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Epiphyte diversity on Scottish aspen – a component of the extended phenotype



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Declaration:

I hereby declare that the work contained in this thesis is my own, unless otherwise acknowledged and cited. This thesis has not in whole or part been previously presented for any degree.

Chantel Davies

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August 26, 2011

The image on the title page is of the expanded male flowers of aspen (*Populus tremula*), and was taken in Scotland by Ernest Emmett (Highland Aspen Group).

Abstract

Species interactions are recognised as an important evolutionary process, with foundation species in particular being of exceptional importance. Foundation species are those species exhibiting dynamic physical traits, under strong genetic control, that shape the natural processes of habitats and ecosystems. These traits lead to extended consequences for the associated organisms in their community. Therefore, intra-specific variation of a foundation species can have important evolutionary consequences for habitats, communities and entire ecosystems.

One such foundation species is aspen (*Populus tremula* L.), which has important conservation value, particularly for the high diversity of associated species. In Scotland aspen exists in fragmented clonal patches, but has been found to contain a high diversity of associated organisms some of which have a UK Biodiversity Action Plan (BAP). One such group of organisms of high diversity and conservation value in Scotland are the epiphytic cryptogams (i.e. mosses, liverworts, lichens). To date more than 300 species have been recorded on aspen in Scotland, comprising approximately 40% of the epiphyte flora of Europe.

The research presented here uses a combination of natural aspen system and two aspen common gardens to test the effects of aspen genetic diversity on physical traits potentially important for epiphyte diversity. The traits investigated were bark texture and bark phenolic chemistry. Bark texture in the wild clones was found vary significantly between clones and under strong genetic control (up to 40%). Bark phenolic chemistry also showed significant genotypic variation, but could not be correlated with patterns of epiphyte species richness and diversity. Nevertheless, epiphytes showed significant patterns related to aspen genotype, particularly along a gradient of bark texture. The results indicate that epiphyte communities are part of the 'extended phenotype' of native aspen populations in Scotland are very important for maintaining current levels of epiphyte diversity. A greater diversity and abundance of aspen genotypes in the landscape are essential for increasing epiphyte species richness and diversity, and for ecosystem health as a whole.

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The use of aspen at Invertromie was authorized by Pete Moore (SNH Achantoul), Karen Sutcliffe and Pete Moore (RSPB Insh Marshes); the use of the experimental aspen clones at Moray and Kilmichael was authorized by Alan Harrison (Forest Research, Bush Estate) with permission from the landowners.

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Fungus took a lichen to an alga…

1 Introduction to ecological effects of interactions at the species level

Ecology is concerned with understanding the distribution and abundance of living organisms, and focuses on the interactions that occur among organisms, and between organisms and their physical environment. A recurring theme in ecology is that the patterns of species distributions and abundances are shaped not only by edaphic and environmental factors, but also by interactions with other organisms. How one species interacts jointly with the environment and with another species has profound extended consequences for the composition of communities and potentially ecosystems (Whitham et al. 2003). A well-researched illustration of this can be found in the predator-prey relationship between bears (*Ursus* spp.) and salmon (*Onchorynchus* spp.). Salmon is a particularly important food source for brown bears (*Ursus arctos*), being positively correlated with female body mass, breeding capability, litter size and population density (Hilderbrand et al. 1999). During spawning season, salmon return from oceanic environments to the rivers they originally spawned from to continue their life cycle. Much of the energy obtained from the ocean is retained within migrating salmon populations and returned to the rivers they spawned from. There is a dramatic increase in body fat (up to 85% increase) and protein (up to 40% increase) from the moment salmon arrive at their destination to senescence several weeks later (Gende et al. 2004). Bears populating these rivers in order to feed have been found to select for larger, male salmon, thus potentially reducing the average body size of salmon that survive long enough to breed. Smaller salmon are generally less fecund, and in the case of no or partial spawning, there is a consequence of reduced paternity for offspring, potentially reducing genetic diversity (Reimchen, 2000; Quinn & Buck, 2001). This energy is either consumed by bear populations near the site with the remains decomposing into the water system, or used for spawning before death occurs.

The commonest way of exploring interactions among organisms and their effects on communities and ecosystems is to focus on interactions that occur between pairs of species. These interactions may involve competition, mutualism, herbivory,

parasitism, mutualism or synergistic interactions and can occur between organisms at the same or different trophic levels. It has been recognised that individuals within species are not uniform, but differ genetically from one another. Genetic variation among individuals within a species can affect phenotypic traits that may be important in determining their interactions with other organisms. For instance genetic variation in secondary chemistry of plants influences their interaction with herbivores with important ecological consequences. A good example of this is provided by recent studies of the effects of monoterpenes in Scots pine (*Pinus sylvestris*) and a range of herbivores, such as slugs (*Arion ater*), capercaillie (*Tetrao urogallus*), bank voles (*Myodes glareolus*) and red deer (*Cervus elaphus*) (Iason et al. 2011). Grazing experiments were carried out on each herbivore type using a range of Scots pine trees that had been verified as having variable monoterpene concentration (high, medium, or low). Of the thirteen monoterpenes selected to test herbivore response, α-pinene, the most abundant monoterpene in Scots pine and with strong genetic correlation to other monoterpenes, successfully deterred slugs and capercaillie. Iason et al. (2011) concluded that by avoiding those pines with higher concentrations of α-pinene, slugs and capercaillie are selecting for higher abundance of a specific monoterpene.

The outcome of interactions is often to change the genetic composition of one species as a consequence of natural selection. Ultimately this may affect the population size of that species e.g. adaptation of pests to pesticides first brings about resistance evolution in the pest population, then an increase in the pest population as resistant individuals rise in frequency in the population. A well-known example of this is the case of myxomatosis-resistance in wild rabbit (*Oryctolagus cuniculus*) populations in Europe and Australia (Ross & Sanders, 1977 & 1984; Aparicio et al. 2004). Originally introduced in the 1950s in Great Britain to control high numbers of wild rabbits, the myxoma virus was incredibly effective in killing an estimated 99% of the total rabbit population that were exposed. By the 1970s increasing resistance had been recorded in some wild British populations (Ross & Sanders, 1984), with a greater level of resistance recorded at least a decade earlier in Australian populations (Aparicio et al. 2004). Several strains of the original virus have evolved, but so too has rabbit resistance to the virus, demonstrating a host-

parasite co-evolutionary process. This evolution of host resistance explained a rise in rabbit population sizes in both areas.

1.1 Ecological interactions and the extended phenotype

Another situation where genetic variation within a species has the potential to affect the distribution and abundance of associated organisms is where that species plays the role of a foundation species in the community. Foundation species are those that are perceived to provide important structure to a community and usually represent the species with the greatest abundance or biomass in the community (Bruno et al. 2003). They may constitute a major food source, such as kelp (*Macrocystis pyrifera*) in ocean environments, possessing physical traits that regulate ecosystem processes and provide a habitat for associated, dependent, communities (Graham, 2004). Kelp forests have a long-standing reputation for providing one of the largest and most important foundation habitats on the planet (Mann, 1973; Steneck et al. 2002), with high diversity of associated species - more than 200 species in southern California alone (North, 1971; Foster & Schiel, 1988). An important consequence of this relationship is that genetic variation in a foundation species could potentially have a large influence on the community of organisms with which it interacts. For instance if the foundation species provides the habitat for a community of dependent organisms, changes in the physical attributes of the foundation species may modify the habitat of the dependent species, and this may alter the community of dependent species that can be supported.

This effect has been demonstrated in common garden experiments involving the flowering plant, goldenrod (*Solidago altissima*). Here genetic variation between goldenrod genotypes led to variation among their associated arthropod communities (Maddox & Root, 1990). Using the same foundation plant species, high genetic diversity was revealed in the phenotypic traits of flowering time and floral abundance, as well as in the extended phenotype traits of flower visitor abundance and richness (Genung et al. 2010). These studies demonstrate the importance of genetic variation in dominant species, and their potential influence for structuring communities of dependent organisms. In the case of pollinators, increased insect

diversity in a natural habitat where *Solidago* is abundant could positively influence pollination for other plant species and promote genetic diversity within the community.

The phenotype of an organism is its outward appearance and is determined by an interaction between its genotype and the environment. For instance aspects of a tree's phenotype would normally be characters such as the shape of its leaves, the morphology of its bark and so on. However if we are dealing with foundation species, and if the genotype of the foundation species influences the community of dependent organisms then the dependent community could be considered as part of the phenotype of the foundation species, or its 'extended phenotype'. For instance if genetic variation affecting leaf shape/chemistry in a tree alters the realised niche for associated insects with community consequences, then the variable insect community found on a tree could be thought of as an aspect of the extended phenotype of that tree. The term 'extended phenotype' was first coined by Richard Dawkins (1982) to describe the effects of an organism's genetic constitution on its community of associated organisms.

1.1.1 Validating and testing the extended phenotype concept

Many studies have been conducted to explore and test the validity of the extended phenotype concept and to assess the importance of genetic variation in foundation species on their communities of dependent organisms. Studies have typically used plants as the foundation species, and insects as the dependent species. Johnson & Agrawal (2005) investigated the importance of plant genotype and environmental factors at differing spatial scales on a diverse arthropod community. Fourteen genotypes of *Oenothera biennis* (total of 926 plants) were planted into five natural habitats representative of the environments in which this plant occurs. Plant genotypic differences accounted for as much as 41% of the variation in arthropod diversity, evenness, richness, abundance and biomass on individual plants, and explained more arthropod community variation than environmental variation across spatial scales. Arthropods appeared to select for certain traits of *O. biennis*, which may lead to important evolutionary changes in associated insect communities should the plants undergo significant genetic change. Bangert et al. (2006a) demonstrated

how genetic variation in hybridizing cottonwood (*Populus* spp.) affects the structure of an associated arthropod community. One important plant trait tested as a possible intermediate link between plant genetics and arthropod community structure was chemical composition of the leaves. In both a common garden experiment of 29 different genotypes (F_1 hybrids and backcrosses) and 25 of the same genotypes within wild populations, trees with similar genetic compositions had similar chemical compositions and similar arthropod compositions.

Barbour et al. (2009b) furthered this research by examining potential genetic effects on the relative thickness of living bark and characteristics of decorticating bark in common garden experiments of *Eucalyptus globulus*. Twenty trees from each of five provenances were selected at random from a common garden trial established in northern Tasmania. Trees were sampled at 17 years old, and 25m in height. Of the ten bark traits measured, significant variation was found in eight of the traits and attributed to genetic differences among provenances (ranging from p <0.001-0.02). Overall, decorticating bark decreased with height up the trunk. Sampling macro-arthropod communities living within the bark revealed a significant effect of host tree genotype on species richness and abundance, with 60% of the overall variation in community attributable to differences in bark structure associated with *Eucalyptus* provenance.

However, contradictory results were found by Tack et al. (2010), who showed how genetic effects of a host plant on associated insect communities tend to be diluted at increasing spatial scales. Herbivorous insects on oak (*Quercus robur*) were sampled in a common garden experiment replicated at three spatial scales: landscape scale (~5 km^2); regional scale (~10,000 km^2); and wild trees at various spatial scales. Their sampling revealed that 32% of the species richness at the landscape scale was explained by spatial correlations, with this effect increasing at the regional and greater scales. Host plant genotype was considered to be of secondary importance.

Genetic effects have been specifically looked for in forest systems where trees are foundation species and associated insects in particular are dependent species. For example, the resistance or susceptibility of *Pinus* spp. (foundation) to grazing by gypsy moth can significantly influence the distribution of nearly 1000 dependent

organisms, including birds, mammals, invertebrates and fungi (Brown et al. 2001; Whitham et al. 2003; Kuske et al. 2003; Diner et al. 2009).

Forest trees are particularly important as foundation species because they provide habitats for many different types of organisms. Zytynska et al. (2011) demonstrated the effects of within-species variation in *Brosimum alicastrum* growing in a tropical forest system, on associated epiphytic plants and arthropod communities. Genetic effects of *Populus* spp. have been found for important ecological processes. Schweitzer et al. (2008) and Madritch et al. (2009) both used a model *Populus* system in a common garden (Schweitzer et al. 2008) and a natural system (Madritch et al. 2009) to evaluate effects of plant genotype on soil processes, such as soil nutrients (i.e., nitrogen) and microbial biomass. In both cases, plant genotype explained variation in soil nitrogen, and microbial activity. In the common garden experiment, genotype explained up to 78% of the variation in microbial biomass. In the natural system, Madritch et al. (2009) used 24 distinct *Populus tremuloides* clones (multiple ramets per clone) spanning a distance of 25km^2 to demonstrate the effects of plant genetic identity on soil processes. Sampling leaf chemistry, soil nutrient content and microbial activity beneath each clone revealed significant variation between genotypes for each measured variable. Genetic distance between clones was correlated with differences in ecosystem processes, suggesting that aspen are capable of modifying their local environment and thereby regulate ecosystem functions.

1.2 Thesis aim

These studies provide emerging evidence that genetic variation within a foundation species can influence a dependent community. Investigations linking the genetics of foundation species with dependent organisms may provide a powerfully integrative framework in community ecology. This thesis extends previous work to a new model system, by considering the effect of host tree genetic structure on cryptogamic epiphytes – an important component of forest biodiversity and ecosystem function in temperate and boreal ecosystems. A single study has provided evidence that a particular aspect of the phenotype, bark texture, influenced the distribution and

abundance of a single, dependent epiphyte species (Lamit et al. 2011). The challenge remaining is to demonstrate not only that genetic variation within a foundation species can influence the community of dependent species, but also to demonstrate how variation in the phenotype of the foundation species is translated into differences in community composition of dependent organisms. This is the major objective of this thesis.

1.3 Study system

An ideal system for achieving this objective comprises the foundation tree, aspen (*Populus tremula*), and its dependent community of epiphytic lichens and bryophytes. Aspen is a particularly suitable foundation species to study because it is known to show extensive genetic variation within Scotland (Easton, 1998). It also has the important attribute of reproducing clonally from suckers. Thus, there are replicate stems possessing the same genotype within a population. This makes it possible to test the significance of differences in phenotypic traits and dependent communities among genotypes within natural populations, using multiple stems per genotype to demonstrate genetic variation. Associated with aspen as dependent species is a particularly rich and important lichen and bryophyte flora with conservation interest (Ellis & Coppins, 2006). More than 300 lichen species have been recorded on Scottish aspen (B. J. Coppins & C. J. Ellis, unpublished data). Of these species, twenty-one are Nationally Scarce (recorded in only 16-100 10km squares); twelve are Nationally Rare (recorded in only 15 or fewer 10km squares); three species are listed on Schedule 8 of the Wildlife and Countryside Act 1981[1]; five species are Red Data Book listed (Vulnerable); two species are Red Data Book listed (Critically Endangered) (Street & Street, 2002). One species, the aspen specialist *Lecanora populicola,* was Red Data Book listed (Extinct) until ca. 2003 when it was discovered in abundance on aspen in Strathspey (Ellis, pers. comm.). Rothero (2001) cites aspen in Scotland as an important habitat for epiphytic bryophytes. Approximately five species of liverwort and twenty-nine species of moss have been recorded on aspen in Scotland. Most noteworthy are those

[1] Protected from intentional picking, uprooting or destruction

belonging to the genus *Orthotrichum.* This genus comprises a group of nationally scarce 'pin-cushion' mosses that can grow in 'tufts' longitudinally on tree trunks. Aspen has been found to host several species of this genus, most notably *O. gymnostomum.* The related species, *O. obtusifolium* is Schedule 8 listed and found on several natural aspen stands in the Strathspey area. Furthermore, aspen in Strathspey are the 'centre of distribution for the nationally rare *Orthotrichum speciosum*.'

Aspen is a foundation species for lichens and bryophytes because it provides the habitat where these epiphytes grow. Specific physical traits of aspen that will influence the habitat relevant to epiphytes are bark texture and bark secondary chemistry. The latter trait is known to have defensive properties and there exists potential for interaction between bark chemistry and epiphytic community structure. In this thesis the objective will be to explore the aspen/epiphyte system to establish:

1. Whether there are differences among aspen clones in important aspects of phenotype such as bark texture and bark chemistry;

2. Whether there are differences among clones in epiphytic communities of lichens and bryophytes, and

3. Whether the measured aspects of aspen phenotype are the cause of any observed differences in epiphyte communities.

1.3.1 Aspen biology, distribution and ecology

Populus tremula L. is a native deciduous tree in the Salicaceae family, with a distribution across Europe (including Britain), North Africa, through temperate Asia and Japan (Brickell, 1999; MacKenzie, 2010). In Scotland, aspen is found in the small areas of the northeastern Highlands (Strathspey). The BSBI hectad distribution map for aspen shows it as being widespread (Fig. 1.1); in reality it is only locally abundant at certain sites, with large populations in Strathspey being unusual in Britain (C. Ellis, pers. comm.). Clapham et al. (1968) and Stace (1997) describe aspen as a tree to 20m, sometimes to 24m, which suckers freely, with pale, greenish-grey bark appearing very smooth on young trees with dark grey diamond-shaped lenticels, becoming dark grey and fissured on older trees. The bark is photosynthetic, sometimes with a whitish or grey bloom of periderm cells in a

constant state of being sloughed off (Covington, 1975), as illustrated in Figure 1.2. Buds are generally glabrous, sometimes with a sticky residue. Leaves of mature shoots are 2.5-6cm, orbicular and generally rounded at the base with serrated edges. Initially leaves are pubescent but becoming glabrous with age; petioles are flattened, giving rise to the characteristic fluttering of the leaves in wind. Leaves of suckering shoots can be up to 15cm, ovate to cordate with greyish pubescence on the underside. The flowers are wind-pollinated catkins produced in early spring before the new leaves appear; they are dioecious, with male and female catkins on different trees. The male catkins are patterned green and brown, 5–10 cm long when shedding pollen; the female catkins are green, 2–4 cm long at pollination, maturing in early summer to bear 10–20 capsules each containing numerous tiny seeds embedded in downy fluff. The fluff assists wind dispersal of the seeds when the capsules split open at maturity. Aspen usually flowers from February to March, and is dioecious having male and female flowers on separate trees. Flowering events are rare in locations where summers are cool, but not unknown, therefore trees may have adapted to reproduce vegetatively by suckers. Examples of mature aspen and aspen suckers are illustrated in Figure 1.3 a-c.

The clonal patch is referred to as a 'genet', with each individual stem known as a 'ramet'. Ramets of suckering *Populus* spp. are initially dependent upon the main rootstock for translocation of all nutrients, solutes and hormones, but will eventually become independent and form their own rootstocks (Barnes, 1966). Within a genet many suckers may be generated, but not all will survive, and as they die off their roots can decay leaving surviving suckers with root systems largely or entirely independent of the root system it originated from; independence varies across genotypes, but can occur as early as 8 years from initial development (Shepperd, 1993), up to 25 years (Barnes, 1966). Evidence for whether ramets remain interconnected or not varies significantly, and may be dependent on disturbance from fire and burrowing/ grazing mammals damaging the root connections (Cottam, 1954; DeByle, 1964; DesRochers & Lieffers, 2001). The average lifespan of a ramet is estimated to be <80 years (Bean, 1976), though ages of up to 150 years have been suggested for populations in the Highlands of Scotland (MacGowan, 1997). Pollen

records indicate that aspen has been present in the UK since ca. 9000 years BP, though it is unknown whether the current populations arrived here after the end of the last glacial period, or if they are relicts of a pre-ice age community (MacKenzie, 2010). Aspen is tolerant of a wide range of soils and environmental conditions (Brickell, 1999), and has many uses in horticulture (i.e., shelterbelts: Bean, 1976), and forestry (pulp, timber, biofuel: Johansson, 2002).

Despite being fragmented and poorly represented in woodlands, aspen has been recognised as being of high conservation value, due to the high diversity of associated species, including epiphytic lichens and bryophytes. Rotheray (2002) recorded 39 insect species on aspen, including 14 Red Data Book species, three of which are Category 1 (endangered). The rare aspen hoverfly (*Hammerschmidtia ferruginea*) is a UK Biodversity Action Plan (BAP) Priority species, and has been recorded in abundance on rotting aspen logs (Rotheray, 2009). Up to 43 Lepidopteran species have been recorded on aspen in Scotland, 17 of which are aspen-specialists (Prescott & Stubbs, 2009). The larvae of one rare species in particular, the Dark-bordered Beauty moth (*Epione vespertaria*), feeds exclusively on the young leaves of suckering aspen and therefore its survival is intimately linked with the presence of aspen in the landscape.

1.3.1.1 Variation within aspen

Due to its reproductive strategy, many *Populus* spp., including Scottish aspen (*Populus tremula*), develop into clonal patches of various sizes. Clones correspond to different genotypes that vary for a range of physical and physiological traits, such as phenology (Yu et al. 2001), nutrient storage (Yu et al. 2001; Lindroth et al. 2002; Donaldson et al. 2006; Osier & Lindroth, 2006), chemical defences (Lindroth & Hwang, 1996; Orians et al. 1996; Osier & Lindroth, 2001, 2004, 2006; Lindroth et al. 2002; Donaldson et al. 2006; Donaldson & Lindroth, 2007), biomass production (Rytter & Stener, 2003; Christersson, 2006) and leaf litter decomposition (Madritch et al. 2006).

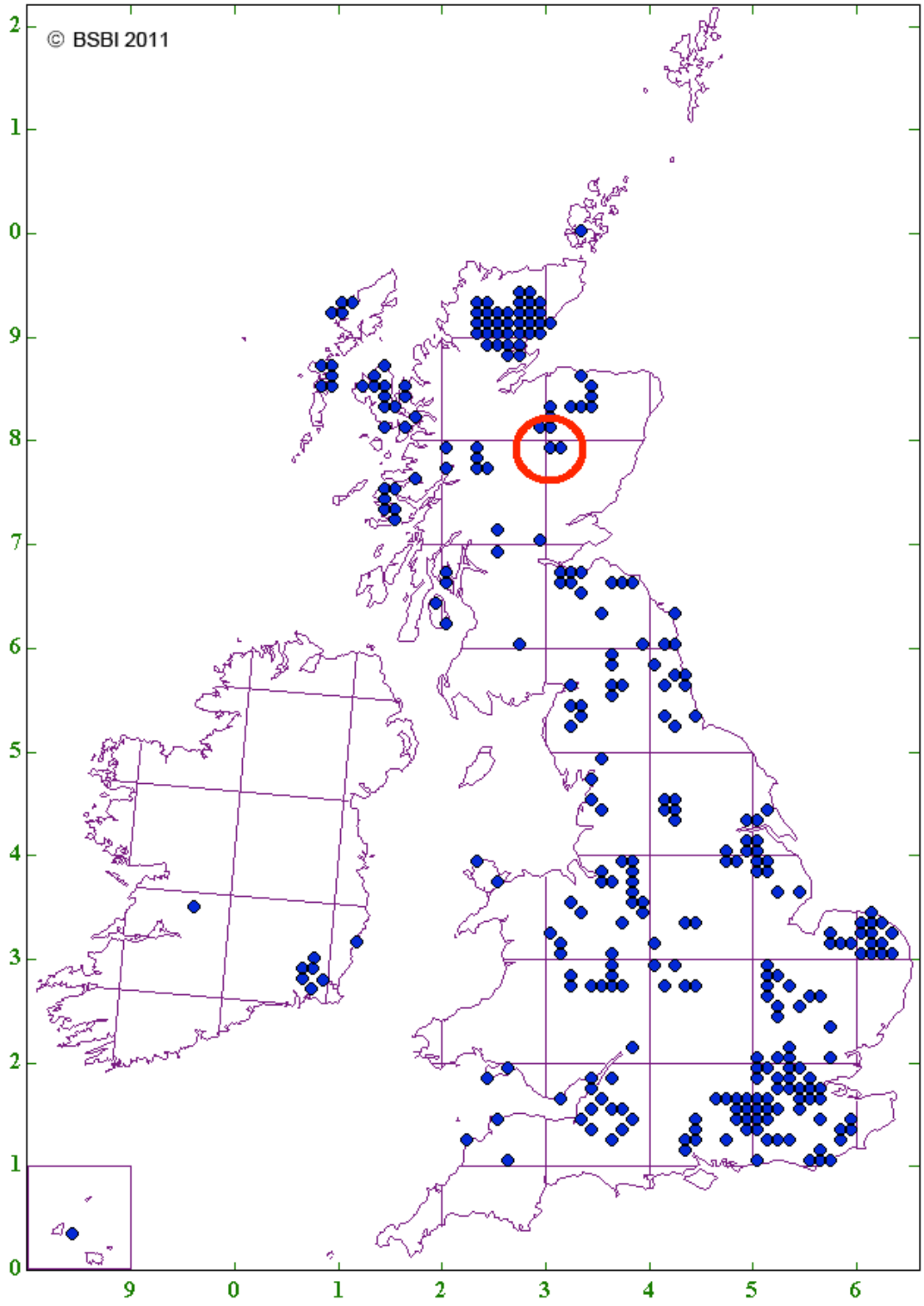

Figure 1.1: BSBI hectad map of aspen (*Populus tremula*), showing the UK distribution; each point represents a population, which can vary from a single ramet to many ramets (from http://www.bsbimaps.org.uk). Approximate location of Strathspey is circled in red.



Figure 1.2: (Top) Aspen with green, photosynthetic bark (right) and slightly thicker, greyish bark, colonized by a few crustose lichens and beginning to show signs of cracking.



a



b



c

Figure 1.3: (a) Mature aspen at Invertromie. Younger aspen are to the left of the picture, displaying greyish-white bark. (b) Young aspen suckers at Invertromie, maybe 3-4 years old. (c) Aspen sucker with a Large Red Damselfly (*Pyrrhosoma nymphula*).

1.4 Epiphytes of aspen

Aspen is naturally colonised by two classes of epiphytes, lichens and bryophytes, of which lichens are by far the most speciose. These two classes are described below.

1.4.1 Lichen epiphytes

Lichens are composite organisms comprising a fungal body and either a green alga or cyanobacterium as the photosynthetic component. The symbiosis brings about some unique physiological and morphological characteristics. Lacking a complex root system, waxy cuticle and stomata, lichens absorb nutrients through deposition from the atmosphere or from its substrate (Williams et al. 1996; Gilbert, 2000). Three main growth forms exist: crustose, foliose and fruticose (Fig. 1.4). Crustose lichens are made up of layers of cortex (fungus), algae/cyanobacteria (beneath the surface of the cortex) and medullary hyphae (fungus), which attach to the substrate; foliose lichens are made up of upper and lower layers of cortex (fungus), algae/cyanobacteria (beneath the surface of the upper cortex) and medullary hyphae (fungus), which may attach to the substrate; fruticose lichens are made up of a central core that attaches to the substrate, around which grows a lower medullary hyphae layer (fungus), a layer of algae or cyanobacteria, and an outer cortex (fungus).

1.4.2 Moss and liverwort epiphytes

Mosses possess distinct stems and spirally arranged leaf scales, similar to vascular plants. In the stems are three distinct layers: epidermis (outer layer), cortex (middle layer), and the central cylinder. The epidermis tends to be mostly one cell thick, but can have more layers. Young cortical cells and the leaves contain chlorophyll, but this diminishes with age. Mosses attach to their substrate via rhizoids – multicellular filaments growing from the base of the stem. Mosses are generally desiccation-tolerant, being able to survive for long periods of time without water, but rapidly rejuvenating when moisture is present (D. Long, pers. comm.; Scott & Brooks, 1943).

Liverworts consist of parenchyma cells with chlorophyll granules occurring in the superficial cells. A unique feature of liverworts is the presence of terpenoids

contained within single membrane-bound bodies. There is a distinct upper and lower surface to the thallus, with the lower surface containing numerous unicellular rhizoids. The epidermis contains a thin cuticle, some of which are thickened with cellulose. Branching tends to be dichotomous, giving rise to the characteristic bilateral branching seen in many liverworts. Unlike mosses, liverworts are more sensitive to desiccation, though some species, such as *Frullania* contain a small sac that retains water and enables them to survive in drier conditions (S. Rubasinghe, pers. comm.; Scott & Brooks, 1943).

1.4.3 Factors influencing epiphyte distribution and abundance

Distribution and abundance of epiphytes on trees differs with aspect, age of stand, phorophyte[2] chemical processes, and phorophyte structure (Gustafsson & Eriksson, 1995; Hemming & Lindroth, 1999; Hedenås & Ericson, 2000; Koopman, 2005; Mistry & Berardi, 2005). Epiphyte community diversity on *Populus* spp. is demonstrably higher than other boreal tree species and its presence in woodlands can positively influence species richness (Uliczka & Angelstam 1999; Goward & Arsenault, 2000; Boudrealt et al. 2002; Hedenås & Ericson, 2000; Jüriado et al. 2003; Lõhmus et al. 2006; Boudrealt et al. 2008). Goward and Arsenault (2000) demonstrated that cyanolichens (lichens with a cyanobacterium as the primary photosynthetic component) grow more abundantly in young- to middle-aged conifer (*Picea* and *Pseudotsuga*) stands when *Populus* is also present, particularly on trees neighbouring *Populus*. Only those trees growing within the drip zone of *Populus* display these differences, which implies an effect of *Populus* on neighbouring tree species.

At broader spatial scales, air quality is one of the most significant factors influencing epiphyte diversity and its effects are well documented (Gilbert, 2000). Lichens especially are useful as bioindicators of pollution, with sensitive species often decreasing in abundance in areas where nitrogen and sulphur dioxide in particular are at high levels (Hawksworth & Rose, 1970; Ferry et al. 1973; Hedenås & Ericson, 2000; Hauck et al. 2003; van Herk et al. 2003; Werth et al. 2006; Mikhailova, 2007).

[2] Living substrate upon which the epiphyte grows, such as plant tissue

Ellis & Coppins (2010) investigated the combined effects of climate change, pollution and woodland quality on epiphyte species composition and richness across 324 sites in Scotland, and found a weaker effect of pollution (i.e., sulphur dioxide, acid deposition, nitrogen deposition, ammonia, nitrogen oxides, and ozone), potentially explaining 3.5-3.7% of the measured composition and richness; with climate accounting for 6.7-8.4%. Thus, pollution may not be a particularly dominant force in influencing composition and richness due to the relatively clean air in northern Scotland, away from urban centres (Gilbert, 2000). In a comprehensive study on the effects of climate and autogenic succession in epiphyte communities on *Populus tremula* populations in Scotland, Ellis & Coppins (2006) demonstrated a significant climatic effect. Rainfall was found to be very important, which in Scotland follows a gradient from the wet, oceanic Atlantic west coast to the drier northeast. However, average tree age at each site was also important, with older trees from drier sites having similar community structure to younger trees at wetter sites. Ultimately, older trees and those in wetter areas tended to have a greater frequency of bryophytes and cyanobacterial lichens, with younger trees and those in drier climates having a greater frequency of crustose and green-algal lichens. Sexual crustose lichens tended to be the earliest colonizers of aspen trunks, gradually being overgrown by larger foliose and fruticose species, along with mosses and liverworts (Ellis, 2009).

More locally, epiphytic mosses and lichens exhibit differences in their preference for light, with certain species possessing the ability to acclimatise rapidly to extreme variations whilst others may fail to thrive (Kershaw & MacFarlane, 1980; Kershaw, 1985). Loppi & Frati (2004) compared the influence of phorophyte and light on lichen diversity in *Tilia platyphyllos* and *Quercus ilex* in Central Italy. The most significant factor determining diversity was the quantity of winter light, which is higher on *Tilia*, being a deciduous tree, and this was reflected in the higher diversity of species colonizing this tree when compared with *Quercus*. Cyanolichens, mosses and liverworts show greater vitality on the northern side of tree trunks in clear-cut systems where a few trees are retained in groups to conserve diversity (Hazell & Gustafsson, 1999; Hedenås et al. 2007).

Epiphytes can be used as food, nesting materials, or camouflage by a range of animals and invertebrates, which may relate to their prevalence during winter when many other food sources are unavailable (Sharnoff & Rosentreter, 1998; Allgaier, 2007). Herbivores can damage lichens and mosses, the amount of damage varying among thallus structures and growth forms, the presence or absence of secondary metabolites, and variation in herbivore preferences (Kumpula, 2001; den Herder et al. 2003; Gauslaa et al. 2006; Nimis & Skert, 2006; Asplund & Gauslaa, 2007); damage to lichen thalli can be caused by slugs via specialised radulae, and the production of saliva and mucus (Fröberg et al. 2006).

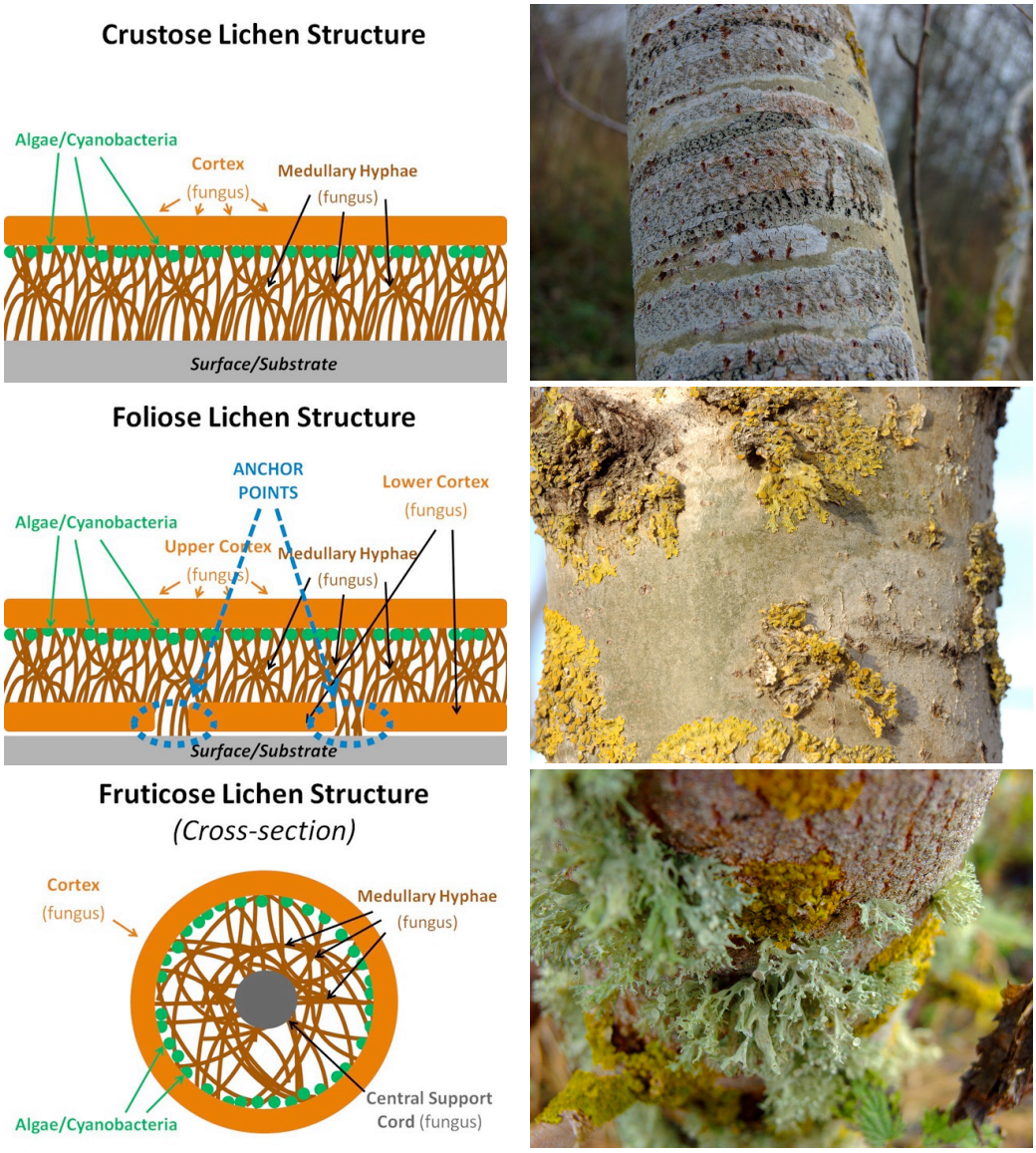

Figure 1.4: (Top left) Diagram of crustose lichen structure (left), showing the layers of cortex (fungus), algae/ cyanobacteria and medullary hyphae (fungus), which attach to the substrate. Crustose lichens growing on aspen in Scotland (right). (Centre left) Diagram of foliose lichen structure (left), showing the upper and lower layers of cortex (fungus), algae/ cyanobacteria and medullary hyphae (fungus), which attach to the substrate. Foliose lichen *Xanthoria parietina* growing on aspen in Scotland (centre right). (Bottom left) Diagram of fruticose lichen structure (left), showing the branch-like layers in cross-section of cortex (fungus), algae/ cyanobacteria and medullary hyphae (fungus), which attaches to the substrate via a central core. Fruticose lichen *Ramalina fraxinea* growing on aspen in Scotland (bottom right). (Lichen structure diagrams used with kind permission from http://watchingtheworldwakeup.blogspot.com/2009/03/stuff-on-rocks-2-all-about-lichen.html; photographs by C. Davies, 2008-2009).

1.5 Variation in aspen with potential effects on epiphytes

1.5.1 Aspen bark and its variability

When aspen bark is first formed it is smooth, thin and photosynthetic (Kaufert, 1937). It comprises the periderm which replaces the epidermis as the protective covering and consists of three structures: 1) the cork or phellem; 2) the cork cambium, or phellogen; and 3) the phelloderm. In smooth bark these layers are thin and difficult to discern. The bark is able to stay smooth by maintaining radial and tangential cell growth in the periderm, concomitant with a 'sloughing off' of the older and inactive periderm cells. A white bloom of dead periderm cells forms protective layers that also varies in consistency between high and low topographies (Covington, 1975).

Rough bark is formed if the integrity of the smooth bark is lost. In these areas of damage new layers of phellogen are induced which produce further phellem, which is forced outwards. The phellem is non-living and comprises inactive suberized cells forming many layers of corky protective tissue. The process of cell differentiation that may result in thickened periderm and rough bark can be complex (Lawson & Poethig 1995; Martín-Trillo & Martínez-Zapater 2002).

A number of different factors can initiate the switch from smooth to rough bark. As a tree ages it will be subject to external stresses which result in greater amounts of damage to the smooth bark, and which then trigger the formation of rough bark. This means that rough bark is more likely to be seen in older bark, located towards the base of the tree, with smoother bark higher up the trunk. Kaufert (1937) indicated that damaged bark of *Populus tremuloides* was limited to aspects of the trunk exposed to storm damage. Colder north-easterly winds and lack of sunlight may cause damage to the periderm and account for the increase in rough bark on the north and eastern aspects. Finally the propensity to form rough bark may vary according to genotype.

Aspen bark can vary quite dramatically from very smooth to very rough, with some clones appearing 'patchy', whilst other clones of similar age have little to no smooth bark for the first 2-3 meters from the ground (personal observation) (Fig. 1.5).



Figure 1.5: (Left) Bark of mature aspen showing variation in smooth, photosynthetic bark, and rougher bark; (right) closer view of rough bark with deep fissuring.

1.5.2 Influence of bark on epiphytes

A major morphological feature of aspen, which is likely to affect establishment of lichens, is the bark texture. This shows striking diversity among clones within single populations (Hyvarinen et al. 1992; Sillett et al. 2000; Boudreault et al. 2008; Lamit et al. 2011). This variation in bark texture facilitates the creation of microsites with differences in, for instance, moisture levels (Sheard & Jonescu, 1974; Aboala et al. 1999; Zhang, 1999; Levia & Herwitz, 2005).

Associations between epiphyte functional groups and phorophyte quality (plant surface colonised by epiphytes) have been documented before in several studies. One of the earliest (Hale Jr., 1950) described communities of foliose and crustose lichens growing on varying bark textures of a range of tree species from the Aton Forest, Connecticut, USA. The tree species surveyed were separated into rough-barked, smooth-barked and intermediate. By examining the lichen flora on these tree species and comparing differences between bark types, the observation was made that a higher proportion of foliose species were found on rough barked trees, a higher proportion of crustose species were found on smooth barked trees (also supported by Pereira et al. 2002), with an intermingling of functional groups on those trees with a mixture of rough and smooth bark. The process of cracking and fissuring with the sloughing off of outer periderm tissue has the capacity to significantly reduce crustose species, whilst providing a niche for foliose species. Tree species with a

tendency to produce rough bark during the process of growth and development were found to have a greater abundance of transitional bark, and therefore a greater mixture of species.

Morley and Gibson (2010) compared lichen communities on smooth- and rough-barked tree species from cool temperate rainforests in two climatically similar regions of Victoria, Australia. Epiphyte communities were sampled on trees up to 2m in height, across an area 20m x 20m. The dominant group of lichen species found on smooth-barked trees were crustose. Conversely, epiphyte community composition on rougher-barked trees changed as the host changed, which the authors attributed to the development of fissuring and rougher bark over time. These fissures provided a habitat for lichen species that were rare on smooth bark.

Previous research supports the phenomenon that rougher, fissured bark provides a more suitable niche for the establishment of foliose and fruticose lichens, though this may differ for individual species. An example of the effect of bark texture on epiphytic lichens was demonstrated by Moxham (1981) and reiterated by Armstrong & Bradwell (2011). Physical variation in bark was cited as having a 'profound influence on the growth', with bark texture recognised as being highly influential in the growth and development of a lichen thallus: the radial growth rate (RaGR) of *Xanthoria parietina* (a common foliose species preferring nutrient rich substrate) increases on smooth bark as opposed to rough bark.

Street & Street (2002) reported patterns of epiphyte diversity associated with the characteristic 'diamond-shaped' structure of bark found on older trees. More rugged areas appeared to favour certain fruticose *Ramalina* species. Smoother sections of bark were found to be more commonly populated by crustose species such as *Lecidella elaeochroma* and *Pertusaria* or *Arthonia* spp., with these smooth areas positively influencing growth of other lichens such as *Xanthoria parietina* (Hyvärinen et al, 1992; Gustafsson & Eriksson, 1995; Hemming & Lindroth, 1999; Hedenås & Ericson, 2000; Street & Street, 2002). The most compelling evidence demonstrating the effect of bark texture influencing epiphyte distribution has been found by Lamit et al. (2011). Replicates of *P. angustifolia* genotypes were obtained via cuttings from a nearby natural population and established in a common garden

experiment for approximately 16 years. After sampling the cover of bark lichen *Xanthomendoza galericulata*, bark roughness, bole circumference and total bark condensed tannins, significant variation in lichen cover was correlated with *Populus* genotype. Bark roughness was found to be under significant genetic variation and the genetically based trait that most influenced lichen cover.

