burnside for all the constructive advice and support that they have given me. Their comments made my thesis far better than it would have otherwise been. I would also like to thank Dr Stephen Waite for all his help in the early stages of my PhD.

I am very grateful to many other academics and technicians within the university for help with different aspects of the project: Dr Andy Overall for his help with both the genetic analysis and with statistics, Dr Chris Joyce for helping me identify unusual plant species and for his advice, Pete Lyons and Chris English for their help, advice and tolerance during the months I spent in their lab carrying out a variety of soil analyses.

I would like to thank the British Bryophyte Society (BBS) whose members helped me out immensely with training and advice. In particular I would like to thank Howard Matcham and Dr David Streeter from the BBS who were always willing to answer my questions. Thanks also go to the Sussex Wildlife Trust for the data provided for me by Penny Green and for the advice given to me by Dr Fran Southgate. I would like to thank The High Weald.org and the Sussex Wildlife Trust for their help and I am also very grateful to all the landowners who gave me permission to survey woodlands on their lands.

I have an enormous debt of gratitude to Dr Francis Rose who sadly is no longer with us, but who had a passion for the Wealden woodlands and whose notebooks made much of my analysis possible.

However, the most important people to help with this project were the volunteers who gave up their free time to help me carry out my surveys, whilst simultaneously donating their blood to the local mosquito populations. Thank you to Kelly Fouchy, Caitrona Donohoe, Karin Johansson, Digby Wheeler and Emma Preston.

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

4th June 2014

Chapter 1 Introduction

1.1 The Wealden ghyll woodlands

The Weald is the name given to the area of south-east England that lies between the parallel chalk escarpments of the North and South Downs. The name Weald is derived from the German ‘wald’ meaning ‘an uncultivated wilderness’ (High Weald Landscape Trust 2014). It was bestowed on the area by Saxons and Kentish Jutes in reference to the immense wooded area that at the time covered much of Surrey, Kent and Sussex (Brandon 2003). The central part of the Weald is known as the High Weald and contains a landscape of ‘rolling hills, studded with sandstone outcrops and cut by streams to form steep-sided ravines; small irregular-shaped fields and patches of heathland, abundant woodlands; scattered farmsteads and sunken lanes and paths’ (Highweald.org 2013). This is surrounded by the Low Weald, a much flatter area of extensively wooded, gently undulating countryside (Brandon 2003).

Today woodland is still the primary land cover within the Weald (Patmore 2000) and much of this woodland occurs within stream valley systems known locally as ghyll woodlands. They are internationally important habitats on the basis of both ecology and geomorphology (Ratcliffe 1968, Rose and Patmore 1997, Burnside et al. 2006). Burnside et al. (2002a) calculated that there were 1130 separate ghyll woodland patches situated throughout the Wealden area. GIS analysis showed 92% of the ghyll systems are located within the High Weald, whilst 6% reside in the Low Weald with the remaining 2% found in the Romney Marshes (Burnside et al. 2002b). The distribution of ghyll woodland throughout the Wealden region is shown in Figure 1.1.

Ghyll woodlands typically occupy steep-sided valleys and contain a stream that over thousands of years has cut through the underlying rock to create the ravine (Robinson and Williams 1984, Pentecost 1991). The deeply incised valleys may be up to 60 metres deep, representing a sizable geological feature in lowland England (Rose and Patmore 1997). Similar features exist in hilly and mountainous areas elsewhere in Britain where they are locally referred to as cloughs, dingles,

gills or glens, but unlike these valleys, the Wealden ghyll valleys are associated with a relatively flat plateau terrain (Rose and Patmore 1997). The sheer-sided nature of the valleys means they are generally inaccessible to livestock and some of the valleys may historically have remained relatively undisturbed and unmanaged (Ratcliffe 1968, Burnside 2002a).

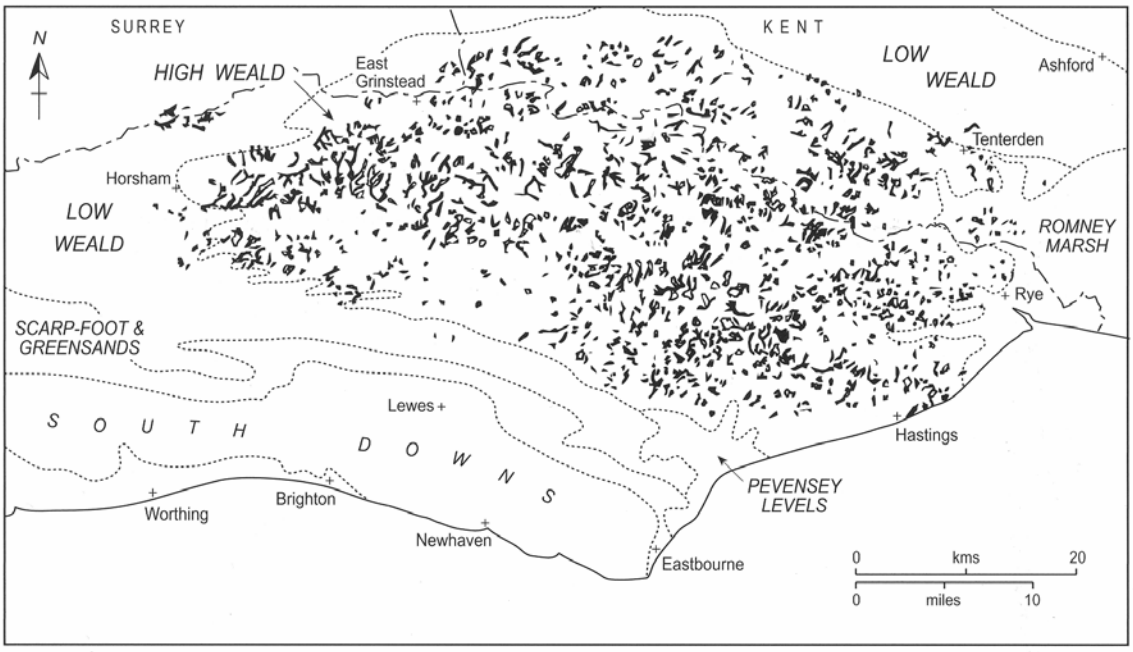


Figure 1.1 The distribution of ghyll woodlands throughout the Weald. Reprinted from Brandon (2003).

The enclosed nature of the valleys is believed to create a warm and moist microclimate similar to conditions found in woodlands in the south of England during the Atlantic period 8000 - 5000 years BP (Ratcliffe 1968, Southgate 2012). Indeed, the lush growths of bryophytes and ferns associated with the ghyll woodlands are reminiscent of flora that existed in southern woodlands during the Atlantic period (Rose and Patmore 1997). Today, similar conditions are absent from eastern and central Britain and are only found in the Atlantic Forests on the western seaboard (Rose and Patmore 1997). The Wealden ghyll woodlands are a unique habitat that supports vascular and non-vascular plant communities that are not found all together anywhere else in Europe (Rose and Patmore 1997).

1.2 The formation of the Wealden ghyll woodlands

1.2.1 The significance of the Wealden geology

The rocks that formed the High Weald were the product of river and sea sediment; deposited in horizontal beds (see Figure 1.2) (Wooldridge and Goldring 1953).

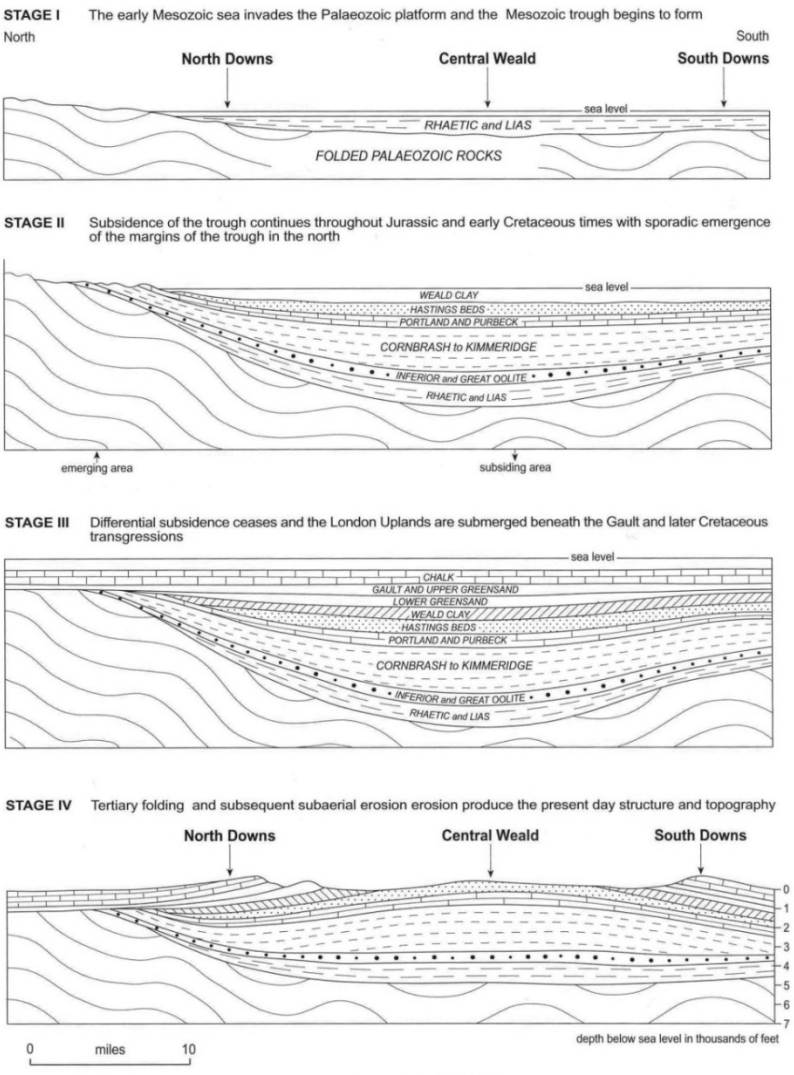


Figure 1.2 Geological evolution of the Weald. Reprinted from Brandon (2003).

The earliest of these beds, known as the Purbeck Limestone Group, were formed in shallow lagoons around 142 million years BP (before present). Further beds were formed that consisted of iron-rich clays and sandstones and were known as

the Hastings Group. The oldest of these beds consisted of Ashdown Sands, which were followed by Wadhurst Clays and then Tunbridge Wells Sands (Wooldridge and Goldring 1953). Rivers and flood-plains continued to deposit sediment creating further beds which became known as the Weald Clay Formation. This process continued until approximately 110 million years BP when the whole area subsided below sea-level (Worssam 1995).

Over the next 35-40 million years new beds were deposited. Initially this consisted of clays which were then followed by sands and then chalk. After this came a long period of uplift that continued until approximately 1.8 million years BP. This created a massive chalk-topped dome reaching around 970 metres OD (ordnance datum) which is nowadays referred to as the Wealden Anticline. Most of this chalk dome was eroded through the actions of rivers and streams leaving a rim which forms the North and South Downs (see Figure 1.2). The erosion of the soft Weald Clay formation in the centre of the anticline left older geologies of the Hastings Group exposed (Wooldridge and Goldring 1953). The rate of erosion differed between the soft clays and harder sandstones that form the Hastings Group.

The Weald is traversed by an east-west trending anticlinorium (a complex fold) (Brandon 2003). Throughout the Wealden region this results in the three substrates that form the Hastings Beds appearing at the surface in close proximity to each other (Rose 1995, Radley 2006). In places, these substrates have been eroded by streams to form valleys and it is these valleys that contain ghyll woodland. Where streams have eroded the sandstone beds they have often cut deeply into the rock substrate to form steep-sided rock walled ravines (Rose and Patmore 1997). Ghyll valleys that have formed on the softer Wadhurst Clays are usually far less steep (Burnside et al. 2006).

The grains forming the sandstone rocks within the Weald are loosely compacted and consequently they have a high water holding capacity (Porley and Hodgetts 2009). Evaporation of water absorbed by these rocks contributes to the humid microclimate that is said to exist within the ghyll valleys (Rose and Patmore 1997). These types of loosely compacted sandstone rocks occur in only three other areas throughout lowland Europe – the Forest of Fontainebleau in France, the Petite

Suisse in Luxembourg and Elbsandstungeberg on the Czech-German border (Brandon 2003).

Ghyll woodland floral diversity is linked to substrate diversity and is highest within ghyll valleys that contain the most geological and geomorphological variation (Rose and Patmore 1997).

1.2.2 The history of the Wealden woodlands

The biodiversity 'value' of the Wealden ghyll woodlands is enhanced by the long ecological continuity of the woodlands which is explained by historical patterns of land-use and woodland management within the Weald.

The 'climax' forest types found in Britain at the end of the Atlantic period (8000 - 5000 BP) were the last that could be described as wholly natural (Peterken 1993). These formed woodlands that covered the majority of the British Isles, the distribution of which was almost completely determined through natural environmental controls (Godwin 1975). Rackham (1986) named this 'natural' woodland the 'Wildwood', a name now widely used to refer to the woodland of this period. Pollen profiles indicate that in around 5000 BP there was a sharp and widespread decrease of Elm (*Ulmus*) and Ivy (*Hedera helix*) (Hinde 1985). Ecologists believed this to be the first evidence of large scale human impact on the natural woodland (Peterken 1993). Ivy was commonly used to feed cattle since grass was scarce and there is little doubt that an increase in domestic livestock was responsible for the decreasing abundance of Ivy (Peterken 1993).

The clearance of the Wildwood continued and extended into higher altitudes and by 2500 years BP over half of the Wildwood in Britain had been cleared (Rackham 2000). At the time of the Domesday Book in 1086 forest cover was down to around 15% (Rackham 2000) and by 1870 less than 5% of the country remained wooded (Smith 2000). In 1919 the Forestry Commission was established to address the problem of Britains rapidly disappearing woodlands. They created a number of initiatives that led to an increase in forest coverage to around 9% by the year 2000 (Smith 2000). Although this figure is a substantial increase on the figures for 1870,

it still makes Britain one of the least wooded countries in Europe (Forestry Commission 1996).

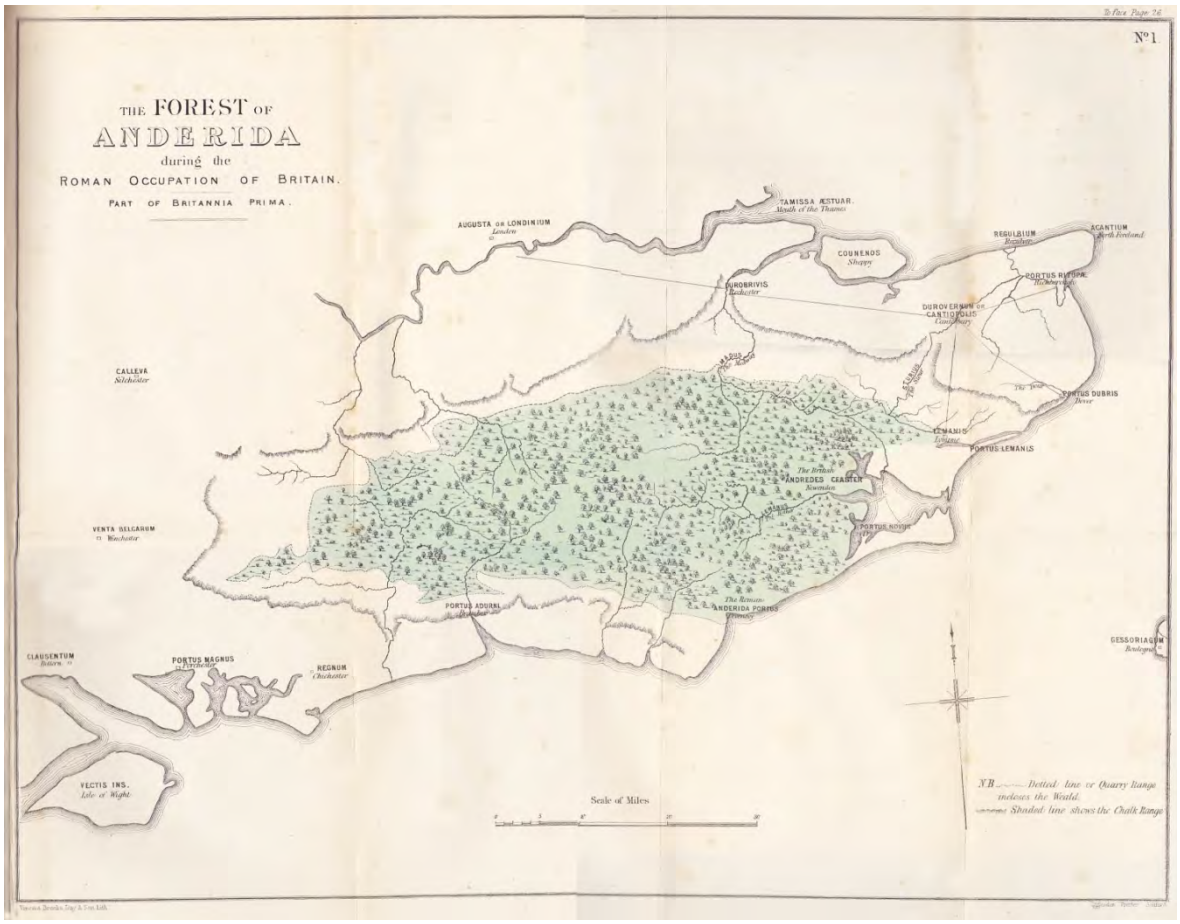


Figure 1.3 A historical map showing woodland coverage in the Weald at the time of the Roman occupation of Britain. Reprinted from Furley and Mackeson (1874).

This woodland clearance did not occur uniformly throughout England, for instance the Wealden area of Kent and Sussex has historically maintained a high level of woodland cover (Brandon 2003). Figure 1.3 shows that at the time of the Roman occupation of Britain (43 - 410 AD) the majority of the Weald was covered in woodland. Figure 1.4 shows a representation of woodland coverage throughout England in 1086 (Rackham 1990) and it can be seen that the Weald contained the largest area of woodland left in England, whilst the more open downs that border the Weald had effectively been cleared much earlier (Brandon 2003). A number of factors contributed to the preservation of the Wealden woodlands. The heavy clay

soils would have made agriculture difficult and the terrain of ridges and ravines would have proved difficult to navigate with merchandise or equipment (Brandon 2003) meaning that historically the area has had a relatively low number of human settlements (Peterken 1993).

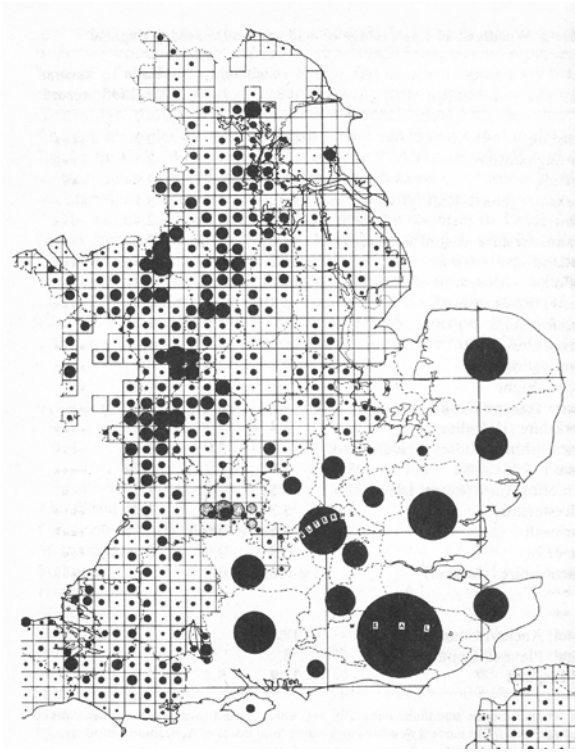


Figure 1.4 The abundance of woodland throughout England as recorded in the Domesday Book of 1086. The black dots represent total woodland area measurements for woodlands in those areas. The large dot in the south-eastern corner of the map is an estimate of the total amount of woodland in the Wealden area. Reprinted from Rackham (1990).

During the 12th and 13th centuries the Wealden countryside changed dramatically as much of the remaining woodland was cleared to provide suitable areas for fields, farms and villages (Brandon 1974, Waller and Marlow 1994, Brandon 2003). The steep-sided, often boggy valleys of the Weald would have been unsuitable for this type of land-use and as such the majority remained wooded (Rose and Patmore 1997).

1.2.2.1 Coppice management in the Weald

During the 16th and 17th centuries, the extensive Wealden iron industry relied heavily on the local woodlands to provide charcoal for its blast furnaces (Brandon

1974). The large amounts of fuel-wood consumed by the furnaces led to concerns about local fuel shortages and a commission of enquiry was set up in 1548 which reported that approximately fifty ironworks existed in East Sussex at this point in time, each consuming around 1,500 loads of 'great wood' per year (Brandon 2003). A large scale industry such as this, running for several centuries, would have exhausted the supply of local fuel-wood unless much of the woodlands were managed for this purpose. To provide a continuous supply of fuel-wood some type of permanent coppice system must have been used to provide charcoal for the Wealden ironworks (Rackham 2000). The extensive and long-term use of coppice management within the Weald meant that large areas of woodland were preserved within the region during a period of widescale woodland clearance throughout the rest of the British Isles (Rackham 1990, Brandon 2003).

Coppice management involves cutting certain tree species down to the base. In southern Britain coppice species traditionally consisted of hazel (*Corylus* sp.), hornbeam (*Carpinus* sp.), beech (*Fagus* sp.), ash (*Sorbus* sp.) or oak (*Quercus* sp.) (Hammersley 1973). As long as a stump or even just a root system is left intact the tree will send out multiple shoots from which crops of poles can eventually be cut (Packham et al. 1992). The rate of shoot growth is very rapid and can exceed five centimetres per day (Rackham 2006). The poles are usually cut when they are between 10 and 25 years old, at which point the cycle begins again and the stool will send up another set of shoots which will eventually form the next crop of poles. This cycle of cutting and re-growth prolongs the life of the trees with carbon dating showing that some trees with a 'natural' life-span of less than 100 years have lived for over 1000 years when managed in this way (Buckley 1992).

Coppicing continued to be the most important woodland management system until its decline during the 20th century. Figure 1.5 shows the dramatic decrease in coppice management in the woodlands of the south-east. In 1947 around 40% of the woodlands in the area were being coppiced; however by 2002 this figure had dropped to around 7% (Forestry Commission 2004). This continued a decline that had begun in the late 19th century (Brandon 2003).

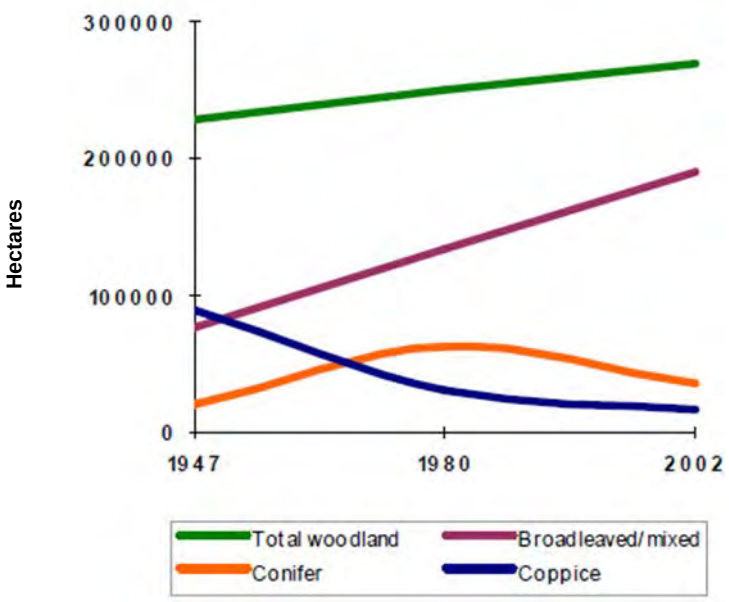


Figure 1.5 Changes in woodland area in south-east England from 1947-2002. The area of woodland being actively coppice managed during the period is also shown. Reprinted from Forestry Commission (2004).

1.3 Ancient woodland within the Weald

Ancient woodlands are defined as areas that have been wooded continuously since 1600 AD (Spencer and Kirby 1992). The threshold date of 1600 AD marks a time when cartographic evidence of the presence of woodland became more widely documented and marked the beginning of the period in which plantation woodlands became more widespread (Spencer and Kirby 1992).

In 2002 the south-east region contained around 270,000 hectares of woodland (Figure 1.5) representing 14.6% of the land area of the south-east and making it the most wooded region in England (Forestry Commission 2004). Approximately 122,700 hectares of these woodlands were classified as ancient woodland which represents around 40% of the total ancient woodland recorded for the whole of the country (Forestry Commission 2004).

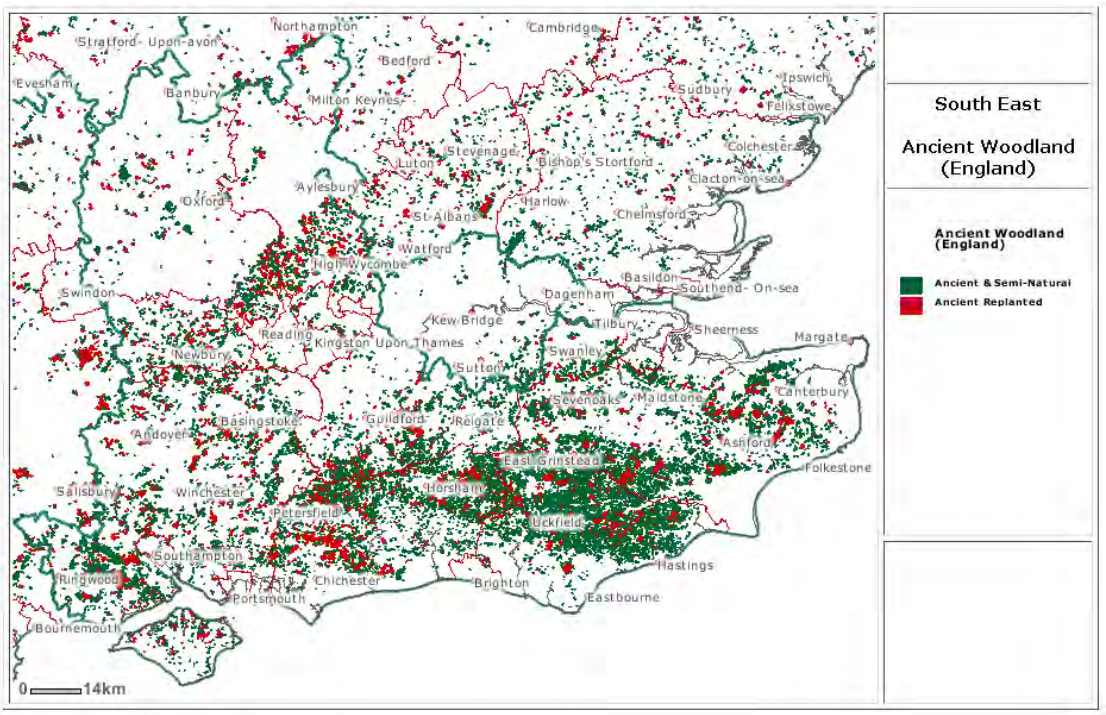


Figure 1.6 The location of Ancient Woodland in the south-east of England. The density of Ancient Woodland is particularly great in the High Weald which is the dense area of woodlands in the south-east of the map. Produced using 'Magic' maps (MAGIC 2011).

Ancient woodlands have been described as the most important category of British woodlands for both biodiversity and nature conservation (Peterken 1993). The woodlands not only contain rich species assemblages of both plants and animals, but also a very high proportion of Britain's rare and endangered woodland species. Many of these rarer species require the stable environment afforded by the long continuity of ancient woodlands (Spencer and Kirby 1992, Bailey et al. 2002, Goldberg et al. 2007). They have been described as reservoirs that have served to maintain the wildlife of the countryside (Goldberg et al. 2007). The continued existence of these woodlands through time means that their species composition may resemble woodland communities that existed in prehistoric times (Goldberg et al. 2007).

It is a general rule that the older the woodland, the more species it will support and the more species supported the more important the site will be for nature conservation (Rose 1999). The richest sites tend to be those that have been continuously wooded for the longest period of time (Rackham 2000). It is therefore possible to use the number of species present as an indication of both age and habitat quality. It is also possible to use the presence of particular species as

indicators of the presence of ancient woodland. As such, a list of vascular plant species that showed a strong affinity for ancient-woodlands in the south of England was compiled by the National Conservancy Council for the purpose of identifying ancient woodland (NCC) (Hornby 1987). Bryophytes and lichens were considered too difficult to identify to be useful as indicator species whilst vascular plants were considered both easy to locate and identify (Rose 1999). The list contained 100 ancient woodland indicator (AWI) species that were believed to be reliable indicators of the presence of ancient woodland in the south of England. Many species commonly found in the Wealden ghyll woodlands are limited to ancient woodland within the region (Bailey et al. 2002). These include the AWI species *Anemone nemorosa* (Wood Anemone), *Hyacinthoides non-scripta* (Bluebell) and *Paris quadrifolia* (Herb Paris), as well as butterflies such as *Boloria euphrosyne* (Pearl-bordered fritillary) and *Argynnis paphia* (Silver-washed fritillary), and small mammals such as dormice (*Muscardinus avellanarius*) and yellow-necked mice (*Apodemus flavicollus*) .

1.4 The biodiversity of ghyll woodlands

1.4.1 Ghyll woodland bryophytes

The Wealden ghyll woodlands support a rich flora of woodland bryophytes (Ratcliffe 1968). They are particularly important for many oceanic species which are restricted in the south-east of England to the ghyll woodlands, and that are hundreds of kilometres from other British populations (Ratcliffe 1968). Oceanic bryophytes can occur in all of the major climatic zones, from the cool temperate to the equatorial tropical (Ratcliffe 1968). Example areas include the Atlantic coasts of Europe and the Americas, the windward sides of many oceanic islands such as the Seychelles, Réunion, Viti Levu in the Fiji Islands, Oahu in Hawaii and others (pers. comms. – Howard Matcham). In these places the vegetation belts usually descend lower (like cloud forests) and bryophytes of otherwise montane distribution can be found at relatively low altitudes. Many bryophyte species such as *Dumortiera hirsuta* (Dumortier's Liverwort) and *Colura calyptrifolia* (Fingered Cowlwort) only occur under these "oceanic" conditions (pers. comms – Howard

Matcham). In Britain and mainland Europe they are sometimes referred to as 'Atlantic' bryophytes due to their geographic distribution in woodlands on the Atlantic seaboard, however, in reference to species occurring in Britain, the terms oceanic bryophyte and Atlantic bryophyte are synonymous (pers. comms – David Streeter and Howard Matcham), hence the term oceanic will be used throughout this report.

In Britain, the main populations of oceanic bryophytes are found in the Atlantic oakwoods on the western seaboard (Ratcliffe 1968). There are a number of characteristics of these woodlands that make them suitable habitats for oceanic species: i) the woodlands are hundreds of years old and have formed dense canopies which prevent turbulent mixing and trap humid air (Rothero 2005); ii) the crags and steep-sided burns found in the Atlantic woodlands are important features that maintain high levels of humidity whilst at the same time hampering intensive management and heavy grazing; iii) most oceanic bryophyte species require firm substrates and the old, hard, often gritty rocks found within the Atlantic forests provide a favourable geology for these species (Rothero 2005).

Southern England is a relatively dry part of the country and as such moisture-loving bryophytes are absent from the majority of the region, the notable exception being the Wealden ghyll woodlands (Paton 1956). The presence of rich assemblages of moisture-loving bryophytes in the ghyll woodlands is explained through the occurrence of suitable geology, topography and humidity, along with the likelihood that the ghylls experienced continuous tree cover during recent periods of deforestation (Ratcliffe 1968, Rose and Patmore 1997, Patmore 2000).

Within many ghyll woodlands, sandstone outcrops and boulders combine with high relative humidity levels to create a damp sandstone substrate that is an internationally rare habitat type (Rose and Patmore 1997). The damp sandstone is home to a number of nationally rare 'sandrock specialist' bryophytes such as the oceanic bryophytes *Orthodontium gracile* (Slender thread-moss) and *Pallavicinia lyellii* (Veilwort) (Rose and Patmore 1997), both of which are 'vulnerable' Red Data Book species (Church et al. 2004). The tropical-oceanic liverwort *Dumortiera hirsute* (Dumortier's liverwort), another nationally rare species, is abundant on the wet sandstone rock faces at Fairlight Glen in Hastings, both at the head of the

ghyll and on wet boulders near the stream (Rose and Patmore 1997). This is the only location east of Devon in which this liverwort occurs (Brandon 2003). A number of other nationally rare bryophytes such as *Tortula freibergii* (Freiberg's screw-moss) and *Fissidens rivularis* (River pocket-moss) have also been found within the Wealden ghyll woodlands (Rose and Patmore 1997).

1.4.2 Ghyll woodland vascular plants

The ghyll valleys also provide a suitable habitat for a number of regionally rare oceanic vascular plant species such as *Wahlenbergia hederacea* (Ivy-leaved Bellflower) and *Sibthorpia europea* (Cornish Moneywort) (Rose and Patmore 1997). The oceanic fern *Dryopteris aemula* (Hay-scented Buckler-fern) is found in over 100 ghyll woodlands throughout the Weald. These are amongst the highest densities of colonies of this species anywhere in Europe (Rose and Patmore 1997). Another oceanic fern, *Hymenophyllum tunbrigense* (Tunbridge Filmy-fern), is restricted within southern England to a small number of sandrock ghylls. Populations of this species are not found elsewhere in eastern or central England and are hundreds of kilometres from other British populations (Rose and Patmore 1997).

Historically the steep-sided nature of the ghyll valleys meant they were difficult to bring under cultivation and so remained under continuous woodland cover. As such most can be classified as ancient woodland and fragments may be remnants of the original ‘wildwood’ (Patmore 2000). GIS analysis indicates that 85% of the Wealden ghyll woodlands lie within ancient woodlands (Burnside et al. 2002b). The ghylls tend to be rich in ancient woodland indicator (AWI) species, with species such as *Cardamine amara* (Large Bittercress) and *Chrysosplenium oppositifolium* (Opposite-leaved Saxifrage) found in nearly every ghyll (Rose and Patmore 1997).

1.4.3 Ghyll woodland fauna

The presence AWI species together with rich liverwort and moss communities suggests that the Wealden ghyll woodlands have remained continuously wooded. As such they are likely to support a high biodiversity of species of conservation concern, particularly terrestrial invertebrates which require woodland continuity and which share similar micro-environmental requirements (Burnside et al. 2006). Because plants and animals interact it is likely that these unique communities of plants support unique communities of fauna at higher trophic levels.

There have been relatively few studies of the fauna associated with the ghyll woodlands. However the studies that have been carried out have recorded a number of nationally rare animals within the ghyll woodlands including the money spider *Diplocephalus protuberans*; the water beetles *Hydraena nigrata, Hydraena pygmaea* and *Hydraena rufipes*; and the moth *Tetheella fluctuosa* (Satin Lutestring) (Rose and Patmore 1997, Hastings Borough Council 2011). Unpublished data collected by Martin Willing and Rendel Williams revealed an oceanic mollusc fauna within some of the ghyll valleys (Rose and Patmore 1997).

1.5 The major threats to Wealden ghyll woodland biodiversity

1.5.1 Changes in woodland management

Rotation coppicing can increase woodland diversity and is beneficial for many woodland species (Peterken 1996). The increased light levels associated with coppicing benefits many AWI species such as; *Primula vulgaris* (Primrose), *Anemone nemorosa* (Wood Anemone) and *Lamiastrum galeobdolon* (Yellow Archangel) (Packham et al. 1992), all of which are commonly found in ghyll woodlands (pers. obs.).

The decline of coppice management in the region, shown in Figure 1.5, will undoubtedly change the composition and structure of the Wealden woodlands. Abandoned coppice will eventually revert to high forest and weedy species, that

during management would have been cleared out, will form a shrub layer and eventually compete with the coppice species already present (Peterken 1993).

Some conservationists would like to see coppice management reintroduced within the ghyll woodlands, because neglected coppice areas tend to have become extremely dense and dark (Rose and Patmore 1997). However, the increased amounts of light and decreased levels of relative humidity may be detrimental to the growth and survival of shade-tolerant and desiccation intolerant species of bryophytes and lichens (Ratcliffe and Staines 2003, Davies 2011). It is unclear how the ghyll woodlands should be managed to maintain or enhance their value in terms of nature conservation. To prevent their high biodiversity value being lost through neglect, it is essential that research is directed towards understanding more about the factors that underpin the biodiversity of the Wealden ghyll woodlands.

1.5.2 Climate change

Studies have shown that the rapid global climate change that began in the early part of the 20th Century has already affected the distributions, physiology, and phenology of a wide variety of organisms (e.g. Spiecker 1999, Frahm and Klaus 2001, Peñuelas and Boada 2003). In the south-east of England mean annual temperatures have increased by approximately 1.7°C over the past 50 years, (UKCIP 2013). Average rainfall remained unchanged during the same period, but patterns of precipitation did change with mean rainfall levels decreasing in the spring and summer months, but increasing in the autumn and winter. Over the same period the average annual and seasonal levels of relative humidity fell by up to 5% in the south and east of England (UKCIP 2013).

The presence of regionally and nationally important vascular and non-vascular plant communities within the Wealden ghylls is attributed to the cool, humid microclimatic conditions associated with the ghyll valleys (Paton 1956, Ratcliffe 1968, Rose and Patmore 1997). The rising temperature levels coupled with the falling relative humidity levels may impact on the persistence of species whose

existence within the ghyll woodlands is closely related to a cool, humid ghyll microclimate.

1.5.3 The effects of habitat loss and woodland fragmentation

Spatial variation in the distribution and abundance of geological processes can result in heterogeneous landscapes containing 'patchily distributed' habitats (Wiens 1997, Collinge 2009). In most terrestrial systems, patchiness involves spatial variation in bedrock, soils, nutrients, or water, and these influence the distributions of plant species (Collinge 2009). The ravines that contain the Wealden ghyll woodlands are geological features that exhibit this type of patchy distribution. Hence, the distribution pattern of ghyll woodlands throughout the Weald is one of naturally occurring patchiness by nature of their topographic associations.

Historically the Wealden ghylls were part of larger contiguous woodlands that covered most of the Wealden area. The Anglo-Saxon chronicle of 892AD describes a 'great wood' called 'Andred' that covered most of the Weald. It recorded the wood as stretching 'east to west 120 miles, or longer, and 30 miles broad' (Brandon 2003). Based on woodland surveys recorded in the Domesday Book, Rackham estimated that in 1086 AD woodland covered approximately 70% of the Wealden region (Rackham 1986). Today woodland cover in the same area is down to approximately 25% (Brandon 2003).

Whilst the south-east remains Britain's most wooded region, it is clear from Figure 1.7 that not only has a large proportion of the woodland been cleared, but also that the woodland that remains is fragmented.

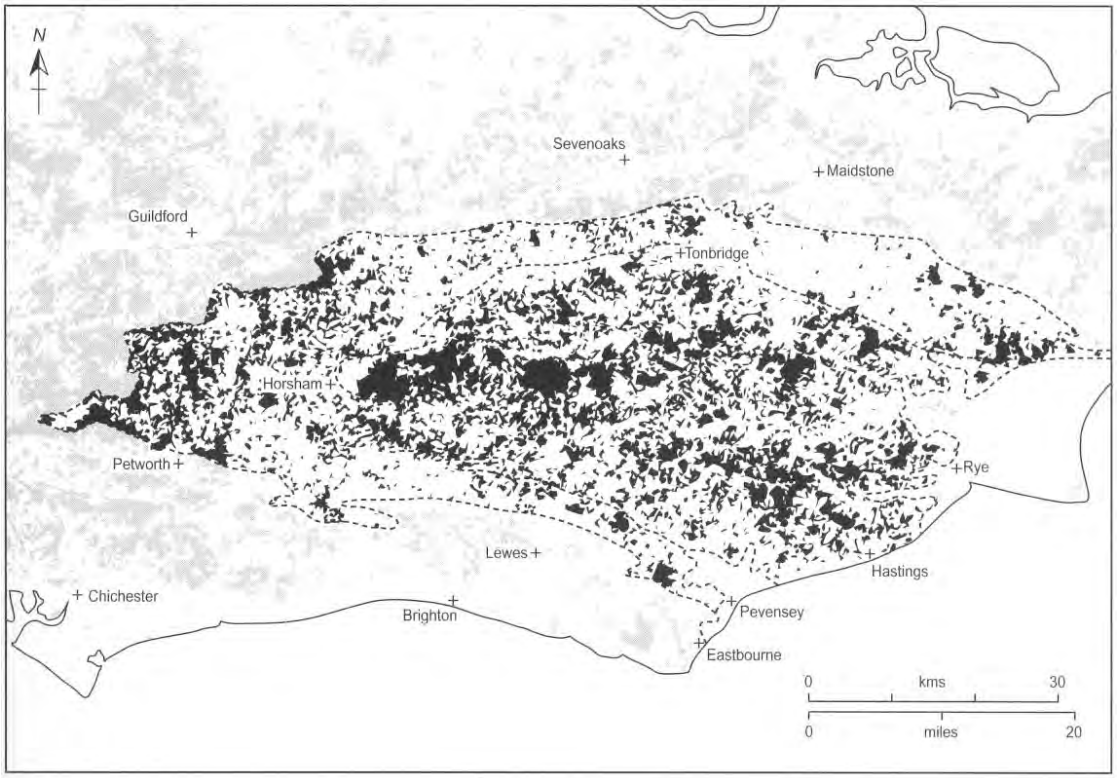


Figure 1.7 The distribution of woodland greater than two hectares in the Weald, 2002 (Forestry Commission). The total area of woodland in the Weald in 2002 was 65,351 hectares. Reprinted from Brandon (2003).

1.5.4 The abundance of ghyll woodland sites in the Weald

Although not a direct threat in itself, one of the major obstacles to ghyll woodland conservation efforts is the sheer density of sites in the Weald that fall under the classification of ghyll woodland. Dr Francis Rose carried out a desk-based study using 1:25,000 OS maps and identified 1130 ghyll woodlands situated throughout the Weald (Rose and Patmore 1997). However, many of these sites are unlikely to contain species considered to be of conservation interest. The most reliable method for identifying sites of conservation concern is to visit and survey each of the ghyll woodland sites. The inaccessibility and remote nature of many ghyll woodlands would make this type of surveying relatively time-consuming and therefore largely impractical. Consequently, less than a third of the ghyll woodland sites have received any ecological surveys and in most cases these were ‘many years ago’ (Patmore 2000). Burnside et al (2006) examined the conservation status of the Wealden ghylls and reported that only about 10% have been given any protection through classification under a recognised conservation designation.

1.6 Study objectives

In light of the perceived biological importance of the Wealden ghyll woodland plant communities, the study will carry out a spatial and temporal analysis of the vegetation associated with the Wealden ghyll woodlands.

Three major objectives have been identified:

a) To identify ghyll woodland and its spatial boundaries through examining and comparing patterns of species occurrence and abundance within ghyll woodlands with those of the surrounding woodlands. The environmental determinants of these patterns will be examined and the presence of sub-communities of ghyll woodlands based on the predominant geology of the site will also be investigated.
b) To investigate temporal changes in ghyll woodland community composition and to identify the environmental correlates of any changes identified.
c) To investigate levels of habitat isolation experienced by the ghyll woodland plant communities resulting from; the patchy distribution patterns of the Wealden ghyll woodland sites, and the loss and fragmentation of woodland in the surrounding region.

1.7 Thesis structure

Chapter 1:

Chapter 1 presents an introduction and background to the Wealden ghyll woodlands, describing the geology, soil, and climate of the region. The chapter examines how the ghyll woodlands were formed both from a geological and an ecological perspective since historically it is a combination of these factors that have created suitable conditions for the regionally important species that occur in these sites. A review of the biodiversity contained within the ghyll woodlands is included. The chapter is based upon a literature review and introduces some conservation issues in the form of the major threats to Wealden ghyll woodland biodiversity.

Chapter 2:

Chapter 2 attempts to determine ghyll woodland, and ghyll woodland types, on the basis of community composition and patterns of species richness and abundance. Multivariate statistical analysis will be used to identify the most important environmental parameters influencing species distributions and plant community composition. The spatial limits of ghyll woodland will be investigated through analysing distributional changes in community composition, species richness patterns and abundance patterns. The chapter attempts to contextualise the biological importance of ghyll woodlands within the region through comparisons with other local ancient woodlands.

Chapter 3:

Chapter 3 presents an analysis of changes in ghyll woodland communities during the post-war period by comparing data collected in surveys carried out during the period 1951-1970 with survey data collected during 1976-1995. The chapter uses archive biological survey data to identify temporal changes in the richness and distribution patterns of ghyll woodland plant communities. The chapter will also examine temporal changes in the distributions and ratios of oceanic bryophytes and ancient woodland indicator (AWI) species; two plant groups that are considered regionally and nationally important. Multivariate analysis will then be used to attempt to reveal the environmental determinants of any changes identified. Plant indicator values will also be used to identify changes in ghyll woodland community composition. The chapter will also examine trends in environmental conditions inferred by changes in the indicator values associated with the ghyll woodland plant communities. The influence of the fragmented distribution patterns of the ghyll woodland sites will be investigated through an examination of plant indicator values, and also through an analysis of the relationship between ghyll woodland species richness and both fragment area and distance to the fragment edge.

Chapter 4:

Chapter 4 investigates the genetic impacts of habitat isolation using four geographically separated ghyll woodland populations of the thalloid liverwort

Conocephalum conicum (Great-scented Liverwort). The extent of molecular divergence within and among populations will be analysed to compare the genetic diversity of the samples and to estimate the level of gene-flow between the survey sites. The relationship between the geographic distance separating the sites and the genetic diversity of the sub-populations will also be examined.

Chapter 5:

Chapter 5 provides a summary and further analysis of the results. The chapter discusses the implications of the study for the future management and conservation of the Wealden ghyll woodlands. The chapter also makes suggestions for future work that would further extend the analysis carried out in this report.

2 A spatial investigation of the richness, abundance and composition of ghyll woodland vegetation

2.1 Introduction

The Wealden ghyll woodlands are an important habitat for many woodland plant species, particularly those that prefer shaded, humid conditions. These include oceanic bryophytes (Ratcliffe 1968, Rose and Patmore 1997), and flowering plants and ferns associated with ancient woodlands (Patmore 2000). The steep-sided valleys containing the ghyll woodlands are impractical for cultivation and most will have remained continuously wooded since the time of the wildwood (Rose and Patmore 1997). This means that the majority of Wealden ghyll woodlands can be considered ancient woodland in the technical sense of this term.

Despite their accepted importance in terms of regional biodiversity, very few studies have focused on the ghyll woodland plant communities (Rose and Patmore 1997). Where studies have occurred the biologically important bryophyte communities, that characterise the ghyll woodlands, have not been included in the analysis (e.g. Burnside et al. 2002b, Burnside et al. 2006, Waite et al. 2010). This study will be the first to examine the diversity of ghyll vegetation in relation to other ancient woodland throughout the region. It will also be the first study to characterise ghyll woodland types based on community associations of both vascular and non-vascular species.

2.1.1 Identifying ghyll woodland plant communities

Community ecologists recognize and classify plant communities either on the basis of their occurrence within discrete habitat boundaries or on the basis of some aspect of the plant assemblage itself such as repeated species associations,

the identification of dominant indicator species, or changes in species richness (Nichols 1923, McVean and Ratcliffe 1962, Rodwell 1991, Gabriel and Bates 2005).

Physically defined communities consist of species found in a particular place or habitat. The boundary of the place or habitat represents the community boundary (Nichols 1923). The sharpness of the community boundary will be a reflection of the sharpness of the habitat boundary, for example, the boundary of a pond is relatively discrete, whereas the transition between forest and savannah may appear gradual, defying a clear spatial delimitation. Floristically defined communities tend to be recognized on the basis of one or more species that either dominate the community or differentiate it from communities that otherwise have a similar composition (McVean and Ratcliffe 1962).

The Wealden ghyll woodlands are identified by most authors as physically defined communities on the basis of their location within 'deeply incised valleys' (e.g. Rose and Patmore 1997, Burnside et al. 2006). However the ghyll woodland boundaries outlined as part of a desk based attempt to map the ghyll woodlands, carried out by Dr Francis Rose for the Sussex Biodiversity Record Centre, frequently included part, or all, of the contiguous woodland surrounding the valleys (SBRC 2000).

In a further attempt to characterise and define these important habitats a series of ghyll woodland surveys were carried out for the 'ghyll woodland characterisation project' (Burnside et al. 2002b). The authors identified ghyll woodland communities based on their floristic composition as well as predominant ghyll valley geologies. Using a variety of multivariate statistical approaches they examined similarities in species assemblages within and between the survey sites. The surveys contained species data for the vegetation groups; canopy, understorey and field layer. The data was collected using a 'uniform walkover survey approach' where only the dominant species from each layer were recorded (Burnside et al. 2002b). Based on this data they were able to show sub-groupings with particularly strong associations, yielding similarity values >80%. These sub-groupings represented the location of ghyll woodlands on either the Ashdown & Tunbridge Sands or the Wadhurst & Fairlight Clays.

A potential weakness of this classification method is that it characterised the woodlands through canopy, understorey and field layer species but not bryophyte species which are acknowledged as important indicators of the presence of ghyll woodland (Ratcliffe 1968, Rose 1995, Rose and Patmore 1997).

2.1.2 The distribution of plant species and the ecological niche

Plant species are found together, in spatially repeating communities, because they have similar abiotic and biotic requirements for environmental factors. These include; light, moisture, temperature, drainage conditions, soil nutrients and the ability to tolerate competition and herbivory (Kent 2012). Environmental levels of these variables will influence both species distributions and community composition (Pignatti 1996, Gurevitch et al. 2006).

Grinnell (1917a, b) was the first to describe species distribution patterns in terms of an ecological niche; however it was Hutchinson (1957) who developed the idea into its current form. Hutchinson outlined a niche as a multidimensional representation of each species' habitat needs, resource requirements and environmental tolerances. A species niche is therefore the set of biotic and abiotic conditions that are necessary for it to survive, grow and reproduce (Crawley 1997). This concept is fundamental to understanding the distribution patterns of plant species. Hutchinson (1957) formulated two basic niche concepts: an organism's fundamental (or pre-interactive) niche and its realized (or post-interactive) niche. The fundamental niche represents those areas within an environment where the species can persist and reproduce in the absence of interactions with any other species (Prinzing et al. 2008). The realized niche represents those areas within an environment that a species actually occupies in the presence of other interacting and competing species (Polechová and Storch 2008, Boulangeat et al. 2012).

2.1.3 Environmental factors influencing the distribution of plant species

Niche theory predicts that the distribution of plant species will be correlated with a range of biotic and abiotic environmental variables. Determining the factors that control the number, abundance and identity of plant species is a central goal of plant ecology (Virtanen et al. 2000). The complexity of the environment has meant that it is customary for plant ecologists to break it down into single factors and to study the effect of these factors on plant distributions (Billings 1952). These types of studies tend to focus on climatic and edaphic parameters that form the primary abiotic environmental influences on the growth and distribution of individual species (Billings 1952). Studies of terrestrial vegetation tend to focus on the climatic variables; temperature, light and relative humidity, along with the edaphic variables; soil moisture content, soil pH, soil nutrient levels and soil type/composition (e.g. Dinsdale et al. 1997, Roem and Berendse 2000, Weiher et al. 2004, Pérez-García et al. 2007).

2.1.3.1 Climatic variables

2.1.3.1.1 Air temperature

Individual species of plants will have an optimum temperature range, where net photosynthesis is maximised. Outside this range, photosynthetic activity will be inhibited. The ability and rate of photosynthesis will directly affect the plants ability to survive, grow and reproduce (Berry and Bjorkman 1980). At low temperatures photosynthesis is slowed and can result in poor plant growth, whilst at high temperatures respiration increases, sometimes higher than the rate of photosynthesis, meaning the plant will be unable to grow (Taiz 2010)

The sheltered ghyll valleys are believed to buffer the ghyll woodlands from temperature fluctuations occurring in the habitat adjacent to them (Rose 1995, Rose and Patmore 1997). This temperature buffering, in conjunction with high levels of relative humidity, provides ideal conditions for bryophyte species such as

bog mosses (*Sphagnum* spp.*)* and thalloid liverworts that are both intolerant of desiccation and also susceptible to frosts (Vanderpoorten and Goffinet 2009).

2.1.3.1.2 Light levels

In mature woodland, light levels are considered to be the most important influence on ground vegetation, mediating all other influences (Peterken 1993, Hooley and Cohn 2003).

Many plant species have become adapted to survive in certain habitat types and in the process they have evolved characteristics that limit their survival in other habitat types. A number of plants commonly found in ghyll woodlands, such as *Circaea lutetiana* (Enchanter's Nightshade) and *Geranium robertianum* (Herb Robert) are examples of shade-tolerant species that are adapted to maximise photosynthesis in shaded woodland. They do this by having leaves that are relatively large, are held horizontally and have a larger number of stomata to increase rates of photosynthesis (Osborn and Taylor 1990, Henry and Aarssen 1997). A problem with these adaptations is that in an open higher-light environment these adaptations would increase rates of evapotranspiration and therefore reduce the fitness of the plant. The oceanic bryophyte community offers furthur examples of shade-tolerant plants found in the ghyll woodlands (Ratcliffe 1968).

2.1.3.1.3 Relative humidity

Levels of relative humidity in the ghyll valleys are thought to be high in relation to other woodland types throughout the region (Ratcliffe 1968, Rose and Patmore 1997, Patmore 2000). This is particularly important for bryophytes as they have no vascular system, instead absorbing water directly through the cell walls (Vanderpoorten and Goffinet 2009). The ability of bryophytes to remain hydrated is therefore primarily regulated by their immediate microclimate (Lee and Roi 1979). Many bryophytes are monoecious and require damp conditions for fertilisation in order for the male cells to migrate through a film of water on the leaf surface to the

archegonia (the female receptacle) (Watson 1981a, Wyatt and Anderson 1984). Atmospheric moisture conditions will therefore have a far greater effect on bryophyte community structure than that of vascular plants (Grytnes et al. 2006).

At a local scale, a number of studies have found that fern species richness tends to increase in humid microhabitats because fern gametophytes are dependent on water for the transportation of gametes (Kessler 2000, Richard et al. 2000, Kluge and Kessler 2006).

Many of the ghyll valleys contain sandstone in the form of outcrops or boulders (Rose and Patmore 1997). The humid microclimate within the ghylls interacts with this sandstone to produce a damp sandstone substrate that is a rare habitat type both on a regional and national scale (Ratcliffe 1968, Rose and Patmore 1997). The damp sandstone provides a suitable substrate for internationally important plant communities such as the nationally rare fern species *Hymenophyllum tunbrigense* (Tunbridge Filmy-fern) which is restricted within the region to a small number of damp, shaded, sandstone outcrops and boulders. It is able to survive in the dry climate of south-east England because of the moisture seeping out of the highly permeable sandrocks which allow the rock surfaces to stay cool and damp (Rich and Rumsey 2004).