1.5.3 Secondary metabolites of aspen

Like most members of the Salicaceae, aspen produces a range of secondary metabolites, which are substances produced and used by plants for defence against herbivory, high levels of UV radiation, and pathogenic attack (Bennett & Walsgrove, 1994; Lieutier et al. 1997; Ockels et al. 2007). One particular class of metabolites are polyphenols. These compounds are synthesized from the amino acid phenylalanine along the shikimate-phenylpropanoid pathway, and undergo many enzymatic changes to produce phenolic glycosides, flavonoid glycosides, and condensed tannins (proanthocyanidins) (Tsai et al. 2006). Phenolic and flavonoid glycosides are a combination of a sugar attached to non-carbohydrate moiety (the glycoside, or glycone) and a simple phenol ring (in the case of phenolic glycosides) or one of many types of flavonoid (in the case of flavonoid glycosides) (Harborne, 1964; Hopkinson, 1969) (Fig. 1.6). These latter components are known formally as the aglycone. Some phenolic glycosides (e.g. salicin and salicortin) occur widely throughout many *Populus* species (Tsai et al. 2006). In recent years, flavonoids in particular have gained a great deal of publicity for their health benefits to humans, particularly as anti-carcinogens and their de-toxifying properties (Ververidis et al. 2007). Large quantities of these compounds are found ubiquitously in plant organs, including bark of *Populus* species (Pearl & Darling, 1968; Lindroth & Hwang, 1996). Production of phenolics has significant costs to plant growth, especially for genotypes producing high quantities of compounds (Osier & Lindroth, 2001, 2004, 2006). Variation in phenolic quantity is largely under genetic control, but varies temporally and spatially according to genotype and environment (Lindroth et al. 1987; Lindroth & Hwang, 1996; Hemming & Lindroth, 1999; Osier & Lindroth 2001; Lindroth et al. 2002; Whitham et al. 2003; Whitham et al. 2005; Donaldson et al. 2006; Rehill et al. 2006; Donaldson & Lindroth, 2007).

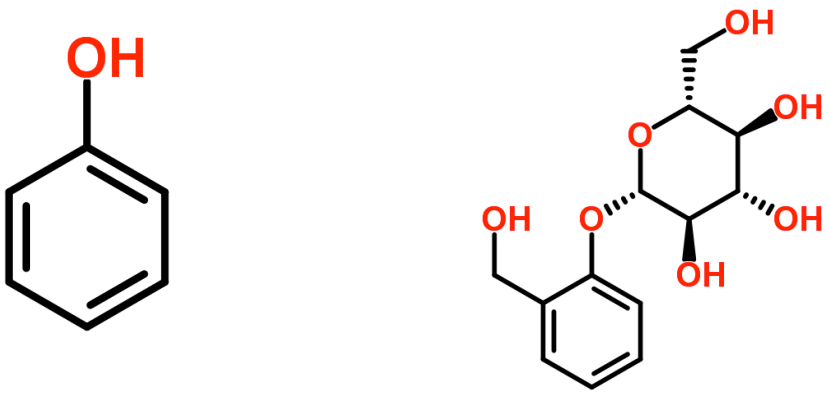

Figure 1.6: (Left) A simple phenol with hydroxyl (OH) bond, and (right) a common phenolic glycoside, salicin, showing the phenol ring at the bottom of the structure. (Chemspider, 2011).

1.5.4 Effects of bark chemicals on lichens

The effects of bark chemicals on epiphytes growing on the bark have not been thoroughly investigated. However, there is evidence of phenolics possessing fungistatic or fungitoxic properties in vitro (Hopkinson, 1969; Gupta et al. 1971; Anaya et al. 1999; Evensen et al. 2000; Wang et al. 2010; Ćirić et al. 2011). Koopman (2005) found evidence linking bark phenolic compounds isolated from *Populus* and patterns in lichen diversity. The research also indicated inhibitory allelopathic effects of lichens on co-occurring cryptogams, including other lichen species and this may mediate differences in the lichen communities associated with aspen via defence reactions initiated after colonization of the bark, as with fungi (Bennett & Wallsgrove, 1994). It is therefore of special interest to determine whether epiphyte communities on aspen vary among clones, and whether this variation in epiphyte communities can be accounted for by differences in physical and chemical properties of the trees.

1.6 Scientific approaches

Two complimentary approaches can be used to adequately address the major research questions. In this thesis physical traits and epiphyte communities of aspen will be sampled in:

1. Natural populations
2. Experimental populations

Each of these approaches has its advantages and disadvantages.

1.6.1 Sampling in natural populations

This approach has the advantage of making measurements under realistic ecological conditions, assessing whether genetic effects are sufficiently important, and whether they have a significant influence on dependent community composition in nature (Tack et al. 2010). It is anticipated that sampling natural populations will provide a realistic representation of how the variation in measured traits may influence epiphyte communities in the wild. Significant results obtained from an experimental trial may not be reproducible in a wild system. However it is important to acknowledge that this approach is subject to a number of limitations that restrict the interpretation of the results obtained from the study. A major limitation is sourcing a sufficient quantity of wild clones, within close proximity to one another, and containing enough ramets to be statistically meaningful. A second limitation is that environmental variation within a given site may confound genetic variation between clones; environmental causes of differences in dependent communities may be difficult to disentangle. To some extent this difficulty can be overcome if clones are intermingled, but the problem remains.

A third limitation of utilising observations in natural populations is that the study will necessarily be restricted to a single site. The genotypes will only be observed under a single set of environmental conditions. This means that it is not possible to draw more general conclusions about the wider importance of any effects of genotypic variation within the structural species on the community composition of dependent species. A fourth problem associated with studies of natural populations is that they only allow determination of effects of genetic variation that exist within the single study population of the dependent species. This genetic variation may be insufficient to have an influence on epiphyte community composition. However genetic variation found between populations may be much larger and more important in determining dependent community composition, as has been found for *Eucalyptus globulus* in south-eastern Australia (Barbour et al. 2009a, 2009b).

A fifth problem with using wild populations is that the different ramets of the same clone may be connected below ground, because of their origin from suckers, and therefore represent non-independent units. It is currently unknown how interconnected the ramets are within their respective clones. However it is believed that independence of ramets from parent trees is likely to occur after 8 years. The aspen clones in the population at Invertromie are 50-80 years old and therefore are likely to have been living on rootstocks independent of the parent ramet for several decades (section 1.3.1). The Highland Aspen Group have also been cutting sections of root away from the Invertromie clones used in this study, further reducing any existing connections between the ramets measured. Taking these facts into consideration the ramets used in chapters 2 and 3 are likely to be largely unconnected, and independent of one another, though the possibility remains that some ramets may be dependent on the same rootstock. For the purposes of the statistical analysis of variation within and among clones, ramets of the same clone will be treated as independent samples of that clone. However it is acknowledged that there is no way to currently establish the degree of connectedness between ramets. If ramets are connected then they will be pseudoreplicates, and the significance of any differences between clones in the statistical analyses will be overestimated.

The final problem with utilising studies from natural populations is that differences in age will exist among the ramets that are compared. Bark characteristics are known to change with tree age. Therefore age may be a confounding and unaccounted for factor in the analysis.

1.6.2 Sampling on experimental populations

In order to overcome the limitations of observations made in natural populations, a more controlled experimental approach may be adopted. Here replicate cuttings of genotypes of the foundation species are taken and planted in trial sites in a randomised block design. The main advantage of this method is that any differences in the epiphyte communities that establish on these different genotypes are due to genetic differences between the clones, not to differences in the environment occupied by the genotypes. A second advantage of this design is that genotypes of

the foundation species can be replicated over a number of sites. This allows us to test whether any genetic effects on community composition of dependent species are reproducible or specific to a particular set of environmental conditions. A third advantage of this design is that it allows cuttings to be taken from a range of different populations of the foundation species to ensure that a broad range of genetic differences within the foundation species are tested. The results are therefore not constrained by the limited amount of genetic variation present within the foundation species at a single site. Finally if the experiments are set up at the same time, age of the ramets does not become a confounding factor affecting epiphyte community composition. However there are some drawbacks to employing this method. Clonal trials using multiple genotypes from diverse areas fail to thoroughly deal with genetic effects at varying spatial scales. Increasing the distance at which the effects of genetic variation in foundation species are studied, from common garden, to local, site and landscape level, can show a significant decrease in host genetic effects on associated communities (Maddox & Cappuccino, 1986; Huston 1999; Tack et al. 2010). In certain cases, genetic effects on community structure can be reduced by environmental variation. Another problem is that removing a genotype associated with a particular area, and using it in a clonal trial in a location where it would not normally grow may attract a suite of dependent organisms not usually associated with that genotype.

1.7 Outline of research objectives and thesis chapters

To summarise, the ultimate objective of this thesis is to determine whether genetic variation in a foundation species, aspen, affects the community composition of its epiphytes, and if so, are these differences caused by clonal differences in either bark texture or secondary chemistry? These questions are tackled both in a natural population of aspen at Invertromie, and in a clonal trial of aspen planted at two contrasting sites in Scotland.

The thesis is arranged into four further chapters whose contents are described below:

1.7.1 Outline of thesis chapters

Chapter 2

Five wild aspen clones were selected from a larger population in Strathspey, Scotland. Bark texture and bark secondary chemistry were measured for each clone to ascertain whether genotypes differ significantly for these traits.

Chapter 3

Using the same aspen clones as for the previous chapter, epiphyte communities were sampled on each clone to ascertain whether genotypes differ significantly for this aspect of their extended phenotype. These results were analysed in conjunction with the results for bark texture and secondary chemistry to discern whether these traits are significant in shaping epiphyte community structure.

Chapter 4

A total of 27 aspen clones, replicated across two sites in Scotland in a randomized block design, were sampled to test for differences between genotypes in bark secondary chemistry and epiphyte community. Variation in bark chemistry was used as an explanatory variable to attempt to explain observed variation in epiphyte community structure between clones.

Chapter 5

This final chapter synthesises results from the preceding experimental chapters (2-4), comparing and contrasting findings of the different systems used and variation observed in bark physical traits and epiphytes. Conclusions are drawn from the main results and suggestions made for further research and conservation strategies.

2 Genetic variation between clones of aspen physical traits potentially important for associated species: bark texture and phenolic chemistry

2.1 Introduction

Communities are assemblages of different organisms, some of which provide a foundation, or habitat, for other (dependent) species that make up the rest of the community. Genetic variability within the foundation species has the potential to influence the expression of physical traits within that species, and in turn influence the dependent species sensitive to these traits. Community genetics is the area of research that investigates how genetic differences in one species, usually a foundation species, affects communities of dependent species.

The study of community genetics can be undertaken in two situations, which were explored in a special issue of *Ecology*. The first is where novel crops are introduced and have immediate impact on the local communities (Neuhauser et al. 2003); the second is in a natural, longer-established ecosystem with greater equilibrium and genetic stability, e.g. woodlands and permanent grasslands (Whitham et al. 2003). In the latter situation a decision needs to be made on which species to include in such studies. We have first to recognize the foundation species, then to define the dependent species. When this has been done we can then identify traits that are likely to be important in their interaction (Chase & Knight, 2003).

In woodland systems chief structural components are the trees. These interact with a wide variety of different dependent communities, such as arthropods, endophytes, epiphytes and micro-organisms. *A priori* we might expect that genetic variation in trees will have effects on these communities; greater than average genetic variation is found within trees is in hybrid zones.

Hybrid zones occur naturally where two or more species overlap within their native range (Stebbins & Anderson, 1954; Ellstrand, 1996; Milne, 2010), and are particularly common in trees e.g. *Quercus*, *Picea*, *Ulmus*, and *Populus*. Trees in hybrid zones segregate for large genetic differences found between species. For

instance, marked differences between clones of *P. tremula* and *P. tremuloides* and their hybrids grown in common garden experiments were observed when examining nutrient content in wood (Rytter & Stener, 2003), growth and phenological traits (Yu et al. 2001), and concentrations of secondary compounds in leaves (Rehill et al. 2005, 2006). There is now a lot of evidence that shows genetically determined differences in hybrid zones of *Populus* (*P. angustifolia* and *P. fremontii*) that affect a wide variety of dependent communities (Whitham et al. 1999). Similar results have come from studies of related hybrid systems e.g., *Eucalytpus* and *Quercus* (Whitham et al. 2006).

The usefulness of hybrid studies to understand the influence of genetics on physical traits and potential (or realized) community structure is well established. However confining studies to the use of hybrid complexes may introduce confounding factors. Interspecific variation is generally greater than intraspecific variation; therefore the recombination of genetic material from two sexually compatible species can form hybrid complexes that manifest a broader range of physical traits capable of influencing communities than the natural range of variation seen within a species. Moreover hybridization can generate novel phenotypes lying outside the range of the parent species. Such hybrids may show lower herbivore resistance and can act as 'sinks' for herbivorous insects even if the parental species are resistant to such attack (Whitham, 1989; Floate et al. 1993). Finally it should be noted that hybrid zones represent only a small fraction of tree populations.

Given these limitations it is important to determine if the community effects described in hybrid systems can be demonstrated within a single species. Preliminary evidence from a range of systems suggests that genetic variation within a species can often be sufficient to affect community structure of dependent organisms (Johnson & Agrawal, 2005; Bangert et al. 2006a, b; Barbour et al. 2009b; Tack et al. 2010).

2.1.1 Variation within and between populations

Genetic variation within a species can come from two sources: between populations and within populations. Genetic differences between populations of a species have

been shown to significantly influence arthropod fauna, taking into consideration a range of spatial scales (Johnson & Agrawal, 2005; Bangert et al. 2006a; Tack et al. 2010). In addition recent research has shown that variation within a foundational species, within a population, has an effect on communities of dependent organisms (Dickson & Whitham, 1996; Wimp et al. 2007; Barbour et al. 2009b; Kanaga, 2009). This suggest that genetic diversity within populations of foundational species particularly play an important role in shaping assemblages of associated species; even small differences between genotypes may be sufficient to affect changes. If these traits are heritable and stable across a range of environmental conditions it would be logical to conclude that each genotype of a foundation species is capable of formulating its own distinct community within a site. Extrapolated to the landscape and ecosystem levels, this could have drastic implications for ecosystem and global biodiversity.

2.1.2 Effects of foundation species on dependent communities

Given the potential importance of these effects it is imperative to broaden our range of studies of the effects of foundation species genotype on dependent species. To do this a model system is needed in which there is an intimate interaction between a foundational species and a dependent community. In the past interactions between trees and arthropods, particularly leaf herbivores, have been chosen (Osier & Lindroth, 2001, 2004, 2006; Bailey et al. 2006; Shuster et al. 2006; Bangert & Whitham, 2007; Tack et al. 2010). However there are other groups that form very important communities on trees that have been overlooked, such as epiphytic lichens (Gustafsson & Eriksson, 1995; Uliczka & Angelstam, 1999; Hedenås & Ericson, 2000; Jüriado et al. 2003). These interact directly with trees. Trees provide microsites within which epiphytes establish and grow (Barkman, 1958; Kantvilas & Jarman, 2004; Fritz & Heilmann-Clausen, 2010). There is also evidence many epiphytes, particularly lichens, penetrate the surface of the tree and interact with chemicals and bark tissues (Ascaso et al. 1980; Ascaso et al. 1983; Ascaso & Rapsch, 1985; Inoue et al. 1987; Legaz et al. 1988; Bouaid & Vicente, 1998; Legaz et al. 2004). Thus there is the potential for genetic variation affecting bark

morphology and bark chemistry of trees to influence the communities of epiphytes that colonize them.

The purpose of this study is to determine whether genetically determined differences in bark morphology and bark chemicals in a foundation tree species influence the composition of its epiphytic lichen community.

2.1.3 Aspen as a foundation species

One such tree species is aspen (*Populus tremula* L.). In many localities aspen is clonal, reproducing from root suckers rather than seed. This leads to the availability of replicate stems within a clone, with the same genotype. Therefore it is possible to assess whether there are significant differences between clones for microsite characters of bark and the chemical composition of bark. Aspen is also amenable to study because of the rich and diverse epiphyte flora (Gustafsson & Eriksson, 1995; Ellis 2008; Jüriado et al. 2009), associated with the high pH of the bark (Hedenås & Ericson, 2000; Street & Street, 2002; Ellis & Coppins, 2007; Jüriado et al. 2009).

A major character of bark likely to affect establishment of lichens on aspen is variation in bark texture (Hyvarinen et al. 1992; Sillett et al. 2000; Boudreault et al. 2008; Lamit et al. 2011). This variation facilitates the creation of microsites with differences in moisture levels (Sheard & Jonescu, 1974; Aboala et al. 1999; Zhang, 1999; Levia & Herwitz, 2005). It is important to establish, in the first instance, whether genetic differences between clones in natural situations are manifested in % smooth bark.

A major chemical family found in bark, particularly knotwood, is the polyphenols (Pietarinen et al. 2005; Neacsu et al. 2007), which include condensed tannins and phenolic glycosides exhibiting strong defences against invasion by pathogens, wood-boring insects and UV radiation (Bennett & Walsgrove, 1994; Lindroth & Hwang, 1996; Lieutier et al. 1997; Ockels et al. 2007). Limited studies have demonstrated that these latter compounds significantly affect lichen growth (Koopman, 2005). Therefore in the second instance it is necessary to determine whether there are clonal differences in phenolic chemistry of bark tissue with which the epiphytic lichens interact. Of course it is likely that bark morphology and chemistry may be affected

by other factors such as age of tree, aspect and height (Levia & Wubbena, 2006; Hamilton et al. 2007; Barbour et al. 2009a). To demonstrate that genetics play a primary role in determining bark morphology and chemical composition, it is important to investigate the effects of aspect and height on % smooth bark and chemistry.

Therefore, to achieve these goals, a natural stand of a minimum of five aspen clones growing in close proximity is required. Using genetic markers and phenology the ramets can be classified into genets. Then individual ramets of the same age can be sampled and scored for % smooth bark and secondary chemical composition; sampling must carried out at different heights and different aspects to determine their effects.

Questions asked are:

1. Are there significant differences between clones in the characters measured?
2. How much of the variation is accounted for by variation between clones?
3. Is any of the residual variation (within clones) accounted for by either height or aspect?

2.2 Materials and methods

2.2.1 Study Site

The study was conducted at RSPB Insh Marshes National Nature Reserve (OS NN774998 or Latitude 57 05 24N, longitude 03 59 48W) was identified as a potential study site: located near Kingussie in the Cairngorms National Park (Fig. 2.1). The reserve lies within the flood plain of the River Spey and is one of the 'largest single units of poor-fen floodplain mire in Britain' (Joint Nature Conservation Committee, 2001). It supports large populations of overwintering wildfowl, such as the Whooper swan (*Cygnus cygnus*) and Greylag goose (*Anser anser*), and assemblages of rare breeding waterfowl, such as teal (*Anas crecca*), lapwing (*Vanellus vanellus*), snipe (*Gallinago gallinago*) and widgeon (*Anas penelope*). Additionally, there are high numbers of nationally rare and scarce aquatic plants and invertebrates owing to the diversity of connected water systems, i.e. a

slow-moving river, a mesotrophic loch and many shingled or gravelled areas. The site has received RAMSAR designation for these reasons. Insh Marshes is approximately 5km long, and 1km wide, lying on either side of the River Spey. Approximately 27% of the total area is peat bog and swamp with a further 25% being seasonally-flooded agricultural land; 20% of the area is either permanent or intermittent freshwater marsh, with the remaining 28% a mixture of permanent lake and river systems, forested peatland, shrub-dominated wetland and drainage canals.

The site comprises a large area of seasonally flooded marsh dominated by sedges and reeds (National Vegetation Classification types S9 *Carex rostrata* swamp; S11 *Carex vesicaria* swamp; S27 *Carex rostrata-Potentilla palustris* tall herb fen; M5 *Carex rostrata-Sphagnum squarrosum* mire; and M15 *Scirpus cespitosus-Erica tetralix* wet heath). To the south of the Spey and west of Loch Insh, and adjacent to the marsh is an area of ground raised approximately 200m above the river with an undulating topography. The soil is a freely draining alluvial derived from glacial erosion. The raised ground is covered with grassland vegetation (NVC type *MG9 Holcus lanatus-Deschampsia cespitosa*) interspersed with patches of broadleaved woodland, predominantly birch and aspen (NVC types W3 *Salix pentandra-Carex rostrata* and W4 *Betula pubescens-Molinia caerulea*; riparian woodland *W7 Alnus glutinosa-Fraxinus excelsior-Lysimachia nemorum*, and small areas of *W11 Quercus petraea-Betula pubescens-Dicranum majus* on less riparian areas). Annual precipitation is approximately 800mm (daily average of 27mm in April (15 days) to 142mm in August (27 days)), with generally mild temperatures ranging from -12°C in January, to 26°C in August. Average sunlight hours vary from 2 hours/ day in January to 6 hours a day in April.

2.2.2 Invertromie aspen population

The aspen stands are located predominantly on the Invertromie section of the reserve (Figures 2.2 and 2.3) and covers an area of roughly 50ha; 20% of the total tree species are aspen (Prescott, 2001). The Invertromie trail is 4.5km long (NN774998) and is a Site of Special Scientific Interest (SSSI). Clones have been putatively identified by the Highland Aspen Group, based on differences in physical traits such as timing of bud burst and autumn senescence, and qualitative differences in autumn

colour. Individual aspen trees are large, with an average height of 20m and diameter at breast height (dbh) of up to 46cm. The density of stems can also be high with 3-4 mature stems per square metre. (Figures 2.4 (a-h)).

This site was deemed most suitable for studying epiphyte communities on wild aspen clones due to the abundance of mature clones of similar age - three cohorts exist from 50-80 years old. The close proximity of the clones across the site would help minimise confounding environmental effects.



Figure 2.1: Map of the United Kingdom of Great Britain and Northern Ireland. The area marked with a black dot shows the location of the Insh Marshes Nature Reserve in the Cairngorms National Park (Joint Nature Conservation Committee, 2001).

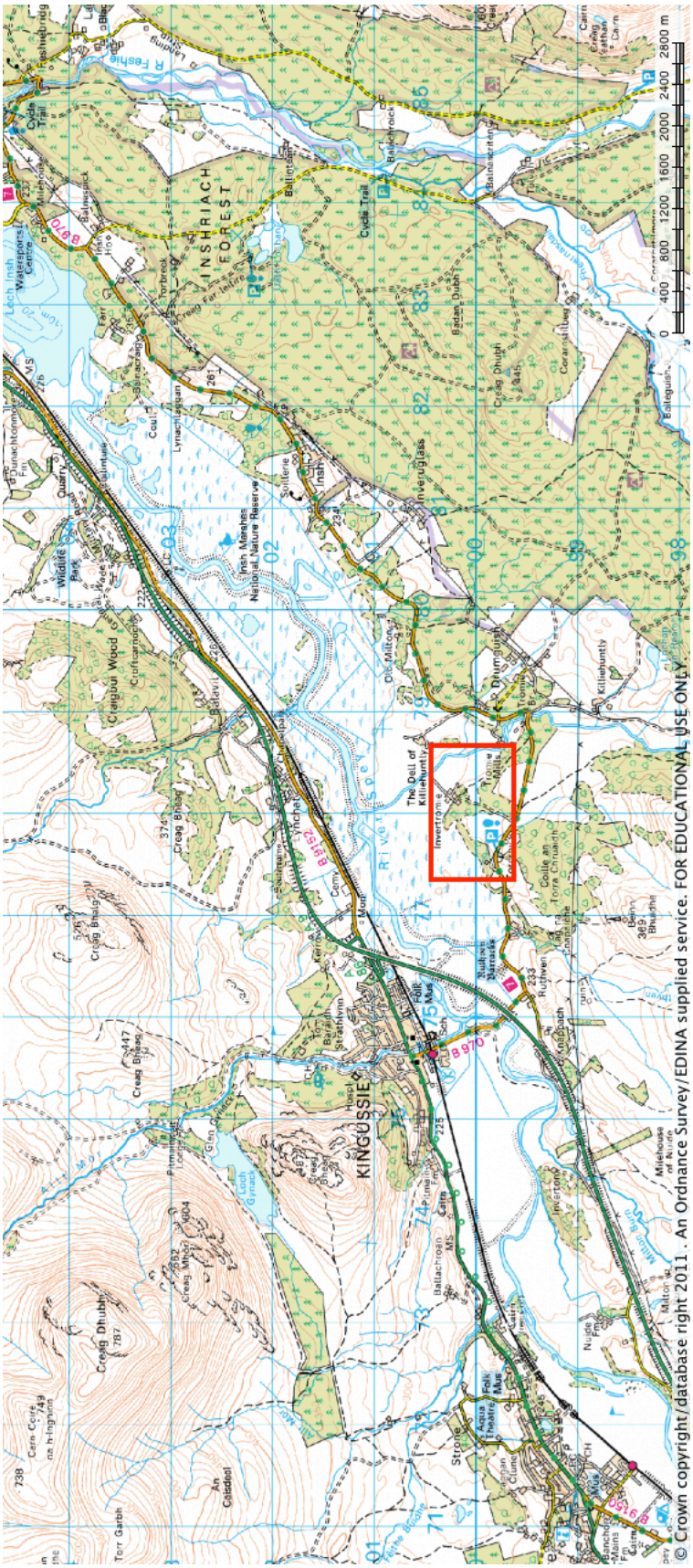

Figure 2.2: Ordinance Survey (2011a). Map of a section of the RSPB Insh Marshes National Nature Reserve and environs, with the location of the Invertromie Trail and aspen clones highlighted in red (scale 1:33925).

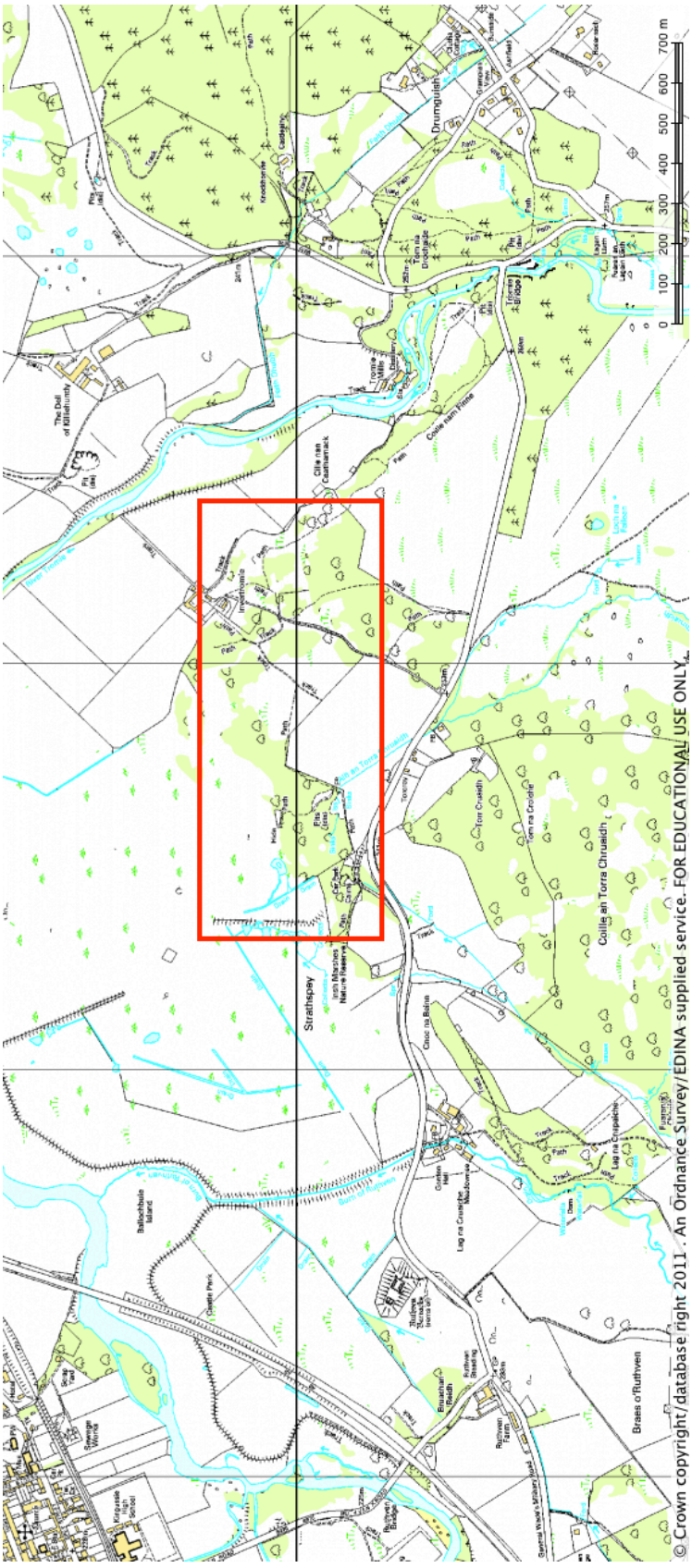

Figure 2.3: Ordinance Survey (2011b). Map of a section of the RSPB Insh Marshes National Nature Reserve, with the location of the Invertromie Trail and aspen clones highlighted in red (scale 1:8481).



a



b



c



d



e



f



g



h

Figure 2.4 (a-h): (a) BD11 (foreground) and the marshes beyond; (b) (c) (d) grassland areas with aspen-birch woodland; (e) small aspen sucker with resting *Pyrrhosoma nymphula*; (f) (g) (h) variation in bark texture from three Invertromie clones (C. Davies, 2008-9)

2.2.3 Potential aspen clones

Previous work by the Highland Aspen Group identified putative clones within Invertromie based on phenological differences, notably timing of bud burst and autumn senescence, together with autumn leaf-colour variation (E. Emmett, pers. comm.). Five of these putative clones were selected for detailed analysis: they covered an area of 500m^2 and comprise approximately 100 stems. In order to confirm that the putative clones represent single genotypes, their clonal identity was confirmed using microsatellite marker analysis.

2.2.4 Bud collection

During January 2008, buds were collected from approximately 100 mature aspen ramets, representing a diversity of sizes (from 15-20m tall and dbh 20-46cm), within the five target clones. Buds were stored in plastic zip-lock bags with silica gel and allowed to dry at approximately 20°C.

2.2.5 Microsatellite analysis and genotyping

Total genomic DNA was extracted from approximately 200mg of bud tissue using a standard CTAB protocol (Doyle and Doyle, 1987) with minor modifications. Six previously developed microsatellite markers were selected for genotype analysis: PMGC2852 (IPGC, 2007); ORPM30, ORPM60, ORPM137 (Tuskan et al. 2004); WPMS 5, WPMS15 (van der Schoot et al. 2000; Smulders et al. 2001). Table 2.1 gives details of the primer pairs used to amplify these loci. Primers were obtained from Applied Biosystems (Cheshire, UK), with the exception of ORPM30 and WPMS5 (Metabion, Germany). Each forward primer was labelled with one of four fluorophores (VIC, FAM, PET or NED).

The primer pairs were combined into groups of three for multiplex PCR[3]. Reactions were run in 25µl total volume with 10ng template DNA, 1x $NH4^+$ reaction buffer, 2.5-4mM $MgCl_2$, 100µM each dNTP, 0.2-0.3µM forward and reverse primer, 0.5

[3] ORPM30 failed to produce trace files and was amplified separately; 2µl of each assay was pooled with 4µl of the corresponding assays from the duplexed PMGC2852 / WPMS15 reactions.

units of *Taq* polymerase, and made up to volume with dH_2O. To reduce non-specific amplification, "hot-start" touchdown PCR was used with an initial denaturation at 95ºC for 3 minutes followed by 1 cycle of 94ºC for 30 sec, final annealing temperature (T_A) + 10ºC for 30 sec and extension at 72ºC for 30 sec. The annealing temperature was reduced by 2ºC during each of the following 5 cycles. Products then went through a further 30 cycles of 94ºC for 30 sec, 48ºC for 30 sec and extension at 72ºC for 30 sec, with a final extension of 20 min. Samples were diluted 20-fold with dH_2O and 1µl of each combined with 9.8µl dH_2O and 0.2µl GS500LIZ (Applied Biosystems, Cheshire, UK) for a total volume of 10µl, and loaded onto bar-coded 96-well plates. Samples were submitted to the University of Edinburgh School of Biological Sciences Sequencing Service (Edinburgh, UK) and run on an ABI 3730 Sequence Analyzer (Applied Biosciences, Cheshire, UK). Fragment analysis was conducted with dnaTools Xplorer v2.4.2 (dnaTools, Inc., Colorado USA).

Table 2.1: Primers used for microsatellite analysis. Primers were separated into two groups, shown A or B, for multiplexing. Forward primers were fluorescently labelled at the 3' end as per the table (ORPM137 (VIC) and WPMS15 (VIC): green; ORPM30 (6FAM) and WPMS5 (6FAM): blue; PMGC2852 (PET): red; and ORPM60 (NED): yellow).

Group	Primer ID	Forward Primer Sequence 3'-5'	Reverse Primer Sequence 3'-5'	Length	Tm (°C)
A	ORPM30	ATGTCCACACCCAGATGACA	CCGGCTTCATTAAGAGTTGG	185	55
A	PMGC2852	ATAATCTCCCTAGCTTAATTCC	GAATAACATGGATAATGTGTTTG	224	55
A	WPMS15	CAACAAACCATCAATGAAGAAGAC	AGAGGGTGTTGGGGGTGACTA	113	61
B	WPMS5	TTCTTTTTCAACTGCCTAACTT	TGATCCAATAACAGACAGAACA	193	53
B	ORPM60	ATAGCGCCAGAAGCAAAAAC	AAGCAGAAAGTCGTAGGTTCG	212	53
B	ORPM137	CCGTGCATCTGCTCACTTTA	GCATTTGCAGATGAAATTGGT	280	53

2.2.6 Mapping clones

On the basis of the results from the microsatellite analysis five clones were identified and the position of the ramets belonging to these clones was mapped with a Garmin GPS 12. In order to determine whether these clones differed in their phenotypic attributes, five ramets were randomly selected from each confirmed clone for detailed measurements of diameter, bark texture (smooth or rough) and bark phenolics.

2.2.7 Sampling scheme

A quadrat method, modified from Asta et al. (2002), was used to record the occurrence, location and abundance of smooth bark on ramets. On each ramet sampling took place at five heights (40cm, 80cm, 120cm, 160 cm and 200cm). On sloping ground measurements were made from the highest side on each tree. For each height the quadrat was placed at cardinal points on the trunk (N, S, E and W) (Fig. 2.5). For scoring the quantity of smooth bark, the 10cm x 10 cm quadrat was divided into subunits of $1cm^2$ and each incidence of smooth bark within these $1cm^2$ subunits was noted. Each quadrat was given a unique identifying label, based on a pre-assigned tree code (601-676), the aspect (N, E, S, W), and height from 1 to 5 (each being 40cm apart) with 1 being the lowest (40cm from the ground) and 5 being the highest (200cm from the ground). For example, the first quadrat on tree 601, at the north aspect, and lowest height would be 601N1. This sampling scheme resulted in data for 20 quadrats per ramet, and 100 quadrats for each of the five clones

The arithmetic means and standard errors (SE) were calculated from raw count data at each experimental unit, i.e., clone, ramet, aspect (N E S W) and height (40cm, 80cm, 120cm, 160cm, 200cm). Each value was transformed to a percentage for analysis.

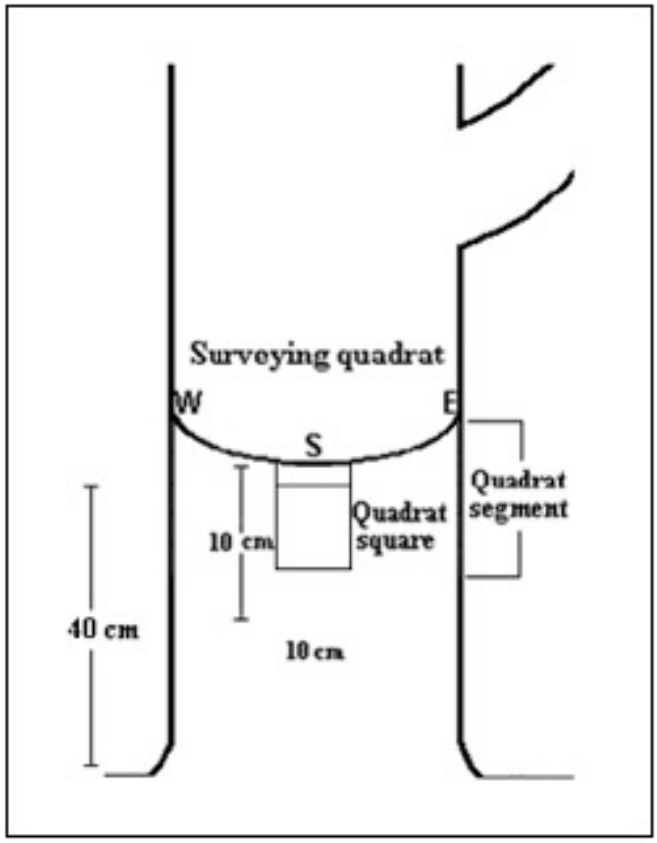

Figure 2.5: Diagram of lichen sampling transects with square quadrats (modified from Asta et al. 2002).

2.2.8 Field measurements

The following measurements were taken in the field:

- Counts of smooth bark per quadrat
- The diameter at breast height (dbh) was recorded by measuring the circumference of each ramet at 132cm from the ground, then dividing by pi to give the diameter to 2 decimal places (d.p.)
- Pieces of outer bark were removed from a section of each quadrat, approximately 2.5cm^2.

2.2.9 Bark phenolic chemistry analysis via HPLC

Bark pieces of approximately 2.5cm^2 were cut with a hard stainless steel knife from the top right corner of each quadrat. Fig. 2.6 shows the layers of bark removed.

Samples were stored in paper packets and left to air-dry at 20C (after Berlizov et al. 2007).

The samples were scraped cleaned of epiphytes prior to collection and any smaller traces of vegetation removed in the lab with a scalpel before ball-milling to a fine powder for 30-60 seconds in a Retsch Mixer Mill MM 400.

To quantify the number and amounts of individual phenolic compounds, 9-11mg of the milled material was extracted using 100% HPLC-grade methanol. Each sample was placed in a 1.5ml Eppendorf tube then 0.6ml of ice-cold methanol with internal standard (2mg/ l of 3,5-dihydroxybenzoic acid) added with 5-20 1mm diameter glass beads. After homogenising the sample for two minutes in a Retsch Mixer Mill MM301, it was left on ice for 15 minutes. Each sample was then homogenised for a further two minutes before being micro-centrifuged (Accuspin Micro, Fisher Scientific) for 3 minutes at 1300 rpm. Supernatant was removed into a 4ml vial with a Pasteur pipette, and the extraction procedure repeated twice more, combining the supernatants each time. These were dried under oxygen gas (only up to sample 622W1A, then nitrogen gas was used) to a residue and stored at -20°C until analysis.

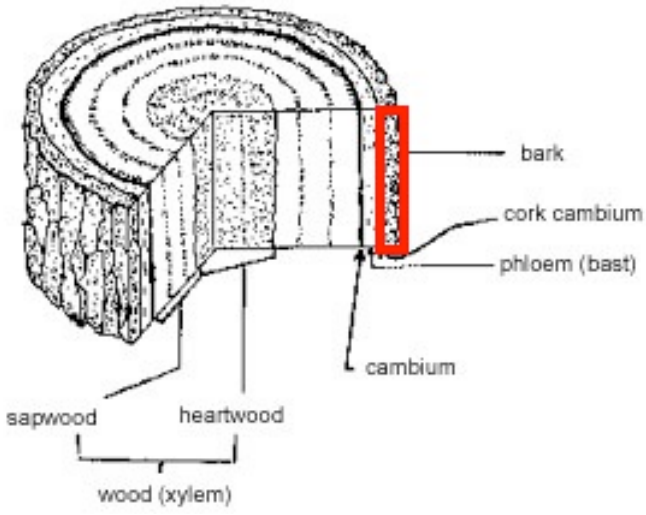

Figure 2.6: Diagram showing basic anatomy of a dicotyledonous tree, with the varying layers of active and inactive tissue. The section of bark removed for phenolic analysis is outlined in red. (RFS, 2010).

Prior to analysis samples were reconstituted using 0.3ml methanol followed by 0.3ml deionised water (only up to sample 601S2B, then 0.6ml of methanol was used). This was due to an unidentified waxy residue appearing in the supernatant that was greatly reduced by the elimination of dH_2O from the samples. Analysis was carried out using a high performance liquid chromatography – diode array detector (HPLC-DAD (G1315B)), collecting wavelengths at 220nm, 270nm, 320nm, 360nm and 280nm; a C18 column (Agilent Zorbax SB-C18 (PN 866953-902; S.N. USDZ011623) rapid resolution 4.6x75mm, 3.5µm) fitted within a Hewlett-Packard Agilent 1100 Series machine (Agilent Technologies, Germany). 10µl of each sample were injected onto the column at a flow rate of 2ml/ min. Two solvents were used: solvent A – aqueous tetrahydrofuran 1.5% (THF) / phosphoric acid H_3PO_4 (0.25%), filtered to 0.2µm; solvent B – HPLC grade methanol (MeOH). Conditions used in HPLC gradient elution are shown in Table 2.2, and are based on a method developed by Meier et al. (1988) and modified by B. Moore (James Hutton Institute, Aberdeen). Spectral libraries for peak identification were provided by R. Julkunen-Tiitto (University of Eastern Finland). A subset of samples was selected due to time constraints. This subset consisted of all bark samples collected from all quadrats from one ramet in each clone, in addition to samples collected from quadrats at heights 40cm and 200cm from the remaining four ramets from each clone.

Table 2.2: Elution gradients for HPLC. Solvent A is filtered nanopure water with 1.5% tetrahydrofuran and 0.25% phosphoric acid; solvent B is 100% HPLC grade methanol.

Time (min)	% solvent A	% solvent B	Flow rate (mil min^{-1})
0	100	0	2
5	95	5	2
10	85	15	2
20	70	30	2
40	50	50	2
60	0	100	2
Rinsing	0	100	2

2.3 Statistical methods

Smooth bark: data were converted to mean percentage with standard errors at each experimental level ('Clone', 'Ramet', 'Aspect' and 'Height') and presented as bar-plots with standard error bars. Raw count data was used for the implementation of

statistical models, with 'ramet' treated as independent variables, as discussed in chapter 1 (pages 26; 41-42).

Chemistry: data were recorded as concentration (µg/ mg) for each compound. These were converted to mean concentrations with standard errors at each experimental level ('Clone', 'Ramet', 'Aspect' and 'Height') and presented as bar-plots with standard error bars. Raw concentration data was used for the implementation of statistical models.

The overall approach involved testing for differences in smooth bark and bark phenolics among clones (Model 1) and subsequently exploring and quantifying the remaining variance within ramet, treating ramet, height and aspect as random effects (Model 2). To elucidate the potential effects of each aspect (i.e., north, east, south and west) and each height level, these factors were tested as fixed components, using 'Ramet' nested within 'Clone' as random components to give the percent residual variation (Model 3). The relative plausibility of each competing model was assessed using Akaike Information Criteria. For each model the ΔAIC and Akaike weight (*w*AIC) were calculated to measure the relative performance each competing model (Wagenmakers & Farrell, 2004). Models with Akaike weights within 10% of the highest candidate model may be considered equally plausible. This is known as the model confidence set and is analogous to a confidence interval for a mean estimate.

2.3.1 Model 1 – Differences between clones

Differences between clones were tested using variation among ramets as a random effect. Generalized linear mixed models (GLMM) were implemented in 'R' version 2.12.0 (R Development Core Team, 2010), using the Laplace approximation method in the lmer function (package 'lme4', version 0.999375-37 (Bates and Maechler, 2010)). A simple GLMM was fitted to the data, in which counts of smooth bark were modelled in relation to 'Clone' as a fixed effect and 'Ramet' as a random effect to account for the nested design. As part of the model-fitting process, the simple model was compared to a null model (i.e. completely random fixed effect, and a simulated random effect following a Poisson distribution) via likelihood ratio tests.

2.3.2 Model 2 – Complete hierarchical model with variance partitioning of nested effects

A second model was designed to examine the variation within ramets – i.e. within stem variation related to height and aspect – in addition to the effect of clone (fixed effect). To do this, ramet, height and aspect were treated as random effects, specifying their contribution to the model as additive, nested, or with an interaction. Models were compared using likelihood-ratio tests, to select models, which were significantly different from the null model. Using this method, three outcomes can be tested:

- If the additive model was selected, one would infer a significant effect of aspect and/or height, but with each of these variables contributing to the model separately.
- If the nested model was selected, one would infer a significant effect of aspect and height, but with the height effect (nested within aspect) related to the hierarchical effect of aspect, across ramets: i.e. the height effect is related to the aspect, which in turn, is related to ramet.
- If the model with an interaction was selected, the effect of aspect and height vary within ramet.

For the best model, according to the AIC and *w*AIC values, the random effects structure ('Aspect', 'Height' and 'Ramet') were used to partition variance, quantifying the contribution (variance explained) of the hierarchical random effects.

2.3.3 Model 3 – Complete hierarchical model, selecting and testing nested fixed effects

The final model for analysis of the effects of height and aspect used height and aspect as fixed effects that were either additive or interacting, and with clone and ramet as nested random effects. The model selection process was identical to the outcome of the variance partitioning for random effects (above). In addition, the additive and nested effect of ramet within clone was tested using likelihood ratio tests and *w*AIC (below).

2.3.4 Variance structure and model tests

Initial plots of the counts of smooth bark in the quadrats were consistent with a Poisson distribution, and for each of the chemicals, a normal distribution. However, this assumption was found to be incorrect when checking the ratio of residual deviance to the residual degrees of freedom. Severe violations of normality were detected in the standardized residuals, and by checking Pearsons residuals over-dispersion was detected for: smooth bark (Pearson = 3.46, significance = $-245.46^{10\text{-}377}$), eriodictyol (Pearson = 9.05, significance = $-1451.87^{10\text{-}37}$) and taxifolin (Pearson = 2.14, significance = -97.54). To deal with these problems a poisson-lognormal model GLMM was implemented by incorporating an individual-level random variable with the model (Elston et. al. 2000). Over-dispersion was not detected for p-coumaric acid (Pearson = 0.25, significance = $-4.08e\text{-}71^{10\text{-}377}$) and therefore a linear mixed model, fitted with REML and assuming a normal distribution, was used.

Wald/ F tests as are considered unreliable compared to likelihood ratio tests (LRT) for hypothesis testing, model selection of random effects in general and for fixed effects without 'large' sample sizes (Bolker et. al. 2009) – though there is no consensus as to what constitutes 'large' or 'small' sample sizes. Wald/ F tests are also not recommended for over-dispersed data. Model selection was therefore based on lowest Akaike Information Criterion (AIC) values (with weighted AIC support), and χ^2_{df} values.

2.4 Results

2.4.1 Microsatellite analysis and genotyping

Of the 100 individuals selected for the initial analysis, 63 were genotyped (Table 2.3). Buds were not collected from 5 ramets (606, 607, 609, 610, 651) due to being inaccessible; buds from 638, 639, 640, 641, 646, 655, 656, 657, 658, 659, 660 and 675 were omitted because the ramets were peripheral to the main clone in the field. Marker ORPM30 revealed a rare allele in both 626 and 630, and a third microsatellite signal was detected in individuals of clone BD5. Of the remaining individuals, 9 did not amplify well enough to confirm their clonal status (616, 620,

628, 666, 667, 668, 670, 671 and 672). Of the six markers used in the analysis of genotypes, three markers produced null signals. Despite this, ramets were successfully identified into clonal groups and mapped (Figures 2.7 & 2.8). Differences in phenological traits, i.e. bud burst, and timing/ quality of autumn senescence, were used in conjunction with the genotyping to support the results of the separation of ramets into their clonal groups.

Table 2.3: Summary of microsatellite markers and signals for each clone. Markers are shown with fluorophore colour tag and loci. Numbers in parentheses are the number of ramets per clone successfully genotyped. 'Null' indicates no microsatellite signal for that marker.

Clones	PMGC2852	WPMS15	ORPM30	ORPM60	ORPM137	WPMS5
	Red 113	Green 193	Blue 224	Yellow 212	Green 185	Blue 280
BD11 (13)	101	Null	185		163	Null
	112		208	209		
BD12 (9)	101		185		163	301
	107	196	208	209		
BD13 (7)	101		185		Null	Null
		196	208	209		
BD14 (6)	Null		185		Null	280
		196	208	209		
BD5 (6)	Null	153	183		181	281
		196	208	209		
			219			

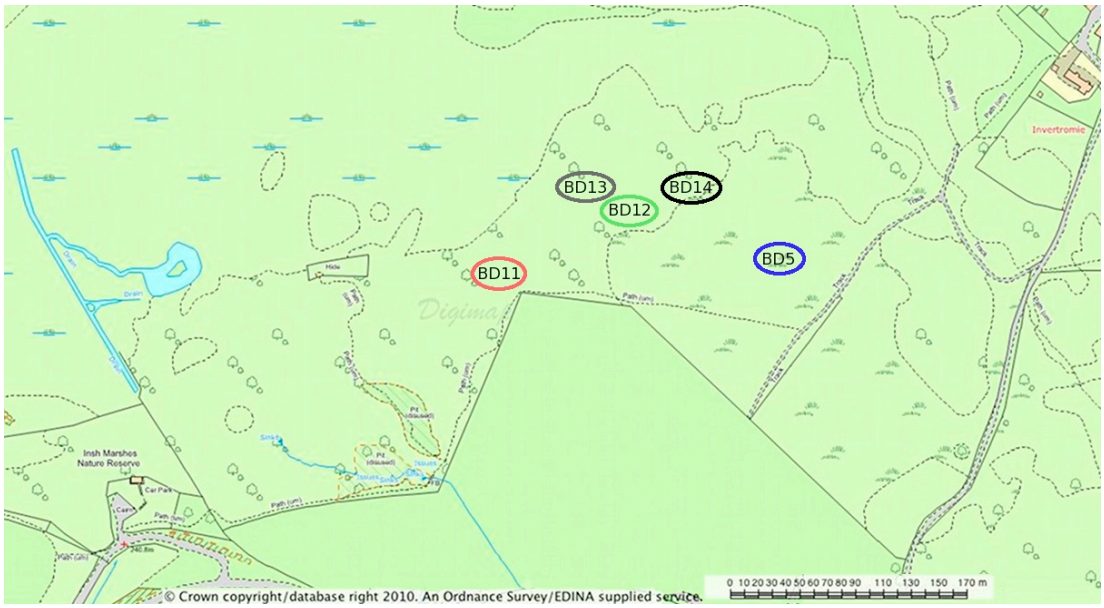

Figure 2.7: Ordinance Survey (2011c). Map of the Invertromie Trail in finer scale, with approximate positions of each clone highlighted by different colours (scale 1:2000).

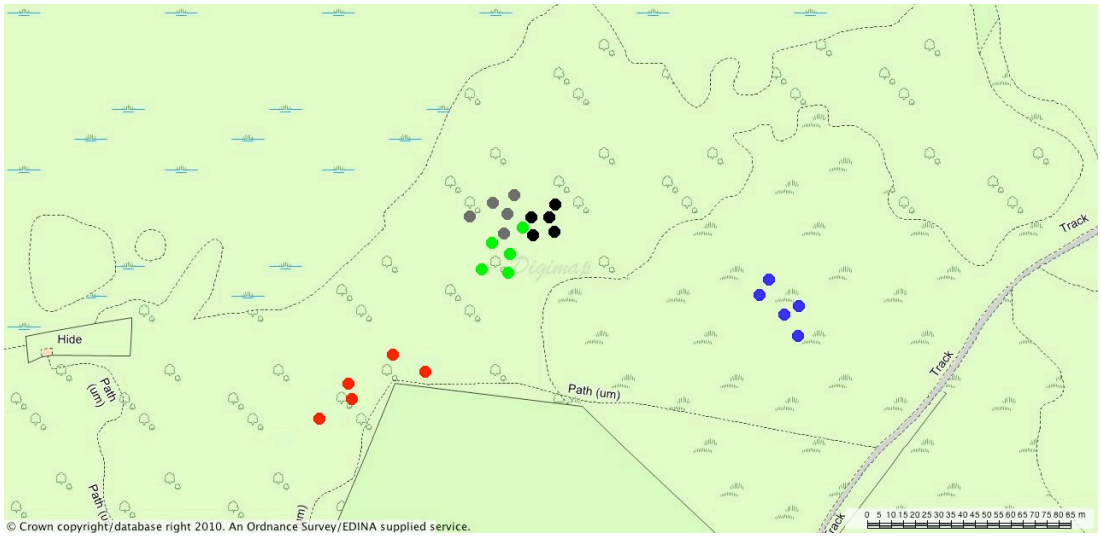

Figure 2.8: Ordinance Survey (2011d). Map showing the Invertromie trail with the position of each ramet colour-coded according to clone – see fig. 2.7 (scale 1:1000).

The dbh of the ramets from each of the clones is shown in Fig. 2.9. ANOVA showed no significant differences in mean dbh between clones ($F_{4, 20}$ 3.86, p 0.07).

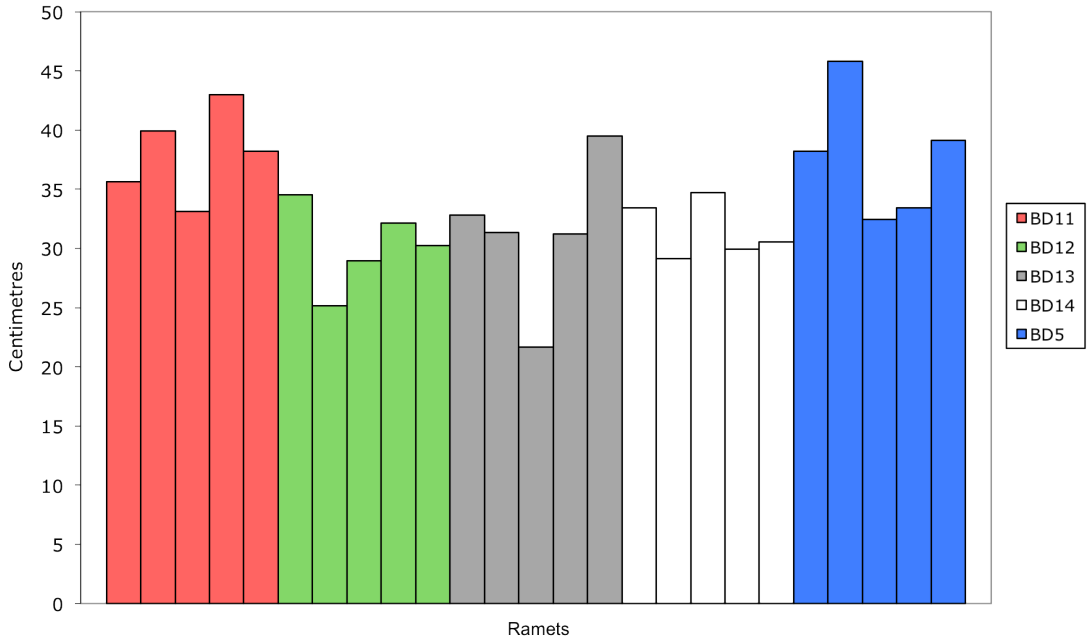

Figure 2.9: dbh of ramets for each clone. Colour-coding coincides with clone positions marked on OS maps.

2.4.2 Bark texture: variation within and between clones

Percentage of smooth bark varied widely between clones: BD11 and BD12 are more similar to each other than either is to clones BD13, BD14 and BD5 (Fig. 2.10) (Model 1: χ^2_6 =18.761, p<0.001). Given the data, the model with 'Clone' as a fixed effect has approximately 217 times greater explanatory evidence (wAIC = 0.995) than the null model (wAIC = 0.005) (Appendix A, Table 1). Thus for this part of the model selection process there is sufficient evidence that the observed variation in smooth bark is due to clone effects. However, clones with a high abundance of smooth bark show considerable intra-clonal variation: according to Model 2 (variance partitioning), ramet as a random effect accounted for 30% of the residual variation seen in % cover of smooth bark across clones (Fig. 2.11).

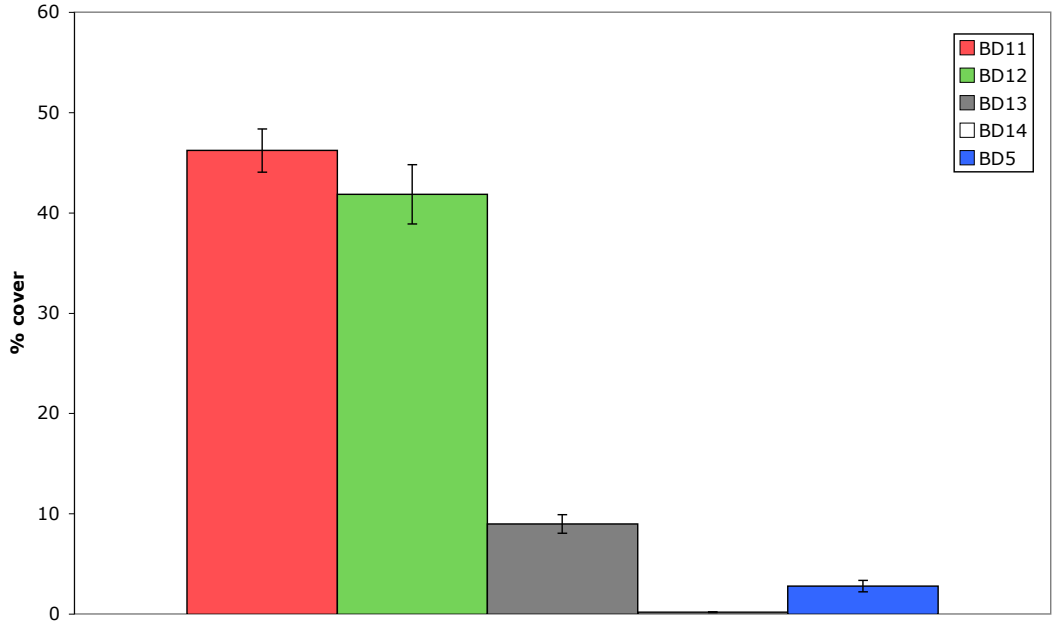

Figure 2.10: % cover (with SE bars) of smooth bark for each clone.

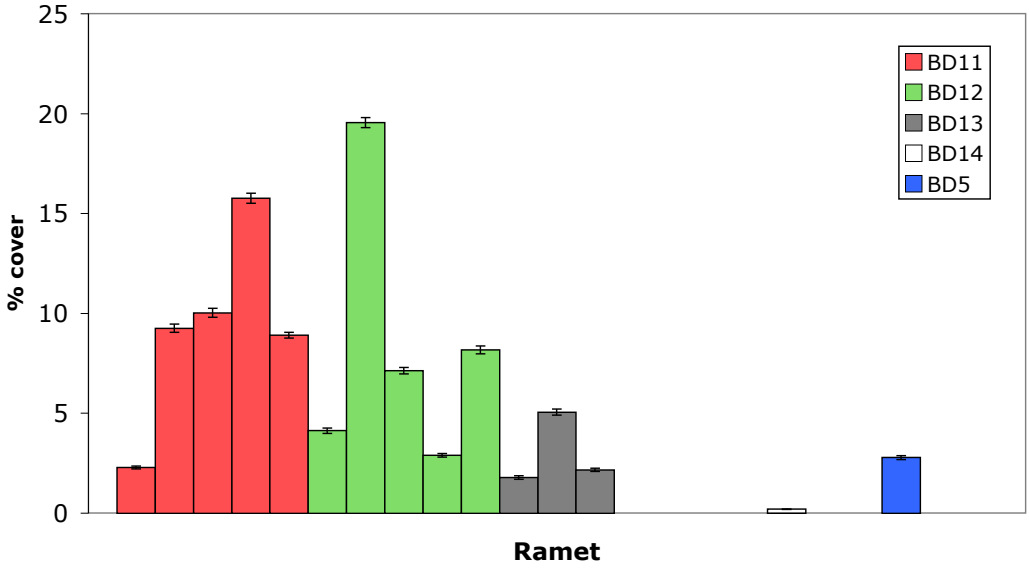

Figure 2.11: % cover (with SE bars) for smooth bark showing range of intra-clonal variation at the level of 'Ramet' for each clone.

2.4.3 Model 2 – variance partitioning of random effects

Implementing Model 2 – variance partitioning with 'Ramet', 'Height' and 'Aspect' as random effects, with 'Clone' as a fixed effect – the optimum model (likelihood ratio and minimised IC) incorporated 'Ramet', 'Aspect' and 'Height' as additive effects: for the best model χ^2_9=48.474, p <0.001, which was superior to the null model (AIC 666.32 vs. 718.66) and the model specifying 'Ramet' as the only random effect (AIC 720.66). Residual variation explained by the random model was partitioned into 'Ramet' (30%, SD 2.42), 'Height' (19%, SD 1.94), and 'Aspect' (19%, SD 1.91).

2.4.4 Full Model – variance partitioning

The optimum variance partitioning model 2 (likelihood ratio and minimised AIC) incorporated 'Ramet', 'Aspect' and 'Height' as additive effects (χ^2_9=48.474, p <0.001). Given the data, the model with 'Ramet', 'Aspect' and 'Height' random additive effects has approximately 11 times greater explanatory power (wAIC = 0.915) than the next best model (wAIC = 0.085) (Appendix A, Table 2). Thus for this part of the model selection process there is sufficient evidence that the additional variation in smooth bark is due to additive effects of 'Ramet', 'Aspect' and 'Height'. Residual variation explained by the random model was partitioned into 'Ramet' (30%, SD 2.42), 'Height' (19%, SD 1.94), and 'Aspect' (19%, SD 1.91).

2.4.5 Full Model – nested fixed effects

Results for Model 3 – the selection and testing of nested fixed effects of Aspect and Height – confirm the additive effects of height and aspect on bark texture. The best model used 'Aspect' and 'Height' as additive fixed effects, which overall indicated a better fitting model and explained more variation when compared to a null model (χ^2_8=9.139, p = 0.03). Given the data, the model with 'Aspect' and 'Height' as fixed additive effects has approximately 4 times greater explanatory power (wAIC = 0.6588) than the next best model (wAIC = 0.1377) (Appendix A, Table 3). However the models with a wAIC value <0.0658 are plausible.

The greatest differences are between N-E (11% and 7%) and S-W (42% and 39%), with 'south' showing the highest percentage, and 'east' the lowest (Fig. 2.12). The percentage of smooth bark increases at each height level from 0% at 40cm to 50% at 200cm (Fig. 2.13).

For the random component 'Ramet' and 'Clone' were highly significant when compared to a null random model (χ^2_{40}=50.589, p< 0.001). Given the data, the model with 'Aspect' and 'Height' as fixed additive effects and 'Clone' and 'Ramet' as nested random effects has approximately 3.37^{+10} times greater explanatory power (*w*AIC = 0.5) than the next best model (*w*AIC = 1.48e-11) (Appendix A, Table 4). Residual variation explained by the random model was partitioned into 'Ramet' within 'Clone' (33%, SD 2.77), and 'Clone' (40%, SD 3.05).

There is no difference between models that specify the random component of the model as nested or additive. Since ramets were selected at random from within each clone it seems appropriate to use the model with nesting effects to understand the nature of the relationship between these variables.

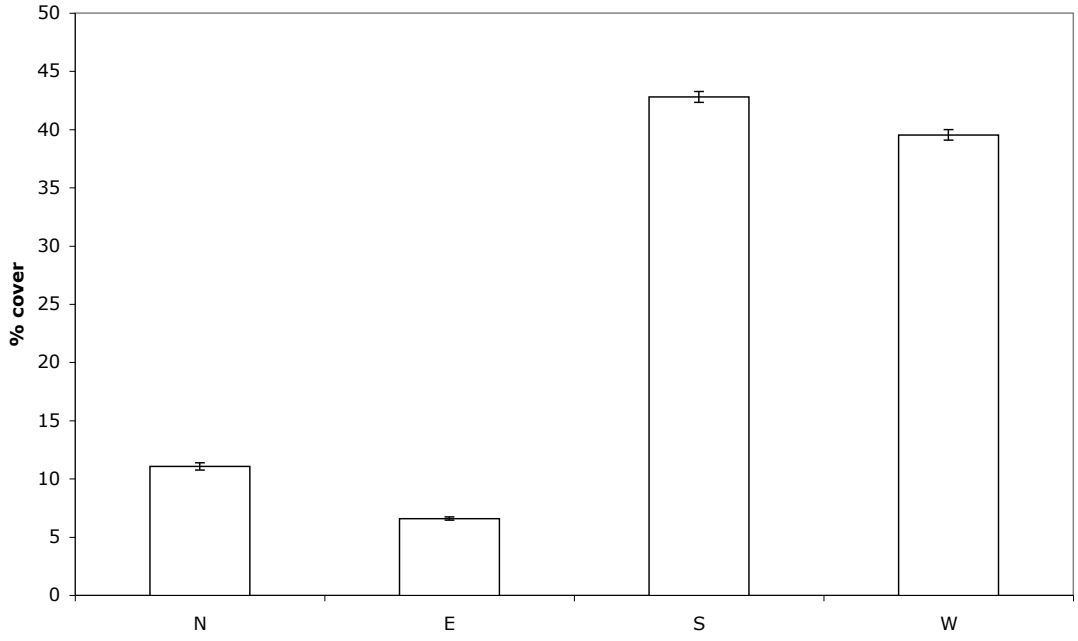

Figure 2.12: % cover (with SE bars) of smooth bark at each aspect.

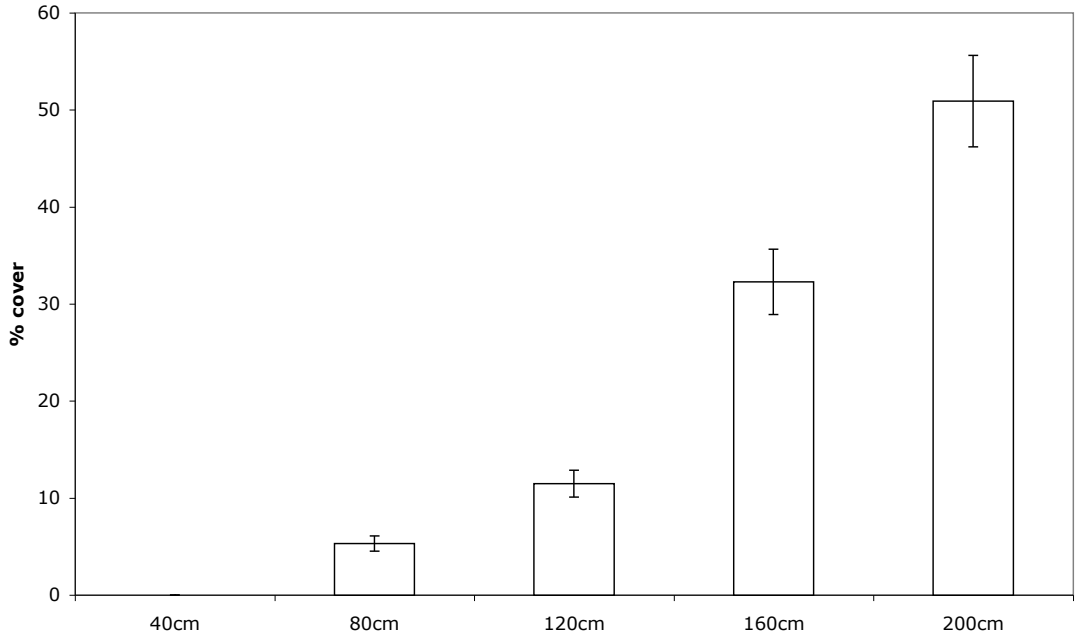

Figure 2.13: % cover (with SE bars) of smooth bark at each height.

There is considerable inter-clonal variation of bark texture at each aspect (Fig. 2.14) and height (Fig. 2.15).

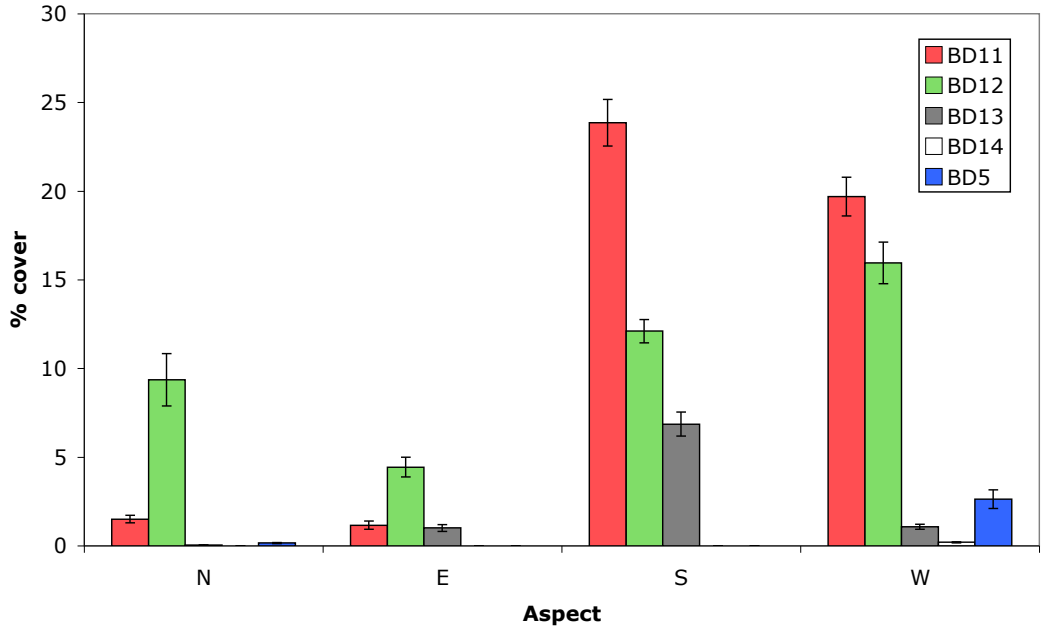

Figure 2.14: % cover of smooth bark (with SE bars) showing range of inter-clonal variation for each aspect: north, east, south and west. The south and west aspects have the largest percentage of smooth bark overall. Significant differences exist with BD11 between aspects N-E and S-W; BD12 between all aspects; BD13 and BD5 between aspects N-E-W and S; BD14 shows no significant differences.

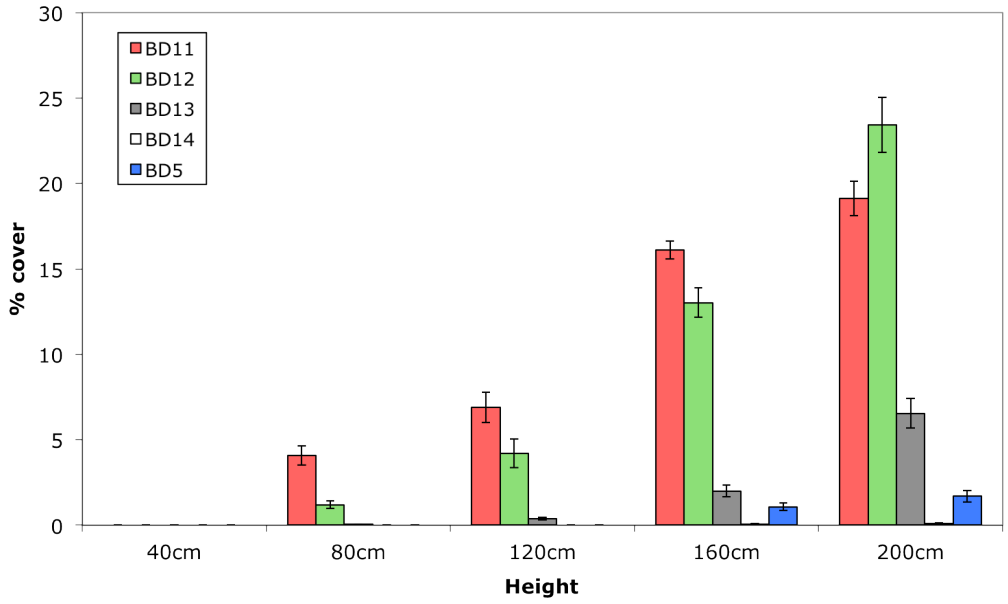

Figure 2.15: % cover of smooth bark (with SE bars) showing range of inter-clonal variation for each height. There is a gradual increase in the percentage of smooth bark with each height level, with the greatest percentage of smooth bark found at 200cm.

2.5 Bark phenolic chemistry

From the subset of samples, a total of 12 compounds were identified: catechol, salicin, (+)-catechin hydrate, chlorogenic acid, o-anisic acid, p-coumaric acid, taxifolin, ampelopsin, naringenin glycoside, eriodictyol, tremulacin, apigenin. Of these, 9 compounds were present in only a few samples and were omitted from the analysis. The remaining compounds, analysed below in detail, were taxifolin ($C_{15}H_{12}O_7$), eriodictyol ($C_{15}H_{12}O_6$) and p-coumaric acid ($C_9H_8O_3$) (Fig. 2.16).

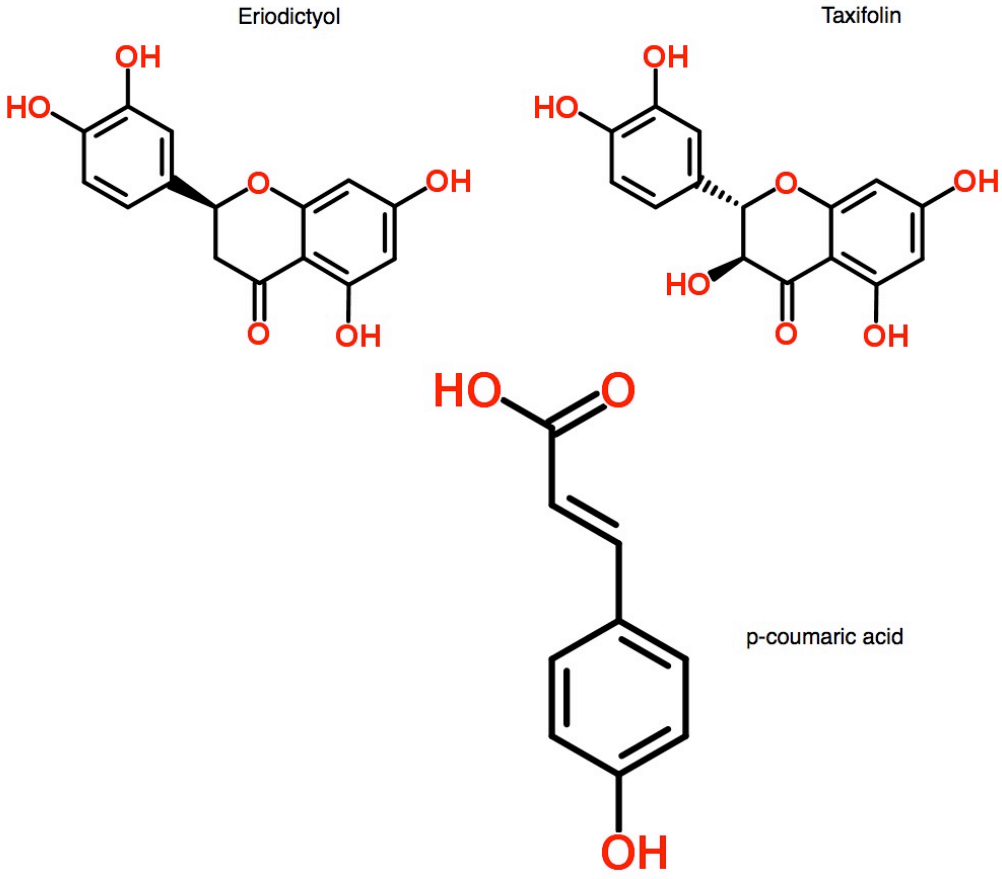

Figure 2.16: Three main compounds identified and quantified in subset of bark samples: eriodictyol (top left); taxifolin (top right); and p-coumaric acid (bottom centre). Chemical structures were obtained from ChemSpider (2010). Eriodictyol and taxifolin are both flavanones – a subgroup of phenol compounds; p-coumaric acid is an isomer of a hydroxy derivative of coumaric acid and the most abundant isomer in nature and a major component of lignocellulose.

2.5.1 Bark phenolic chemistry: variation between clones

Mean concentration of each compound differed between clones (Fig. 2.17).

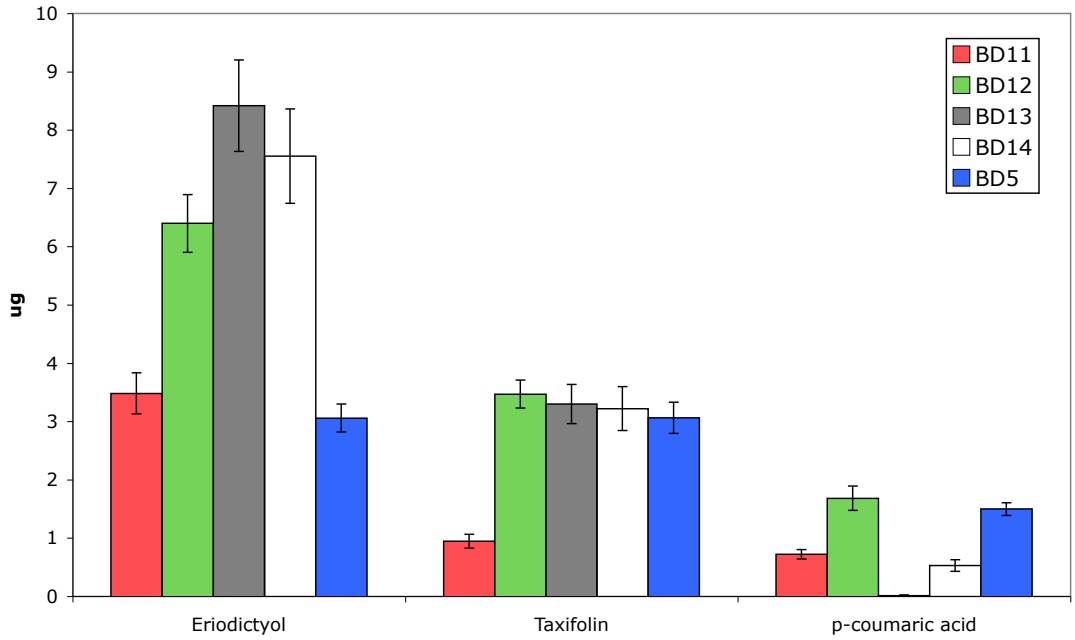

Figure 2.17: Mean concentration µg/mg (with SE bars) of each compound for each clone.

Eriodictyol: as a fixed effect, 'Clone' is significant (χ^2_6=56.197, p<0.001) for explaining differences observed when compared to a null model. Given the data, the model with 'Clone' as a fixed effect has approximately 2.91e^{+10} times greater explanatory power (*w*AIC = 1) than the null model (*w*AIC = 3.43e^{-11}) (Appendix A, Table 5). Thus for this part of the model selection process there is sufficient evidence that the observed variation in eriodictyol is due to clone effects.

BD11 and BD12 are more similar to each other than either is to clones BD13, BD14 and BD5.

Taxifolin: as a fixed effect, 'Clone' is significant (χ^2_6=60.835, p<0.001) for explaining differences observed when compared to a null model. Given the data, the model with 'Clone' as a fixed effect has approximately 2.98e^{+11} times greater explanatory power (*w*AIC = 1) than the null model (*w*AIC = 3.36e^{-12}) (Appendix A, Table 6). Thus for this part of the model selection process there is sufficient evidence that the observed variation in taxifolin is due to clone effects.

p-coumaric acid: as a fixed effect, 'Clone' is significant (χ^2_6=19.816, p<0.001) for explaining differences observed when compared to a null model. Given the data, the model with 'Clone' as a fixed effect has approximately 369 times greater explanatory power (*w*AIC = 0.9973) than the null model (*w*AIC = 0.0027) (Appendix: Chapter 2, Table 7). Thus for this part of the model selection process there is sufficient evidence that the observed variation in p-coumaric acid is due to clone effects.

2.5.2 Full Model – variance partitioning

Implementing Model 2 – variance partitioning with 'Ramet', 'Height' and 'Aspect' as random effects, with 'Clone' as a fixed effect – the optimum model (likelihood ratio and minimised AIC) incorporated only 'Ramet' as a random effect for p-coumaric acid (40%, SD 0.58); none of the measured effects of 'Ramet', 'Aspect' or 'Height' were significant for eriodictyol or taxifolin.

2.5.3 Full Model – nested fixed effects

Results for Model 3 – the selection and testing nested fixed effects – confirmed a slight effect of aspect on eriodictyol. The best model was marginally significant when compared to a null model (χ^2_5=7.828, p = 0.05). Given the data, the model with 'Clone' as a fixed effect has approximately 2.4 times greater explanatory power (*w*AIC = 0.6589) than the next best model which uses a randomly estimated fixed effect (*w*AIC = 0.2652), and 12 times more explanatory power than the model specifying 'Aspect' and 'Height' as additive fixed effects (Appendix A: Table 8). However the models with a *w*AIC value <0.066 are plausible.

For the random component 'Clone' was highly significant when compared to a null random model (χ^2_6=38.434, p< 0.001). Given the data, the model with 'Aspect' as a fixed effect and 'Clone' as a random effect has approximately 2.2 times greater explanatory evidence (*w*AIC = 0.5205) than the next best models (*w*AIC = 0.2398) (Appendix A: Table 9). However the models with a *w*AIC value <0.052 are plausible. 'Clone' accounted for 29% (SD 0.36) of the residual variance.

Taxifolin: as fixed effects, neither 'Aspect' nor 'Height' were significant when compared to a model specifying a null fixed component.

For the random component 'Clone' was highly significant when compared to other models (χ^2_3=39.648, p< 0.001). Given the data, the model with a randomly estimated fixed effect and 'Clone' as a random effect has approximately 2.1 times greater explanatory power (*w*AIC = 0.5358) than the next best model (*w*AIC = 0.2518), and 2.5 times greater explanatory power than the third model (Appendix A: Table 10). However the models with a *w*AIC value <0.053 are plausible. 'Clone' accounted for 53% (SD 0.44) of the residual variance.

p-coumaric acid: as fixed effects, neither 'Aspect' nor 'Height' were significant when compared to a model specifying a randomly estimated fixed component. For the random component, 'Ramet' nested within 'Clone' was significant when compared to a null random model (χ^2_4=6.589, p 0.01). Given the data, the models with a randomly estimated fixed effect with 'Clone' and 'Ramet' as random effects has approximately 9.9 times greater explanatory power (*w*AIC = 0.4760) than the next best model (*w*AIC = 0.0480) (Appendix A: Table 11). However the models with a *w*AIC value <0.0476 are plausible. Residual variation explained by the random model was partitioned into 'Ramet' within 'Clone' (27%, SD 0.58) and 'Clone' (33%, SD 0.42).

There is a marginally significant effect of 'Aspect' on eriodictyol, which may be attributable to a slightly higher concentration of this chemical found in the Northern quadrats. No significant 'Aspect' effect was found for taxifolin or p-coumaric acid (Fig. 2.18). Height was not a significant effect for any of the chemicals measured (Fig. 2.19).

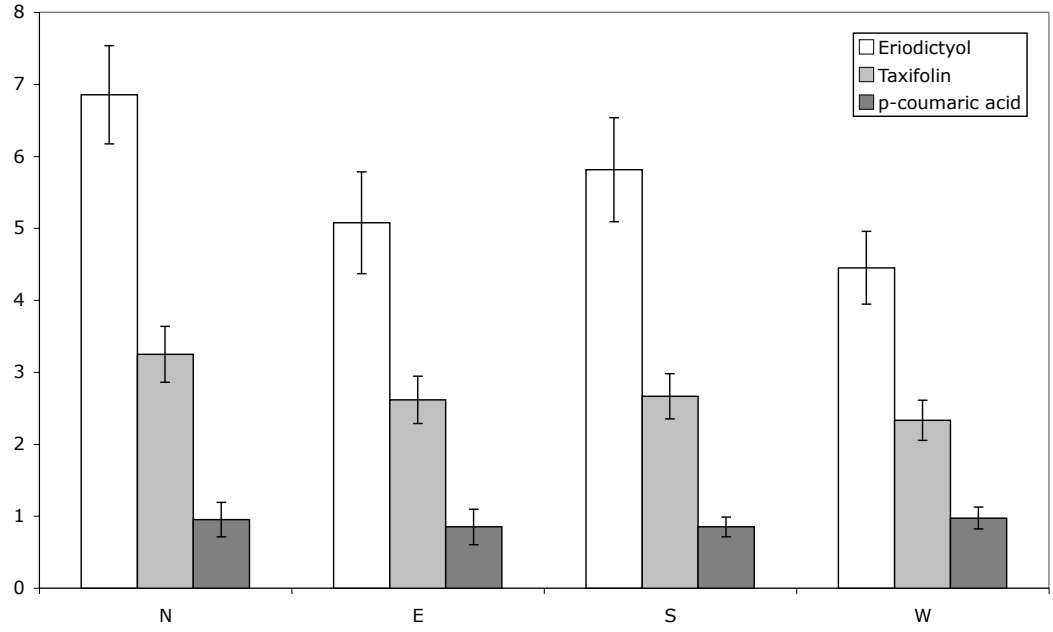

Figure 2.18: Mean concentration µg/mg (with SE bars) of each chemical at each aspect. Estimates and SE of effects from the GLMM indicate overlap between aspects for each chemical.