The ghyll woodland bryophyte and lichen communities contain a strong oceanic component. For many of these oceanic species the ghyll communities represent their main stronghold in south-east England (Rose and Patmore 1997). The species differ in their precise ecological needs, but almost all require humid sheltered conditions (Church et al. 2004). The distribution patterns of oceanic bryophyte species shows them to be strongly correlated with the wetter parts of Britain such as the west coasts of Wales and Scotland. Most oceanic bryophytes are not dependent on excessive ground moisture for survival, but rely on continual atmospheric dampness (Ratcliffe 1968). In drier regions (such as south-east England), oceanic species tend to be limited to specialized habitats where the necessary humid conditions are maintained only locally (Ratcliffe 1968).

2.1.3.2 Edaphic variables

2.1.3.2.1 Soil moisture

Vascular plant species have differing soil moisture level thresholds. Below these thresholds plants experience drought-stress leading to a reduction in the efficiency of processes and functions (Holsten et al. 2009). Too much soil moisture may fill pore spaces in the soil thereby causing oxygen availability to decrease and again the plant efficiency may be reduced (Taiz 2010). Soil moisture levels are also important for the germination and establishment of many species (Kaplan and Muñoz-Carpena 2011).

Studies have shown a positive correlation between moisture levels and bryophyte diversity and abundance (Lee and Roi 1979, Frisvoll and Prestø 1997, Gould and Walker 1999, Turner et al. 2006). Bryophyte community composition is frequently differentiated along soil moisture/atmospheric humidity gradients (Fuertes et al. 1996, Odasz 1996, Gabriel and Bates 2005). Soil moisture gradients have also been shown to influence the distribution patterns of vascular plants (Breshears et al. 1997, Breshears and Barnes 1999).

Patterns of soil moisture are seen in the majority of studies of the distributional structure of riparian vegetation and can be generalized as transverse gradients perpendicular to the water channel (Malanson 1993).

2.1.3.2.2 Soil type and composition

Surface soils consist of a mixture of organic and inorganic particles which vary in size and shape. The size of these mineral particles will influence both soil moisture levels and also rates of nutrient cycling (Zhuang et al. 2001). Soil particle size determines the total surface area that is available for absorption and therefore influences the absorption rates of organic and inorganic substances (Naiman et al. 2005). Medium-sized sand particles have around 7000 particles per gram with a combined surface area of around 0.013m^2, whilst clay particles have around 400 billion particles per gram with a combined surface area of 1 to 10m^2 (Fisher and Binkley 2000). These vast differences in surface area per gram (spanning three

orders of magnitude) have a dramatic effect on water potential, organic matter binding and general biotic activity. Particle size strongly influences plant growth, but this effect is indirect, manifesting through the influence of factors such as water-holding capacity, aeration and organic matter retention (Zhuang et al. 2001).

An important component of the soil is the organic matter content. This consists of plant and animal residues in various stages of decomposition (Richardson and Vepraskas 2001). This enters the soil as particulates that decompose into soluble form and can then either be absorbed into mineral surfaces, be precipitated out of the soil or get taken up by plants (Richardson and Vepraskas 2001). Soil organic matter influences both soil water retention and levels of soil aeration, whilst also increasing levels of root penetration and plant production (Gartzia-Bengoetxea et al. 2009). Soil organic matter is the most important source of nutrients for plants, containing 95% of the nitrogen and sulphur, and 25% of the available phosphorous found in surface soils (Zech et al. 1997, Fisher and Binkley 2000).

Plant community composition is heavily influenced by factors related to soil composition and soil type (Daubenmire 1968, Tuomisto and Poulsen 1996). Studies have found high substrate specificity for both ferns (Tuomisto and Ruokolainen 1994, Tuomisto and Poulsen 1996) and flowering plant species (Pavlik and Manning 1993).

2.1.3.2.3 Soil pH

Soil pH levels affect the amount of soil nutrients that are available to plants. For most plants a pH of between 5.5 and 7 will allow optimum soil nutrient availability. Going up or down from this range increases the availability of certain nutrients whilst decreasing the availability of others (Agren and Andersson 2012). Highly acidic soils (pH<4) may result in high, potentially toxic, concentrations of ions of aluminium, manganese and iron. At the other pH extreme, in highly alkaline soils (pH>9), many essential plant nutrients become insoluble and inaccessible to plants (Jones 2004).

Studies focusing on other wetland systems occurring in steep-sided valleys have shown strong correlations between pH and plant distributions. Analysis of the

Buffalo National River system found a strong pH gradient across the riparian landscape (Sagers and Lyon 1997). The study found that species composition in the riparian forest was influenced by environmental gradients dominated by pH and local elevation above river level. A study of the Ozark National Scenic River system also found the dominant environmental gradients influencing tree and herb riparian vegetation distributions were elevation and soil pH (Lyon and Sagers 1998). Studies examining bryophyte distribution patterns in relation to environmental gradients have also found strong correlations with changes in pH (Watson 1981b, Virtanen et al. 2000, Hokkanen 2006).

2.1.3.2.4 Soil nutrient levels

Individual species of plants will respond differently to variations in nutrient levels. They require not just different levels of nutrients for optimum growth but also different ratios (Clarke 1993, Quinn and Keough 2002). Regional variations in nutrient levels are a major influence on plant distributions and community composition (e.g. Verhoeven et al. 1996, Braakhekke and Hooftman 1999). Studies of bryophyte species show strong individualistic responses to nutrient levels in accordance with each species ecological niche (Kellner 1993, Turkington et al. 1998).

The six macronutrients; nitrogen, phosphorous, potassium, magnesium, calcium and sulphur are the most important nutrients in terms of growth requirements and are major influences of species distribution patterns (Dinsdale et al. 1997, Marschner and Marschner 2011). The analysis of soil nutrients in this chapter will therefore focus on these six macronutrients.

2.2 Aims

The chapter aims to investigate whether ghyll woodlands can be separated from other types of ancient woodland in the Weald on the basis of species richness, abundance and community composition.

The analysis aims to determine the spatial limits of the ghyll woodlands through the use of biotic and abiotic data collected within the ghyll valleys and within the surrounding woodlands.

A further aim is to identify and quantify the major environmental correlates of ghyll woodland plant distributions.

Substrate specificity of ghyll woodland species will be analysed with the aim of revealing sub-communities of ghyll woodlands based on their location on clay or sandstone substrates; the two predominant geological substrates within the region.

2.3 Methods

2.3.1 Study area

The study sites are located in the Wealden region of south-east England (Figure 2.1). Following on from four weeks of pilot studies, the field surveys took place over an eight week period commencing in the second week of July 2012.

2.3.2 Site selection

A digital map containing the location data of all of the Wealden ghyll woodlands was supplied by the Sussex Biological Record Centre (SBRC 2000). A separate digitized map containing soil information for the Wealden area was supplied by the National Soil Resource Institute (NSRI 2001).

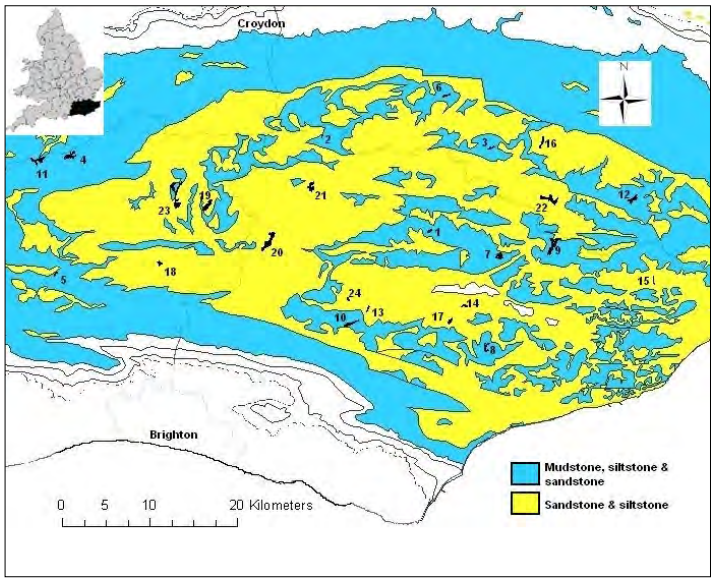


Figure 2.1 The geological composition of the Weald. The location of the ghyll woodland survey sites are shown on the map. 1. Cinderhill Wood; 2. Great Wood; 3. Hayden Wood; 4. Horseghyll Wood; 5. Joles Farm Wood; 6. Knowles Bank; 7. Park Wood; 8. Reed Wood; 9. Silverhill Wood; 10. Slay's Wood; 11. Tickfold Ghyll; 12. Windmill Wood; 13. Bryckden Place; 14. Dallington Forest; 15. Flatropers Wood; 16. Forstal Wood; 17. Foul Mile; 18. New England Wood; 19. Philpots Wood; 20. Pollardsland Wood; 21. Posingford Wood; 22, Tanyard Ghyll; 23. The Warren; 24. Tickerage Wood.

Eighty-five percent of ghyll woodland sites are associated with areas of ancient woodland (Burnside et al. 2006). To examine spatial boundaries it was necessary that the ghyll woodlands selected for surveying were all located within contiguous ancient woodland; therefore the 15% of sites that did not meet this criterion were removed from the list of potential survey sites.

Using the soil substrate map, the remaining ghyll woodland sites were divided into two groups based on whether sites were located on soils classified as predominantly clay (referred to as 'Mudstone, siltstone & sandstone' in Figure 2.1) or located on soils classified as predominantly sandstone (referred to as 'Sandstone & siltstone' in Figure 2.1). Random number generation (MS Excel 2010) was used to select 12 survey sites from each substrate group. The locations of the 24 ghyll woodland sites selected are shown in Figure 2.1. Representative photographs of the survey sites are shown in Appendix 1.

Sites	Location of Ghyll valleys	Mapped ghyll area (h)*	Ghyll length (m)	Stream status
Clay sites				
Cinderhill Wood	N560736 E128620	6.1	588	Flowing
Great Wood	N548438 E139479	3.9	369	Bed damp
Hayden Wood	N567872 E138160	5.7	605	Bed damp
Horseghyll Wood	N519557 E137207	23.0	1529	Flowing
Joles Farm Wood	N517922 E123769	3.9	901	Flowing
Knowles Bank	N562659 E144139	5.4	965	Flowing
Park Wood	N568620 E125794	24.8	475	Standing
Reed Wood	N567168 E115328	11.6	1005	Dry
Silverhill Wood	N574611 E126451	59.4	1786	Flowing
Slay's Wood	N551366 E117990	17.2	1352	Flowing
Tickfold Ghyll	N515643 E136707	22.2	1797	Flowing
Windmill Wood	N584348 E132412	22.1	1339	Dry
Sandstone sites				
Bryckden Place	N553706 E119749	4.1	679	Standing
Dallington Forest	N565175 E120637	5.8	1609	Flowing
Flatropers Wood	N586411 E122789	3.2	937	Bed damp
Forstal Wood	N573489 E138505	12.2	1209	Flowing
Foul Mile Wood	N563090 E118284	11.6	715	Bed damp
New England Wood	N529963 E124888	12.4	631	Flowing
Philpots Wood	N535028 E132091	35.9	1611	Dry
Pollardsland Wood	N542162 E127054	74.8	2703	Flowing
Posingford Wood	N547257 E133990	32.4	1269	Flowing
Tanyard Wood	N574615 E132381	42.4	2133	Flowing
The Warren	N532076 E132356	59.5	3322	Flowing
Tickerage Wood	N551163 E120792	4.4	404	Flowing

*The mapped ghyll area frequently did not correspond to the boundaries of the ghyll valleys, but also contained quite large areas of the contiguous woodland

Table 2.1 Locations of the 24 ghyll valley survey sites. These locations also represent the locations of the 24 ancient woodland sites surveyed that were adjacent to ghyll valleys. Ghyll area and length measurements calculated with ArcMap v.10 using the Sussex Biological Record Centre ghyll woodland dataset.

At each of the ghyll survey sites a separate survey was carried out in the ancient woodland surrounding the ghyll valley. A further 12 ancient woodlands were randomly selected for surveying from a digital map of ancient woodland within the region, also supplied by the SBRC.

Sites	Location	Area (h)
Broad Street Wood	N586132 E116371	7.2
Elphicks Wood	N569346 E137515	59.1
Marlpost Wood	N514314 E125602	60.5
Plashetts Wood	N545851 E115994	24.2
Southover Wood	N565229 E125524	35.8
Upper Parrock Wood	N545503 E134340	27.4
Burgh wood	N572405 E127429	72.9
Burnthouse Wood	N572863 E118091	154.6
Hawkhurst Common Wood	N553049 E119216	35.9
Herrings Farm Wood	N556514 E122352	35.4
Paiges Wood	N531814 E124779	20.4
Westlands Wood	N533225 E138213	51.9

Table 2.2 Location of the 12 regional ancient woodland survey sites. Area measurements were carried out with ArcMap v.10 using the Sussex Biological Record Centre ancient woodland digital dataset.

The selection criteria for these sites was that they were spatially separated from the 24 ancient woodlands already selected (i.e. not part of the same contiguous

woodland), and were at least 200 metres from any known ghyll woodland sites. The details of the ancient woodland survey sites are shown in Table 2.2.

Hence, a total of 60 woodland surveys were carried out between July and September 2012.

2.3.3 Survey Methods

2.3.3.1 Sampling units

All of the sites were surveyed using 2m x 2m square quadrats. Quadrats are the most common method used to sample vegetation for floristic description and analysis (Rodwell 1991, Fowler et al. 1998, Magurran 2004). The quadrat size used in the surveys matched the size of those used for the herbaceous vegetation surveys carried out as part of the British National Vegetation Classification (NVC) surveys (Rodwell 1991, Pigott et al. 1992, Haslam 1996). Species accumulation curves were constructed to assess the optimum number of quadrats for sampling vegetation (Appendix 2) (Kent 2012). 'Running means' were calculated to gauge the optimum number of environmental measurements that needed to be taken at each site (Appendices 3-7). The plateaux at which accumulative means varied by less than 5% was selected as the appropriate sample size (Waite 2000). The results indicated that a minimum of 15 quadrats would need to be surveyed at each site. The running mean analysis indicated a minimum of 13 environmental readings would need to be taken for each environmental variable being studied to ensure data used for the analysis was representative of 'typical' conditions within the ghyll.

2.3.3.2 Location of sampling units

To investigate the spatial limits of ghyll woodland vegetation, lateral changes in species distributions were examined using a stratified random sampling scheme. The study site was divided into four equal strata. Six quadrats were then randomly placed within each stratum (Sutherland 2006, Kent 2012). Therefore, a total of 24

quadrats were used to survey each ghyll. The four strata ran the length of the ghyll, parallel to the stream, along one of the valley sides. The first stratum was the area from the stream edge to 2 metres up the valley side. The next three strata formed strips that were; 2 to 4 metres, 4 to 6 metres and 6 to 8 metres from the edge of the stream. The length of each ghyll was calculated in advance (ArcMap v.10) and each stratum was divided into metres. The location of each quadrat along the length of the stratum was then chosen through the use of random number generation. Where the chosen location was inaccessible the quadrat was placed within the stratum as close as possible to the proposed location. Ghyll streams tend to be meandering by nature, with streams frequently changing orientation; hence aspect was not recorded.

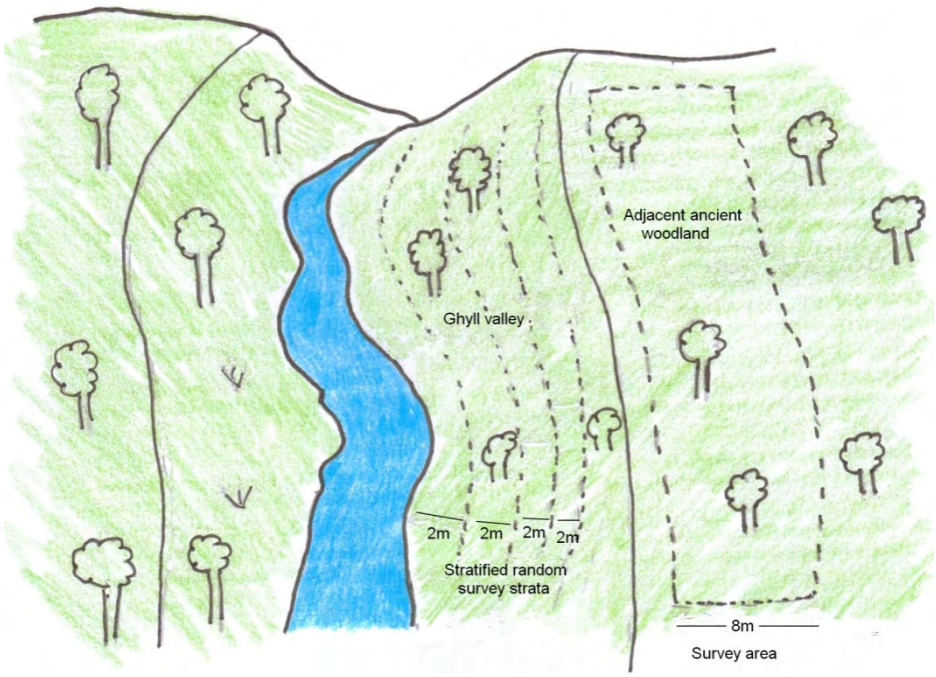


Figure 2.2 The layout of the survey area at each ghyll woodland site. Within the ghyll valley one of the banks is divided into 4 x 2 m strata. The ancient woodland survey area is located parallel to and at the edge of the ghyll valley.

The average survey ghyll length was 1250 metres (Table 2.1). The total width of the survey area was 8 metres meaning that an average ghyll valley survey area totalled 10,000 m^2 (1 hectare). Surveying was carried out on the basis of equal sampling effort at all survey sites. Therefore within all of the ancient woodlands, 24 quadrats were randomly located within a 200 x 50 metre (1 hectare) survey area.

The location of quadrats was decided in advance by creating coordinates through random number generation. Where ancient woodland adjacent to the ghyll valleys was being surveyed, the edge of the ancient woodland survey area was located at the edge of the ghyll valley and ran parallel to the valley for 200 metres (Figure 2.2). The ghyll survey and the ancient woodland survey were both carried out on the same side of the valley at each site.

2.3.3.3 Species data

Percentage abundances for each flowering plant, bryophyte and fern species were recorded within each quadrat. Where possible, plants were identified in the field through the use of vegetation keys (Rose et al. 2006, Merryweather 2007, Atherton et al. 2010). Where species could not be identified 'on-site', a sample of each species was taken back to the laboratory for identification. Many bryophytes can only be 'keyed-out' in the field when the sporophyte is present (Atherton et al. 2010). Unfortunately the sporophyte generation was absent from many of the bryophytes encountered meaning a large number of samples were collected at each site for microscopic identification. A small sample of the bryophyte would be removed and placed into a labelled envelope. The samples rapidly desiccated at room temperature and were stored in this condition in the laboratory for later analysis. Samples were rehydrated using tap water prior to examination (Watson 1981a). Identification was based on Smith (2006) for Bryophyta (mosses) and Paton (1999) for Marchantiophyta (liverworts). Current nomenclature was checked using Atherton (2010).

2.3.3.4 Environmental data

Edaphic and atmospheric environmental variables were measured during the surveys in order to examine their influence on species distributions and ghyll woodland community composition. These measurements were taken from the centre of every quadrat.

During the pilot studies it became clear that atmospheric variable readings are liable to change rapidly in response to changes in the prevailing weather conditions. In order for results to be comparable, readings were taken simultaneously within the ghyll valleys and within the surrounding woodland using identical equipment.

Atmospheric variables (temp., light, humidity, etc.) were not directly comparable between survey sites due to temporal variations in atmospheric conditions. To overcome this problem, atmospheric readings recorded at each site were measured within the valleys themselves and simultaneously within the surrounding woodlands. Site averages for each variable were calculated relative to an average figure of the readings taken in the surrounding woodland. These relative variables were only used for comparisons of ghyll woodland atmospheric variables.

Relative humidity and air temperature were measured using a Hanna Instruments HI8064 hygrometer. Light levels were measured using a LX-101 luxmeter supplied by Lutron Electronic Enterprise Co Ltd. Soil moisture, temperature and salinity readings were measured using HH2 moisture meters fitted with WET2 sensor probes, both supplied by Delta-T Devices Ltd.

2.3.3.4.1 Soil samples: collection and analysis

The methodology for collection and storage of soil samples followed the protocols outlined in Carter (1993).

Plant availability of the soil macronutrients; potassium, phosphorous, sulphur, magnesium and calcium were all measured using the Mehlich 3 soil nutrient extractant method (Zhang et al. 2010). The preparation of Mehlich 3, and the extractant procedure, followed the SOP outlined by Zhang et al. (2010). The method estimates plant availability of macronutrients in soils using a dilute acid-flouride-EDTA solution of pH 2.5. Extracted nutrient levels were analysed using the ‘Inductively Coupled Plasma Spectroscopy’ technique (ICP-OES/MS) in a Perkin Elmer Optical Emission Spectrometer, Optima 2100DV using ‘Perkin Elmer Winlab 32 for ICP’ software V.4.

Soil pH levels were measured using a Hanna Instruments pH209 pH meter. The analysis followed the protocol BS1377: Part 3: 1990: 9 (Head and Keeton 2010). Soil nitrate was extracted from the soil samples into an aqueous solution using the 'Calcium Sulphate Extraction' method (Dahnke 1980). These samples were then analysed for nitrate levels using the 'Cadmium Reduction' Method described in the Hach Company DR2400 Portable Spectrometer handbook. Analysis of loss on ignition was carried out on the soil samples following the 'Alternate Procedure' outlined in the text 'Recommended Soil Organic Matter Tests' (Schulte 1995).

2.3.4 Data analysis

2.3.4.1 Determining ghyll woodland on the basis of community composition and species richness

Several methods were used to investigate whether the ghyll woodlands could be distinguished from regional ancient woodlands on the basis of species composition. The species composition of ghyll woodlands located on clay was compared to the composition of ghylls located on sandstone, to examine whether ghyll woodland community composition could be distinguished on the basis of the predominant substrate present. Modular Analysis of Vegetation Information System (MAVIS) (Smart 2000) software was used to classify the vegetation data within each substrate group on the basis of the National Vegetation Classification system (NVC) (Rodwell 1991). The NVC system is one of the most widely used methods for classifying British woodland. It is a phytosociological classification system, classifying vegetation solely on the basis of floristic composition and is based on extensive sampling of woodlands throughout Britain (Rodwell 1991).

Community composition was further investigated through the use of Analysis of Similarities (ANOSIM) (CAP v.4 PISCES Conservation Ltd) testing which provides a way to test whether there is a significant difference between the species abundance and composition of one group, to that of one or more other groups, using the Bray-Curtis measure of similarity (Chapman and Underwood 1999, Wimp et al. 2004). ANOSIM is generally used for ecological 'taxa-in-samples' data, where groups of samples are being compared (PISCES 2013). For this analysis

the survey sites were separated into five groups, each containing 12 sites. The five groups were; i) clay ghyll woodlands, ii) ancient woodlands adjacent to clay ghyll woodlands, iii) sandstone ghyll woodlands, iv) ancient woodlands adjacent to sandstone ghyll woodlands and v) regional ancient woodlands.

To test for significance ANOSIM ranks similarity within and between the groups and compares this with the similarity that would be generated by chance. The samples are assigned into random groups one-thousand times and a value (r) is calculated for each permutation (PISCES 2013). The value of r will lie between -1 and 1. The observed value of r is then compared against that of the random distribution to determine if it is significantly different from the value that would occur randomly. A significant, positive, r value indicates that the samples within groups are more similar than would be expected by random chance. The value r=0 would occur if the high and low similarities are perfectly mixed and bear no relationship to the group. Because pair-wise tests were carried out on the five habitat groups a total of 10 ANOSIM tests were carried out on each taxa and so the significance levels were Bonferroni corrected to $P<0.005$.

Species richness was also analysed to examine whether ghyll woodlands located on clay soils could be differentiated from those on sandstone soils. Species data was found to be normally distributed and of equal variance (SPSS v.20) and therefore differences in species richness, within and between the five woodland groups, were investigated using one-way ANOVA (SPSS v.20) analysis. Because of the known importance of the ghyll woodlands for bryophytes (Ratcliffe 1968, Rose and Patmore 1997) the data was divided along taxonomic lines into bryophytes, ferns & flowering plants and the ANOVA analysis repeated for each species group. The Tukey test option, selected as part of the one-way ANOVA analysis, is a common test used by biologists when comparing all of the groups being analysed with each other (Tabachnick and Fidell 2012). Significance levels for this test were again Bonferroni corrected to $P<0.005$.

Rank shift analysis (Scott et al. 2006) was carried out to quantify differences in the number of sites occupied by each species (site occupancy) within the clay and sandstone ghyll woodlands. Site occupancy figures for species recorded in clay ghyll woodland sites were pooled and compared with pooled occupancy data from

the sandstone ghyll sites. The total number of sites occupied by each species was used to rank the species within each group with the species occupying the most sites given a ranking of one, the next a rank of two, etc. Species occupying the same number of sites were assigned the same rank. Where species only occurred in either sandstone or clay sites a rank shift percentage could not be calculated. These species were listed in the results table if their occupancy ranking was 60 or higher within the group that they occurred.

Species richness varied between the groups and therefore the number of ranks within each group varied. To compensate, the rank shift figure was worked out as a percentage based on the number of ranks within each group.

The equation used to work this out was as follows;

$$\left(\left(\frac{Species\ y\ rank\ in\ group\ A}{Total\ ranks\ in\ group\ A}\right) - \left(\frac{Species\ y\ rank\ in\ group\ B}{Total\ ranks\ in\ group\ B}\right)\right) \times 100 = rank\ shift\ \%\ of\ species\ y\ in\ group\ B$$

To examine the influence of substrate on ghyll woodland community structure, Indicator Species Analysis (ISA) was carried out using PC-ORD v.5.33. The analysis identified species that characterise sandstone ghyll communities and those that characterise clay ghyll woodland communities. ISA calculates a series of values for each species based on its abundance and frequency within a particular group (De Cáceres et al. 2010). Statistical significance is determined by a Monte Carlo randomization test with the null hypothesis that the species are of no value as indicator species. Indicator values calculated for each species range from 0 to 100. A species would be assigned a value of 100 if it were classified as a perfect indicator species, occurring in all samples within one group, and absent from all other groups (Kent 2012).

To attempt to explain which environmental variables were driving patterns of species richness and community composition within the sites, a number of abiotic variables were measured during each survey and their relationship with species richness and community composition was examined.

An Anderson-Darling test indicated that with the exception of the loss-on-ignition data, all of the environmental variables exhibited non-parametric distributions. Spearman rank correlation analysis (Minitab v.16) showed no significant correlations between the explanatory variables. The medians of the environmental variables measured were compared through Kruskal-Wallis testing. Mann-Whitney U *post-hoc* testing was then used to carry out pair-wise analysis of the habitat groups for the environmental variables whose medians were identified as significantly different in the Kruskal-Wallis (Minitab v.16). The ten environmental variables analysed through Kruskal-Wallis analysis were; loss-on-ignition, angle of slope, nitrates, potassium, phosphorous, sulphur, magnesium, calcium, sodium, pH. The significance levels for both the Kruskal-Wallis analysis and the Mann-Whitney U analysis were Bonferroni corrected to $P<0.005$.

The species richness values for the complete set of sites were regressed against the set of environmental variables used in the Kruskal-Wallis analysis using a linear regression. A second set of linear regressions were carried out, but this time only the ghyll woodland sites were included. The environmental variables used in the ghyll woodland regressions also included the atmospheric environmental variables that had been recalculated to give values that were 'relative' to measurements taken at the same time in the surrounding woodland. These additional environmental variables were; air temperature, relative humidity, light, soil moisture and soil temperature.

All linear regressions were carried out within a Generalized Linear Modelling (GLM) framework (SPSS v.20). Within the GLM the dependent variable was modelled using a Poisson distribution and log-link function (McCullagh and Nelder 1989). To derive the minimal adequate model a forwards stepwise-model procedure was used (Quinn and Keough 2002). All predictor variables were initially entered separately into the model. Variables with the greatest deviance values were entered consecutively until a non-significant model at the 95% confidence level was reached (Quinn and Keough 2002, Scott et al. 2012). Linear regression analysis was carried out using all of the species data and was then repeated using the three taxonomic groups separately.

2.3.4.2 Examining the spatial boundaries of ghyll woodland vegetation

To examine the spatial boundaries of ghyll vegetation, species distributions within the 24 ghyll sites were examined. Species richness totals were calculated for each stratum at each site by pooling the data from the six quadrats within the stratum. Each of the 24 ghylls therefore had four species richness totals (corresponding to the four strata). The species richness totals from the corresponding strata at each of the ghyll woodland sites were treated as replicates and combined into groups to form four groups (the four strata) each with 24 species richness totals. This first dataset contained species richness totals for all species. Three more datasets were constructed in exactly the same way but using species richness totals based on the taxonomic groups bryophytes, ferns and flowering plants. ANOVA tests (SPSS v.20) were carried out on each taxonomic group to analyse whether significant differences existed between the species richness recorded in the four ghyll stratum. An examination of the residuals showed a non-parametric distribution for the bryophyte and fern data so the bryophyte and fern groups were retested using Kruskal-Wallis analysis (MINITAB v.16).

2.4 Results

A total of 144 species were recorded during the surveys. They consisted of 60 flowering plant species, 70 bryophytes and 14 species of fern.

2.4.1 Characterisation of the Wealden ghyll woodlands

The ghyll woodland survey data was analysed to examine whether ghyll woodland could be differentiated from both the surrounding woodlands and also from a sample of broadleaved ancient woodland in the region. The analysis showed that species richness is clearly higher in ghyll woodlands than in any of the ancient woodland groups. The clay ghyll woodlands contained a total of 92 species whilst the sandstone ghylls contained 95 (Table 2.3). Species richness totals recorded for the ancient woodland groups varied between 62 in the sandstone ancient

woodlands and 69 in the clay ancient woodlands, whilst the regional ancient woodland group contained a total of 67 species. The average number of species recorded within the two ghyll groups was similar with 30 (±6.5) species recorded at clay ghyll sites and 27.5 (±7.7) at sandstone ghyll sites (Table 2.3). The clay and sandstone ancient woodlands again contained a similar number of species with an average of 15.7 (±4) species recorded at clay sites and an average of 15 (±5.3) at sandstone sites.

	Clay ghylls	Clay AW's	Sandstone ghylls	Sandstone AW's	Regional AWs
Species richness totals					
Bryophytes	45	36	42	29	22
Flowering plant	35	26	40	24	36
Ferns	12	7	13	9	9
Total	92	69	95	62	67
Site averages (SD)					
Bryophytes	13.9 (±2.7)	7.9 (±1.7)	10.8 (±3)	7.5 (±3.7)	7.8 (±4.5)
Flowering plant	12.7 (±4.8)	6.5 (±3.3)	11.5 (±5.5)	4.8 (±3.5)	10.3 (±4.8)
Ferns	3.4 (±2.4)	1.3 (±1.7)	5.5 (±1.5)	2.7 (±1.7)	2.5 (±2.5)
Total	30 (±6.5)	15.7 (±4.0)	27.5 (±7.7)	15 (±5.3)	20.5 (±8.9)

Table 2.3 Species richness totals for the ghyll woodland and ancient woodland survey sites. Standard deviations for site averages are in brackets.

The regional ancient woodland group contained an average of 20.5 (±8.9) species which was greater than the other ancient woodland groups due to the relatively high number of field layer species recorded at these sites. The mean number of flowering plant species recorded at the clay ancient woodland sites was 6.5 (±3.3), and within sandstone ancient woodlands was 4.8 (±3.5). A mean of 10.3 (±4.8) species was recorded for the regional ancient woodland group of sites which was considerably higher than the two other ancient woodland sites and closer to the 12.7 (±4.8) average recorded for the clay ghyll group and the 11.5 (±5.5) figure recorded for sandstone ghylls. The species richness totals show that the figure of 22 bryophyte species recorded within the regional ancient woodland group was the lowest number recorded within any group. However, the 36 flowering plant species recorded within this same group was the second highest field layer total recorded (Table 2.3).

Species richness within the ghyll woodland and ancient woodland groups was compared using one-way ANOVA analysis (Table 2.4). The analysis indicated that

in all four tests at least two of the group means differed significantly at the P<0.001 significance level.

Source	DF	SS	MS	F	Significance
a) all Species					
Between Groups	4	2454.76	613.69	16.36	P<0.001
Within Groups	55	2062.16	37.49		
Total	59	4516.92			
b) bryophytes					
Between Groups	4	439.90	109.97	14.93	P<0.001
Within Groups	55	405.08	7.36		
Total	59	844.98			
c) flowering plants					
Between Groups	4	627.50	156.87	9.19	P<0.001
Within Groups	55	938.83	17.07		
Total	59	1566.33			
d) ferns					
Between Groups	4	117.90	29.47	7.51	P<0.001
Within Groups	55	215.83	3.92		
Total	59	333.73			

Table 2.4 One-way ANOVA analysis comparing the species richness means of the five habitat groups based on a) all species, b) bryophytes only, c) flowering plant only, d) ferns only.

Post hoc Tukey testing was used to identify which of the group species richness means were significantly different from each other (Table 2.5). The analysis indicated there were no significant differences between the species richness means of the two ghyll groups (sandstone and clay) within any of the plant groups and no significant differences between the means calculated for the ancient woodland groups (Table 2.5). An examination of all species (Table 2.5a) indicated that species richness was significantly higher in the clay ghyll woodland group than in any of the ancient woodland sites. Sandstone ghyll woodlands were also significantly richer in species than both clay and sandstone ancient woodlands, but showed no significant difference in species richness compared to the regional ancient woodland group of sites. Bryophyte species richness (Table 2.5b) was significantly higher in the clay ghyll woodland sites than in any of the ancient woodland groups. Bryophyte richness was also significantly higher in sandstone ghyll woodlands than in both the sandstone and regional ancient woodland groups, but showed no significant difference when compared with the species richness within the clay ancient woodlands. The analysis of flowering plants showed that the species richness of both clay and sandstone ghyll woodlands was

significantly different to the clay ancient woodlands and the sandstone ancient woodlands (Table 2.5c). The Tukey analysis indicated that sandstone ghyll fern communities are significantly richer than those recorded within the clay ancient woodland and the regional ancient woodland sites (Table 2.5d).

a) All species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	14.83**			
Sandstone Ghylls	NS	-11.92**		
Sandstone AWs	15.91**	NS	13.00**	
Regional AWs	11.17**	NS	NS	NS

NS indicates mean difference was not significant at P<0.005.
*P<0.005, **P<0.001

b) Bryophyte species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	6.00**			
Sandstone Ghylls	NS	NS		
Sandstone AWs	6.83**	NS	4.25*	
Regional AWs	6.75**	NS	4.17*	NS

NS indicates mean difference was not significant at P<0.005.
*P<0.005, **P<0.001

c) Flowering plant species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	7.08*			
Sandstone Ghylls	NS	NS		
Sandstone AWs	8.33**	NS	7.08*	
Regional AWs	NS	NS	NS	NS

NS indicates mean difference was not significant at P<0.005.
*P<0.005, **P<0.001

d) Fern species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	NS			
Sandstone Ghylls	NS	-4.25**		
Sandstone AWs	NS	NS	NS	
Regional AWs	NS	NS	3.00*	NS

NS indicates mean difference was not significant at P<0.005.
*P<0.005, **P<0.001

Table 2.5 *Post hoc* Tukey tests showing significant differences between group species richness means. The significance level was Bonferroni corrected to P<0.005 for all tables

MAVIS software was used to analyse community composition and assign NVC classifications to each group of sites. The highest three coefficients for each group are shown in table 2.6.

	NVC type – Highest 3 coefficients for each habitat type		
	1	**2**	**3**
Clay ghylls	W9a	W9	W8
Clay AWs	W9	W9a	W8
Sandstone ghylls	W9a	W8	W9
Sandstone AWs	W10	W10e	W10a
Regional AWs	W9a	W9	W8

Table 2.6 National Vegetation Classifications of communities in the survey habitat groups as identified by MAVIS software. Classifications are based on flowering-plant, bryophyte and fern species. NVC communities identified as present; W8 Fraxinus excelsior – Acer campestre – Mercurialis perennis woodland; W9 Fraxinus excelsior – Sorbus aucuparia - Mercurialis perennis woodland; W9a typical subcommunity; W10 Quercus robur – Pteridium aquilinum – Rubus fruticosus woodland; W10a typical subcommunity; W10e Acer pseudoplatanus – Oxalis acetosella subcommunity

The three NVC classifications W9, W9a & W8 were assigned to all of the groups with the exception of the sandstone ancient woodland group which was assigned the classifications W10, W10e and W10a (Table 2.6).

a) All species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	NS			
Sandstone Ghylls	0.538**	0.409**		
Sandstone AWs	0.560*	0.201*	0.298**	
Regional AWs	0.335**	NS	0.273**	NS

NS indicates r value was not significant at P<0.005.
*P<0.005, **P<0.001

b) Bryophyte species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	0.180*			
Sandstone Ghylls	0.416**	0.498**		
Sandstone AWs	0.241**	NS	0.160*	
Regional AWs	0.227*	NS	0.452**	NS

NS indicates r value was not significant at P<0.005.
*P<0.005, **P<0.001

c) Flowering plant species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	NS			
Sandstone Ghylls	0.276*	NS		
Sandstone AWs	0.512**	NS	NS	
Regional AWs	0.258*	NS	NS	NS

NS indicates r value was not significant at P<0.005.
*P<0.005, **P<0.001

d) Fern species

	Clay Ghylls	Clay AWs	Sandstone Ghylls	Sandstone AWs
Clay AWs	0.602**			
Sandstone Ghylls	0.656**	0.756**		
Sandstone AWs	0.622**	0.600**	0.348**	
Regional AWs	0.401**	0.456**	0.350**	0.360**

*P<0.005, **P<0.001

Table 2.7 Pairwise Analysis of Similarity (ANOSIM) of the habitat groups using Community Analysis Package v4 PISCES Conservation Ltd. Significance level was Bonferroni corrected to P<0.005 for all tables

ANOSIM was used to further compare species composition and abundance between the site groups. Significant 'r values' were interpreted according to the manual for the software package PRIMER which interprets r values between 0.501 and 0.750 as 'overlapping but clearly different' and r>0.250 as 'barely separable' (Clarke 2001). The analysis of all species together (Table 2.7a) showed the community composition of clay ghylls and sandstone ghylls to be 'overlapping but clearly different' (r=0.538, P<0.001). The clay ghyll communities were also 'clearly different' in comparison to sandstone ancient woodlands (r=0.560, P<0.005). The analysis indicated that clay ghyll bryophyte composition was 'barely separable' from the composition of bryophyte communities in any of the ancient woodland sites (all comparisons r<0.250) (Table 2.7b). Flowering plant community composition within clay ghyll woodlands was 'overlapping but clearly different' when compared to the composition of flowering plants in the sandstone ancient woodland sites (r=0.512, P<0.001) (Table 2.7c).

The analysis of fern composition showed a significant difference in composition between all groups (P<0.001) (Table 2.7d). Fern community composition in the clay ghyll woodland sites 'overlapped but was clearly different' to the clay ancient woodland sites (r=0.602, P<0.001), the sandstone ghyll woodland sites (r=0.656, P<0.001) and the sandstone ancient woodland sites (r=0.622, P<0.001). The fern composition of the sandstone ghyll woodlands was found to be 'well separated' in comparison with the fern composition of clay ancient woodlands (r=0.756, P<0.001).

2.4.2 Examining associations between the predominant site substrate and ghyll woodland species distributions and composition

Burnside et al. (2006) used cluster analysis to analyse soil associations of the dominant vascular ghyll plant species and identified sub-groupings of species with strong associations with either sandstone or clay geologies. To investigate the influence of substrate on the distribution patterns of ghyll woodland vegetation, six oceanic plant species that in south-east England are mainly restricted to ghyll woodlands (Rose and Patmore 1997) were examined (Table 2.8). Survey records for these species were obtained from the Sussex Biological Records Centre (SBRC). These species are representative of oceanic species occurring within ghyll woodlands but are not a complete species list of ghyll woodland oceanic vegetation.

Species	Type	Number of times survey species has been recorded in Wealden ghyll woodlands	Substrate of each survey site species was recorded at	
			Clay	Sandstone
Jungermannia pumila	Bryophyte	23	1	22
Fissidens rivularis	Bryophyte	14	0	14
Dryopteris aemula	Fern	84	6	78
Cardamine bulbifera	Field layer	64	50	14
Wahlenbergia hederacea	Field layer	28	0	28
Sibthorpia europaea	Field layer	5	0	5

Table 2.8 The distribution patterns within ghyll woodlands of six 'oceanic' plants that Rose & Patmore (1997) describe as strongly associated with ghyll woodland. Table based on the ghyll woodland survey records held by the Sussex Biological Records Centre (SBRC) on the 15/3/12.

Five of the six species were found to be associated with sandstone soils. The remaining species, *Cardamine bulbifera* (Coralroot), was strongly associated with clay substrates with 50 of the 64 recordings of this species occuring in surveys of

clay ghyll woodlands. The fern *Dryopteris aemula* (Hay-scented Buckler-fern) was identified as an indicator species of sandstone ghyll woodlands in Table 2.8. The SBRC data contained 84 survey records for *D. aemula* and of those 78 occurred on sandstone sites.

To attempt to identify associations between community composition and ghyll substrates, Indicator Species Analysis (ISA) was carried out on the clay and sandstone ghyll woodlands. ISA identified eight species that were significant indicators of clay ghyll woodland and 10 species that were indicators of sandstone ghyll woodland (Table 2.9).

	Plant group	Observed Indicator Value (IV)	IV from randomised groups (S. Dev)	P value
Clay ghyll species				
Atrichum undulatum	Bryophyte	67.5	50.4 (±8.47)	0.0442
Brachythecium rutabulum	Bryophyte	67.3	44.2(±10.57)	0.0240
Eurhynchium striatum	Bryophyte	66.8	33.8 (±9.11)	0.0042
Viola riviniana	Flowering plant	66.0	29.6 (±9.16)	0.0040
Geum urbanum	Flowering plant	65.0	33.3 (±8.55)	0.0048
Thamnobryum alopecurum	Bryophyte	63.4	29.1 (±8.64)	0.0042
Mercurialis perennis	Flowering plant	60.0	34.2 (±9.32)	0.0148
Fissidens taxifolius	Bryophyte	58.6	31.9 (±9.18)	0.0136
Sandstone ghyll species				
Pellia epiphylla	Bryophyte	89.4	37.7 (±8.70)	0.0002
Blechnum spicant	Fern	83.0	33.9 (±9.15)	0.0002
Dryopteris dilatata	Fern	79.4	47.8 (±8.36)	0.0026
Dryopteris aemula	Fern	75.9	47.6 (±8.19)	0.0050
Rubus fruticosus agg.	Flowering plant	72.2	50.3 (±8.54)	0.0202
Dryopteris carthusiana	Fern	69.4	30.6 (±8.14)	0.0012
Polytrichum commune	Bryophyte	51.4	31.3 (±8.76)	0.0278
Pseudotaxiphyllum elegans	Bryophyte	45.9	25.2 (±8.99)	0.0480
Pteridium aquilinum	Fern	41.7	19.4 (±7.58)	0.0362
Hypnum andoi	Bryophyte	41.1	22.0 (±7.90)	0.0340

Table 2.9 Indicator species analysis examining clay and sandstone Ghyll Woodlands only.

Sub-communities identified in Table 2.9;

i. *Atrichum undulatum – Brachythecium rutabulum – Eurhynchium striatum* community; indicative of clay ghyll woodland. This sub-community consisted of five bryophyte species and three flowering plant species.

ii. *Pellia epiphylla - Blechnum spicant – Dryopteris dilatata* community; indicative of the presence of sandstone ghyll woodland. The 10 indicator species identified for this sub-community consisted of five ferns, four bryophytes and one flowering plant species.

Rank-shift analysis was then used to compare the species composition of ghyll woodlands located on clay with ghyll woodlands on sandstone (Table 2.10).

Clay ghylls		Clay ghyll occupancy rank (sandstone ghyll rank)	Plant type	Rank shift % (+)	Sandstone ghylls		Sandstone ghyll occupancy rank (clay ghyll rank)	Plant type	Rank shift % (+)
Allium ursinum		35	flowering		*Ctenidium molluscum*		27	bryophyte	
Hypnum jutlandicum		56	bryophyte		*Euphorbia amygdaloides*		50	flowering	
Isothecium alopecuroides		60	bryophyte		*Heracleum sphondylium*		53	flowering	
Plagiochilla asplenoides		46	bryophyte		*Hypnum resupinatum*		44	bryophyte	
Platyhypnidium riparoides		41	bryophyte		*Impatiens glandulifera*		30	flowering	
Primula elatior		42	flowering		*Isopterygium elegans*		37	bryophyte	
Primula vulgaris		50	flowering		*Juncus conglomeratus*		32	flowering	
Rhynchostegiella pumila		25 (90)	bryophyte	72	*Plagiothecium curvifolium*		55	bryophyte	
Viola riviniana	IS	11 (75)	flowering	71	*Polystichum setiferum*		41	bryophyte	
Thamnobryum alopecurum	IS	13 (60)	bryophyte	52	*Pteridium aquilinum*	IS	11	fern	
Plagiothecium undulatum		33 (70)	bryophyte	40	*Stellaria holostea*		38	flowering	
Fissidens bryoides		22 (58)	bryophyte	39	*Blechnum spicant*	IS	4 (72)	fern	77
Geranium robertianium		31 (67)	flowering	39	*Hypnum andoi*	IS	36 (88)	bryophyte	60
Amblystegium serpens		57 (90)	bryophyte	35	*Pellia epiphylla*	IS	5 (51)	bryophyte	52
Fissidens taxifolius	IS	24 (56)	bryophyte	35	*Dryopteris carthusiana*	IS	14 (54)	fern	46
Eurhynchium striatum	IS	19 (49)	bryophyte	33	*Pseudotaxiphyllum elegans*	IS	17 (54)	bryophyte	42
Rhynchostegium confertum		15 (42)	bryophyte	30	*Polytrichum commune*	IS	20 (51)	bryophyte	36
Geum urbanum	IS	27 (53)	flowering	28	*Polystichum aculeatum*		30 (58)	bryophyte	33
Geum rivale		39 (65)	flowering	28	*Rumex sanguineus*		51 (75)	flowering	29
Brachythecium rutabulum	IS	4 (29)	bryophyte	28	*Lonicera periclymenum*		26 (49)	flowering	27
Mercurialis perennis	IS	2 (23)	flowering	23	*Dicranella heteromalla*		43 (65)	bryophyte	26
Conocephalum conicum		48 (68)	bryophyte	21	*Athyrium filix-femina*		10 (32)	fern	25
Dryopteris affinis borreri		21 (40)	fern	21	*Dryopteris aemula*	IS	8 (29)	fern	24
Teucrium scorodonia		47 (65)	flowering	19	*Urtica dioica*		28 (44)	flowering	19
Thuidium tamariscinum		5 (21)	bryophyte	18	*Dryopteris dilatata*	IS	2 (18)	fern	18
Glechoma hederacea		22 (35)	flowering	14	*Dryopteris affinis*		16 (28)	fern	14
Lysimachia nemorum		34 (47)	flowering	14	*Ajuga reptans*		34 (44)	flowering	12
Isothecium myosuroides		43 (56)	bryophyte	13	*Rubus fruticosus agg.*	IS	1 (10)	flowering	10
Polytrichum formosum		9 (18)	bryophyte	10	*Hypnum cupressiforme*		51 (58)	bryophyte	9
Brachythecium rivulare		36 (45)	bryophyte	9	*Pellia endiviifolia*		24 (30)	bryophyte	7
Oxyrrhynchium hians		36 (45)	bryophyte	9	*Plagiothecium nemorale*		83 (85)	bryophyte	4
Carex pendula		17 (25)	flowering	8	*Brachythecium velutinum*		61 (63)	bryophyte	4
Leucobryum glaucom		69 (78)	bryophyte	8	*Mnium hornum*		3 (6)	bryophyte	3
Hedera helix		25 (33)	flowering	8	*Chrysosplenium oppositifolium*		19 (20)	flowering	2
Atrichum undulatum	IS	8 (15)	bryophyte	8	*Circaea lutetiana*		13 (14)	flowering	1
Cardamine pratensis		40 (47)	flowering	7					
Oxalis acetosella		3 (9)	flowering	7					
Dicranum scoparium		51 (58)	fern	6					
Dryopteris filix-mas		16 (22)	fern	6					
Lamiastrum galeobdolon		1 (6)	flowering	6					
Dryopteris cambrensis		72 (78)	fern	5					
Stachys sylvatica		65 (68)	flowering	2					
Asplenium scolopendrium		72 (75)	fern	2					

Table 2.10 Rank shift analysis comparing flora recorded in sandstone ghyll woodlands with flora recorded in clay ghyll woodlands. The occupancy rank column shows the rank of each species within a substrate class (clay or sandstone) with the species rank in the alternate substrate class in parentheses. Changes in the occupancy rates of clay and sandstone ghyll woodland are expressed as a rank shift percentage. If the rank shift % column is blank then the species was not recorded in the ghylls on the alternate substrate. IS = Indicator species identified in Table 2.9.

The species with the biggest rank shift within the clay ghyll group was the moss *Rhynchostegiella pumilla* (Dwarf Feather-moss) which was ranked 25th in the clay group and 90th in the sandstone ghyll group, a positive rank shift of 72% (Table 2.10). The species showing the biggest rank shift in the sandstone ghyll group was the fern *Blechnum spicant* (Hard Fern) which was the 4th most abundant species in the sandstone sites a positive rank shift of 77% when compared with its rank of 72nd within the clay group. Indicator Species Analysis had previously identified *B. spicant* as the 2nd most important indicator species of sandstone ghyll woodland (Table 2.9).

Seven of the 15 species with the highest rank shift figures for clay ghylls (Table 2.10) had previously been identified as clay ghyll indicator species (Table 2.9). Bryophytes appear to be the most important species in terms of increased abundance in the clay group. Of the 15 clay species with the highest rank shift values, 10 were bryophytes and five were flowering plant species (Table 2.10). Within the sandstone ghylls the 15 species with the highest rank shift values consisted of six bryophyte species, three flowering plant species, but also six ferns, indicating the relative importance of ferns within this group (Table 2.10). Eight of these 15 species had previously been identified as sandstone ghyll woodland indicator species (Table 2.9).

2.4.3 The determinants of species richness

Multivariate analysis was used to identify the principal environmental factors influencing patterns of species distributions and abundance. A number of generalized linear models (GLMs) were constructed using all of the sites and using edaphic and landscape variables recorded at the sites as predictor variables (Table 2.11). Angle of slope was identified as a positive predictor within all of the GLM models constructed using data from all of the survey sites. Angle of slope therefore appears to be an important influence on species richness (Table 2.11). Slope angel was also identified as a predictor in the ghyll woodland GLM analysis (Table 2.12). With the exception of the fern only model, pH was identified as a significant predictor in all of the GLM models analysing all sites (Table 2.11). The positive relationship indicates that increases in species richness reflect increasing pH levels within sites. The variables; air temperature, relative humidity, soil temperature, soil moisture and light were liable to change based on the prevailing atmospheric conditions on the day of the survey. In order to control for this, readings were taken simultaneously in the ghyll valley and in the surrounding woodland and the difference between the two values was used. These values were used as predictors in a second set of GLMs that focused solely on ghyll woodland sites (Table 2.12). None of the atmospheric variables were identified as significant predictors within any of the ghyll woodland GLM models. Loss-on-ignition (LOI) explained the most deviance in the model examining all of the

species together (Table 2.12a) and also in the bryophyte only model (Table 2.12b). The relationship was negative, indicating that species richness decreases with increasing levels of soil organic matter within the ghyll woodland soils.

a) All species

All species, all sites model: Slope + pH + Ca + LOI$^{\Delta}$ + P + S
Model deviance: 116.638 d.f. 53

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
pH	P<0.001	14.9	0.736
Slope	P<0.001	14.1	0.017
Ca	P<0.001	4.9	-0.003
P	P<0.05	2.7	-0.128
LOI$^{\Delta}$	P<0.05	2.5	0.056
S	P<0.05	2.4	0.058
Total deviance explained by model		41.5%	

$^{\Delta}$LOI = loss on ignition

b) Bryophytes only

Bryophytes only, all sites model: Slope + pH
Model deviance: 57.167 d.f. 57

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
pH	P<0.001	18	0.313
Slope	P<0.001	16	0.017
Total deviance explained by model		34%	

c) flowering plant species only

Flowering plants only, all sites model: pH + S + slope + K
Model deviance: 114.290 d.f. 55

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
pH	P<0.001	22.6	0.686
S	P<0.05	4	0.104
Slope	P<0.05	3.9	0.012
K	P<0.05	2.5	-0.019
Total deviance explained by model		32.1%	

d) ferns only

Ferns only, all sites model: Slope + Ca
Model deviance: 114.078 d.f. 59

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
Slope	P<0.001	10.5	0.026
Ca	P<0.05	3.8	-0.002
Total deviance explained by model		14.3%	

Table 2.11 Generalized linear models of all sites; examining the determinants of species richness

Soil acidity was identified as a negative predictor of fern species richness, meaning low pH (acidic) readings were associated with increased fern species richness (Table 2.12d). This correlates with the findings of the Tukey test (Table 2.5d) which indicated that fern species richness was significantly higher within the relatively acidic sandstone ghyll sites in comparison to the other sites.

a) all species

All species, ghyll Woodland model: LOI$^{\Delta}$ + slope
Model deviance: 29.333 d.f. 21

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
LOI$^{\Delta}$	P<0.05	17.4	-0.071
Slope	P<0.05	10.9	-0.010
Total deviance explained by model		28.3%	

$^{\Delta}$LOI = loss on ignition

b) bryophytes only

Bryophytes only, ghyll Woodland model: LOI$^{\Delta}$
Model deviance: 57.167 d.f. 57

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
LOI$^{\Delta}$	P<0.05	25.4	-0.084
Total deviance explained by model		25.4%	

$^{\Delta}$LOI = loss on ignition

c) flowering plants only

Flowering plants only, ghyll Woodland model: nitrate
Model deviance: 20.961 d.f. 22

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
Nitrate	P<0.05	16.8	-0.217
Total deviance explained by model		16.8%	

d) ferns only

Ferns only, ghyll Woodland model: pH
Model deviance: 25.751 d.f. 22

Predictor variables	Significance level	% deviance explained by predictor	Parameter estimation
pH	P<0.05	22.8	-0.484
Total deviance explained by model		22.8%	

Table 2.12 Generalized linear models based on ghyll sites only, examining the determinants of species richness

The environmental variables; angle of slope, potassium (K), magnesium (Mg), calcium (Ca), sodium (Na) and pH were analysed using Kruskal-Wallis analysis. Variables whose medians tested as significantly different (P<0.005) amongst the habitat groups were then tested *post hoc* using Mann-Whitney U analysis. This indicated that the average levels of Ca, Na, Mg, K and pH differed significantly between clay and sandstone ghyll woodlands (Table 2.13). In each case the value calculated for the clay ghyll woodlands was significantly higher than the figure calculated for the sandstone ghyll sites. Levels of Ca, Na, Mg, K and pH also differed significantly between the clay and sandstone ancient woodland sites. Again the levels were always significantly higher in the clay ancient woodlands than the sandstone ancient woodlands.

Environmental variables within the regional ancient woodland group did not vary significantly between the clay or sandstone ancient woodland groups, with the exception of pH where the figure for clay ancient woodlands was significantly higher than the sandstone figure. The pH levels measured within the clay ghyll woodlands were significantly higher than those in the clay ancient woodland sites (Table 2.13).

The angle of slope calculated for both ghyll woodland groups was significantly higher than the slope angles calculated for the ancient woodland groups (Table 2.13).

	Median *mg/l*	**P value**		**Median** *mg/l*	**P value**
Calcium			*Sodium*		
Clay ghylls	159		Clay ghylls	10.8	
Sandstone ghylls	29.3	P<0.001	Sandstone ghylls	8.2	P<0.005
Clay ghylls	159		Clay ghylls	10.8	
Sandstone AWs	10.5	P<0.001	Sandstone AWs	7.7	P<0.001
Clay AWs	66.3		Clay ghylls	10.8	
Sandstone AWs	10.5	P<0.001	Regional AWs	7.7	P<0.001
Sandstone AWs	10.5		Clay AW's	10	
Regional AWs	56.4	P<0.005	Sandstone AW's	7.7	P<0.005
Magnesium			*Potassium*		
Clay ghylls	20.9		Clay ghylls	16.7	
Sandstone ghylls	4.2	P<0.001	Sandstone ghylls	5.9	P<0.001
Clay ghylls	20.9		Clay ghylls	16.7	
Sandstone AWs	3.1	P<0.001	Sandstone AWs	5.7	P<0.001
Clay ghylls	20.9		Clay ghylls	16.7	
Regional AWs	8.7	P<0.005	Regional AWs	8	P<0.001
Sandstone ghylls	4.2		Clay AWs	13	
Clay AW's	13.6	P<0.005	Sandstone ghylls	5.9	P<0.001
Clay AWs	13.6		Clay AWs	13	
Sandstone AWs	3.1	P<0.001	Sandstone AWs	5.7	P<0.001
pH			*Slope angle*		
Clay ghylls	5.3		Clay ghylls	17.5	
Clay AWs	4.7	P<0.001	Clay AWs	6.6	P<0.001
Clay ghylls	5.3		Clay ghylls	17.4	
Sandstone ghylls	4.3	P<0.001	Sandstone AWs	9.5	P<0.001
Clay ghylls	5.3		Clay ghylls	17.4	
Sandstone AWs	4	P<0.001	Regional AWs	4.4	P<0.001
Clay ghylls	5.3		Sandstone ghylls	20.2	
Regional AWs	4.5	P<0.005	Clay AWs	6.5	P<0.001
Sandstone ghylls	4.3		Sandstone ghylls	20.2	
Clay AWs	4.7	P<0.005	Sandstone AWs	9.5	P<0.005
Clay AWs	4.7		Sandstone ghylls	20.2	
Sandstone AWs	4	P<0.001	Regional AWs	4.4	P<0.001
Sandstone AWs	4				
Regional AWs	4.5	P<0.005			

Table 2.13 Mann-Whitney analysis of the variance of environmental medians. Significance levels for each variable were Bonferroni corrected to P<0.005. AWs = ancient woodlands.

2.4.4 The distribution of species within the ghyll valleys

ANOVA and Kruskal-Wallis analysis carried out on the four plant groups; all species, bryophytes, flowering plants and ferns, found no significant difference in the species richness of the four strata.

2.5 Discussion

2.5.1 Examining the composition and spatial limits of ghyll woodland plant communities

Around 85% of the Wealden ghyll woodlands are designated as ancient woodlands (Burnside et al. 2006). These include all 24 of the ghyll woodlands surveyed for this chapter. The analysis indicates that ghyll woodlands are significantly richer in flowering plant and bryophyte species relative to other ancient woodland within the region. Fern species richness was significantly higher in the sandstone ghyll sites than in the ancient woodlands adjacent to them (Tables 2.3, 2.4 & 2.5). The richness of flowering plant, bryophyte and fern communities is regarded by many as perhaps the main component that determines the presence of ghyll woodland within the Weald (Ratcliffe 1968, Rose and Patmore 1997, Burnside et al. 2006). This study is the first to compare vascular and non-vascular ghyll woodland plant assemblages with those of ancient woodlands within the region. Such an overview serves to inform the debate on the regional biodiversity value of the Wealden ghyll woodlands.

NVC classifications assigned to the habitat groups on the basis of bryophyte, flowering plant and fern species composition, were compared to determine whether ghyll woodland could be distinguished from ancient woodland on the basis of community composition (Table 2.6). With the exception of the sandstone ancient woodland sites, the NVC woodland classifications that most closely matched the community composition at each site were W9, W9a & W8 woodland communities (Ash-Maple variations). The sandstone ancient woodland communities were distinct from the other sites and were assigned the classifications W10, W10a & W10e woodlands, which represent varieties of Oak-Birch woodland (Rodwell 1991).

W9 & W9a woodlands are commonly found next to streams in the cool, damp uplands of western and northern Britain (Rodwell 1991). The combination of mild winter temperatures and high levels of relative humidity produce a community that is markedly oceanic in character containing an abundance of ferns and bryophytes (Rodwell 1991). It is clear from the distribution map in Figure 2.3 that W9

woodland is not a woodland type associated with the warm, dry, south-east of England. The identification of moisture-loving, oceanic communities within the ghyll woodlands may indicate the presence of a mild, humid microclimate (Ratcliffe 1968). The W9 and W9a woodland classifications were also assigned to the clay ancient woodland and the regional ancient woodland sites (Table 2.6). This appears to indicate that oceanic communities are not restricted to the ghyll valleys but are present throughout the ancient woodlands within the region. The analysis indicates that ghyll woodlands cannot be distinguished from other ancient woodland in the region based on NVC classifications assigned using flowering-plant, bryophyte and fern species data.

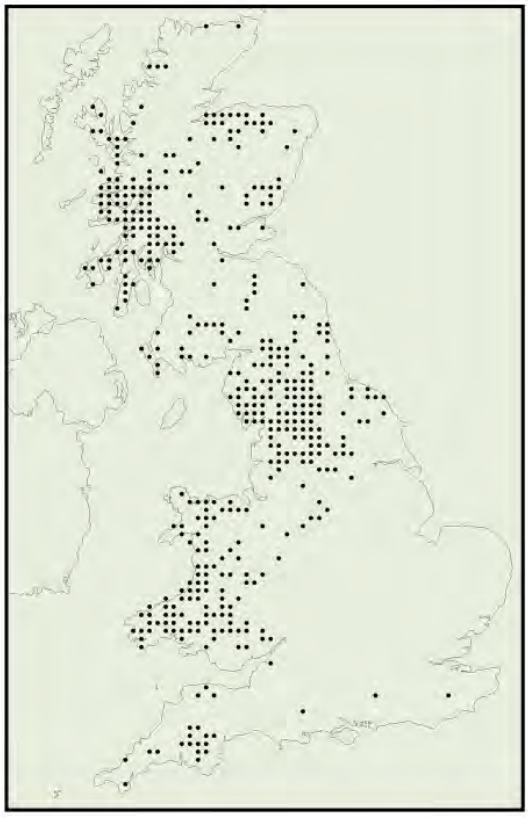


Figure 2.3 Distribution of W9 *Fraxinus excelsior – Sorbus aucuparia – Mercurialis perennis* woodland throughout Britain. Reprinted from Rodwell (1991)

ANOSIM analysis showed that fern composition within ghyll woodlands was clearly distinct from the composition of ferns in the ancient woodland sites (Table 2.7). The presence of a humid microclimate within the ghyll valleys may be responsible

for the differences in fern composition. Many fern species require relatively high humidity levels for growth and reproduction (Tryon 1986, Barrington 1993). Fern diversity tends to increase both within ravines and beside streams due to the high humidity levels found close to water bodies (Kessler 2010). The analysis found the composition of bryophyte and flowering plant communities within the ghyll woodlands was 'barely separable' from the composition of the surrounding ancient woodlands (Table 2.7).

A study of ghyll woodland vascular plant communities by Burnside et al. (2006) classified 47% of the sites analysed as W10 *Quercus robur – Pteridium aquilinum – Rubus fruticosus* woodland and a further 43% as W8 *Fraxinus excelsior – Acer campestre – Mercurialis perennis* woodland. The remaining areas comprised W7, W12 & W15 with smaller pockets of W4, W6 and W16 woodland. A survey of ancient woodland in the Weald found W10 woodland was the most common type of ancient woodland in the Wealden District (Westaway 2006). Other NVC types mentioned in the report were W8 & W16 woodland communities and small patches of wet alder woodland (primarily NVC W6 & W7) which were 'common along-side streams within the Weald'. The NVC communities identified in Burnside et al's ghyll woodland surveys (2006) and in Westaway's ancient woodland surveys (2006) appear very similar containing the same dominant NVC community types (W10 & W8) and sharing three of the six less widespread NVC categories (W7, W6 & W16). These studies also appear to indicate that ghyll woodlands cannot be separated from ancient woodlands on the basis of NVC communities.

The W9 & W9a communities identified in Table 2.6 do not appear to have been found in either the ghyll woodlands studied by Burnside et al (2006) or in the ancient woodlands studied by Westaway (2006). A major difference between the surveys was the type of data used. Both Burnside et al. and Westaway used NVC classifications based on vascular plant species which included understorey and canopy species, but they did not include bryophytes in their analyses. By definition, the influence of microclimatic conditions on ghyll vegetation is likely to be a function of scale. Larger species, such as those in the canopy and understory, are less likely to be affected by a microclimate than ground layer and field layer species. The species composition recorded within the two studies did not include the oceanic bryophyte communities and therefore appears to simply

reflect the typical composition of W10 & W8 woodland associated with higher plants throughout the warm and dry south-east region.

The ghyll woodlands contain oceanic bryophyte communities that are not found elsewhere in eastern or central England and which are hundreds of kilometres from other British populations (Patmore 2000). The studies carried out by Burnside et al (2006) and Westaway (2006) indicate that if bryophyte species are excluded then ghyll woodland communities cannot be distinguished from other ancient woodland communities on the basis of NVC communities. The analysis of NVC communities carried out for this chapter (Table 2.6) did include the bryophyte component of the woodlands and assigned NVC types that reflected these biologically important oceanic communities. The analysis illustrates that any attempt to classify the Wealden ghylls on the basis of vegetation must include the bryophyte component that typifies this internationally important habitat type.

An aim of the analysis was to determine whether ghyll woodlands could be separated from ancient woodland on the basis of species richness or community composition. The results indicate that the ghylls can be distinguished from ancient woodland based on bryophyte and flowering plant richness, which were significantly higher in ghyll woodland than in ancient woodland (Table 2.5b & 2.5c). The analysis also indicated that ghylls located on sandstone could be separated from all of the ancient woodland groups on the basis of the significantly higher fern species richness occurring within the sandstone ghyll woodlands (Table 2.5d). The study found that although moisture loving bryophytes are important for classifying ghyll woodland NVC communities, the same oceanic NVC community types were also assigned to the majority of ancient woodland sites studied (Table 2.6). Based on these results the ghylls could not be separated from ancient woodlands on the basis of community composition.

A further aim was to identify the spatial limits of ghyll woodland vegetation. The surveys of ancient woodland immediately adjacent to the valley edges (Table 2.5) allowed species richness within the valleys to be compared with richness levels immediately outside the valley edge. The significantly higher mean species richness levels recorded within the valleys relative to the surrounding ancient woodlands (Table 2.5) indicates that ghyll woodland vegetation is confined to the

stream valleys and does not appear to extend into the woodland surrounding the valleys. Defining spatial boundaries may facilitate a true analysis of the extent and character of these internationally important habitats.

2.5.2 The influence of substrate on species composition and abundance

Species richness levels showed no significant variation between the two ghyll substrate groups, indicating that species richness cannot be used to differentiate clay and sandstone ghyll woodlands. A comparison of community composition assigned the same 'top three' NVC woodland types to both groups, indicating that similar species lists were recorded within both sandstone and clay ghylls. However ANOSIM analysis, which takes into account species abundance as well as species richness, indicated that community composition was distinguishable between the two groups of sites. The flowering plant and bryophyte communities within the sandstone and clay ghylls were significantly different to each other, but it was again the fern community that showed the clearest distinction, with the analysis indicating that the two communities were overlapping but clearly separated in terms of fern species composition (Table 2.7).

The importance of fern species in differentiating clay and sandstone ghyll communities was also apparent in the results of the indicator species analysis with ferns constituting half of the species identified as indicators of sandstone communities (Table 2.8). Many fern species are closely associated with specific rock or soil types (Mehltreter et al. 2010). For example *Blechnum spicant* (Hard Fern) identified as the 2nd most important indicator species and ranked 4th for abundance within the sandstone ghyll group, is strongly associated with acidic woodland soils (Merryweather 2007) and in Europe many *Asplenium* species are confined to either limestone, serpentine or acidic rock (Vogel et al. 1999). Studies in the tropics have shown that differences in soil substrates may explain many of the changes in fern community composition. Studies of the ferns *Adianthum* and *Polybotrya* in Amazonia and studies of tree ferns in Costa Rica have shown that individual species have distinct preferences for specific soil conditions (Tuomisto and Ruokolainen 1994, Tuomisto and Poulsen 1996, Poulsen et al. 2006). The

specificity of fern communities to soil substrates makes them suitable as indicators of forest type (Ruokolainen et al. 1997, Mehltreter et al. 2010) and as proxy indicators of the distribution patterns of other substrate sensitive plant groups (Vormisto et al. 2000).

The fern *Dryopteris aemula* (Hay-scented Buckler-fern) was identified as an indicator species of sandstone ghyll woodlands. This was one of the six oceanic species that Rose and Patmore (1997) used as examples of species that are strongly associated with the Wealden ghyll woodlands (Table 2.9). Data supplied by the Sussex Biological Records Centre showed 84 separate populations of *D. aemula* had been identified within the Wealden ghyll woodlands. These represent some of the densest colonies of this fern anywhere in Europe. Elsewhere within the UK colonies of the fern are only found in these densities in Devon, Cornwall and Western Ireland (Rose and Patmore 1997).

Indicator species analysis identified the thalloid liverwort *Pellia epiphylla* (Overleaf Pellia) as the most important indicator species of sandstone ghyll sites (Table 2.8). This species is associated with damp habitats and is often found on stream-banks in dense bands just above the water level (Atherton et al. 2010). The availability of moisture from the stream makes the moisture-holding capacity of the substrate far less important for this type of desiccation intolerant species. *P. epiphylla* grows on neutral to acidic substrates, which may explain its strong associations with sandstone ghyll sites. It is replaced by *Pellia endiviifolia* (Endive Pellia) in base rich sites, where it again can be found growing in very close proximity to the watercourse (Atherton et al. 2010).

Of the eight clay ghyll woodland indicator species identified through indicator species analysis (Table 2.8), five were bryophytes, indicating that bryophytes appear to be the most important taxonomic group for identifying the presence of clay ghyll woodland. All of the bryophytes identified as clay ghyll indicator species are species that have a broad pH tolerance (Atherton et al. 2010) and therefore it seems unlikely that soil acidity is the primary influence on the distribution of these species. A study of bryophytes in New South Wales examined bryophyte abundance on both clay and sandstone soils (Downing et al. 2007). The authors explained bryophyte distribution patterns on the basis of the capacity of the

underlying substrate to hold water. Clay based soils have a better capacity to retain moisture than free-draining sandstone based soils because of the vast increase in particle surface area per gram (Section 2.1.2.2.2). Most bryophyte species are able to cope with periods of extended desiccation (Proctor et al. 2007), however critical constraints upon niche occupation may operate during establishment from spores (Wiklund and Rydin 2004). The occurrence and abundance of these bryophyte species may therefore be correlated with their ability to establish, rather than their ability to persist once established. The capacity of soils to retain moisture may be an important factor in bryophyte establishment because spore germination requires a damp, humid environment (Goffinet and Shaw 2009). Bryophytes can also colonise sites via vegetative spread, however, the success of this process has also been shown to be moisture dependent (Cleavitt 2002). A practical example of this is the technique developed for the restoration of *Sphagnum* on peat beds after the peat has been harvested. The fragments of *Sphagnum* used are covered by a layer of damp straw mulch to prevent the fragments becoming desiccated before the plants have become properly established (Rochefort and Lode 2006). This highlights the importance that substrate moisture levels play in the vegetative spread of bryophytes.

2.5.3 The influence of the physical environment

Regression modelling identified pH as the primary environmental variable influencing the species richness patterns observed for both bryophyte and field layer species in the GLMs that used all of the sites. Soil acidity was also identified as a predictor of fern species richness in the analysis that only included the ghyll woodland sites.

A number of studies of vascular plants and bryophytes, both in Britain and throughout Europe, have also found a strongly positive correlation between plant diversity and soil acidity levels (Watson 1981b, Brunet et al. 1996, Corney et al. 2004, Pärtel et al. 2004, Hokkanen 2006). Highly acidic soils (pH<4) may result in high, potentially toxic, concentrations of aluminium ions, manganese ions and iron ions (Section 2.1.3.2.3). The soil acidity levels recorded within the sandstone ghyll

sites and sandstone ancient woodland sites were frequently less than pH 4, with the lowest reading recorded in the sandstone ghylls being pH 3.5 and the lowest within the sandstone ancient woodlands group being pH 3.4. Species able to colonise and survive soils with a pH<4 are known as acidophilic. These plants have complex biochemical and physiological pathways that allow them to survive the harsh chemical environment induced by the high soil acidity (Ehrenfeld et al. 2005). The high levels of acidity in the sandstone sites will effectively aid colonization by acidophilic species through the elimination of competitors unable to tolerate highly acidic sites. Once established, a number of acidophilic bryophytes exhibit a plant-soil feedback system that ensures the soil remains highly acidic. Probably the best example of this is the effect of the mosses in the *Sphagnum* genus. Decomposition of plant tissues containing polyuronic acids can lead to a rapid decrease in soil pH levels by two or more units within the first few years of establishment (Ehrenfeld et al. 2005). The presence of areas with extremely acidic soils within the sandstone ghylls would provide a suitable habitat for acidophilic species, potentially leading to increased diversity within these sites.

Most plant species found in the mid-range of pH values tend to be capable of growing under a broad range of soil acidity (Crawley 1997). Species with a narrow range of pH tolerance tend to be species that are characteristic of either extremely acidic or extremely base soils (Crawley 1997). Threatened vascular plant species tend to have a restricted pH tolerance range while non-threatened species tolerate a wider range of soil pH (Pärtel et al. 2004). It is possible therefore, that areas of extremely acidic soils would not just increase species richness within the ghylls, but also provide a suitable habitat for rare acidophilic species.

The GLM model containing just the ghyll valley sites showed a negative association between angle of slope and species richness (Table 2.12a). The steep sided nature of the Wealden ghyll woodlands has been cited as the reason that much of the woodland within these valleys has historically remained ‘untouched, undisturbed and ancient’ (Southgate 2012). If this is the case then it might be expected that there would be a negative correlation between angle of slope and the amount of management that has historically been carried out at a site. With the potential for ‘old growth’ species to persist in relatively untouched ancient woodland sites, it might therefore also be expected that species richness would

increase with increasing slope angle rather than decrease. Slope angle itself is unlikely to directly restrict or influence plant growth. It is more likely it is an indirect or distal variable (Austin 2007). Angle of slope can affect soil moisture levels, organic matter build up, levels of erosion, levels of light etc. Studies of riparian plant distributions have frequently found soil moisture to be the principle variable influencing distribution patterns (Malanson 1993, Naiman and Decamps 1997, Coroi et al. 2004, Stewart and Mallik 2006). However, levels of a number of climatic and edaphic variables, including light and soil moisture, were included in the models but were not identified alongside slope angle as significant predictors in the ghyll woodland regression models (Table 2.12).

Loss on ignition is an indication of soil organic matter content. Bryophyte species richness within the ghyll woodland sites was therefore negatively correlated to the amount of organic matter within the soil (Table 2.12b). Decaying organic matter provides a major source of plant nutrients (Gosz et al. 1976, Zech et al. 1997) and therefore low levels of organic matter within soils have been found to correlate with low soil fertility (Tiessen et al. 1994). Bryophytes have been shown to respond negatively to increased levels of soil nutrients. A study of the effects of nine years of N,P,K, fertilization on boreal forest vegetation in north-western Canada showed a decrease in bryophyte species richness in response to increased nutrient availability (Turkington et al. 1998, Virtanen et al. 2000). Experiments have shown that increasing nitrogen levels can lead to increased plant growth in terms of biomass, but can also lead to decreasing levels of species richness as dominant species ‘shade out’ subordinate species both inhibiting survival of the subordinate and preventing its’ seedlings from germinating (Foster and Gross 1998, Wilson and Tilman 2002). Bryophytes have a number of mechanisms that allow them to cope with low soil nutrient levels. Bryophytes lack the root systems of vascular plants and instead sustain their nutritional needs through the assimilation of minerals dissolved in rainwater and aerosols (wet deposition) and through the assimilation of atmospheric gases such as ammonia, and oxides of nitrogen and dust (dry deposition) (DeLuca et al. 2002, Hokkanen 2006, Vanderpoorten and Goffinet 2009). Bryophytes have a very effective mechanism for translocating nutrients from old to new cells and therefore the cycling of nutrients within bryophytes is highly efficient (Brown and Bates 1990). Studies have even shown

considerable growth in some bryophyte species during temporary suspensions to the input of inorganic nutrients (Wells and Brown 1996, Bates 1997). Bryophytes are therefore able to survive in sites with relatively low levels of nutrients because although bryophytes have the same nutrient requirements as vascular plants (Bates 2000), they are able to fulfil most of their nutrient needs from sources other than the soil (Vanderpoorten and Goffinet 2009).

The source of soil organic matter is predominantly through the decomposition of leaf litter and therefore levels of soil organic matter may be an indication of levels of leaf litter in the system (Facelli and Pickett 1991). This could also provide an explanation for the negative correlation between loss on ignition and bryophyte species richness within the ghyll valleys. Aside from providing a nutrient source, leaf litter directly and indirectly alters abiotic conditions and plays a major role in structuring plant communities (Kostel-Hughes et al. 2005). The accumulated litter intercepts sunlight, thereby shading seedlings and seeds, whilst also potentially reducing soil temperature (Xiong and Nilsson 1999). The litter can form a barrier, reducing water evaporation from the soil and therefore maintaining damp soil conditions. Conversely, the litter may reduce water penetration into soils through absorbing and retaining a large proportion of precipitation (Facelli and Pickett 1991). Litter can also limit the emergence of plants by creating a physical barrier which prevents the emergence of some seedlings. This leaf litter 'barrier' might also prevent seeds and spores from reaching the soil (Donath et al. 2006, Natalia et al. 2008).

Valley floodplains tend to be nutrient rich in comparison to the surrounding habitats because they are depositional (Peterson and Rolfe 1982), with the abundance of nutrients tending to decrease with elevation (van Coller et al. 2000). Frequent periodic flooding will mean that substrates around the stream, as well as emergent substrates within the stream itself, are regularly inundated with water. This is important for many riparian valley species, including some ferns and bryophytes (Watson 1981a, Patmore 2000). The area directly adjacent to the stream often contains species not found elsewhere in the valley (Haase and Gläser 2009). Indeed, Patmore (2000) states that within the Wealden ghyll woodlands, 'rocks in and near streams provide suitable habitat for the internationally important lower plants'. Analysis of species distributions within

riparian valleys frequently show a gradient in species richness, highest directly adjacent to the watercourse and decreasing with increased distance from the water's edge (Harris 1988, Gregory et al. 1991, Coroi et al. 2004). However, Kruskal-Wallis analysis of species distributions within the ghyll valleys indicated that the ghyll woodland vegetation was not significantly richer in the area surrounding the stream, nor did there appear to be any gradient in species richness across the valleys.

The effect of nutrient levels on plant survival and growth will be species specific, but as a general rule species richness tends to be greatest where nutrients are in short supply as plants will then not grow tall enough to create competition for light (Grime 1979, Roem and Berendse 2000). Levels of the nutrients; calcium, sodium, magnesium and potassium were all significantly higher in the clay ghylls than in the sandstone ghylls (Table 2.13). Species richness appeared unaffected by this and did not vary significantly between the two groups.

The presence of a humid microclimate within the ghyll valleys has been widely reported (Ratcliffe 1968, Rose 1995, Patmore, 2000). Extensive environmental readings were recorded both within the valleys and in the surrounding woodland, including relative humidity, air temperature, soil moisture and soil temperature. Analysis of these figures indicated no significant differences between the atmospheric readings within the valleys and those taken outside the valleys (Table 2.13). Therefore, despite the identification of rich oceanic communities associated with shaded, humid habitats (Table 2.6), the results of the environmental variable analysis did not show evidence of the existence of a shaded, humid microclimate within the ghyll woodlands (Table 2.12).

2.6 Conclusions

NVC woodland classifications assigned on the basis of flowering plant and bryophyte species composition were more characteristic of the humid, cool, Atlantic forests of north-west Scotland, rather than the warm and dry woodland typically associated with south-east England. A number of authors have

highlighted the presence of regionally rare oceanic bryophyte and field layer species within the ghyll woodlands (e.g. Ratcliffe 1968, Rose 1995, Rose and Patmore 1997) and it appears to be this oceanic component of the ghyll flora that is producing these 'Atlantic forest' classifications.

The study indicated that ghyll woodland can be distinguished from other ancient woodland within the region on the basis of species richness. Flowering plant and bryophyte richness is significantly higher in the ghyll woodland sites irrespective of substrate, whilst fern species richness is significantly higher in the sandstone ghyll sites than in the adjacent woodlands. The oceanic vegetation classifications assigned to the ghyll woodlands are associated with damp, humid microclimatic conditions (Table 2.6). However, the analysis of environmental variables found no significant difference between the variables measured within the ghyll valleys and those in the surrounding woodlands (Table 2.13). Therefore despite the generally held view that species richness within the ghylls is correlated with a humid microclimate (e.g. Paton 1956, Ratcliffe 1968, Rose and Patmore 1997) no physical evidence of this microclimate was found. Instead fern species richness appears correlated to soil pH, whilst the level of organic matter content within the soil appears correlated to bryophyte species richness and flowering plant species richness is strongly correlated with nitrate levels (Table 2.12). It is interesting that the oceanic NVC community classifications (W9 & W9a) assigned to the ghylls were also assigned to the ancient woodland sites located on clay, and also to the regional group of ancient woodlands (Table 2.6). This may be an indication that shaded, humid, microclimatic conditions exist in ancient woodlands throughout the region and would explain why no significant difference was found between climatic conditions within the ghyll valleys and those in the surrounding woodlands (Table 2.13).

Spatial analysis of the Sussex Biological Records Centre data indicates the Wealden ghylls cover an area of 9332 hectares (Burnside et al. 2006). The accuracy of this figure has been questioned since the mapped boundaries of many ghyll woodlands include areas of woodland beyond the valley boundaries (Southgate 2012). The analysis within this chapter has shown that species richness within the valleys is significantly higher than in surrounding woodlands. If ghyll woodland is distinguished from other woodland within the region on the basis

of higher levels of species richness then this study indicates that ghyll woodland is confined to the valleys themselves. If this is the case then the boundaries of many ghyll woodlands would need to be redrawn and the estimated area for ghyll woodland within the region would need revising.

Analysis indicated that ghyll communities located on clay and on sandstone are similar in terms of species richness, but can be differentiated on the basis of community composition and species abundance. Fern species composition and abundance appears particularly important in separating the two communities and to a lesser extent so do the bryophyte and field-layer components. Indicator species analysis identified an *Atrichum undulatum – Brachythecium rutabulum – Eurhynchium striatum* community indicative of the presence of clay ghyll woodland and a *Pellia epiphylla - Blechnum spicant – Dryopteris dilatata* community indicative of sandstone ghyll woodland. These could potentially be used to confirm the presence of one of the two sub-communities, although this has not been tested in the field.

This research has shown that ghyll woodlands appear significantly higher in bryophyte, field layer and fern species richness than other ancient woodland within the region emphasising the value of ghyll woodlands in terms of regional biodiversity.

3 An investigation of temporal trends in Wealden ghyll woodland plant communities

3.1 Preamble

The previous chapter found that ghyll woodlands could be distinguished from other woodland within the region on the basis of the significantly higher plant species richness found in the ghylls. This chapter will investigate whether the richness and composition of ghyll woodland communities has changed significantly during the post-war period.

Chapter 2 also examined community composition through assigning National Vegetation Classifications. The NVC types identified indicated that the ghyll woodland bryophyte communities have strong oceanic affiliations. In response to these findings Chapter 3 will separate oceanic bryophyte species and examine them separately, along with another group of biologically important species associated with the ghyll woodlands; ancient woodland indicator (AWI) species (Rose and Patmore 1997).

3.2 Introduction

Around 5000 years ago the majority of Britain was covered in woodlands that today are collectively referred to as the 'Wildwood' (Rackham 1986). Due to the large-scale woodland clearance that occurred throughout Britain over the last 3000 years (Section 1.2.2) only 5% of Britain's land surface remained wooded by 1900 (Rackham 2006). Initiatives were introduced after the First World War to promote woodland conservation and restoration. This led to a simple UK woodland policy of bringing as much land as possible under productive woodland cover (Farmer and Nisbet 2004). The policy helped increase net woodland cover to its current level of around 9% (Smith and Gilbert 2003). However, this increase reveals only part of

the picture, masking both gains and losses in different woodland types, as well as changes in woodland structure (Hopkins and Kirby 2007). The majority of the increased woodland coverage was due to the establishment of large-scale conifer plantations (Forestry Commission 2004). These conifer plantations were usually planted as commercial crops and tended to be mono-cultures, usually containing even-aged Sitka spruce (*Picea sitchensis*) (Farmer and Nisbet 2004). This lack of species diversity within the canopy, together with the low structural diversity associated with even-aged conifer monocultures, leads to low levels of biodiversity in comparison with broadleaf woodlands (Rackham 2006). Whilst net woodland cover increased in Britain during the 20th century, the amount of ancient woodland decreased (Smith and Gilbert 2003). From 1935 to 1985 around 7% of the UK's ancient broadleaf woodlands were destroyed and a further 38% were converted into conifer plantations (Spencer and Kirby 1992). Today the southeast of England contains around 40% of the country's ancient woodland (Forestry Commission 2004). The analysis documented in Section 2.4.1 (Tables 2.3 & 2.5) of this report indicates that the species richness of the Wealden ghyll woodlands is significantly higher than other ancient woodlands within the region and underlines the importance of the Wealden ghylls in terms of regional biodiversity. Global concerns over losses in biodiversity have led to the formulation of national and local action plans aimed at conserving and increasing biodiversity (e.g. Maddock 2008, UK BAP 2010). The protection of the Wealden ghyll woodlands is central to the habitat action plans of all of the regional authorities covering the Wealden area (Surrey Biodiversity Partnership 2001, Tunbridge Wells Borough Council 2008, Sussex Biodiversity Partnership 2010, Kent County Council 2013). The focus of these habitat action plans appears to be the preservation of the current ghyll woodland boundaries rather than the preservation of ghyll woodland species composition. In the period since the end of the Second World War, a number of environmental changes have occurred that may influence species composition within the Wealden woodlands. These include changes in woodland management, climate change, land-use changes and the effects of relatively recent habitat loss and fragmentation (Hopkins and Kirby 2007). It is important that species composition within the ghyll woodlands is monitored to help with the conservation of biologically important species and species assemblages (Cramer and Whittaker 1999). This chapter uses archive plant survey data to reveal temporal changes in

environmental indicator scores proposed by Ellenberg (1974) and Preston and Hill (1997) along with changes in indicator scores based on species specific dispersal characteristics and habitat preferences (Grime et al. 1988, Davies et al. 2004, Hill et al. 2007). The analysis will seek to identify temporal changes in ghyll woodland species composition and explore the determinants of any changes identified.

3.2.1 Using archived survey data to reveal temporal changes in species composition

The high levels of biodiversity losses occurring today are a cause of great concern (Butchart et al. 2010, Cardinale et al. 2012). As such, the collection of data that can be used for monitoring and gauging changes in biodiversity through time is essential (Magurran et al. 2010).

To accurately measure changes in species distributions, and to identify the environmental correlates of these changes, it is necessary to observe communities over relatively long periods of time (Magurran et al. 2010). The length of each monitoring period will depend on the ecology of the species being studied. Species such as bryophytes, that produce a generation each year, will respond far more quickly to changes in the environment than woody species whose generation times may be several decades or more (Snall 2005). A major advantage of carrying out long term monitoring surveys is that they identify real changes in real communities. The biggest disadvantage is that they generally require many years to identify trends, by which time deleterious patterns of species change may be irreversible (Morison and Morecroft 2008). An alternative to long-term monitoring programmes is the use of archive survey data, which again enable researchers to examine real changes through time in real plant communities, whilst obtaining the results relatively rapidly.

A limitation of archive data is that many long-term datasets contain variations in sampling methodology, intensity and interval, often due to changing research priorities and changing researchers (Magurran et al. 2010). But providing these limitations are acknowledged and the methodology is designed accordingly then

archive survey data is extremely useful for rapidly identifying temporal changes (Magurran et al. 2010).

To reveal post-war changes in the ranges and abundance of Wealden ghyll woodland vegetation and to attempt to determine the causes of any observed changes, archived survey data from a series of surveys carried out over a 53 year period was analysed.

3.2.2 Changes in woodland management and structure

For at least the past 2000 years the majority of Britain's woodlands have been under some type of woodland management (Rackham 1980). Up until the 20th century most ancient woodland in south-east England was managed using some form of coppice system (Rackham 1990, Brandon 2003). Although coppicing was largely abandoned towards the end of the 19th century, the increased demands for wood during the First and Second World Wars meant coppicing was reintroduced in the woodlands during these periods (Forestry Commission 1952, Richards 2003). Consequently in 1947 around 40% of the southeast woodlands were being coppiced (Forestry Commission 2004). However, coppicing was again abandoned after the war and by 2002 this figure had fallen to 7%.

3.2.2.1 The ecological implications of coppicing

Coppicing dramatically increases light penetration within woodlands. In full daylight light levels on the woodland floor can increase from around 5% before coppicing to as much as 100% afterwards (Peterken 1993). Levels of light then decrease over a number of years as the canopy slowly closes. Coppice rotations were usually less than 30 years, and this sequence of a few years of light, followed by several decades of increasing shade was repeated with each coppice cycle. Many shade tolerant perennials, such as *Anemone nemorosa* (Wood anemone) and *Primula vulgaris* (Primrose), need this cycle of coppicing. They grow most strongly in the years of light but require the years of shade to suppress grasses and other weedy type plants that would out-compete them in high-light environments (Barkham

1992b, Van Calster et al. 2008). The cycle of coppice rotation meant that there were always open areas of young-growth woodland that could be colonized by herbaceous ground flora, either from the soil seed-bank or through dispersal from neighbouring areas (Buckley 1992). Vascular plant diversity will increase in the relatively open early coppice (Barkham 1992a) and then decline as woody species re-grow and re-establish their monopoly of the available light (Mitchell and Kirby 1989). These open, early coppice stands are considered highly valuable for biodiversity (Buckley 1992, Fuller et al. 1993).

The large-scale abandonment of coppice management has meant that many former coppice woodlands are now classified as high forest (Evans 1984, Kirby et al. 2005, Hopkins and Kirby 2007, Amar et al. 2008). National figures show that in 1947, 51% of woodland area in Britain was classified as high forest, but by 2002 this figure had increased to 97% (Hopkins and Kirby 2007). The abandonment of coppice management and the subsequent change into high forest woodland types will inevitably reduce the availability of open or young-growth stands. Kirby et al. (2005) found a negative association between ground flora species richness and the increasing basal area of trees (associated with high forests). This relationship was stronger for woodland specialists than for generalist species. They found that species that increased in numbers within high forests tended to be species associated with shaded or semi-shaded habitats.

As well as affecting light levels, coppicing causes relative humidity levels to fall and temperatures to increase (Thomas and Packham 2007). Levels of soil nutrients and pH are also likely to change (Hölscher et al. 2001). These environmental changes may impact upon the distribution patterns of species sensitive to microclimatic changes.

3.2.3 Habitat fragmentation

3.2.3.1 The effects of habitat loss

Historically, the steep, frequently boggy, ghyll woodlands have been considered unsuitable for cultivation or settlement (Rose and Patmore 1997). As such they

have been left wooded whilst much of the surrounding woodland has been cleared. In some cases the adjacent woodland has been cleared right up to the edges of the ghyll valleys and where this occurs the remaining woodland forms a linear habitat whose dimensions and shape are determined by the valley boundaries (Burnside et al. 2002a). It is more common for woodland to be cleared on one side of the valley only, whilst the land on the other side continues to be wooded (pers. obs.). Where this occurs the ghyll valley forms part of the boundary of larger, contiguous woodland.

Numerous studies have shown that habitat loss impacts negatively on levels of biodiversity (Fahrig 2003). Species have a minimum habitat size threshold below which they cannot survive; this is termed the extinction threshold (Flather and Bevers 2002, Fahrig 2003). Smaller areas generally contain less species than larger areas of the same habitat type; a pattern known as the 'species-area relationship' (MacArthur and Wilson 1967, Saunders et al. 1991). Where habitat loss has reduced the size of the woodland patches, this will impact on the number of species that the woodland can support. The survival of species that utilise both the ghyll valleys themselves and the surrounding woodlands may be affected by the loss of woodland surrounding the ghyll valleys if woodland clearance has reduced the total habitat size to a level below the species minimum habitat threshold.

Losses of woodland in the surrounding landscape can also lead to increasing levels of 'patch isolation' (Gibbs 2001). Small 'patches' of habitat are often inhabited by subpopulations of species that form larger 'metapopulations' that span several patches (Levins 1970). These patches are genetically connected through the dispersal of individuals or genetic material (such as pollen and spores) (Levins 1970). The loss of woodland in the surrounding landscape may result in increased levels of isolation because the distance between patches becomes too great for the dispersal of individuals or genetic material (Andrén 1994, Lindenmayer and Fischer 2006). Changes in land-use that occur in the matrix between patches and which increase the 'hostility' of the matrix for the dispersing species may create a barrier to dispersal and could therefore increase levels of genetic isolation (Cushman et al. 2006, Holderegger et al. 2010). Isolation is therefore not just the distance between patches, but a combination of distance,

species dispersal mechanisms and the 'permeability' of the landscape between sites (Jeffries 2006).

Species isolation can lead to a number of genetic problems, particularly for species whose numbers are low (Franklin 1980). Small isolated populations may lack the genetic variation to cope with future changes in the environment. They will also suffer an increased likelihood of mating with closely related individuals which will reduce the fitness of the population through the deleterious effects of inbreeding depression (Soulé and Simberloff 1986). A figure of between 500 and 1000 has been proposed as the minimum viable population (MVP) size necessary for most species to avoid genetic problems in completely isolated populations (Franklin and Frankham 1998). Population size may be a particular problem for ghyll woodland biodiversity since the distributions of species that are cited as being of a 'high' biodiversity value are often restricted to a small number of ghyll woodland sites (Ratcliffe 1968, Porley and Hodgetts 2009). For example, the Red List thalloid liverwort *Pallavicinia lyellii* (Veilwort) is known to occur in only two, well separated, single sex, sandrock sites in the Weald (Church et al. 2004, Porley and Hodgetts 2009). Other species whose distributions are limited to a small number of ghyll woodland sites include the Red List bryophytes *Orthodontium gracile* (Slender Thread-moss) and *Jungermannia leiantha* (Long-leaved Flapwort) (Church et al. 2004), the fern *Hymenophyllum tunbrigense* (Tunbridge Filmy-fern), the forb *Cardamine bulbifera* (Coralroot) and the grass *Festuca altissima* (Wood Fescue) (Rose and Patmore 1997).

3.2.3.2 'Edge effect'

The widespread woodland clearance that has occurred in the landscape surrounding the ghyll woodlands has meant that many ghylls are now bordered by grasslands, agricultural fields, roads or other non-woodland land-use types (pers. obs.). The removal of these woodlands will have caused significant changes in physical fluxes across the landscape (Saunders et al. 1991). The cleared areas are likely to experience higher daytime temperatures and lower night temperatures, as well as experiencing an increased incidence of frost (Geiger 1965). With the 'buffer' of the surrounding woodland removed ghyll woodlands

become exposed to the climatic conditions associated with these more 'open' habitat environments. As a result, light penetration and wind shear will increase within the ghyll woodland border zone, temperatures will fluctuate more widely, and levels of relative humidity and soil moisture will decrease (Camargo and Kapos 1995, Didham and Lawton 1999). Table 3.1 shows a comparison of environmental conditions recorded within the interior of a virgin pine forest in northern Idaho with those recorded within an adjacent clearing (Larsen 1922). The table illustrates the contrast between conditions within a forest fragment and those in the surrounding habitat.

Factor		Forest	Clearing
Air temperature (°C)	Maximum	25.9	30.0
	Minimum	7.4	3.9
	Range	18.5	26.1
Mean relative humidity at 5pm (%)		38.8	35.2
Mean daily evaporation (ml)		14.1	36.1
Mean soil temperature at 15 cm (°C)		12.8	17.0
Mean soil moisture at 15 cm (%)		32.0	43.2

Table 3.1 Comparison of certain atmospheric and edaphic environmental variables in a virgin pine forest in northern Idaho and in an adjacent clearing. Data for the month of August. (Larsen 1922)

Conditions within woodland edges are intermediate, influenced by conditions associated with the woodland interior, and the climatic conditions associated with the surrounding land-use (Forman 1995, McCollin 1998). Many rarer ghyll woodland species, such as some oceanic bryophytes and fern species, are intolerant of ground frost and are associated with mild, humid, shaded microclimatic environments (Merryweather 2007, Atherton et al. 2010). It is widely believed that these microclimatic conditions are characteristic of the ghyll woodlands (e.g. Paton 1956, Ratcliffe 1968, Rose 1995, Rose and Patmore 1997, Burnside et al. 2006) although no physical evidence of this type of microclimate was found in the analysis in Chapter 2 (Table 2.13). Any species that are reliant on oceanic conditions within the ghyll valleys may not be capable of tolerating the changed climatic conditions within a woodland 'edge' zone. Species not traditionally associated with ghyll woodlands, but that are either associated with the surrounding land-use types or associated with the new climatic conditions found within the edge 'ecotone', may invade and establish within the zone, where

they will compete with native ghyll woodland species (Yahner 1988). Many ancient woodland species are weakly competitive (Hermy et al. 1999) and are unlikely to be able to compete with the fast growing weedy species that commonly invade these edge environments (Honnay et al. 2002).

Collinge (2009) suggests that the width of the edge zone depends upon which 'edge variable' is being measured. Microclimatic changes will only persist over relatively small distances whereas changes in the abundance and distributions of woodland fauna may persist over several kilometres (Collinge 2009).

Island biogeography theory predicts that immediately after woodland clearance the residual habitat remnants will have more species than they are capable of maintaining (Simberloff 1976). Through time species will invade the edges of the remnants, whilst many native species will disappear. Eventually vegetation will develop at the edges of the forests which will moderate the climatic extremes within the edge zones (McCollin 1998). In tropical forests a thick barrier of vegetation may develop within five years, however in temperate forests side canopy closure is far slower and may take in excess of 50 years (Williams-Linera 1990, Matlack 1994). The consequences of edge effect and woodland fragmentation have an important bearing on any proposals for future woodland clearance or restoration efforts.

The modification of climate through plant cover is greatest within woodland interiors. Consequently, the effects of environmental changes are likely to be greatest at the woodland edge. The impact of climatic changes on ghyll woodland vegetation is likely to be affected by the location of the ghyll woodland within the wider woodland fragment and in particular the proximity of the ghyll valley to the woodland edge.

3.2.4 Climate change

Climate is a major influence on the geographical distribution of biological species. By implication, ghyll community assemblages are likely to change in response to future climatic changes. Whilst there may be some opportunities to be gained from

a changing climate, it is expected that the majority of changes will be negative for the natural environment (Jenkins et al. 2009). Predicting the effects of climatic changes on plant species and communities is a major goal for the conservation and restoration of species deemed to be of biological importance (e.g. Harris et al. 2006, Hannah et al. 2007, Heller and Zavaleta 2009). Identifying trends in plant distribution patterns during recent rapid climate change may provide a basis for predicting community responses to predicted future climate change scenarios (Berry et al. 2002).

3.2.4.1 Evidence of recent climate change in South-east England

Records indicate that in south-east England, from 1961-2006, average temperatures increased by 1.77°C during the summer months and by 2.00°C during the winter (Jenkins et al. 2007). Over the same period the diurnal temperature range decreased with minimum daily temperatures increasing more rapidly than maximum daily temperatures (Jenkins et al. 2007). The annual number of air frost days, defined as days when the temperature 1 metre above ground level reached 0°C or below, decreased by approximately 20 days between 1961 and 2006 (Jenkins et al. 2007). Annual mean precipitation in England and Wales has not changed significantly since records began in 1766 (Jenkins et al. 2007), however seasonal rates of precipitation have altered. During the period 1961-2006 summer precipitation in south-east England decreased by 13.1% whilst winter rainfall increased by 23.3%. During the same period levels of relative humidity in south-east England fell by 4.7% during the summer months and by 3.3% in the winter (Jenkins et al. 2007).

In order to put in place measures to minimize the negative effects of climate change, the UK Climate Impacts Programme was created by the Met Office to make predictions about future levels of climatic changes. This culminated in the publication of the 'UK Climate Projections: Briefing report' (Jenkins et al. 2009). Because of the high probability that climatic changes are occurring largely as the result of anthropogenic 'greenhouse gas' emissions and the uncertainty of future emission levels, the report gives three sets of climate projections based on three emission scenarios. For each scenario the report gives a range of climate change

projections in order to take into account natural variability such as changes in the amount of volcanic atmospheric particles or changes in the energy levels received from the sun (Jenkins et al. 2009). A central estimate of change (those at the 50% probability level) is given for each climate variable, followed in brackets by changes that are likely to be exceeded (those at the 10% probability level) and those unlikely to be exceeded (those at the 90% probability level). The projections are predicted to occur by 2080 and figures are relative to a 1961-1990 baseline. Under the medium emission scenario, mean summer temperatures are predicted to rise in southern England by 4.2°C (2.2 to 6.8°C). Over the same period annual precipitation in the south is predicted to remain relatively unchanged. However annual precipitation patterns are predicted to change, with winter precipitation increasing by up to 33% (9 to 70%) whilst summer levels are predicted to decrease by around 40% (-65 to -6%). Summer relative humidity levels are predicted to fall by around 9% (-20 to 0%) in the south by 2080, whilst winter levels are predicted to remain approximately the same.

3.2.4.2 The response of terrestrial species and ecosystems to recent climate change

To accurately measure the species responses to environmental changes it is necessary to monitor target species over a relatively long period of time. The length of the monitoring period will depend on the ecology of the species being studied (Magurran et al. 2010). Species with short generation times, such as bryophytes that produce a generation each year, will respond more quickly to changes in local climatic conditions than woody species whose generation times may be several decades (Pharo and Zartman 2007). A number of long-term monitoring approaches have been used to obtain information on recent vegetation trends in response to climate change. The use of historical distribution maps, long-term field studies and the use of archive field survey data has enabled a number of recent trends to be identified (Hickling et al. 2005, Franco et al. 2006, Hickling et al. 2006). Observed changes in range boundaries, community composition, species abundance and in the timing of phenological events may represent responses to climate change (Hopkins and Kirby 2007).

During the 20th century, scientists observed a northward range shift in many taxonomic groups that have their northern or southern range boundaries in Britain. These included birds, butterflies, and flowering plant species (e.g. Parmesan et al. 1999, Thomas and Lennon 1999, Parmesan 2001, Hickling et al. 2005, Franco et al. 2006, Hickling et al. 2006). These sorts of range shifts may lead to changes in community composition as species invade rapidly from lower latitudes whilst the native species tend to shift northward far more slowly (Walther et al. 2002). This may result in a temporary increase in species richness.

Phenological events occurring during the life-history of plants, such as the timing and duration of flowering events, tend to follow seasonal cycles (Bonan and Bonan 2008). A number of these phenological phases have been monitored in the past with some studies covering many decades. These studies provide long-term data that can be used to track temporal phenological changes that relate to changes in environmental conditions (Walther 2010). A 2002 study of nearly 400 plant species distributed throughout England showed that the average date of first flowering had advanced by 4.5 days over the previous decade (Fitter and Fitter 2002). The International Phenological Gardens are a network of European sites where genetically identical tree and shrub species have been planted to make large-scale comparisons among the phenological phases of plants (Schnelle and Volkert 1974). Data from this network for the period 1959-1996 revealed that spring events, such as leaf unfolding, advanced by an average of 6.3 days, whereas autumn events, such as leaf colouring, were delayed by an average of 4.5 days (Menzel 2000). The data indicates that the average annual growing season has lengthened by 10.8 days since 1959. These changes appear to be a response to recent climate change (Walther et al. 2002).

3.2.4.3 The use of oceanic bryophytes as bio-indicators

The biological richness and importance of the Wealden ghyll woodland bryophyte communities has been discussed in Chapter 1. Many oceanic bryophyte species found in the woodlands of southern England have either their main strongholds within the Wealden ghylls, or in some cases are solely confined to the ghyll valleys and most are at the easternmost edge of their geographic range (Rose and

Patmore 1997). The importance of mild, humid, microclimatic conditions for the survival of 'niche specialist' oceanic bryophyte species has been highlighted by a number of authors (e.g. Paton 1956, Ratcliffe 1968, Rose 1995, Rose & Patmore 1997, Patmore 2000). Bryophytes are poikilohydric relying on atmospheric precipitation for the majority of their water and nutrient uptake. They therefore react rapidly to changes in atmospheric water availability and temperature (Désamoré et al. 2012). The high sensitivity and rapid response of bryophytes to specific environmental conditions makes them ideally suited for ecological field studies designed to monitor changes in local climatic conditions (Raabe et al. 2010). Although the analysis carried out for Chapter 2 did not show evidence of a ghyll microclimate, a number of authors believe the presence of oceanic bryophyte species within the ghylls is the result of, and is therefore indicative of, a mild, humid and shaded habitat that is occurring within a warm, dry region (Paton 1956, Ratcliffe 1968).

The short life-cycle of bryophytes will mean that any changes occurring in response to fluctuating environments will be quickly apparent (Raabe et al. 2010). Species such as oceanic bryophytes, whose distributions are limited to relatively narrow moisture or temperature ranges, are likely to be amongst the first species impacted by small changes in local climatic conditions (Parry 1991). The potential for the richness and abundance of ghyll woodland oceanic bryophyte communities to alter rapidly in response to changing microclimatic conditions make it important that these biologically important communities are closely monitored. The response of the ghyll oceanic bryophyte communities to observed and projected climate change may depend on the balance between the favourable effects of warmer winters and the adverse effects of drier summers (Tuba et al. 2011).

3.2.4.4 The use of ancient woodland indicator species as bio-indicators

Over 85% of the Wealden ghyll woodlands are associated with ancient woodland (Burnside et al. 2006), a woodland type described as the most important category of British woodlands in terms of nature conservation and biodiversity (Peterken 1993). Ancient woodlands tend to contain rich assemblages of both flora and fauna, including a high proportion of Britain's rare and endangered woodland

species (Spencer and Kirby 1992). The results of the analysis carried out for Chapter 2 of this report indicate that ghyll woodlands are significantly richer in bryophyte, flowering plant and fern species than other ancient woodlands within the region. Ancient woodland indicator (AWI) species are an important component of the Wealden ghyll woodlands (Rose and Patmore 1997). Hermy et al. (1999) compared the ecological characteristics of ancient and other woodland plant species through comparing Ellenberg indicator values, plant strategies and phytosociological associations. They found ancient woodland species tended to be more shade-tolerant in comparison with other woodland plant species. They tend to avoid dry and wet sites, and are typical of sites with intermediate pH and nitrogen availability. They also found that ancient woodland species tended to have limited dispersal abilities, low rates of diaspore production and low competitive abilities, making them very poor at colonising new woodlands. The ability of species to migrate at a sufficient rate to keep up with changing climatic conditions will depend on the dispersal abilities of individual species (Pearson and Dawson 2003). The species will need to successfully colonise new habitats at least as fast as they become extinct within existing habitats (Hermy et al. 1999). Many AWI species exist only in sites that have a long history of ecological continuity and are extremely slow colonisers of new sites (Peterken 1974, Rackham 1980). Numerous studies have shown that climate-induced range shifts are likely to alter community composition because phenotypes such as AWI species that have poor dispersal characteristics and relatively narrow ecological niches are likely to have low colonisation rates (Pearson and Dawson 2003, Walther 2003, Thuiller et al. 2008, Hannah 2010). Many AWI species are restricted to ancient woodland in the south-east because their specific ecological requirements are only met within this type of woodland (Rackham 1980). In south-east England the summers are becoming progressively dryer and winters progressively wetter (Jenkins et al. 2007). AWI species tend to avoid overly dry or wet conditions, so changes in precipitation patterns may influence the richness and abundance of some AWI species (Hermy et al. 1999). Parry (1991) describes how a shift in temperature of 1°C within the UK is the equivalent of a latitudinal shift of between 200-300 kilometres. Based on this estimate it is possible that the increase in average temperatures that occurred in southern England between 1961 & 2006 of 1.77°C in the summer and 2.00°C in the winter (Section 3.1.4.1)

led to an increase in migrant species arriving in southern England from mainland Europe. Range shifts like these typically occur at the expense of native species that have poor competitive abilities such as the AWI species (Hermy et al. 1999). The sensitivity of many AWI species to changes in the precipitation regime and their inability to compete with migrant species means they are useful as bio-indicators of changing climatic conditions.

3.2.5 The use of plant indicator scores for assessing the prevailing environmental conditions experienced by the survey species

The influence of a range of selected environmental variables on the occurrence and distributions of ghyll woodland plant species were investigated using plant indicator values. These indicator values are usually applied to data 'post-survey' during 'secondary' analysis (e.g. McCollin et al. 2000b, Godefroid 2001) and are therefore particularly valuable for predicting environmental conditions when environmental readings were not recorded as part of the original surveys.