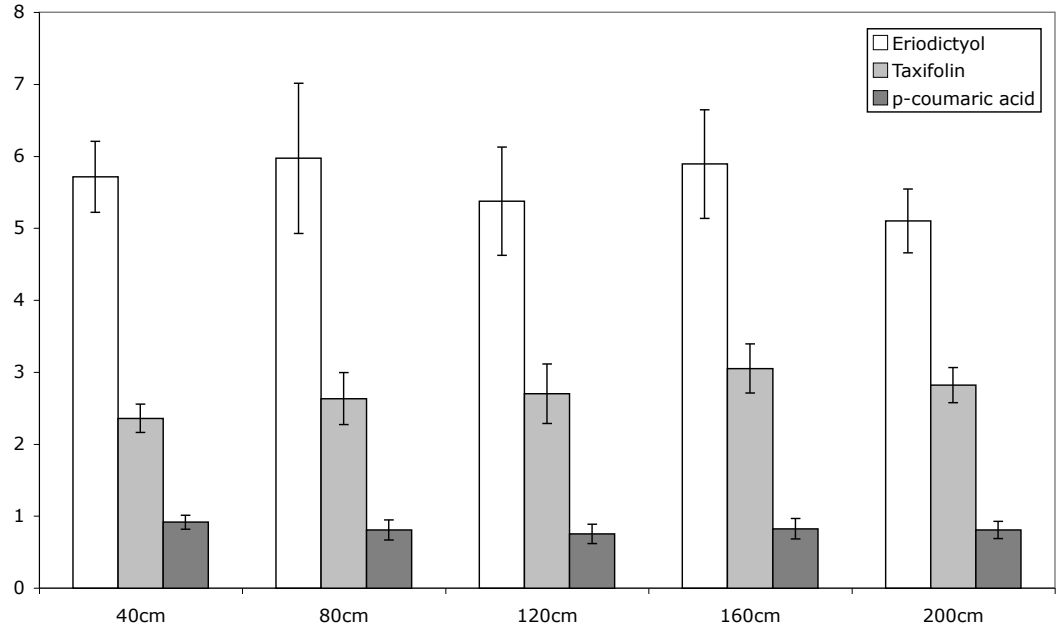

Figure 2.19: Mean concentration µg/mg (with SE bars) of each chemical at each aspect. Estimates and SE of effects from the GLMM indicate overlap between aspects for each chemical.

A summary of the models used, with the fixed and random components is shown in Table 2.4:

Table 2.4: Model selection process with selection of significant effects for each response variable measured. Fixed and random components are indicated relative to model and response variable.

	Response	Fixed	Random	Distribution
Model 1	Smooth bark	Clone	Random	lognormal poisson
Model 2	Smooth bark	Clone	Ramet+Aspect+Height	lognormal poisson
Model 3	Smooth bark	Aspect+Height	Clone/Ramet	lognormal poisson
Model 1	Eriodictyol	Clone	Random	lognormal poisson
Model 2	Eriodictyol	Clone	Random	lognormal poisson
Model 3	Eriodictyol	Aspect	Clone	lognormal poisson
Model 1	Taxifolin	Clone	Random	lognormal poisson
Model 2	Taxifolin	Clone	Random	lognormal poisson
Model 3	Taxifolin	Random	Clone	lognormal poisson
Model 1	p-coumaric acid	Clone	Random	normal
Model 2	p-coumaric acid	Clone	Ramet	normal
Model 3	p-coumaric acid	Random	Clone/Ramet	normal

2.6 Discussion

This research has shown that in a natural population of aspen in Scotland significant differences have been found among five clones for two sets of bark characteristics. The percentage of smooth bark varied significantly between clones (χ^2_6=18.761, p>0.001), as did the phenolic composition of the bark (eriodictyol: χ^2_6=56.197, p<0.001; taxifolin: χ^2_6=60.835, p<0.001; p-coumaric acid: χ^2_6=19.816, p<0.001). In both cases the proportion of variation explained by clonal difference was high. Forty percent of the variation in abundance of smooth bark observed was attributable to 'Clone' effects. For the phenolic compounds eriodictyol, taxifolin, and p-coumaric acid the variation attributable to clones were 29%, 53%, and 33% respectively.

For the smooth bark character, there were significant effects of both height and aspect, each accounting for 19% of the variation. These variables did not account for any significant variation in phenolic compounds.

Overall percentage of smooth bark increased with height, with an absence of smooth bark at 40cm for all clones. The south and west aspects had the greatest abundance of smooth bark, 40-45%, compared with the north and east, 7.5-10%.

2.6.1 Bark texture and developmental processes

When bark is first formed it is smooth, thin and photosynthetic. It comprises the periderm which replaces the epidermis as the protective covering and consists of three structures: 1) the cork or phellem; 2) the cork cambium, or phellogen; and 3) the phelloderm. In smooth bark these layers are thin and difficult to discern. The bark is able to stay smooth by maintaining radial and tangential cell growth in the periderm, concomitant with a 'sloughing off' of the older and inactive periderm cells. A white bloom of dead periderm cells forms protective layers that also varies in consistency between high and low topographical elevations (Covington, 1975).

Rough bark is formed if the integrity of the smooth bark is lost. In these areas of damage new layers of phellogen are induced which produce further phellem, which is forced outwards. The phellem is non-living and comprises inactive suberized cells forming many layers of corky protective tissue. The process of cell differentiation that may result in thickened periderm and rough bark can be complex (Lawson & Poethig 1995; Martín-Trillo & Martínez-Zapater 2002).

A number of different factors can initiate the switch from smooth to rough bark. As a tree ages it will be subject to external stresses that result in a greater amounts of damage to the smooth bark, which then triggers the formation of rough bark. This means that rough bark is more likely to be seen in older bark, located towards the base of the tree. This is a likely explanation for the finding that smooth bark increases with height. The amount of damage suffered by bark is likely to be related to aspect. Kaufert (1937) indicated that damaged bark of *Populus tremuloides* was limited to aspects of the trunk exposed to storm damage. In the present case rough bark was confined to the northern and eastern aspects. Colder north-easterly winds and lack of sunlight may cause damage to the periderm and account for the increase in rough bark on the north and eastern aspects. Finally the propensity to form rough bark may vary according to genotype. This may reflect the inability of some

genotypes to retain smooth bark in the event of being damaged. Older trees with a greater quantity of smooth bark may have a limited capability for the cell differentiation processes that result in suberized and lignified cells.

Genetic control of bark smoothness has been demonstrated in other studies where environment has been controlled. Lamit et al. (2011) quantified differences in bark texture between 25 different genotypes (replicated two to nine times) of *Populus angustifolia* grown in a common garden environment at the Ogden Nature Center in Ogden, Utah, USA. Trees were grown from cuttings collected from wild riparian stands close to the site and cultivated in randomized blocks with genotypes of *P. fremontii* and natural hybrids. Bark texture was classified as 'rough' or 'smooth' and recorded from vertical quadrats, 10cm by 1m tall, placed at ground level on the north side of each tree. Broad-sense heritability (H^2) estimates indicated that 35% of the variance in rough bark was explained by genotype (log-likelihood ratio test: χ^2=15.17, p<0.001).

In studies from other genera Hamilton et al. (2007) examined bark characters, including thickness, among populations of *Eucalyptus globulus*. Genotype and provenance effects were tested across 248 trees of 10 difference subraces [genotypes] growing on a population progeny trial in north-western Tasmania. There were significant effects of both height and provenance on bark thickness: overall bark thickness decreased with height.

Barbour et al. (2009a, b) furthered this research by examining potential genetic effects on the relative thickness of living bark and characteristics of decorticating bark in common garden experiments of *E. globulus*. Twenty trees each from five genotypes were selected at random from a common garden trial established in northern Tasmania. Trees were sampled at 17 years old, and 25m in height. Of the ten bark traits measured, significant variation was found in eight of the traits and attributed to genotype (ranging from p <0.001-0.02). Overall, decorticating bark decreased with height up the trunk.

Though none of these bark traits specifically relate to those measured on the Invertromie aspen clones, the results demonstrate that intra-specific variation in bark

characteristics may not only be under significant genetic control, but with measurable height effects.

It has been demonstrated that variation in bark roughness is under a significant amount of genetic control (up to 40% in *Populus* spp., including this study). Hypotheses for how this roughness is initiated have been proposed but not sufficiently investigated so as to be conclusive. The extent of any protective or defensive traits manifested by a tree will be determined genetically, i.e., the degree of susceptibility to pathogenic invasion and the rate and depth of cell division in the cortical layers, will vary according to the specific genotype of that species. Whether these defensive traits are initiated or not will depend on the individual's degree of exposure to 'attack', and the availability of resources in the environment to supply the tree with sufficient energy to produce extra cellular layers in the periderm.

2.6.2 Bark phenolic chemistry synthesis and partitioning

Apart from the analysis of bark morphology we also studied phenolic chemistry in the bark. Three major compounds found in the bark were:

Eriodictyol ($C_{15}H_{12}O_6$) **–** originally isolated from *Eriodictyon californicum* (Tutin, 1910) but found in a wide range of other plant species (Sakushima et al. 1983; Conde et al. 1996; Lee et al. 1998; Encarnación et al. 1999; Lee et al. 2003; Sudjaroen et al. 2005). It has been shown to possess anti-microbial and anti-inflammatory properties in addition to being fungistatic or fungitoxic in vitro (Anaya et al. 1999; Wang et al. 2010; Ćirić et al. 2011).

Taxifolin ($C_{15}H_{12}O_7$) – originally isolated from *Pseudotsuga taxifolia*, and found in a wide range of other plant species (Pew 1948; Barton & Gardner 1958; Hergert & Goldschmid, 1958), possessing similar anti-microbial and anti-inflammatory properties as Eriodictyol (Gupta et al. 1971; Evensen et al. 2000).

p-coumaric acid ($C_9H_8O_3$) – a ubiquitous plant metabolite, being a major component of cell walls (Pan et al. 1998; Boudet et al. 2003), with important anti-oxidant activity (Luceri et al. 2007) and functioning as an intermediate compound in the formation of phenols (Clifford 2000; Sachan et al. 2006).

Of the three compounds the greatest variation between clones was observed for eriodictyol, then p-coumaric acid. Four of the five clones did not vary significantly for mean concentrations of taxifolin overall. The greatest mean concentration was found in eriodictyol (approx. 6µg/mg), which was 2-3 times greater than taxifolin (approx. 2.5µg/mg) and approximately six times greater than p-coumaric acid (approx. 1µg/mg).

The biosynthetic pathways and biological activity of phenolic compounds in plants are well documented (Harborne 1977; Stafford 1991; Dixon and Paiva, 1995; Orians et al. 1996; Häkkinen, 2000; Winkel-Shirley, 2001; Métraux, 2002; Willför et al. 2003; Taylor & Grotewald, 2005), including specifically *Populus* spp. (Meyermans et al. 2000; Harding et al. 2005; Tsai et al. 2006; Diouf et al. 2009). The primary phenolic compounds in *Populus* spp. are synthesized from salicylic acid via the shikimate-phenylpropanoid pathways, and contain esters of salicin (Pierpoint, 1994). They are stored ubiquitously in the apoplast or within cell vacuoles. As periderm tissues are formed via the cork cambium and become inactive the phenolic compounds may remain within these tissues. Since p-coumaric acid is a major component of lignin, it is an important compound utilized in the formation of cambial layers.

A great diversity of phenolic compounds have been identified from several species, such as *P. deltoides* (Picard & Chenault, 1994: leaves); *P. davidiana* (Zhang et al. 2006: stem bark); *P. balsamifera* (Pearl & Darling 1971: leaves; Mattes et al. 1987: winter buds, internodes), *P. sieboldii* (Greenaway et al. 1991a), *P. ciliata* (Greenaway & Whatley 1991b: bud exudate), *P. angustifolia* (Greenaway & Whatley 1990: bud exudate); *P. trichocarpa* (Pearl & Darling 1971: leaves), and *P. tremula* (Neacsu et al. 2007: knotwood).

Of the three major compounds quantified from the Invertromie aspen bark, eriodictyol has also been identified in bud exudate of *P. sieboldii*, taxifolin has been identified in bud exudate of *P. angustifolia* and knotwood of *P. tremula*, and p-coumaric acid has been identified in knotwood of *P. tremula* and bark of *P. tremuloides*. A major phenolic compound found within most tissues of *Populus*, salicin, was not identified in the five aspen clones from this study. It is likely that

this compound has previously been esterified into the compounds in the samples. Most phenolic compounds are highly unstable and minor changes in temperature or UV levels can be sufficient to affect transitions along their biosynthetic pathway, or allow for the structures to be degraded.

2.6.3 Genetic variation in the concentrations of phenolic compounds

Genetic effects on phenolic compounds *Populus* spp. have received a great deal of attention (Pearl et al. 1958; Tsai et al. 2006). Many studies have investigated relationships between phenolics and plant genetics by analysing leaf chemistry and its effects on arthropod community composition (Bailey et al. 2006; Bangert et al. 2006a & 2006b; Hemming & Lindroth, 1995 & 1999; Hwang & Lindroth, 1997 (and references within); Osier & Lindroth, 2001& 2004; Wimp et al. 2007), mammalian grazing (Diner et al. 2009), and endophytic colonisation (Holeski et al. 2009)

Hwang & Lindroth (1997) found 'striking intraspecific variation in concentrations of phenolic glycosides' when comparing leaf material collected from thirteen different clones (eight ramets per clone) of *Populus tremuloides*. Two chemicals were quantified via high-performance thin-layer chromatography (HPTLC): salicortin and tremulacin. Significant differences in the variation of these phenolics were observed and attributable genotype. A regression analysis of foliar chemistry and overall growth revealed a strong negative correlation (r=-0.764, p 0.002) suggesting costs involved the production of secondary metabolites. These results confirm other studies comparing phenolic concentrations between various *Populus* clones (Hemming & Lindroth1995; Osier & Lindroth 2001, 2004 & 2006).

The majority of studies have concentrated predominantly on several compounds found abundantly in *Populus tremuloides* leaves: tremulacin, salicortin, salicin and tremuloidin. These compounds were not discernible in the *P. tremula* bark samples used for this study, possibly due to structural changes in the compounds over time. Strong differences attributable to genetics were found in those phenolics that were detected and quantified. The measured effects of 'Aspect' and 'Height' did not account for any residual variation, which may be explained by the effects of

stemflow washing solutes and other matter down the outer layers of bark. It is logical to conclude that there are unmeasured environmental effects influencing the residual variation observed for each compound.

The five aspen clones used in this study are randomly scattered across one small site. When sampling 'wild' systems it is important to try to control for random environmental variation as much as possible, but it is highly likely that this cannot be completely eliminated, leading to localised differences on a micro scale. Even minor climatic and edaphic deviations between and within clone sites could be sufficient to alter phenolic chemistry via soil nutrients, timing and rate of leaf decomposition, mammalian/ insect grazer selection of clones based on existing chemical expression, rainfall, stemflow, and subtle differences in community structure of related organisms between each clone. Confounding of effects can be overcome by applying a similar sampling strategy to Scottish aspen clones grown as randomly planted replicates in a common garden experiment. Sampling from such clones would allow environmental effects to be controlled for thus enabling the elucidation of genetic effects, and *vice versa*.

Two sets of aspen physical traits have been measured and are found to vary significantly between clones, suggesting an underlying genetic effect. Considering the close physical nature of the relationship between aspen bark and epiphytic species, it is possible that epiphyte communities are influenced by the variation seen in bark traits. In the following chapter the same five aspen clones will be used to determine whether there are observable differences in epiphyte community structure.

3 Epiphyte community structure profoundly influenced by genetically-based trait variation in a natural aspen stand

3.1 Introduction

Communities are assemblages of different organisms, some of which provide a foundation, or habitat, for other (dependent) species that make up the rest of the community. Genetic variability within the foundation species has the potential to influence the expression of physical and chemical traits within that species, and in turn influence the dependent species' sensitive to these traits. Community genetics is the area of research that investigates how genetic differences in foundation species affect communities of dependent species. Preliminary evidence from a range of systems suggests that genetic variation within a species can be sufficient to affect significantly the community structure of dependent organisms (Johnson & Agrawal, 2005; Bangert et al. 2006a, b; Barbour et al. 2009b; Tack et al. 2010).

In woodland systems the chief structural components are the trees. These interact with a wide variety of different dependent communities, such as arthropods, endophytes, epiphytes and micro-organisms. *A priori* we might expect that genetic variation in trees will have effects on these dependent communities. In the past, studies of these interactions have primarily focused on invertebrate communities, particularly insects (Floate et al. 1993; Dickson & Whitham, 1996) and gastropods (Asplund et al. 2010), but mammals have also been investigated (Dickson & Whitham, 1996; Bailey et al. 2004). In this study I focus on the effects of genetic variation within tree species on their lichen and bryophyte epiphyte community. The same chemical and physical traits that can influence, or even shape dependent communities already studied, include known controls of non-vascular epiphytes (Hyvärinen et al. 1992; Hauck & Javkhlan, 2009).

Epiphytic lichens and bryophytes form very important and often species rich communities on trees (Gustafsson & Eriksson 1995; Uliczka & Angelstam 1999; Hedenås & Ericson 2000; Jüriado et al. 2003). Trees provide microsites within which

epiphytes establish and grow (Barkman 1958; Kantvilas & Jarman 2004; Fritz & Heilmann-Clausen 2010) and therefore it could be said that epiphytes constitute dependent species. There is also evidence that many epiphytes, particularly lichens, penetrate the surface of the tree and interact with chemicals and bark tissues (Ascaso et al. 1980; Ascaso et al. 1983; Ascaso & Rapsch 1985; Inoue et al. 1987; Legaz et al. 1988; Bouaid & Vicente 1998; Legaz et al. 2004). Thus there is the potential for genetic variation affecting both bark morphology and bark chemistry of trees to influence the communities of epiphytes that colonize them. For instance the allelopathic effects of phenolic compounds (salicortin and tremulacin) extracted from *Populus canadensis* bark have been tested on the foliose lichen *Physcia tenella* and found to inhibit growth *in vitro* (Koopman, 2005).

The purpose of this study is to determine whether genetically determined differences in bark morphology and bark chemicals in a foundation tree species, aspen, influence the composition of its epiphytic community, which is composed of lichens, mosses and liverworts.

3.1.1 Aspen as a foundation species

An important attribute of aspen (*Populus tremula* L.) is that in many localities it reproduces from root suckers rather than from seed, forming extensive clones. This means that replicate stems are available within a clone, all possessing the same genotype. Using these replicate stems it is possible to assess whether there are significant differences in epiphyte community structure among aspen genotypes. A further reason for choosing aspen for this study is that it possesses a rich and diverse epiphyte flora (Gustafsson & Eriksson 1995; Ellis 2008; Jüriado et al. 2009), associated with the high pH of the bark (Hedenås & Ericson, 2000; Ellis & Coppins 2007; Jüriado et al. 2009). This means that the aspen system is sensitive for monitoring differences in epiphyte community structure caused by genetic variation in the foundation tree species.

A major morphological feature of aspen, which is likely to affect establishment of lichens, is the bark texture. This shows striking diversity among clones within single populations (Hyvarinen et al. 1992; Sillett et al. 2000; Boudreault et al. 2008; Lamit

et al. 2011). This variation in bark texture facilitates the creation of microsites with differences in, for instance, moisture levels (Sheard & Jonescu 1974; Aboala et al. 1999; Zhang 1999; Levia & Herwitz 2005). An important objective of this research is therefore to establish whether community differences in epiphyte species composition exist between clones in natural situations and whether these relate to observed differences in bark texture among clones.

A major family of chemicals found in the bark of aspen is the polyphenols (Pietarinen et al. 2005; Neacsu et al. 2007). These include condensed tannins and phenolic glycosides, chemicals that are implicated in defence against invasion by pathogens and wood-boring insects (Bennett & Walsgrove 1994; Lindroth & Hwang 1996; Lieutier et al. 1997; Ockels et al. 2007). Limited studies have demonstrated that these compounds may also significantly affect lichen growth (Koopman 2005). Therefore in the second instance it is necessary to determine whether differences in epiphyte communities can be accounted for by clonal differences in the phenolic chemistry of bark tissue.

Of course it is likely that epiphyte community distribution will be affected by other factors unrelated to the genotype of the tree such as the age of the ramet, and the aspect and height of sampling on the ramet (Levia & Wubbena 2006; Hamilton et al. 2007; Barbour et al. 2009a, b). To assess the overall importance of genetic effects on epiphyte community structure in natural situations it is therefore helpful to compare the size of these genetic effects with those caused by environmental differences related to height and aspect of sampling.

Taking these considerations into account, this research has three main aims. The first is to determine whether genetic variation within the foundation tree species aspen influences the composition of its epiphyte communities. The second objective is to establish whether any variation in epiphyte community composition detected among clones could be accounted for by differences in texture or chemical composition of the bark. The final aim is to compare the relative magnitude of differences in community composition caused by genetic variation between clones with variation caused by differences in height and aspect of sampling within a clone.

To achieve these goals, epiphyte communities were compared among five aspen clones growing in close proximity in a natural stand at Invertromie in the Scottish Highlands. Using a stratified quadrat sampling method, the presence of epiphyte species was recorded on aspen clones at different heights and on different aspects of the stems. For each sampling quadrat information was also gathered on bark texture and phenolic composition of the aspen bark. Data on individual species were used to calculate species richness and diversity measures which could be compared among clones. These data were also used in cluster and ordination analysis to look for differences in epiphyte community composition among clones, and relate these to differences in bark texture and phenolic chemistry. In further analyses of community composition, given the huge diversity of species present on aspen bark, epiphytes were classified into biologically meaningful 'functional groups', based on growth form. These growth forms were crustose lichens, fruticose lichens, foliose lichens, mosses and liverworts Analysis of functional groups was used to investigate how the ecological attributes of epiphytes were related to their distribution with respect to clone genotype and differences in height and aspect of sampling. These relationships would be difficult to establish if the analyses were conducted on individual species rather than on functional groups.

3.2 Materials and methods

3.2.1 Study Site

The study was conducted in the Invertromie section of the RSPB Insh Marshes National Nature Reserve (OS NN774998 or Latitude 57 05 24N, longitude 03 59 48W) located near Kingussie in the Cairngorms National Park. Aspen trees used in the study were located on an area of ground raised approximately 200m above the River Spey. Here grassland vegetation (NVC type *MG9 Holcus lanatus-Deschampsia cespitosa*) is interspersed with patches of broadleaved woodland, predominantly birch and aspen (NVC types W3 *Salix pentandra-Carex rostrata* and W4 *Betula pubescens-Molinia caerulea*; riparian woodland *W7 Alnus glutinosa-Fraxinus excelsior-Lysimachia nemorum*, and small areas of *W11 Quercus petraea-Betula pubescens-Dicranum majus* on less riparian areas).

3.2.2 Aspen clones

At the Invertromie site the spatial locations of ramets of five putative aspen clones (BD5, BD11, BD12, BD13, BD14) were mapped, and microsatellite analysis was used to confirm the identity of these five clones as described in Chapter 2. The spatial location of the five clones within the Invertromie reserve is shown in Fig 3.1. Clones BD5 and BD11 are located on their own, away from other aspen clones, while the ramets of the remaining clones, BD12, BD13 and BD14 are intermingled within the same area of the site. From each clone five ramets in the age bracket 50 to 80 years of age were chosen for detailed assessment of epiphytes and environmental variables associated with the ramets (Fig. 3.1).

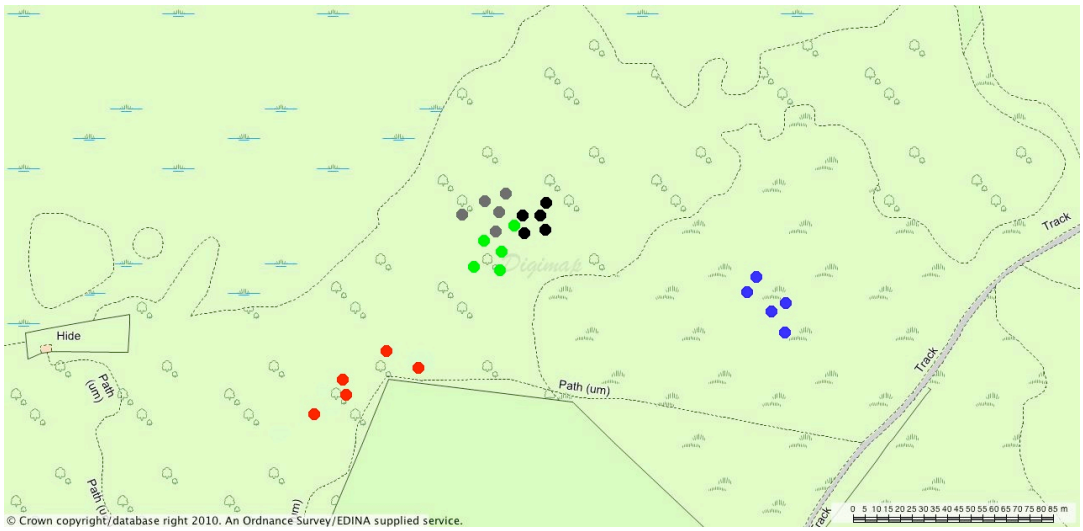

Figure 3.1: Ordinance Survey (2011d). Location of the aspen clones sampled for epiphytes on the Invertromie reserve. Clones are colour coded: Red = BD11, Grey = BD13, Green = BD12, Black = BD14, Blue = BD5. Individual dots show approximate locations of the five ramets of each clone sampled for epiphytes.

In order to interpret differences in epiphyte communities among clones, a number of environmental measurements were taken. For each ramet sampled, the location was recorded using a hand held GPS and from this X, Y coordinates were calculated. The diameter at breast height of each ramet was measured in cm. As well as measurements on ramets, recordings were taken of the proportion of smooth bark within each of the epiphyte sampling quadrats (see later). In addition a 5cm x 5cm piece of outer bark was removed from each epiphyte sample quadrat. These bark

samples were analysed for the proportion of three major phenolic compounds, Taxifolin, Eriodictyol, and p-coumaric acid using the methods described in chapter 2.

3.2.3 Epiphyte community assessment

A quadrat method, modified from Asta et al. (2002), was used to record the frequency, location and abundance of epiphyte species on ramets. Species were identified in the field with a x10 hand lens; specimens that could not be identified with certainty were collected and returned to the Royal Botanic Garden Edinburgh for chemical spot tests, comparison with herbarium specimens, and/ or microscope sections for examination. Lichen specimens that lacked sufficient identification features were classified as 'sterile crusts'. Specimens of *Physcia tenella* and *Physcia adscendens* could not often be distinguished due to a lack of reproductive structures and were grouped together.

On each ramet sampling took place at five heights (40cm, 80cm, 120cm, 160 cm and 200cm). On sloping ground measurements were made from the highest side on each tree. For each height the quadrat was placed at cardinal points on the trunk (N, S, E and W) (Fig. 3.2). Stratified sampling in this manner facilitates the testing of aspect and height effects on epiphyte species. For scoring the abundance of each epiphyte, the 10cm x 10cm quadrat was divided into subunits of $1cm^2$ and the incidence of each species within each these $1cm^2$ subunits was noted. Each quadrat was given a unique identifying label, based on a pre-assigned tree code (601-676), the aspect (N, E, S, W), and height from 1 to 5 (each being 40cm apart) with 1 being the lowest (40cm from the ground) and 5 being the highest (200cm from the ground). For example, the first quadrat on tree 601, at the north aspect, and lowest height would be 601N1. This sampling scheme provided data for 20 quadrats per ramet, and 100 quadrats for each of the five clones.

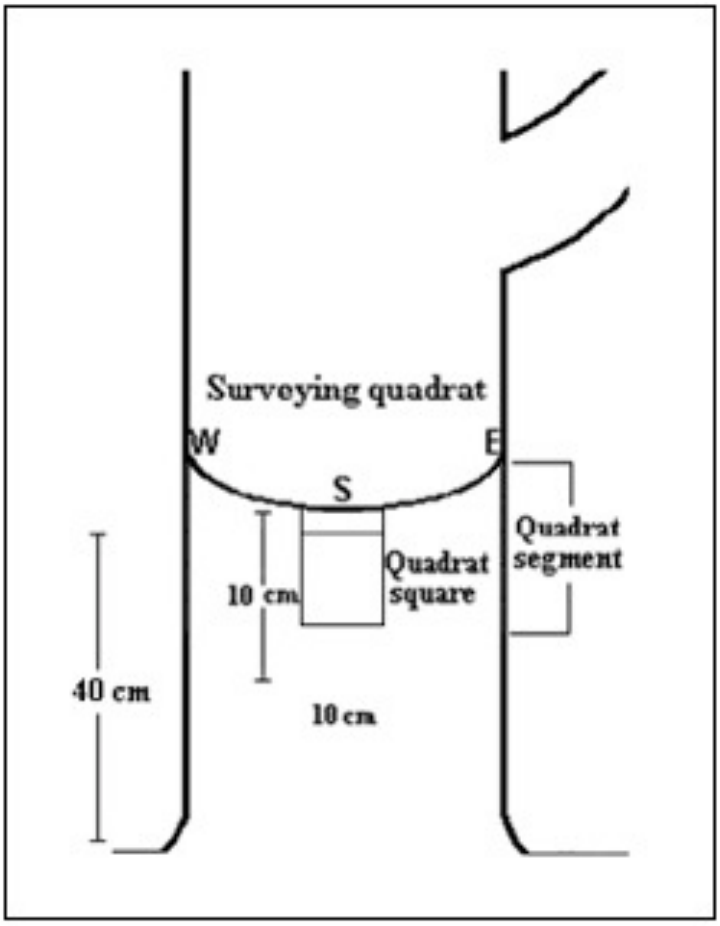

Figure 3.2: Diagram of lichen sampling transects with square quadrats (modified from Asta et al., 2002).

3.3 Statistical analysis

3.3.1 Species richness and Shannon diversity measures

The statistical analyses of differences among clones, heights and aspects used the 10cm x 10 cm quadrat as the sample unit. For each of these quadrats, the total number of epiphytic species recorded was calculated (Total Species Richness). Epiphyte species were then split into five different functional groups (crustose lichens, fruticose lichens, foliose lichens, mosses and liverworts) and the total number of species within each functional group was estimated for each quadrat (Functional Group Species Richness). Finally, based on the number of 1cm x 1cm squares in which species occurred, the frequency of occurrence of each epiphyte species in each 10cm x 10 cm quadrat was estimated. From these data the Shannon Diversity Index H' for total epiphyte species was calculated as:

$$H' = -\sum p_i * \ln(p_i)$$

where p_i is the proportion of the total sample in the quadrat belonging to the *i*th species. This index takes into account species evenness and decreases if a few species dominate.

3.3.2 Analysis of differences among clones, heights and aspects

In order to assess effects of clone, ramet, aspect and height on epiphyte richness and diversity, ANOVA was applied using as response variables: 1. total species richness per quadrat; 2. functional group species richness per quadrat; and 3. Shannon Diversity Index per sample quadrat. Sampling was hierarchical (four aspects and five heights measured on each ramet, with five ramets from each clone). Therefore the analysis tested the effects of clone against ramets within clones, while aspect and height (and their interaction) were tested against the within ramet error term. Ramets were treated as independent variables, as discussed in chapter 1 (pages 26; 41-42).

3.3.3 Community dynamics and distribution

3.3.3.1 Hierarchical cluster analysis

Central to the concept of cluster analysis is the separation of a collection of variables or objects into discrete groups based on similarities (or dissimilarities). The initial step is to standardize the response variables by dividing the mean of each response by the standard deviation. A dissimilarity matrix is then calculated using a distance measure to compute distances between objects. The most straight-forward way of computing distances in multi-dimensional space is the Euclidean distance, which uses geometric distance and is not subject towards bias of new objects to the analysis, or outliers. It is not entirely necessary to standardize raw data prior to calculation of a distance matrix that uses the Euclidean distance.

Geometric distance is computed as:

$$\text{distance}(x,y) = \sqrt{\sum_{i=1}^{n}(y_i - x_i)^2}$$

where x and y are points in Euclidean *n*-space.

When each object has its own putative cluster, the distances are defined by the distance measure. To determine whether two clusters are similar enough to be linked together a linkage rule needs to be specified. Several possibilities exist, such as nearest neighbour, or single linkage; furthest neighbour, or complete linkage; a range of pair-group averaging methods; and Ward's method (Ward 1963). This last method uses an analysis of variance approach to evaluate the distances between clusters by minimizing the sum of squares (SS) of any pair of clusters formed at each step. It is considered to be the most parsimonious method and very efficient.

Using R function 'Hclust' in standard package 'stats', similarities between ramets within each clone based on epiphyte communities were assessed via hierarchical cluster analysis. Euclidean distances of species count data were used with Ward's linkage method. Approximately Unbiased (AU) *p*-values were generated in R v 2.12.0 package 'pvclust' (Suzuki & Shimodaira, 2011) by multi-scale bootstrap resampling for each node and were based on 10,000 replications. AU values above 95% indicate strongly supported groupings.

3.3.3.2 Ordination

Ordination was used to summarise the major variation among sampled epiphyte communities, before relating this to variation in the measured environmental variables. This was done first using unconstrained ordination – which derives axes of community variation first, and then relates these to the environment – and constrained ordination, which seeks to identify only the variation that can be explained by specific environmental variables. Two contrasting approaches were used: 1. Nonmetric multidimensional scaling (unconstrained ordination), which optimally maps sample dissimilarities in community structure, and 2. RDA and/or CCA (constrained ordination), each of which aligns samples in ordination space

based on specific assumptions about the species response, i.e. linear or unimodal, respectively.

Unconstrained analysis: Non-metric multidimensional scaling (NMDS) is a method of optimally mapping observed community dissimilarities into ordination space. The ordination result is selected to reduce dimensionality, while retaining to the best possible effect the structure of a pairwise dissimilarity matrix. This is achieved using a random starting configuration of *n* dimensions (axes), and calculating a measure of stress: i.e. the ordination distances among all pairwise observations in ordination space are plotted against the matrix dissimilarities, and the stepwise deviation from a monotonic gradient is calculated:

$$S = \sqrt{\sum (d^* - d)^2 / \sum d^{*2}}$$

An iterative process recurrently nudges points in ordination space in the direction decreased stress, aiming towards a stable solution with minimum stress. Lower stress values are desirable, though this will depend on the initial size of the data matrix.

In operation, a community data matrix is first tested to find the most parsimonious number of 'dimensions', or axes. A standard method of iterative searching in NMDS is to use several random starts to generate the ordination result as the iteration can often become 'stuck' and fail to reach the best possible solution. Using R v. 2.12.0 with package 'vegan' (Oksanen et al. 2010), an unconstrained NMDS analysis was carried out on the epiphyte community matrix using raw count data for each ramet. The matrix was tested for optimal dimensions before applying the function 'metaMDS' which automates the random iterations to find the solution with the best stress value. Data were automatically square root transformed, then submitted to Wisconsin double standardization, i.e. responses divided by the maxima, and standardized to equal totals. The 'Bray-Curtis' dissimilarity was used, which calculates the total dissimilarity between two sites by dividing the total number of species unique to any two sites by total species at those sites. Twenty random starts were used. In the final result, the solution is rotated so that the largest variance of site scores is on the first dimension. Species and site scores were given equal variances.

Two methods were used to examine the extent to which difference among communities in ordination space were related to and explained by the measured environmental variables.

1. Vector fitting. Measured environmental variables were projected onto the NMDS results via vector fitting, in order to gain insight into their potential effects on epiphyte community structure. The direction of travel of the vector, from the origin on the ordination plot, is related to the environmental correlation with sample axis scores, and the length shows the relative strength of these correlations. The measured variables are: dbh, all aspects, all heights, all clones, bark texture (% smooth bark), and concentration of the phenolic compounds Taxifolin, Eriodictyol, and p-coumaric acid in the bark. These variables are represented in the NMDS plot as lines with arrows.
2. Mantel tests. Mantel tests were used to account for spatial autocorrelation in community structure, by exploring the relative importance of spatial position (proximity) and environment in structuring the dissimilarity among samples. Three Mantel tests were performed (100,000 randomisations) using a reduced set of environmental vectors selected on the basis of potential significance for influencing epiphyte communities, i.e. bark texture, taxifolin, eriodictyol and p-coumaric acid (PC-ORD v 4.41 (McCune & Mefford, 1999)). The three tests involved:

   (i) directly comparing the environmental distance, to the distance between ramets in ordination space analogous to standard indirect ordination analysis,

   (ii) comparing the distance between ramets in ordination space, to the pairwise geographic distances between ramets, to examine spatial autocorrelation in epiphyte community structure, and

   (iii) using a partial Mantel test to compare the distance between ramets in ordination space to environmental distance, when controlling for the effect of geographic distance.

Constrained analysis: Redundancy Analysis (RDA) is related to principal components analysis in making the assumption of linear species' response.

Here RDA was used to examine the direct effect of explanatory variables in controlling epiphyte community structure. First, Monte Carlo permutation (9999 replications) was combined with forward selection to indicate those environmental variables best for explaining epiphyte communities among ramets. Second, spatial coordinates, and the interaction between ramet X and Y location variables, were included as a dummy covariate, and environmental variables were selected after partialling out the spatial effect; in both cases with and without spatial effects.

Analyses were performed for ramets across all clones, and separately for ramets within the three overlapping clones. A one-way ANOVA was applied on the axis 1 scores of the RDA analyses (of all ramets and overlapping ramets respectively) to test whether clones were aligned along a gradient of bark texture.

3.3.3.3 Indicator species analysis

An indicator species analysis can be used to test whether species distributions are significantly different from random, with respect to a grouping variable, i.e. 'clone identity'. The count data for each species was averaged across all ramets and fidelity and relative abundance of each species on each ramet were calculated. The first step is to calculate the mean abundance of species in each group with the following formula:

$$x_{kj} = \frac{\sum_{i=1}^{n_k} a_{ijk}}{n_k}$$

where **A** = sample unit × species matrix; a_{ijk} = abundance of species j in sample unit i of group k; n_k = number of sample units in group k; g = total number of groups. The next step is to calculate the relative abundance (RA_{jk}) of each species in each group:

$$RA_{jk} = \frac{x_{kj}}{\sum_{k=1}^{g} x_{kj}}$$

Following this, the proportional frequency of the species in each group (the proportion of sample units in each group that contain that species) were calculated by first transforming **A** to a matrix of presence-absence, **B**:

$$b_{ij} = a_{ij}^{0}$$

then calculating relative frequency RF_{kj} of species j in group k:

$$RF_{kj} = \frac{\sum_{i=1}^{n_k} b_{ijk}}{n_k}$$

Finally, the two proportions calculated in the results from the first two steps are combined by multiplying them; and the overall result expressed as a percentage, yielding an indicator value (IV_{kj}) for each species j in each group k:

$$IV_{kj} = 100\,(RA_{kj} \times RF_{kj})$$

Using species scores from each of the ordination methods, the significantly non-random species (those found only on one ramet, or in great abundance on one ramet) were plotted onto their respective ordination. Plots therefore highlight those species associated with particular ramets or environmental variables.

3.4 Results

3.4.1 Overview of the epiphyte community

The epiphyte community scored in quadrats on aspen is made up of 57 species comprising 50 lichens, 5 mosses, 1 liverwort and 1 bracket fungus. Species involved are shown in Table 3.1. Four of the lichen species (*Caloplaca flavorubescens, Catillaria nigroclavata, Lecanora persimilis, Lecanora populicola*) have

conservation designations. The presence of the parasitic bracket fungus, *Phellinus tremulae* was noted on some ramets, but was excluded from all subsequent analyses. Principally this is because the species is parasitic and ecologically very different from the epiphytes being studied. Moreover sampling the bark surface with quadrats does not provide information about the biomass/area/abundance of *Phellinus* on/in a given aspen. The sampled lichens fall into three functional groups, crustose, fruticose and foliose. Number of species in each of these and the remaining functional groups (mosses and liverworts) and % abundance of each functional group, in terms of relative number of 1cm x 1cm quadrats in which they were scored, is given in Table 3.2 and illustrated in Figure 3.2.

Table 3.1: List of epiphyte species recorded for the five aspen clones at Invertromie. Lichens are divided into three main morphological groups: crustose, fruticose and foliose. [1 & 6]Red Data Book critically endangered Schedule 8, nationally scarce, [2 & 3]Nationally scarce, [4]Formerly extinct in Red Data book; [5]nationally rare.

Lichens			**Bryophytes**		**Fungi**
Crustose	**Fruticose**	**Foliose**	**Mosses**	**Liverworts**	**Bracket**
Arthonia didyma	Bryoria fuscescens	Evernia prunastri	Dicranum scoparium	Frullania dilatata	Phellinus tremulae[1]
Arthonia radiata	Cladonia squamules	Hypogymnia physodes	Hypnum andoi		
Buellia disciformis	Ramalina farinacea	Hypogymnia tubulosa	Hypnum cupressiforme		
Buellia griseovirens	Ramalina fraxinea	Melanelixia fuliginosa subsp. glabratula	Leucodon sciuroides		
Caloplaca flavorubescens[2]	Usnea subfloridana	Melanelixia subaurifera	Orthotrichum speciosum[6]		
Caloplaca holocarpa		Parmelia saxatilis			
Caloplaca sericea		Parmelia sulcata			
Candelariella cf. reflexa		Phaeophyscia orbicularis			
Catillaria nigroclavata[3]		Physcia aipolia			
Cliostomum griffithii		Physcia leptalia			
Lecania naegelii		Physcia tenella/adscendens			
Lecanora carpinea		Physconia distorta			

Lichens			Bryophytes		Fungi
Crustose	**Fruticose**	**Foliose**	**Mosses**	**Liverworts**	**Bracket**
Lecanora cf. intumescens		Platismatia glauca			
Lecanora chlarotera		Pseudevernia furfuracea			
Lecanora expallens		Xanthoria parietina			
Lecanora persimilis[4]					
Lecanora populicola[5]					
Lecanora pulicaris					
Lecidella elaeochroma					
Leptorhapis atomaria					
Megalaria grossa					
Mycoblastus fucatus					
Ochrolechia androgyna					
Ochrolechia microstictoides					
Pertusaria armara					
Pertusaria pertusa					
Phlyctis argena					
Sclerophora pallida					
Unid.crust					
Tephromela atra					

Table 3.2: Functional groups of epiphyte species recorded at Invertromie, showing the total number of species within each group and the relative abundance (%).

Functional Group	Number of Species	Relative Abundance (%)
Crustose lichens	30	26
Fruticose lichens	5	13
Foliose lichens	15	47
Moss	5	3
Liverwort	1	10
Bracket Fungus	1	1

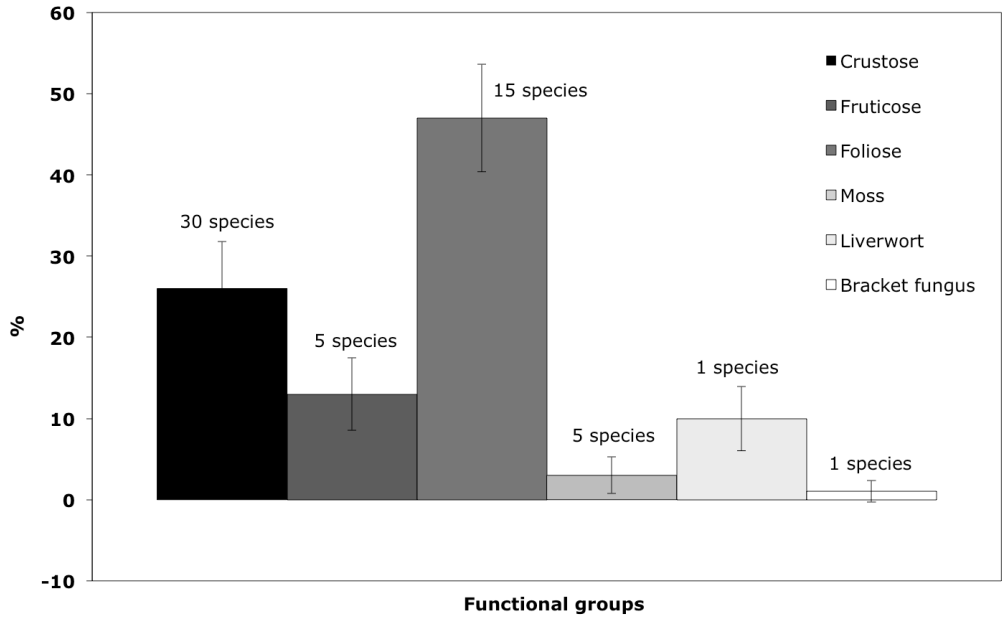

Figure 3.3: Percent relative abundance (SE) of each functional group showing number of species representing each group recorded on aspen at Invertromie.