Scientists in central Europe have examined the occurrence and abundance of different plant species in relation to environmental conditions and have assigned semi-quantitative 'indicator values' to each species. Ellenberg indicator values provide autoecological information on the field responses of a wide range of vascular and non-vascular plants to climatic and edaphic conditions in central Europe (Ellenberg 1974, Ellenberg et al. 1991, Hermy et al. 1999). They are based on a simple ordinal classification of plants in relation to the position of their realised ecological niche along an environmental gradient (Ellenberg 1974). Ellenberg indicator values were originally developed for the German flora but have since been adapted to represent the field response of UK vascular plants (Hill et al. 1999) and bryophytes (Hill et al. 2007). These indicator values characterise integrated signals of plant-environment associations in response to climatic conditions (Ellenberg 1974). Microclimatic conditions within a site are liable to fluctuate rapidly throughout the day. Therefore 'spot-measurements' taken with field equipment may not accurately represent typical conditions at that location (Scherrer and Körner 2011). The community of plants growing at a particular site are largely a response to, and will reflect, typical environmental conditions

occurring within the site. Therefore, characterising climatic conditions using plant Ellenberg indicator values may provide more accurate results than the use of 'spot-measurements' (Scherrer and Körner 2011). Indicator values signifying climate change and based on the distribution patterns of British flowering plant species were also included in the analysis (Preston and Hill 1997). The first set of indicator values reflected the presence of each species in one or more of four major biome categories (MBC) (Arctic-montane, Boreal-montane, Temperate and Southern). Preston & Hill (1997) also classified species based on their eastern distributional limit (ELC) (Oceanic, Sub-oceanic, European, Eurosiberian, Eurasian and Circumpolar). The ability of a species to respond to environmental changes may be dependent on certain seed-related attributes of the species (Graae and Sunde 2000). Indicators of the dispersal characteristics of ghyll woodland flowering plant and fern species were based on their abundance in the soil seedbank and the average dispersule weight of each species (Grime et al. 1988). The distribution patterns of each flowering plant species were analysed based on the number of habitats (Mire, Skeletal, Arable, Pasture, Spoil, Wasteland and Woodland) that the species occupies within the UK. The potential number of habitats occupied ranged from one (specialist species) to seven (generalists) (Grime et al. 1988). The distribution patterns of bryophytes were analysed using indicator scores based on the number of substrates and the number of habitats that each species was associated with (Davies et al. 2004, Hill et al. 2007).

3.3 Aims

The chapter will use archive survey data with the aim of identifying temporal changes in richness and composition of ghyll woodland species during the post-war period through a comparison of surveys carried out between 1951-1970 with a second group carried out from 1976-1995.

Oceanic bryophytes and ancient woodland indicator species are bio-indicator groups since they are restricted to particular habitat types due to their specialist ecological niches. Temporal changes in environmental conditions within the ghyll woodlands are likely to be reflected by changes in the distributions of these

biologically important species groups. This chapter aims to identify temporal changes in the distributions and composition of oceanic bryophytes and AWI species during the survey period and examine how these changes relate to changes in the environment.

The chapter will use plant indicator values to characterise habitat preferences with the aim of identifying temporal changes in the composition of ghyll woodland plant communities.

The indicator values will be used along with landscape variables, measured using a GIS, to examine the environmental and landscape correlates of changes in ghyll woodland species and communities.

3.4 Methods

3.4.1 Study area and survey data

The dataset used for this analysis contained survey data on bryophytes, flowering plants and ferns. The data was collected from ghyll woodlands and ancient woodlands located throughout the Wealden area by the renowned botanist Dr. Francis Rose MBE during the period 1945–1998. The survey results were recorded in a series of field notebooks which were later transcribed and digitised by the Sussex Biological Record Centre. The digital records are available for public access via the National Biodiversity Network Gateway.

Each survey was undertaken over a one day period, during which time a thorough search of the site was conducted and the presence of bryophyte, fern and flowering plant species were recorded (pers. comms. - Howard Matcham and Dr David Streeter).

The dataset included National Grid coordinates identifying the location of each survey site to a 1 kilometre grid square. Some survey records also contained survey notes further identifying the locations of the survey sites. Only sites clearly identified as either ghyll woodland or ancient woodland were used in the analysis. The location of each site was cross-checked against a digital map of the Wealden

ghyll woodlands (SBRC 2000) and a separate map of ancient woodland within the region (Natural England 2013).

The notebook contained two periods, 1946-1950 and 1971-1975, during which only one ghyll woodland survey was carried out. These limitations of survey continuity are recognised and have been addressed by excluding these time periods from the analysis through selecting the two 20 year survey periods 1951-1970 and 1976-1995 for the study. By doing this the periods in which significantly fewer surveys were carried out have been avoided. The number of sites and plant records used in the analysis are shown in Table 3.2. A full list of the names and locations of sites is shown in appendices 8-11.

Survey period	Bryophytes		Flowering plants		Ferns	
	Total no. of survey sites	Total no. of plant records	Total no. of survey sites	Total no. of plant records	Total no. of survey sites	Total no. of plant records
Ghyll woodlands						
1951-1970	35	575	23	429	21	87
1976-1995	35	508	27	535	25	79
Ancient woodlands						
1951-1970	26	396	17	429	19	106
1976-1995	25	352	17	422	17	95

Table 3.2 The total number of surveys and total number of plant records in each taxonomic group in the ghyll woodland and ancient woodland surveys during each 20 year time period. Each plant record represents one species recorded at one site.

Bryophyte nomenclature continues to evolve relatively rapidly. Where species in the survey dataset could not be found in the indicator score datasets, all known synonyms of that species were determined and checked using Smith (2006), Paton (1999) and Atherton (2010).

3.4.2 Assessment of change

3.4.2.1 A temporal examination of ghyll woodland species richness

Both habitat fragmentation and climate induced range shifts can cause alterations in species richness levels. The numbers of bryophyte, fern and flowering plant

species recorded during each survey were calculated to give a list of species richness totals for the periods 1951-1970 and 1976-1995. These were compared using one-way ANOVA analysis (Minitab v.16) to investigate temporal changes in species richness. Separate ANOVA's were carried out for bryophytes, flowering plants and ferns for both ghyll woodlands and ancient woodlands.

3.4.2.2 Examining changes in the frequencies of bio-indicator species

Oceanic bryophyte species recorded during the periods 1951-1970 and 1976-1995 were identified using Ratcliffe (1968). The frequencies of oceanic bryophytes in each time period (in relation to the total number of bryophytes recorded during each period) was compared using chi-squared analysis (Minitab v.16). The analysis was then repeated for oceanic bryophytes recorded in the ancient woodland surveys.

Flowering plant and fern AWI species for south-east England were identified using Rose et al. (2006). Analysis of the frequencies of AWI species within both ghyll woodland and ancient woodland survey sites followed the method used for oceanic bryophytes.

3.4.2.3 Analysing changes in the distributions of bio-indicator species

The relative abundance (%) of survey sites occupied by each oceanic bryophyte species during 1951-1970 and 1976-1995 was calculated for both the ghyll woodland and ancient woodland sites using the following equation:

$$relative\ abundance\ of\ sp.\ x = \frac{no.of\ sites\ sp.x\ recorded}{total\ no.of\ sites}\ \text{x}\ 100$$

Relative abundance figures were used in order to account for the difference in the total number of ancient woodland sites surveyed during the two time periods

(Graae and Sunde 2000). Species classified as 'vulnerable' in the current British bryophyte Red Data Book (Church et al. 2004) were identified and highlighted in the list of abundance results.

3.4.3 Examining temporal changes in the environmental correlates of species composition

3.4.3.1 Changes in environmental conditions examined using plant indicator values

The influence of a range of environmental variables on the composition of ghyll woodland and ancient woodland vegetation was investigated using two separate methods, both based around the use of plant 'indicator scores'. It is expected there will be a strong relationship between the results of the two approaches. The initial method, developed by McCollin et al. (2000a), involved calculating a single 'index of change' value for each species that represents the changing status of that species over the two survey periods 1951-1970 and 1976-1995. Abundance figures were calculated for each time period based on the number of sites in which each species was recorded. The abundance figures were then used as variables in a linear regression (SPSS v.20). The standardised residual figures calculated for each species in the linear regression analysis were used to represent an index of change. These residuals characterise the deviation of the observed abundance values from their expected values, against a background of the changes occurring in all of the species being analysed (McCollin et al. 2000b). Correlations between the standardised residuals and a range of environmental indicator values were investigated using Spearman's rank correlation analysis (Minitab v.16).

Ecological variables used in the analysis are listed in Table 3.3. The 'Modified British' Ellenberg values were used throughout the analysis (Hill et al. 2004, Hill et al. 2007). Ellenberg scores for light, soil-moisture, salt, reaction (pH) and nitrogen were used in the analysis of all of the survey species (Ellenberg 1974, Ellenberg et al. 1991). A further Ellenberg indicator category, based on tolerance to heavy metal contamination, was used in the bryophyte analysis (Hill et al. 2007). Indicator values based on the occurrence of the species in one of four 'major

terrestrial biomes' (MBC) and on each species 'eastern distributional limit' (ELC) (Preston and Hill 1997) were also used in the analysis.

Variable	Description	Categories
Bryophytes, flowering plants & ferns		
Ellenberg's – L[a]	Light	*Bryophytes* ~ 0, plant in complete darkness, to 9, plant in full light *Flowering plants & ferns* ~ 1, plant in deep shade, to 9, plant in full light
Ellenberg's – F[a]	Soil Moisture	*Bryophytes* ~ 1, indicator of extreme dryness, to 12, normally submerged *Flowering plants & ferns* ~ 1, indicator of extreme dryness, to 9, submerged
Ellenberg's – R[a]	Reaction	*Bryophytes* ~ 1, indicator of extreme acidity, to 9, on substrata with free calcium carbonate *Flowering plants & ferns* ~ 1, indicator of extreme acidity, to 9, indicator of basic reaction
Ellenberg's – N[a]	Nitrogen	*Bryophytes* ~ 1, extremely infertile sites, to 7, richly fertile sites *Flowering plants & ferns* ~ 1, extremely infertile sites, to 9, richly fertile sites
Ellenberg's – S[a]	Salt	*Bryophytes* ~ 0, absent from saline sites, to 5, upper edge of saltmarshes and obligate halophytes of cliffs receiving regular salt spray *Flowering plants & ferns* ~ 0, absent from saline sites, to 9, species of extremely saline conditions
MBC[b]	Major biome category	*Bryophytes* ~ Scale 1, arctic-montane, to 9, Mediterranean-Atlantic *Flowering plants & ferns* ~ Scale 1, arctic-montane, to 9, southern
ELC[b]	Eastern limit category	*Bryophytes* ~ Scale 0, Hyperoceanic to 6, circumpolar *Flowering plants & ferns* ~ Scale 1, oceanic to 6, circumpolar
Bryophytes only		
Ellenberg's – HM[a]	Heavy Metal	Scale 0, species absent from substrates with moderate or high concentrations of HM, to 5 species confined to substrates with moderate or high concentrations of HM
NSUBSTR	Substrate class	Sum of substrates in the UK on which species occurs, such as rock, rotting wood or soil (range 1-14)
EUNIS	EUNIS habitat classes	Sum of EUNIS habitat classes throughout the UK in which species occurs (range 1-31)
Flowering plants & ferns only		
NUKHAB[c]	Number of UK habitats	Sum of major primary habitats throughout the UK in which species occurs (range 1-7)
SEEDB[c]	Seed bank	1. Most seed germinating shortly after being shed 2. Most seed persistent only until start of next growing season 3. A small amount of seed persists in the soil but concentrations only high after seed shed 4. Large persistent seed bank throughout the year
WGHT[c]	Dry weight of seed, achene or other (mg)	1. Too small to be measured easily 2. ≤0.20 3. 0.21-0.50 4. 0.51-1.00 5. 1.01-2.00 6. 2.01-10.00 7. ≥10.00

Table 3.3 Explanation of variables used in the Spearman rank correlation analysis to investigate the determinants of temporal changes in ghyll woodland species distributions and abundance. Variables taken from: [a] Ellenberg et al. (1974) adapted for UK vascular plants by Hill et al. (1999) and for UK bryophytes by Hill et al. (2007) [b] Preston and Hill (1997) and [c] Grime et al. (1988). Table adapted from McCollin et al. (2000a)

MBC and ELC indicator values have been specifically developed for the British flora and are indicators of climate change (McCollin et al. 2000b). Indicator values

relating to habitat and dispersal characteristics were also included. For flowering plants and ferns these were extracted from Grime et al. (1988) and consisted of scores based on the number of major primary habitats throughout Britain that a species was associated with (NUKHAB), seedbank characteristics of the species (SEEDB) and the species mean dried seed weight (WGHT). For bryophyte species, traits relating to habitat and dispersal were taken from Hill et al. (2007). These consisted of scores based on the number of substrates a species is associated with (NSUBSTR) and the number of EUNIS (European Nature Information System - Davies et al. 2004) habitat classes throughout the UK that the species is associated with. Species were excluded from the analysis where indicator data was unavailable.

A series of tests were carried out to identify whether there were significant differences between average indicator values calculated for each of the two survey periods. The indicator values used are based on ordinal scores and therefore the non-parametric Mann-Whitney U analysis was used in the study.

3.4.3.2 Investigating the effects of environmental/ecological changes on bio-indicator species

To reveal the environmental determinants of community change within the bio-indicator groups; oceanic bryophytes and AWI species, indicator trait scores recorded in the 1951-1970 and 1976-1995 surveys were compared using Mann-Whitney U analysis (Prach and Pyšek 1999, Graae and Sunde 2000, Godefroid 2001). Histograms were produced for each trait that the Mann-Whitney U analysis indicated as differing significantly over the survey periods.

3.4.4 Investigating the effects of habitat fragmentation on ghyll woodland species richness

3.4.4.1 Examining evidence of an 'edge effect'

Changes in species richness were analysed in relation to the proximity of the ghyll woodlands to the edge of the contiguous woodland fragments. This analysis used

a digital map of Wealden streams and rivers (Environment Agency 2013) in conjunction with the ghyll woodland digital map (SBRC 2000) to identify the ghyll woodland streams within the survey sites. Random points were selected along each ghyll stream in ARCMAP v.10 and the distance to the nearest woodland edge was measured. To work out the optimum number of measurements that needed to be taken, a running mean of 'distance to woodland edge' was calculated after each measurement until additional measurements altered the running mean by less than 5%. Based on these calculations 20 measurements were carried out for each site. Pearson's correlation analysis was used to examine whether ghyll species richness was correlated with distance of the ghyll from the nearest border of the contiguous woodland fragment. The analysis was carried out for all species, and also separately for the taxonomic groups; bryophytes, flowering plants and ferns.

3.4.4.2 Species-area relationship: examining the relationship between ghyll woodland size and species richness

Against a background of habitat fragmentation, rapid losses of biodiversity and environmental changes on a global scale, understanding the correlation between species diversity and area is a fundamental prerequisite of current ecological research (Ney-Nifle and Mangel 2000, Rosenzweig 2003, Drakare et al. 2006, Bogich et al. 2012). The relationship between species and area is considered by many to be the principal factor in determining species diversity (MacArthur and Wilson 1967, Rosenzweig 1995, Brown and Lomolino 1998, Mitchell and Ryan 1998, Lomolino 2000, 2001) and has been described as 'one of community ecology's few laws' (Schoener 1976, Yu et al. 2008). Regardless of the taxonomic group or type of ecosystem being considered, species number tends to increase with increasing area. It persists over areas both large and small and with both flora and fauna (Harte et al. 2005, Shen et al. 2009).

Inaccuracies appear to exist within the ghyll woodland digital maps concerning the boundaries of the ghyll woodland sites, with mapped boundaries containing areas beyond the edges of the ghyll valleys (see Section 2.1.3 for more detail). Ghyll woodlands are linear features and therefore ghyll area is likely to be highly

correlated with ghyll length (Burnside et al. 2002b, Burnside et al. 2006). Therefore to attempt to overcome the inaccuracies in the digital map, ghyll stream length was used in this analysis as a proxy variable for ghyll area. The length of the ghyll streams was measured using the south-east England 'rivers and streams' map (Environment Agency 2013) within a GIS framework (ARCMAP v.10). Pearson's correlation analysis was then used to examine whether ghyll species richness was correlated to ghyll length. Again, the analysis was carried out for all species, and also separately on the taxonomic groups; bryophytes, flowering plants and ferns.

3.5 Results

A comparison of the sites surveyed during the 1951-1970 period with those surveyed from 1976-1995 indicated species richness did not change significantly within either the ghyll woodland or ancient woodland sites.

Time Period	No. of bioindicator plants observed	expected	No.of non-bioindicator plants observed	expected	Chi-squared	d.f.	Significance
Oceanic bryophytes							
Ghyll Woodland							
1951 - 1970	45	57.55	574	561.45			
1976 - 1995	82	69.45	665	677.55	5.52	1	P<0.05
Ancient Woodland							
1951 - 1970	17	19.76	470	467.24			
1976 - 1995	20	17.24	405	407.76	0.86	1	ns
Flowering plant AWI spp.							
Ghyll Woodland							
1951 - 1970	139	156.38	418	400.62			
1976 - 1995	313	295.62	535	757.38	4.10	1	P<0.05
Ancient Woodland							
1951 - 1970	59	58.31	118	118.69			
1976 - 1995	53	53.69	110	109.31	0.03	1	ns
Fern AWI species							
Ghyll Woodland							
1951 - 1970	49	45.76	86	89.24			
1976 - 1995	51	54.24	109	105.76	0.64	1	ns
Ancient Woodland							
1951 - 1970	175	177	470	467.24			
1976 - 1995	175.75	176.25	405	407.76	0.01	1	ns

Table 3.4 Comparison of the frequencies of oceanic bryophytes and ancient woodland indicator species between the time periods 1951–1970 and 1976-1995 using chi-squared goodness of fit tests. ns = not significant

Analysis of the taxonomic groups; bryophytes, flowering plants and ferns, also showed no significant temporal changes in species richness within either the ghyll

woodland or the ancient woodland sites. Although species richness did not change significantly, chi-squared analysis indicated that the composition of the ghyll woodland sites did alter over the two time periods, with a significant increase ($P<0.05$) in the frequencies of the bioindicator plant groups; oceanic bryophytes and flowering plant AWI species (Table 3.4). In contrast, the frequencies of oceanic bryophytes and flowering plant AWI species recorded in the ancient woodland surveys did not vary significantly between the two periods. The frequency of fern AWI species also showed no significant difference between the two periods within either ghyll woodland or ancient woodland sites.

Oceanic bryophyte species	No of sites	Relative abundance %	Oceanic bryophyte species	No of sites	Relative abundance %
Ghyll woodlands 1951-1970			Ghyll woodlands 1976-1995		
Hookeria lucens	10	29	*Scapania gracilis*	12	34
Campylopus flexuosus	6	17	*Bazzania trilobata*	9	26
Heterocladium h. var. flaccidum	4	11	*Harpanthus scutatus*	8	23
Harpanthus scutatus	3	9	*Hookeria lucens*	8	23
Scapania gracilis	3	9	*Orthodontium gracile (VU)*	7	20
Bazzania trilobata	2	6	*Dicranum scottianum*	6	17
Campylostelium saxicola	2	6	***Pallavicinia lyellii*** *(VU)*	6	17
Calypogeia arguta	1	3	***Scapania umbrosa***	4	11
Campylopus brevipilus	1	3	*Cephalozia catenulata*	3	9
Cephalozia catenulata	1	3	*Fissidens rivularis*	3	9
Cololejeunea minutissima	1	3	*Heterocladium heteropterum*	3	9
Dicranum scottianum	1	3	*Saccogyna viticulosa*	3	9
Dumortiera hirsute (VU)	1	3	*Calypogeia arguta*	2	6
Fissidens celticus	1	3	*Dumortiera hirsute (VU)*	2	6
Fissidens rivularis	1	3	*Fissidens celticus*	2	6
Heterocladium heteropterum	1	3	*Hypnum cupressiforme var. resup.*	2	6
Hypnum cupressiforme var. resup.	1	3	***Metzgeria conjugata***	1	3
Lophocolea fragrans	1	3	***Trichostomum tenuirostre***	1	3
Odontoschisma sphagni	1	3			
Orthodontium gracile (VU)	1	3			
Saccogyna viticulosa	1	3			
Tortula cuneifolia *(VU)*	1	3			
Ancient woodlands 1951-1970			Ancient woodlands 1976-1995		
Hookeria lucens	7	17	***Campylopus flexuosus***	5	17
Calypogeia arguta	4	11	*Calypogeia arguta*	3	10
Campylostelium saxicola	2	5	*Harpanthus scutatus*	2	7
Fissidens celticus	1	3	*Hookeria lucens*	2	7
Harpanthus scutatus	1	3	***Scapania gracilis***	2	7
Heterocladium heteropterum	1	3	***Cephalozia catenulata***	1	3
Heterocladium var. heteropterum	1	3	***Dicranum scottianum***	1	3
			Heterocladium heteropterum	1	3
			Heterocladium h. var. flaccidum	1	3
			Metzgeria fruticulosa	1	3
			Orthodontium gracile (VU)	1	3

Table 3.5 Oceanic bryophytes species recorded in ghyll woodland and ancient woodland sites during the two 20 year time periods. Species in bold italics were unique to the time period they are listed under within either ghyll woodlands or ancient woodlands. The relative abundance worked out for each species is the percentage of sites surveyed in the time period within which the species was recorded. Relative abundance figures were rounded up or down to the nearest integer. (VU) indicates the species status in Britain is classified as vulnerable in the British Red Data Book for Mosses and Liverworts (Church et al. 2004).

An examination of the oceanic bryophyte species recorded during the surveys showed that 22 oceanic bryophytes were recorded in the ghyll woodland sites

during the 1951-1970 surveys compared with 18 during the 1976-1995 surveys (Table 3.5). Eight species were recorded in the period 1951-1970 that were not recorded during the period 1976-1995 and four species were found in the 1976-1995 period that had not been previously recorded in the 1951-1970 surveys.

Three species recorded in the ghyll woodlands during the 1951-1970 surveys; *Dumortiera hirsute* (Dumortier's Liverwort), *Orthodontium gracile* (Slender Thread-moss) and *Tortula cuneifolia* (Wedge-leaved Screw-moss), are classified as vulnerable in the British Red Data Book for Mosses and Liverworts (Church et al. 2004). Each of these three species was recorded at only one site during this time period. *O. gracile* and *D. hirsute* were also recorded in the ghyll woodlands during the 1976-1995 period along with a further 'vulnerable' Red List species; *Pallavicinia lyellii* (Veilwort). *O. gracile* was recorded at a single ghyll woodland site during the 1951-1970 surveys, but was recorded in seven ghyll woodlands during the 1976-1995 surveys. This represented 20% of all the sites surveyed during the 1976-1995 period. *P. lyellii* was not recorded in either the ghyll sites or the ancient woodland sites during the 1951-1970 surveys but was found at six ghyll woodland sites during the 1976-1995 survey period, which represented 17% of the ghyll sites surveyed. *D. hirsute*, found in one ghyll woodland during the 1951-1970 surveys was found in two ghyll sites during the 1976-1995 surveys.

In the ancient woodland surveys seven oceanic bryophyte species were recorded in the surveys carried out during 1951-1970 whilst eleven were recorded during the 1976-1995 survey period (Table 3.5). The Red List species *O. gracile* was absent from the 1951-1970 ancient woodland surveys but was recorded in one ancient woodland site during the 1976-1995 survey period. The 'shade-tolerant' liverwort *Hookeria lucens* (Shining Hookeria) (Atherton et al. 2010) was the most widespread oceanic bryophyte recorded in the 1951-1970 ancient woodland surveys and was recorded in seven (17%) of the sites surveyed during this period, but was found in only two (7%) ancient woodland sites surveyed from 1976-1995.

Table 3.5 shows that in the ghyll woodland surveys, *Campylopus flexuosus* (Rusty Swan-neck Moss) was the second most abundant oceanic bryophyte recorded in the period 1951-1970, appearing in 17% of the survey sites. This species was not recorded in the ghyll woodland surveys carried out from 1976-1995 and was also

absent from the surveys carried out in the ancient woodlands surveyed from 1951-1970. However, *C. flexuosus* was the most abundant oceanic bryophyte recorded in the ancient woodland surveys carried out from 1976-1995.

Temporal changes in the environmental requirements of ghyll communities based on residuals from linear regression analysis are displayed in Table 3.6. Fern species were also analysed but revealed no significant correlations and are therefore not included in the table.

	Spearman's correlations		
Indicator	**Bryophytes**	**Flowering plants**	**All species**
Ghyll woodland			
Light	**-0.36 *** **	**-0.40 *** **	**-0.36 *** **
Moisture	ns	**-0.20** **	**-010* **
Reaction	ns	**0.16* **	ns
Nitrogen	ns	**0.26*** **	**0.14** **
Salt	**-0.14* **	ns	**-0.10* **
Heavy Metal	ns	~	~
MBC	~	ns	~
ELC	~	ns	~
NSUBSTR	**0.32*** **	~	~
EUNIS	ns	~	~
NUKHAB	~	ns	~
SEEDB	~	**-0.19* **	~
WGHT	~	**0.24** **	~
Ancient woodland			
Light	**-0.20*** **	ns	ns
Moisture	**-0.18* **	ns	ns
Reaction	ns	**-0.18*** **	ns
Nitrogen	ns	ns	ns
Salt	ns	ns	ns
Heavy Metal	**0.17* **	~	~
MBC	~	ns	~
ELC	~	ns	~
NSUBSTR	**0.26** **	~	~
EUNIS	**0.24** **	~	~
NUKHAB	~	ns	~
SEEDB	~	ns	~
WGHT	~	ns	~

Table 3.6 The estimated contribution of environmental/ecological factors thought to be responsible for changes in species occurrence in the 1976-1995 surveys in comparison to species occurring during 1951-1970. The contribution of each variable was measured using Spearman ranking analysis to compare the indicator values for each species against the residuals of a linear regression produced through comparing the number of sites each species was recorded at in each of the time periods 1951-1970 & 1976-1995. Light, Soil moisture, reaction, nitrogen, salt and heavy metal are all Ellenberg indicator values (Ellenberg et al. 1991). MBC = major biome category and ELC = eastern limit category (Preston and Hill 1997). NSUBSTR = number of substrates (Hill et al. 2007) and EUNIS = number of habitat classes (Davies et al. 2004). NUKHAB = number of primary UK habitats, SEEDB = seedbank characteristics and WGHT = weight of dried dispersule (Grime et al. 1988). Spearman correlations were also calculated for fern species using the same indicator value classes as flowering plant species but no significant correlations were found. ns = not significant. ~ = indicator values not available for this taxonomic group.

Temporal analysis of Ellenberg light scores for the ghyll woodland plant communities showed a strong negative correlation between the light scores and

the abundance of both bryophyte (r_s = -0.36, P<0.001) and flowering plant species (r_s = -0.40, P<0.001) (Table 3.6). Ellenberg light scores for bryophytes recorded in the ancient woodland surveys also showed a negative correlation between light values and bryophyte abundance (r_s = -0.20, P<0.001). The results indicate an increase in the occurrence of 'shade-tolerant' bryophyte (ghyll woodlands & ancient woodlands) and flowering plant species (ghyll woodlands only) in the 1976-1995 surveys, and a decrease in the occurrence of bryophyte and flowering plant species associated with higher levels of light and therefore more open environments.

The negative soil moisture correlations produced for ghyll woodland flowering plant occurrence and ancient woodland bryophyte occurrence indicated that the ratio of moisture-loving species within these communities decreased in the period 1976-1995, in comparison with the 1951-1970 surveys. Ellenberg nitrogen scores for ghyll woodland flowering plant communities were strongly correlated with the regression residuals (r_s=0.26, P<0.001) indicating a change in community composition with an increase in the relative abundance of species with higher nitrogen requirements. Indicator scores representing the number of substrates (NSUBSTR) that bryophyte species were associated with were highly significant for both ghyll woodlands (r_s = 0.32, P<0.001) and ancient woodlands (r_s = 0.26, P<0.005) indicating an increasing ratio of 'generalist' species associated with multiple habitats within these communities and a decrease in more 'specialist' species associated with only a small number of habitat types. Other environmental correlates of change for ghyll woodland flowering plants were; reaction (r_s = 0.16, P<0.05), seed-bank characteristics (SEEDB) (r_s = -0.19, P<0.05) and dried seed weight (WGHT) (r_s=0.24, P<0.005). Ellenberg salt tolerance scores were significantly correlated with ghyll woodland bryophyte occurrence (r_s = -0.14, P<0.05).

Table 3.7 shows temporal changes in species ecological requirements identified through Mann-Whitney U analysis. The analysis indicated that significant temporal changes had occurred in the light requirements of flowering plant, bryophyte and fern communities recorded within both ghyll woodlands and within ancient woodlands when comparing the 1951-1970 survey communities with those recorded in the 1976-1995 surveys. Average bryophyte Ellenberg indicator scores

for light, recorded over the two time periods, fell from a median of five for the ghyll woodland surveys carried out during the 1951-1970 period to four for the surveys carried out during 1976-1995 (P<0.001). The median average light value for ancient woodland flowering plants reduced from six during 1951-1970 to five during the 1976-1995 period (P<0.001). Indicator values representing salt tolerance values (P<0.05) and the number of substrates (NSUBSTR) (P<0.001) were both identified as indicator values that changed significantly within ghyll woodland bryophyte communities during the survey period. The Mann-Whitney U analysis (Table 3.7) identified the same environmental indicator variables for ghyll woodland bryophytes (light, salt & NSUBSTR) as those identified in the correlation analysis based on the regression residuals (see Table 3.6).

	Indicator score medians (no.of samples)		
Indicator	**1951-1970**	**1976-1995**	**Significance**
Bryophytes			
Ghyll Woodland			
Light	5 (592)	4 (708)	P<0.001
Salt	0 (592)	0 (708)	P<0.05
NSUBSTR	6 (590)	7 (708)	P<0.001
Ancient Woodland			
Light	5 (470)	5 (375)	P<0.05
Moisture	6 (470)	6 (375)	P<0.05
Flowering plants			
Ghyll Woodland			
Light	6 (465)	5 (629)	P<0.001
Moisture	6 (465)	6 (629)	P<0.001
Reaction	6 (465)	6 (629)	P<0.001
Nitrogen	5(465)	5(629)	P<0.001
SEEDB	3(391)	3(554)	P<0.001
WGHT	2(397)	2(559)	P<0.05
Ancient Woodland			
Light	6 (529)	6 (476)	P<0.05
Nitrogen	5 (529)	5 (476)	P<0.05
Ferns			
Ghyll Woodland			
Light	5 (91)	5 (105)	P<0.05
All species			
Ghyll Woodland			
Light	5 (1148)	5 (1411)	P<0.001
Moisture	6 (1148)	6 (1411)	P<0.05
Reaction	5 (1148)	5 (1411)	P<0.05
Nitrogen	4 (1148)	4 (1411)	P<0.001
Salt	0 (1148)	0 (1411)	P<0.05

Table 3.7 Significant changes in the ecological requirements of the survey species based on Mann-Whitney U comparisons of the indicator scores for the survey periods 1951-1970 and 1976-1995. Only significant results are shown. Indicator values used to make pairwise comparisons are the same as in Table 3.6.

The Mann-Whitney U analysis (Table 3.7) indicated a significant temporal change in Ellenberg soil moisture values for bryophytes recorded in the ancient woodlands (P<0.05) and for flowering plants recorded in ghyll woodlands (P<0.001). Ellenberg nitrogen values for flowering plants recorded during the ghyll woodland (P<0.001) and the ancient woodland surveys (P<0.05) also altered significantly over the survey period. Ghyll woodland flowering plant indicator scores for reaction (P<0.001), seed-bank characteristics (SEEDB) (P<0.001) and dried seed weight (P<0.05) all changed significantly over the survey period. The five ghyll flowering plant environmental determinates, that were shown in Table 3.7 to have changed significantly over the two survey periods, were the same as those identified for ghyll woodland flowering plants in the Spearman ranking analysis detailed in Table 3.6.

Analyses focusing solely on oceanic bryophyte communities indicated that within the ghyll woodland surveys the Ellenberg indicator scores for light, reaction and nitrogen all reduced significantly over the two survey periods (1951-1970 and 1976-1995) (Table 3.8 and Figures 3.1a & d).

	Medians		Mann-Whitney U test statistic
	1951-1970	1976-1995	
Ghyll woodlands			
Oceanic bryophytes			
Light	$4_{(45)}$	$3_{(86)}$	3112.5*
Reaction	$4_{(45)}$	$3_{(86)}$	3480*
Nitrogen	$3_{(45)}$	$2_{(86)}$	3368.5*
Flowering plant AWI spp.			
Seed weight	$3_{(118)}$	$4_{(220)}$	18324*
Light	$5_{(150)}$	$5_{(253)}$	32774.5*
Moisture	$6_{(150)}$	$5_{(253)}$	33258*
Reaction	$5_{(150)}$	$6_{(253)}$	27956.5*
Nitrogen	$5_{(150)}$	$5_{(253)}$	27149**
Fern AWI species			
MBC	$7_{(50)}$	$7_{(52)}$	2307*
Light	$5_{(50)}$	$5_{(52)}$	2753.5*

	Medians		Mann-Whitney U test statistic
	1951-1970	1976-1995	
Ancient woodlands			
Oceanic bryophytes			
NSUBSTR	$4_{(17)}$	$6.5_{(20)}$	248.5*
Light	$3_{(17)}$	$3.5_{(20)}$	248*
Moisture	$7_{(17)}$	$6_{(20)}$	388*
Reaction	$5_{(17)}$	$3_{(20)}$	418*
Nitrogen	$4_{(17)}$	$2_{(20)}$	400.5*

Table 3.8 Statistically significant changes in plant indicator scores between the two survey periods 1951-1970 and 1976-1995 identified using Mann-Whitney U analysis. The number of plant records used to carry out the Mann-Whitney analysis for each variable within each time period is recorded in brackets after the indicator score medians. *= P<0.05; **= P<0.005; ***= P<0.001

Analysis of oceanic bryophytes in the ancient woodland sites showed indicator scores for the number of substrates species were associated with increased over

the two survey periods, as did average Ellenberg light values, whilst Ellenberg soil moisture, reaction and nitrogen scores all decreased.

Analysis focused solely on the ghyll flowering plant AWI species indicated that Ellenberg values for reaction and nitrogen increased significantly over the two survey periods, as did the indicator value for seed weight (Table 3.8 & Figure 3.1b). Ellenberg light and soil-moisture scores for ghyll woodland communities of AWI flowering plants both decreased. Ellenberg light scores for fern AWI species recorded in the ghyll surveys also decreased during the survey period (Table 3.8 & Figure 3.1c).

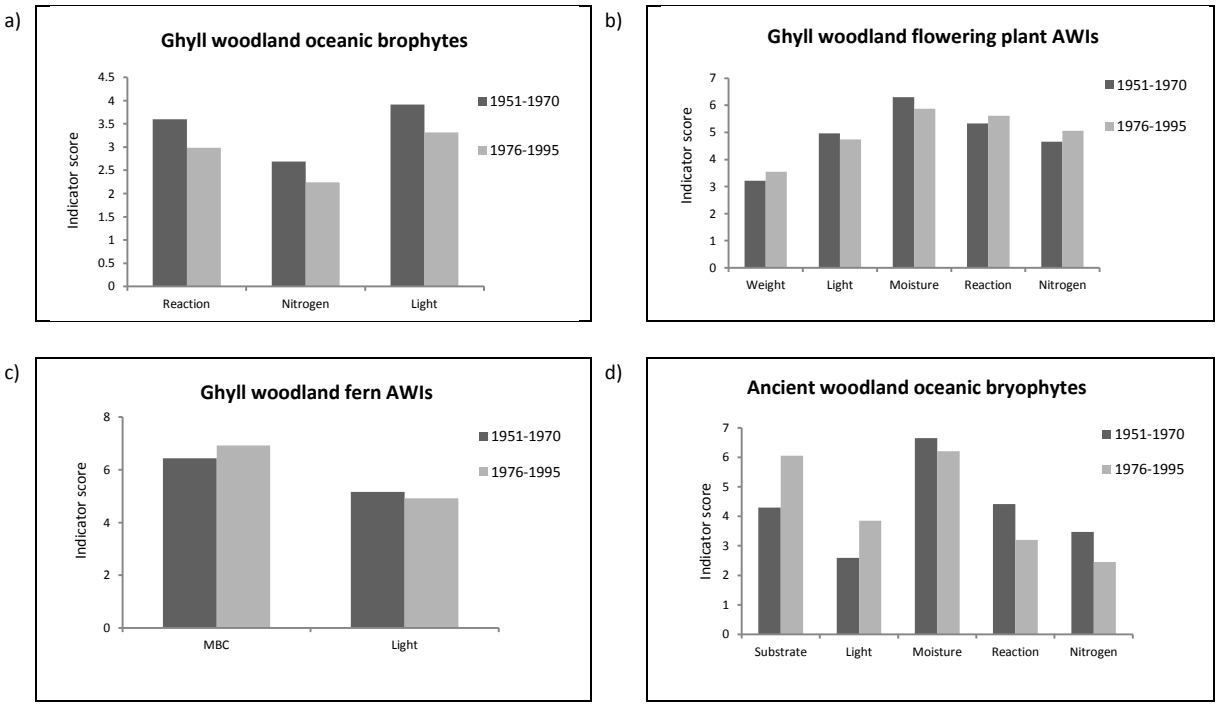


Figure 3.1 Comparison of mean indicator scores for the environmental/ecological variables that tested as significantly different in the Mann-Whitney U analysis shown in Table 3.9.

Analysis of the relationship between ghyll woodland length and bryophyte species richness (Table 3.9) showed no significant relationship for the 1951-1970 period but a strong correlation was revealed for the 1976-1995 surveys.

Analysis of ghyll woodland fern species showed no correlation between species richness and ghyll length for the period 1951-1970, but a significant negative

correlation for the period 1976-1995, indicating a decrease in fern diversity with increasing ghyll length (Table 3.9).

Time period	Pearson's correlations Edge	Length
All species		
1951-1970 (85)	ns	ns
1976-1995 (103)	ns	ns
Bryophytes		
1951-1970 (37)	ns	ns
1976-1995 (35)	ns	0.486***

Time period	Pearson's correlations Edge	Length
Flowering plants		
1951-1970 (25)	ns	ns
1976-1995 (31)	ns	ns
Ferns		
1951-1970 (23)	ns	ns
1976-1995 (31)	ns	-0.431*

Table 3.9 Pearson's correlations between site species richness and the mean distance from the ghyll stream to the nearest edge of contiguous woodland, and correlations between site species richness and ghyll length. Numbers in brackets are the number of sites used for each piece of analysis. *= P<0.05; ***= P<0.001

Analysis of the relationship between species richness and the distance of the ghylls from the closest woodland edge showed no significant correlations indicating that ghyll species richness does not appear to be affected by the proximity of the ghyll woodland to the edge of the woodland fragment in which they are located.

3.6 Discussion

3.6.1 Analysing temporal changes in ghyll species richness

A number of environmental changes occurring in southern England during the post-war period might be expected to impact upon species richness levels within the Wealden ghyll woodlands.

Climate has a major influence on species distributions and as such the rapid increase in regional temperatures during the post-war period, together with changes in precipitation regimes in the area (Jenkins et al. 2009), are likely to impact on the diversity of ghyll woodland plant communities. It is predicted that in cool-temperate regions, such as the Weald, climate change will initially lead to an increase in species richness as changing climatic conditions lead to increasing levels of invasion by exotic species. Species no-longer suited to the changed

environment will not be lost immediately, but their potential lack of reproductive success and recruitment will lead to extinction over a longer timescale (Parry 1991, Hawkins et al. 2003, Thuiller et al. 2005). Changes that have occurred in post-war woodland management may also have impacted on levels of species richness within the regions woodlands. The wide-scale abandonment of coppice management in the post-war period will have altered the structure and possibly the composition of many of the regions woodlands. Light levels in early coppice stands are high and consequently early coppice is associated with high plant diversity (Barkham 1992a). Diversity then tends to decrease through time as the canopy closes and light levels fall (Mitchell and Kirby 1989). The impact of climate change and the wide-scale abandonment of coppice management in the region might therefore be expected to impact on species richness within the woodlands. Therefore one of the study aims was to identify temporal changes in both species richness and composition occurring during the post-war study period. However, the study found no significant changes in flowering plant, bryophyte or fern species richness within the ghyll woodland or the ancient woodland surveys during the survey period.

3.6.2 Changes in community composition

Although species richness remained relatively constant throughout the study period, the composition of the ghyll bryophyte and flowering plant communities changed significantly.

3.6.2.1 Changes in species distribution patterns

An examination of oceanic bryophyte species that declined over the study period (Table 3.5) showed that *Campylopus flexuosus* (Rusty Swan-neck Moss) was the second most abundant bryophyte recorded within the ghyll woodland sites surveyed during the 1951-1970 period (recorded in 17% of sites) but was absent from the ghyll woodlands surveyed during 1976-1995. *C. flexuosus* was also absent from the ancient woodland sites surveyed between 1951-1970, but it was

the most abundant species recorded in the ancient woodland surveys carried out between 1976-1995, occurring in 17% of the sites. It is unclear why this species that is associated with damp sandstone (Atherton et al. 2010) appears to have shifted its range from the ghyll valleys to the ancient woodland sites. It is possible that abandoned coppice maturing may be creating favourable microclimatic conditions for oceanic bryophytes within the wider ancient woodland.

The most widely distributed ghyll woodland oceanic bryophyte species in the 1976-1995 surveys was *Scapania gracilis* (Western Earwort) which was found in 9% of the sites in 1951-1970 and 34% of the sites in the 1976-1995 surveys. *S. gracilis* is common in woodlands in the west of England, but rare in the south-east where it usually grows on boulders or trees in dry but humid sites (Atherton et al. 2010).

The oceanic bryophyte *Orthodontium gracile* (Slender Thread-moss), classified as vulnerable in the 2004 bryophyte red data book (Church et al. 2004), was recorded in a single ghyll site in the surveys carried out during 1951-1970 (Table 3.5) but appears to have dispersed more widely in the second period where it was recorded in seven ghyll sites (20%). *Pallavicinia lyellii* (Veilwort), another red data book oceanic bryophyte species, was absent from the 1951-1970 ghyll woodland surveys, but was recorded in six (17%) of the 1976-1995 ghyll survey sites. The spread of these 'vulnerable' oceanic bryophytes, along with the increased distribution of *S. gracillis*, another oceanic bryophyte species, indicates that conditions within the ghyll woodlands may be becoming increasingly favourable for oceanic bryophytes. It appears that species that are on the eastern edge of their range boundaries (Ratcliffe 1968) are successfully colonising new sites and expanding their distributions.

3.6.2.2 Changes in the frequencies of bio-indicator species

It is important to gather information regarding temporal changes in the frequencies of biologically important species so that scientists are able to predict future trends and subsequently set conservation priorities (Telfer et al. 2002). Oceanic bryophytes and ancient woodland indicator (AWI) species are both species groups

that are restricted to particular habitat types because of their narrow ecological niches (Hutchinson 1957). They are considered biologically important species groups within the region (e.g. Ratcliffe 1968, Spencer and Kirby 1992, Peterken 1993, Rose and Patmore 1977) and are both associated with ghyll woodlands. A further aim of this chapter was to identify temporal changes in the distributions and frequencies of these bio-indicator groups. Analysis of the ghyll bryophyte communities indicated a temporal increase in the ratio of oceanic bryophytes. Oceanic bryophytes are 'niche specialists' that are strongly associated with shaded, humid environments (see Section 1.4.1). The increase in oceanic bryophytes may be correlated to the overriding pattern of climate change within the region which is one of warmer, dryer summers and warmer, wetter winters (Jenkins et al. 2007). Bryophytes are far better at tolerating high (or very low) temperatures when they are dry rather than wet (Proctor 2009). They are therefore less sensitive to rises in summer temperatures when conditions are dryer because these warmer periods coincide with periods when the bryophytes desiccate, during which time they are physiologically inactive (Frahm and Klaus 2001). Many bryophytes growing in temperate latitudes have their main growth periods during the winter months (Tuba et al. 2011). Frahm and Klaus (2001) consider increases in winter temperature and precipitation, together with the decrease in the number of winter air frost days, to have been the most likely cause of the range expansions recorded for 34 species of central European bryophytes occurring between 1981-2001.

3.6.2.3 The ecological determinants of temporal changes in community composition

3.6.2.3.1 Climatic and edaphic determinants

The ratio of ghyll woodland flowering plant AWI species also increased significantly over the study period (Table 3.5). Most flowering plant species associated with ancient woodlands prefer more shaded conditions than those associated with other woodland types (Hermy et al. 1999).

Analyses of the ecological requirements of ghyll woodland vegetation over the study period showed a significant decrease in the light requirements of both bryophyte and flowering plant communities (Table 3.6 & 3.7). This would indicate an increase in the ratio of shade-tolerant bryophytes and flowering plants over the study period and a decrease in the ratio of light-demanding species. This trend occurred during a period of climatic change when regional temperatures were rising and the summer growing period was lengthening (Jenkins et al. 2007). Since increased temperatures and a longer growing period are strongly correlated with increasing seasonal and annual light levels (Bonan and Bonan 2008) it might be expected that shade-tolerant species would decrease and light-demanding species would increase during this period, but the results show the opposite trend. Ellenberg light values for bryophytes and flowering plants are a reflection of light intensity at the forest floor and this will be moderated by the structure of the forest itself. The trend towards more shade-tolerant and less light-demanding bryophytes and flowering plant communities within the ghyll valleys may be related to the effects of post-war changes in woodland management on woodland structure. Abandoned coppice woodland will eventually revert to high forest and the woodland canopy will close (Peterken 1996). As shading increases, species that are reliant on the relatively high light levels associated with coppice woodlands will become less prolific and some may disappear entirely, whilst at the same time shade-tolerant species may re-establish or increase their abundance within the sites (Packham and Harding 1982). The abandonment of management and the subsequent increase in shade within the trunk-space provides an explanation for the increase in the abundance of shade-tolerant bryophytes and flowering plants within the ghyll woodlands indicated by the change in Ellenberg light values over the survey period (Table 3.6 & 3.7). Analysis of the ecological requirements of the bio-indicator groups; oceanic bryophytes (Table 3.8 & Figure 3.1a) and flowering plant AWI species (Table 3.8 & Figure 3.1b), showed a similar pattern of an increase in the relative abundance of shade-tolerant species over the study period. Studies of temporal changes in vascular plant communities within abandoned coppice woodlands in Germany have shown that light-demanding species decreased significantly whilst shade-tolerant species increased significantly (Trautmann 1976, Wilmanns et al. 1986). Another temporal study of changes occurring in abandoned coppice woodlands, this time in Switzerland, involved re-

visiting previously sampled plots after a 30-50 year period (Känzig-Schoch 1996). The Swiss study found that average Ellenberg light indicator values decreased in almost every plot and that many light-demanding species had disappeared 'due to the increased darkening of forests' attributed by the study authors to the previous abandonment of coppice management within the forests (Wohlgemuth et al. 2002). The soil moisture indicator values associated with the ghyll woodland flowering plant communities decreased significantly over the two study periods (Tables 3.6 & 3.7). This would be consistent with both rising regional temperatures and an increase in canopy density associated with abandoned coppice (Buckley 1992). The density of woodland canopies has been shown to be negatively correlated with soil moisture levels because an increasing percentage of the precipitation falling on the woodland is intercepted by the canopy and evaporates without ever reaching the ground (Thompson 1970, Moore and Vankat 1986, Giacomin and Trucchi 1992). The increase in the frequency of moisture loving oceanic bryophytes within the ghyll valleys (Table 3.4) appears to conflict with the environmental conditions indicated by the flowering plant soil moisture scores which decreased over the survey period (Tables 3.6 & 3.7). This may be explained by the importance of atmospheric moisture, rather than soil-moisture, in fulfilling the water and nutrient requirements of many bryophyte species (Désamoré et al. 2012). Studies have shown that woodland canopies generally form a barrier trapping atmospheric water within the woodland and leading to increased levels of relative humidity (Larsen 1922, Rackham 1975). The increase in the ratio of moisture-loving, shade-tolerant bryophyte species (Ratcliffe 1968) within the ghyll woodlands (Table 3.4) would be consistent with increasing canopy density that will have occurred in many of the regions woodlands as a result of the widespread abandonment of coppice management during the post-war period (Mitchell 1992).

3.6.2.3.2 Seed-size and species distributions

Small-seeded species have a greater dispersal capacity and are produced in greater quantities than large-seeded species, meaning that species with smaller seeds tend to occupy a greater number of sites (e.g. Venable and Brown 1988, Westoby et al. 1996, Guo et al. 2000). McCollin et al. (2000b) ventured that within

a fragmented habitat system, small-seeded species might do better than heavier-seeded ones. However, the analysis of plant indicator values for dried seed weight indicated that the ratio of ghyll flowering plant species with larger seeds had increased over the survey, whilst species with small seeds were becoming less common (Table 3.6 & 3.7). Interestingly, McCollin et al. (2000b) identified a similar pattern in their temporal analysis of the flora of Northamptonshire. Salisbury (1942) analysed the influence of light levels on the seed mass of various British species by comparing the mean seed mass of 23 open habitat species with 23 shade-tolerant congeners. The shade-tolerant species had an average seed mass 3.3 times greater than the open habitat group indicating a negative association between the seed size of British species and light levels. A number of studies have also found that species whose seedlings establish within shaded environments tend to have larger seeds (Grime and Jeffrey 1965, Westoby et al. 1992, Leishman and Westoby 1994a). The increase in large-seeded plants may therefore be correlated to an increase in shade-tolerant species within the ghyll woodlands. Salisbury (1942) and Baker (1972) both argue that plants recorded in environments associated with a high risk of drought during seed germination, tended to be species with larger seeds compared to plants found in lower risk environments. Leishman and Westoby (1994b) found seedlings from species with larger seeds performed better under drought conditions than those with smaller seeds in a manipulated environment within a greenhouse. If seed-size is related to the ability of species to establish under drought conditions then the increase in species with large-seeds may be linked to the decrease in soil moisture requirements measured over the study period (Tables 3.6, 3.7 & 3.8) and may therefore be linked to both climate change and increasing canopy density.

3.6.2.3.3 Substrate-specificity and bryophyte distribution patterns

Indicator values representing the number of substrates in the UK that bryophyte species are associated with were significantly correlated to changes in plant distributions (Tables 3.6 & 3.7). The ratio of ‘generalist’ species associated with a wide range of substrates increased whilst the ratio of ‘specialist’ species associated with a narrow range of substrates decreased. This appears to be

reflected in the analysis of oceanic bryophyte species richness (Table 3.5). Eight oceanic bryophyte species were recorded in the 1951-1970 period but not in the 1976-1995 period, whilst four species recorded in the 1976-1995 surveys had not been recorded during the 1951-1970 period. The oceanic bryophyte species recorded only in the 1951-1970 surveys were associated with an average of 2.75 substrate types, whereas the oceanic bryophyte species recorded only in the second period were associated with an average of 3.5 substrate types. This indicates that the increase in the frequency of oceanic bryophyte species (Table 3.4) is the result of an increase in 'generalist' species associated with multiple substrate types and a decrease in more 'substrate specific' species.

A number of studies of bryophyte distribution patterns highlight substrate specificity as a causal mechanism that limits plant species dispersal abilities and reduces population densities in isolated habitats (Cleavitt 2001, Pharo and Zartman 2007). Substrate specificity has been cited as a potential explanation of vascular species rarity, particularly for species reliant on fragmented habitats (Rabinowitz et al. 1986, Fiedler and Ahouse 1992). If substrate specificity is correlated with bryophyte rarity then the decrease in 'substrate specific' species may indicate a corresponding decrease in rare bryophyte species.

3.6.2.3.4 Examining the effects of woodland fragmentation on ghyll species richness

Analysis of the ghyll woodland habitat patches found no relationship between species richness and the distance of the ghyll from the nearest woodland edge and therefore no evidence of an edge effect. This may indicate that the ghyll valleys buffer the woodlands from the surrounding environment as a number of authors have speculated (Rose and Patmore 1997, Patmore 2000, Burnside et al. 2006). There is also the possibility that ghylls located at different distances from the woodland edge did not display significant differences in species richness because the majority of the ghyll woodlands consist entirely of edge habitat. A study of the borders of forest fragments in Pennsylvania and Delaware found that changes in humidity and leaf-litter moisture penetrated approximately 50 metres into the forest interiors (Matlack 1993). A study of bryophyte distributions in the

edge zones of temperate rainforest fragments in British Columbia showed an increase in the richness of clearing-affiliated bryophytes up to 45 metres from the rainforest edge and a decrease in old-growth associated bryophyte species within the same area (Baldwin 2004). Baldwin states that this implies the influence of the forest edge for old-growth bryophyte species extends at least 45 metres into the forest fragments. The width of an edge zone will remain constant irrespective of woodland size. Therefore, as the size of the woodland decreases, the proportion of edge habitat increases (Collinge 2009). Analysis carried out by Burnside *et al*. (2002a) found that the ghyll woodlands predominantly form small linear patches of woodland. Linear habitats usually have longer boundaries, and therefore more edge habitat, than relatively square or circular shaped habitats (Hunter 2002). In many cases the surrounding woodland has been cleared up to the edge of the linear ghyll valleys (pers. obs.). If ghyll woodland is restricted to the valley boundaries, as the analysis in Chapter 2 indicates, then 45 metres is greater than the total width of some of the ghyll woodlands surveyed in Chapter 2. Therefore, it is conceivable that for old-growth bryophyte species, a number of the ghyll valleys are entirely edge habitat.

On the basis that ghyll woodlands are linear features, the length of the ghyll stream was used as a proxy to represent ghyll area (Table 3.9). A relationship between species and area has been widely observed in the type of fragmented habitat 'islands' that the ghylls represent (Mitchell and Ryan 1998, Lawson and Jensen 2006, Shen et al. 2009, Lomolino et al. 2010). The analysis of ghyll bryophytes showed no relationship between species richness and length for the 1951-1970 period, however analysis of the sites surveyed in the 1976-1995 period revealed a highly significant ($P>0.001$) positive relationship between bryophyte richness and ghyll length. It is unclear why this relationship only occurs during the second survey period. The analysis also showed that a negative relationship existed between fern species richness and ghyll length for the sites surveyed during the 1976-1995 period.

A detectable response in species richness levels to fragmentation pre-supposes that populations have had sufficient time to respond to the landscape changes. The lack of a relationship during the 1951-1970 period for both bryophytes and

ferns may simply be that insufficient time had elapsed since levels of fragmentation passed a critical threshold for significant changes to have occurred.

The significant changes in ghyll species composition in relation to seed size, number of substrates and species richness, relative to ghyll length (area), are all variables indicative of the effects of habitat fragmentation. However, it is clear from the significant changes in the indicator values of other variables such as soil moisture and light (Tables 3.7 & 3.8) that there are a number of environmental changes occurring within the ghyll woodlands and it is difficult to separate the effects of fragmentation from other landscape-wide changes such as changes in woodland management regimes and regional climate change that may be causing changes in the composition of ghyll plant communities.

3.6.3 The use of archive survey data

At a time when plant communities are experiencing increasing environmental changes and increasing rates of biodiversity loss (e.g. Scheffer et al. 2001, Biggs et al. 2009, Morecroft et al. 2009) it is essential to monitor changes in biodiversity. However, the absence of information on background rates (and directions) of change can make it difficult to assess the levels and impacts of these changes. Long-term datasets have become increasingly important to provide baseline data against which efforts to moderate rates of biodiversity loss can be measured (Magurran et al. 2010). The use of archival sources offers a valuable method of studying temporal changes in biodiversity, but it is important that potential problems involved with this type of dataset are recognised and their impact on the quality of the data assessed.