3.4.2 Distribution of epiphyte species on aspen clones

Of the 57 species encountered, 26 were found on all 5 aspen clones, nine were present on 4 of the clones, 6 were found on 3 clones, 8 were found on only two clones, while 7 species were detected only on one aspen clone. Clone BD13 had two unique species; clone BD11 had 3 unique species, whilst clones BD5 and BD12 each had only one unique epiphytic species.

A comparison of rank abundance of epiphyte species in the whole sample and on individual clones indicates some differences in the composition of the epiphyte community on different clones. Most and least abundant species across all clones,

and on each clone are listed in Table 3.3. The most abundant species, occurring in 384 quadrats, is the fruticose lichen, *Ramalina farinacea.* Of the five clones, this species is also the most abundant on BD13 and BD14, and one of the top five most abundant species on BD12 and BD5. Other abundant species recorded across the site are foliose species *Physcia tenella/ adscendens* (also on BD11, BD12, BD13, and BD5), *Parmelia sulcata* (also abundant on all clones), *P. saxatilis* (also abundant on BD13, and BD5), and the liverwort *Frullania dilatata* (also abundant on BD11). Species recorded as abundant on the other clones were: crustose *Tephromela atra* (BD11 and BD12); foliose *Hypogymnia physodes* (BD11 and BD14); foliose *Phaeophyscia orbicularis*; and crustose *Ochrolechia androgyna* (BD14).

The least abundant species, occurring in one quadrat, is the crustose lichen, *Arthonia didyma.* This species was recorded on BD13. Other species of low abundance recorded across the site are crustose *Lecanora intumescens* (recorded on BD11), *Dicranum scoparium* (recorded on BD11; one of the least abundant species on BD13), foliose *Physcia leptalia* (also least abundant on BD12, BD13, BD14, and BD5), and crustose *Leptorhapis atomaria* (recorded on BD11 and BD12). Other species recorded as low abundance on the other clones were: fruticose *Usnea subfloridana* (BD11, BD12, BD13, and BD14); sterile crustose (BD11); *Sclerophora pallida* (BD11, BD12, and BD5); fruticose *Ramalina fraxinea* (BD11); foliose *Platismatia glauca* (BD11); crustose *Ochrolechia microstictoides* (BD12, BD14 and BD5); *Dicranum sciuroides* (BD12, BD13 and BD5); *Hypnum andoi* (BD13 and BD14); foliose *Phaeophyscia orbicularis* (BD14); and *Ochrolechia androgyna* (BD5). The foliose *Phaeophyscia orbicularis* was one of the most abundant species on BD12 and BD14, but not recorded on BD14. Likewise, the crustose *Ochrolechia androgyna*, whilst abundant on BD14, was not recorded on BD11 or BD5.

Mean percent cover for each epiphytic species across each of the five clones is shown in Appendix B, Table 1. Certain species are more abundant on some clones than others, such as *Megalaria grossa* (abundant on BD11 and BD12, scarce on the other three clones) and *Frullania dilatata* (highly abundant on BD11).

Table 3.3: Top five most and least abundant epiphyte species recorded on Invertromie aspen, comparing the overall abundant species (column labelled 'All'), and each of the five clones.

	All	**BD11**	**BD12**	**BD13**	**BD14**	**BD5**
Most abundant	Ramalina farinacea	Frullania dilatata	Physcia tenella/ adscendens	Ramalina farinacea	Ramalina farinacea	Parmelia saxatilis
	Physcia tenella/ adscendens	Physcia tenella/ adscendens	Parmelia sulcata	Parmelia sulcata	Parmelia sulcata	Ramalina farinacea
	Parmelia sulcata	Parmelia sulcata	Ramalina farinacea	Physcia tenella/ adscendens	Ochrolechia androgyna	Phaeophyscia orbicularis
	Frullania dilatata	Tephromela atra	Tephromela atra	Frullania dilatata	Parmelia saxatilis	Parmelia sulcata
	Parmelia saxatilis	Hypogymnia physodes	Phaeophyscia orbicularis	Parmelia saxatilis	Hypogymnia physodes	Physcia tenella/ adscendens
Least abundant	Arthonia didyma	Usnea subfloridana	Usnea subfloridana	Usnea subfloridana	Usnea subfloridana	Sclerophora pallida
	Lecanora intumescens	Sterile crust	Sclerophora pallida	Physcia leptalia	Physcia leptalia	Physcia leptalia
	Hypnum andoi	Sclerophora pallida	Physcia leptalia	Dicranum scoparium	Phaeophyscia orbicularis	Ochrolechia microstictoides
	Physcia leptalia	Ramalina fraxinea	Ochrolechia microstictoides	Leucodon sciuroides	Ochrolechia microstictoides	Ochrolechia androgyna
	Leptorhapis atomaria	Platismatia glauca	Dicranum scoparium	Hypnum andoi	Leucodon sciuroides	Dicranum scoparium

3.4.3 Analysis of species richness and species diversity of epiphytes

3.4.3.1 Variation among aspen clones

Significant differences were found in species richness of the total epiphyte community. Species richness of crustose (p <0.05), fruticose (p <0.001) and foliose (p <0.001) lichens, and liverworts (p <0.001) differed significantly among clones (Tables 3.4 & 3.5; Figures 3.3-3.5), though no discernable differences in species richness of mosses. There were also significant differences in species diversity index among clones (p <0.01) (Tables 3.4 & 3.5). The greatest species richness was found on BD12 (10.04, SE 0.284), the least on BD14 (6.50, SE 0.317). Of the functional groups, BD12 had the greatest number of crustose lichens (4.87, SE 0.279); BD5 had the greatest richness of fruticose and foliose lichens (1.11, SE 0.053 and 4.55, SE 0.129); BD11 had the greatest richness of liverwort (0.79, SE 0.041); and BD13 had the greatest mean number of mosses (0.44, SE 0.057). Lowest species richness of crustose lichen diversity was found on BD14 (2.58, SE 0.219); lowest fruticose and foliose richness was on BD11 (0.50, SE 0.054 and 2.39, SE 0.158); lowest species richness of liverworts and mosses were on BD14 (0.08, SE 0.027); and BD12 (0.23, SE 0.045) respectively. The overall species diversity index for epiphytes (Shannon Index) was greatest on BD12 (2.260, SE 0.032), and least on BD11 (1.808, SE 0.061).

3.4.3.2 Variation among sampling heights

For all variables measured (total species richness, species richness for each functional group and Shannon diversity of functional groups) apart from species richness of mosses, there were significant differences in epiphyte communities among sampling heights, specifically in the total species richness (p <0.001), species richness of crustose (p <0.001), foliose (p <0.001), and fruticose (p <0.01) lichens, and liverworts (p <0.01) (Table 3.6; Figures 3.6-3.8). Shannon diversity indices were also highly significantly different at different heights (p <0.001). There were no significant differences for the mosses. The highest total species richness was found at 200cm (8.81, SE 0.352), and the least at 40cm (6.82, SE 0.362). For the functional groups, the greatest richness of crustose lichens (8.81, SE 0.352) was at

200cm; 120cm had the most fruticose lichen and liverwort species (0.93, SE 0.046 and 0.40, SE 0.049 respectively); the highest numbers of foliose lichen species were found at 80cm (3.54, SE 0.168); moss diversity was greatest at 40cm (0.37, SE 0.056). Lowest species richness of crustose, fruticose and foliose lichen was found at 40cm (6.82, SE 0.362; 0.69, SE 0.053, and 2.64, SE 0.187); lowest numbers of liverwort and moss species were at 160cm (0.25, SE 0.044) and at 80cm (0.25, SE 0.052) respectively. The greatest Shannon Diversity was found at 80cm (2.061, SE 0.048), whilst the least was found at 40cm (1.730, SE 0.069).

3.4.3.3 Variation among aspects

Significant differences were found among aspect for total species richness of epiphytes, species richness of all epiphyte types (p <0.001), except fruticose lichens (Table 3.7; Figures 3.9-3.11) and for overall species diversity. The highest number of species was found on the South aspect (9.456, SE 0.285), the least on the East (6.848, SE 0.332). Among the functional groups, South had the highest richness of crustose lichens (4.584, SE 0.244); North had the highest richness of fruticose lichens (0.888, SE 0.043); West had the highest richness of foliose lichens (3.688, SE 0.149); East had the highest number of liverwort and moss species (0.48, SE 0.045 and 0.456, SE 0.051). The greatest species diversity was found at the South aspect (2.168, SE 0.041), whilst the least was found on the Eastern aspect (1.737, SE 0.061).

Table 3.4: Results of Analysis of Variance testing for effects of clones, height and aspect on various parameters of epiphyte community on aspen clones. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant.

Source of Variation	df	MS Total species	MS Crustose lichens	MS Fruticose lichens	MS Foliose lichens	MS Liverworts	MS Mosses	MS Shannon Index
Among Clones	4	200.93 **	83.09 n.s.	4.78 ***	78.57 ***	8.05 ***	0.89 n.s.	4.77 **
Among Ramets	20	34.837 n.s.	24.01	0.52 n.s.	6.43 n.s.	0.75 n.s.	1.01 n.s.	0.89 n.s.
Height	4	63.583 ***	25.4 ***	0.83 ***	12.74 ***	0.52 ***	0.25 n.s.	2.032 ***
Aspect	3	160.637 ***	112.75 ***	0.37 n.s.	34.24 ***	1.58 ***	2.386 ***	4.279 ***
Height x Aspect	12	14.462 *	15.41 ***	0.17 n.s.	0.78 n.s.	0.23 *	0.17 n.s.	0.25 n.s.
Error	456	7.258	4.45	0.19	1.62	0.12	0.21	0.19

Table 3.5: Epiphyte community diversity for each clone based on mean total species per quadrat/ clone; mean species per functional group/ clone; and Shannon Index based on mean species per quadrat (with SE). Results of ANOVA are indicated by significance values: *** p <0.001; ** p <0.01; p <0.05; n.s. not significant.

Clone	Total species	Crustose lichens	Fruticose lichens	Foliose lichens	Liverworts	Mosses	Shannon Index
BD11	7.07 (0.328)	3.01 (0.229)	0.50 (0.054)	2.39 (0.158)	0.79 (0.041)	0.38 (0.059)	1.808 (0.061)
BD12	10.04 (0.284)	4.87 (0.279)	0.85 (0.039)	3.78 (0.120)	0.31 (0.047)	0.23 (0.045)	2.260 (0.032)
BD13	8.15 (0.368)	3.66 (0.276)	0.88 (0.038)	2.78 (0.165)	0.39 (0.049)	0.44 (0.057)	1.955 (0.064)
BD14	6.50 (0.317)	2.58 (0.219)	0.80 (0.043)	2.79 (0.144)	0.08 (0.027)	0.25 (0.046)	1.741 (0.056)
BD5	8.91 (0.270)	2.88 (0.249)	1.11 (0.053)	4.55 (0.129)	0.12 (0.033)	0.25 (0.044)	2.139 (0.032)
Significance	**	*	***	***	***	n.s.	**

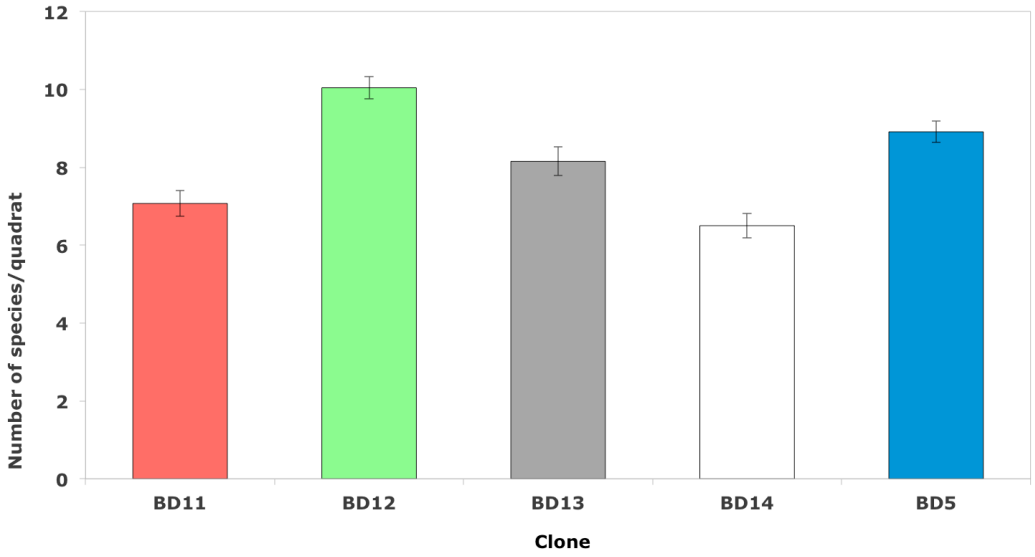

Figure 3.4: Mean number of epiphyte species per quadrat (with SE) in each of the clones.

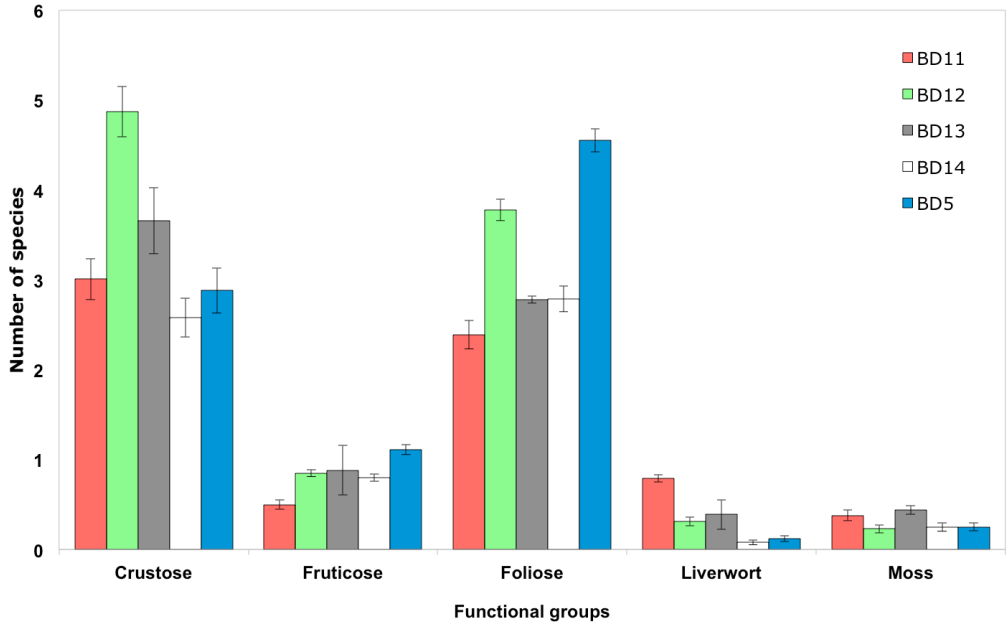

Figure 3.5: Mean number of epiphyte species within different functional groups per quadrat (with SE) for each of the clones.

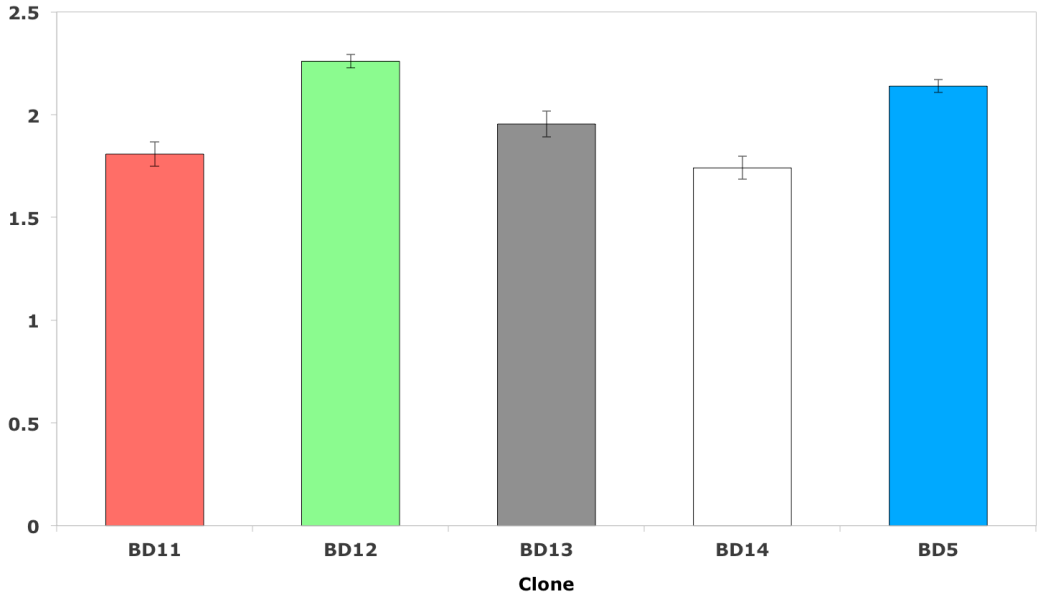

Figure 3.6: Shannon Index of total species within quadrats for each of the clones.

Table 3.6: Epiphyte community diversity for each sampling height based on mean total species per quadrat/ height; mean species per functional group/ height; and Shannon Index based on mean species per height. Results of ANOVA significance values: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant.

Height	Total species	Crustose lichens	Fruticose lichens	Foliose lichens	Liverworts	Mosses	Shannon Index
40cm	6.82 (0.362)	2.74 (0.250)	0.69 (0.053)	2.64 (0.187)	0.38 (0.049)	0.37 (0.056)	1.730 (0.069)
80cm	8.61 (0.317)	3.55 (0.231)	0.88 (0.041)	3.54 (0.168)	0.39 (0.049)	0.25 (0.052)	2.061 (0.048)
120cm	7.97 (0.305)	3.03 (0.257)	0.93 (0.046)	3.29 (0.133)	0.40 (0.049)	0.32 (0.047)	1.992 (0.043)
160cm	8.46 (0.322)	3.71 (0.271)	0.84 (0.051)	3.39 (0.148)	0.25 (0.044)	0.27 (0.049)	2.035 (0.052)
200cm	8.81 (0.352)	3.97 (0.292)	0.80 (0.055)	3.43 (0.168)	0.27 (0.045)	0.34 (0.052)	2.076 (0.050)
Significance	***	***	**	***	**	n.s.	***

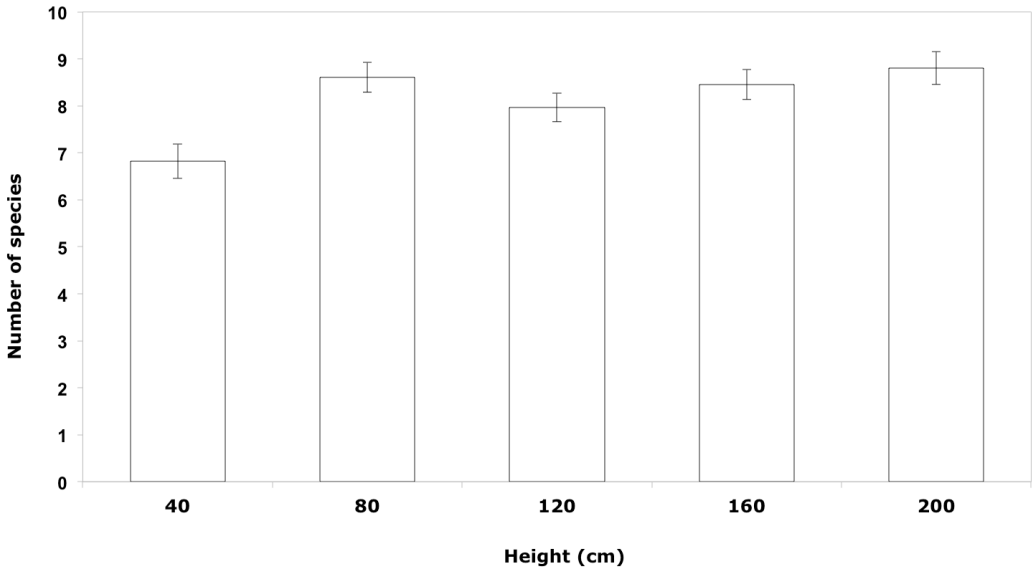

Figure 3.7: Mean number of epiphyte species within different functional groups per quadrat (with SE) for each of the sampling heights.

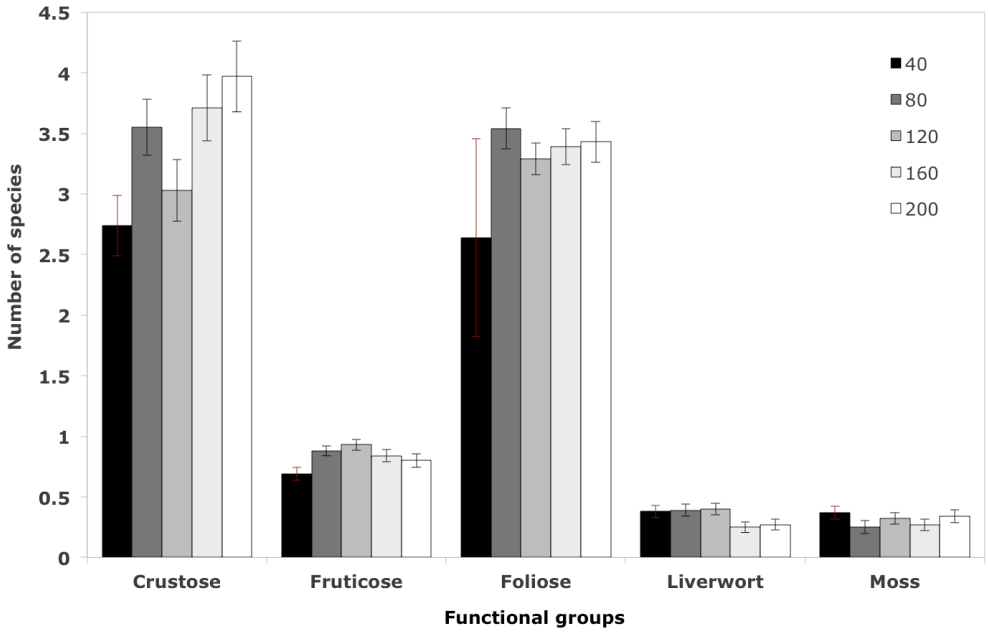

Figure 3.8: Species richness within quadrats at each sampling height for each functional group.

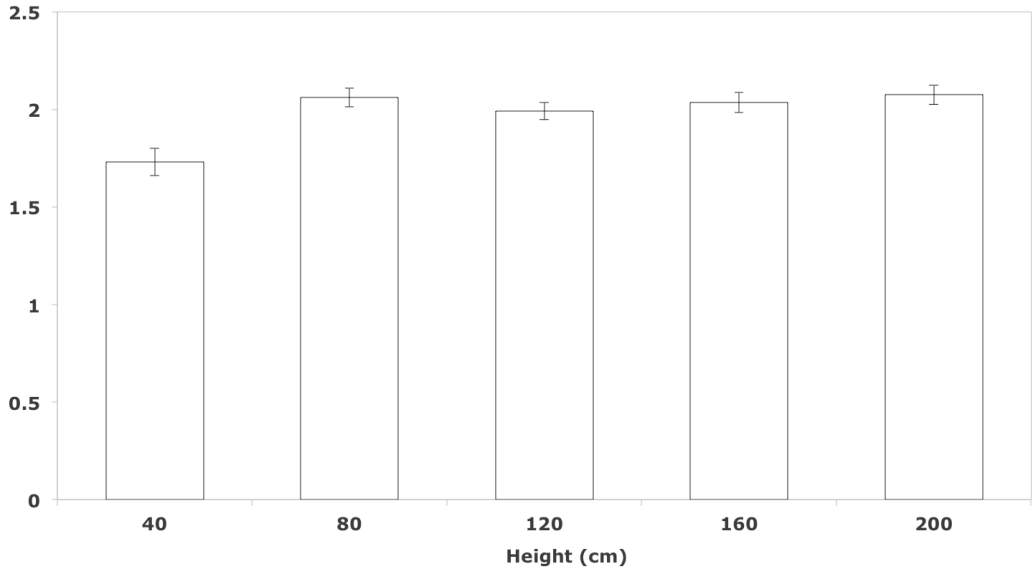

Figure 3.9: Shannon Index of total species within quadrats for each sampling heights.

Table 3.7: Epiphyte community diversity for each aspect based on mean total species per quadrat/ aspect; mean species per functional group/ aspect; and Shannon Index based on mean species per quadrat. Results of ANOVA significance values: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant.

Aspect	Total species	Crustose lichens	Fruticose lichens	Foliose lichens	Liverworts	Mosses	Shannon Index
North	7.640 (0.260)	2.616 (0.190)	0.888 (0.043)	3.400 (0.143)	0.352 (0.043)	0.384 (0.051)	1.945 (0.041)
East	6.848 (0.332)	2.648 (0.231)	0.768 (0.047)	2.496 (0.151)	0.48 (0.045)	0.456 (0.051)	1.737 (0.061)
South	9.456 (0.285)	4.584 (0.244)	0.856 (0.042)	3.448 (0.122)	0.312 (0.042)	0.265 (0.042)	2.168 (0.041)
West	8.592 (0.281)	3.752 (0.229)	0.80 (0.045)	3.688 (0.149)	0.208 (0.036)	0.144 (0.032)	2.066 (0.041)
Significance	***	***	n.s.	***	***	***	***

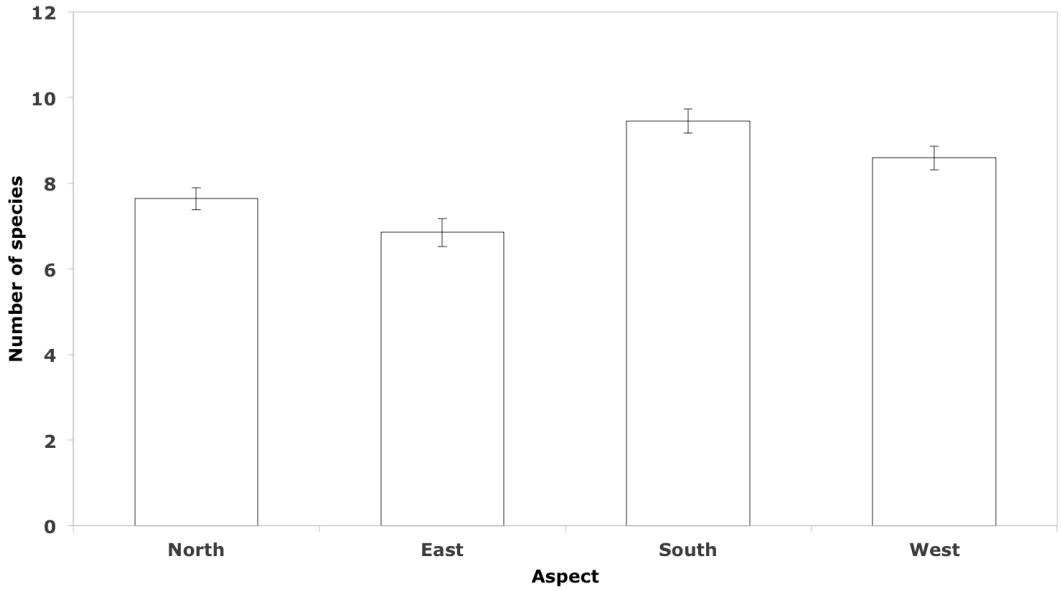

Figure 3.10: Total species richness of epiphytes within quadrats for each aspect.

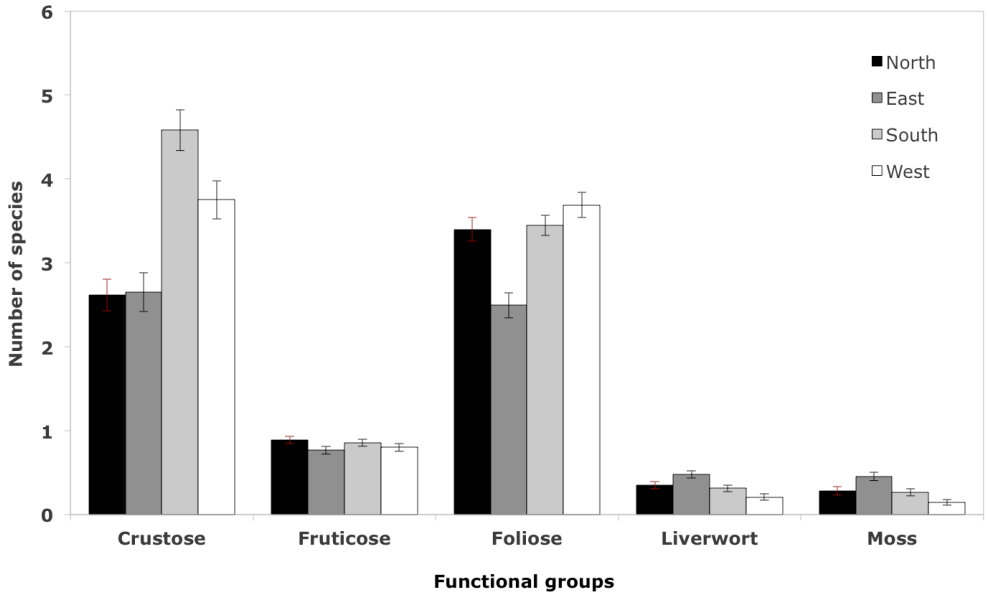

Figure 3.11: Species richness of epiphytes within each functional group for each aspect.

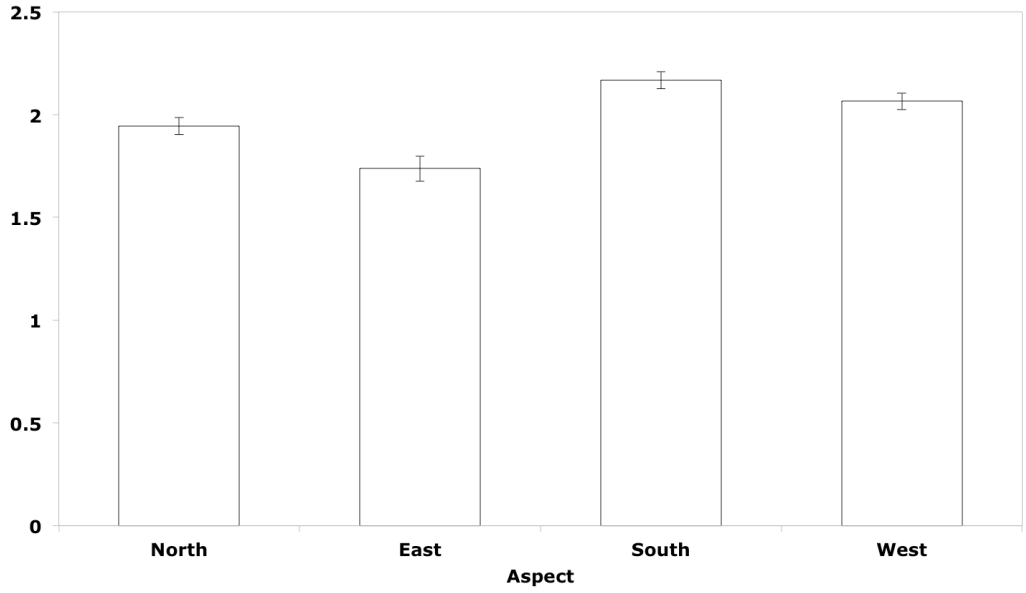

Figure 3.12: Shannon's diversity index for epiphyte species within quadrats for each aspect.

3.4.4 Hierarchical cluster analysis

Five main clusters were produced by the 'Hclust' analysis (Fig. 3.12). These comprised: 1. four ramets from BD5, with one ramet from BD12, though the AU support was weak (74%); 2. two ramets each from BD13 and BD14 with a ramet from BD5 and BD12, with moderate AU support (91%); 3. three ramets from BD14 and two ramets from BD13, but with no AU support (55%); 4. three ramets from BD12 with very strong AU support (99%); and 5. five ramets from BD11, with one ramet from BD13 with strong AU support (97%). Those clusters with AU support <95% were not consistently reproduced during resampling and may adopt alternate configurations. Ramets from BD11 form the strongest cluster, and to a lesser extent, so do BD12 and BD5. BD13 and BD14, which are part of the intermingled clones (together with BD12) form diffuse groups. This is to be expected, as some epiphytes are ubiquitous across the clones and indicates an environmental influence on community composition. However, there nevertheless appears to be an effect of clone on epiphyte communities.

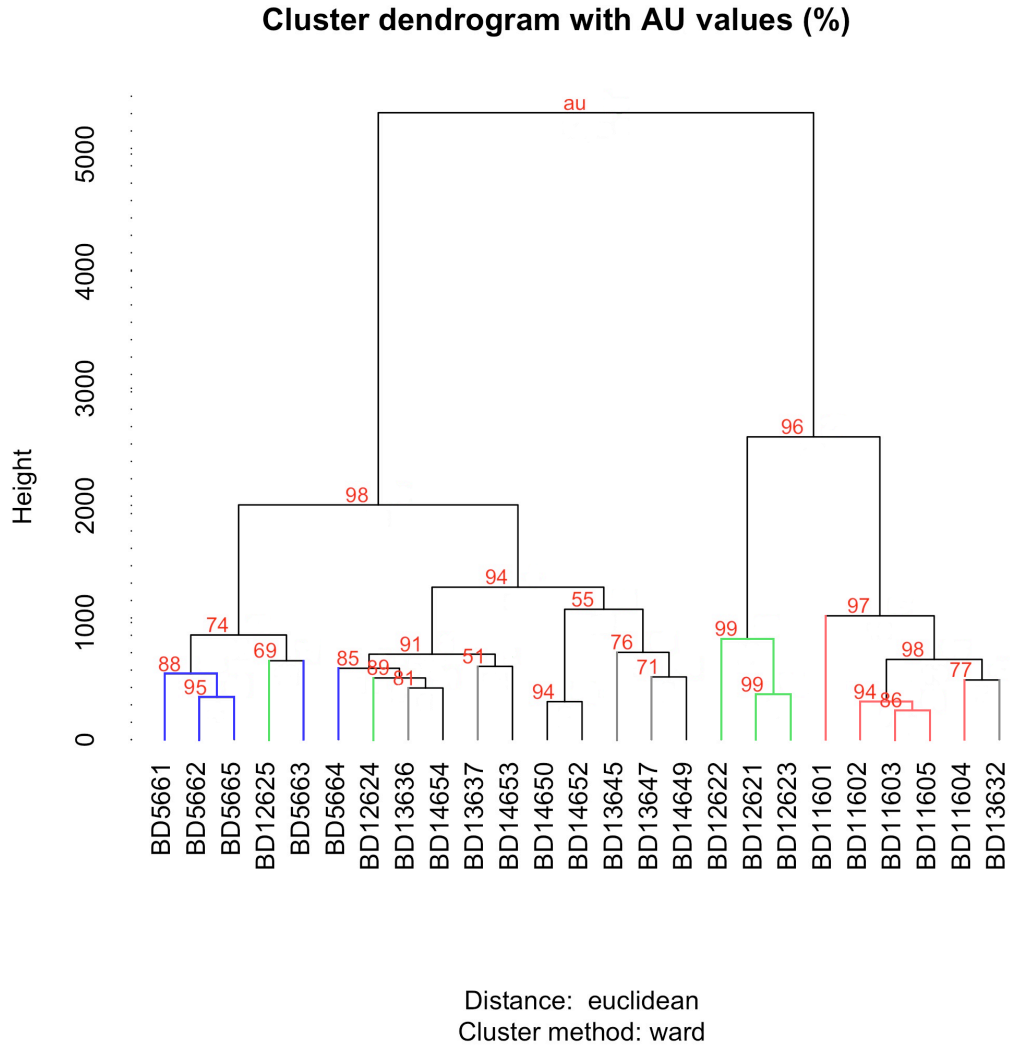

Figure 3.13: Dendrogram showing separation of ramets based on species count data. Branches are colour-coded by clone. Approximately Unbiased (AU) *p*-values are shown at nodes and are based on 10,000 replications. AU values above 95% indicate strongly supported groupings.

3.4.5 Unconstrained ordination – NMDS

The model with three dimensions was found to be the most parsimonious whilst still explaining much of the variation in the epiphyte community dataset. Minimum stress value was 17.49.

3.4.6 Epiphyte community variation and measured effects

On the ordination plot distinct epiphyte communities are manifest between clones (Fig. 3.13), with some intermingling due to common species shared amongst the clones. BD11 appears most distinct from the other clones. Of the measured

variables (i.e. bark texture and phenolic chemistry: eriodictyol, taxifolin, p-coumaric acid), the strongest gradient for explaining separation of epiphyte communities along the first axis is bark texture ($r^2 = 0.218$, $p < 0.001$), followed by p-coumaric acid ($r^2 = 0.1043$, $p < 0.001$) along the second axis. Of much less importance is taxifolin ($r^2 = 0.0784$, $p < 0.001$) and dbh ($r^2 = 0.0784$, $p < 0.001$). Of no apparent significance is the phenolic compound eriodictyol ($r^2 = 0.0127$, p 0.196).

The greatest abundance of smooth bark was recorded on clones BD11 and BD12, the least on BD13, BD14 and BD5. Along this gradient are several species revealed to be of significance in the Indicator Species Analysis: crustose *Tephromela atra*, *Catillaria nigroclavata*, *Caloplaca flavorubescens*, *Caloplaca holocarpa*, and foliose *Physcia aipolia*. Conversely, clones BD13 and BD14 had significantly less smooth bark and were strongly associated with crustose *Ochrolechia androgyna* and moss *Hypnum cupressiforme*. Other species associated with lower abundances of smooth bark are foliose *Parmelia saxatilis*, *Evernia prunastri*, *Melanelixia fuliginosa* subsp. *glabratula*, fruticose *Ramalina fraxinea*, *Bryoria fuscescens*, and two crustose species: *Lecanora expallens* and *Lecanora persimilis* (Fig. 3.14). Of the phenolics, concentrations of eriodictyol were most variable between clones (3-8.5 μg/mg) with BD12, BD13 and BD14 having the greatest concentrations (>6 μg/mg). Taxifolin was the least variable with BD11 having the lowest concentration (<1μg/mg) and the other four clones ±3μg/mg. The phenolic compound p-coumaric acid varied between clones from 0.1-1.75 μg/mg across clones (see Chapter 2 for complete results). Clone BD11 is associated with the liverwort *Frullania dilatata* and the moss *Leucodon sciuroides*.

3.4.7 Epiphyte functional groups and measured effects

Plotting epiphyte species as functional groups shows a strong relationship of mostly crustose species along the gradient of bark texture (Figures 3.13 and 3.14, dimension 1) indicating a preference for smooth bark by this functional group. The foliose and fruticose functional groups are more closely associated with rougher bark. Along the second dimension, epiphytes are separated along a gradient of p-coumaric acid, with the majority of species positively associated with this variable. The factors of clone,

aspect and height (all levels) were tested for effect on separation of functional groups. In addition to the results for the measured effects of bark texture and phenolic chemistry, clone was found to explain the most variation ($r^2 = 0.3494$, p 0.001), followed by height ($r^2 = 0.1019$, p 0.001) and aspect ($r^2 = 0.0512$, p 0.003). In order to determine the unique and specific effects of variables of interest (bark texture and phenolic chemistry) on epiphyte communities it is necessary to constrain further ordination to these variables and apply RDA.

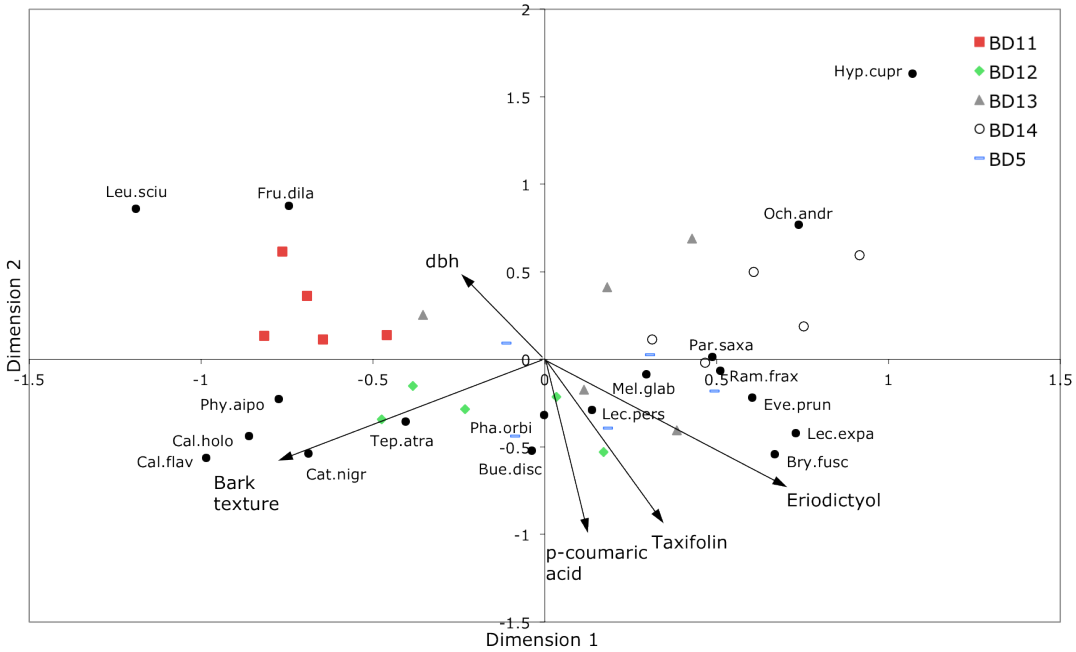

Figure 3.14: NMDS biplot showing separation of clones (colour-coded) based on epiphyte community composition. Environmental effects on epiphyte communities are indicated by length and direction of lines. Significant species from an Indicator Species Analysis are represented by black dots; species key: Bry.fusc *Bryoria fuscescens*; Bue.disc *Buellia disciformis*; Cal.flav *Caloplaca flavorubescens*; Cal.holo *Caloplaca holocarpa*; Cat.nigr *Catillaria nigroclavata*; Eve.prun *Evernia prunastri*; Fru.dila *Frullania dilatata*; Lec.expa *Lecanora expallens*; Lec.pers *Lecanora persimilis*; Leu.sciu *Leucodon sciuroides*; Mel.glab *Melanelixia fuliginosa* subsp. *glabratula*; Och.andr *Ochrolechia androgyna*; Par.saxa *Parmelia saxatilis*; Pha.orbi *Phaeophyscia orbicularis*; Phy.aipo *Physcia aipolia*; Ram.frax *Ramalina fraxinea*; Tep.atra *Tephromela atra*.

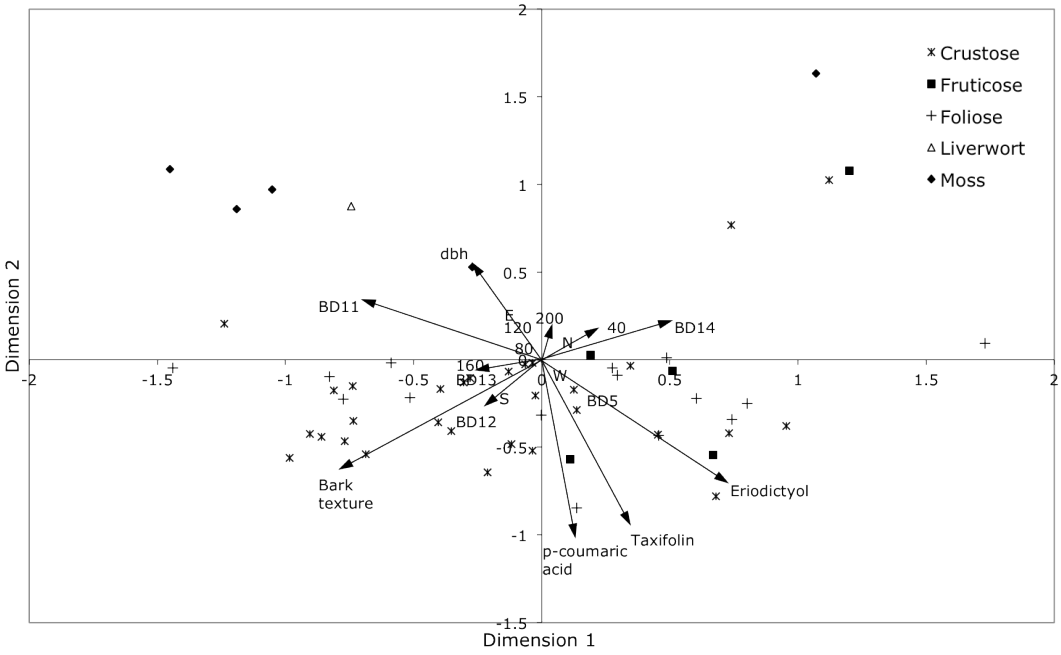

Figure 3.15: NMDS biplot showing epiphyte groups (symbol-coded). Environmental effects on epiphyte communities are indicated by length and direction of arrows. Of the factors fitted against the ordination, clone was accountable for the greatest variation (r^2 = 0.3494, *p* 0.001), followed by height (r^2 = 0.1019, *p* 0.001) and aspect (r^2 = 0.0512, *p* 0.003).

3.4.8 Constrained ordination – RDA

The epiphyte community dataset was analysed using DCA. Gradient length for DCA axis one is used as a standard bench mark to select contrasting linear response or unimodal response tools, with gradient lengths < 3-4 indicating low species turnover along the first axis, consistent with a linear response. The observed gradient length in this study was < 3 (2.438), indicating that RDA was an appropriate tool for constrained ordination. All measured environmental variables were selected into a constrained ordination based on a Monte Carlo test with 9999 permutations. As with the NMDS, epiphytes separate into clonal groups (Fig. 3.15) along a gradient of bark texture (Axis 1: 50.4%; *F* = 3.91, *p* <0.001) with BD11 and partly BD12 with most smooth bark and this being the main factor attributable to observed variation. Taxifolin and eriodictyol show significant effects (*F* 3.82 and 3.21 respectively, *p* <0.001). Concentration of taxifolin was lowest in BD11; concentration of eriodictyol was lowest in BD5, then BD12. The least variation is attributed to p-coumaric acid (*F* 3.12, *p* 0.05), which is correlated with BD12 and BD5 in this analysis. Previous results indicate that these clones had the greatest concentration of

p-coumaric acid. All factors combined accounted for 46% of the variation in epiphyte community (Appendix B, Table 2).

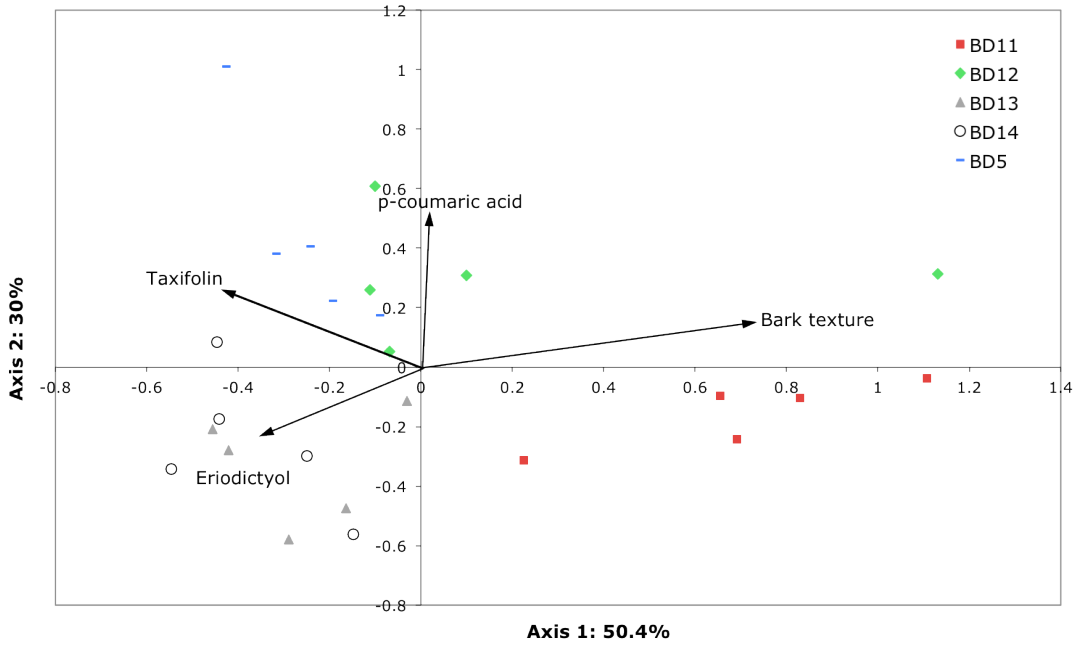

Figure 3.16: RDA of all ramets and epiphyte species: biplot for the first two canonical axes of sites, species and environmental variables. Eigenvalues were 0.234 (axis 1) and 0.139 (axis 2). Cumulative percentage variance for the first two axes was 80.4%. Bark texture was the strongest gradient (*F* 3.91, *p* <0.001) separating epiphyte communities into clonal groups (axis 1); Taxifolin and Eriodictyol were significant variables (*F* 3.82, *p* <0.001 and *F* 3.12, *p* <0.001 respectively) secondary to bark texture, and allied with clones BD13 and BD14. Axis 2 separated epiphyte communities into clones BD12/BD5 and BD11/BD13/BD14 along a gradient of p-coumaric acid (*F* 3.12, *p* 0.05).

Clones are separated on the ordination plot, indicating community differences that can be ascribed to bark variation and chemical differences among clones.

3.4.9 Accounting for spatial autocorrelation

One potential explanation for the observed differences in epiphyte communities among clones is that they are not due to community genetic effects, but to spatial proximity of ramets within a clone. That is, because clones are often spatially clumped, the clone effect may include autocorrelation in unmeasured environmental factors, or with community similarity owing to dispersal constraints. If this were the case, similarity in epiphyte communities of individual ramets would be explicitly associated with the physical distance between them. To test for a link between the structure of epiphyte communities in NMDS ordination and physical space, mantel

tests were performed using 100,000 randomisations. Significant spatial autocorrelation for epiphyte community ordination scores was found both when all five clones ($r = 0.464$, $p < 0.001$), and when only the intermingled clones BD12, BD13 and BD14 ($r = 0.488$, $p < 0.001$) were included in the analysis (Table 3.8).

NMDS combined with mantel tests indicated that the effect of bark chemistry is spatially structured and cannot be distinguished from alternative explanations for autocorrelation in epiphyte community structure. Thus, bark chemistry is a significant factor when considering distant clones (not mixed), but is no longer significant having accounted for spatial effects, or when considering the mixed clones only. However, this not the case for bark texture, which appears to be a significantly important explanatory factor for community composition, even having accounted for spatial effects.

Table 3.8: Comparison of the epiphyte community relationships in ordination space and environmental variables. Significant correlations of measured aspen physical traits are shown for all clones, and intermingled clones, with and without spatial effects. Significant correlations are shown in bold.

	All Clones		**Mixed Clones Only**	
	Without spatial effect	With spatial effect	Without spatial effect	With spatial effect
Bark texture	**r = 0.272**	**r = 0.220**	**r = 0.459**	**r = 0.450**
	p = 0.010	**p = 0.036**	**p = 0.000**	**p = 0.002**
Taxifolin	**r = 0.265**	r = 0.057	r = -0.181	r = -0.090
	p = 0.004	p = 0.243	p = 0.105	p = 0.309
Eriodictyol	**r = 0.210**	r = 0.108	r = 0.143	r = 0.212
	p = 0.013	p = 0.121	p = 0.111	p = 0.057
p-coumaric acid	r = 0.348	r = 0.023	r = 0.101	r = 0.053
	p = 0.425	p = 0.445	p = 0.215	p = 0.376

Conversely, it is also possible that effect of community genetic processes is subject to environmental effects that are spatially structured. This makes it very difficult to identify a unique spatial versus chemical effect in a field setting. Thus, Mantel tests indicate that the effect of bark chemistry (taxifolin and eriodictyol) is important when considering distant clones (clones in different environmental settings), but is not significant when accounting for spatial effects, or when considering mixed clones only (clones within similar environmental settings).

The importance of bark texture effect is confirmed using RDA with X and Y co-ordinates treated as dummy variables (Figure 3.16); having accounted for spatial effects, only bark texture was a significant explanatory variable (F 4.31, p 0.001), based on a Monte Carlo test with 9999 permutations.

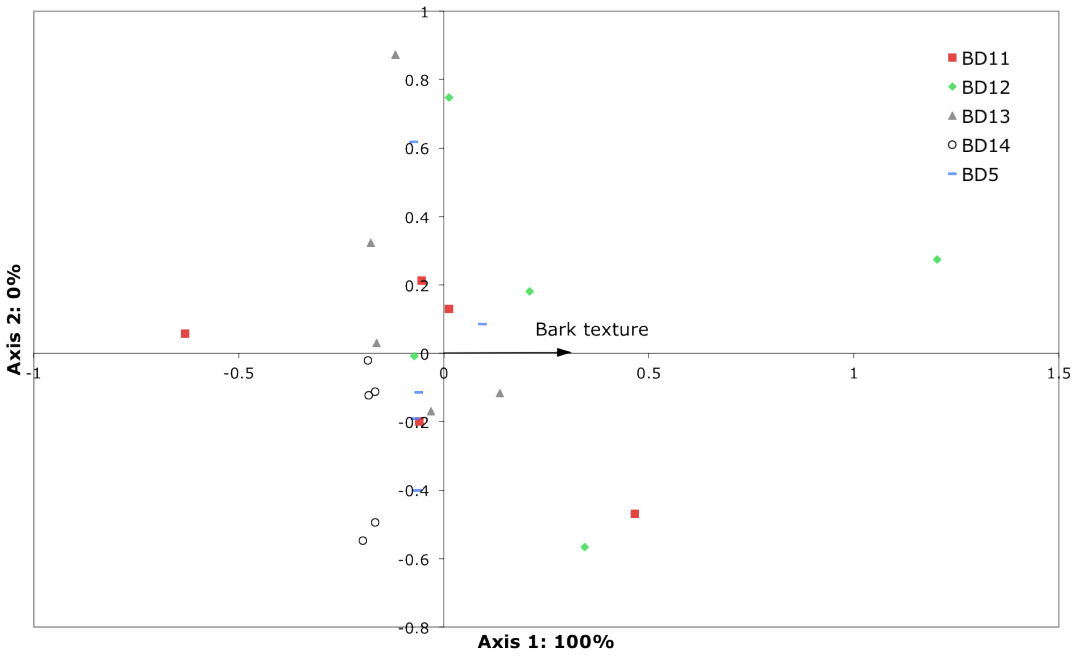

Figure 3.17: RDA with X and Y variables from previous analysis used as dummy variables: biplot for the first two axes using bark texture as the environmental effect. Eigenvalues were 0.136 (axis 1) and 0.101 (axis 2). Cumulative percentage for these two axes was 100. A one-way ANOVA of axis 1 scores showed no significant differences between all clones ($F_{4, 20}$ 2.256, p 0.10).

Accounting for linear spatial effects (distance along X or Y co-ordinates) negates the chemical effect, again indicating spatial structure in ramet chemistry. Bark texture accounted for 10% of the variation in epiphyte community amongst the five clones (Appendix B, Table 3).

Having removed spatial effects, and using bark texture as a single variable in constrained ordination, community differences attributable to bark texture are positioned along axis one, with remaining ordination axes showing residual (unconstrained) variation. On this basis, ramets do not separate into their respective clones along axis one, and a one-way ANOVA of axis 1 scores showed no significant differences between all clones ($F_{4, 20}$ 2.256, p 0.10: Table 3.9). This suggests that community differences attributed to bark texture are not determined by a clone effect, having accounted for spatial structure.

Again, applying an equivalent RDA on the intermingled clones, using all chemical effects and bark texture as explanatory variables, once again only bark texture was significant (F 5.05, p <0.001) based on a Monte Carlo test with 9999 permutations. Therefore, the analysis was repeated using only bark texture as an environmental effect (Fig. 3.17). Bark texture accounted for 28% of the variation in epiphyte community between the three intermingled clones (Appendix B, Table 4).

With bark texture as a single constrained variable, aligned along axis one, community differences among ramets from intermingled clones (BD12, BD13 and BD14) are significantly associated with a gradient in bark texture. A one-way ANOVA of axis 1 scores showed significant differences between all clones ($F_{2, 12}$ 6.041, p 0.02: Table 3.10). This demonstrates that epiphyte community differences amongst the intermingled clones are determined by bark texture, having accounted for spatial structure (i.e. using the spatial structure of the mixed clones to 'break' any spatial effects).

Table 3.9: One-way ANOVA using axis 1 scores from RDA – XY co-ordinates as dummy variables – to test whether clones are aligned along a gradient of bark texture.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Clone	4	0.78312	0.195780	2.2567	0.09912
Residuals	20	1.73512	0.086756		

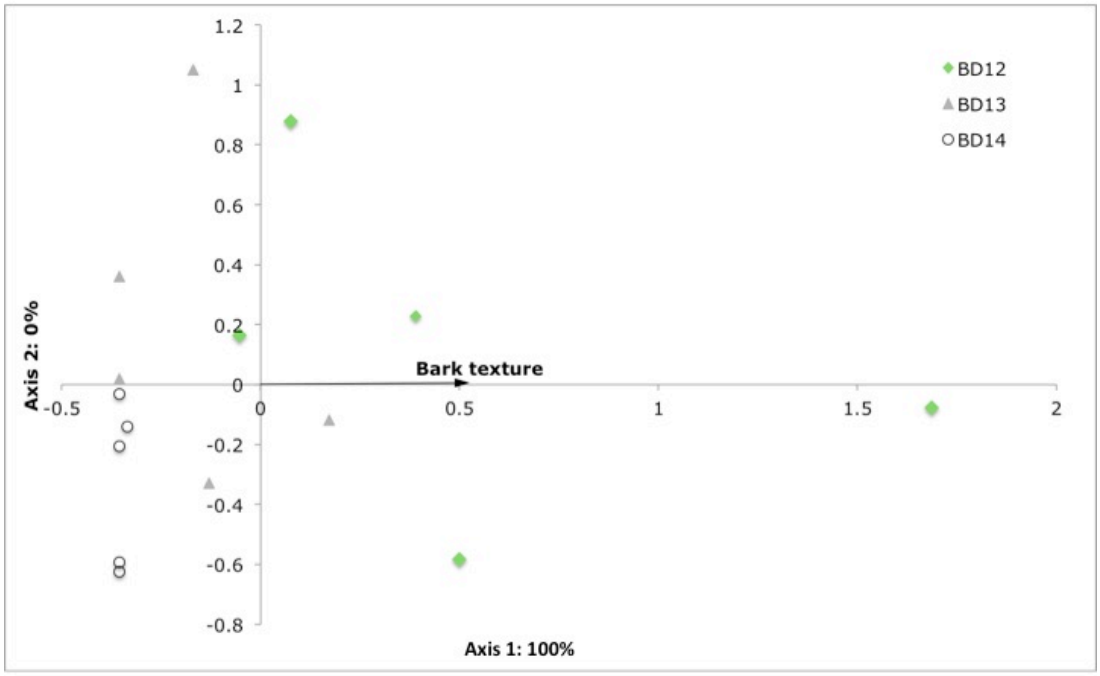

Figure 3.18: RDA of all ramets from intermingled clones and epiphyte species: biplot for the first two axes using bark texture as the environmental effect. Eigenvalues were 0.280 (axis 1) and 0.139 (axis 2). Cumulative percentage for these axes was 100. A one-way ANOVA of axis 1 scores showed significant differences between all clones ($F_{2, 12}$ 6.041, p 0.02).

Table 3.10: One-way ANOVA of axis 1 scores, testing whether epiphyte communities on intermingled clones are separated along a gradient of bark texture.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Clone	2	2.1059	1.05295	6.0408	0.01531
Residuals	12	2.0917	0.17431		

3.5 Discussion

This research has shown that in a natural population of aspen in Scotland there are significant differences in the epiphyte communities present on five naturally distributed clones. Clones differ both in their total species richness, species richness of functional groups and total species diversity of epiphytes. Evidence from ordination analysis suggests an association between bark texture of the clones and epiphyte community composition, but not between bark phenols and epiphyte communities. Clones with a higher proportion of smooth bark tend to have a greater number and diversity of crustose lichen species. This association between bark texture and epiphyte community composition is still observed even when the effect of non-random spatial location of ramets from the same clone is taken into account. The effects of differences between clones are large accounting for 35% of the separation between clones in ordination space, whereas height of sampling and aspect sampled account for only 10% and 5% respectively of this separation among clones.

3.5.1 Effect of phorophyte on epiphyte communities

Associations between epiphyte functional groups and phorophyte (plant surface colonised by epiphytes) quality have been documented before in several studies. One of the earliest (Hale, 1950) described communities of foliose and crustose lichens growing on varying bark textures of a range of tree species from the Aton Forest, Connecticut, USA. The tree species surveyed were separated into rough-barked, smooth-barked and intermediate. By examining the lichen flora on these tree species and comparing differences between bark types, the observation was made that a higher proportion of foliose species were found on rough barked trees, a higher proportion of crustose species were found on smooth barked trees (also supported by Pereira et al. 2002), with an intermingling of functional groups on those trees with a mixture of rough and smooth bark. The process of cracking and fissuring with the sloughing off of outer periderm tissue has the capacity to significantly reduce crustose species, whilst providing a niche for foliose species. Tree species with a tendency to produce rough bark during the process of growth and development were

found to have a greater abundance of transitional bark, and therefore a greater mixture of species.

Morley and Gibson (2010) compared lichen communities on smooth- and rough-barked tree species from cool temperate rainforests in two climatically similar regions of Victoria, Australia. Epiphyte communities were sampled on trees up to 2m in height, across an area 20m x 20m. The dominant group of lichen species found on smooth-barked trees were crustose. Conversely, epiphyte community composition on rougher-barked trees changed as the host changed, which the authors attributed to the development of fissuring and rougher bark over time. These fissures provided a habitat for lichen species that were rare on smooth bark. However crustose species such as *Coccotrema curcurbitula* and *Graphis tenella*, abundant on smooth-bark, were also frequently found on the smoother areas of the predominantly rougher-barked trees. Older, rougher-barked trees were also home to more rare lichen species not recorded on smaller trees or smoother bark trees.

Ellis (2008) surveyed stands of Scottish aspen (*Populus tremula*) in Strathspey to determine the composition and diversity of epiphyte communities with the purpose of assessing aspen's role in supporting and maintaining lichen species. It was suggested that changes in bark texture of aspen might have an influence on the changes in epiphyte community composition over time. This has also been recorded on *Fagus sylvatica* (Fritz et al. 2009). Sexually reproductive crustose lichens (e.g. *Lecania naegelii*, *Lecanora populicola*, *Lecidella elaeochroma*) tend to be pioneer species and early colonisers of smooth aspen bark, gradually being taken over by asexual crustose lichens, foliose lichens and bryophytes. Species interactions could also account for changes over time.

Not only were the Invertromie aspen clones significantly different in epiphyte community composition they were also significantly different in species richness. This too could be partially attributed to the percentage of smooth bark as species diversity, principally of crustose species, is greater on smooth than on rough bark. Clone BD11 had the highest percentage of smooth bark up to 2m, but was the third most species rich clone after BD12, BD5 and BD13. This may be accounted for by the abundance of the liverwort *Frullania dilatata* on BD11, which was found to

dominate the northern and eastern aspects of the ramets in this clone. Clone BD14 was recorded as having the lowest percentage of smooth bark up to 2m, and was also the least species rich (mean 6.5, SE 0.317), with the lowest diversity of crustose species (mean 2.58, SE 0.219). Fruticose and foliose species diversity was highest on BD5, which had the second lowest percentage of smooth bark.

Explanations for the observed association between epiphyte group and bark texture have been studied by a number of groups. Previous research supports the phenomenon that rougher, fissured bark provides a more suitable niche for the establishment of foliose and fruticose lichens, though this may differ for individual species. An example of the effect of bark texture on epiphytic lichens was demonstrated by Moxham (1981) and reiterated by Armstrong & Bradwell (2011). Physical variation in bark was cited as having a 'profound influence on the growth', with bark texture recognised as being highly influential in the growth and development of a lichen thallus: the radial growth rate (RaGR) of *Xanthoria parietina* (a common foliose species preferring nutrient rich substrate) increases on smooth bark as opposed to rough bark.

It has been demonstrated that development of bark texture in *Populus tremula* may be under genetic control with up to 40% of variation in the field attributable to a clone effect (see Chapter 2 Results and Discussion). Putting these results together with the data from the present study suggests that genetic differences in bark texture among aspen clones plays a significant role in shaping epiphyte communities. In the previous chapter it was also demonstrated that bark phenolic chemistry differs between clones of aspen at Invertromie (Chapter 2 Results and Discussion). However while initial analysis suggested an effect of phenolic chemistry on epiphyte communities, this result is possibly confounded by unmeasured environmental variables, which vary spatially with the aspen clones. Analysis of the intermingled clones alone failed to show an effect of phenolic chemistry on epiphyte communities, suggesting that when spatial effects are accounted for the chemical effect disappears. To demonstrate a significant effect of phenolic chemistry on an epiphyte community it may be necessary both to directly demonstrate the effects of bark phenolic compounds on epiphyte growth, and to analyse an experiment in which clonal ramets

are randomly arranged spatially to avoid problems with spatial correlation between the chemical variation and some unmeasured environmental variable.

3.5.2 Aspect and height effects may be a function of variation in bark texture

Of the five sampling heights used in this study, the greatest species richness and diversity was found at 80cm and 200cm; the least at 40cm. At 40cm moss was most abundant, but not the most species rich of the functional groups, and may explain the low richness and diversity at this sampling height. Moss can often overgrow epiphytic lichens in addition to acting as a moisture-rich habitat for slugs that emerge to graze on lichen thalli, thus influencing the vertical distribution of lichens on trees (Asplund et al. 2010). Understory vegetation may also act as a shading influence on epiphytic lichens, and reduce their photosynthetic capability. The presence of smooth bark varied at each height level for clones BD11 and BD12, and may explain the greater diversity and richness at 80cm and 200cm. Crustose lichens were most abundant and diverse at 200cm; the physiology of a crustose thallus is more conducive to survival in colder, drier and more exposed environments than their foliose/ fruticose counterparts (Armstrong & Bradwell, 2010). Raup (1930) conducted a preliminary investigation of the distribution of non-vascular epiphytes (predominantly lichen species) on two mature *Picea canadensis*. By recording epiphyte species' abundance, lichen flora was classified into two major zones, with an intermediate zone between: the lower zone (up to 2m) was predominantly foliose species, while the higher zone (above 6m) was predominantly crustose species; the intermediate zone consisted of a mixture of these main functional groups. Patterns of height distribution for foliose species on Invertromie aspen were also observed in a study by John (1991). An increase in total percent cover of foliose species was concomitant with an increase in height found on 34 individual of *Quercus rubra* growing in four separate stands, 1km apart, at the University of Michigan Biological Station.

Of the four aspects sampled here, 'south' and 'west' were the most species rich and diverse, with crustose lichens having the highest abundance and richness; the lowest diversity was found on the eastern and northern aspects. All functional groups were

significantly different at each aspect, except for the fruticose lichens, though this may be due to the abundance of rough bark across all clones that provide niches in which this group can thrive. Crustose lichens are generally more desiccation-tolerant, with diminutive thalli and can cope better in sunnier, drier and more exposed areas (Hedenås et al. 2007). Thus they are expected to be more abundant on southern aspects.

Previous studies that looked at the effect of aspect on epiphyte distribution have not revealed such significant effects as found here. In one of the most detailed studies of epiphyte communities on *Populus tremuloides* in Western Canada, Case 1977 revealed some similarities between species also found on Invertromie aspen, but also some contradictions. This may be due to differences in the stand structure of *Populus* in the two studies, with the stands at Invertromie being much more fragmented than those in western Canada. In other studies the influence of aspect was only found for two or three species (Griffin & Conran, 1994), or the effects were suggestive (Peck et al. 2004), or were confined to species-specific responses (Case 1977; Pentecost 1979; Griffin & Conran 1994).

3.5.3 Implications for conservation

Evolution by natural selection requires genetically based intra-specific variation of significant physical traits and here it has been shown that epiphyte communities vary between different genotypes of *Populus tremula* in a natural system. Thus epiphyte community composition can be seen as a variable and genetically determined trait of aspen, which has consequences for epiphyte populations if the frequency of aspen genotypes is altered within populations. The clonal composition of aspen in woodlands will have important effects on epiphyte communities, specifically on the proportion of functional types, species diversity and species richness. Restricting or reducing clonal diversity will reduce these aspects of epiphyte diversity. Currently, challenges to increasing aspen clone diversity exist in obtaining diverse planting stock due to the paucity of flowering events and lack of seed; propagation from root cuttings is the optimal method, yet laborious and the results are slow and unpredictable. Even so it is imperative to include as much diversity of aspen genotypes as possible to ensure diversity in associated epiphyte communities.

As yet there have been no studies addressing the question of *Populus* genetic variation and extended consequences for epiphyte evolution. Some of the lichen epiphyte species, such as *Xanthoria parietina*, are found in abundance on other tree species and substrates (e.g. rocks), whereas *Lecanora populicola* favours aspen as a niche habitat. Certain other rare species, such as *Caloplaca flavorubescens* tend to be found in woodlands containing aspen as a primary component (C. Ellis, pers. comm.). Other *Populus* species are also regularly colonised by unique and rare lichens not commonly found on neighbouring trees of other species (Uliczka & Angelstam, 1999; Hedenås & Ericson 2000). It may not be true of all lichen species that selective pressures on physical traits of aspen will have extended consequences, though the possibility exists for rare, endangered or specialist species.

My detailed study of epiphytes has shown significant differences attributed to clonal variation in bark texture. The assumption is that differences between clones are genetic, but could also be environmental. Further study of naturally colonised genotypes randomised under common conditions is required in order to elucidate the genetic effects of aspen clones on epiphyte communities. The usefulness of natural aspen clones for studying genetic effects on associated epiphytes has already been described in chapter 1. However, the research conducted in chapters 2 and 3 show the limitations of employing natural systems, i.e. confounding of genetic effects with environmental effects that cannot be controlled for. Thus common garden trials containing multiple native aspen clones grown across at least two very different sites would facilitate understanding of genotype effects, controlling for environmental influences.

4 Multi-site common gardens of aspen genotypes demonstrate significant effects of genetic variation on their physical traits and epiphyte communities

4.1 Introduction

In recent years the discipline of ecology has recognised that foundation species such as trees within forest ecosystems are often variable within as well as between species, for both physical and chemical traits likely to affect the dependent species with which they interact (Whitham et al. 2003). Although morphological and chemical traits respond to environmental factors, these traits may be stable in time, and the variation may be largely genetically determined, having a profound influence on the composition of the dependent communities with which the foundation species co-exist. Substantial evidence from a range of systems suggests that genetic variation within a foundation species can often be sufficient to affect community structure of dependent organisms (Johnson & Agrawal, 2005; Bangert et al. 2006a; Barbour et al. 2009a; Tack et al. 2010).

In the past, studies of these interactions have primarily focused on the interaction of trees (foundation species) with invertebrates (dependent species), particularly insects (Floate et al. 1993; Dickson & Whitham, 1996). However it has also been demonstrated that interaction with higher plants, birds and mammals may be affected by genetic variation in traits possessed by foundation species (Dickson & Whitham, 1996; Bailey et al. 2004; Iason et al. 2005). This study will focus on lichens as species that are dependent on trees because similar chemical and physical traits that can influence, or even shape, insect dependent communities have also been shown to be potentially relevant for non-vascular epiphytes (Hyvärinen et al. 1992; Hauck & Javkhlan, 2009).

Epiphytes form a significant component of the species richness of temperate forests (Gustafsson & Eriksson 1995; Uliczka & Angelstam 1999; Hedenås & Ericson 2000; Jüriado et al. 2003). Trees provide microsites within which epiphytes establish and grow (Barkman 1958; Kantvilas & Jarman 2004; Fritz & Heilmann-Clausen 2010)

and therefore it is clear that epiphytes are dependent upon trees within the community. There is also evidence that many epiphytes, particularly lichens, penetrate the surface of the tree and interact with chemicals and bark tissues (Ascaso et al. 1980; Ascaso et al. 1983; Ascaso & Rapsch 1985; Inoue et al. 1987; Legaz et al. 1988; Bouaid & Vicente 1998; Legaz et al. 2004). Thus there is the potential for host genetic variation affecting the physical and chemical traits of trees, such as growth rate and bark secondary chemistry, to simultaneously influence the communities of epiphytes that colonize them. For instance the allelopathic effects of phenolic compounds (salicortin and tremulacin) extracted from *Populus canadensis* bark have been tested on the foliose lichen *Physcia tenella* and found to inhibit growth *in vitro* (Koopman, 2005).

4.1.1 Aspen as a study system

One tree species that is particularly suited to such a study is aspen (*Populus tremula* L.). In many localities aspen is clonal, reproducing from root suckers rather than seed. This leads to the availability of replicate stems within a clone, with the same genotype. Therefore it is possible to assess whether there are significant differences between clones for epiphyte community structure. Aspen is also particularly amenable to study because of its rich and diverse epiphyte flora (Gustafsson & Eriksson 1995; Ellis 2008; Jüriado et al. 2009), due to its particular bark characteristics (Hedenås & Ericson, 2000; Ellis & Coppins 2007; Jüriado et al. 2009). Furthermore aspen clones vary for both physical and chemical attributes of the bark likely to affect the establishment of lichens (Hyvärinen et al. 1992; Sillett et al. 2000; Boudreault et al. 2008; Lamit et al. 2011; Sheard & Jonescu 1974; Aboala et al. 1999; Zhang 1999; Levia & Herwitz 2005). Of particular potential importance are polyphenols (Pietarinen et al. 2005; Neacsu et al. 2007), including condensed tannins and phenolic glycosides, which have been shown to provide strong defences against invasion by pathogens and wood-boring insects (Bennett & Walsgrove 1994; Lindroth & Hwang 1996; Lieutier et al. 1997; Ockels et al. 2007). More limited studies have demonstrated that some of these compounds significantly affect lichen growth (Koopman 2005). It is therefore of great interest to determine whether epiphyte communities on aspen vary among clones, and whether this variation in

epiphyte communities can be accounted for by differences in physical and chemical properties of the trees.

4.1.2 Elucidating genetic effects via common garden experiments

One approach to investigating this question is to make observations of epiphyte communities on naturally occurring clones in the wild, and determining whether epiphyte community differences are associated with bark physical and chemical differences expressed by the aspen clones in the field. This is the approach that was adopted in chapters 2 and 3. This approach has the advantage of making measurements under realistic ecological conditions, and assessing whether genetic effects are sufficiently important, relative to environmental effects, that they have a significant influence on dependent community composition in nature (Tack et al. 2010). However it is important to acknowledge that this approach is subject to a number of limitations that restrict the interpretation of the results obtained from the study.

The first of these is that although the ramets of a single clone in a natural population represent replicates of that clone, the ramets are rarely randomised in space, indeed they are highly likely to be clumped with replicate stems tending to be located within similar environments. Genetic differences between clones may therefore be confounded with environmental differences, and genetic and environmental causes of differences in dependent communities cannot therefore be disentangled. To some extent this difficulty can be overcome if clones are intermingled (as for three of the clones at Invertromie), but the problem remains substantial.

The second problem with utilising observations in natural populations is that the study will necessarily be restricted to a single site. The genotypes will only be observed under a single set of environmental conditions. This means that it is not possible to draw more general conclusions about the wider importance of any effects of genotypic variation within the structural species on the community composition of dependent species. The third problem associated with studies of natural populations is that they only allow us to determine the effects of the genetic variation that exists within the single study population of the dependent species. This genetic variation

may be insufficient to have an influence on epiphyte community composition. However genetic variation found between populations may be much larger and more important in determining dependent community composition, as has been found for *Eucalyptus globulus* in south-eastern Australia (Barbour et al. 2009a, 2009b). The final problem with utilising studies from natural populations is that differences in age will exist among the ramets that are compared. Bark characteristics are known to change with tree age, and therefore age may be a confounding and unaccounted for factor in the analysis.

In order to overcome the limitations of observations made in natural populations, a more controlled experimental approach may be adopted. Here replicate cuttings of genotypes of the foundation species are taken and planted in trial sites in a randomised block design. This ensures that any differences in the epiphyte communities that establish on these different genotypes are due to genetic differences between the clones, not to differences in the environment occupied by the genotypes. A second advantage of this design is that genotypes of the foundation species can be replicated over a number of sites. This allows us to test whether any genetic effects on community composition of dependent species are reproducible or specific to a particular set of environmental conditions. A third advantage of this design is that it allows cuttings to be taken from a range of different populations of the foundation species to ensure that a broad range of genetic differences within the foundation species are tested. The results are therefore not constrained by the limited amount of genetic variation present within the foundation species at a single site. Finally if the experiments are set up at the same time, age of the ramets does not become a confounding factor affecting epiphyte community composition.

This chapter makes use of a replicated common garden approach for studying the influence of foundation species genotype on epiphyte community composition. Aspen clones collected from a wide range of populations throughout Scotland have been planted in a replicated and randomised design at each of two contrasting sites in Scotland. Using a stratified quadrat sampling method, naturally colonising epiphyte species have been recorded on the ramets after the same period of time has elapsed. The experiment allows us to estimate the effects of genetic variation within Scottish

aspen on the community composition of dependent epiphytes independently of environmental or temporal variation. Making observations on different aspects of each ramet, and on different blocks within each of two sites allows the strength of genetic effects on dependent community composition relative to both small scale and large scale environmental effects to be evaluated. As part of the analysis epiphytes have been classified into biologically meaningful 'functional groups', based on growth form (in the case of lichens: crustose, fruticose and foliose), and moss, liverwort, and fungi. This allows us to investigate whether different functional groups respond differently to genotypic differences within the foundation species. Responses are easily quantified by the use of percentage cover, species richness and species diversity measures.

The questions asked in this chapter are:

1. Do aspen clones collected from throughout Scotland differ genetically for growth rate and secondary bark chemistry, and is this consistent within and between experimental sites?
2. Do aspen clones collected from throughout Scotland and grown in the trial differ genetically in their associated lichen communities, and is this consistent within and between experimental sites?
3. How much of the variation in growth rate, bark chemistry and epiphyte community composition is accounted for by variation between clones, sites, blocks within sites, and aspects within ramets?
4. Can variation in community composition of dependent epiphyte communities be accounted for by growth rate or bark chemistry differences between aspen clones?

4.2 Materials and methods

4.2.1 Study sites

The study was conducted at two experimental sites: Kilmichael, Argyll (OS NR884956 or latitude 56 06 22N, longitude 05 24 15W) and Moray (OS NJ167618

or latitude 57 38 18N, longitude 03 23 48W) (Fig. 4.1). Both sites were formerly cultivated agricultural land, most recently used as pasture for sheep and cattle.

The Moray site (Figs. 4.2 and 4.3) is approximately 20-30m above sea level, situated adjacent to Burnside Farm, approximately 2km south of Newton and 5km west of Elgin in a very exposed site. This region is classified geologically as Eastern Moine Highlands, consisting of pure sandstone and upper old red sandstone. Soil is poorly drained, sandy alluvium, of moderate to poor quality having been part-grazed and part-arable in the past. Vegetation is largely mesotrophic grass/ herb mixture (*Epilobium* spp*., Urtica dioica*). Annual precipitation is approximately 750mm (daily average of 36mm in April (15 days) to 71mm in August (17 days)), with generally mild temperatures ranging from -8°C to 6°C from December to February, and from 10°C to 22°C in July and August. Average sunlight hours vary from 1 hour/ day in December and January to 5 hours a day from May to July.

The Kilmichael site (Figs. 4.4 and 4.5) is approximately 30m above sea level, in the south-west Dalradian Highlands and situated in a well-sheltered flood plain in the Add River valley. This region is classified geologically as Dalradian, comprising metamorphic rock, primarily varieties of schist, quartzite and limestone. Soil is mainly typical brown earth with peaty podzols, peaty gleys and peat. Vegetation is largely Atlantic and Boreal heather moor, with heath-rush-fescue grassland, rush pastures and blanket bog. Annual precipitation is approximately 1800mm (daily average of 59mm Apr/May (16 days) to 142mm Oct (23 days)), with generally mild temperatures ranging from -2°C to 7°C in December and January, to 10°C to 17°C in July and August. Average sunlight hours vary from 1 hour/ day in December and January to 7 hours a day in May.

4.2.2 Aspen experimental trials

Root cuttings from 27 genotypes of *Populus tremula* were collected from 26 source locations across Scotland (Table 4.1; Fig. 4.1) in 1993 by Forest Research (Scottish Forestry Commission) and cultivated for one year before planting out into the field sites in 1994 (Moray) and 1995 (Kilmichael) (Figs 4.2-4.6 (a-s)). The purpose of this work was to understand the potential variation within native aspen populations

and its potential for use as small roundwood in Scottish forestry applications. At Moray the 27 clones were replicated five times in a randomized block design. At the Kilmichael site the same 27 clones were replicated four times in a randomized block design. Each block was subdivided into plots, each containing four plants of the same clone spaced 3m apart. Sites were initially treated with propyzamide (Kerb granules) to subdue weed populations. The largest, healthiest replicate from each block for each clone was selected for sampling.

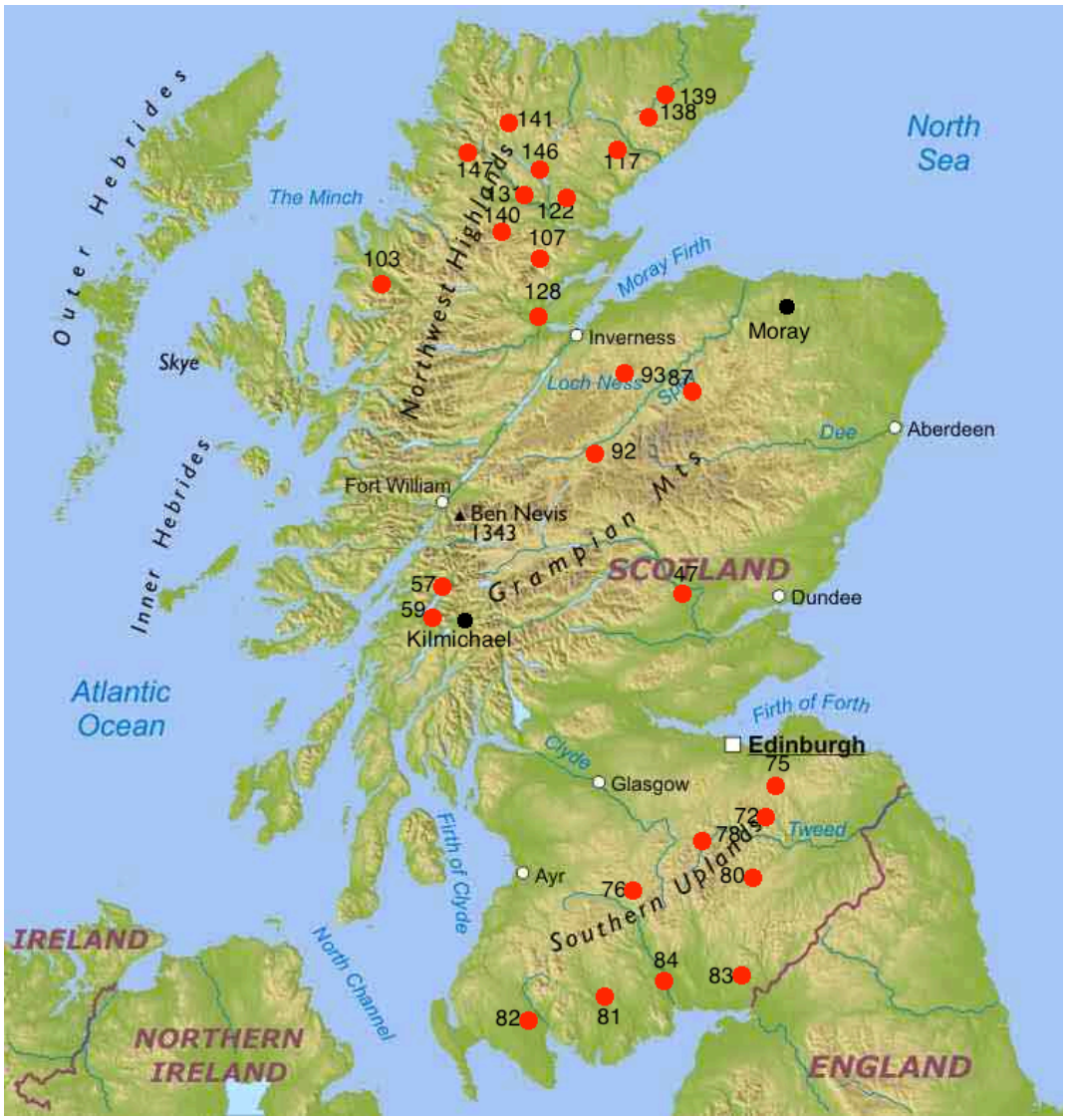

Figure 4.1: Map of Scotland indicating provenance of each clone (red points and clone number). The areas marked with black dots show the locations of the aspen clone garden sites at Moray and Kilmichael (map modified from http://www.freeworldmaps.net/europe/united-kingdom/scotland/map.html)

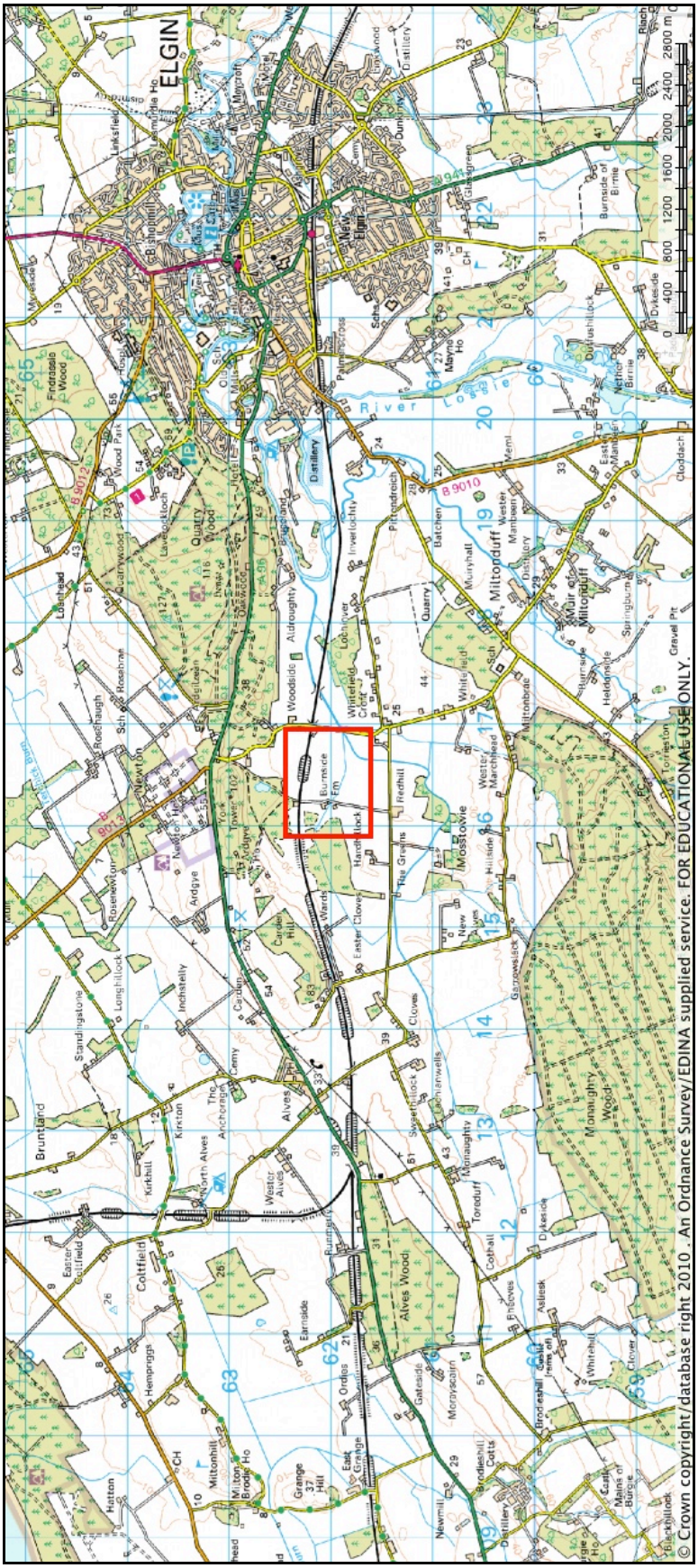

Figure 4.2: Ordinance Survey (2011e). Map of a section of the Moray aspen clones experimental site (outlined red) and environs (scale 1:33926).