The long-term nature of many archive datasets is what makes the data so valuable, but the long length of time covered by the studies can also cause significant problems. The sample effort, sampling frequency, spatial extent and the taxonomic groups focused upon, can vary markedly throughout long-term studies, particularly where changes in surveyors have occurred during the study (Magurran et al. 2010). Temporal changes in survey methodologies can cause major issues of data comparability. One of the reasons that the ‘Dr Francis Rose Notebook’

data is so valuable is that it is extremely rare for survey data spanning such a long time period (five decades) to have been collected in surveys that were all led by a single individual. As such the methodology throughout the surveys appears to have remained constant. Although the methodology may have remained constant, it is likely that Dr Rose's botany skills and habitat knowledge would have improved over such a long time period, during which time he carried out hundreds of surveys. His abilities to locate and identify the rarer species associated with the ghyll woodlands may have improved over the course of the surveys and therefore it is possible that the increase in the ratios of oceanic bryophytes could be the result of an improved ability to locate these biologically important species rather than an actual increase in frequency. However, the increase in bio-indicator species was only recorded in the ghyll woodlands. Therefore, no significant change was found in the frequency of oceanic bryophytes or flowering plant AWI species recorded in the ancient woodland group of surveys and it would be expected that any increasing expertise at locating these bio-indicator species would be replicated within these sites too.

3.7 Conclusion

The study found no significant temporal changes in the species richness of ghyll woodland bryophyte, flowering plant or fern communities. Analysis of ghyll woodland species composition showed a significant increase in the ratios of oceanic bryophytes and flowering plant AWI species (Table 3.4). Both of these bio-indicator groups are associated with relatively shaded woodlands (Ratcliffe 1968, Hermy et al. 1999). The increase in the ratio of these shade tolerant species groups occurred during a period of climate change during which the growing season was lengthening and average light levels within the Wealden region were increasing (Jenkins et al. 2007, UKCIP 2013). The increase in the ratio of these bio-indicator groups was restricted to the ghyll woodlands and no significant change was recorded in the ancient woodland surveys. The results indicate an increase in the frequencies of shade-tolerant species which may be diagnostic or indicative of a move to more shaded conditions within the ghyll woodlands.

Analysis of the Ellenberg moisture scores assigned to the oceanic bryophyte group indicated an increase in the ratio of species associated with higher levels of moisture and a decrease in the levels of species associated with dryer conditions. Conflictingly, analysis of the moisture indicator values for ghyll woodland flowering plant AWI species indicated the opposite trend, with the ratio of 'moisture-loving' plants decreasing in the ghyll woodland communities. Bryophytes rely on atmospheric moisture for the majority of their water intake (Tuba et al. 2011), whilst flowering plants rely on their root systems to extract water from the soil. It may be that whilst soil moisture levels have decreased, as is indicated by the flowering plant moisture indicator values, relative humidity within the valleys has increased and the apparent increase in 'moisture-loving' bryophyte species is a response to relative humidity levels and not soil moisture levels. The increase in the ratio of these 'moisture-loving', shade-tolerant bryophyte species would be consistent with microclimatic conditions likely to occur as abandoned coppice matures and canopy density increases (Wohlgemuth et al. 2002).

The temporal changes in indicator values for dried seed size and number of substrates, along with the significant relationship between species richness and ghyll length, are indicators of the effects of habitat fragmentation. The consequences of ghyll woodland habitat fragmentation will be examined further in Chapter 4.

Climatic changes experienced in the region over the past century are relatively mild in comparison with those predicted to occur over the next 70 years (UKCIP 2013). Conservation strategies may be needed to protect important ghyll woodlands from the rapid climatic changes predicted to occur this century. Understanding past biotic responses to climate change is central for assessing future impacts on biodiversity (Overpeck et al. 2005). Whilst the rate of future change may differ from that in the past, inferences on the mechanisms and speed of response may be drawn from the analysis of archival data (Botkin et al. 2007). Understanding the impacts of previous climate change on patterns of vegetation may enable the development of models that allow future shifts in species, communities and ecosystems to be predicted (Webb III 1992, Lowe and Walker 1997).

4 RAPD analysis of the genetic diversity and population structure of *Conocephalum conicum* within the fragmented Wealden ghyll woodlands

4.1 Preamble

The analysis in Chapter 2 indicated that ghyll woodlands form a fragmented habitat separated from other woodlands in the region on the basis of the high species richness of the ghyll woodland flowering plant and bryophyte communities. Chapter 3 used temporal data to analyse species indicative of these communities and examine whether the composition and distributions of these communities are changing through time. Temporal analysis of plant indicator values showed significant temporal changes in indicator values for dried seed weight, an environmental variable that McCollin et al. (2000b) describe as being 'indicative of the effects of habitat fragmentation'. The chapter also revealed a significant relationship between both bryophyte and fern species richness and ghyll length (a proxy for ghyll area). A significant species-area relationship is also indicative of habitat fragmentation (Wilcove et al. 1986). This chapter will investigate the effects of habitat fragmentation on the genetic structure of ghyll woodland communities. To examine and predict levels of genetic isolation and geneflow, the chapter will compare molecular markers of four populations of *Conocephalum conicum*, a common ghyll woodland bryophyte species.

4.2 Introduction

It has been widely observed that habitat isolation can lead to a decrease in the genetic variability of species within those habitats (Hedrick 2005, Primack 2006,

Allendorf and Luikart 2007, Hoglund 2009, Frankham et al. 2010), which is in turn linked to the persistence of populations (Wright 1931, Gugerli et al. 2008). The analyses in this section seeks to provide evidence that physical isolation has resulted in genetic isolation, through a reduction in gene-flow, and consequently a reduction in within subpopulation genetic diversity.

4.2.1 Habitat fragmentation and the Wealden ghyll woodlands

Habitat fragmentation is one of the biggest threats to biodiversity in the 21st century and understanding the effects of habitat fragmentation and loss is a crucial part of modern conservation studies (Fahrig 2003, Harrison and Bruna 1999). The effects of fragmentation on biodiversity occur at different scales, from large regional effects on entire ecosystem processes and patterns (Virtaten and Oksanen 2007) to local scale effects of isolation or habitat loss on individual populations of species (Van der Ree et al. 2004, Cushman 2006).

The Wealden ghyll woodlands were in fact once part of larger woodlands that covered most of the Wealden area (see Section 1.2.2). Many of the ghyll woodlands now exist within isolated fragments of woodland, surrounded by habitats that are less hospitable to woodland species (Ratcliffe 1968, Streeter 1983). The loss of woodland regionally and the fragmentation of the woodland that remains would have caused the local extinctions of many plant populations. Some of the populations of plants found in the ghyll woodlands may survive as parts of a metapopulation (Levins 1970) exchanging genetic material with other populations of the species located in the wider woodlands. Under these circumstances it is possible the loss of populations in the wider woodlands would lead to increased levels of genetic isolation within the ghyll woodland populations. This is because gene-flow is usually negatively correlated with the geographical distance between subpopulations (Beebee and Rowe 2004, Freeland 2005). The majority of the woodland that covered large areas of the Weald existed up until the 12th and 13th century (Brandon 2003). It is likely therefore that any genetic isolation of ghyll woodland plant populations would have occurred during, or after this period, i.e. within the last 800yrs. The effects of genetic drift will potentially increase with each

generation, therefore the more generations that have occurred since the period of genetic isolation, the greater the chances that genetic changes will have occurred within the subpopulations of that species. For species that have a long generation time, such as Pedunculate Oak (*Quercus robur*), the 800 year period since isolation would only be long enough for perhaps 16-20 generations, whereas a species that matures relatively rapidly, such as Silver Birch (*Betula pendula*), might produce 80-160 generations over the same 800 year time period. Many bryophyte species produce a new generation annually and would therefore have undergone as many as eight hundred generations in the same time period. The rapid generation times of most bryophyte species offers a unique opportunity to examine the genetic effects of habitat fragmentation within relatively short time periods (Snall 2005). The utilization of bryophyte populations for studying the evolutionary consequences of fragmentation may provide valuable information about the long term impacts of habitat isolation and would therefore be potentially helpful for developing general conservation priorities for the plant groups within the fragmented habitat (Pharo and Zartman 2007).

4.2.2 The consequences of genetic isolation

Allele frequencies of small isolated populations will change from one generation to the next, merely through the random union of gametes, even without selection, migration and mutation, this process is known as 'genetic drift' (Wright 1929). In the absence of selection, gene-flow and mutation, the random changes of allele frequencies within the gene-pool of small populations can lead to a fixation at all loci and a complete absence of genetic variability within the populations (Wright 1978). An allele occurring at a low frequency has a significant chance of being lost in subsequent generations (Wright 1929, Lande and Kirkpatrick 1988, Allendorf and Luikart 2007). For example, if a rare allele occurs in 10% of all individuals in a haploid population then, in a large population of 100,000 individuals, 10,000 will carry the allele. Assuming that the population has approximately the same allele frequencies from one generation to the next then the allele will not be lost from the population, but will be maintained at the same low level (10%) in the next generation. In this large population a random chance event, such as a carrier of

the rare allele failing to produce offspring, or a carrier failing to pass the allele on to their offspring would be balanced by others that pass the allele on to a greater than average number of offspring. In a population of only 10 individuals, where again 10% carry the rare allele, only one individual carries the allele. If, by chance, that individual fails to produce any surviving offspring, or the offspring don't inherit the allele, then the allele will be lost from the population (Beebee and Rowe 2004, Primack 2006, 2008, Peixoto et al. 2010). Small isolated populations undergoing genetic drift have an increased vulnerability to the degenerative genetic process known as inbreeding depression (Darwin 1876, Angeloni et al. 2011). This is the mating of closely related individuals and is characterized by increased offspring mortality rates, less offspring, or weak, sterile offspring with reduced mating success (Darwin 1868, 1876). They can also suffer a decrease in evolutionary adaptability, potentially limiting the population's ability to respond to changes in the environment from such things as new diseases, climate change, etc. (Frankham 2005). These processes may lead to a reduction in population size, causing a further loss of fitness through increased inbreeding, and leading to an increased risk of extinction (Franklin 1980, Frankham 2005, Brook et al. 2008). Populations trapped in this deleterious spiral are said to be caught in an 'extinction vortex' (Williams et al. 1990, Fagan and Holmes 2006, Brook et al. 2008).

4.2.3 RAPD genetic analysis

The protocol for this analysis followed the protocol used by Zhu et al. (2007) who studied the genetic diversity of the moss *Brachythecium rivulare* (River Feather-moss) in the Foping Nature Reserve in Shaanxi, China, using the random amplified polymorphic DNA (RAPD) technique. This technique is based on DNA amplification by the polymerase chain reaction (PCR) using primers of short (typically <10bp) arbitrary sequence (Williams et al. 1990). It is a widely used method that has proved very useful for assessing genetic relationships in a variety of species (e.g. Li and Jin 2006, Tang et al. 2007, Na et al. 2009, Sharma et al. 2009, Przyborowski and Sulima 2010). It has advantages over some other techniques of genetic analysis in that prior knowledge of the targeted genome sequence is not required, a large number of samples can be quickly and easily

evaluated, and only small quantities of DNA are required (Zhu et al. 2007). A disadvantage of using the RAPD technique is that it cannot separate a heterozygote (Aa) from a homozygote (AA), but this is not an issue when studying bryophytes as they can be collected during the haploid phase of their life-cycle (Pharo and Zartman 2007).

Zhu et al. (2007) sampled five separate subpopulations of bryophytes. However, a number of studies have used either three or four subpopulations to carry out similar types of analysis (e.g Kirsten et al. 1998, Tansley and Brown 2000, Tang et al 2007, Julio et al. 2008). Four subpopulations of the target species *Conocephalum conicum* were sampled for this study.

4.2.3.1 Bryophyte reproduction and dispersal

Gene flow between subpopulations of bryophytes that reproduce sexually is perpetuated through sperm, spore or propagule dispersal (Korpelainen et al. 2005). Studies have found that bryophyte sperm dispersal distances are relatively short, often just millimeters or centimeters (Wyatt and Anderson 1984). Shimamura *et al* (2008) measured the maximum distance travelled by sperm during a fertilization event in the liverwort species *C. conicum* (the species selected for this study) and could detect no sperms greater than 1 metre from the source. There have only been a limited number of studies on frequency and dispersal distance of asexual propagules, however there is general agreement that spores are able to disperse further from the source than larger asexual diaspores (Kimmerer 1994, Sundberg 2005). Gene flow between separate subpopulations of bryophytes usually occurs through the long distance dispersal of spores (Van Der Velde and Bijlsma 2003, Miller and McDaniel 2004, Sundberg 2005). A primary factor in choosing the species for this study was the potential spore dispersal distance of the target bryophyte species. A bryophyte species capable of producing millions of relatively small spores, capable of travelling large distances, would be less likely to be affected, in terms of a reduction in gene-flow through fragmentation on the scale of the Wealden woodlands, than a species with a

relatively small number of large spores such as the study species *C. conicum* (see Figure 4.1) (Goffinet and Shaw 2009, Vanderpoorten and Goffinet 2009).

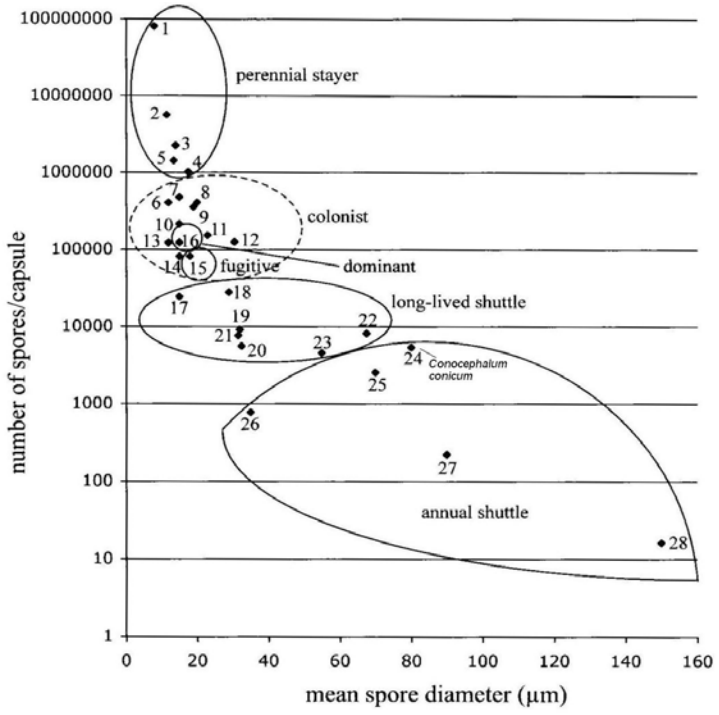


Figure 4.1 Life history strategies of a sample of bryophyte species. The graph axis show spore diameter and number of spores per capsule. Adapted from Vanderpoorten & Goffinet (2009).

The spore dispersal range of bryophytes is heavily influenced by the 'life strategy' of each species. Habitat patches may become unsuitable for a species of bryophyte through competition, herbivory, or for seasonal reasons such as summer dessication events caused by seasonal temperature increases (Vanderpoorten and Goffinet 2009). The habitat patch itself may only be temporary; as in the case of boggy areas on river floodplains after periods of excessive rain (Soderstrom and Herben 1997). The duration that bryophytes occupy a habitat can range from weeks, in the case of boggy floodplains, to thousands of years on some forest floors (Vanderpoorten and Goffinet 2009). The best life strategy for any organism would appear to be to have a high rate of reproduction throughout the entire length of its life, whilst at the same time the organism would produce offspring that are ideally adapted to survive a range of environmental conditions and competition, and would have a relatively long life

expectancy. In practice however the longevity and competitiveness of an organism is usually correlated with size (MacArthur and Wilson 1967, Simberloff and Wilson 1969, Pizo et al. 2006, Ramírez-Valiente et al. 2009). There is usually competition for the limited resources available and so there is a trade-off involving how these resources are exploited by the organism (Vanderpoorten and Goffinet 2009).

Strategy	Life Span (yrs)	No and size of spores	Reproductive effort	Typical habitat
Fugitive	<1	numerous, <20µm	high	very temporary
Colonist	few	numerous, <20µm	average	temporary
Perennial stayers	many	numerous, <20µm	low	Stable habitats
Annual shuttle	<1	few, >20µm	high	Cyclic, temp. habitats
Dominants	many	few, >20µm	low	Open, stable habitats

Table 4.1 The bryophyte life history strategy categories identified by During (1992). The table details the trade-offs involved in spore production. Large spores will have a lower dispersal capacity but better chances of successful establishment. The potential life-span of the bryophytes will be negatively correlated with reproductive effort. Reprinted from Vanderpoorten and Goffinet (2009).

Spore or seed size is not only linked to offspring survivorship, but in many cases to dispersal ability. Bryophyte spores are dispersed by the wind and therefore, due to the weight of the spores, large spores will have a lower dispersal capacity than small spores (During 1992, Vanderpoorten and Goffinet 2009). However, larger spores will have a greater chance of successfully establishing than smaller spores and will also survive for a longer period of time in the soil or surface substrate (Vanderpoorten and Goffinet 2009). During (1992) divided bryophytes into five broad life strategy categories based on each species life span and stability of habitat (Table 4.1).

The study species *Conocephalum conicum* is an annual shuttle species (Table 4.1). This group of bryophyte species is usually associated with cyclic habitats that disappear at certain times of year and reappear at the same location (During 1992, Vanderpoorten and Goffinet 2009). This category of bryophyte species creates ephemeral gametophytes that die-off during periods of extreme stress such as severe dessication events or frosts. The resources allocated for reproduction are characteristically high for annual shuttle species and they typically produce a relatively small number of large spores (Kurschner et al. 2007). It would seem

reasonable therefore to hypothesize that since the spores of annual shuttle species are less widely distributed than many other species of bryophytes, they would be more likely to suffer from a reduction in geneflow as a result of habitat isolation. Each generation of annual shuttle species lives for less than a year and will usually produce a new generation each year (Vanderpoorten and Goffinet 2009). Species from this category will therefore have the potential to evolve more rapidly and as such the consequences of genetic isolation, in terms of levels of genetic drift, are likely to be more pronounced in this group of species.

Figure 4.2 Photograph of the survey species *Conocephalum conicum.* Photograph taken from Szweykowski et al. (2005).

The annual shuttle liverwort *Conocephalum conicum* (Figure 4.2) chosen for this study is a bryophyte that is associated with damp, humid environments such as forest floors or wet rocks found in and by streams (Watson 1981a) and has been recorded in a number of ghyll woodland surveys. It is a thalloid liverwort that is unable to survive prolonged periods of dessication. *C. conicum* was chosen because it is a fairly common species within ghyll woodlands and it was felt that rarer ghyll species, existing in smaller populations and in fewer sites, might be experiencing levels of genetic isolation irrespective of landscape changes. It is also a species whose populations have been shown to contain low intraspecific variation along with weak inter-population differentiation (Wyatt 1985) meaning

any significant genetic variations identified between separate populations are unlikely to be the result of natural genetic variation between sites.

4.3 Aims

The chapter aims to investigate the genetic impacts of habitat isolation using four geographically separated ghyll woodland populations of the thalloid liverwort *Conocephalum conicum*.

The extent of molecular divergence within and among populations will be analysed with the aim of comparing the genetic diversity of the samples and to estimate the level of gene-flow between the survey sites.

The relationship between the geographic distance between populations and the genetic diversity of the survey sites will also be examined.

4.4 Methods

The use of RAPD markers, in conjunction with the relevant statistical analyses, has become a widely used method for examining both inter- and intra-population genetic variability among bryophyte populations (Selkirk et al. 1997, Skotnicki et al. 2004a, Skotnicki et al. 2004b, Liu et al. 2006). The analysis only requires very small amounts of plant material, which therefore means the environmental impact is minimal (Zhu *et al*. 2007). The major problem encountered with RAPD's is the reproducibility of the amplification results (Hallden et al. 1996, Karp et al. 1996, Li and Jin 2006, Li et al. 2008). To ensure that the RAPD banding patterns were reproducible the analysis was duplicated for each sample. The results of this analysis are therefore considered to be reliable.

4.4.1 Study sites

Genetic material was collected from four gorge-shaped ghyll woodlands. Samples of the liverwort C. conicum were collected from the ghyll woodlands at Fairlight Glen in Hastings, Marline Valley in Hollington, Fore Wood in Crowhurst and Brick Kiln Wood in Mayfield.

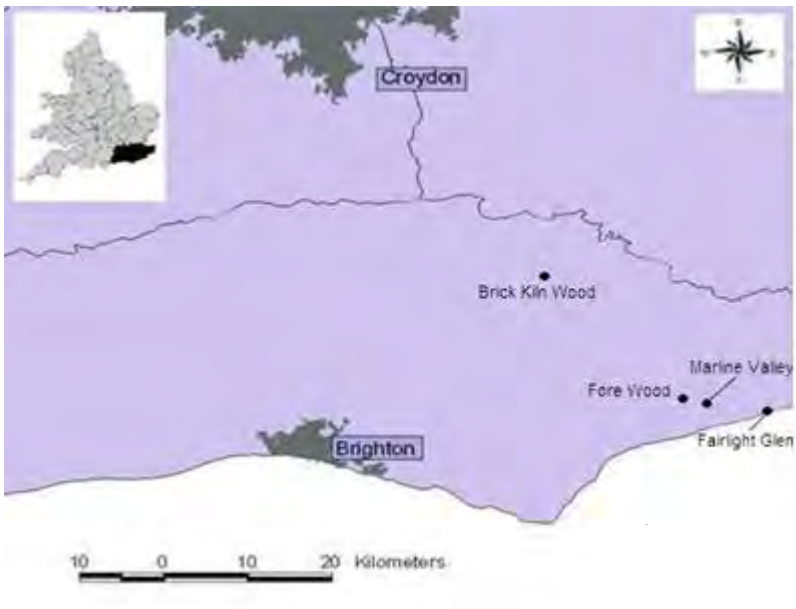


Figure 4.3: The locations of the four ghyll woodlands survey sites. Twelve samples of the liverwort *Conocephalum conicum* were collected from each of these sites.

Distance measurements between the survey sites are recorded in Table 4.2. The closest sites geographically were Fore Wood and Marline Valley which were only 1.7 kilometres apart. The two sites that were furthest apart were Brick Kiln Wood and Fairlight Glen which were 32.1 kilometres apart.

	Fairlight Glen	Marline Valley	Fore Wood
Fairlight Glen	-		
Marline Valley	6.6	-	
Fore Wood	9.5	1.7	-
Brick Kiln Wood	32.1	25.4	23.2

Table 4.2 Geographical distance, in kilometres, between each of the survey sites. Distances were measured using ARCMAP v.9. Distance measurements are the shortest straight-line distances between the borders of each of the woodlands using boundary data supplied digitally by the Sussex Biodiversity Records Centre.

Environmental and landscape variables measured in a GIS (ARCMAP v.9) using the digital ghyll woodlands map (SBRC 2000) are recorded in Table 4.3. Fore Wood was the longest of the survey ghylls at 590 metres long whilst Marline Valley was the shortest ghyll with a length of only 200 metres. Brick Kiln Wood was the largest survey site with an area measurement of 43.2 hectares whilst Fairlight Glen was the smallest with only 8.2 hectares of woodland.

Site	Length (m)	Area (h)	Adjacent land-use	Contiguous woodland around ghyll (hectares)	No. of woodlands within 5km
Brick Kiln Wood	300	43.2	bm, un, im	121.7	23
Fore Wood	590	10.6	bm, im, ga, he, rd	27.4	44
Marline Valley	200	12.4	bm, im, he	92.6	52
Fairlight Glen	570	8.2	bm, im, ar, he	38.3	65

Table 4.3 Table containing environmental and landscape variables and measurements related to the four survey sites. Length, area and adjacent land-use information is taken from the BERG dataset (Burnside et al. 2006). Figures for contiguous woodland around ghyll and number of woodlands within 5km calculated using ARCMAP v.9 using woodland data provided in digital form by the Sussex Biodiversity Records Centre. Adjacent land-use types are bm = broadleaved woodland, un = unimproved grassland, im = improved grassland, ga = garden, he = hedgerow, rd = road, he = heathland.

All of the sites where bordered, at some point, by broadleaved woodland and improved grassland. All of the sites were also part of larger woodlands. The woodland to which Brick Kiln Wood was attached was the largest of the woodlands at 121.7 hectares. The woodland to which Fore Wood was attached was the smallest at 27.4 hectares. Brick Kiln Wood had 23 woodlands within 5 kilometres of its border, which was the fewest number recorded for all of the survey sites. Fairlight Glen had 52 woodlands within 5 kilometres of its borders and this was the largest number recorded for all of the survey sites.

Figure 4.4 OS map 1:25,000 showing the location of the ghyll woodland at Fairlight Glen, Hastings.

Fairlight Glen (TQ852107) is part of Hastings Country Park which has been designated a Special Area of Conservation (SAC), a Site of Special Scientific Interest (SSSI) and is a proposed Nature Reserve. It lies within the High Weald area of Outstanding National Beauty. The woodland at this site supports many rare mosses and liverworts (Hastings Borough Council 2011).

Figure 4.5 OS map 1:25,000 showing the location of the ghyll woodland at Marline Valley, Hollington

Marline valley (TQ780122) is a SSSI and has also been classified as a Local Nature Reserve. The SSSI status is due in part to the diverse communities of rare liverworts and mosses which grow alongside the ghyll stream (Hastings Borough Council 2010).

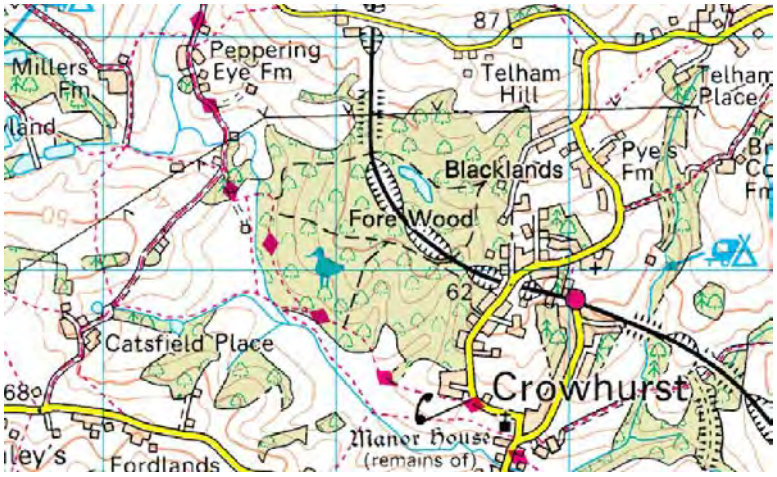


Figure 4.6 OS map 1:25,000 showing the location of the ghyll woodland at Fore Wood, Crowhurst

Fore Wood (TQ754128) is a SSSI and is one of the largest blocks of semi-natural Ancient Woodland remaining in East Sussex (ESCC 2005). This site features some very steep sided sandstone ravines that contain a number of rare ferns (RSPB 2010).

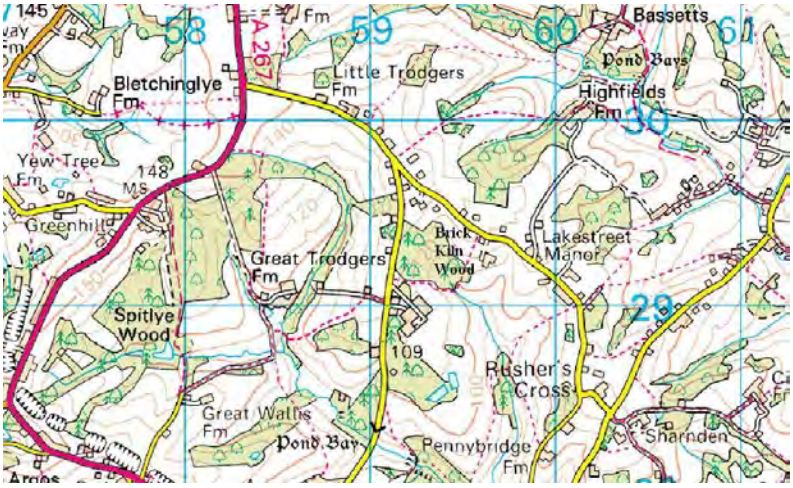


Figure 4.7 OS map 1:25,000 showing the location of the ghyll woodland at Brick Kiln Wood, Blackboys

Brick Kiln Wood (TQ582292) lies within an area designated an Area of Outstanding Natural Beauty (AONB).

The four ghylls selected were all gorge-shape valleys. The deep, steep-sided nature of these valleys is believed to buffer the interiors from temperature changes and helps produce a mild, humid microclimate (Rose and Patmore 1997). The

analysis in Chapter 2 of this study did not find direct physical evidence of this microclimate, however the richness of moisture-loving, shade-tolerant oceanic bryophyte communities within the ghyll valleys measured in Chapter 3 (Table 3.5) does indicate the presence of a shaded, humid ghyll woodland microclimate. Gorge-shaped ghyll woodlands are therefore associated with many of the rarer ghyll species that rely on shaded, humid conditions to survive (Ratcliffe 1968, Rose and Patmore 1997). It is these rarer species that are most likely to be particularly sensitive to the effects of genetic isolation. Their populations are likely to be relatively small and they often have narrow ecological niches, increasing the probability of subpopulation extinction, both in the ghylls themselves and in the surrounding matrix, through the processes that lead to the 'extinction vortex' (Green 2003).

Sandrock outcrops are found in all four of the survey sites. The damp humid environment associated with these gorge-shaped ghylls, coupled with the presence of sandstone, provides a perfect environment for internationally important lower plant communities (see Section 1.2.1) (Rose and Patmore 1997).

4.4.2 Sample collection

During August 2008 samples of the liverwort *Conocephalum conicum* were collected from four separate gorge-shaped ghyll woodlands. Twelve samples were collected from along the length of the watercourse at each of the sites. Samples consisted of the growing tip of a 'leaf' from an individual plant. Each sample was approximately 1cm^2. At all four sites the liverwort was found in small isolated patches, usually either on the stream bank or on wet rocks within the stream. These isolated patches were distributed approximately 1 to 3 metres apart within all the survey sites with the exception of Brick Kiln Wood where it was less common and patches were approximately 5 to 10 metres apart. Because the species can spread clonally it was decided that to try to avoid pseudo-replication all samples collected should be at least 5 metres from each other. The samples were stored in clean sealable plastic sample bags until DNA was extracted in the

laboratory, after which the samples were discarded. DNA extraction took place within 24 hours of the samples being collected.

4.4.3 Protocol for sample analysis

Zhu et al (2007) studied the moss *Brachythecium rivulare* (River Feather-moss) and included in their paper a full list of the genetic primers that they had successfully used to carry out their analysis, along with a comprehensive protocol for analysing the data. Following preliminary studies the methodology of Zhu et al. (2007) was adapted and successfully applied to the liverwort *C. conicum*. Forty-eight samples of *C. conicum* from four ghylls (12 samples from each) were used in the analysis for this project.

4.4.4 DNA extraction and preparation for PCR

The samples were cleaned by agitation in distilled water. Excess water was removed by placing samples between two clean paper towels and applying gentle pressure.

The DNA was extracted using GE Healthcare Whatman FTA plant cards following the protocol supplied with the cards (GE Healthcare 2011). The leaf section was placed onto the plant card and covered using the cover sheet attached to the card. A porcelain pestle was used to apply pressure to the leaf tissue by using a rolling motion on top of the cover sheet and the cell walls of the plant tissue were burst in this way. The lysis of the plant cells releases the nucleic acid and it becomes entrapped among the cellulose fibres within the plant card. Pressure was applied until enough lysate was extracted to penetrate through to the underside of the card. Any large pieces of plant tissue that had become attached to the plant card were gently removed by scraping with a scalpel. The cards were then left to dry at room temperature for 1 hour. The nucleic acids remain immobilized and stabilized within the card ready either for processing immediately or for storing for use at a later date. Provided the cards are kept dry the DNA can be stored indefinitely in this form.

A small disc was removed from the sample card using a biopsy punch. The disc was added to a 1.5µl micro centrifuge tube along with 100µl of purification reagent. The tube was vortexed at maximum for 5 seconds, using a Stuart Scientific Auto Vortex SA9. The tube was then vortexed every 30 seconds for a period of 5 seconds and after 2 minutes the purification reagent was discarded. Another 100µl of reagent was added to the tube and the procedure was repeated. This process was repeated twice more, but this time using 100ul of TE-1 buffer instead of the purification reagent.

4.4.5 DNA amplification

The DNA sample was prepared for thermocycling by using the protocol from the 'puRe *Taq* Ready-to-Go' PCR bead product handbook (GE Healthcare 2007). The disc punched from the FTA card was added to a 0.5ml microcentrifuge tube containing a 'puRe *Taq* Ready-to-Go' PCR bead. 20µl of pure grade autoclaved scientific water was then added to the tube along with 5µl of a 10bp random primer, supplied by Eurofins mwg/operon. The final volume of 25ul contains 2.5 units of puReTaq^{TM} DNA polymerase, 10mM Tris-HCl (pH 9.0 at room temperature), 50mM KCl, 1.5mM $MgCl_2$, 200µM dATP, dCTP, dGTP, and dTTP, and stabilisers including BSA. The tube was then briefly centrifuged for 5 seconds on short spin in order to ensure both the FTA Plant Card disc and the liquids were at the base of the tube.

DNA amplification was achieved through the use of the polymerase chain reaction (PCR) technique. This technique amplifies a specific DNA sequence through enzymatic replication (Moody 2007). Each strand of DNA generated is itself used as a template for further replication forming a chain reaction in which the DNA template is, theoretically, amplified exponentially (McPherson and Moller 2006). Using this method millions of copies of the target DNA sequence can be created in a matter of hours. The PCR reaction was carried out in a Hybaid Touchdown Thermal Cycler using the following cycle (after Zhu et al 2007):

Denaturation: The solution containing the DNA was heated to 94°C for 5 minutes, followed by 35 cycles of 45 seconds, again at 94°C. This melted the hydrogen

bonds holding the complementary bases of the DNA strand together resulting in two single strands of DNA.

- *Annealing:* After each 45 seconds denaturation stage the thermocycler was programmed to rapidly lower the temperature of the solution to 36°C for 45 seconds. This allowed the primer, contained in the solution, to anneal to sections of complementary bases on the single-stranded DNA template.
- *Extension/elongation:* After each annealing step the thermocycler raised the temperature rapidly to 72°C, and the DNA polymerase extended along the single stranded template DNA in the 5′ to 3′ direction, creating a new complementary strand of DNA.

Where the genotype of an organism has been sequenced, or a library has been developed, primers can be designed that complement, and will therefore attach to, specific sections of the single DNA strands. Where no sequence is known, as is the case with *C. conicum,* a PCR technique known as random amplification polymorphic DNA (RAPD) can be used. This technique uses arbitrary primer sequences, usually about 10-20 base pairs long, which act as both forward and reverse primers (Allendorf and Luikart 2007). The primer will bind to sites along the genome where it encounters a sequence of complementary base pairs. However, forward and reverse sites must be less than approximately 2000 base pairs apart for amplification to take place (Hughes and Moody 2007). The primers will usually bind to a number of different regions of the genome and therefore a variety of sized DNA fragments will be amplified. These fragments appear as bands when separated on an agarose gel using electrophoresis (McPherson and Moller 2006). The relative lengths of the amplicons (pcr products) will in turn determine the distance it travels through the gel (Moody 2007). If the complementary primer sequences of the genome vary from that of the primer, then annealing is unlikely to occur and this genomic sequence is not amplified, resulting in an absent band for that sample (Newton and Graham 1997, McPherson and Moller 2006, Moody 2007, Frankham et al. 2010).

The product of the PCR amplification was analysed using gel electrophoresis in an eight well plate tank using a standard protocol (Ausubel et al. 2002). 10µl of the product was mixed with 1µl of loading dye and then pipetted into wells in a 1.5%

agarose gel which was stained with ethidium bromide. The gel electrophoresis was run at 80v for 1 hour in 1 x TBE buffer. Samples were selected that gave clear banding patterns and the whole process was repeated to ensure that the banding patterns produced were reproducible. Initially approximately 40 samples from each study site were analysed. Samples that produced banding patterns that were not reproducible were discarded. Once samples were identified that produced reproducible banding patterns, the remaining 15µl left from the PCR reaction was frozen. This was repeated until 12 working samples from each population had been collected. To analyse the RAPD results the set of 48 samples for each site were defrosted at room temperature for 1hr. 2µl of loading dye was added to the tube containing each sample. A 25 well plate tank was used for the gel electropheresis; the gel was double combed and was therefore able to hold two rows of samples. In this way, all 48 samples for each primer (12 samples for each population) could be run at the same time on one large gel. 15µl of each sample was pipetted into each well with the exception of the middle lane of both rows. Molecular weights were estimated using Fisher Bioreagents exACTGene 100bp PCR DNA ladder, 10µl of which was added to the middle lanes that had been left empty. The gels were photographed and observed in an Alpha Innotech Fluorchem Multi Image Light Cabinet.

4.4.6 Data analysis

RAPD amplified fragments that had the same mobility, and therefore the same length (bp), were manually scored for presence (1) or absence (0) of bands. Only products with bright, clear bands were scored as being present.

The computer programme Popgene version 1.32 (Yeh and Yang 1999) was used to carry out a range of analyses on the presence/absence spreadsheet in order to examine levels of genetic diversity. The program was used to calculate the percentage of polymorphic loci (PPL) within each population. The PPL represents the percentage of loci that have more than one allele per locus.

One of the primary effects of the subdivision of populations in terms of genetic diversity is that observed heterozygosity (H) is reduced, through genetic drift, in comparison with the expected H.

Wright (Wright 1921) attempted to quantify levels of inbreeding resulting from subdividing populations of cattle. His method has come to be termed the fixation index. The index is a measure of the loss of heterozygosity through genetic drift, as populations become more subdivided. The fixation index is abbreviated as an F with subscripts that indicate what level of subdivision is being examined: F_{IS}, also known as the Inbreeding Coefficient, attempts to measure the mean loss of H for an individual resulting from non-random mating within a subpopulation. F_{IT} is a measurement of the average loss of H for an individual in relation to the population as a whole. The most inclusive measure of the subdivision of populations is the fixation index F_{ST} (Hartl and Clark 2007). F_{ST} measures the mean loss in H within a subpopulation, in relation to the total population, resulting from genetic drift among subpopulations. The F_{ST} value for the subdivided populations of *Conocephalum conicum* was calculated using Arlequin v.3.5 (Excoffier and Lischer 2010).

There are a number of alternative methods of estimating F_{ST}, but the most commonly used equation is (Frankham et al. 2010);

$$Fst = \frac{\text{var (p)}}{p(1-p)}$$

(p = the mean frequency of the alleles)

The variance in p is standardized by the maximum variance over the subpopulations. If the subpopulations all have the same allele frequencies then both var (p) and F_{ST} will be zero (Weir 1996, Frankham et al 2010).

Another measurement of the levels of heterozygosity within and between populations, based upon allele frequencies, is Nei's coefficient of gene variation known as G_{ST} (Nei 1973, 1978):

$$G_{ST} = D_{ST}/H_T$$

(D_{ST} is the average gene diversity among subpopulations and H_T is the gene diversity in the total population)

Popgene was used to work out Nei's coefficient of gene differentiation (G_{ST}). Nei (1978) states that where there are only two alleles at a locus, as was the case for the RAPD analysis, G_{ST} is equivalent to F_{ST}. However, because the algorithm used to calculate these values is slightly different, the value produced for G_{ST} by Popgene and that produced for overall F_{ST} by Arlequin will be similar, but not exactly the same. Therefore both G_{ST} and F_{ST} fixation indices have been included.

The loss of genetic diversity caused by the isolation of populations can be substantially reduced, or even negated, by the introduction of individuals from other genetically distinct populations (Freeland 2005), each individual that joins the population is known as a migrant. Once the G_{ST} value had been calculated it was then possible to estimate the level of genetic flow between haploid populations using the equation:

$$Nm = \left(\frac{1}{2}\right)\left[\left(\frac{1}{Gst}\right) - 1\right]$$

(N_m = number of migrants per generation).

Sewall Wright found that one migrant per generation was adequate to prevent complete differentiation of laboratory populations irrespective of population size (Wright 1969, Frankham et al. 2010).

Nei's genetic identity (*I*) values are used to look at the genetic similarity of populations based on the proportion of alleles the populations have in common.

The values were calculated for the sample sites using the following equation (Weir 1996):

Equation for Nei's genetic identity; $I = \frac{\tilde{q}_{12}}{\sqrt{\tilde{Q}_1 \tilde{Q}_2}}$

(*q* = proportion of alleles that are alike between the subpopulations, *Q* = proportion of alleles that are alike within the subpopulations).

Genetic identity values range from 0 to 1. If the allele frequencies of two populations are similar then the value for *I* will approach 1, whilst two completely dissimilar populations, with no common alleles, would have a genetic identity value of 0 (Freeland 2005). The genetic identity values were then used to estimate genetic distance values. Nei's genetic distance (*D*) values provide a quantitative measure of genetic divergence between populations (Nei 1972, 1973, 1978). Genetic distance measures the differences in locus-specific allele frequencies for two or more populations (Jin and Chakraborty 1994). The mean genetic distance of several individual alleles is then used to calculate an overall genetic distance. Values for *D* range from 0 to infinity. Measurements of *D* for populations with similar allele frequencies will be approximately 0, and for populations with no alleles in common *D* will be infinite (Weir 1996). Nei's genetic distance assumes that genetic differences occur due to both genetic drift and mutations, whereas the coefficient of gene differentiation (G_{ST}) assumes genetic divergence of populations is solely the result of genetic drift.

Equation for Nei's genetic distance; $D = -ln(I)$

(*ln* = natural logorithm)

Arlequin v.3.5 was used to examine the genetic variability and structure among populations through the analysis of molecular variance (AMOVA) producing pairwise F_{ST} values for the populations (Excoffier et al. 1992). Although not originally designed for use with RAPD analysis, AMOVA has proved successful in many other similar studies (Julio et al. 2008, Lambertini et al. 2008, An et al. 2009, Hend et al. 2009, Katsiotis et al. 2009, Bobes et al. 2010).

4.5 Results

Zhu et al (2007) listed 12 random primers that they used successfully with the moss *Brachythecium rivulare.* These were tested initially on four randomly selected samples of the liverwort *Conocephalum conicum* and four primers were selected for their distinct and reproducible banding patterns. Primer sequences for the primers chosen are shown in Table 4.4.

Primer	Sequence (5' – 3')	No. of loci	No. of polymorphic loci
S4	GGACTGGAGT	17	9
S5	TGCGCCCTTC	11	11
S10	CTGCTGGGAC	12	7
S1091	GTCACGTCCT	15	7

Table 4.4 RAPD primer sequences and the bands generated from the 48 samples of *C. conicum.*

Reactions were conducted with all 48 samples and the banding patterns generated for each primer are shown in Figures 4.8, 4.9, 4.10 and 4.11. The first 12 lanes in the top left section of each gel are the samples from Marline Valley. The last 12 lanes in the top right section are the samples from Fore Wood. The 12 lanes in the bottom left are from Fairlight Glen and the 12 in the bottom right are from Brick Kiln Wood. The selected primers produced 55 unambiguous bands of which 34 (62%) were polymorphic across all four populations.

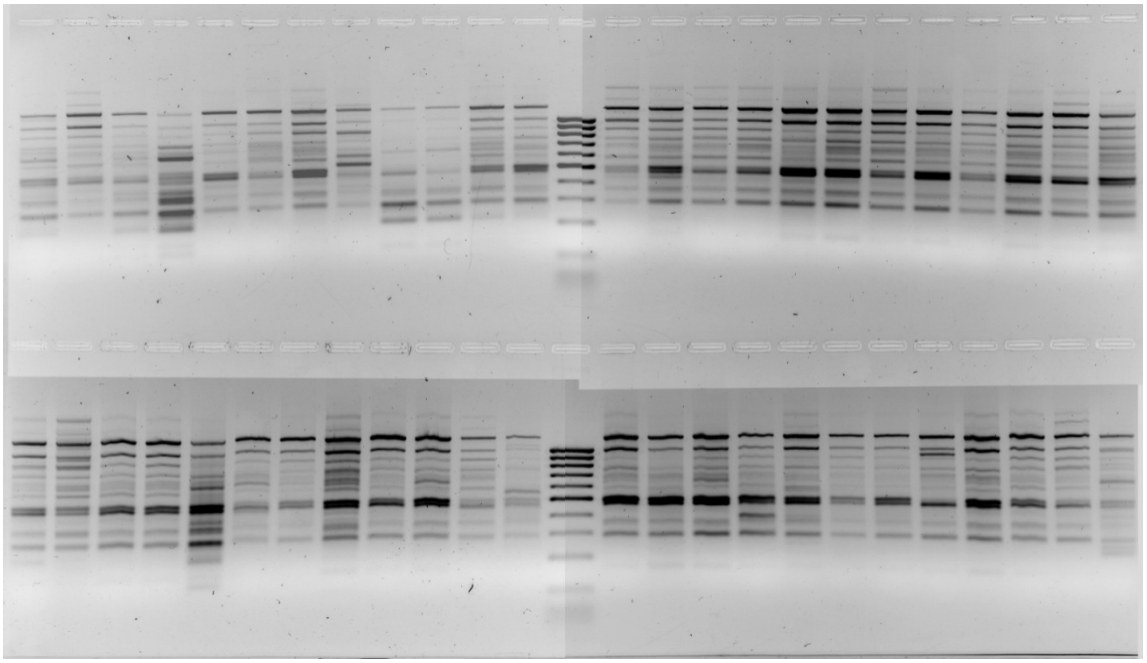


Figure 4.8 RAPD banding patterns produced by primer S4 for samples from all survey sites.

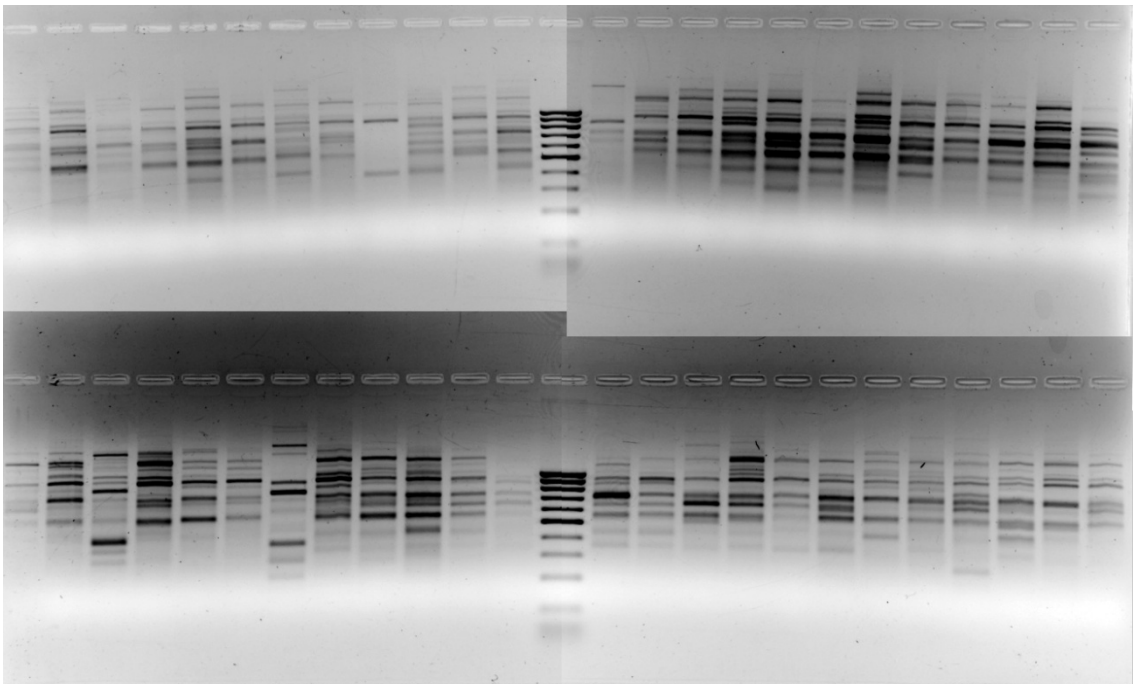


Figure 4.9 RAPD banding patterns produced by primer S5 for samples from all the survey sites.

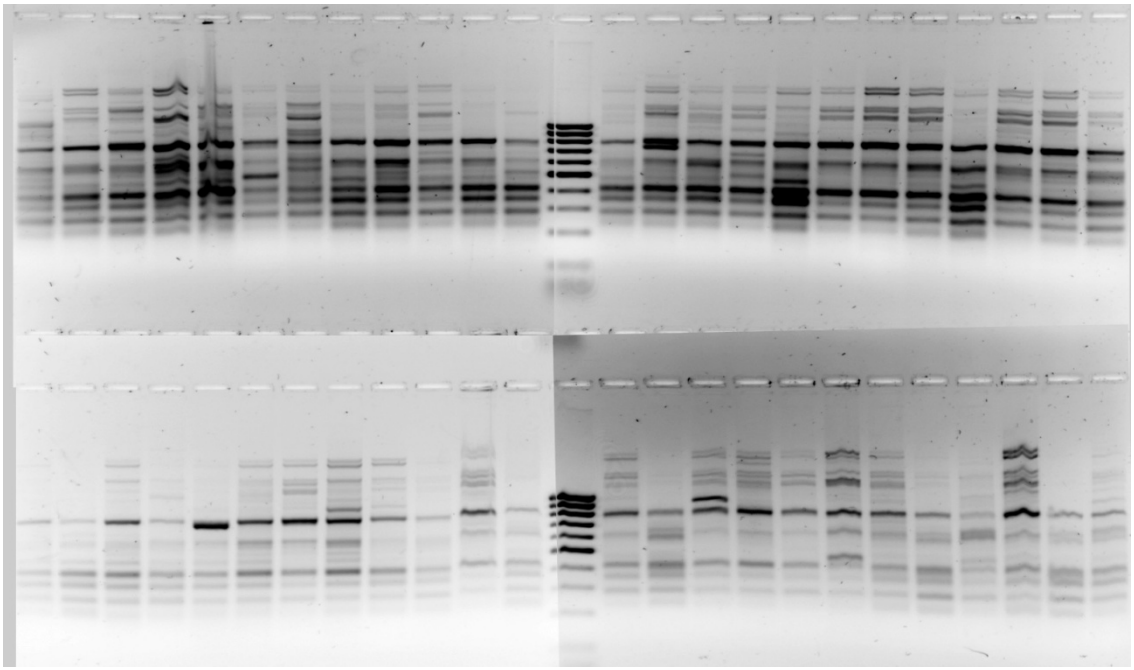


Figure 4.10 RAPD banding patterns produced by primer S10 for samples from all the sites.

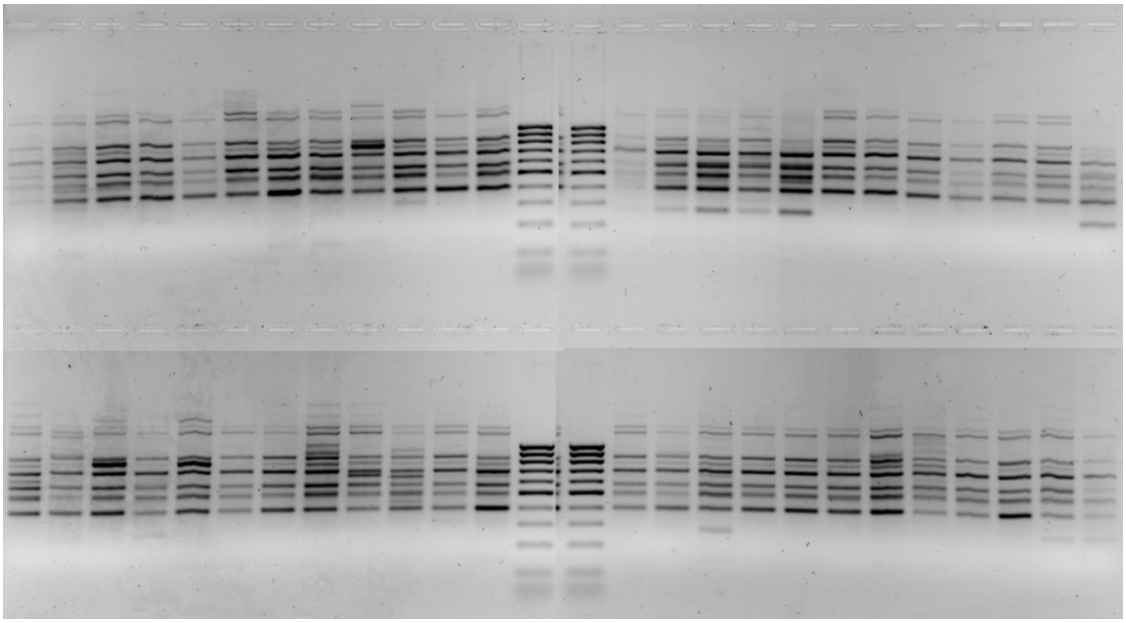


Figure 4.11 RAPD banding patterns produced by primer S1091 for samples from all the survey sites.

Table 4.5 shows the results of a number of RAPD studies looking at the genetic diversity of various bryophyte species. This study of the ghyll woodlands species *C. conicum* is shown on the first line of the table. In the various studies the percentage of polymorphic loci for a single population ranged from 9.1% for *Thuidium cymbifolium* (Wang et al. 2006) to 70.5% for the population of *C. conicum* from Fairlight Glen. The F_{ST} figure for *C. conicum* of 5.24 was the lowest recorded in the surveys shown in Table 4.4, whilst the figure of 33.1 for *Trichocolea tomentella* (Pohjamo et al. 2008) was the highest.

Brophyte species	PPL (%)	NGD	G_{ST}	Nm	F_{ST}	I	Study authors
Conocephalum connicum	44.1 -70.5	0.1099-0.1826	0.1019	4.4074	5.24	0.9669 -0.9788	Flint 2014
Brachythecium rivulare	53.3 -63.1	0.0624-0.1478	0.2168	1.8066	8.80	0.8626-0.9395	Zhu et al. 2007
Thuidium cymbifolium	9.10 -14.5	*	0.647	*	*	0.8780-0.9510	Wang et al. 2006
Trichocolea tomentella	21.1 -57.9	0.045-0.111	*	*	33.1	*	Phojamo et al. 2008
Trichocolea tomentella	26.3 -63.2	0.039-0.132	*	*	21.5	*	Phojamo et al. 2008
Trichocolea tomentella	26.3 -68.4	0.078-0.172	*	*	14.5	*	Phojamo et al. 2008
Trichocolea tomentella	*	*	*	*	19.8	*	Phojamo et al. 2008

Table 4.5 Results from previous papers studying the genetic effects of habitat fragmentation on bryophytes. * = statistic was not included in the paper. PPL = Percentage of polymorphic loci. NGD = Nei's genetic distance. G_{ST} = Coefficient of gene differentiation. Nm = number of migrants per generation. F_{ST} = The fixation index (the proportion of genetic diversity due to allele frequency differences among populations). I = Nei's genetic identity.

The level of gene flow (*Nm*), recorded in Table 4.5, was calculated at 4.4074 migrants per generation. This was done by manipulating the relationship:

$$Gst = \frac{1}{1 + (4Nm)}$$

To produce the equation:

$$Nm = \left(\frac{1}{2}\right)\left[\left(\frac{1}{Gst}\right) - 1\right]$$

The F_{ST} values produced in the AMOVA analysis (Table 4.6) show 94.76% of the total genetic variation occurred within the populations and 5.24% was explained through genetic variation between the populations. These values were both highly significant ($p<0.001$).