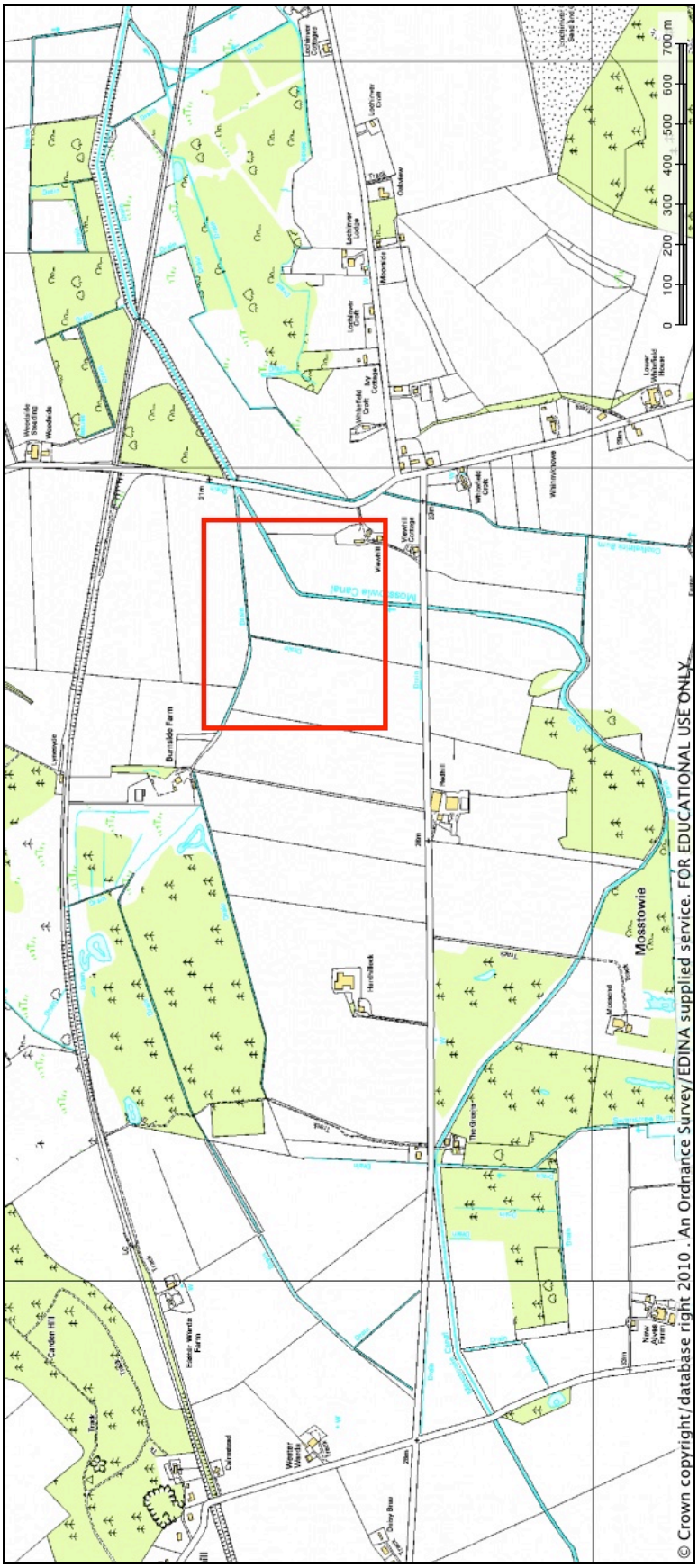

Figure 4.3: Ordinance Survey (2011f). Map of a section of the Moray aspen clones experimental site (outlined red) and environs (scale 1:8481).

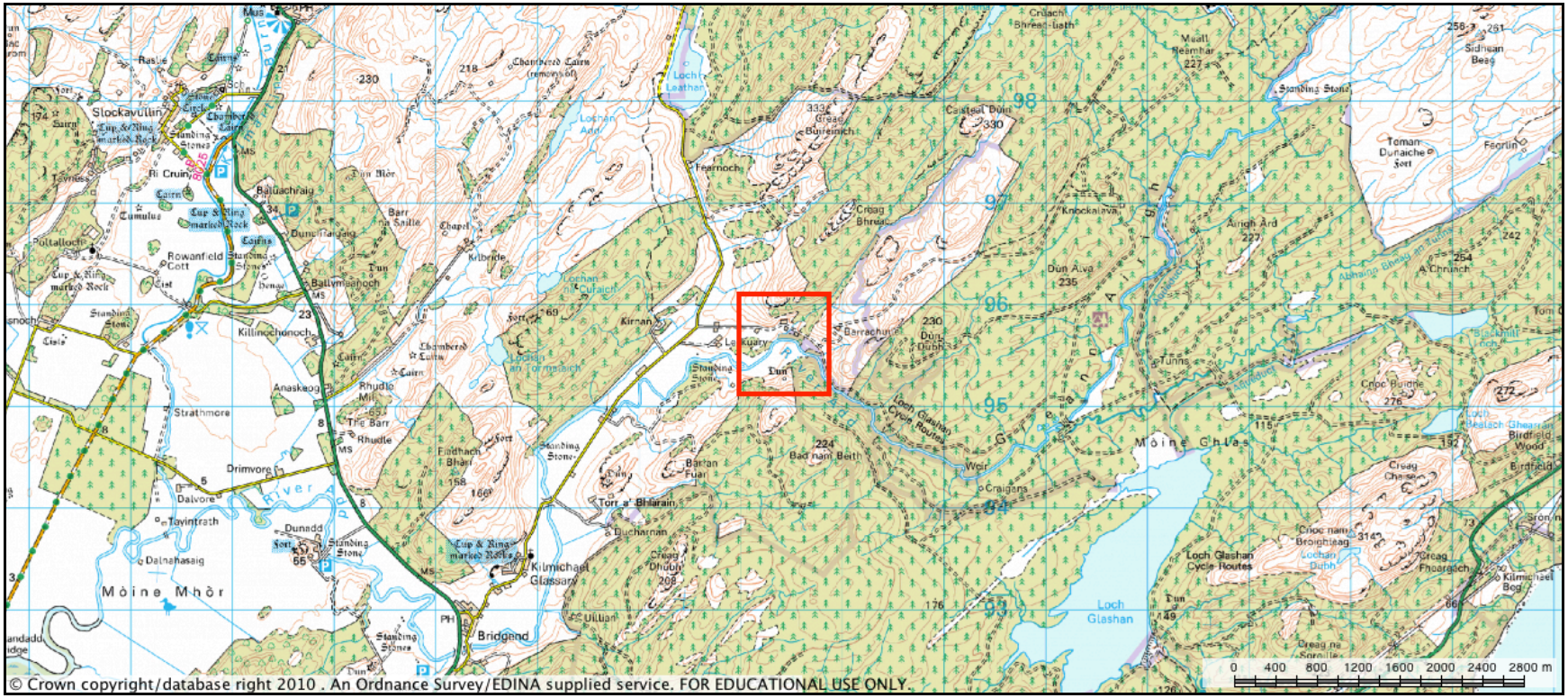

Figure 4.4: Ordinance Survey (2011g). Map of a section of the Kilmichael aspen clones experimental site (outlined red) and environs (scale 1:33925).

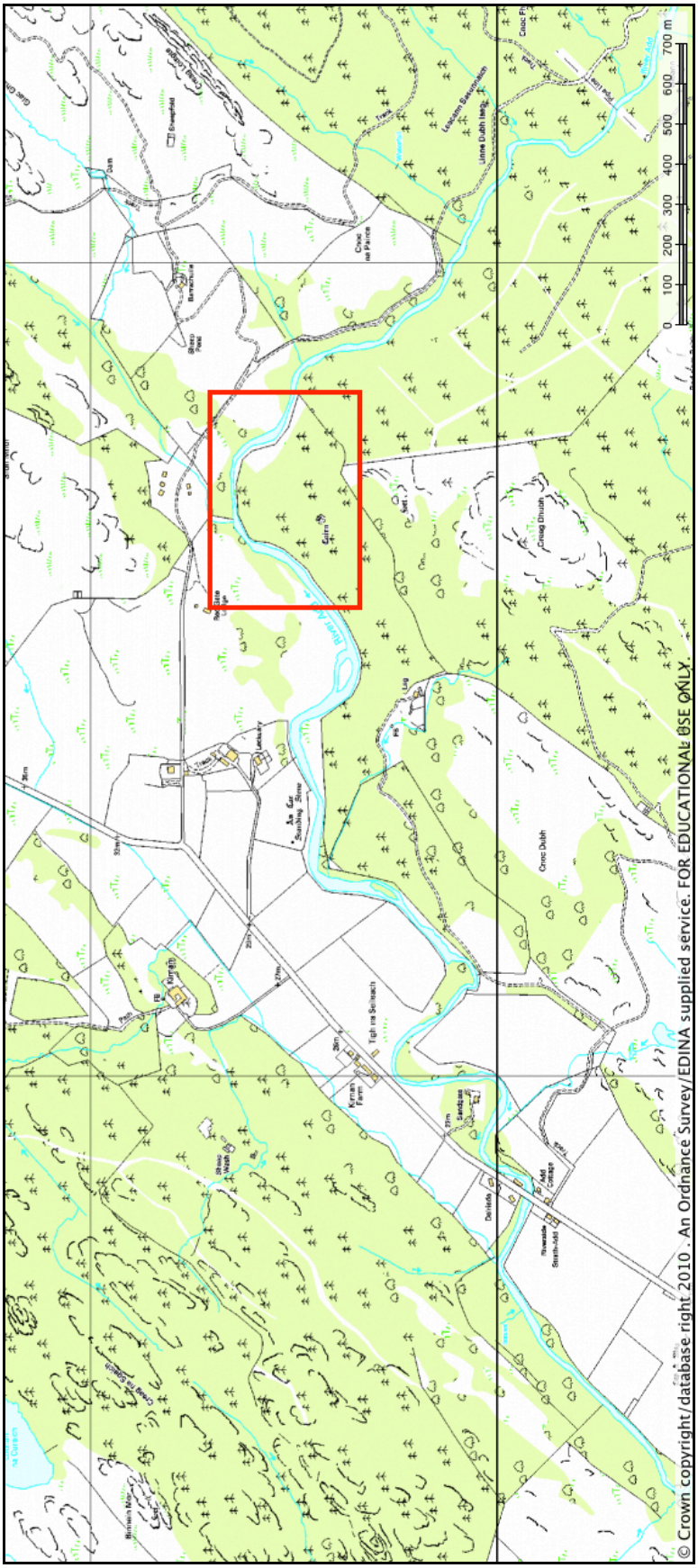

Figure 4.5: Ordinance Survey (2011h). Map of a section of the Kilmichael aspen clones experimental site (outlined red) and environs (scale 1:8481).

4.1.2.1 Moray aspen site



a



b


c


d


e


f


g


h


i


j



k



l

4.1.2.2 Kilmichael aspen clones



m



n



o



p



q



r


s

Figure 4.6: Moray (a-l) and Kilmichael (m-t) aspen clone sites. (a-c): view of the Moray site from the adjacent field, and aspen clones arranged in rows within plots; (d-f): contrast of smooth-barked clone, colonised by *Xanthoria parietina*, and a clone with fissuring bark, colonised by crustose species; (g): slugs nibbling on the thallus of *Xanthoria parietina*, *Ramalina* spp., *Evernia* spp., and several crustose lichens can be seen; (h) a tessellation of crustose species on the bark of an aspen clone with some well-developed *Ramalina* and *Evernia*; (i-l): four different clones with colonisation by sterile crusts (image i), *Xanthoria parietina* and some sterile crusts (image j), a mixture of mature crustose and foliose lichens (image k), and no visible epiphytes at all (image l); (m-n): two views of the River Add at Kilmichael, which had to be crossed to reach the clones; (o): a diversity of crustose lichens and a large patch of bryophytes compete for space on the bark; (p) the hills of Kilmichael Glassary add an interesting backdrop to the site, but attention needs to be kept on the ground to avoid scrape mounds and drainage ditches; (q): smooth bark beginning to crack at the lenticels; (r): as with the Moray site, clones are planted in plots of four replicates, across several blocks; (s): *Hypnum* and *Peltigera* battle for supremacy at the base of a clone.

Table 4.1: Provenance information for each clone, including OS grid reference, description of the clones, general location and elevation (meters) above sea level. Clone information supplied by A. Harrison (Forest Research, Bush Estate, Scotland).

Clone	Grid Ref.	Tree description	Location	Elev.
47	NN993465	20 small trees to 8m/25cm	W of B898 N of Woodinch	60
57	NM982446	8 trees variable size	N shore Loch Creran	-
59	NM953323	Several trees	W edge Fearnoch forest	60
72	NT421336	small trees by burn	Glenkinnon burn	200
75	NT471667	as 602 nr Beech Expt.		-
76	NS786124	in mixed wood	Backwood SSSI Valley	-
78	NT233203	many trees in plantation	Oxcleugh	350?
80	NT278116	many suckers by river	By Tima water Meerlees	260
81	NX687694	sm roadside stand	Loch Ken Hensol Estate	-
82	NX294671	10-20 trees	Along burn Drumnabrennan	100
83	NY108880	sm stand in marsh	Perchall Loch	-
84	NX961929	sm. grp.roadside.comp5150	100m up from road junct & grid ref.Ae.	-
87	NH912184	6 small stands all ages	Around and N of Kinveachy	230
92	NH807016	Two large stands good	W of Insh N of B970	220
93	NJ025283	Many trees in mixed wood	Glen Beg Grantown	240
103	NG831721	2 lge + suckers	betw road & river R.Kerry Gairloch	-
107	NH511926	6 trees; red autumn foliage	N of river Braelangwell	52
117	NC986186	80+ trees 12m good form	Birchwood Lodge	35
122	NH556977	40 old trees to 56 cm	Rhelonie	10
128	NH517589	20 trees 2 groups	In copse, Fodderty	-
131	NC480012	Single fine tree 19m	Rosehall wood	30
138	ND113225	11 old trees to 52cm DBH	By Langwell Berriedale	-
139	ND156308	100-200 scattered trees	Along riverside Dunbeath	10
140	NC561163	single 12-13mx53cm	Rhian F.C woodpecker nest	-
141	NC573343	2-3 doz medium	Altnaharra	-
146	NC572175	single	E of rd opp. boat hse L.Shin	-
147	NC395134	N/I	Glenmuick	-

4.2.3 Sampling scheme

A ladder quadrat method, modified from Asta et al. (2002), was used to record the frequency, location and abundance of epiphyte species on ramets (Fig. 4.7) Species were identified in the field with a x10 hand lens; specimens that could not be identified with certainty were collected and returned to the Royal Botanic Garden

Edinburgh for chemical spot tests, comparison with herbarium specimens, and/ or microscope sections for examination. Lichen specimens that lacked sufficient identification features were classified as 'sterile crusts'. Specimens of *Physcia tenella* and *Physcia adscendens* could not be distinguished due to a lack of reproductive structures and were grouped together.

On each ramet, sampling took place at breast height (130cm) at each cardinal point on the trunk (N, S, E and W) (Figure 4.7). Sampling at breast height was considered optimal because branching patterns at greater heights were found to be obstructive. At lower heights weed cover had a potential shading effect on the stems and epiphytes, therefore potentially confounding the results.

Stratified sampling in this manner facilitates the testing of aspect effects on epiphyte species. For scoring the abundance of each epiphyte, the 5cm x 25cm quadrat was divided into sub-quadrats of 5cm x 5cm, each with $1cm^2$ subunits and each species within these $1cm^2$ subunits was noted. For estimating percent cover of epiphyte species, the total number of subunits/ per sub-quadrat containing epiphytes was recorded and divided by the total number of subunits.

4.2.4 Field measurements

In addition to recording presence, frequency and abundance of epiphyte species, the following measurements and procedures were conducted in the field:

- The diameter at breast height (dbh) was recorded by measuring the circumference of each ramet at 130cm from the ground, then dividing by π to give the diameter to 2 decimal places (d.p.)
- Pieces of outer bark measuring approximately 15cm x 15cm were removed from the southern aspect, approximately 1.6m from the base of the trunk. Samples were scraped cleaned of epiphytes at the time of collection. Samples were stored in paper packets and left to air-dry at 20^oC (after Berlizov et al. 2007).

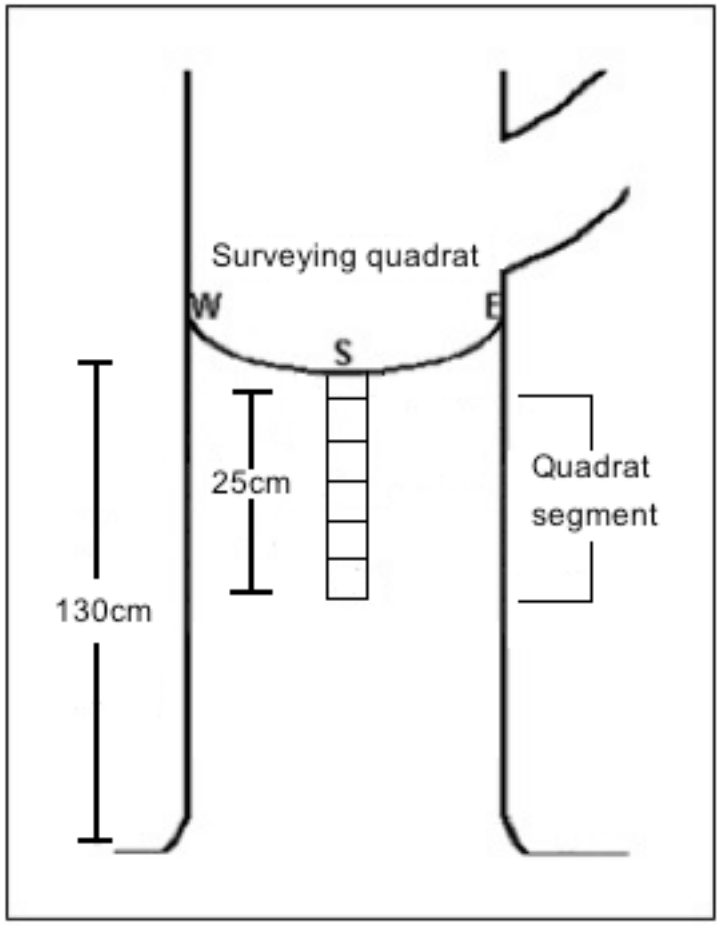

Figure 4.7: Diagram of lichen sampling transects with ladder quadrats (modified from Asta et al. 2002).

4.2.5 Bark phenolic chemistry analysis via HPLC

Any small traces of vegetation were removed from the bark sample in the lab with a scalpel before ball-milling to a fine powder for 30-60 seconds in a Retsch Mixer Mill MM 400. To quantify the number and amounts of individual phenolic compounds, 9-11mg of the milled material was extracted using 100% HPLC-grade methanol. Each sample was placed in a 1.5ml Eppendorf tube then 0.6ml of ice-cold methanol with internal standard (2mg/ l of resorcinol) added with 5-20 1mm diameter glass beads. After homogenising the sample for two minutes in a Retsch Mixer Mill MM301, it was left on ice for 15 minutes. Each sample was then homogenised for a further two minutes before being micro-centrifuged (Accuspin Micro, Fisher Scientific) for 3 minutes at 1300 rpm. Supernatant was removed into a 4ml vial with a Pasteur pipette, and the extraction procedure repeated twice more, combining the

supernatants each time. These were dried under nitrogen gas to a residue and stored at 4°C until analysis. Prior to analysis samples were re-dissolved in 0.6ml methanol. Analysis was carried out using a high performance liquid chromatography – diode array detector (HPLC-DAD (G1315B)), collecting wavelengths at 220nm, 270nm, 320nm, 360nm and 280nm; a C18 column (Agilent Zorbax SB-C18 (PN 866953-902; S.N. USDZ011623) rapid resolution 4.6x75mm, 3.5µm) fitted within a Hewlett-Packard Agilent 1100 Series machine (Agilent Technologies, Germany). 10µl of each sample were injected onto the column at a flow rate of 2ml/ min. Two solvents were used: solvent A – aqueous tetrahydrofuran 1.5% (THF) / phosphoric acid H_3PO_4 (0.25%), filtered to 0.2µm; solvent B – HPLC grade methanol (MeOH). Conditions used in HPLC gradient elution are shown in Table 4.2, and are based on a method developed by Meier et al. (1988) with modifications by B. Moore (James Hutton Institute, Aberdeen); spectral libraries for peak identification were provided by R. Julkunen-Tiitto (University of Eastern Finland).

Table 4.2: Elution gradients for HPLC. Solvent A is filtered nanopure water with 1.5% tetrahydrofuran and 0.25% phosphoric acid; solvent B is 100% HPLC grade methanol.

Time (min)	% solvent A	% solvent B	Flow rate (mil min^{-1})
0	100	0	2
5	95	5	2
10	85	15	2
20	70	30	2
40	50	50	2
60	0	100	2
Rinsing	0	100	2

4.3 Statistical methods

4.3.1 Aspen traits: dbh

Diameter at breast height: mean dbh (with SE) was calculated for each clone based on measurements of the stem circumference (value of circumference divided by *pi*) for each clone per block and presented as a barplot with error bars. An ANOVA was fitted to the data, testing effects of 'Clone', 'Site', and 'Provenance' ('Block' was

tested but is perfectly nested within site and may be a redundant variable). Interaction effects of clone x site were tested, with clone nested within provenance.

4.3.2 Aspen traits: chemistry

Chemistry: data were recorded as concentration (µg/ mg) for each compound. These were converted to mean concentrations with standard errors at each experimental level ('Clone', and 'Site') and presented as bar-plots with standard error bars. Concentration data for each clone/ block was used for the implementation of statistical models.

4.3.3 Analysis of differences among clones, sites and clone provenance

In order to assess effects of clone, site and clone provenance on variation in concentration of phenolic compounds, an ANOVA was fitted using concentration (µg/ mg) of each compound as the response variable. The analysis tested the effects of clone, site, provenance, block, and dbh as a covariate, and interactions between clone and site, and block within site.

4.3.4 Correlation tests

To test for potentially significant correlations between the phenolic compounds, a matrix of Pearson's *r* correlation coefficients for all possible pairs were calculated. Missing values were deleted in pairs rather than deleting all rows of any missing variables. Analysis was performed using the 'Hmisc' package (ver. 3.8-3, Harrell Jr. 2010) in the R programming environment.

4.3.5 Ordination

A principal component analysis was implemented on a matrix of concentrations or correlations between individual phenolic compounds via function 'prcomp' in R package 'stats'. Missing values were excluded, and variables were scaled (dividing the columns of x by their standard deviations) to have unit variance before the analysis took place. An ANOVA was fitted to scores for each of components 1 and 2 to test for differences between clone, site, and a clone × site interaction.

4.3.6 GLMM and variance components of phenolic chemistry

Variance component testing was carried out on each phenolic compound and dbh via GLMM to understand the amount of variation attributable to each effect of clone, site, block, and provenance. Understanding the influence of each of these effects was considered to be important, therefore two rounds of model-fitting were applied to each of the response variables (i.e., each phenolic compound and dbh).

The overall approach involved testing for differences in bark phenolics and dbh among clones and exploring and quantifying the variance of site, block, and provenance, as random effects (Model 1). To elucidate the variation attributable to the effects of site, block and provenance, these factors were tested as fixed components, using clone as the random component to give the percent variation attributable to clone effects, and percent residual variation (Model 2).

4.3.6.1 Model 1 – Complete model, variance partitioning of effects

Generalized linear mixed models (GLMM) were implemented in 'R' version 2.12.0 (R Development Core Team, 2010), using the REML approximation method in the lmer function (package 'lme4', version 0.999375-37 (Bates and Maechler, 2010)). A model was designed to examine the variation between clones in relation to site, block and provenance. To do this site, block and provenance were treated as random additive effects.

4.3.6.2 Model 2 – Complete model, testing contribution of 'Clone'

The second model to quantify of the effects of site, block and provenance specified them as fixed effects that were additive, and with clone as the random effect. Results are presented as a table showing each response with the percent variation attributable to each effect tested.

4.3.7 Epiphyte community

4.3.7.1 Species richness and diversity measures

An overall estimate of epiphyte colonisation was obtained by estimating epiphyte cover within each 5cm x 25cm quadrat and converting to a percentage. Statistical

analyses sought to determine epiphyte community differences between clones, effects of aspect, site, and blocks, and whether ecological traits of the epiphyte species were related to these effects. To this end, epiphytes were initially classified into functional groups: lichens – foliose, fruticose, and crustose; moss; and liverwort.

Species presence/ absence measures that yield measures of species richness are useful for providing an overview of beta diversity, or differences at the metapopulation level. Therefore raw counts for each species within each group were converted to presence/ absence ('0', '1') within the appropriate sample unit, and total species were calculated for each clone, aspect and site. Using these data, the Shannon index was calculated for clone, aspect and site based on total number of species:

$$H' = -\sum p_i * \ln(p_i)$$

where p_i is the proportion of the total sample belonging to the *i*th species. This index measures evenness and decreases if a few species dominate. Results are presented as barplots of means with SE bars.

4.3.8 Analysis of differences among clones, sites and aspects

In order to assess effects of clone, aspect, site and provenance on epiphyte functional groups, an ANOVA was applied using as response variables: 1. total number of species per sample quadrat (species richness); 2 total number of species within each functional group per sample quadrat; 3. the Shannon index of total species diversity per sample quadrat; and 4. percent cover. The analysis tested the effects of clone, site, blocks, aspect and site with nesting of block within site and the interactions of clone x site and clone x aspect. The effect of mean dbh per clone on mean % cover of epiphytes per clone was tested using linear regression and Pearson correlation.

4.3.9 Community dynamics and distribution

4.3.9.1 Ordination

Unconstrained ordination first identifies the major variation in a dataset before relating this variation to observed environmental variables. Constrained ordination

seeks to identify only the variation that can be explained by specific environmental variables.

Constrained analysis: Redundancy Analysis (RDA) is related to principal components analysis, using Euclidean distances and makes an assumption of linear species' response. The axes of the final ordination show linear combinations of species data and environmental variables. Here RDA (CANOCO v4.3) was used to examine the direct effect of explanatory variables in controlling epiphyte community structure. First, all rows with missing data for epiphytes and chemistry were removed, and all rows with total epiphyte count of 'zero'. The column containing counts for the fruticose lichen, *Ramalina fraxinea*, was removed as it was only recorded on one clone (47, block 2) at Kilmichael and massively skewed the results. Second, Monte Carlo permutation (9999 replications) was combined with forward selection to indicate those environmental variables best explaining epiphyte communities among clones. RDA was used to estimate the unique effects of individual variables, and their shared effects. Regression analysis of RDA axis 1 scores was carried out by first extracting a mean score for each clone and using this as the response variable against: 1. mean concentration of each phenolic compound for each clone; 2. mean dbh for each clone; 3. mean score for each clone from PCA axes 1 and 2 (testing clonal variation in phenolic chemistry)

In addition, mean clone values for species richness, mean % cover and Shannon diversity were each modelled as a response to mean PCA axis scores 1 and 2 for each clone (from phenolic chemistry analysis), and as a response to mean concentration of each compound.

4.3.10 GLMM and variance components of epiphyte community composition

Variance component testing was carried out on the mean score for each clone from axis 1 of the RDA analysis and using these as the response variable in a GLMM to understand the amount of variation attributable to each effect of clone, site, block, and provenance. Understanding the influence of each of these effects was

considered to be important, therefore two rounds of model-fitting were applied to each of the response variable.

The overall approach involved testing for differences in epiphyte community structure among clones and exploring and quantifying the variance of site, block, and provenance, as random effects (Model 1). To elucidate the variation attributable to the effects of site, block and provenance, these factors were tested as fixed components, using clone as the random component to give the percent variation attributable to clone effects, and percent residual variation (Model 2).

4.3.10.1 Model 1 – Complete Model, Variance Partitioning of Effects

Generalized linear mixed models (GLMM) were implemented in 'R' version 2.12.0 (R Development Core Team, 2010), using the REML approximation method in the lmer function (package 'lme4', version 0.999375-37 (Bates and Maechler, 2010)). A model was designed to examine the variation between clones in relation to site, block and provenance. To do this site, block and provenance were treated as random additive effects.

4.3.10.2 Model 2 – Complete Model, Testing Contribution of 'Clone'

The second model for analysis of the effects of site, block and provenance were specified as fixed effects that were additive, and with clone as the random effect. Results are presented as a table showing each response with the percent variation attributable to each effect tested.

4.4 Results

4.4.1 Clonal differences: DBH of clones

Mean dbh of the replicates from each of the clones is illustrated in Figure 4.8 (Appendix C, Table 1). ANOVA showed highly significant differences between clones overall ($F = _{26, 193}$ 7.28, $p = <0.001$). Differences in observed variation of dbh accounted for approximately 50% (r^2 0.50, $p <0.001$) of the variation between clones. Mean dbh between clones was not significantly different when testing for site, provenance or block effects.

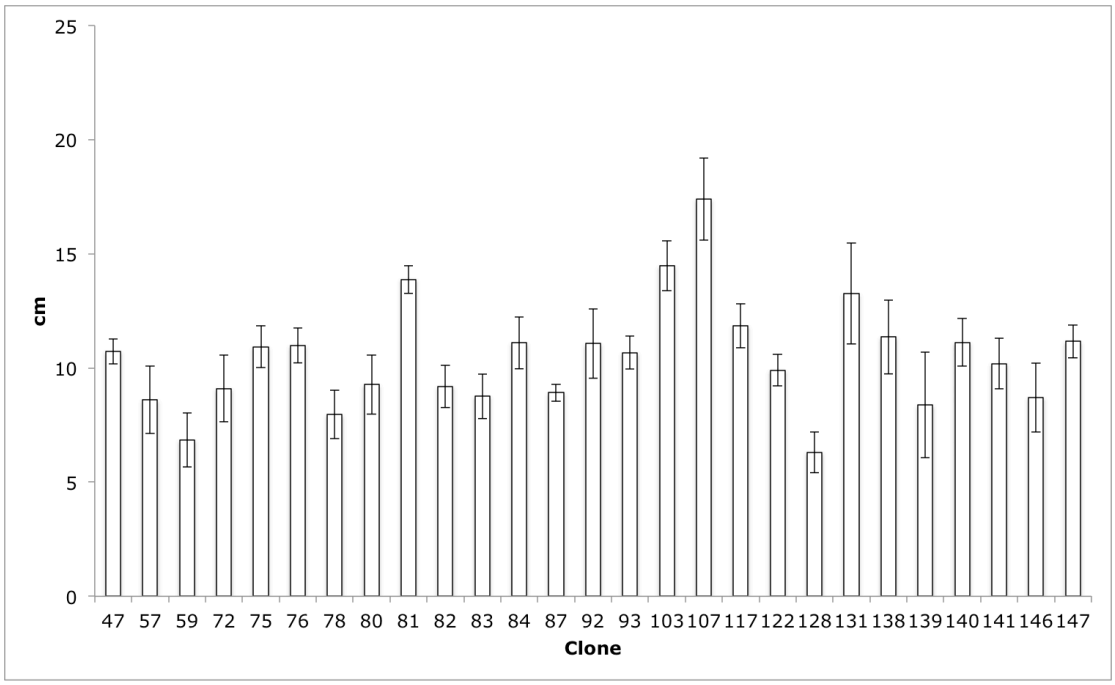

Figure 4.8: Mean dbh (with SE) per clone.

4.4.2 Clonal differences: phenolic chemistry of aspen bark sections

Five compounds were identified and quantified in the samples of bark: salicin ($C_{13}H_{18}O_7$), (+)-catechin ($C_{15}H_{16}O_7$), taxifolin ($C_{15}H_{12}O_7$), eriodictyol ($C_{15}H_{12}O_6$), and catechol ($C_6H_6O_2$) (Figure 4.9).

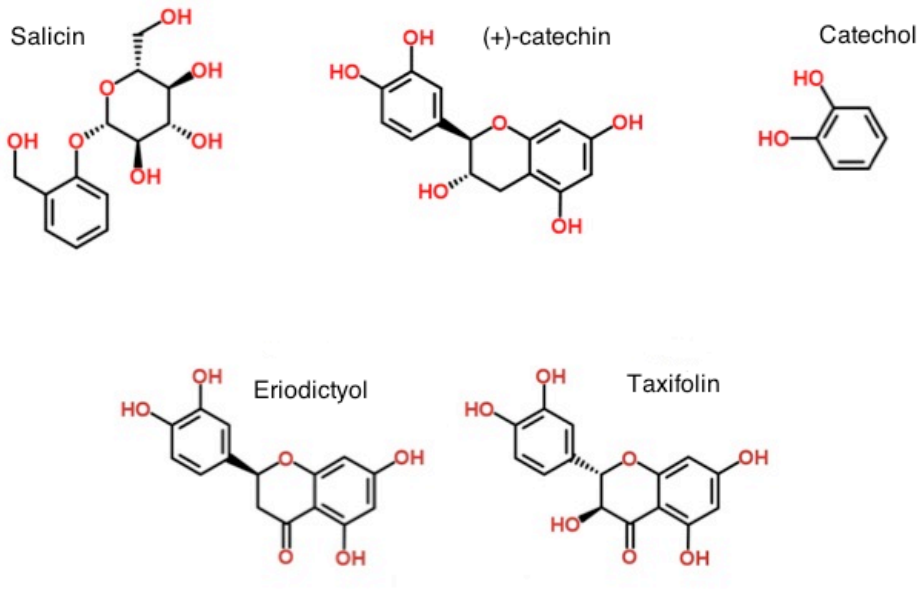

Figure 4.9: Five main compounds identified and quantified in subset of bark samples: salicin (top left), (+)-catechin (top centre), catechol (top right), eriodictyol (bottom left); taxifolin (bottom right). Chemical structures were obtained from ChemSpider (2010). Salicin is an alcoholic ß-glucoside found in the bark and leaves of *Salix* spp., and *Populus* spp., and is a precursor to aspirin; (+)-catechin is a natural antioxidant, and can have effects ranging from antibiotic to herbicidal; eriodictyol and taxifolin are both flavanones – a subgroup of phenol compounds; catechol is a naturally occurring substance found in kino, fruits and vegetables.

4.4.3 Variation between aspen clones

Mean concentration of each compound for each clone is shown in Appendix C, Table 2, and in Figures 4.10-4.14. Significant differences (p <0.001) were found in mean concentration of all phenolic compounds among clones (Table 4.3). The most abundant compound salicin was found in the highest concentration on clone 128 (4.66µg/ mg, SE 0.50), and the lowest concentration on clone 81 (0.59µg/ mg, SE

0.19); (+)-catechin was the second most abundant compound, found in the highest concentration on clone 128 (3.80µg/ mg, SE 0.82), and the lowest concentration on clone 147 (0.60µg/ mg, SE 0.09); taxifolin was found in the highest concentration on clone 117 (3.80µg/ mg, SE 1.50), and not detected in ten clones (47, 57, 59, 72, 76, 78, 82, 83, 93 and 122); eriodictyol was found in the highest concentration on clone 117 (3.57µg/ mg, SE 1.13) and the lowest concentration in clone 93 (0.01µg/ mg, SE 0.01); catechol was the least abundant of the phenolic compounds, detected in the highest concentration in clone 139 (0.93µg/ mg, SE 0.35) and not detected in 15 clones.

4.4.4 Variation between sites

Significant differences were found in concentrations of some phenolic compounds between sites (Table 4.3; Fig. 4.15), specifically in salicin (p <0.05), (+)-catechin (p <0.001), eriodictyol (p <0.001) and taxifolin (p <0.001). There were no significant site differences in concentrations of Catechol. Salicin was the compound in the highest concentration, occurring at the Moray site at a concentration of 2.51µg/ mg, (SE 0.16), followed by (+)-catechin at Kilmichael (2.23µg/ mg, SE 0.11). The lowest concentration of compound was for catechol and both Moray and Kilmichael (0.11µg/ mg, SE 0.03, and 0.13µg/ mg, SE 0.04 respectively). For blocks within site, catechol was the only compound that differed significantly among blocks within site (p <0.01).

4.4.5 Clone x Site interactions

All compounds showed significant clone x site interaction effects (Table 4.3), specifically: salicin and catechol (p <0.001), (+)-catechin (p <0.01), taxifolin and eriodictyol (p <0.05). Concentration of (+)-catechin decreased by an average of 1.5mg per clone from Kilmichael to Moray. Concentration of taxifolin decreased by an average of 1.5mg per clone from Kilmichael to Moray, except for a 0.5µg increase from Kilmichael to Moray for one clone. Catechol concentration varied by 0.25µg from Kilmichael to Moray for four clones and vice versa for four clones; the remaining four clones showed no differences between sites. Salicin and eriodictyol were the most variable for the clones across the two sites; interactions were often

attributable to clones behaving in opposite ways in response to changes in site, some clones increasing, others decreasing the concentration of a particular phenolic (Fig. 4.16, 4.17).