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation (F_{ST})	*P* value
Between populations	3	13.604	0.15078	5.24	<0.001
Within populations	44	119.917	2.72538	94.76	<0.001

Table 4.6 Hierarchical analysis of molecular variance (AMOVA) of the *Conocephalum connicum* populations.

The percentage of polymorphic loci (PPL) measured within the ghyll populations ranged from 44.1% at Brick Kiln Wood to 70.5% at Fairlight Glen (see Table 4.7). Nei's genetic distance (D) ranged from 0.1099 at Fore Wood to 0.1826 at Marline Valley, with an average value of 0.1636.

	Sample Size		na	ne	NGD	No. polymorphic loci	Percentage polymorphic loci (PPL)
Fairlight Glen	12	Mean	1.7059	1.2695	0.1793	24	70.5%
		St. Dev	0.4625	0.2849	0.1551		
Marline Valley	12	Mean	1.6471	1.2812	0.1826	22	64.7%
		St. Dev	0.4851	0.2931	0.1683		
Fore Wood	12	Mean	1.5882	1.1469	0.1099	22	58.8%
		St. Dev	0.4996	0.1924	0.1155		
Brick Kiln Wood	12	Mean	1.4412	1.1795	0.1160	15	44.2%
		St. Dev	0.5040	0.2812	0.1591		
All Sites	48	Mean	2.0000	1.2343	0.1636		
		St. Dev	0.0000	0.2578	0.1315		

Table 4.7 Measures of genetic diversity within the survey sites (Popgene v.1.32). na = observed number of alleles. ne = effective number of alleles (Kimura and Crow 1964). NGD = Nei's (1973) genetic distance.

Population pairwise analysis (Table 4.8) showed that the genetic identity (*I*) among the four populations ranged from 0.9669 between Marline Valley and Fore Wood, to 0.9788 between Fairlight Glen and Fore Wood.

	Fairlight Glen	Marline Valley	Fore Wood	Brick Kiln Wood
Fairlight Glen	-	0.9761	0.9788	0.9750
Marline Valley	0.0242	-	0.9669	0.9751
Fore Wood	0.0214	0.0337	-	0.9749
Brick Kiln Wood	0.0253	0.0253	0.0254	-

Table 4.8 Nei's (1978) original measures of pairwise genetic identity (*I*) (above diagonal) and pairwise genetic distance (*D*) (below diagonal) between the *C. conicum* populations (Popgene 1.32).

The mean *I* value for all sites was 0.9745. A value of 1 for genetic identity (*I*) would represent a pair of subpopulations that are genetically identical. The values obtained through this analysis indicate that the subpopulations are all relatively similar genetically. Genetic distances (*D*) between populations ranged from 0.0214 between Fore Wood and Fairlight Glen, to 0.0337 between Fore Wood and Marline Valley, with a mean genetic distance for all sites of 0.0259 (Table 4.8).

Values for *D* are close to 0 indicating that the subpopulations have similar locus-specific allele frequencies and again this indicates the subpopulations are genetically very similar. The *D* values for these subpopulations were considerably smaller than the *D* values obtained by Zhu et al. (2007) for *B. rivulare* who recorded values that ranged from 0.0624 to 0.1478 (see Table 4.4).

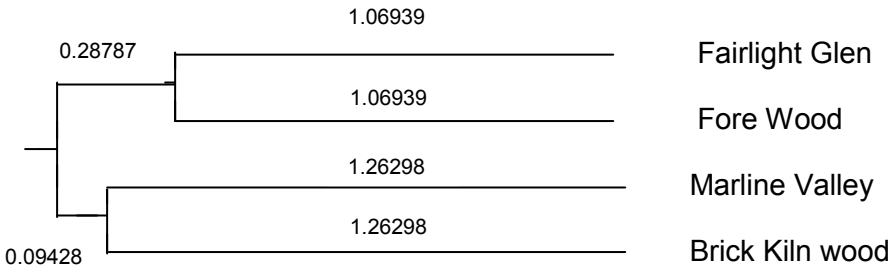


Figure 4.12 Dendrogram generated by UPGMA based on Nei's (1978) Genetic Distance showing the relationships among the four populations of *C. conicum.* (Popgene 1.32)

UPGMA cluster analysis was used to create a dendrogram based on the Nei's genetic distance results from Table 4.8. The dendrogram (Figure 4.12) represents the relationships between the populations. The four populations were clustered

into two groups with Fairlight Glen and Fore Wood in one group and Marline Valley and Brick Kiln Wood in the other.

A Mantel test was carried out to examine the relationship between genetic distance and geographic distance (Table 4.9) (Mantel 1967, Escudero et al. 2003, Telles et al. 2007, Fatemi and Gross 2009).

Mantel two-tailed test	
r(AB)	0.060
p-value (two-tailed)	0.982
alpha	0.05

Table 4.9 Testing the relationship between genetic and geographical distance using a Mantel test. The Mantel test used the genetic distance values which were calculated in the Popgene analysis (see Table 4.8) and used the geographic distance measurements from Table 4.2 as the other input data.

The null hypothesis was that there was no correlation between the matrices for genetic distance and geographical distance. As P>0.05 the null hypothesis was accepted. The risk to reject the null hypothesis while it is true is 98.2%.

4.6 Discussion

It is generally accepted that long-term habitat fragmentation, at both regional and local scales, is a primary influence on levels of genetic diversity (O'Grady et al. 2006, Taylor et al. 2007, Shao et al. 2009). Species whose distributions are geographically restricted tend to exhibit less genetic variability than more widespread species of the same genus (Gitzendanner and Soltis 2000). This study contains the first analysis of the effects that the fragmentation of the woodland within the Weald is having on the genetic diversity of ghyll woodland plant species.

The amount of variation between the four populations of *C. conicum* was analysed through examining the percentage of polymorphic loci (PPL) (Table 4.7). The PPL varied greatly between populations, with Brick Kiln Wood having a figure of only

44.12% whereas Fairlight Glen had a figure of 70.59% (Table 4.7). Fairlight Glen is a coastal community that is on the very edge of the Wealden area. There would clearly be limited geneflow entering the site from the coastal direction. However, the PPL measurements indicate that the population of the coastal community at Fairlight Glen is the most genetically diverse. Moving away from the coast and towards the Wealden interior the populations get less genetically diverse, culminating in the least diverse population of Brick Kiln Wood (Figure 4.3). The PPL figures from all of the sites indicate a high level of genetic variation within all the sub-populations, particularly within Fairlight Glen. This level of within-site variation is similar to other RAPD studies of bryophytes such as; Phojami et al. (2008) who studied sub-populations of the species *Trichocolea tomentella* in Finland, Lithuania and the UK, and reported PPL ranges of 21.1% to 57.9%, 26.3% to 63.2% and 26.3% to 68.4% respectively and Zhu et al. (2007) who studied *B. rivulare* and recorded a range of 53.3% to 63.1% (Table 4.5).

Nei's genetic diversity analysis (Table 4.7) produced a different pattern of results, with the Marline Valley community being the most genetically diverse and Fore Wood the least. Population pairwise analysis indicated that the sites Marline Valley and Fore Wood were the most genetically dissimilar, whilst Fairlight Glen and Fore Wood had the smallest genetic distance between the populations. Both of these relationships were highly significant ($P<0.001$). The UPGMA cluster analysis (Figure 4.12) clustered Fairlight Glen and Fore Wood into one group and Marline Valley and Brick Kiln Wood into another. For most species the level of geneflow between populations is negatively correlated to the distance between the populations (Freeland 2005), this is known as isolation by distance (IBD) (Wright 1969, Barrès et al. 2008, Björklund et al. 2010). To test for a correlation between the genetic and geographic distances of the sample populations a Mantel test was conducted (Freeland 2005, Ranganathan et al. 2010). The Mantel test (Table 4.9) indicated no significant relationship between the geographic distance separating individual ghyll woodlands and the genetic distance between them. This appeared to be confirmed by the pairwise genetic distances recorded in Table 4.8 and the geographic distances recorded in Table 4.2 which did not appear to be correlated. Fairlight Glen and Fore Wood, which the UPGMA analysis clustered together, are around 9.5km apart whilst Marline Valley and Brick Kiln Wood, also clustered

together, are separated by approximately 25.5km. If the genetic similarity of the sites was based purely on their proximity to each other, then one would expect the analysis to have clustered Fore Wood and Marline Valley together since they are only 1.7km apart.

In theory, F_{ST} has a minimum value of 0 (no genetic diversity) and a maximum of 1 (complete fixation of alternative alleles in different sub-populations), when the number of alleles are equal to the number of subpopulations. However, a value of 1 would only ever be possible when analysing two sub-populations since all individuals with one allele would be in one subpopulation and all individuals without the allele would be in the other subpopulation. With >2 subpopulations some of the subpopulations will have identical alleles at fixation, so the maximum theoretical value may only be 0.5 (Hartl and Clark 2007).

Wright (1978) suggested that G_{ST} and F_{ST} values should be interpreted using the following guidelines;

- 0 to 0.05 indicates little genetic differentiation
- 0.05 to 0.15 indicates moderate genetic differentiation
- 0.15 to 0.25 indicates great genetic differentiation
- Above 0.25 indicates very great genetic differentiation

The value calculated for G_{ST} was 0.102 (see Table 4.5) and for F_{ST} was 0.052 (Table 4.6). The G_{ST} value produced indicates around 10% of the genetic variation existed among the populations and around 90% of the variation exists within the populations. The figures indicate that there is a moderate amount of genetic variation between the subpopulations of *C. conicum*. Although the subpopulations of the liverwort appear separated in terms of their geographic locations, they may still be exchanging genetic material. Dispersal facilitates geneflow and therefore reduces the genetic impact of habitat fragmentation. If the level of dispersion and subsequently the level of geneflow are large enough, then separate subpopulations can effectively be viewed genetically as a single large population of the total size of all the sub-populations involved (Levins 1970, Beebee and Rowe 2004, Frankham et al. 2010). Wright (1969) found that among idealized populations 1 migrant per generation was sufficient to prevent fixation of alleles.

The level of genetic diversity calculated in this study was consistent with 4.4 migrants per generation entering the sampled population. Despite Wright's findings for idealized populations the figures for F_{ST} and G_{ST} in this study do indicate that gene-flow is hindered in some way and that these populations are displaying the effects of genetic drift.

Although situated in the Wealden interior, the PPL analysis, recorded in Table 4.7, indicates that the Brick Kiln Wood subpopulation appears to be the most affected site in terms of genetic drift. The PPL figure of 44.2% indicates it is the most homogenous of the populations. Nei's genetic diversity (NGD) value calculated for Brick Kiln Wood (Table 4.7) is also relatively low; however the figure for Fore Wood is the lowest of all the sites. The figures for PPL and for NGD indicate that both Marline Valley and Fairlight Glen would appear to be the sites least affected by genetic drift.

So far levels of genetic diversity within and between the populations of *C. conicum* have been analysed using estimates of geneflow based on genetic distance values and by using frequency measurements of polymorphic loci. The next stage was to attempt to identify the causes of the level of geneflow identified through the genetic distance analysis. Environmental variables were recorded for the sites as a component of other parts of the study. However, it was felt that the number of sites surveyed was too low for correlation analysis to be reliable.

Habitat isolation has been put forward as the primary factor causing the levels of genetic drift found in these fragmented populations. However, it is difficult to separate the genetic effects of habitat isolation per se. from a number of other processes that occur as the woodlands surrounding the ghylls gets smaller. As the woodlands become more isolated the processes within each ghyll will inevitably change and this in itself will produce selection pressures that are separate from genetic drift. For instance, it has been discussed already that the ghylls have become increasingly isolated as a result of changes in surrounding land-use. Often the ghyll valleys have been left untouched and wooded because the steep sides and boggy ground within the ghylls would have made cultivation difficult and uneconomic (Rose and Patmore 1997, Burnside et al 2002a, Burnside et al 2006). In many cases the land surrounding the ghylls has been cultivated up to the edge

of the ghyll valleys. Table 4.3 includes information regarding the surrounding land use for each ghyll. Although all four of the ghylls surveyed have broadleaf or mixed woodland adjacent to them somewhere along their length, they are all also bordered by improved grassland at other points. Brick Kiln Wood is also bordered by unimproved grassland, Marline Valley by heathlands, Fore Wood by heathlands, a garden and a road, whilst Fairlight Glen is also bordered by heathland, arable land and improved grassland. At points therefore the linear nature of the ghylls themselves has meant that the ghyll woodland forms a thin strip of woodland bordered by a differing land use type.

The genetic diversity of isolated subpopulations will change at different rates as a result of differences in site-specific selection pressures within those populations. Fairlight Glen, for instance, is a coastal site and may therefore be more humid and suffer fewer periods where the subpopulation becomes dessicated. It may also have higher levels of ground and airborne salinity. It is therefore extremely difficult to accurately separate out the influence of selection from the effects of random genetic drift when employing markers of unknown effect (Speiss 1977).

Rising regional temperatures, resulting from climatic changes, are likely to become an increasingly important factor determining the distribution of flora both locally and nationally (Kelly and Goulden 2008, Engler and Guisan 2009). Poikilohydric species, such as the thalloid liverworts associated with ghyll woodlands, are sensitive to changes in ambient humidity (Vanderpoorten and Goffinet 2009). Increasing regional temperatures may also have an indirect effect on ghyll species through drying out smaller areas of damp habitats that may be acting as ‘stepping-stones’ allowing gene-flow between ghyll woodlands (Keitt et al. 1997). It would seem likely that increasing regional temperatures would in turn increase the genetic isolation of these species through the loss of these ‘stepping stone’ habitats (Keller et al. 2010, Lindenmayer et al. 2010).

4.7 Conclusion

Maintaining genetic variation is a major goal for the conservation of rare and threatened species (Luan et al. 2006). Measurements of genetic variation within, and between populations represent an important measurement of the extent of habitat fragmentation and genetic isolation (Milligan et al. 1994).

C. conicum is widely distributed in ghylls and therefore the application of DNA fingerprinting of this species provides a simple tool for examining the degree of isolation of individual ghyll woodlands. Where conservation or restoration efforts are already being targeted at specific ghylls this type of analysis would reveal the importance of directing some of this effort into increasing geneflow. This may be through creating wildlife corridors, buffer zones or through restoring 'stepping-stone' woodland habitats in the surrounding habitat matrix to facilitate geneflow between genetically isolated ghyll woodlands.

During this analysis many of the samples failed to produce banding patterns at all, possibly due to problems with the DNA extraction, and others produced unstable banding patterns that were not reproducible. Limitations in terms of both funding and time meant that it was therefore only possible to analyse samples from four separate ghyll woodlands. This meant some potentially very useful and interesting analysis was not carried out purely on the basis that the limited number of survey sites would have meant it was unreliable. It is therefore recommended that future surveys of this nature should include a greater number of survey sites to allow analysis to be undertaken using the landscape and environmental variables available for these sites. It is also recommended that future attempts to analyse genetic diversity, using this method, should include some form of abundance analysis during the initial surveys. This would attempt to ensure that population size is eliminated as a possible causal variable influencing levels of genetic drift.

5 General Discussion

The study presented here has concentrated on flowering plant, bryophyte and fern species since it is the presence of rarer species belonging to these taxonomic groups that make the Wealden ghyll woodlands important in terms of regional biodiversity (Ratcliffe 1968, Rose and Patmore 1997). Bryophytes are relatively small plants that tend to be difficult to differentiate, often requiring microscopic examination for identification; hence the recording of bryophytes has been carried out far less frequently than the recording of vascular plant species (Hodgetts 1992). Previous studies that have attempted to examine and characterise the Wealden ghylls have neglected to include this bryophyte community (e.g. Burnside et al. 2002b, Burnside et al. 2006, Waite et al. 2010). This study therefore denotes the first formal attempt to characterise and differentiate ghyll woodlands using bryophyte richness, abundance and composition in addition to vascular plant species.

5.1 The identification of ghyll woodland plant communities

The analysis in Chapter 2 represents the first attempt at identifying ghyll woodland communities on the basis of species richness, composition, and distributions. The study showed that bryophyte and flowering plant species richness was significantly higher within the ghyll woodlands than in the other ancient woodlands surveyed within the region. Therefore ghyll woodlands can be distinguished from other ancient woodlands in the region on the basis of the high levels of bryophyte and flowering plant species richness. The rich diversity of species recorded within the ghyll woodlands indicates that these sites may be important both for individual plant species and also in terms of regional biodiversity.

NVC classifications assigned on the basis of species composition indicated that, with the exception of sandstone ancient woodlands, the three classifications assigned to each site were W8, W9 and W9a (typical sub-community). The

classifications W9 and W9a communities consist primarily of moisture loving species that are associated with the mild, damp, humid Atlantic woodlands on the west coast of Britain where they grow adjacent to streams (Rodwell 1991). They are not species that are generally associated with woodlands in the warm, dry south-east of England (Rodwell 1991).

Based on surveys of 48 ghyll woodlands, Burnside et al. (2006) found 47% predominantly consisted of W10 woodlands and 43% were dominated by W8 woodlands. The remaining 10% consisted of various woodland types that did not include either W9 or W9a woodland. A survey of ancient woodland throughout the Weald (Westaway 2006) also found the main woodland types were W8 and W10 woodlands. However, the NVC classifications in both these reports were based on datasets that contained vascular species but did not include bryophytes. This reiterates the importance of including bryophytes in any attempt to examine the community composition of the Wealden ghyll woodlands. However, the W9, W9a and W8 classifications assigned to the ghyll sites was also assigned to two-thirds of the ancient woodland sites (Table 2.6). If the NVC classifications assigned to the ghyll woodlands in Chapter 2 are the result of the abundance of oceanic species within the ghyll woodlands, then the NVC classifications assigned to the surrounding ancient woodlands (W9, W9a and W8) indicate that oceanic species were also widely found in these woodlands too. The apparent similarity in species composition between ghyll woodlands and the surrounding ancient woodlands indicates that the woodlands cannot be differentiated from each other on the basis of NVC classifications.

A further aim of Chapter 2 was to identify the environmental/ecological factors responsible for patterns of species richness, distributions and abundance within the ghyll woodlands. Burnside et al. (2006) used cluster analysis to examine geological components of the ghyll woodlands and found sub-groupings with strong associations which reflected the presence of ghylls on substrates classified as predominantly clay or sandstone. Based on their findings the analysis in Chapter 2 of this report compared the richness and composition of vascular and non-vascular plant communities from ghyll woodlands located on clay and sandstone substrates. The results indicated that flowering plant, bryophyte and fern communities occurring in sandstone and clay ghyll woodlands differed

significantly from each other. Substrate specificity was particularly strong within the fern species group. Indicator Species Analysis (ISA) identified 10 species whose presence was indicative of sandstone ghyll woodlands and five of these indicator plants were fern species (Table 2.8). A number of studies have shown that ferns generally tend to be highly substrate specific (e.g. Tuomisto and Ruokolainen 1994, Tuomisto and Poulsen 1996). Young & Leon (1989) studied 61 fern species and fern allies along an edaphic gradient in an Amazonian rain forest. They found 43 of these species were exclusive to either clay or sandy-loam soils. The correlation of fern species and soil types is so strong that in another rainforest study, Tuomisto & Ruokolainen (1994) reported an almost complete turnover in fern species at a soil-clay transition. Some ghyll woodlands also traverse boundaries between sand and clay geologies and at these transition zones an abrupt change in vegetation occurs (Rose and Patmore 1997). General linear modelling (GLM) identified the distributions of fern species as being strongly correlated with substrate pH (Table 2.12). This would explain the separation of fern species on the basis of ghyll substrate, with individual species primarily associated with either the highly acidic sandstone ghyll woodlands or the less acidic clay ghyll sites (Table 2.14).

Bryophytes appear to be the most important species group for identifying the presence of clay ghyll woodlands contributing five of the eight indicator species identified through ISA (Table 2.8). The bryophytes identified as indicators of clay ghyll woodland are all intolerant of highly acidic substrates (Atherton et al. 2010) and this may explain their relatively low occurrence within the sandstone sites (Table 2.10). Overall, the analysis indicated that species composition is influenced by substrate and that substrate specific sub-communities can be used to identify and separate sandstone and clay ghyll woodlands.

The rich species assemblages associated with the Wealden ghylls have been widely attributed to the presence of a shaded, humid microclimate within the ghyll valleys (e.g. Ratcliffe, 1968, Rose, 1995, Patmore, 2000, Burnside, 2006). Analysis of environmental measurements recorded within the valleys did not identify any microclimatic variables as determinants of ghyll species richness. Chapter 2 therefore found no direct physical evidence that the rich species assemblages associated with the Wealden ghyll woodlands are the product of

microclimatic conditions within the valleys. It is however possible that atypical weather conditions occurring during the survey period may have affected the results of this analysis. The surveys were carried out during the summer of 2012 which Met Office data shows to be the wettest summer for 100 years (Met Office 2013). The humid ghyll microclimate is primarily due to the presence of streams within the steep sided ghyll valleys (Rose and Patmore 1997). Although surveys were never carried out during rain events, it is accepted that soil-moisture levels are likely to have been unseasonably high during this period of high precipitation. In combination with warm summer temperatures this may have produced high levels of relative humidity throughout all of the woodlands irrespective of the presence of running water. If relative humidity levels were similar throughout the survey sites then it would be unlikely that humidity would be identified as a predictor of species richness.

5.2 Investigating temporal changes in the ghyll woodland plant communities

Chapter 3 contains the first temporal study of changes in the richness and composition of Wealden ghyll woodland bryophyte, flowering plant and fern communities. The chapter is also the first study to utilise the Dr Francis Rose Notebook data which contains the largest known dataset of ghyll woodland vegetation surveys carried out by a single individual.

The analysis in Chapter 2 indicated that the ghyll woodlands could be differentiated from other woodlands within the region on the basis of the significantly higher species richness of ghyll woodland plant communities (Table 2.4). Chapter 3 extended the analysis to examine species richness through time and found no significant change in species richness over the course of the study period. Although species richness appeared stable, the analysis indicated community composition had changed significantly during the study. The ratio of ghyll woodland oceanic bryophytes, a plant group requiring mild, humid, shaded conditions (Ratcliffe 1968), increased over the survey period, as did another group of bioindicator species associated with shaded woodlands; ghyll woodland

flowering plant AWI species (Hermy et al. 1999) (Table 3.4). The increase in the ratio of these shade tolerant species indicates that light levels at the woodland floor appear to have decreased over the survey period. Analysis of Ellenberg indicator values for light appears to confirm this with an increase in the ratio of shade tolerant bryophyte and flowering plant species recorded over the course of the survey period (Table 3.6 & 3.7). The ratios of oceanic bryophytes and flowering plant AWI species in the ancient woodland sites did not change significantly over the course of the survey (Table 3.4 & 3.5), indicating that the increase in these bioindicator species was limited to the ghyll woodlands. The analysis of Ellenberg light values associated with the oceanic bryophytes showed that the significant increase in the ratio of shade-tolerant species within ghyll woodlands did not occur in the ancient woodland sites, but instead the ancient woodlands appear to have suffered a significant decrease in shade tolerant oceanic bryophyte species during the post-war period (Table 3.9 and Figure 3.2a & 3.2d). This analysis indicates that ghyll woodlands appear to be important habitats within the region for the conservation of shade-tolerant oceanic bryophyte species, a plant group that a number of authors have highlighted as being particularly important in terms of regional biodiversity (e.g. Ratcliffe 1968, Rose 1995, Burnside et al. 2006 etc.). The post-war period saw the large-scale abandonment of coppice management throughout the Weald (Forestry Commission 2004) and the increase in the ratio of shade tolerant bryophyte species would be consistent with increased canopy density associated with abandoned coppice and a move towards a high forest woodland type (Wohlgemuth et al. 2002). The increase in abundance of both oceanic bryophytes and flowering plant AWI species during a period of increasing light and temperature (Jenkins et al. 2007, UKCIP 2013) may indicate that changes in environmental variables associated with the canopy closure of abandoned coppice are counteracting the negative effects of climate change for these plant groups. A number of authors have stated that the Wealden ghyll valleys have a ‘buffering effect’, sheltering ghyll vegetation from temperature and humidity fluctuations and creating a ghyll microclimate indicative of Atlantic forests on the Western seaboard (Rose 1995, Rose and Patmore 1997, Burnside et al. 2006), however prior to this study, microclimatic conditions within the ghylls had not been scientifically investigated. The analysis in Chapter 2 of this report failed to find any evidence of

a ghyll microclimate. However, the increase in the ratio of shade-tolerant, moisture-loving species is certainly indicative that a microclimate does exist within the Wealden ghyll valleys. The results of this study indicate ghyll woodlands may be acting as refuges for species unable to survive climatic changes occurring within the surrounding woodlands. Mean annual temperatures in the south-east rose by 1.7°C between 1961 and 2006 (UKCIP 2013). This rise however, is relatively small-scale in comparison with the changes predicted to occur over the course of the present century (Section 3.2.4.1). It is predicted that by 2080 mean winter temperatures will rise by up to 3.7°C whilst summer temperatures will rise by as much as 6.5°C (UKCIP 2013). It is unclear what effect climatic changes on this scale will have on rarer species within the regions woodlands, many of which have relatively narrow climatic niches. If the sheltered ghyll valleys afford protection for woodland plants suffering deleterious effects from climate change then the ghyll woodlands may become even more valuable within the region in future years, providing refuges for regionally important species threatened by changing climatic conditions.

A consequence of woodland fragmentation is usually an increase in the ratio of woodland 'edge' habitat. Therefore, not only will a fragmented habitat usually cover less area than it did pre-fragmentation, but the ratio of area representing 'edge' habitat will also increase at the expense of 'interior' habitat (Laurance 2004). The contrast in microclimatic conditions between this woodland edge 'ecotone' and the conditions within the forest interior will be heavily influenced by climatic conditions associated with the surrounding habitat type. To examine the impact of 'edge effect' on species richness within the fragmented ghyll woodlands, the relationship between ghyll species richness and proximity to the woodland edge was examined, however no evidence of a relationship was found. The degree of similarity between the land-use within a habitat fragment and the land-use in the matrix adjacent to that fragment may strongly influence the flow of materials, nutrients and energy, and may therefore affect the persistence of plant and animal species in the habitat fragment itself (Stamps et al. 1987, Forman 1995, Collinge 1996). Martin et al. (2006) investigated the extent to which surrounding land-use influenced bird assemblages within linear riparian habitats. They concluded that landscape context was an important determinant of the

relative abundance of approximately half the bird species examined. Similar results have been revealed in other studies examining floral assemblages occurring within fragmented woodland habitats (Paine and Ribic 2002, Murphy and Lovett-Doust 2004). Burnside et al. (2006) examined the spatial distributions of the ghyll woodland sites and found that over 85% lay within larger ancient woodlands. It appears likely that the relative similarities in physical structure and climate between the ghyll woodlands and the surrounding ancient woodlands is to some extent protecting the ghyll woodlands from many of the physical changes associated with edge effect (see Section 3.1.4.2). If the rich floral assemblages within the ghyll woodlands are being preserved partly as a result of the presence of an ancient woodland 'buffer' which surrounds the majority of ghyll woodland patches then any measures aimed at preserving ghyll woodland plant communities may have limited value unless this woodland buffer is also preserved. This may be particularly important for sites containing rarer ghyll woodland plant species or communities that are particularly sensitive to microclimatic changes (Ratcliffe 1968).

The surveys carried out for Chapter 2 of this study indicated that ghyll woodland vegetation was restricted to the ghyll valleys themselves. However the ghyll woodland boundaries created for the digital map used for the analysis in Chapter 3 included woodland beyond the valley boundaries and therefore could not be used to produce accurate area figures for the woodlands. Since ghyll woodlands are linear features (Burnside et al. 2006) ghyll length is likely to be correlated to area and so ghyll length was used as a proxy variable to examine the relationship between species richness and ghyll fragment size. The relationship between ghyll woodland length and species richness was examined for both of the two time periods 1951-1970 and 1976-1995. Analysis of the bryophyte group revealed no significant correlation between species richness and ghyll length for the period 1951-1970, but species richness was positively correlated with length for the period 1976-1995 (Table 3.9). One of the most widely encountered ecological patterns is the species-area relationship (see Section 3.2.3.1) (MacArthur and Wilson 1967). The pattern of increasing bryophyte species richness in relation to increasing area was therefore not unexpected. However, the lack of a species area relationship during the 1951–1970 survey period was more surprising. It is

possible that woodland fragmentation had occurred relatively recently prior to the first survey period and losses of species had not had time to occur before the first set of surveys took place. If that is the case then the rapid generation times of bryophyte species would explain the presence of a species-area relationship for bryophytes but not for flowering plants during the second period of surveying (Table 3.9).

The analysis of plant indicator values showed an increase in the ratio of species with larger seeds as well as an increase in species associated with many habitat types and a decrease in species associated with few habitats (Table 3.6 & 3.7). Indicator values for dried seed size and number of substrates are both variables that are 'indicative of the effects of habitat fragmentation' (McCollin et al. 2000b). Therefore, the significant values calculated for these indicator groups may be indicative that the ghyll woodlands are suffering from the effects of habitat fragmentation.

5.3 Examining levels of genetic isolation within ghyll woodland 'patches'

The Wealden ghyll woodlands form a network of habitat patches situated within larger fragmented woodlands (Section 3.2.3). As stated above, the analysis in Chapter 3 identified significant changes in a number of variables indicative of habitat fragmentation. Chapter 4 sought to extend this analysis to identify and quantify levels of genetic isolation associated with fragmented habitats (Wright 1921). This is the first study to examine the effects of habitat fragmentation on these biologically important communities. The chapter investigated the genetic impacts of ghyll woodland fragmentation and isolation on the bryophyte *Conocephalum conicum*, a species commonly found in ghyll woodlands throughout the Weald (Ratcliffe 1968). In theory, habitat fragmentation constricts the genepool of surviving populations by reducing population size, whilst also increasing the average distance between the surviving populations (Pharo and Zartman 2007). Regional-scale changes occurring as a result of habitat fragmentation are expected to involve a decrease in local genetic variability and an

increase in genetic differentiation between populations as a result of the increased likelihood of breeding with closely related individuals (Franklin and Frankham 1998). The extent of molecular divergence within and among populations was used to examine levels of genetic isolation across the four populations. Coefficients of genetic diversity (G_{ST} and F_{ST} values) indicate the degree of differentiation among fragments and can be used to estimate levels of geneflow. Dispersal rates between populations typically reduce with distance in both plants and animals (Frankham 2010). Consequently genetic differentiation is often related to geographic distance between populations. This is known as isolation by distance (Wright 1969). For this reason, although similar studies have been carried out to assess levels of isolation in other bryophyte species, it is difficult to compare the *C. conicum* study with these other studies since they have typically been carried out over much larger areas (see Table 5.1).

Bryophyte species	**Survey area**	**G_{ST}**	**F_{ST}**	**Authors**
Conocephalum conicum	The Weald	0.102	0.052	This study
Pleurochaete squarrosa	Mediterranean basin	0.094		(Grundmann et al. 2007)
Porella canariensis	Macronesian archipelagos	0.603		(Freitas and Brehm 2001)
Plagiomnium ciliare	United States	0.248		(Wyatt et al. 1989)
Grimmia montana	Worldwide		0.320	(Vanderpoorten et al. 2008)
Sphagnum lindbergii	Norway & Newfoundland		0	(Stenøien and Såstad 1999)
Sphagnum angustifolium	Norway & Newfoundland		0.132	(Stenøien and Såstad 1999)
Sphagnum fallax	Norway & Newfoundland		0.536	(Stenøien and Såstad 1999)
Brachythecium rivulare	Mt. Qinling, China	0.217		(Zhu et al. 2007)

Table 5.1 The results of similar RAPD studies examining the genetic differentiation among fragmented populations of bryophyte species based on G_{ST}/F_{ST} values. The value is a measure of the loss of heterozygosity through genetic drift, as populations become more subdivided (Wright 1921) and is therefore an index of genetic isolation

Differences in the genetic diversity of fragmented populations are expected to be low where distances between populations are small and dispersal rates are high (Young and Clarke 2000). The ghyll woodland *C. conicum* populations would potentially fit into this category of species distributions since the fragmented ghyll woodland habitats are small but 'closely packed' within the Weald, with a mean distance from each ghyll to its nearest neighbour of only 206 metres (Burnside 2002b). *C. conicum* also produces large quantities of small, wind-dispersed spores (Vanderpoorten and Goffinet 2009) (see Figure 4.1). As has been suggested earlier, care should be taken when interpreting comparisons between the

bryophyte studies listed in Table 5.1, but it is interesting that the G_{ST} figure of 0.102 calculated for the ghyll woodland *C. conicum* populations was very similar to the G_{ST} figure of 0.094 calculated for the bryophyte *Pleurochaete squarrosa* (Grundmann et al. 2007). This is despite the fact that the populations of *C. conicum* analysed were all within 32km of each other whilst many of the survey populations of *P. squarrosa* occurred on separate oceanic islands 100's of kilometres apart and spread over an area over 2000 kilometres wide.

The values calculated for G_{ST} (0.102) and F_{ST} (0.052) in the ghyll survey (Tables 4.5 & 4.6) indicated moderate genetic differentiation between populations (Wright 1978) and a restriction in geneflow between sites. These results may be evidence that the woodland fragmentation and loss that has occurred throughout the Wealden area (Section 1.2.2) has increased the genetic isolation of ghyll woodland plant populations. *C. conicum* is a poikilohydric species and like most thalloid liverworts it is very sensitive to changes in microclimatic conditions (Vanderpoorten and Goffinet 2009). Analysis in Chapter 3 (Table 3.4) indicated that the numbers of shade-tolerant bryophyte species have been increasing in ghyll woodlands, possibly as a result of changes in woodland management. The bryophyte *C. conicum* is an example of a shade-tolerant bryophyte species and its ghyll woodland populations would be likely to benefit from a maturing canopy structure within the ghyll valleys. Smaller populations of *C. conicum* also exist in damp areas within the woodland matrix surrounding the ghylls (Atherton et al. 2010 and pers. obs.), however the increase in shade tolerant species identified in Chapter 3 was only recorded in the ghyll valleys and not in the surrounding ancient woodlands (Table 3.4). This may mean that *C. conicum* populations within the ghylls have been less affected by the negative effects of climate change than populations in the surrounding woodlands. If the populations in the adjacent woodlands are acting as 'stepping stones' facilitating geneflow between ghyll woodlands (Levins 1970), then any losses in these populations could in turn increase the genetic isolation of the ghyll woodland populations. In this case conservation efforts aimed at protecting the ghyll populations may need to involve the protection, or even restoration, of woodland populations in the surrounding habitat matrix rather than solely concentrating on bryophyte populations within the ghyll sites themselves (Lindenmayer et al. 2010).

As a general rule, the level of geneflow between populations is negatively correlated to the distance between the populations (Freeland 2005). Nei's genetic diversity analysis indicated that Marline Valley and Fore Wood were the most genetically distinct sites, whilst Fairlight Glen and Fore Wood were the most similar. A mantel test carried out to look for a correlation between the genetic and the geographic distances of the sample populations indicated that no significant relationship existed between geographic and genetic distance (Freeland 2005, Ranganathan et al. 2010). The results of the mantel test indicate that the isolation detected in the *C. conicum* populations is unrelated to distance and therefore it may be related to the ecology of the species itself. Geneflow between populations of *C. conicum* is through sperm or spore dispersal (Watson 1981a). Sperm dispersal occurs over very short distances, for most mosses this dispersal distance averages less than 10cm (e.g. Bedford 1940, Riemann 1972, Anderson and Lemmon 1974, Reynolds 1980). Very little is known about the dispersal distance of spores, but data gathered on spores of the moss *Atrichum angustatum* (Lesser Smoothcap) showed a leptokurtic distribution pattern with over 97% of all spores travelling less than 2 metres away from the parent plant and only around 1% of the spores travelling greater than 15 metres. Further avenues of geneflow between bryophyte sub-populations are through the dispersal of asexual propagules, which occurs in up to 40% of leafy liverworts in the UK (Watson 1981a), and also through vegetative spread. Very little analysis has been carried out on the dispersal distances of propagules or fragments, or the frequency with which these types of dispersals occur, but observations carried out by Miller and Ambrose (1976) suggest that distances are relatively small and that the wind dispersal of bryophyte fragments is frequent and possibly more important than the dispersal of spores for establishing new colonies (Wyatt 1985). It is possible that the genetic differences identified between spatially separated populations and the lack of geneflow between the populations is due to the tendency of *C. conicum* to spread vegetatively over short distances which would effectively restrict geneflow between more distant populations.

5.4 Conclusions and recommendations

The study indicated that environmental changes occurring over the survey period did not appear to have adversely affected the richness of the plant groups examined (Section 3.4). However, this does not mean that future climatic changes will not impact negatively on biologically important species associated with the ghyll woodlands. It is clear that many of the ghylls have been coppice managed up until the mid-20th century (Rose and Patmore 1997, Brandon 2003). The increasing density of ghyll woodland canopies in areas of abandoned coppice appears to have benefited oceanic bryophytes and flowering plant AWI species, the frequencies of both increasing over the study period.

The increased light levels and shrubby re-growth that coppicing produces has been shown to benefit many woodland plants such as the AWI species *Anemone nemorosa* (Wood anemone) and *Primula vulgaris* (Primrose) (Barkham 1992), as well as benefiting species from other taxonomic groups such as; fritillary butterflies (Warren 1984, Collier et al. 1986), breeding birds (Stuttard and Williamson 1971, Fuller and Warren 1991) and woodland invertebrates (Warren and Key 1991). Coppice woodlands are therefore considered highly valuable in terms of biodiversity (Buckley 1992, Fuller et al. 1993) and as such a number of authors have suggested that a reintroduction of coppicing within the regions woodlands would benefit regional biodiversity (Simmons 2001, Westaway 2006). However, coppicing is generally damaging for woodland bryophyte and lichen flora because of increased light and wind exposure, the removal of dead wood during the process and a reduction in the area of habitat suitable for epiphytic species (Kirby 1992, Ódor and Standovár 2001). The analysis in Chapter 3 indicates that canopy closure within the ghylls may be benefitting oceanic bryophytes and flowering plant AWI species, both species groups that contain rare species considered to be important in terms of regional, and in some cases national, biodiversity (Ratcliffe 1968, Rose and Patmore 1997, Church et al. 2004). It is clear that a reintroduction of coppicing within ghyll woodlands may benefit some species whilst negatively affecting others. It is therefore recommended that before sites are considered for coppicing, they are surveyed for bryophytes, ferns and field-layer species. Where rare species or communities are encountered it is important that the impact of

coppicing is considered if damage to biologically important species is to be avoided.

The investigation of geneflow between ghyll woodland patches carried out in Chapter 4 showed that populations of the bryophyte *C. conicum* were displaying the effects of genetic drift with F_{ST} and G_{ST} statistics indicating a moderate amount of genetic isolation (Tables 4.5 & 4.6). The analysis in Chapter 2 indicated that the presence and abundance of a number of ghyll woodland species appeared correlated with ghylls located on either sandstone or clay substrates (Tables 2.8, 2.9 & 2.10). For species limited to ghyll woodland 'patches' within the region, this type of substrate specificity will limit the number of ghyll woodlands that a species can potentially occupy. Within an ecological context, patches can be defined as a discontinuity in an ecological variable (such as substrate) that affects the distribution patterns of an organism (Wiens 1976). Studies of bryophyte species in relation to patchily distributed substrates have found that bryophytes are particularly sensitive to reduced habitat density (i.e. to increased distance between suitable patches) (Herben and Söderström 1992, Pharo and Zartman 2007). Herben and Söderström concluded that for bryophytes the most serious effect of habitat fragmentation is the increased isolation of habitat patches. Species whose distributions are restricted to either clay or sandstone ghyll woodlands will potentially experience greater levels of habitat fragmentation and isolation than species whose distribution patterns are not affected by the predominant substrate upon which the woodlands are located. It is important therefore that populations of rarer, substrate-specific species are investigated to examine levels of gene-flow between populations in order to ensure that genetic drift does not threaten the future survival of these populations.

Rose identified 1130 ghyll woodlands in the Wealden area using a desk-based approach (SBRC 2000). Since the majority of sites have never been field surveyed it is not possible to determine the proportion of ghyll woodlands that contain biologically important species or populations. This may explain why Burnside et al. (2006) state that the ghyll woodlands are afforded a relatively weak level of protection despite the acknowledged high biodiversity value of a number of sites. In order to protect the most important sites it may be necessary to accept that the ghyll woodlands do not uniformly warrant the same level of protection. In order to

ensure the protection of rare species and communities associated with some of the ghyll woodlands, it is vital that ghylls likely to contain biologically important species or communities are identified. Conservation efforts targeted at either individual woodlands or small networks of woodlands are far more likely to gain funding and be sustainable in the long-term, and therefore more likely to ultimately be successful than efforts that merely seek 'blanket protection' for the ghyll woodlands as a whole. One of the aims of this project was to attempt to identify key components of the Wealden ghylls that would indicate that a particular site had the potential to be important in terms of biodiversity value. In this way it was hoped that the numbers of potentially high-biodiversity sites could be targeted to such an extent that surveying ghyll woodland sites would become practical both in terms of man-hours and in terms of funding. Analysis in Chapter 2 showed that ghyll woodlands were restricted to the ghyll valleys and sub-communities could be identified on the basis of the predominant substrate upon which the ghyll valley is located. This finding may prove useful to better target surveys in order to locate rare species or communities where substrate specificity can be identified.

The analysis in Chapter 2 indicated that ghyll woodlands are particularly diverse, containing significantly higher numbers of bryophyte and flowering plant species than the ancient woodlands surrounding the ghyll sites (see Table 2.5). Given the rapid environmental changes currently occurring and those anticipated for the near future (resulting from climate change and woodland fragmentation), the species rich, old-growth ghyll woodlands may play an important evolutionary role within the region as reservoirs of genetic diversity and reproductive fitness (Mosseler et al. 2003).

5.5 Suggestions for future study

The presence of a humid microclimate was investigated through the measurement of environmental variables measured inside and outside the ghyll valleys. No physical evidence of a microclimate was found in the ghyll woodlands surveyed. However, the analysis in Chapter 3 found the ghylls to be rich in species associated with humid, shaded environments, indicating that a humid, shaded

microclimate does in fact exist. The study searched for evidence of a ghyll microclimate based on 'point readings' which were measured during each ghyll survey and which would therefore have been affected by the climatic conditions occurring on the day the readings were taken. It is possible that the damp, humid ghyll microclimate, cited by a number of authors (e.g. Ratcliffe 1968, Rose and Patmore 1997), occurs seasonally and/or intermittently depending on the prevailing weather conditions. To further investigate the presence and importance of microclimatic conditions, future work would involve the long-term installation of data-logging equipment in selected ghyll woodlands. This method would allow the identification and analysis of microclimatic conditions that occur intermittently.

Chapter 2 found that plant species richness was particularly high within the ghyll woodlands in comparison with similar non-ghyll ancient woodland throughout the region. The high plant species richness means more energy is available for species at higher trophic levels and therefore it is possible the ghyll woodlands contain rich communities of woodland fauna. Future work would involve surveying ghyll woodlands and the surrounding woodlands for woodland fauna to assess the biological importance of the ghyll woodlands for animal species within the region.

Southgate (2012) describes how the Sussex region is believed to have lost a 'staggering proportion' of its wetland habitats, with over 60% of Sussex wetlands thought to have been drained between 1960 & 1980. However, the counties wetland areas have never been properly inventoried and as such there is no way of knowing how much of these valuable resources have been lost or damaged, and how much currently exists within the region today (Southgate 2012). In order to estimate the current area covered by ghyll woodland within the region, as well as to monitor future losses and gains it is important that a baseline figure for the area covered by this resource is calculated. Current estimates of ghyll woodland area are based on ghyll woodland boundaries that were estimated from paper maps that did not show valley boundaries and the resultant boundaries frequently contained large areas of the surrounding woodland. The study carried out in Chapter 2 indicated that ghyll woodland could be identified on the basis of the rich bryophyte and field-layer species communities which were significantly higher than those recorded in the surrounding woodlands. The increased species richness was limited to the ghyll valleys themselves leading to the conclusion that ghyll

woodlands are confined to the stream valleys. The identification of ghyll woodland boundaries should enable a more accurate estimation of the total current extent of ghyll woodland cover. Total ghyll woodland cover could be estimated through DGPS mapping of the valley boundaries of a representative sample of ghyll woodlands. The unreliability of DGPS mapping within woodlands might necessitate a different approach. An estimate of total ghyll area could also be obtained through repeated measurements of valley width along the length of the valleys during field visits to a representative sample of ghyll valleys. These could then be used to produce an average width measurement which could be applied to all of the Wealden ghyll woodlands within a GIS framework.

The analysis carried out in Chapter 3 has shown that the ratio of oceanic bryophyte species and flowering plant AWI species increased over the survey period (see Tables 3.5 & 3.4). This suggests that changes in ghyll woodland canopy structure appear to be buffering oceanic bryophyte and flowering plant AWI species from the deleterious effects of climate change. The regional and in some cases national importance of these species groups means that it is important that this is examined further. Future work would involve growing ghyll woodland oceanic bryophyte and flowering plant species in the laboratory under conditions that simulate different 'stages' of the coppice cycle right through to high forest conditions. Results could then be compared to establish the influence of canopy structure on ghyll woodland oceanic bryophytes and flowering plants.

The genetic analysis carried out in Chapter 4 was based on the genetic samples collected from a fairly common ghyll woodland bryophyte. To properly assess levels of geneflow in the rare oceanic species that are the focus of conservation efforts it would be more relevant to carry out the analysis using these rare oceanic species. The RAPD technique requires removing a very small amount of plant material (a small section of a single leaf is sufficient) and therefore it would be possible to repeat this analysis on a rare ghyll woodland species without damaging the population itself. Future work would therefore entail repeating the genetic analysis using a rare oceanic bryophyte species.

Bibliography

Agren, G. I. and F. O. Andersson. 2012. Terrestrial Ecosystem Ecology. The Forestry Chronicle **88**:214-214.

Allendorf, F. W. and G. Luikart. 2007. Conservation and the Genetics of Populations. Blackwell Publishing Ltd, 350 Main Street, Malden, MA 02148-5020, USA.

Amar, A., C. Hewson, R. Thewlis, K. Smith, R. Fuller, J. Lindsell, G. Conway, S. Butler, and M. MacDonald. 2008. What's Happening to Our Woodland Birds?RSPB. http://www.rspb.org.uk/ourwork/projects/details.aspx?id=220427

An, N., H.-b. Guo, and W.-d. Ke. 2009. Genetic Variation in Rhizome Lotus (*Nelumbo nucifera Gaertn.* ssp. *nucifera*) Germplasms from China Assessed by RAPD Markers. Agricultural Sciences in China **8**:31-39.

Anderson, L. E. and B. E. Lemmon. 1974. Gene flow distances in the moss, Weissia controversa Hedw. Journal of the Hattori Botanical Laboratory. **38**:67-90

Andrén, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos:355-366.

Angeloni, F., N. J. Ouborg, and R. Leimu. 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. Biological Conservation **144**:35-43.

Atherton, I., S. D. Bosanquet, and M. Lawley. 2010. Mosses and liverworts of Britain and Ireland: A field guide. British Bryological Society Plymouth.

Austin, M. 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. Ecological Modelling **200**:1-19.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 2002. Short Protocols in Molecular Biology. 5th edition. Wiley.

Bailey, S. A., R. H. Haines-Young, and C. Watkins. 2002. Species presence in fragmented landscapes: modelling of species requirements at the national level. Biological Conservation **108**:307-316.

Baker, H. G. 1972. Seed weight in relation to environmental conditions in California. Ecology. **53**:997-1010.

Baldwin, L. K. 2004. Seeing the Forest for the Bryophytes: The Effects of Forest Fragmentation on the Bryophyte Community in Coastal Temperate Rainforests of British Columbia. PhD Thesis. University of British Columbia. http://circle.ubc.ca/handle/2429/16196

Barkham, J. 1992a. pp.115-146 *in*:The effects of coppicing and neglect on the performance of the perennial ground flora. Edited by G. P. Buckley. Springer.

Barkham, J. P. 1992b. The effects of management on the ground flora of ancient woodland, Brigsteer Park Wood, Cumbria, England. Biological Conservation **60**:167-187.

Barrès, B., F. Halkett, C. Dutech, A. Andrieux, J. Pinon, and P. Frey. 2008. Genetic structure of the poplar rust fungus Melampsora larici-populina: Evidence for isolation by distance in Europe and recent founder effects overseas. Infection, Genetics and Evolution **8**:577-587.

Barrington, D. S. 1993. Ecological and historical factors in fern biogeography. Journal of biogeography **20**:275-279.

Bates, J. 1997. Effects of intermittent desiccation on nutrient economy and growth of two ecologically contrasted mosses. Annals of Botany **79**:299-309.

Bates, J. 2000. Mineral nutrition, substratum ecology, and pollution. Bryophyte biology **248**:311.

Bedford, T. 1940. The fruiting of Breutelia arcuata Schp. Naturalist **1940**:113-115.

Beebee, T. and G. Rowe. 2004. An Introduction to Molecular Ecology. Oxford University Press.

Berry, J. and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology **31**:491-543.

Berry, P., T. Dawson, P. Harrison, and R. Pearson. 2002. Modelling potential impacts of climate change on the bioclimatic envelope of species in Britain and Ireland. Global ecology and biogeography **11**:453-462.

Biggs, R., S. R. Carpenter, and W. A. Brock. 2009. Turning back from the brink: detecting an impending regime shift in time to avert it. Proceedings of the National Academy of Sciences **106**:826-831.

Billings, W. D. 1952. The environmental complex in relation to plant growth and distribution. The Quarterly Review of Biology **27**:251-265.

Björklund, M., S. Bergek, E. Ranta, and V. Kaitala. 2010. The effect of local population dynamics on patterns of isolation by distance. Ecological Informatics **5**:167-172.

Bobes, R. J., G. Fragoso, M. d. R. Reyes-Montes, E. Duarte-Escalante, R. Vega, A. S. de Aluja, G. Zúñiga, J. Morales, C. Larralde, and E. Sciutto. 2010. Genetic diversity of Taenia solium cysticerci from naturally infected pigs of central Mexico. Veterinary Parasitology **168**:130-135.

Bogich, T. L., G. M. Barker, K. Mahlfeld, F. Climo, R. Green, and A. Balmford. 2012. Fragmentation, grazing and the species–area relationship. Ecography **35**:224-231.

Bonan, G. and G. Bonan. 2008. Ecological climatology: concepts and applications. Cambridge University Press.

Botkin, D. B., H. Saxe, M. B. Araujo, R. Betts, R. H. Bradshaw, T. Cedhagen, P. Chesson, T. P. Dawson, J. R. Etterson, and D. P. Faith. 2007. Forecasting the effects of global warming on biodiversity. BioScience **57**:227-236.

Boulangeat, I., D. Gravel, and W. Thuiller. 2012. Accounting for dispersal and biotic interactions to disentangle the drivers of species distributions and their abundances. Ecology letters **15**:584-593.

Braakhekke, W. G. and D. A. P. Hooftman. 1999. The resource balance hypothesis of plant species diversity in grassland. Journal of Vegetation Science **10**:187-200.

Brandon, P. 1974. The Sussex Landscape. Hodder & Stoughton.

Brandon, P. 2003. The Kent and Sussex Weald. Phillimore & Co. Ltd. Manor Barn, Chichester, West Sussex. England.

Breshears, D. D. and F. J. Barnes. 1999. Interrelationships between plant functional types and soil moisture heterogeneity for semiarid landscapes within the grassland/forest continuum: a unified conceptual model. Landscape Ecology **14**:465-478.

Breshears, D. D., P. M. Rich, F. J. Barnes, and K. Campbell. 1997. Overstory-imposed heterogeneity in solar radiation and soil moisture in a semiarid woodland. Ecological applications **7**:1201-1215.

Brook, B. W., N. S. Sodhi, and C. J. A. Bradshaw. 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution **23**:453-460.

Brown, D. H. and J. W. Bates. 1990. Bryophytes and nutrient cycling. Botanical Journal of the Linnean Society **104**:129-147.

Brown, J. H. and M. V. Lomolino. 1998. Biogeography. 2nd edition. Sinaeur Associates, Sunderland, M.A.

Brunet, J., U. Falkengren-Grerup, and G. Tyler. 1996. Herb layer vegetation of south Swedish beech and oak forests--effects of management and soil acidity during one decade. Forest Ecology and Management **88**:259-272.

Buckley, G. P. 1992. Ecology and Management of Coppice Woodlands. Chapman and Hall.

Burnside, N. G., N. J. Carrett, D. Metcalfe, and S. Waite. 2002a. GIS Analysis of Ancient and Ghyll Woodlands within East Sussex., Biogeography & Ecology Research Group. University of Brighton. A Report for East Sussex County Council.

Burnside, N., N. Carrett, D. Metcalfe, and S. Waite. 2002b. The determination of Ghyll woodland types in the Weald: a GIS approach.

Burnside, N. G., D. J. Metcalfe, R. F. Smith, and S. Waite. 2006. Ghyll woodlands of the Weald: characterisation and conservation. Biodiversity and Conservation **15**:1319-1338.

Butchart, S. H., M. Walpole, B. Collen, A. van Strien, J. P. Scharlemann, R. E. Almond, J. E. Baillie, B. Bomhard, C. Brown, and J. Bruno. 2010. Global biodiversity: indicators of recent declines. Science **328**:1164-1168.

Camargo, J. L. C. and V. Kapos. 1995. Complex edge effects on soil moisture and microclimate in central Amazonian forest. Journal of Tropical Ecology **11**:205-221.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, and D. A. Wardle. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59-67.

Carter, M. R. 1993. Soil sampling and methods of analysis. CRC Press.

Chapman, M. and A. Underwood. 1999. Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests. Marine ecology progress series **180**:257-265.

Church, J. M., N. G. Hodgetts, C. D. Preston, and N. F. Stewart. 2004. British Red data Books: mosses and liverworts. JNCC.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian journal of ecology **18**:117-143.

Clarke, K. R. G. 2001. PRIMER v5: User Manual/Tutorial, PRIMER-E. Plymouth UK.

Cleavitt, N. 2001. Disentangling moss species limitations: the role of physiologically based substrate specificity for six species occurring on substrates with varying pH and percent organic matter. The Bryologist **104**:59-68.

Cleavitt, N. L. 2002. Stress tolerance of rare and common moss species in relation to their occupied environments and asexual dispersal potential. Journal of Ecology **90**:785-795.

Collier, R., N. C. Council, G. Britain, and I. Branch. 1986. The conservation of the chequered skipper in Britain. NHBS. Joint Nature Conservation Committee.

Collinge, S. K. 1996. Ecological consequences of habitat fragmentation: implications for landscape architecture and planning. Landscape and Urban Planning **36**:59-77.

Collinge, S. K. 2009. Ecology of fragmented landscapes. John Hopkins University Press.

Corney, P., M. Le Duc, S. Smart, K. Kirby, R. Bunce, and R. Marrs. 2004. The effect of landscape-scale environmental drivers on the vegetation composition of British woodlands. Biological Conservation **120**:491-505.

Coroi, M., M. S. Skeffington, P. Giller, C. Smith, M. Gormally, and G. O'Donovan. 2004. Vegetation diversity and stand structure in streamside forests in the south of Ireland. Forest Ecology and Management **202**:39-57.

Cramer, W. and R. J. Whittaker. 1999. Changing the surface of our planet—results from studies of the global ecosystem. Global ecology and biogeography **8**:363-365.

Crawley, M. J. 1997. Plant Ecology. 2nd edition. Blackwell Science.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation **128**:231-240.

Cushman, S. A., K. S. McKelvey, J. Hayden, and M. K. Schwartz. 2006. Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. The American Naturalist **168**:486-499.

Dahnke, W. C. 1980. Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station, North Dakota State University.

Darwin, C. 1868. The variation of animals and plants under domestication. John Murray.

Darwin, C. 1876. The effects of cross and self fertilisation in the vegetable kingdom. J. Murray.

Daubenmire, R. 1968. Plant communities. A textbook of plant synecology.

Davies, A. L. 2011. Long-term approaches to native woodland restoration: Palaeoecological and stakeholder perspectives on Atlantic forests of Northern Europe. Forest Ecology and Management **261**:751-763.

Davies, C. E., D. Moss, and M. O. Hill. 2004. EUNIS habitat classification revised 2004. Report to: European Environment Agency-European Topic Centre on Nature Protection and Biodiversity:127-143.

De Cáceres, M., P. Legendre, and M. Moretti. 2010. Improving indicator species analysis by combining groups of sites. Oikos **119**:1674-1684.

DeLuca, T. H., O. Zackrisson, M.-C. Nilsson, and A. Sellstedt. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature **419**:917-920.

Désamoré, A., B. Laenen, M. Stech, B. Papp, L. Hedenäs, R. G. Mateo, and A. Vanderpoorten. 2012. How do temperate bryophytes face the challenge of a changing environment? Lessons from the past and predictions for the future. Global Change Biology **18**:2915-2924.

Didham, R. K. and J. H. Lawton. 1999. Edge Structure Determines the Magnitude of Changes in Microclimate and Vegetation Structure in Tropical Forest Fragments1. Biotropica **31**:17-30.

Dinsdale, J., P. Dale, and M. Kent. 1997. The biogeography and historical ecology of Lobelia urens L.(r)(the heath lobelia) in southern England. Journal of biogeography **24**:153-175.

Donath, T. W., N. Hölzel, and A. Otte. 2006. Influence of competition by sown grass, disturbance and litter on recruitment of rare flood-meadow species. Biological Conservation **130**:315-323.

Downing, A., E. Brown, R. Oldfield, P. Selkirk, and R. Coveny. 2007. Bryophytes and their distribution in the Blue Mountains region of New South Wales. Cunninghamia **10**:225-254.

Drakare, S., J. J. Lennon, and H. Hillebrand. 2006. The imprint of the geographical, evolutionary and ecological context on species–area relationships. Ecology letters **9**:215-227.

During, H. J. 1992. Ecological classification of bryophytes and lichens. Oxford: Clarendon Press.

Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant-soil system. Annu. Rev. Environ. Resour. **30**:75-115.

Ellenberg, H. 1974. Indicator values of vascular plants in central Europe. Scripta Geobotanica **9**.

Ellenberg, H., H. Weber, R. Duell, V. Wirth, W. Weber, and D. Paulissen. 1991. Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica **18**:1-248.

Engler, R. and A. Guisan. 2009. MigClim: Predicting plant distribution and dispersal in a changing climate. Diversity and Distributions **15**:590-601.

Environment Agency. 2013. Detailed River Network Layer - Wealden/South-east rivers and streams GIS data. Contains Environment Agency information (c) Environment Agency and database right.

ESCC. 2005. East Sussex County Council. Walks4Life. Crowhurst Walk. http://www.walk4life.info/walk/crowhurst-walk

Escudero, A., J. M. Iriondo, and M. E. Torres. 2003. Spatial analysis of genetic diversity as a tool for plant conservation. Biological Conservation **113**:351-365.

Evans, J. 1984. Silviculture of broadleaved woodland. Forestry Commission.

Excoffier, L. and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources.

Excoffier, N. C., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among haplotypes: application to human mitochondrial DNA restriction data. Genetics **131**:479-491.

Facelli, J. M. and S. T. Pickett. 1991. Plant litter: its dynamics and effects on plant community structure. The Botanical Review **57**:1-32.

Fagan, W. F. and E. E. Holmes. 2006. Quantifying the extinction vortex. Ecology letters **9**:51-60.

Fahrig, L. 2003. Effects of Habitat Fragmentation on Biodiversity. Annual Review of Ecology, Evolution, and Systematics **34**:487-515.

Farmer, R. and T. Nisbet. 2004. An overview of forest management and change with respect to environmental protection in the UK. Hydrology and Earth System Sciences Discussions **8**:279-285.

Fatemi, M. and C. L. Gross. 2009. Life on the edge - High levels of genetic diversity in a cliff population of *Bertya ingramii* are attributed to *B. rosmarinifolia* (Euphorbiaceae). Biological Conservation **142**:1461-1468.

Fiedler, P. L. and J. J. Ahouse. 1992. Hierarchies of cause: toward an understanding of rarity in vascular plant species. Pages 23-47 Conservation Biology. Springer.

Fisher, R. F. and D. Binkley. 2000. Ecology and Management of Forest Soils. John Wiley and Sons Inc.

Fitter, A. and R. Fitter. 2002. Rapid changes in flowering time in British plants. Science **296**:1689-1691.

Flather, C. H. and M. Bevers. 2002. Patchy reaction-diffusion and population abundance: the relative importance of habitat amount and arrangement. Amateur Naturalist **159**:40-56.

Forestry Commission. 1952. Census of woodlands, 1947-1949, by forestry commission. HMSO.

Forestry Commission. 1996. Woodland Creation: Needs and oppurtunities in the English Countryside: A Discussion paper.
Forestry Commission/Countryside Commission.

Forestry Commission. 2004. England's most wooded region - Background information/research summary No. 3. http://www.forestry.gov.uk/pdf/englandsmostwoodedregioninventory.pdf/$FILE/englandsmostwoodedregioninventory.pdf

Forman, R. T. T. 1995. Land Mosaics. Cambridge University Press.

Foster, B. L. and K. L. Gross. 1998. Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. Ecology **79**:2593-2602.

Fowler, J., L. Cohen, and P. Jarvis. 1998. Practical Statistics for Field Biology. 2nd edition. John Wiley & Sons.

Frahm, J.-P. and D. Klaus. 2001. Bryophytes as indicators of recent climate fluctuations in Central Europe. Lindbergia:97-104.

Franco, A., J. Hill, C. Kitschke, Y. Collingham, D. Roy, R. Fox, B. Huntley, and C. Thomas. 2006. Impacts of climate warming and habitat loss on extinctions

at species' low-latitude range boundaries. Global Change Biology **12**:1545-1553.

Frankham, R. 2005. Genetics and extinction. Biological Conservation **126**:131-140.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2010. Introduction to Conservation Genetics. Cambridge University Press, The Edinburgh Building, Cambridge. CB2 8RU. UK.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages pp135-150 *in* M. E. Soule and B. A. Wilcox, editors. Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer, Sunderland, MA.

Franklin, I. R. and R. Frankham. 1998. How large must populations be to retain evolutionary potential? Animal Conservation **1**:69-70.

Freeland, J. R. 2005. Molecular Ecology. John Wiley & Sons Ltd.

Freitas, H. and A. Brehm. 2001. Genetic diversity of the Macaronesian leafy liverwort Porella canariensis inferred from RAPD markers. Journal of Heredity **92**:339-345.

Frisvoll, A. A. and T. Prestø. 1997. Spruce forest bryophytes in central Norway and their relationship to environmental factors including modern forestry. Ecography **20**:3-18.

Fuertes, E., A. Burgaz, and A. Escudero. 1996. Pre-climax epiphyte communities of bryophytes and lichens in Mediterranean forests from the Central Plateau (Spain). Vegetatio **123**:139-151.

Fuller, R. and M. Warren. 1991. Conservation management in ancient and modern woodlands: responses of fauna to edges and rotations. http://agris.fao.org/agris-search/search.do?recordID=GB9124497

Fuller, R., M. S. Warren, N. C. Council, and G. Britain. 1993. Coppiced woodlands: their management for wildlife. Joint Nature Conservation Committee.

Furley, R. and H. B. Mackeson. 1874. A History of the Weald of Kent, with an Outline of the Early History of the County. Igglesden.

Gabriel, R. and J. Bates. 2005. Bryophyte community composition and habitat specificity in the natural forests of Terceira, Azores. Plant Ecology **177**:125-144.

Gartzia-Bengoetxea, N., A. González-Arias, A. Merino, and I. Martínez de Arano. 2009. Soil organic matter in soil physical fractions in adjacent semi-natural and cultivated stands in temperate Atlantic forests. Soil Biology and Biochemistry **41**:1674-1683.

GE Healthcare. 2007. Illustra puReTaq Ready-To-Go PCR Beads - Product Booklet. GE Healthcare UK Ltd.

GE Healthcare. 2011. Whatman FTA for plant DNA. GE Healthcare UK Ltd.

Geiger, R. 1965. The Climate near the Ground, 611 pp. Cambridge, Mass.

Giacomin, A. and P. Trucchi. 1992. Rainfall interception in a beech coppice (Acquerino, Italy). Journal of Hydrology **137**:141-147.

Gibbs, J. P. 2001. Demography versus habitat fragmentation as determinants of genetic variation in wild populations. Biological Conservation **100**:15-20.

Gitzendanner, M. A. and P. S. Soltis. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany **87**:783-792.

Godefroid, S. 2001. Temporal analysis of the Brussels flora as indicator for changing environmental quality. Landscape and Urban Planning **52**:203-224.

Godwin, H. 1975. History of the British Flora. 2nd edition. Cambridge University Press.

Goffinet, B. and A. J. Shaw. 2009. Bryophyte Biology. Cambridge University Press.

Goldberg, E., K. Kirby, J. Hall, and J. Latham. 2007. The ancient woodland concept as a practical conservation tool in Great Britain. Journal for Nature Conservation **15**:109-119.

Gosz, J. R., G. E. Likens, and F. H. Bormann. 1976. Organic matter and nutrient dynamics of the forest and forest floor in the Hubbard Brook Forest. Oecologia **22**:305-320.

Gould, W. A. and M. D. Walker. 1999. Plant communities and landscape diversity along a Canadian Arctic river. Journal of Vegetation Science **10**:537-548.

Graae, B. J. and P. B. Sunde. 2000. The impact of forest continuity and management on forest floor vegetation evaluated by species traits. Ecography **23**:720-731.

Green, D. M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. Biological Conservation **111**:331-343.

Gregory, S. V., F. J. Swanson, W. A. McKee, and K. W. Cummins. 1991. An ecosystem perspective of riparian zones. BioScience **41**:540-551.

Grime, J. and D. Jeffrey. 1965. Seedling establishment in vertical gradients of sunlight. The Journal of Ecology **63**:621-642.

Grime, J. P. 1979. Plant Strategies and Vegetation Processes. Wiley, New York.

Grime, J. P., J. G. Hodgson, and R. Hunt. 1988. Comparative plant ecology. A functional approach to common British species. Unwin Hyman Ltd.

Grinnell, J. 1917a. Field tests of theories concerning distributional control. American Naturalist:115-128.

Grinnell, J. 1917b. The niche-relationships of the California Thrasher. The Auk **34**:427-433.

Grundmann, M., S. W. Ansell, S. J. Russell, M. A. Koch, and J. C. Vogel. 2007. Genetic structure of the widespread and common Mediterranean bryophyte *Pleurochaete squarrosa* (Brid.) Lindb.(Pottiaceae)—evidence from nuclear and plastidic DNA sequence variation and allozymes. Molecular ecology **16**:709-722.

Grytnes, J. A., E. Heegaard, and P. G. Ihlen. 2006. Species richness of vascular plants, bryophytes, and lichens along an altitudinal gradient in western Norway. Acta-oecologica **29**:241-246

Gugerli, F., T. Englisch, H. Niklfeld, A. Tribsch, Z. Mirek, M. Ronikier, N. E. Zimmermann, R. Holderegger, and P. Taberlet. 2008. Relationships among levels of biodiversity and the relevance of intraspecific diversity in conservation - a project synopsis. Perspectives in Plant Ecology, Evolution and Systematics **10**:259-281.

Guo, Q., J. H. Brown, T. J. Valone, and S. D. Kachman. 2000. Constraints of seed size on plant distribution and abundance. Ecology **81**:2149-2155.

Gurevitch, J., S. M. Scheiner, and G. A. Fox. 2006. The ecology of plants. 2nd edition. Sinauer Associates Incorporated.

Haase, D. and J. Gläser. 2009. Determinants of floodplain forest development illustrated by the example of the floodplain forest in the District of Leipzig. Forest Ecology and Management **258**:887-894.

Hallden, C., M. Hansen, N. O. Nilsson, A. Hjerdin, and A. Sall. 1996. Competition as a source of errors in RAPD analysis. Theoretical and Applied Genetics **93**:1185-1192.

Hammersley, G. 1973. The charcoal iron industry and its fuel, 1540–1750. The Economic History Review **26**:593-613.

Hannah, L. 2010. Climate change biology. Academic Press. UK.

Hannah, L., G. Midgley, S. Andelman, M. Araújo, G. Hughes, E. Martinez-Meyer, R. Pearson, and P. Williams. 2007. Protected area needs in a changing climate. Frontiers in Ecology and the Environment **5**:131-138.

Harris, J. A., R. J. Hobbs, E. Higgs, and J. Aronson. 2006. Ecological restoration and global climate change. Restoration Ecology **14**:170-176.

Harris, R. R. 1988. Associations between stream valley geomorphology and riparian vegetation as a basis for landscape analysis in the eastern Sierra Nevada, California, USA. Environmental Management **12**:219-228.

Harrison, S., Bruna, E. 1999. Habitat fragmentation and large-scale conservation: what do we know for sure? Ecography **22**:225-232.

Harte, J., E. Conlisk, A. Ostling, J. L. Green, and A. B. Smith. 2005. A theory of spatial structure in ecological communities at spatial scales. Ecological Monographs **75**:179-197.

Hartl, D. L. and A. G. Clark. 2007. Principles of Population Genetics. 4th edition. Sinauer Associates, Inc. Sunderland, Massachusetts.

Haslam, S. 1996. British plant communities. Vol. 4. Aquatic communities, swamps and tall-herb fens: Edited by JS Rodwell. University Press, Cambridge.

Hastings Borough Council. 2010. Hastings Online - Countryside and Nature Conservation. http://www.hastings.gov.uk/community_living/places_spaces_facilities/allotments_parks_beaches/countryside/countryside_nature/

Hastings Borough Council. 2011. HCP Management Plan Woodland. http://wildhastings.files.wordpress.com/2010/12/hcpnr-man-plan-2010-2015-with-site-description.pdf

Hawkins, B. A., R. Field, H. V. Cornell, D. J. Currie, J.-F. Guégan, D. M. Kaufman, J. T. Kerr, G. G. Mittelbach, T. Oberdorff, and E. M. O'Brien. 2003. Energy, water, and broad-scale geographic patterns of species richness. Ecology **84**:3105-3117.

Head, K. H. and G. P. Keeton. 2010. Manual of Soil Laboratory Testing. Whittles Publishing.

Hedrick, P. W. 2005. Genetics of populations. 3rd edition. Jones and Bartlett, Sudbury. MA.

Heller, N. E. and E. S. Zavaleta. 2009. Biodiversity management in the face of climate change: a review of 22 years of recommendations. Biological Conservation **142**:14-32.

Hend, B. T., B. Ghada, B. M. Sana, M. Mohamed, T. Mokhtar, and S. Amel. 2009. Genetic relatedness among Tunisian plum cultivars by random amplified polymorphic DNA analysis and evaluation of phenotypic characters. Scientia Horticulturae **121**:440-446.

Henry, H. A. L. and L. W. Aarssen. 1997. On the relationship between shade tolerance and shade avoidance strategies in woodland plants. Oikos **80**:575-582.

Herben, T. and L. Söderström. 1992. Which habitat parameters are most important for the persistence of a bryophyte species on patchy, temporary substrates? Biological Conservation **59**:121-126.

Hermy, M., O. Honnay, L. Firbank, C. Grashof-Bokdam, and J. E. Lawesson. 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biological Conservation **91**:9-22.

Hickling, R., D. Roy, J. Hill, R. Fox, and C. Thomas. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology **12**:450-455.

Hickling, R., D. Roy, J. Hill, and C. Thomas. 2005. A northward shift of range margins in British Odonata. Global Change Biology **11**:502-506.

High Weald Landscape Trust. 2014. High Weald Area of Outstanding Natural Beauty.

Highweald.org. 2013. High Weald AONB. http://www.highweald.org/.

Hill, M. O., J. Mountford, D. Roy, and R. Bunce. 1999. Ellenberg's indicator values for British plants. ECOFACT Volume 2 Technical Annex. Institute of Terrestrial Ecology.

Hill, M. O., C. Preston, S. Bosanquet, and D. Roy. 2007. BRYOATT: attributes of British and Irish mosses, liverworts and hornworts. Centre for Ecology and Hydrology.

Hill, M. O., C. D. Preston, and D. Roy. 2004. PLANTATT-attributes of British and Irish plants: status, size, life history, geography and habitats. Centre for Ecology & Hydrology.

Hinde, T. 1985. Forests of Britain. Victor Gollancz Ltd.

Hodgetts, N. 1992. Guidelines for selection of biological SSSIs: non-vascular plants. Joint Nature Conservancy Committee.

Hoglund, J. 2009. Evolutionary Conservation Genetics. Oxford University Press, Great Clarendon Street, Oxford. OX2 6DP. UK.

Hokkanen, P. J. 2006. Environmental patterns and gradients in the vascular plants and bryophytes of eastern Fennoscandian herb-rich forests. Forest Ecology and Management **229**:73-87.

Holderegger, R., D. Buehler, F. Gugerli, and S. Manel. 2010. Landscape genetics of plants. Trends in plant science **15**:675-683.

Hölscher, D., E. Schade, and C. Leuschner. 2001. Effects of coppicing in temperate deciduous forests on ecosystem nutrient pools and soil fertility. Basic and Applied Ecology **2**:155-164.

Holsten, A., T. Vetter, K. Vohland, and V. Krysanova. 2009. Impact of climate change on soil moisture dynamics in Brandenburg with a focus on nature conservation areas. Ecological Modelling **220**:2076-2087.

Honnay, O., K. Verheyen, and M. Hermy. 2002. Permeability of ancient forest edges for weedy plant species invasion. Forest Ecology and Management **161**:109-122.

Hooley, J. and E. Cohn. 2003. Models of field layer vegetation interactions in an experimental secondary woodland. Ecological Modelling **169**:89-102.

Hopkins, J. J. and K. J. Kirby. 2007. Ecological change in British broadleaved woodland since 1947. Ibis **149**:29-40.

Hornby, R. J. R., F. 1987. The use of vascular plants in evaluation of ancient woodlands for nature conservation in southern England. Nature Conservancy Council.

Hughes, S. and A. Moody. 2007. PCR. Scion Publishing Limited.

Hunter, M. L. 2002. Fundamentals of Conservation Biology. 2nd edition. Blackwell Science Ltd.

Hutchinson, G. E. 1957. Concluding remarks. Pages 415-427 *in* Symposium on Quantitative Biology, Cold Spring Harbour.

Jeffries, M. J. 2006. Biodiversity and Conservation. 2nd edition. Routledge. Taylor and Francis Group.

Jenkins, G. J., Murphy, J.M., Sexton, D.M.H., Lowe, J.A., Jones, P. & Kilsby, C.G. 2009. UK Climate Projections: Briefing report. Met Office Hadley Centre.

Jenkins, G. J., Perry, M.C., and Prior, M.J.O. 2007. The climate of the United Kingdom and recent trends. Met Office Hadley Centre.

Jin, L. and R. Chakraborty. 1994. Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. Molecular Biology and Evolution **11**:120-127.

Jones, A. M. 2004. Environmental Biology. Routledge, London and New York.

Julio, N., A. Sobral, J. R. Dueñas, J. Di Rienzo, D. Renison, and I. Hensen. 2008. RAPD and ISSR markers indicate diminished gene flow due to recent fragmentation of Polylepis australis woodlands in central Argentina. Biochemical Systematics and Ecology **36**:329-335.

Känzig-Schoch, U. 1996. Artenschutz im Wald: zur Verbreitung, Vergesellschaftung und Ökologie von fünf gefährdeten Pflanzenarten im Berner Mittelland. Eidgenössische Forschungsanstalt für Wald. Schnee und Landschaft.

Kaplan, D. and R. Muñoz-Carpena. 2011. Complementary effects of surface water and groundwater on soil moisture dynamics in a degraded coastal floodplain forest. Journal of Hydrology **398**:221-234.

Karp, A., O. L. E. Seberg, and M. Buiatti. 1996. Molecular Techniques in the Assessment of Botanical Diversity. Annals of Botany **78**:143-149.

Katsiotis, A., N. Nikoloudakis, A. Linos, A. Drossou, and T. Constantinidis. 2009. Phylogenetic relationships in Origanum spp. based on rDNA sequences and intra-genetic variation of Greek O. vulgare subsp. hirtum revealed by RAPD. Scientia Horticulturae **121**:103-108.

Keitt, T. H., D. L. Urban, and B. T. Milne. 1997. Detecting critical scales in fragmented landscapes. Conservation Ecology **1**:4

Keller, D., S. Brodbeck, I. Flöss, G. Vonwil, and R. Holderegger. 2010. Ecological and genetic measurements of dispersal in a threatened dragonfly. Biological Conservation **143**:2658-2663.

Kellner, O. 1993. Effects on associated flora of sylvicultural nitrogen fertilization repeated at long intervals. Journal of applied ecology **30**:563-574.

Kelly, A. E. and M. L. Goulden. 2008. Rapid shifts in plant distribution with recent climate change. Proceedings of the National Academy of Sciences **105**:11823-11826.

Kent County Council. 2013. Biodiversity. Action for Kent's wildlife. Kent BAP. http://www.kentbap.org.uk/

Kent, M. 2012. Vegetation Description and Data Analysis: A Practical Approach. Wiley-Blackwell.

Kessler, M. 2000. Elevational gradients in species richness and endemism of selected plant groups in the central Bolivian Andes. Plant Ecology **149**:181-193.

Kessler, M. 2010. Biogeography of ferns. Fern ecology. Edited by K. Mehltreter, L.R. Walker and J. M. Sharpe. Cambridge University Press. pp22-60.

Kimmerer, R. W. 1994. Ecological consequences of sexual vs. asexual reproduction in Dicranum flagellare. The Bryologist **97**:20-25.

Kimura, M. and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics **49**:725-738.

Kirby, K. 1992. Accumulation of dead wood—a missing ingredient in coppicing? pp. 99-112 In: Ecology and Management of Coppice Woodlands. Edited by G.P. Buckley. Springer Publishing.

Kirby, K., S. Smart, H. Black, R. Bunce, P. Corney, and R. Smithers. 2005. Long term ecological change in British woodland (1971-2001). English Nature research reports **653**.

Kirsten, J. H., C. J. Dawes, and B. J. Cochrane. 1998. Randomly amplified polymorphism detection (RAPD) reveals high genetic diversity in *Thalassia testudinum banks ex König* (Turtlegrass). Aquatic Botany **61**:269-287.

Kluge, J. and M. Kessler. 2006. Fern endemism and its correlates: contribution from an elevational transect in Costa Rica. Diversity and Distributions **12**:535-545.

Korpelainen, H., M. Pohjamo, and S. Laaka-Lindberg. 2005. How efficiently does bryophyte dispersal lead to gene flow? Journal of the Hattori Botanical Laboratory:195-205.

Kostel-Hughes, F., T. P. Young, and J. D. Wehr. 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation toseed size 1. The Journal of the Torrey Botanical Society **132**:50-61.

Kurschner, H., M. Stech, M. Sim-Sim, S. Fontinha, and W. Frey. 2007. Life form and life strategy analyses of the epiphytic bryophyte communities of Madeira's laurel and ericaceous forests. Botanische Jahrbucher **127**:151-164.

Lambertini, C., M. H. G. Gustafsson, J. Frydenberg, M. Speranza, and H. Brix. 2008. Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. Aquatic Botany **88**:160-170.

Lande, R. and M. Kirkpatrick. 1988. Ecological speciation by sexual selection. Journal of Theoretical Biology **133**:85-98.

Larsen, J. 1922. Effect of removal of the virgin white pine stand upon the physical factors of site. Ecology **3**:302-305.

Laurance, W. F. 2004. Forest-climate interactions in fragmented tropical landscapes. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **359**:345-352.

Lawson, D. and H. J. Jensen. 2006. The species-area relationship and evolution. Journal of Theoretical Biology **241**:590-600.

Lee, T. D. and G. H. Roi. 1979. Bryophyte and understorey vascular plant beta diversity in relation to moisture and elevation gradients. Vegetatio **40**:29-38.

Leishman, M. and M. Westoby. 1994a. The role of large seed size in shaded conditions: experimental evidence. Functional Ecology **8**:205-214.

Leishman, M. R. and M. Westoby. 1994b. The role of seed size in seedling establishment in dry soil conditions--experimental evidence from semi-arid species. Journal of Ecology **82**:249-258.

Levins, R. 1970. Extinction. Lectures on mathematics in the life sciences **2**:75-107.

Li, F., S. Gan, Q. Weng, X. Zhao, S. Huang, M. Li, S. Chen, Q. Wang, and F. Shi. 2008. RAPD and morphological diversity among four populations of the tropical tree species *Paramichelia baillonii* (Pierre) Hu in China. Forest Ecology and Management **255**:1793-1801.

Li, J.-M. and Z.-X. Jin. 2006. High genetic differentiation revealed by RAPD analysis of narrowly endemic *Sinocalycanthus chinensis* an endangered species of China. Biochemical Systematics and Ecology **34**:725-735.

Lindenmayer, D. B. and J. Fischer. 2006. Habitat fragmentation and landscape change: an ecological and conservation synthesis. Island Press.

Lindenmayer, D. B., W. Steffen, A. A. Burbidge, L. Hughes, R. L. Kitching, W. Musgrave, M. Stafford Smith, and P. A. Werner. 2010. Conservation strategies in response to rapid climate change: Australia as a case study. Biological Conservation **143**:1587-1593.

Liu, L., Y. Q. Zhu, and Y. F. Wang. 2006. The morphological and RAPD analyses of different *Myuroclada maximowiczii* populations. Acta Botanica Yunnanica **28**:570-574.

Lomolino, M. V. 2000. Ecology's most general, yet protean pattern: the species-area relationship. Journal of biogeography **27**:17-26.

Lomolino, M. V. 2001. The species-area relationship: new challenges for an old pattern. Progress in Physical Geography **25**:1-21.

Lomolino, M. V., B. R. Riddle, R. J. Whittaker, and J. H. Brown. 2010. Biogeography. 4th edition. Sinauer Associates, Inc. Sunderland, Massachusetts.

Lowe, J. J. and M. J. Walker. 1997. Reconstructing quaternary environments. Longman Harlow.

Luan, S., T. Chiang, and X. Gong. 2006. High Genetic Diversity vs. Low Genetic Differentiation in *Nouelia insignis* (Asteraceae), a Narrowly Distributed and Endemic Species in China, Revealed by ISSR Fingerprinting. Annals of Botany **98**:583-589.

Lyon, J. and C. Sagers. 1998. Structure of herbaceous plant assemblages in a forested riparian landscape. Plant Ecology **138**:1-16.

MacArthur, R. H. and E. O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press.

Maddock, A. 2008. UK Biodiversity Action Plan; Priority Habitat Descriptions. UK Biodiversity Action Plan. http://jncc.defra.gov.uk/PDF/UKBAP_PriorityHabitatDesc-Rev2010.pdf

MAGIC. 2011. Ancient Woodland in the South-east of England. DEFRA.

Magurran, A. E. 2004. Measuring Biological Diversity. Blackwell Science Ltd.

Magurran, A. E., S. R. Baillie, S. T. Buckland, J. M. Dick, D. A. Elston, E. M. Scott, R. I. Smith, P. J. Somerfield, and A. D. Watt. 2010. Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends in Ecology & Evolution **25**:574-582.

Malanson, G. P. 1993. Riparian Landscapes. Cambridge University Press.

Mantel, N. 1967. The detection of disease clustering and a generalizated regression approach. Cancer Research **27**:209-220.

Marschner, H. and P. Marschner. 2011. Marschner's mineral nutrition of higher plants. Access Online via Elsevier. http://www.sciencedirect.com/science/book/9780123849052

Martin, T. G., S. McIntyre, C. P. Catterall, and H. P. Possingham. 2006. Is landscape context important for riparian conservation? Birds in grassy woodland. Biological Conservation **127**:201-214.

Matlack, G. R. 1993. Microenvironment variation within and among forest edge sites in the eastern United States. Biological Conservation **66**:185-194.

Matlack, G. R. 1994. Vegetation dynamics of the forest edge--trends in space and successional time. Journal of Ecology **82**:113-123.

McCollin, D. 1998. Forest edges and habitat selection in birds: a functional approach. Ecography **21**:247-260.

McCollin, D., J. I. Jackson, R. G. H. Bunce, C. J. Barr, and R. Stuart. 2000a. Hedgerows as habitat for woodland plants. Journal of Environmental Management **60**:77-90.

McCollin, D., L. Moore, and T. Sparks. 2000b. The flora of a cultural landscape: environmental determinants of change revealed using archival sources. Biological Conservation **92**:249-263.

McCullagh, P. and J. A. Nelder. 1989. Generalized linear models (Monographs on statistics and applied probability 37). Chapman Hall, London.

McPherson, M. and S. Moller. 2006. PCR. 2nd edition. Taylor & Francis Group.

McVean, D. N. and D. A. Ratcliffe. 1962. Plant communities of the Scottish High-lands. A study of Scottish mountain, moorland and forest vegetation. Plant communities of the Scottish High-lands. A study of Scottish mountain, moorland and forest vegetation. Monographs of the nature conservancy No. 1.

Mehltreter, K., L. R. Walker, and J. M. Sharpe. 2010. Fern Ecology. Cambridge University Press.

Menzel, A. 2000. Trends in phenological phases in Europe between 1951 and 1996. International Journal of Biometeorology **44**:76-81.

Merryweather, J. 2007. The fern guide: a field guide to the ferns, clubmosses, quillworts and horsetails of the British Isles. Field Studies Council.

Met Office. 2013. Summer 2012 was the wettest in 100 years. http://www.metoffice.gov.uk/news/releases/archive/2012/second-wettest-summer

Miller, N. G. and L. H. Ambrose. 1976. Growth in culture of wind-blown bryophyte gametophyte fragments from Arctic Canada. Bryologist **79**:55-63.

Miller, N. G. and S. F. McDaniel. 2004. Bryophyte dispersal inferred from colonization of an introduced substratum on Whiteface Mountain, New York. American Journal of Botany **91**:1173-1182.

Milligan, B. G., J. Leebens-Mack, and A. E. Strand. 1994. Conservation genetics: beyond the maintenance of marker diversity. Molecular ecology **3**:423-435.

Mitchell, K. and R. Ryan. 1998. The Species Area Relationship. UMAP Journal **19**:139-170.

Mitchell, P. 1992. Growth stages and microclimate in coppice and high forest. pp31-35. *in* Ecology and Management of Coppice Woodlands. Edited by G.P. Buckley. Springer Publishing Ltd.

Mitchell, P. and K. Kirby. 1989. Ecological effects of forestry practices in long-established woodland and their implications for nature conservation. http://agris.fao.org/agris-search/search.do?recordID=GB19930107513

Moody, A. 2007. An introduction to the polymerase chain reaction. Pages pp.1-20 *in* S. Hughes, Moody, A., editor. PCR. Scion Publishing Limited.

Moore, M. R. and J. L. Vankat. 1986. Responses of the herb layer to the gap dynamics of a mature beech-maple forest. American Midland Naturalist **115**:336-347.

Morecroft, M., C. Bealey, D. Beaumont, S. Benham, D. Brooks, T. Burt, C. Critchley, J. Dick, N. Littlewood, and D. Monteith. 2009. The UK Environmental Change Network: Emerging trends in the composition of plant and animal communities and the physical environment. Biological Conservation **142**:2814-2832.

Morison, J. I. and M. D. Morecroft. 2008. Plant growth and climate change. Blackwell Publishing.

Mosseler, A., J. Major, and O. Rajora. 2003. Old-growth red spruce forests as reservoirs of genetic diversity and reproductive fitness. Theoretical and Applied Genetics **106**:931-937.

Murphy, H. T. and J. Lovett-Doust. 2004. Context and connectivity in plant metapopulations and landscape mosaics: does the matrix matter? Oikos **105**:3-14.

Na, A. N., G. U. O. Hong-bo, and K. E. Wei-dong. 2009. Genetic variation in rhizome lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) germplasms from China assessed by RAPD markers. Agricultural Sciences in China **8**:31-39.

Naiman, R. J. and H. Decamps. 1997. The ecology of interfaces: riparian zones. Annual review of ecology and systematics:621-658.

Naiman, R. J., H. Decamps, and M. E. McClain. 2005. Riparia: Ecology, Conservation and Management of Streamside Communities. Elsevier Academic Press.

Natalia, S., V. J. Lieffers, and S. M. Landhäusser. 2008. Effects of leaf litter on the growth of boreal feather mosses: implication for forest floor development. Journal of Vegetation Science **19**:253-260.

Natural England. 2013. GIS Digital Boundary Datasets.*in* E. Nature.

Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America **70**:3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583-590.

Newton, C. R. and A. Graham. 1997. PCR. 2nd edition. BIOS Scientific Publishers Limited.

Ney-Nifle, M. and M. Mangel. 2000. Habitat Loss and Changes in the Species-Area Relationship. Conservation Biology **14**:893-898.

Nichols, G. E. 1923. A working basis for the ecological classification of plant communities. Ecology **4**:154-179.

NSRI. 2001. Soils of the South East of England Datalease No. LC0056/001.*NSRI*

O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation **133**:42-51.

Odasz, A. M. 1996. Bryophyte vegetation and habitat gradients in the Tikhaia Bay Region, Hooker Island, Franz Josef Land, arctic Russia. Bryologist **99**:407-415.

Ódor, P. and T. Standovár. 2001. Richness of bryophyte vegetation in near-natural and managed beech stands: the effects of management-induced differences in dead wood. Ecological bulletins No. 49 pp.219-229.

Osborn, J. M. and T. N. Taylor. 1990. Morphological and Ultrastructural Studies of Plant Cuticular Membranes. I. Sun and Shade Leaves of Quercus velutina (Fagaceae). Botanical Gazette **151**:465-476.

Overpeck, J., J. Cole, and P. Bartlein. 2005. A “paleoperspective” on climate variability and change. pp.91-108. *in* Climate Change and Biodiversity. T.E. Lovejoy and L. Hannah. Yale University Press, New Haven, Connecticut.

Packham, J. R. and D. J. Harding. 1982. Ecology of Woodland Processes. Edward Arnold (Publishers) Limited.

Packham, J. R., D. J. L. Harding, G. M. Hilton, and R. A. Stuttard. 1992. Functional Ecology of Woodland and Forests. Chapman & Hall.

Paine, L. K. and C. A. Ribic. 2002. Comparison of riparian plant communities under four land management systems in southwestern Wisconsin. Agriculture, ecosystems & environment **92**:93-105.

Parmesan, C. 2001. Detection of range shifts: general methodological issues and case studies of butterflies. pp.57-76 *in* “Fingerprints” of Climate Change. Edited by G.R. Walther., C.A. Burga and P.J. Edwards. Springer.

Parmesan, C., N. Ryrholm, C. Stefanescu, J. Hill, C. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, and T. Tammaru. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature **399**:579-583.

Parry, M. L. 1991. The potential effects of climate change in the United Kingdom. HMSO.

Pärtel, M., A. Helm, N. Ingerpuu, Ü. Reier, and E.-L. Tuvi. 2004. Conservation of Northern European plant diversity: the correspondence with soil pH. Biological Conservation **120**:525-531.

Patmore, J. M. 2000. High weald Natural Area Profile. http://www.naturalareas.naturalengland.org.uk/Science/natural/profiles%5CnaProfile72.pdf

Paton, J. A. 1956. Bryophyte Succession on the Wealden Sandstone Rocks.

Paton, J. A. 1999. The liverwort flora of the British Isles. Harley Books Colchester.

Pavlik, B. M. and E. Manning. 1993. Assessing limitations on the growth of endangered plant populations, Experimental demography of *Erysimum capitatum* ssp. *Angustatum* and *Oenothera deltoides* ssp. *Howellii*. Biological Conservation **65**:257-265.

Pearson, R. G. and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global ecology and biogeography **12**:361-371.

Peixoto, M. G. C. D., C. F. Poggian, R. S. Verneque, A. A. Egito, M. R. S. Carvalho, V. M. Penna, J. A. G. Bergmann, M. A. Viccini, and M. A. Machado. 2010. Genetic basis and inbreeding in the Brazilian Guzerat (*Bos indicus*) subpopulation selected for milk production. Livestock Science **131**:168-174.

Pentecost, A. 1991. The weathering rates of some sandstone cliffs central Weald, England. Earth Surface Processes and Landforms **16**:83-91.

Peñuelas, J. and M. Boada. 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). Global Change Biology **9**:131-140.

Pérez-García, B., A. Mendoza-Ruiz, M. E. Sánchez-Coronado, and A. Orozco-Segovia. 2007. Effect of light and temperature on germination of spores of four tropical fern species. Acta Oecologica **32**:172-179.

Peterken, G. F. 1974. A method for assessing woodland flora for conservation using indicator species. Biological Conservation **6**:239-245.

Peterken, G. F. 1993. Woodland Conservation and Management. 2nd edition. Chapman & Hall, London.

Peterken, G. F. 1996. Natural woodland: ecology and conservation in northern temperate regions. Cambridge University Press.

Peterson, D. L. and G. L. Rolfe. 1982. Seasonal Variation in Nutrients of Floodplain and Upland Forest Soils of Central Illinois1. Soil Sci. Soc. Am. J. **46**:1310-1315.

Pharo, E. J. and C. E. Zartman. 2007. Bryophytes in a changing landscape: The hierarchical effects of habitat fragmentation on ecological and evolutionary processes. Biological Conservation **135**:315-325.

Pignatti, S. 1996. Ecograms for phytosociological tables based on Ellenberg's Zeigerwerte. Annali di Botanica (Roma) **54**:5-14.

Pigott, C., J. Rodwell, N. C. Council, and G. Britain. 1992. British Plant Communities: Grasslands and Montane Communities; Edited by JS Rodwell. Cambridge University Press.

PISCES. 2013. Software from PISCES Conservation Ltd. Lymington, UK.

Pizo, M. A., C. Von Allmen, and L. P. C. Morellato. 2006. Seed size variation in the palm Euterpe edulis and the effects of seed predators on germination and seedling survival. Acta Oecologica **29**:311-315.

Pohjamo, M., H. Korpelainen, and N. Kalinauskaitė. 2008. Restricted gene flow in the clonal hepatic *Trichocolea tomentella* in fragmented landscapes. Biological Conservation **141**:1204-1217.

Polechová, J. and D. Storch. 2008. Ecological niche. Encyclopedia of ecology **2**:1088-1097.

Porley, R. and N. G. Hodgetts. 2009. Mosses and Liverworts. Collins.

Poulsen, A. D., H. Tuomisto, and H. Balslev. 2006. Edaphic and Floristic Variation within a 1-ha Plot of Lowland Amazonian Rain Forest. Biotropica **38**:468-478.

Prach, K. and P. Pyšek. 1999. How do species dominating in succession differ from others? Journal of Vegetation Science **10**:383-392.

Preston, C. D. and M. O. Hill. 1997. The geographical relationships of British and Irish vascular plants. Botanical Journal of the Linnean Society **124**:1-120.

Primack, R. B. 2006. Essentials of Conservation Biology. 4th edition. Sinauer Associates, Inc.

Primack, R. B. 2008. A Primer of Conservation Biology. 4th edition. Sinauer Associates Inc. Sunderland, MA 01375 USA.

Prinzing, A., W. Durka, S. Klotz, and R. Brandl. 2008. Geographic variability of ecological niches of plant species: are competition and stress relevant? Ecography **25**:721-729.

Proctor, M., editor. 2009. Physiological ecology. 2nd edition.

Proctor, M. C., M. J. Oliver, A. J. Wood, P. Alpert, L. R. Stark, N. L. Cleavitt, and B. D. Mishler. 2007. Desiccation-tolerance in bryophytes: a review. The Bryologist **110**:595-621.

Przyborowski, J. A. and P. Sulima. 2010. The analysis of genetic diversity of *Salix viminalis* genotypes as a potential source of biomass by RAPD markers. Industrial Crops and Products **31**:395-400.

Quinn, G. G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press.

Raabe, S., J. Müller, M. Manthey, O. Dürhammer, U. Teuber, A. Göttlein, B. Förster, R. Brandl, and C. Bässler. 2010. Drivers of bryophyte diversity allow implications for forest management with a focus on climate change. Forest Ecology and Management **260**:1956-1964.

Rabinowitz, D., S. Cairns, and T. Dillon. 1986. Seven forms of rarity and their frequency in the flora of the British Isles. pp.182-204. *in* Conservation Biology the science of scarcity and diversity. Edited by D. Rabinowitz., S. Cairns and T. Dillon. Sinauer Associates.

Rackham, O. 1975. Hayley Wood: Its history and ecology. Cambridgeshire and Isle of Ely Naturalists' Trust Cambridge.

Rackham, O. 1980. Ancient woodland, its history, vegetation and uses in England. Ancient woodland, its history, vegetation and uses in England. Castlepoint Press.

Rackham, O. 1986. The history of the countryside. JM Dent Publishers. London.

Rackham, O. 1990. Trees and woodland in the British landscape. J. M. Dent & sons Ltd.

Rackham, O. 2000. The History of the Countryside. Phoenix Press, London.

Rackham, O. 2006. Woodlands. Harper Collins Publishers.

Radley, J. D. 2006. A Wealden guide I: the Weald Sub-basin. Geology Today **22**:109-118.

Ramírez-Valiente, J. A., F. Valladares, L. Gil, and I. Aranda. 2009. Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber L*.). Forest Ecology and Management **257**:1676-1683.

Ranganathan, J., J. Krishnaswamy, and M. O. Anand. 2010. Landscape-level effects on avifauna within tropical agriculture in the Western Ghats: Insights for management and conservation. Biological Conservation **143**:2909-2917.

Ratcliffe, D. A. 1968. An Ecological Account of Atlantic Bryophytes in the British Isles. New Phytologist **67**:pp.365-439.

Ratcliffe, P. and B. Staines. 2003. Deer management requirements for the delivery of Natura 2000 objectives in Atlantic oakwoods. Restoring Natura Forest Habitats pp.35-40.

Reynolds, D. N. 1980. Gamete dispersal in *Mnium ciliare*. Bryologist **83**:73-77.

Rich, T. and F. Rumsey. 2004. Plate 490. *Hymenophyllum tunbrigense* Hymenophyllaceae. Curtis's Botanical Magazine **21**:70-76.

Richard, M., T. Bernhardt, and G. Bell. 2000. Environmental heterogeneity and the spatial structure of fern species diversity in one hectare of old-growth forest. Ecography **23**:231-245.

Richards, E. G. 2003. British forestry in the 20th century Policy and achievements. Koninklijke Brill Leiden, The Netherlands.

Richardson, J. L. and M. J. Vepraskas. 2001. Wetland Soils: Genesis, Hydrology, Landscapes and Classification. Lewis Publishers.

Riemann, B. 1972. On the Sex-Distribution and the Occurence of Sporophytes in *Rhytidiadelphus triquetrus* (Hedw.) Warnst. in Scandinavia. Lindbergia **1**:219-224.

Robinson, D. A. and R. Williams. 1984. Classic landforms of the Weald. Geographical Association in conjunction with the British Geomorphological Research Group.

Rochefort, L. and E. Lode. 2006. Restoration of degraded boreal peatlands. pp.381-423 *in* Boreal peatland ecosystems. Edited by R.K. Weider and D.H. Vitt. Springer Publishing.

Rodwell, J. 1991. British Plant Communities, Vol. 1. Woodlands. Cambridge University Press, Cambridge, UK.

Roem, W. and F. Berendse. 2000. Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. Biological Conservation **92**:151-161.

Rose, F. 1995. The Habitats and Vegetation of Sussex. Brighton Borough Council.

Rose, F. 1999. Indicators of ancient woodland - the use of vascular plants in evaluating ancient woods for nature conservation. British Wildlife **10**:241-251.

Rose, F., C. O'Reilly, D. Smith, and M. Collings. 2006. The wild flower key: how to identify wild flowers, trees and shrubs in Britain and Ireland. Frederick Warne.

Rose, F. and J. M. Patmore. 1997. Gill Woodlands in the Weald. English Nature, Peterborough.

Rosenzweig, M. L. 1995. Species Diversity in Space and Time. Cambridge University Press.

Rosenzweig, M. L. 2003. Reconciliation ecology and the future of species diversity. Oryx **37**:194-205.

Rothero, G. 2005. Oceanic bryophytes in Atlantic oakwoods. Transactions and Proceedings of the Botanical Society of Edinburgh and Botanical Society of Edinburgh Transactions **57**:135-140.

RSPB. 2010. About Fore Wood. https://www.rspb.org.uk/reserves/guide/f/forewood/

Ruokolainen, K., A. Linna, and H. Tuomisto. 1997. Use of Melastomataceae and pteridophytes for revealing phytogeographical patterns in Amazonian rain forests. Journal of Tropical Ecology **13**:243-256.

Sagers, C. L. and J. Lyon. 1997. Gradient analysis in a riparian landscape: contrasts among forest layers. Forest Ecology and Management **96**:13-26.

Salisbury, E. J. 1942. The reproductive capacity of plants. Studies in quantitative biology. The reproductive capacity of plants. Studies in quantitative biology.

Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological Consequences of Ecosystem Fragmentation: A Review. Conservation Biology **5**:18-32.

SBRC. 2000. Ghyll Woodland data created by Sussex Biodiversity Record Centre using the expert knowledge of Dr. Francis Rose. © Crown Copyright. All rights reserved 2013.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature **413**:591-596.

Scherrer, D. and C. Körner. 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. Journal of biogeography **38**:406-416.

Schnelle, F. and E. Volkert. 1974. International phenological gardens in Europe the basic network for international phenological observations. Pages 383-387 Phenology and seasonality modeling. Springer.

Schoener, T. W. 1976. The Species-area relationship within archipelagoes: Models and evidence from island birds. pp.629-642 *in* XVI International Ornithological Congress 6.

Schulte, E. 1995. Recommended soil organic matter tests. Recommended soil testing procedures for the northeastern United States. Northeast Regional Bull **493**:47-56.

Scott, D., F. Southgate, A. Overall, S. Waite, and B. Tolhurst. 2012. The Eurasian water shrew: an unsuitable candidate species for a vertebrate bio-indicator of aquatic pollution. Journal of Zoology **286**:30-37.

Scott, D. M., D. Brown, S. Mahood, B. Denton, A. Silburn, and F. Rakotondraparany. 2006. The impacts of forest clearance on lizard, small mammal and bird communities in the arid spiny forest, southern Madagascar. Biological Conservation **127**:72-87.

Selkirk, P. M., M. L. Skotnicki, K. D. Adam, M. B. Connett, T. Dale, T. W. Joe, and J. Armstrong. 1997. Genetic variation in Antarctic populations of the moss *Sarconeurum glaciale*. Polar Biology **18**:344-350.

Shao, J.-W., W.-L. Chen, Y.-Q. Peng, G.-P. Zhu, and X.-P. Zhang. 2009. Genetic diversity within and among populations of the endangered and endemic species *Primula merrilliana* in China. Biochemical Systematics and Ecology **37**:699-706.

Sharma, A. K., M. J. Mendki, S. N. Tikar, K. Chandel, D. Sukumaren, B. D. Parashar, V. Veer, O. P. Agarwal, and S. Prakash. 2009. Genetic variability in geographical populations of *Culex quinquefasciatus* Say (Diptera:

Culicidae) from India based on random amplified polymorphic DNA analysis. Acta Tropica **112**:71-76.

Shen, G., M. Yu, X.-S. Hu, X. Mi, H. Ren, I.-F. Sun, and K. Ma. 2009. Species–area relationships explained by the joint effects of dispersal limitation and habitat heterogeneity. Ecology **90**:3033-3041.

Shimamura, M., T. Yamaguchi, and H. Deguchi. 2008. Airborne sperm of *Conocephalum conicum* (Conocephalaceae). Journal of plant research **121**:69-71.

Simberloff, D. 1976. Experimental Zoogeography of Islands: Effects of Island Size. Ecology **57**:629-648.

Simberloff, D. S. and E. O. Wilson. 1969. Experimental Zoogeography of Islands. The Colonization of Empty Islands. Ecology (Washington D C) **50**:278-296.

Simmons, E. 2001. The Weald of Kent and Sussex, England. Threatened landscapes: conserving cultural environments:105.

Skotnicki, M. L., A. Mackenzie, and P. M. Selkirk. 2004a. Mosses surviving on the edge: Origins, genetic diversity, and mutation in Antarctica. Pages 388-403 *in* B. Goffinet, V. Hollowell, and R. Magill, editors. Molecular Systematics of Bryophytes. Missouri Botanical Garden, St Louis.

Skotnicki, M. L., A. M. Mackenzie, and J. A. Ninham. 2004b. High levels of genetic variability in the moss *Ceratodon purpureus* from continental Antarctica, subantarctic Heard and Macquarie Islands, and Australasia. Polar Biology **27**:687-698.

Smart, S. 2000. Modular analysis of vegetation information system (MAVIS). Centre for Ecology and Hydrology, Lancaster. URL http://www. ceh. ac. uk/products/software/cehsoftware-mavis. htm.

Smith, A. J. E. 2006. The Moss Flora of Britain. 2nd with corrections edition. Cambridge University Press.

Smith, S. 2000. The National Inventory of Woodland and Trees - England. http://www.forestry.gov.uk/pdf/frnationalinventory0001.pdf/$FILE/frnationalinventory0001.pdf

Smith, S. and J. Gilbert. 2003. National inventory of woodland and trees: Great Britain. National inventory of woodland and trees: Great Britain. http://www.forestry.gov.uk/forestry/INFD-86XC6C

Snall, T., Ehrlen, J. & Rydin, H. 2005. Colonization-extinction dynamics of an epiphyte metapopulation in a dynamic landscape. Ecology **86**:106-115.

Soderstrom, L. and T. Herben. 1997. Dynamics of bryophyte metapopulations. Pages 205-240 *in* R. E. Longton, editor. Advances in Bryology; Population studies.

Soulé, M. E. and D. Simberloff. 1986. What do genetics and ecology tell us about the design of nature reserves? Biological Conservation **35**:19-40.

Southgate, F. 2012. The Wetland Potential of Sussex. The Sussex Wildlife Trust.

Speiss, E. B. 1977. Genes in Populations. John Wiley & Sons.

Spencer, J. W. and K. J. Kirby. 1992. An inventory of ancient woodland for England and Wales. Biological Conservation **62**:77-93.

Spiecker, H. 1999. Overview of recent growth trends in European forests. Water, Air, and Soil Pollution **116**:33-46.

Stamps, J. A., M. Buechner, and V. Krishnan. 1987. The effects of edge permeability and habitat geometry on emigration from patches of habitat. American Naturalist **129**:533-552.

Stenøien, H. K. and S. M. Såstad. 1999. Genetic structure in three haploid peat mosses (Sphagnum). Heredity **82**:391-400.

Stewart, K. J. and A. U. Mallik. 2006. Bryophyte responses to microclimatic edge effects across riparian buffers. Ecological applications **16**:1474-1486.

Streeter, D. A. 1983. Biogeography, Ecology and Conservation in Sussex. *in* Sussex: Environment, Landscape and Society. Edited by Alan Sutton.

Stuttard, P. and K. Williamson. 1971. Habitat requirements of the Nightingale. Bird Study **18**:9-14.

Sundberg, S. 2005. Sporophyte size positively influences short-range spore dispersal in *Sphagnum*, but what happens further away? Oikos **108**:115-124.

Surrey Biodiversity Partnership. 2001. Woodland: A Habitat Action Plan for Surrey. http://www.surreybiodiversitypartnership.org/xwiki/bin/view/Woodland/ActionPlan

Sussex Biodiversity Partnership. 2010. Woodland Habitat Action Plan. http://www.biodiversitysussex.org.uk/habitats/woodland

Sutherland, W. J. 2006. Ecological census techniques: a handbook. Cambridge University Press.

Szweykowski, J., K. Buczkowska, and I. J. Odrzykoski. 2005. *Conocephalum salebrosum* (Marchantiopsida, Conocephalaceae) - a new Holarctic liverwort species. Plant Systematics and Evolution **253**:133-158.

Tabachnick, B. G. and L. Fidell. 2012. Using Multivariate Statistics: International Edition. Pearson.

Taiz, L. a. Z., E. 2010. Plant Physiology. 5th edition. Sinauer Associates Inc. Sunderland, Massachusetts USA.

Tang, S., Y. Li, Y. Geng, G. Zhang, L. Wang, and Y. Zhong. 2007. Clonal and spatial genetic structurein natural populations of Luohanguo (*Siraitia grosvenorii*), an economic species endemic to South China, as revealed by RAPD markers. Biochemical Systematics and Ecology **35**:557-565.

Tansley, S. A. and C. R. Brown. 2000. RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. Biological Conservation **95**:39-48.

Taylor, P. J., S. M. Eppley, and L. K. Jesson. 2007. Sporophytic inbreeding depression in mosses occurs in a species with seperate sexes but not in a species with combined sexes. American Journal of Botany **94**:1853-1859.

Telfer, M. G., C. Preston, and P. Rothery. 2002. A general method for measuring relative change in range size from biological atlas data. Biological Conservation **107**:99-109.

Telles, M. P. C., J. A. F. Diniz-Filho, R. P. Bastos, T. N. Soares, L. D. A. Guimaraes, and L. P. Lima. 2007. Landscape genetics of *Physalaemus cucieri* in Brazilian Cerrado: Correspondence between population structure and patterns of human occupation and habitat loss. Biological Conservation **139**:37-46.

Thomas, C. D. and J. J. Lennon. 1999. Birds extend their ranges northwards. Nature **399**:213-213.

Thomas, P. A. and J. R. Packham. 2007. Ecology of Woodlands and Forests. Cambridge University Press.

Thompson, F. 1970. Seasonal rainfall interception by Oak coppice (*Quercus robur L.*) in the leafy and leafless state in southern England. Aspects of forest climates. pp.E1-E11.

Thuiller, W., C. Albert, M. B. Araújo, P. M. Berry, M. Cabeza, A. Guisan, T. Hickler, G. F. Midgley, J. Paterson, and F. M. Schurr. 2008. Predicting global change impacts on plant species' distributions: future challenges. Perspectives in Plant Ecology, Evolution and Systematics **9**:137-152.

Thuiller, W., S. Lavorel, M. B. Araújo, M. T. Sykes, and I. C. Prentice. 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences of the United States of America **102**:8245-8250.

Tiessen, H., E. Cuevas, and P. Chacon. 1994. The role of soil organic matter in sustaining soil fertility. Nature **371**:783-785

Trautmann, W. 1976. Changes in the flora of woods and in the woodland vegetation of the Federal Republic of Germany in recent decades. Schriftenr. Vegetationsk **10**:91-108.

Tryon, R. 1986. The biogeography of species, with special reference to ferns. The Botanical Review **52**:117-156.

Tuba, Z., N. G. Slack, and L. R. Stark. 2011. Bryophyte ecology and climate change. Cambridge University Press.

Tunbridge Wells Borough Council. 2008. Tunbridge Wells Borough Council Biodiversity Habitat Action Plan. http://www.tunbridgewells.gov.uk/residents

/planning/conservation-and-heritage/natural-conservation-and-wildlife-sites/2

Tuomisto, H. and A. D. Poulsen. 1996. Influence of edaphic specialization on pteridophyte distribution in neotropical rain forests. Journal of biogeography **23**:283-293.

Tuomisto, H. and K. Ruokolainen. 1994. Distribution of Pteridophyta and Melastomataceae along an edaphic gradient in an Amazonian rain forest. Journal of Vegetation Science **5**:25-34.

Turkington, R., E. John, C. Krebs, M. Dale, V. Nams, R. Boonstra, S. Boutin, K. Martin, A. Sinclair, and J. Smith. 1998. The effects of NPK fertilization for nine years on boreal forest vegetation in northwestern Canada. Journal of Vegetation Science **9**:333-346.

Turner, P., J. Kirkpatrick, and E. Pharo. 2006. Bryophyte relationships with environmental and structural variables in Tasmanian old-growth mixed eucalypt forest. Australian journal of botany **54**:239-247.

UK BAP. 2010. UK Biodiversity Action Plan. List of priority species: www.ukbap.org.uk.

UKCIP. 2013. Climate Change Impacts. http://www.ukcip.org.uk/category/news/climate-change-impacts/

Van Calster, H., L. Baeten, K. Verheyen, L. De Keersmaeker, S. Dekeyser, J. E. Rogister, and M. Hermy. 2008. Diverging effects of overstorey conversion scenarios on the understorey vegetation in a former coppice-with-standards forest. Forest Ecology and Management **256**:519-528.

van Coller, A. L., K. H. Rogers, and G. L. Heritage. 2000. Riparian vegetation-environment relationships: complimentarity of gradients versus patch hierarchy approaches. Journal of Vegetation Science **11**:337-350.

Van der Ree, R., A. F. Bennett, and D. C. Gilmore. 2004. Gap-crossing by gliding marsupials:thresholds for use of isolated woodland patches in an agricultural landscape. Biological Conservation **115**:241-249.

Van Der Velde, M. and R. Bijlsma. 2003. Phylogeography of five Polytrichum species within Europe. Biological Journal of the Linnean Society **78**:203-213.

Vanderpoorten, A., N. Devos, B. Goffinet, O. J. Hardy, and A. J. Shaw. 2008. The barriers to oceanic island radiation in bryophytes: insights from the phylogeography of the moss Grimmia montana. Journal of biogeography **35**:654-663.

Vanderpoorten, A. and B. Goffinet. 2009. Introduction to Bryophytes. Cambridge University Press.

Venable, D. L. and J. S. Brown. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. American Naturalist **131**:360-384.

Verhoeven, J. T. A., W. Koerselman, and A. F. M. Meuleman. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends in Ecology & Evolution **11**:494-497.

Virtanen, R., A. Johnston, M. Crawley, and G. Edwards. 2000. Bryophyte biomass and species richness on the Park Grass Experiment, Rothamsted, UK. Plant Ecology **151**:129-141.

Virtaten, R. and J. Oksanen. 2007. The effects of habitat connectivity on cryptogam richness in boulder metacommunity. Biological Conservation **135**:415-422.

Vogel, J. C., F. J. Rumsey, J. J. Schneller, J. A. Barrett, and M. Gibby. 1999. Where are the glacial refugia in Europe? Evidence from pteridophytes. Biological Journal of the Linnean Society **66**:23-37.

Vormisto, J., O. Phillips, K. Ruokolainen, H. Tuomisto, and R. Vásquez. 2000. A comparison of fine-scale distribution patterns of four plant groups in an Amazonian rainforest. Ecography **23**:349-359.

Waite, S. 2000. Statistical Ecology: A practical guide. Prentice Hall, Harlow, UK.

Waite, S., N. Burnside, and A. Flint. 2010. Habitat diversity determines ghyll woodland species diversity. Adding complexity to the species area relationship, a new predictive model? http://eprints.brighton.ac.uk/8051/

Waller, M. P. and A. D. Marlow. 1994. Flandrian Vegetational History of Southeastern England - Stratigraphy of the Brede Valley and Pollen Data from Brede Bridge. New Phytologist **126(2)**:369-392.

Walther, G.-R. 2003. Plants in a warmer world. Perspectives in Plant Ecology, Evolution and Systematics **6**:169-185.

Walther, G.-R. 2010. Community and ecosystem responses to recent climate change. Philosophical Transactions of the Royal Society B: Biological Sciences **365**:2019-2024.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature **416**:389-395.

Wang, Z., S. An, H. Liu, J. Feng, F. Zhang, and X. Leng. 2006. Effect of stand age and management regime on genetic diversity of *Thuidium cymbifolium* in western China. Biological Conservation **129**:551-557.

Warren, M. 1984. The biology and status of the wood white butterfly, *Leptidea sinapis*(L.)(Lepidoptera: Pieridae), in the British Isles. Entomologist's Gazette **35**:207-224.

Warren, M. and R. Key. 1991. Woodlands: past, present and potential for insects. The conservation of insects and their habitats. pp.155-211. Proceedings of the 15th Symposium of the Royal Entomological Society of London. Edited by N.M. Collins and J. A. Thomas.

Watson, E. V. 1981a. British Mosses and Liverworts. 3rd edition. Cambridge University Press.

Watson, M. A. 1981b. Patterns of microhabitat occupation of six closely related species of mosses along a complex altitudinal gradient. Ecology **62**:1067-1078.

Webb III, T. 1992. Past changes in vegetation and climate: lessons for the future. pp.59-75 *in* Global warming and biological diversity. Edited by R.L. Peters and T.E. Lovejoy.

Weiher, E., S. Forbes, T. Schauwecker, and J. B Grace. 2004. Multivariate control of plant species richness and community biomass in blackland prairie. Oikos **106**:151-157.

Weir, B. S. 1996. Genetic Data Analysis II. Sinauer Associates Inc.

Wells, J. and D. Brown. 1996. Mineral nutrient recycling within shoots of the moss *Rhytidiadelphus squarrosus* in relation to growth. Journal of Bryology **19**:1-17.

Westaway, S. 2006. Weald Ancient Woodland Survey. A revision of the Ancient Woodland Inventory for Wealden District, East Sussex. Final Report 2006. Wealden District Council with High Weald AoNB Unit, Flimwell, East Sussex.

Westoby, M., E. Jurado, and M. Leishman. 1992. Comparative evolutionary ecology of seed size. Trends in Ecology & Evolution **7**:368-372.

Westoby, M., M. Leishman, J. Lord, H. Poorter, and D. J. Schoen. 1996. Comparative ecology of seed size and dispersal [and discussion]. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **351**:1309-1318.

Wiens, J. A. 1976. Population responses to patchy environments. Annual review of ecology and systematics **7**:81-120.

Wiens, J. A. 1997. The emerging role of patchiness in conservation biology. Pages 93-107 *in* The ecological basis of conservation. Edited by S.P.A. Pickett., R.S. Ostfeld., M. Shachak. and G.E. Likens. Springer.

Wiklund, K. and H. Rydin. 2004. Colony expansion of *Neckera pennata*: modelled growth rate and effect of microhabitat, competition, and precipitation. The Bryologist **107**:293-301.

Wilcove, D. S., C. H. McLellan, and A. P. Dobson. 1986. Habitat fragmentation in the temperate zone. Conservation Biology **6**:237-256.

Williams-Linera, G. 1990. Vegetation structure and environmental conditions of forest edges in Panama. The Journal of Ecology **78**:356-373.

Williams, J. G. K., A. R. Kubelik, and K. J. Livak. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research **18**:6231-6235.

Wilmanns, O., A. Bogenrieder, and W. Müller. 1986. Der Nachweis spontaner, teils autogener, teils immissionsbedingter Änderungen von Eichen-Hainbuchenwäldern. Eine Fallstudie im Kaiserstuhl/Baden. Natur landsch **61**:415-422.

Wilson, S. D. and D. Tilman. 2002. Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. Ecology **83**:492-504.

Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Keim, and T. G. Whitham. 2004. Conserving plant genetic diversity for dependent animal communities. Ecology letters **7**:776-780.

Wohlgemuth, T., M. Bürgi, C. Scheidegger, and M. Schütz. 2002. Dominance reduction of species through disturbance—a proposed management principle for central European forests. Forest Ecology and Management **166**:1-15.

Wooldridge, S. W. and F. Goldring. 1953. The Weald. Collins, London.

Worssam, B. 1995. The Geology of Wealden Iron. *in* The Iron Industry of the Weald. Edited by H. Cleere and D. Crossley. Merton Priory Press Ltd.

Wright, S. 1921. Systems of Mating. II. the Effects of Inbreeding on the Genetic Composition of a Population. Genetics **6**:pp124-143.

Wright, S. 1929. The evolution of dominance. The American Naturalist **63**:556-561.

Wright, S. 1931. Evolution in Mendelian populations. Genetics **16**:97.

Wright, S. 1969. Evolution and the Genetics of Populations, vol 2, The Theory of Gene Frequencies. Chicago University Press.

Wright, S. 1978. Evolution and the Genetics of populations. Vol. 4: Variability within and among Natural populations. University of Chicago Press, Chicago.

Wyatt, R. 1985. Species concepts in bryophytes: input from population biology. Bryologist **88**:182-189.

Wyatt, R. and L. E. Anderson. 1984. Breeding Systems of Bryophytes. pp.39-64. *in* The Experimental Biology of Bryophytes. Edited by D.H. Brown., A.F. Dyer. and J.G. Duckett. Academic Press NY.

Wyatt, R., I. J. Odrzykoski, and A. Stoneburner. 1989. High levels of genetic variability in the haploid moss *Plagiomnium ciliare*. Evolution **43**:1085-1096.

Xiong, S. and C. Nilsson. 1999. The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology **87**:984-994.

Yahner, R. H. 1988. Changes in wildlife communities near edges. Conservation Biology **2**:333-339.

Yeh, F. C. and R. C. Yang. 1999. POPGENE 1.32, Microsoft Windows based freeware for Population Genetic Analysis. University of Alberta and Tim Boyle, Centre for International Forestry Research.

Young, A. G. and G. M. Clarke. 2000. Genetics, demography and viability of fragmented populations. Cambridge University Press.

Young, K. R. and B. León. 1989. Pteridophyte species diversity in the central Peruvian Amazon: importance of edaphic specialization. Brittonia **41**:388-395.

Yu, F.-H., B. Krüsi, M. Schütz, J. J. Schneller, and O. Wildi. 2008. Plant communities affect the species-area relationship on *Carex sempervirens* tussocks. Flora - Morphology, Distribution, Functional Ecology of Plants **203**:197-203.

Zech, W., N. Senesi, G. Guggenberger, K. Kaiser, J. Lehmann, T. M. Miano, A. Miltner, and G. Schroth. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma **79**:117-161.

Zhang, H., Hardy, D., & Wang, J. 2010. Mehlich 3 Extraction for Macro- and Micro-nutrients in Soil test methods for the southeastern United States http://www.clemson.edu/agsrvlb/sera6/MethodsManualFinalSERA6.pdf

Zhu, Y.-Q., L. Liu, Y.-F. Wang, and X.-M. Shao. 2007. Genetic diversity and population structure of *Brachythecium rivulare* Schimp. (Brachytheciaceae) from Foping Nature Reserve, Shaanxi, China, detected by RAPD markers. Journal of Bryology **29**:104-110.

Zhuang, J., Y. Jin, and T. Miyazaki. 2001. Estimating Water Retention Characteristic From Soil Particle-Size Distribution Using A Non-Similar Media Concept. Soil Science **166**:308-321.

Appendix 1: Representative photos of some of the ghyll woodlands surveyed for Chapter 2

Horsegill Wood Ghyll streambed

Dallington Forest Ghyll stream

Joles Farm Ghyll stream

Windmill Wood Ghyll streambed

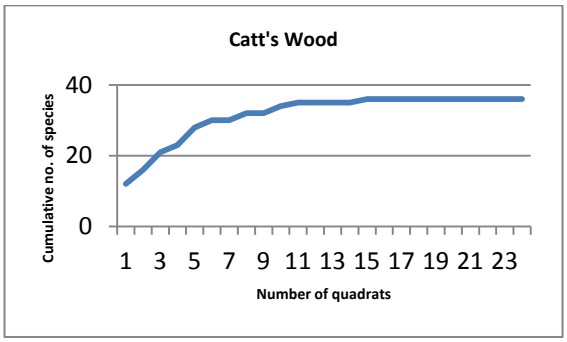
Pollardsland Wood Ghyll stream

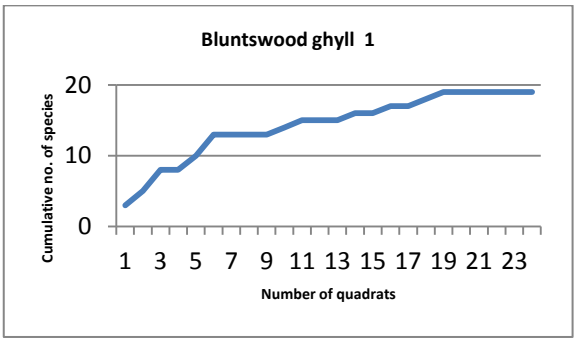
Cinderhill Wood Ghyll stream

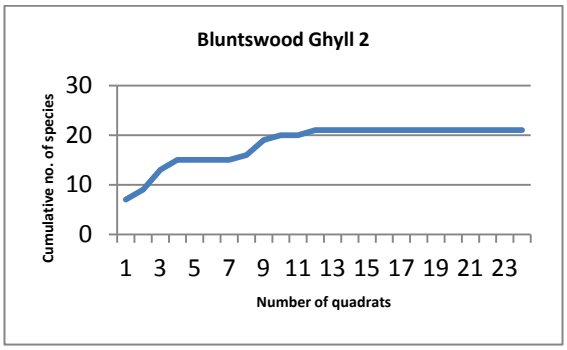
Forstal Wood Ghyll streambed

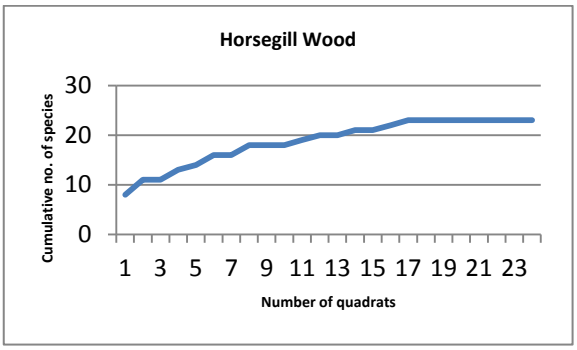
New England Wood Ghyll stream

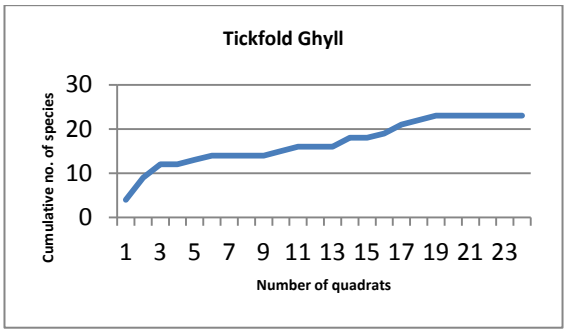
Appendix 2: Species accumulation curves – pilot study

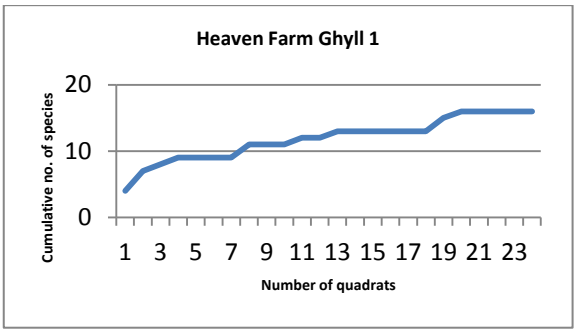


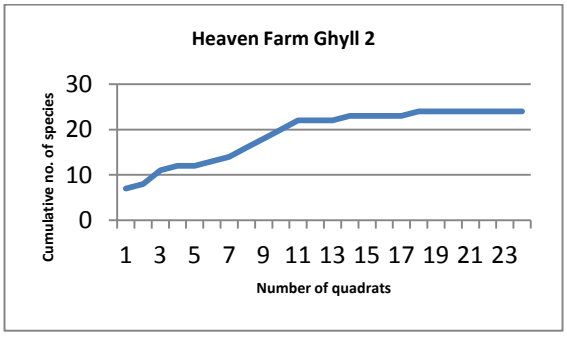


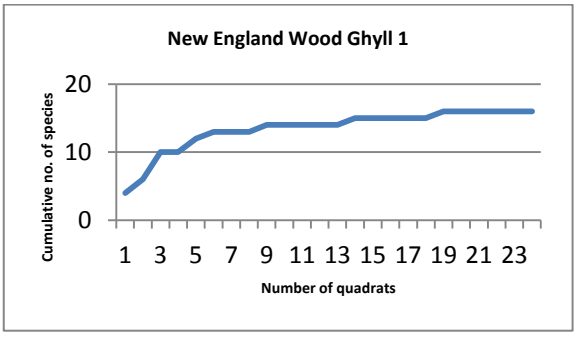


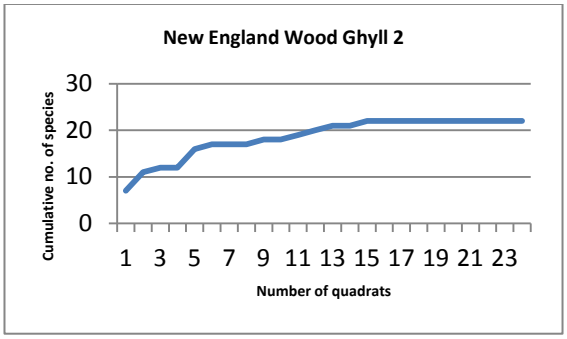


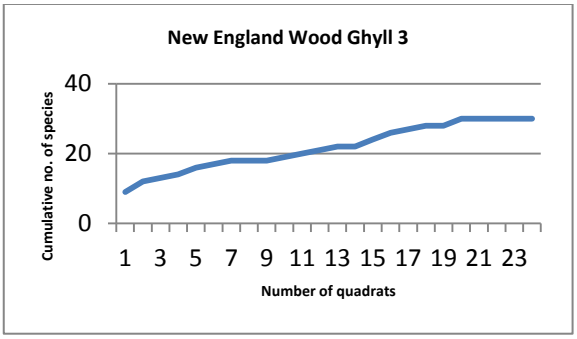




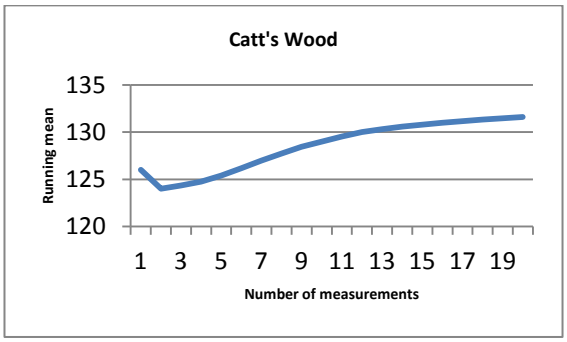


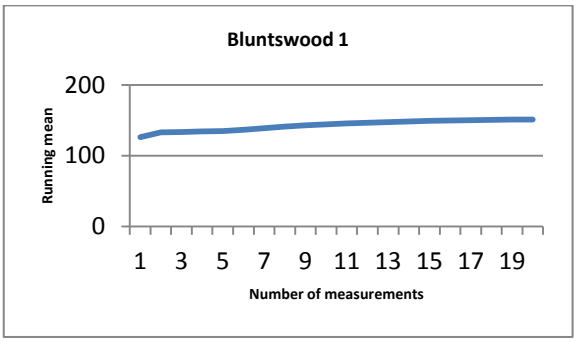


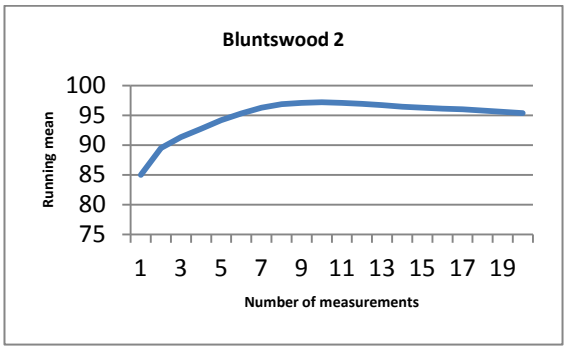


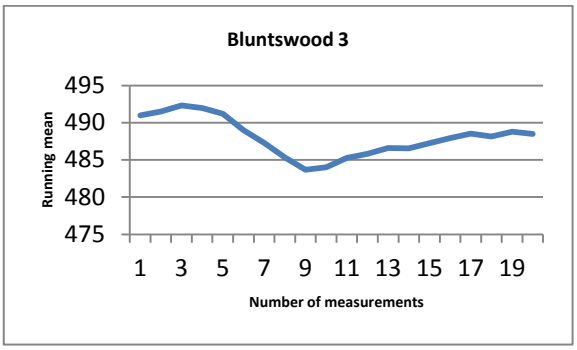


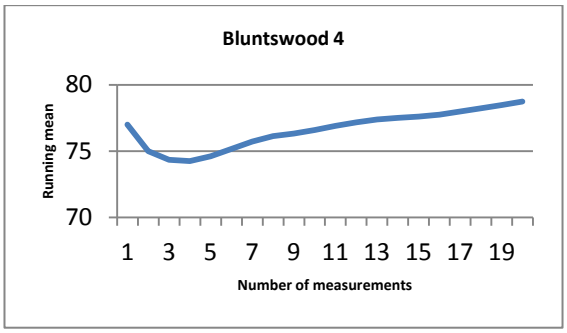
Appendix 3: Light levels – running mean pilot study

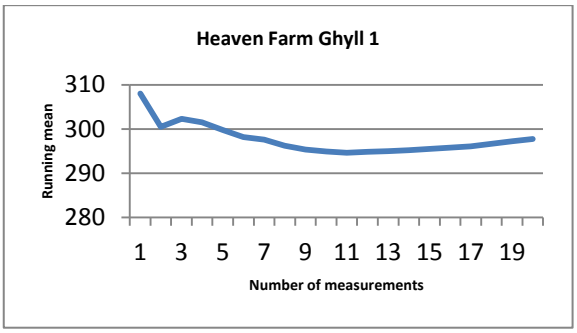


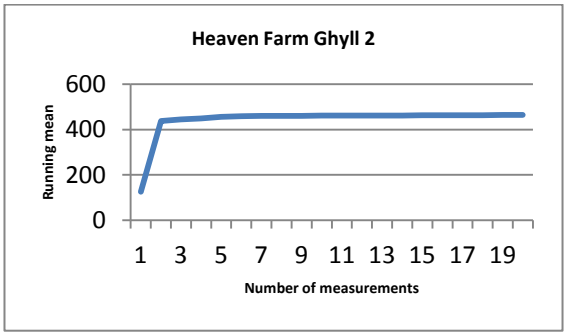


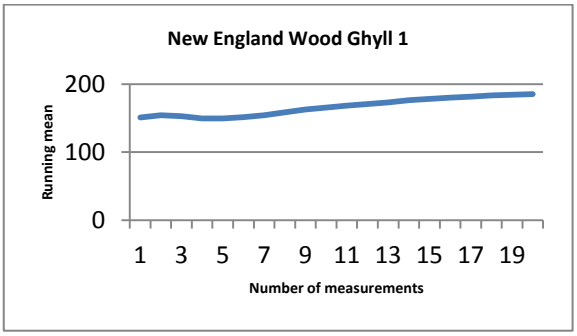


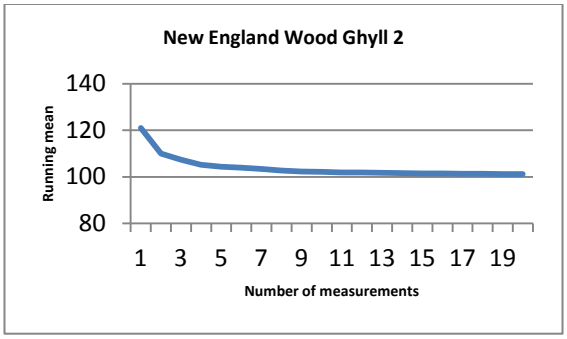


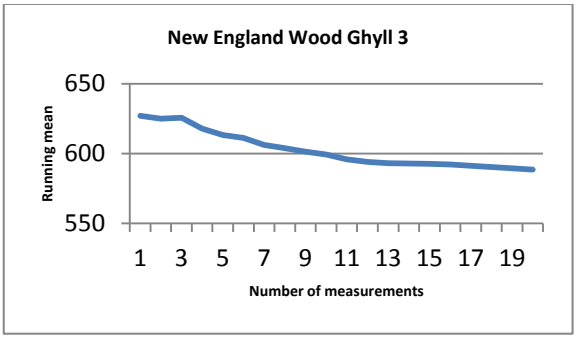




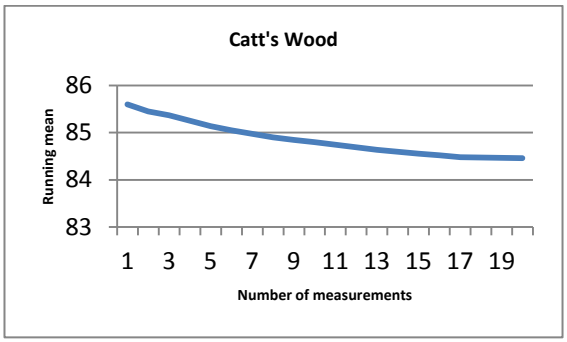


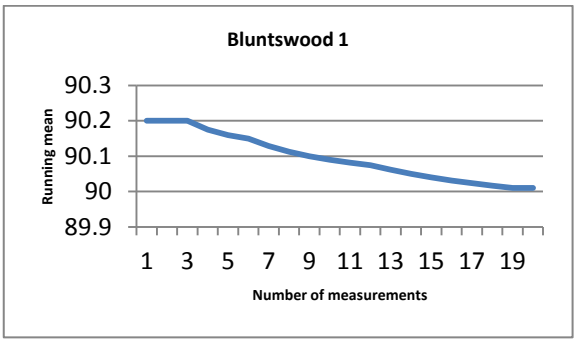


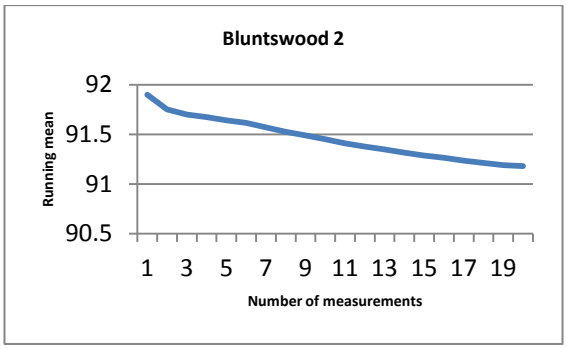


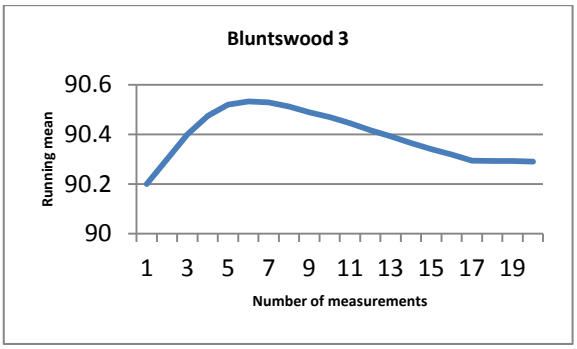


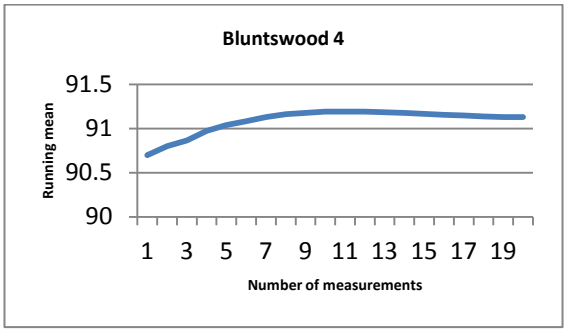
Appendix 4: Relative humidity levels – running mean pilot study

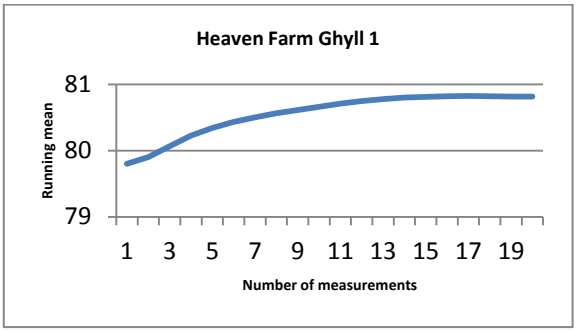


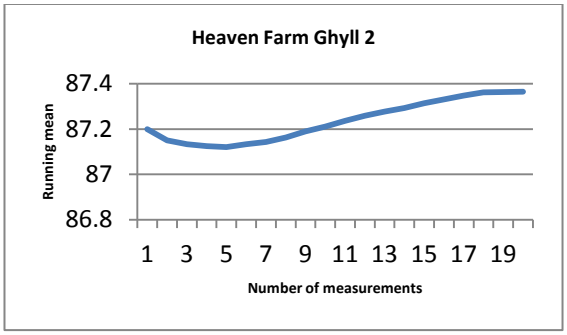


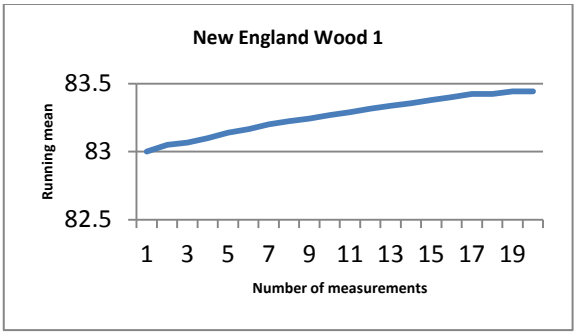


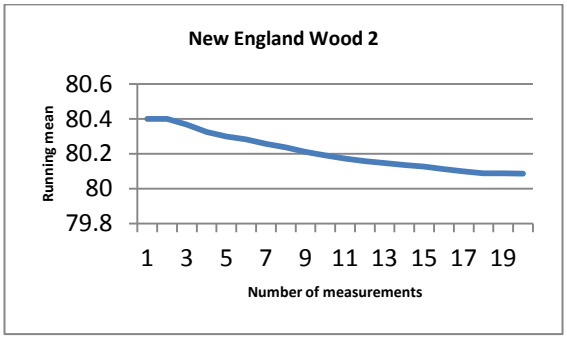


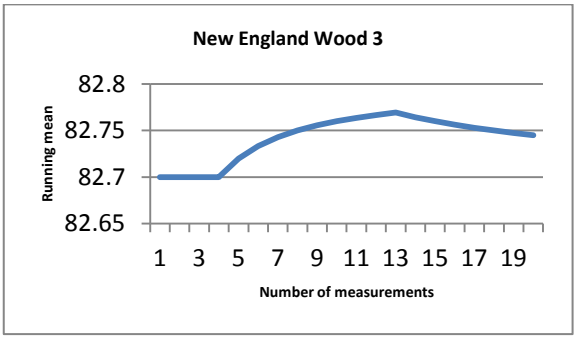




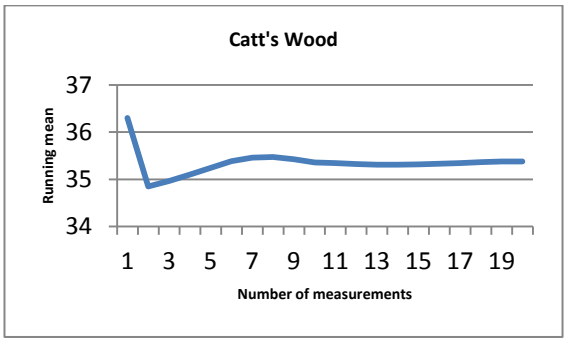


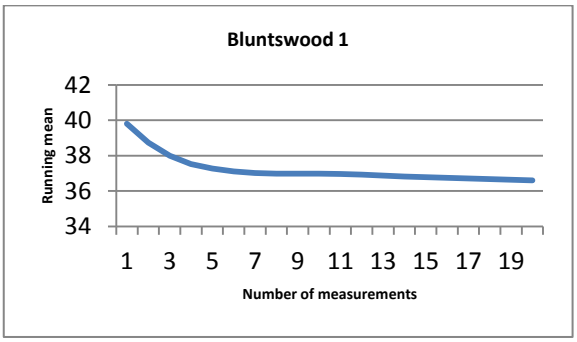


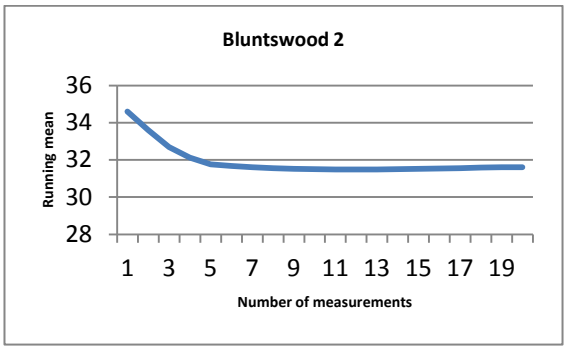


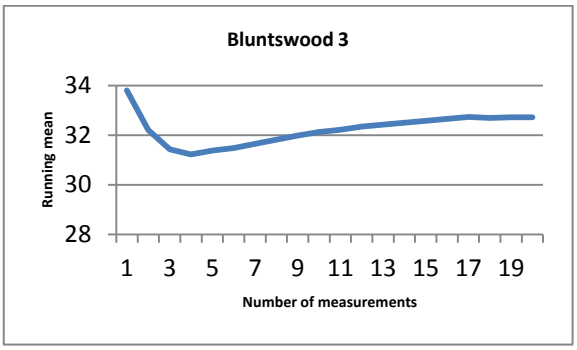


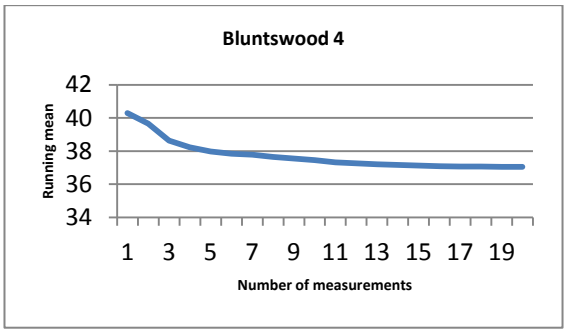
Appendix 5: Soil moisture levels – running mean pilot study

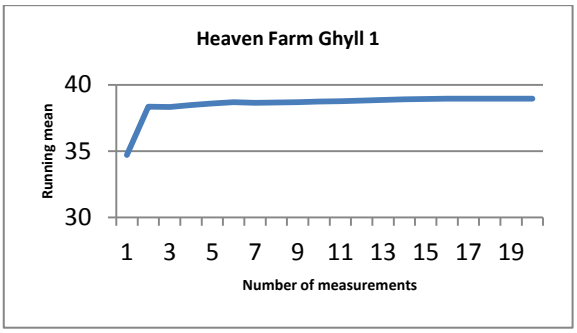


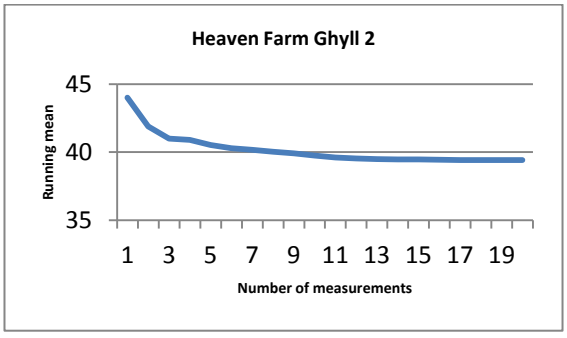


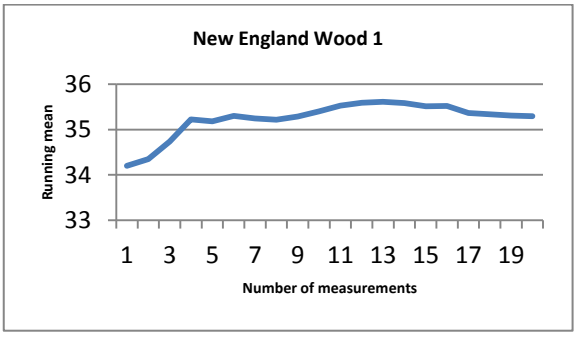


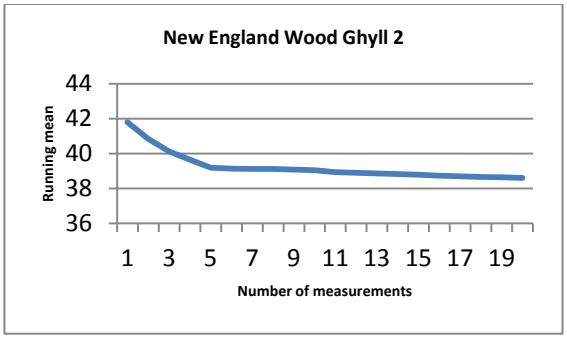




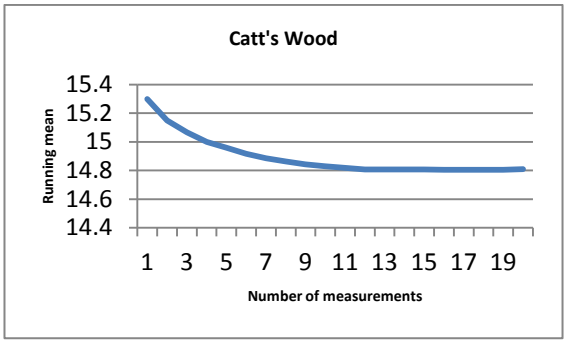


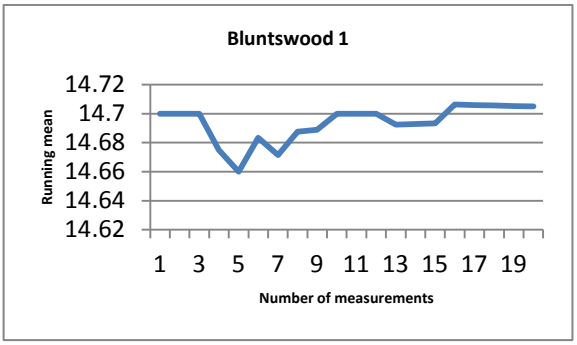


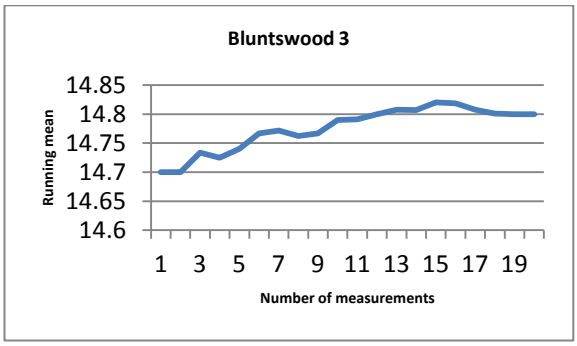


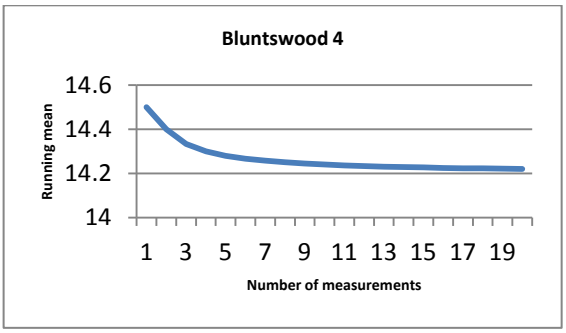


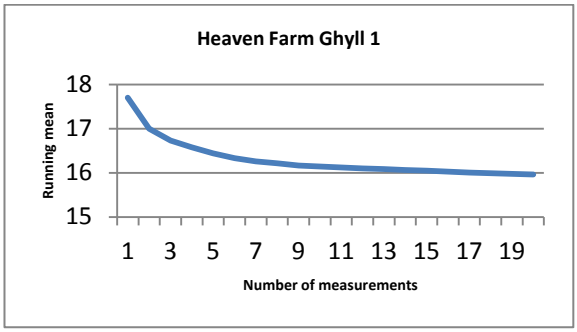
Appendix 6: Soil temperature levels - running mean pilot study

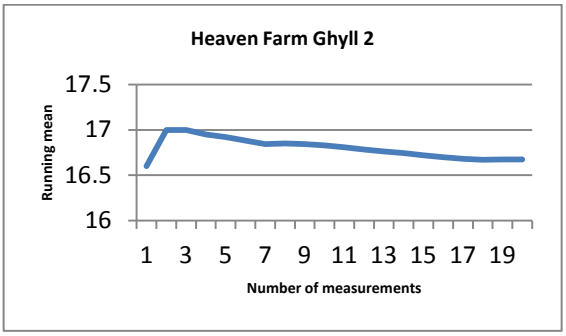


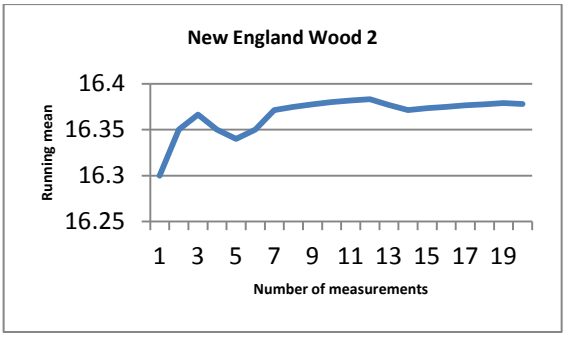




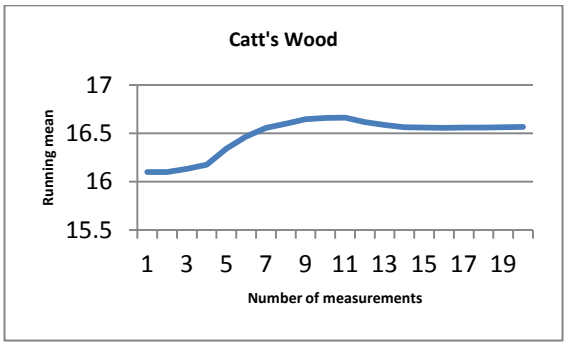


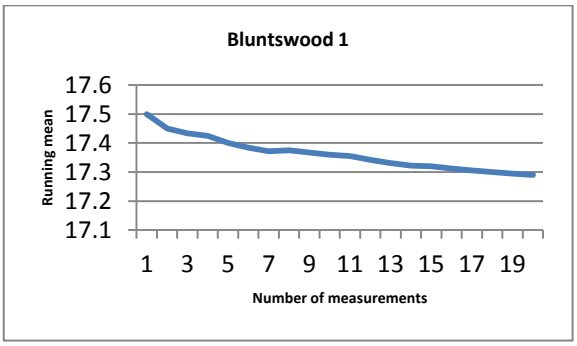


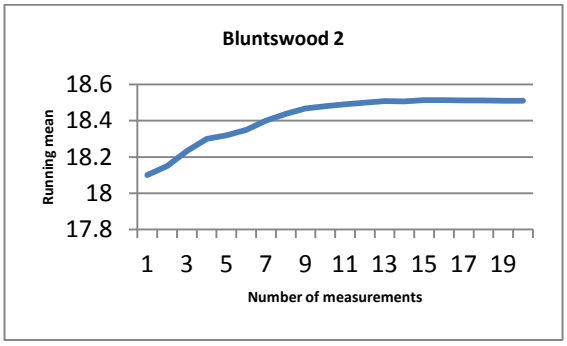


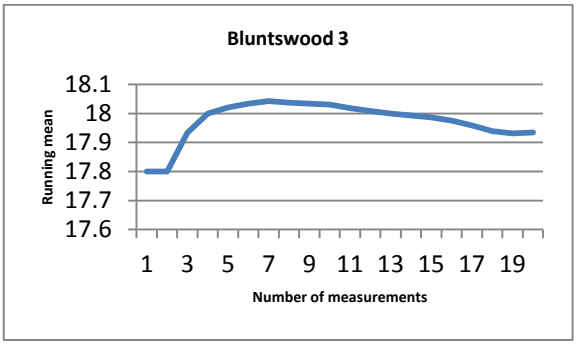


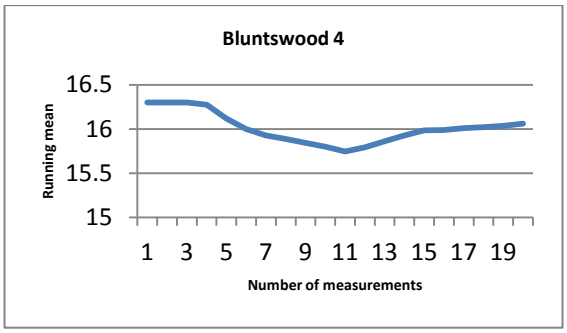
Appendix 7: Air temperature levels – running mean pilot study

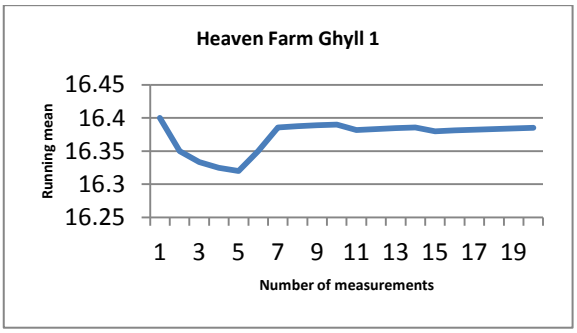


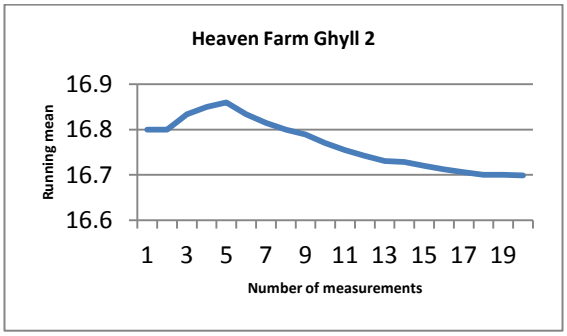


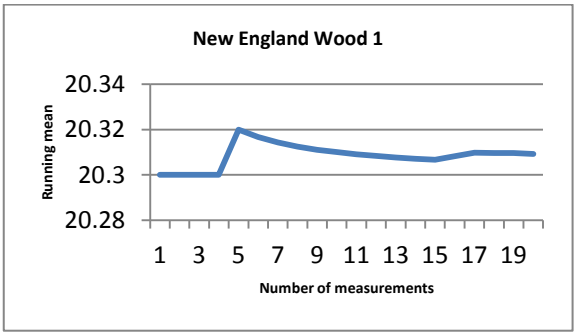


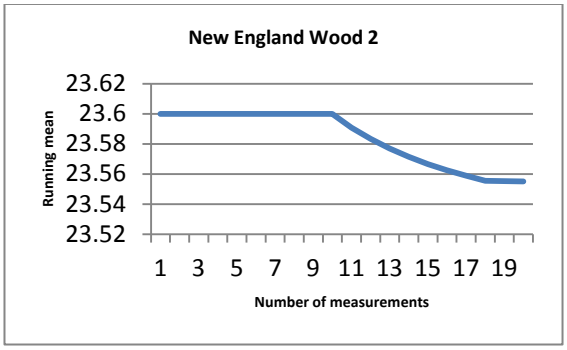












Appendix 8: Ghyll woodland survey sites 1951-1970

Survey site	Grid Ref	Year of survey
Bream Wood	TQ5232	1951
Duddlesworth Forest	TQ4528	1951
Nap Wood	TQ5832	1953
Balcombe Mill	TQ3130	1954
Hollybush Woods	TQ4327	1954
Old Mill Farm	TQ5822	1957
Sheffield Forest	TQ4226	1960
Old Mill Farm	TQ5822	1961
The Warren	TQ3131	1962
Great Wood	TQ5933	1963
Masketts Wood	TQ4227	1963
Burwash Weald	TQ6422	1965
Brenchley Wood	TQ6442	1966
Chiddingstone Hoath	TQ4942	1966
Hurst Wood	TQ7927	1966
Nap Wood	TQ4942	1966
Marline Wood	TQ7712	1966
Sandpit Wood	TQ8535	1966
Ashburnham Park	TQ6814	1967
Bassets Gill	TQ4941	1967
Bingletts Wood	TQ6221	1967
Brenchley wood	TQ6442	1967
Fairlight Glen	TQ8510	1967
Great Maytham Park	TQ8430	1967
Philpotts	TQ3532	1967
Sheepwash Gill	TQ2029	1967
Warren Glen, Fairlight	TQ8010	1967
Ashburnham Park	TQ6814	1968
Beauport Park	TQ7814	1968
Eridge Park	TQ5030	1968
Heathfield Park	TQ5921	1968
Hyde Gill	TQ2430	1968
St Leonards Forest	TQ2429	1968
Ashburnham Park	TQ6814	1969
Brenchley Wood	TQ6442	1969
Brook House Park	TQ3529	1969
Fairlight Glen	TQ8510	1969
Heathfield Park	TQ5920	1969
Paddockhurst Wood	TQ3233	1969
Lordship Wood	TQ7622	1970

Appendix 9: Ghyll woodland survey sites 1976-1995

Survey site	Grid Ref	Year of survey
Cow Wood	TQ2629	1976
Fairlight Glen	TQ8510	1976
Eridge Park	TQ5030	1977
Chiddingly Wood	TQ3432	1982
Chiddingly Wood	TQ3432	1983
Great Maytham Park	TQ8432	1984
Marline Wood	TQ7712	1984
Wakehurst	TQ3030	1984
West Wood	TQ3331	1984
Fore Wood	TQ7513	1985
Balcombe Mill	TQ3130	1986
Birchen Wood	TQ7217	1986
Four Acre Wood	TQ7010	1986
Keywards Wood	TQ5032	1986
Paddockhurst Park	TQ3233	1986
Wakehurst	TQ3332	1986
Ashes Wood	TQ7217	1987
Dallington Forest	TQ6520	1987
Sheffield Forest	TQ4226	1987
Cow Wood	TQ2629	1988
Eridge Park	TQ5030	1988
Furnace Wood	TQ4726	1988
Philpotts	TQ3532	1988
St Leonards Forest	TQ2629	1988
Wakehurst	TQ3030	1988
Ashburnham Park	TQ6814	1989
Ashdown Forest	TQ4328	1989
Sheepwash Gill	TQ2122	1989
Tickfold Gill	TQ1636	1989
Fairlight Glen	TQ8510	1990
The Spinney	TQ5619	1990
Fairlight Glen	TQ8510	1991
Wakehurst	TQ3030	1991
Darwell Wood	TQ7020	1992
Philpotts	TQ3532	1992
Paddockhurst Park	TQ3233	1994
Philpotts	TQ3532	1994
Wakehurst	TQ3030	1994

Appendix 10: Ancient woodland survey sites 1951-1970

Survey site	Grid Ref	Year of survey
Bream Wood	TQ5232	1951
Ashdown Forest	TQ4733	1952
Worth forest	TQ3032	1952
Hollybush Woods	TQ4327	1954
Worth forest	TQ3032	1954
Worth Forest	TQ3032	1954
Hoth Wood	TQ5631	1955
Roughets Wood	TQ5432	1955
Roughets Wood	TQ5432	1955
Darwell Wood	TQ6919	1957
Old Mill Farm	TQ5822	1957
Quarry Wood	TQ3235	1957
Rock Wood	TQ4726	1957
Mayfield	TQ5020	1961
Masketts Wood	TQ4228	1962
Waldron Down	TQ5322	1962
Foxholes Wood	TQ6223	1965
Birch Wood	TQ9140	1966
Brogues Wood	TQ8435	1966
Catsfield	TQ7213	1966
Finchbourne Wood	TQ9030	1966
Honeyfield Wood	TQ9030	1966
Parsonage Wood	TQ7932	1966
Smallmans Wood	TQ9030	1966
The Forest	TQ9342	1966
Waste Wood	TQ5223	1966
Widehurst Wood	TQ7442	1966
Bedgebury	TQ7234	1967
Brenchley Wood	TQ6442	1967
Burgh Wood	TQ7227	1967
Butness Wood	TQ9531	1967
Combwell Wood	TQ7133	1967
Kilndown Wood	TQ7035	1967
Broomham	TQ8515	1968
Buckhurst Park	TQ4834	1968
Collins Wood	TQ7020	1968
Combwell Wood	TQ7133	1968
Great Bounds	TQ5743	1968
Hyde Hill Wood	TQ2030	1968
St Leonards Forest	TQ2231	1968

Appendix 11: Ancient woodland survey sites 1976-1995

Survey site	Grid Ref	Year of survey
Ashburnham Park	TQ6814	1976
Worth forest	TQ3032	1976
Hungershall Woods	TQ5030	1979
Kilndown Wood	TQ7035	1979
Clayhill Wood	TQ6537	1980
Etchingly Wood	TQ5416	1981
Chiddingly Wood	TQ3432	1982
Idehurst	TQ0020	1983
Mills Rocks	TQ4137	1983
Sheffield Park	TQ4424	1983
Vert Wood	TQ5114	1983
Brinkwells	TQ0221	1984
Coomb Wood	TQ8927	1984
Wakehurst	TQ3331	1984
Ashdown Forest	TQ4332	1985
Fore Wood	TQ7512	1985
Ashes Wood	TQ7217	1987
Dallington Forest	TQ6020	1987
Forestry Commission Wood	TQ6020	1987
Saxonbury Hill	TQ5733	1987
Sheffield Forest	TQ4226	1987
Ditchling	TQ3010	1988
Furnace Wood	TQ4726	1988
Streat Hill	TQ3513	1988
Wakehurst	TQ3331	1988
Asburnham Park	TQ6814	1989
Bayham Stubbs Wood	TQ6537	1990
Chingley Wood	TQ6833	1990
Combwell Wood	TQ7133	1990
Coombe Holt	TQ1312	1990
The Mens	TQ0222	1990
The Spinney	TQ5619	1990
Copthorne Common	TQ3238	1991
Lodge Hill	TQ3215	1991
Old Deer Park	TQ2225	1993