Salicin

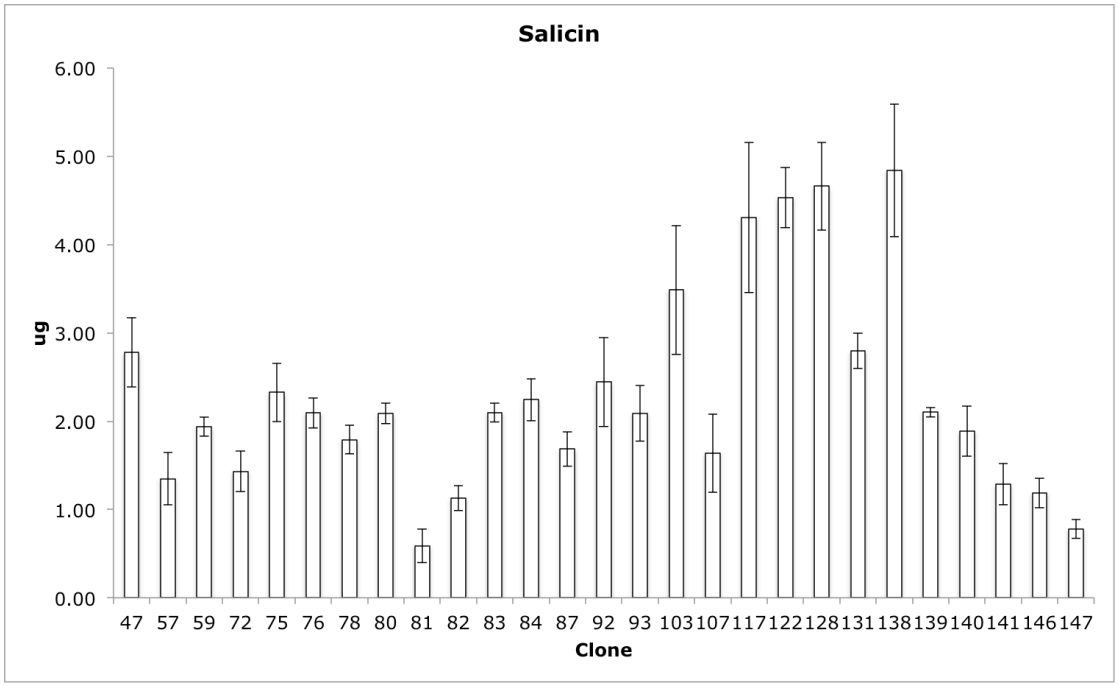

Figure 4.10: Barplot of mean (with SE) concentrations (µg) of salicin per clone

(+)-catechin

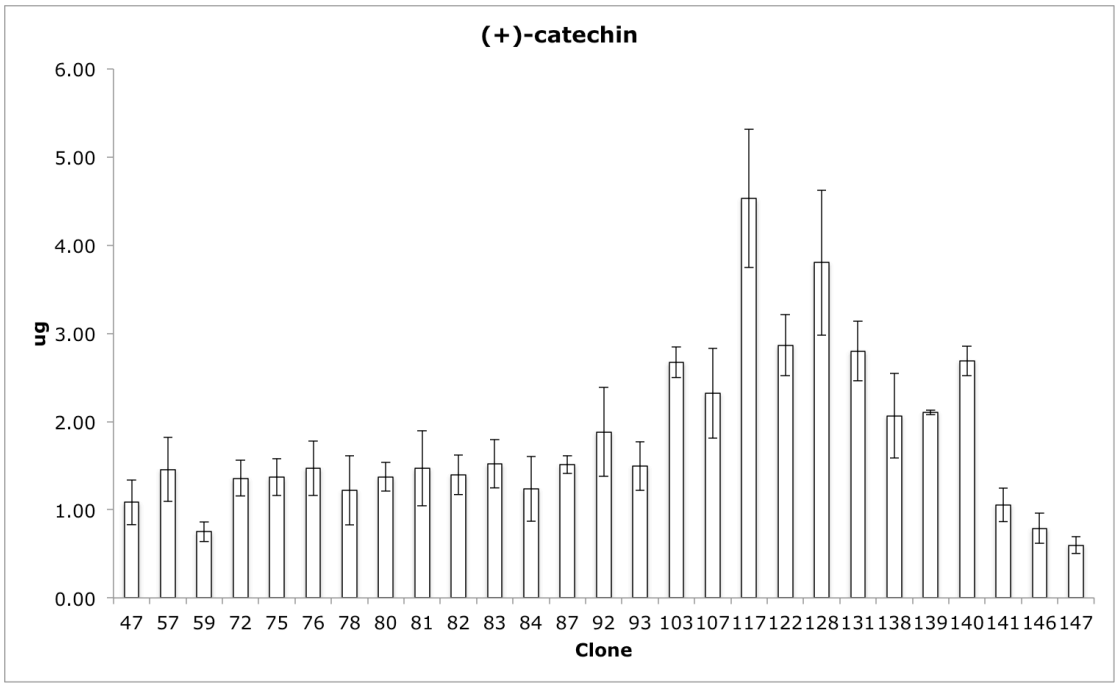

Figure 4.11: Barplot of mean (with SE) concentrations (µg) of (+)-catechin per clone

Taxifolin

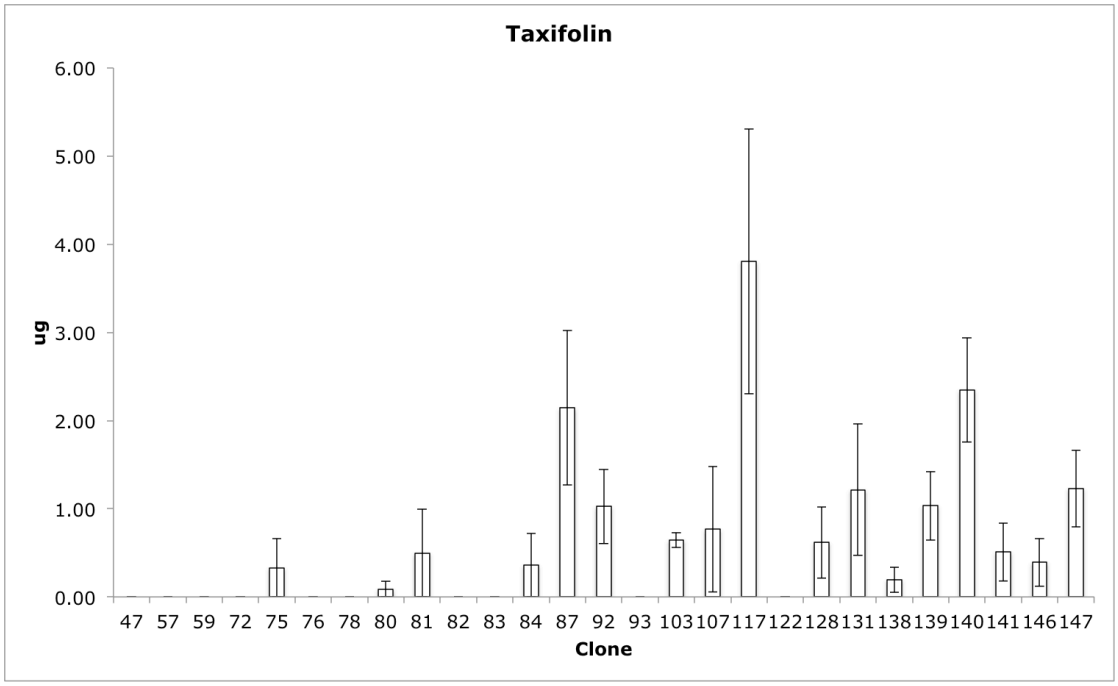

Figure 4.12: Barplot of mean (with SE) concentrations (µg) of taxifolin per clone

Eriodictyol

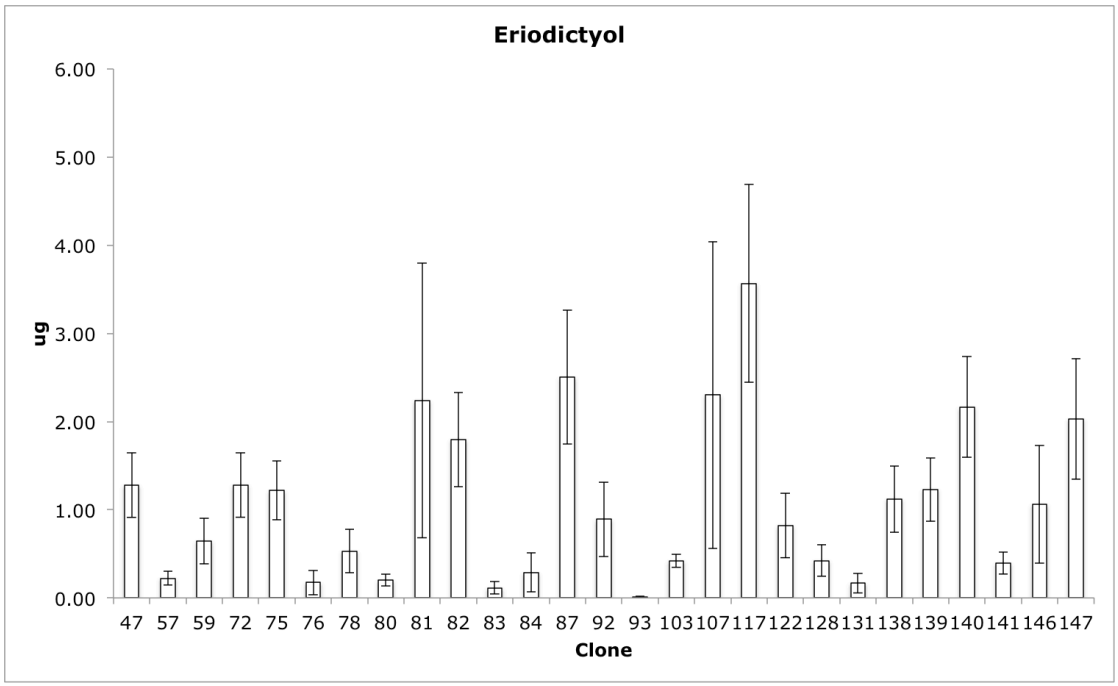

Figure 4.13: Barplot of mean (with SE) concentrations (µg) of eriodictyol per clone

Catechol

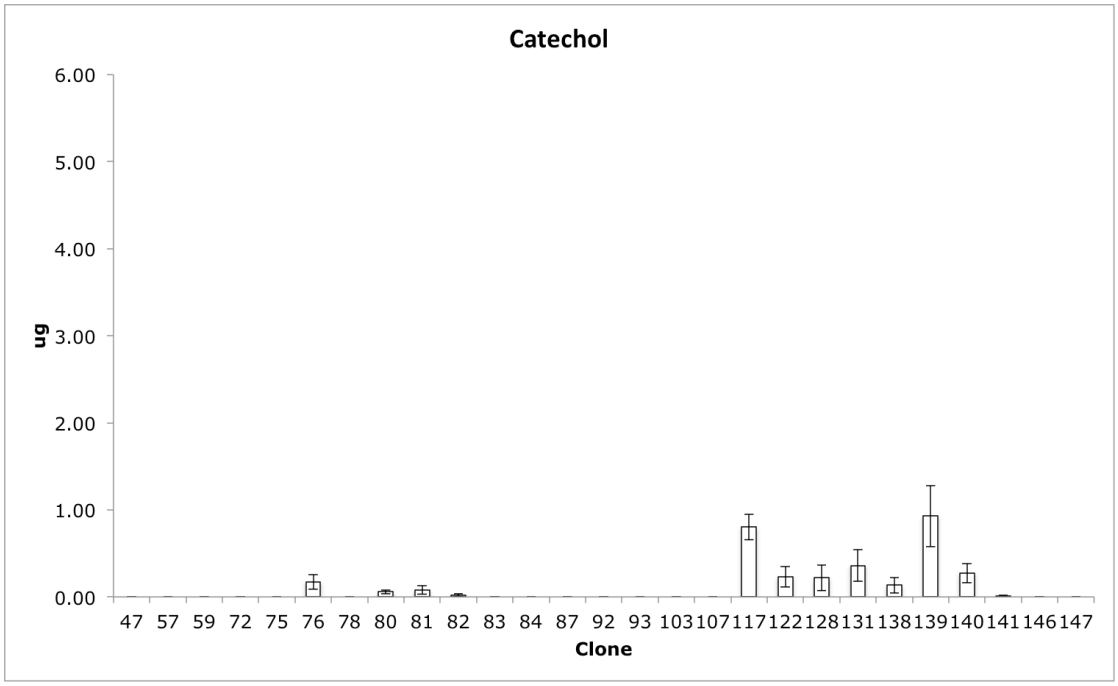

Figure 4.14: Barplot of mean (with SE) concentrations (µg) of catechol per clone

4.4.6 Site differences

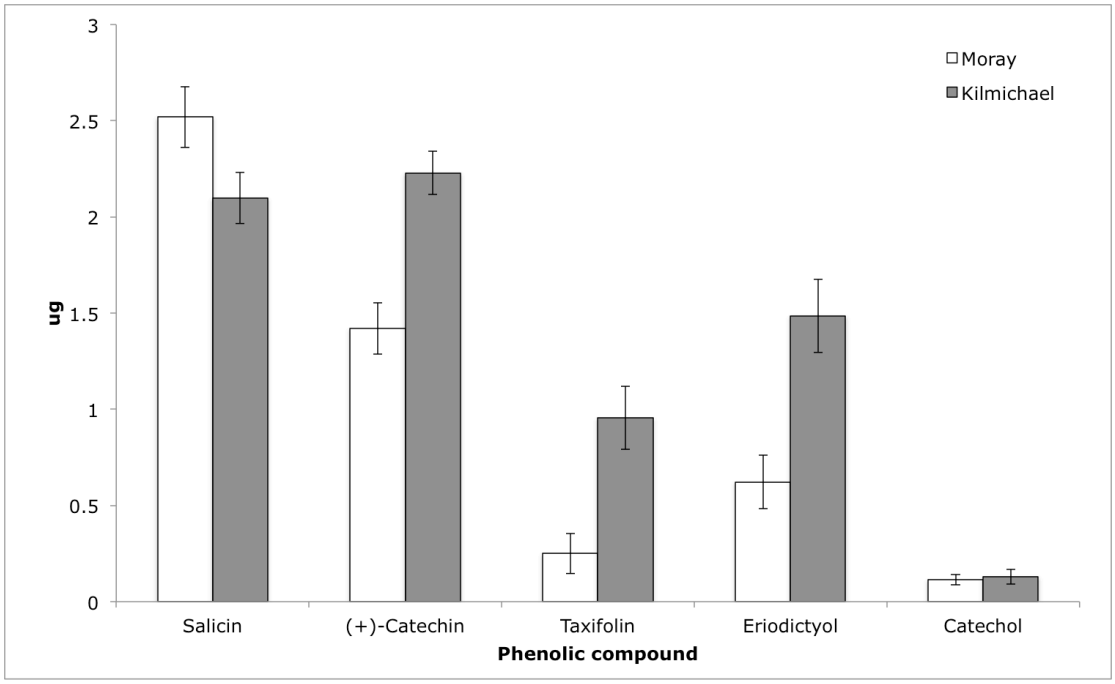

Figure 4.15: Mean (with SE) phenolic concentration (µg) comparing between the two sites of Moray and Kilmichael.

Table 4.3: Results of Analysis of Variance testing for effects of clones, sites, blocks and interactions on various parameters of phenolic chemistry and dbh on aspen clones. *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Source of Variation	df	MS Salicin	MS (+)-catechin	MS Taxifolin	MS Eriodictyol	MS Catechol	MS dbh
Among Clones	26	11.26 ***	6.88 ***	6.24 ***	6.35 ***	0.45 ***	45.22 ***
Among Sites	1	5.71 *	40.95 ***	24.81 ***	37.99 ***	0.04 n.s.	4.92 n.s.
Among Blocks	4	4.09 **	3.68 ***	2.63 n.s.	1.60 n.ss	0.01 n.s.	4.19 n.s.
Block within Site	7	0.72 n.s.	0.98 n.s.	0.96 n.s.	1.39 n.s.	0.10 *	4.47 n.ss
Clone x Site	26	2.08 ***	1.67 **	2.07 *	4.08 *	0.32 ***	3.46 n.s.
Error	156	0.89	0.74	1.29	1.62	0.03	6.75

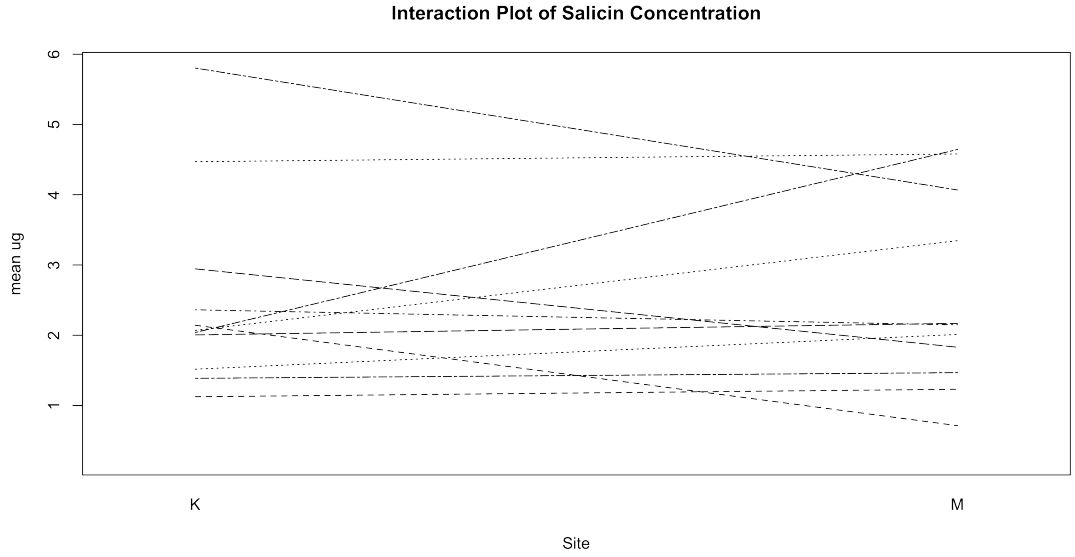

Figure 4.16: Interaction plot for concentration of Salicin for each clone and the variation between the sites Moray (M) and Kilmichael (K).

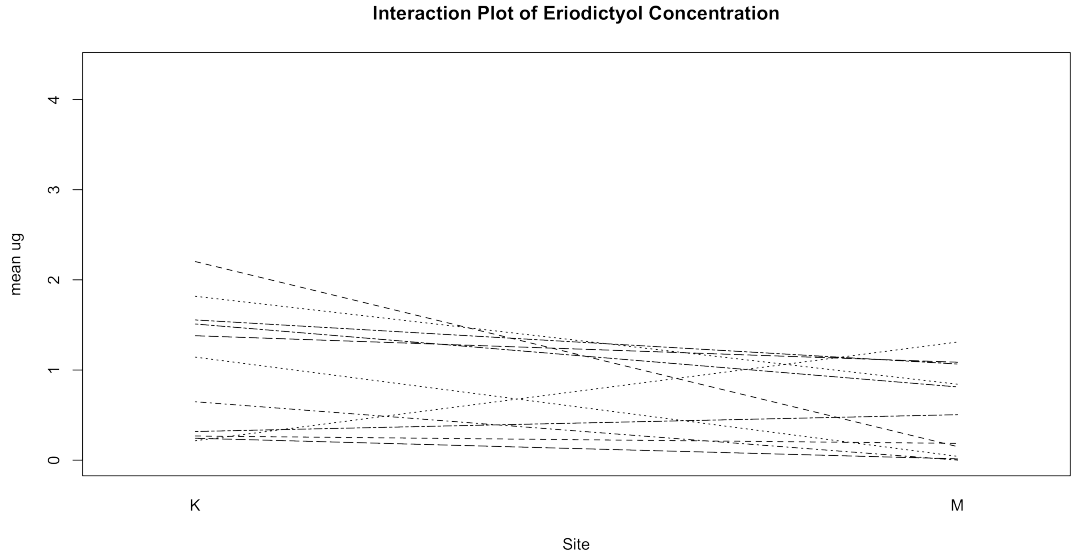

Figure 4.17: Interaction plot for concentration of Eriodictyol for each clone and the variation between the sites Moray and Kilmichael

4.4.7 Correlation matrix of phenolics

Significant positive correlations were found between (+)-catechin and salicin (p <0.001), (+)-catechin and taxifolin (p <0.001), (+)-catechin and catechol (p <0.001), taxifolin and eriodictyol (p <0.001), and taxifolin and catechol (p <0.001); and between dbh, taxifolin and eriodictyol (both p <0.05). A significant negative correlation was found for salicin and eriodictyol (p <0.01) (Table 4.4).

4.4.8 PCA of phenolic chemistry

A principal component analysis of mean concentration of each phenolic compound for each clone/ block revealed a highly aggregated cluster towards the centroid with some clones showing distinct differences (not shown). The first axis accounts for 49% of the overall variation, along a gradient of eriodictyol and taxifolin; the second axis accounts for 18% of the overall variation, along a gradient of salicin (Table 4.5). Testing each set of axis scores via ANOVA showed significant effects of clone (p <0.001), site (p <0.001) and a clone x site interaction (p <0.05) for axis 1 and significant effects of clone (p <0.001), site (p <0.01) and a clone × site interaction (p <0.001) for axis 2 (Table 4.6). Of the measured effects, clone accounted for the greatest variation in phenolic concentrations along axis 1 (r^2=0.29, p <0.001) and axis 2 (r^2=0.75 p <0.001). The effect of dbh on phenolic concentrations was not significant.

Table 4.4: Pearson correlation matrix of phenolic compounds and dbh. *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

	Salicin	**(+)- Catechin**	**Taxifolin**	**Eriodictyol**	**Catechol**	**dbh**
Salicin	-	0.22***	-0.10	-0.16**	0.10	0.01
Catechin		-	0.26***	0.11	0.32***	0.04
Taxifolin			-	0.69***	0.24***	0.15*
Eriodictyol				-	0.11	0.14*
Catechol					-	0.00
dbh						-

Table 4.5: PCA scores for first two components for each phenolic compound

Phenolic compound	**PC1**	**PC2**
Salicin	-0.8195	2.92368
(+)-catechin	0.7207	1.95508
Taxifolin	2.7716	0.46138
Eriodictyol	3.6333	-0.08784
Catechol	0.1409	0.19508

Table 4.6: Mean squares and significance of results for ANOVA of PCA axis 1 and axis 2 scores for phenolic chemistry, comparing clone, site and interactions: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

	Df	MS PC1	MS PC2
Clone	26	0.5059***	1.3127***
Site	1	3.7528***	0.3361**
Clone x Site	26	0.2199*	0.1592***
Residuals	163	0.1400ns	0.0419ns

4.4.9 GLMM and variance components

Of the effects specified in the models, clone accounted for the greatest variation in concentrations of phenolic compounds (15-51%) and dbh (44%) (Table 4.7). Clone effects were strongest for salicin (51%), then (+)-catechin and catechol (34% and 33%), then taxifolin (23%) and eriodictyol (15%). Site effects were greatest for (+)-catechin (13%), then taxifolin and eriodictyol (both 9%), bur not for salicin, catechol and dbh.

Table 4.7: Variance components of GLMM, combining the results of Model 1 and Model 2, indicating approximate variation attributed to the effects of clone, site, block and provenance on each phenolic compound and dbh

Compound	Clone	Site	Block	Provenance	Residual
Salicin	51	0	5	3	40
(+)-catechin	34	13	6	4	43
Taxifolin	23	9	2	5	62
Eriodictyol	15	9	0	6	70
Catechol	33	0	0	8	58
dbh	44	0	0	0	56

4.5 Epiphyte community results

4.5.1 Overview of the epiphyte community

The epiphyte community scored in quadrats on aspen is made up of 26 species comprising 23 lichens, 2 mosses and 1 liverwort (Table 4.8). One species has a conservation designation of 'nationally rare'. Nineteen species were present at Moray and thirteen at Kilmichael. Twelve species were common between the two sites. Species not recorded from Moray were: *Arthonia radiata*, *Pertusaria leioplaca*, *Parmelia sulcata*, *Platismatia glauca*, *Hypnum cupressiforme*, *Orthotrichum speciosum*, *Frullania dilatata*; and species not recorded from Kilmichael were: *Buellia disciformis*, *Candelariella* cf. *reflexa*, *Ramalina fastigiata*, *Parmelia saxatilis*, *Physcia tenella/adscendens*, *Physconia distorta*, *Xanthoria parietina*.

The lichens found can be further subdivided into functional groups – crustose, fruticose and foliose. The number of species in each of these and the remaining functional groups and % abundance of each functional group, in terms of relative

number of 5cm x 25cm quadrats in which they were scored, is given in Table 4.9 and illustrated in Figure 4.18.

Table 4.8: List of epiphyte species recorded for the twenty-seven aspen clones at Moray and Kilmichael. Lichens are divided into three main morphological groups: crustose, fruticose and foliose. [1]Nationally rare. Species distributions between sites are marked K (Kilmichael only), M (Moray only) or B (Both sites).

Lichens			**Bryophytes**	
Crustose	**Fruticose**	**Foliose**	**Mosses**	**Liverworts**
Arthonia radiata K	Ramalina farinacea B	Evernia prunastri B	Hypnum cupressiforme K	Frullania dilatata K
Buellia disciformis M	Ramalina fastigiata M	Parmelia saxatilis M	Orthotrichum speciosum K	
Caloplaca holocarpa B	Ramalina fraxinea K	Parmelia sulcata K		
Candelariella cf. reflexa M		Phaeophyscia orbicularis B		
Lecania cyrtella B		Physcia aipolia B		
Lecanora carpinea B		Physcia tenella/adscendens M		
Lecanora chlarotera B		Physconia distorta M		
Lecanora populicola B[1]		Platismatia glauca K		
Lecidella elaeochroma B		Xanthoria parietina M		
Pertusaria leioplaca K				
Sterile crust B				

Table 4.9: Functional groups of epiphyte species recorded at Moray and Kilmichael, showing the total number of species within each group and the relative abundance (%)

Functional Group	Number of Species	Relative Abundance (%)
Crustose lichens	11	67
Fruticose lichens	2	4
Foliose lichens	9	13
Moss	2	9
Liverwort	1	6

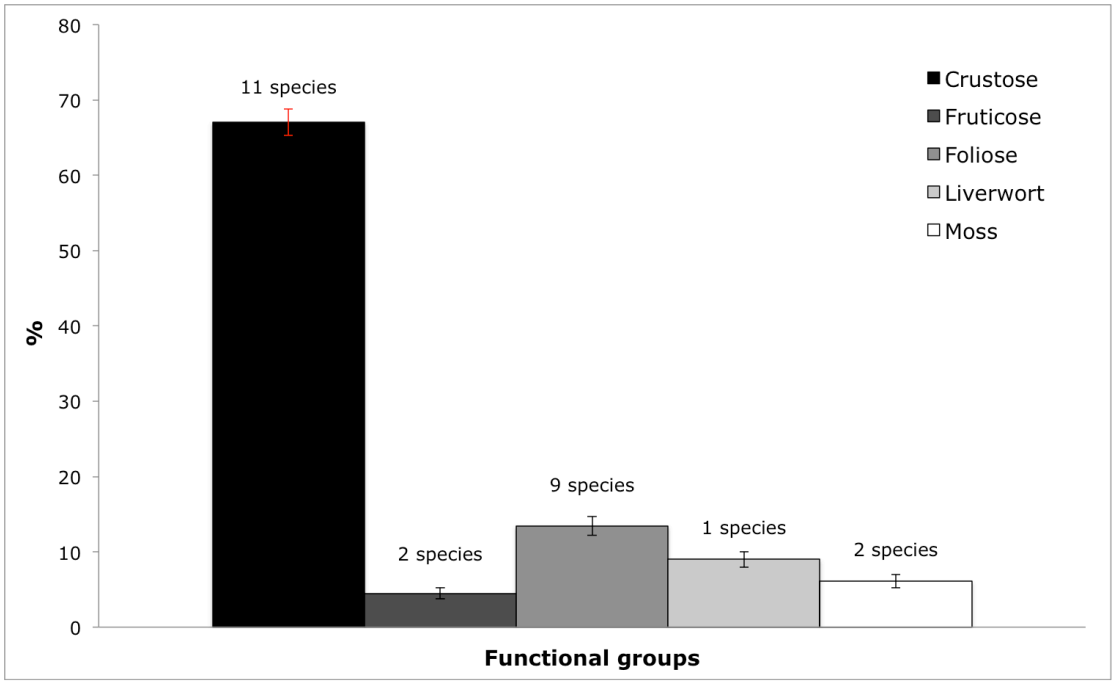

Figure 4.18: Percent relative abundance (SE) of each functional group showing number of species representing each group recorded on aspen at Moray and Kilmichael

4.5.2 Differences in mean cover between clones, aspects and sites

Clones were significantly different (p <0.001) in the % cover of epiphytes (Fig. 4.19; Appendix C, Table 4). There were no significant differences in mean % epiphyte cover between sites (Fig. 4.20; Appendix C, Table 5). Significant differences (p <0.001) were also found in the mean % cover of epiphytes across aspects (Fig. 4.21; Appendix C, Table 6), with the highest cover on the north (59.89%, SE 3.33) and east (54.98%, SE 3.39) aspects and the lowest on the south (40.39%, SE 3.34) and west (38.13%, SE 3.30) aspects. Regression analysis and

Pearson correlation of mean dbh per clone and mean % cover per clone revealed no significant effect (r^2 <0.001, *p* 0.923 and 0.01, *p* ns, respectively).

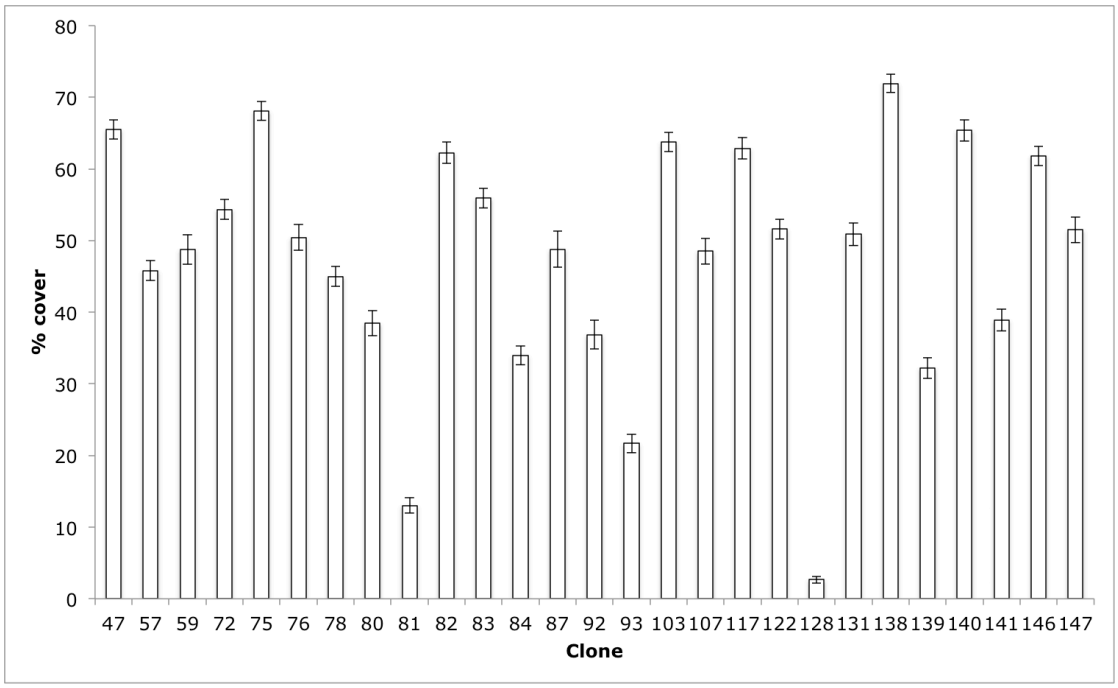

Figure 4.19: Barplot of mean % cover (SE) of total epiphytes per clone, showing significant variation.

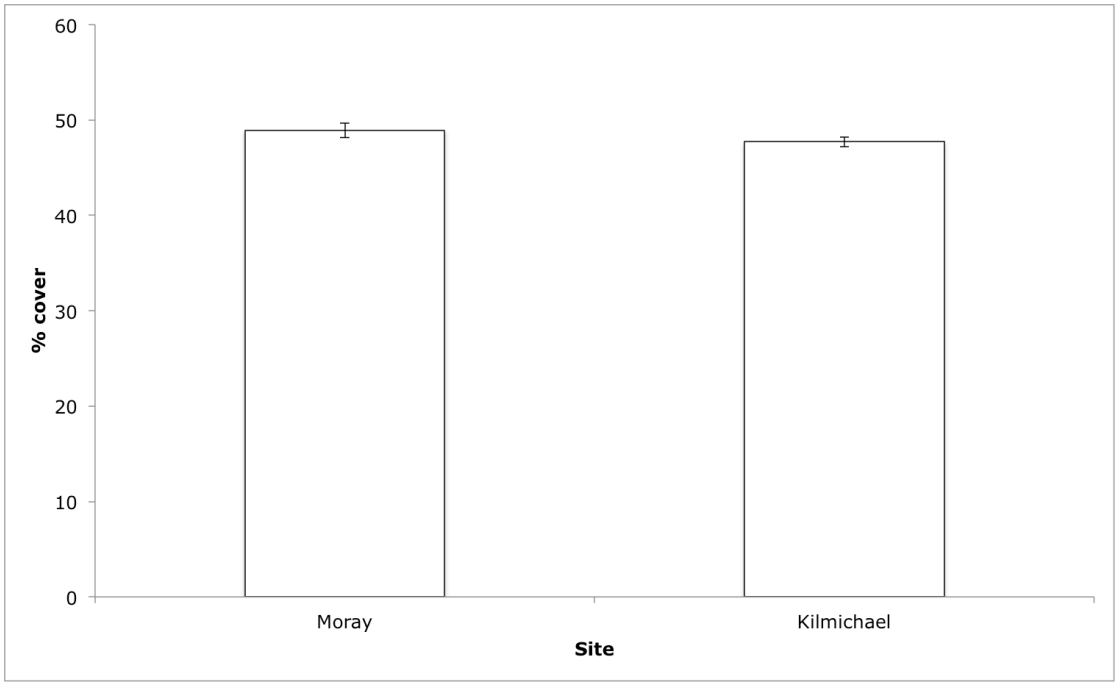

Figure 4.20: Barplot of mean % cover (SE) of total epiphytes per site (Moray and Kilmichael), showing no significant differences.

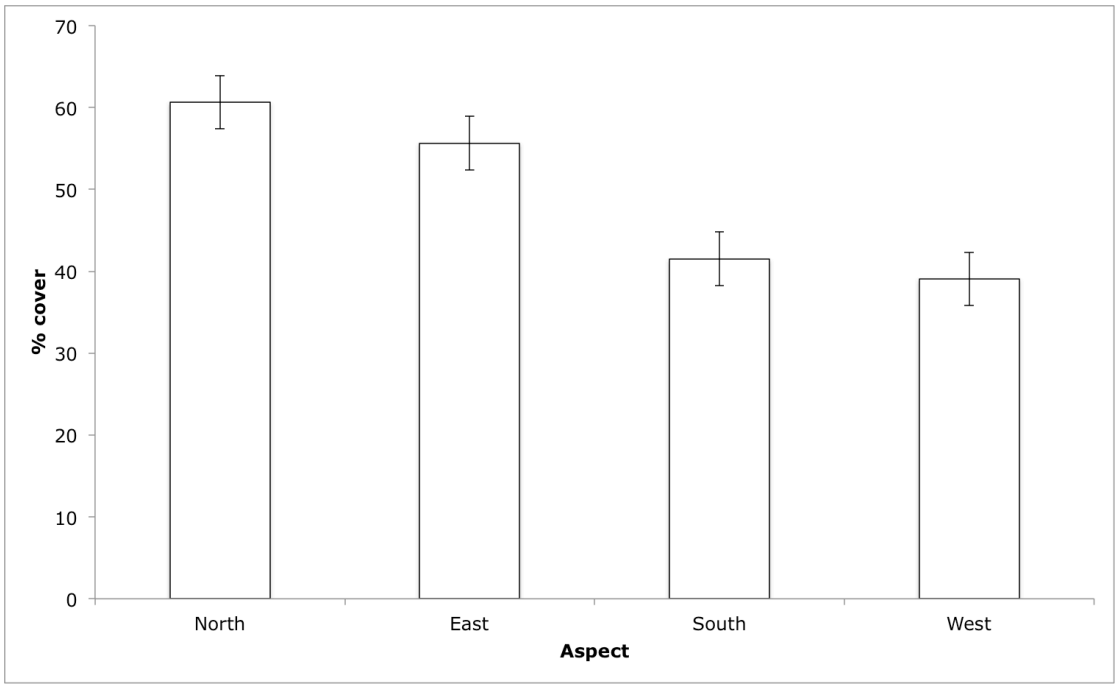

Figure 4.21: Barplot of mean % cover (SE) of total epiphytes per aspect, showing significant differences.

4.5.3 Distribution of epiphyte species on aspen clones

Of the 26 species encountered, only sterile crusts were found on all 27 aspen clones. One species (*Lecanora chlarotera*) was present on 26 of the clones. Only one species, *Arthonia radiata*, was recorded on only one clone. For each species, mean percent cover was calculated and combined with rank abundance (the total number of quadrats recorded in across both sites for all clones), (Table 4.10 and Fig. 4.22).

Table 4.10: Rank abundance of each species recorded from Moray and Kilmichael aspen with mean % cover and total number of quadrats recorded for each species.

Species	Mean % cover	Total number of quadrats
Arthonia radiata	1.00	1
Phaeophyscia orbicularis	1.70	5
Physconia distorta	1.88	6
Parmelia sulcata	2.00	2
Parmelia saxatilis	2.67	2
Buellia disciformis	2.73	12
Evernia prunastri	2.80	3
Physcia tenella/ adscendens	2.84	16
Candelariella cf. reflexa	3.94	6
Orthotrichum speciosum	4.24	44
Hypnum cupressiforme	4.33	5
Pertusaria leioplaca	5.12	16
Lecanora populicola	5.41	27
Ramalina farinacea	5.44	29
Physcia aipolia	5.79	16
Lecidella elaeochroma	6.09	68
Lecanora carpinea	7.53	10
Caloplaca holocarpa	7.82	19
Ramalina fastigiata	8.07	5
Lecania cyrtella	8.90	9
Lecanora chlarotera	9.39	116
Sterile crust	9.64	165
Platismatia glauca	10.38	3
Xanthoria parietina	11.95	40
Frullania dilatata	12.98	45
Ramalina fraxinea	15.14	3

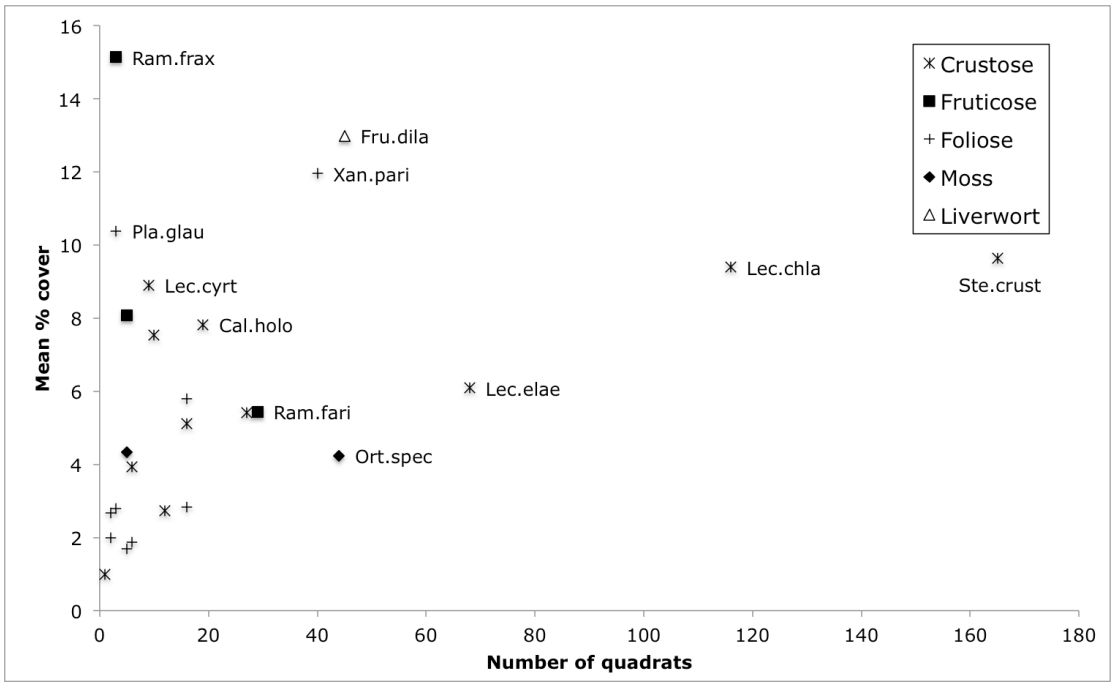

Figure 4.22: Scatterplot of species presence (x axis – number of quadrats) against mean % cover (y axis). Species names are abbreviated and are coded according to functional group.

Whilst sterile crusts were found in the greatest abundance (165 quadrats), *Ramalina fraxinea* occupied the greatest area when present (15.14%), despite being one of the least abundant species. The crustose species *Lecanora chlarotera* and *Lecidella elaeochroma* are also amongst the most abundant (116 and 68 quadrats respectively). Mean percent cover for each functional group is shown in Appendix C, Table 7.

4.5.4 Analysis of species richness and functional group diversity in sample quadrats

Within each 5cm x 25cm quadrat, total species richness, species richness of each functional type and Shannon's index of diversity based on the proportions of individual species within the quadrat were calculated. Data were analysed to test for differences for each of these variables among clones, and in relation to both site and aspect of sampling (Table 4.11). Tables of means and the results of the ANOVAs used to conduct the analyses are given in Appendix C, Tables 8-10.

4.5.5 Variation between aspen clones

Significant differences were found in total species richness of the epiphyte community among clones (p <0.001) specifically in the diversity of crustose (p<0.001), fruticose (p <0.001) and foliose (p <0.001) lichens, mosses (p <0.001) and liverworts (p <0.001). Clonal differences in a diversity index measure (Shannon) were also statistically significant (p <0.001) (Figures 4.23-4.28). The greatest diversity of species was found on clone 75 (3.72, SE 1.59), the least on clone 128 (0.31, SE (0.38). Of the functional groups, clone 138 had the greatest diversity of crustose lichens (2.61, SE 0.22); clones 122 and 141 had the greatest diversity of fruticose lichens (0.32, SE 0.12 and 0.32, SE 0.13); clone 72 had the greatest diversity of foliose lichens (0.67, SE 0.16); clone 75 had the greatest diversity of liverwort (0.31, SE 0.08); and clone 87 had the greatest diversity of mosses (0.40, SE 0.11). Lowest crustose lichen diversity was found on clones 81 and 128 (0.31, SE 0.09 and 0.31, SE 0.08); fruticose species were not recorded on ten clones (78, 80, 81, 83, 84, 92, 93, 128, 138 and 139); foliose species were not recorded on four clones (84, 87, 92 and 128); liverworts were not recorded on seven clones (78, 80, 81, 83, 92, 128, 141); and mosses were not recorded on six clones (78, 80, 83, 92, 107 and 128). The greatest diversity of species (Shannon Index) was found on clone 75 (1.19, SE 0.08), whilst clone 128 was found to be the least diverse (0.00, SE 0.00), colonised only by a small quantity of sterile crusts.

4.5.6 Variation between sites

For all variables measured (total species richness, species richness for functional groups and Shannon diversity), there were significant differences in epiphyte communities among sites, specifically in the total species richness (p 0.05), richness of crustose (p <0.001), foliose (p <0.001), and fruticose (p <0.01) lichens, liverworts (p <0.001) and mosses (p <0.001). Site differences for the Shannon diversity index measure were significant (p <0.05) (Figures 4.30-4.32; Appendix C, Table 9). The most total species were found at Moray (2.25, SE 0.10), and the least at Kilmichael (1.98, SE 0.08). Of the functional groups, Moray had the greatest richness of crustose lichens (1.71, SE 0.07), fruticose lichens (0.12, SE 0.02) and foliose lichens

(0.43, SE 0.03); the highest richness of liverworts and mosses were recorded from Kilmichael (0.28, SE 0.02 and 0.27, SE 0.02 respectively). Lowest total species, and crustose, fruticose and foliose lichen diversity was found at Kilmichael (1.98, SE 0.08; 1.29, SE 0.05; 0.06, SE 0.01; and 0.08, SE 0.02). Liverworts and mosses were not recorded from the Moray site. The greatest richness of species (Shannon Index) was found at Moray (0.65, SE 0.03), whilst the least was found at Kilmichael (0.53, SE 0.03).

4.5.7 Variation between aspects

Significant differences were found in total species richness of the epiphyte community among aspects for total species richness, species diversity (Shannon Index), liverworts and mosses (p <0.001), crustose and fruticose lichens (p <0.05), but not for foliose lichens (Figures 4.33-4.35; Appendix C, Table 10). The highest species richness was found on the North aspect (2.49, SE 0.14), the least at West (1.79, SE 0.12). Of the functional groups, North had the highest richness of crustose lichens (1.66, SE 0.08), fruticose lichens (0.14, SE 0.03), liverworts (0.19, SE 0.03) and mosses (0.20, SE 0.03); North and East together had the highest richness of foliose lichens (0.30, SE 0.05 and 0.30, SE 0.04). Lowest total species were found on the West (1.79, SE 0.12); lowest richness was found on the West aspect for crustose (1.35, SE 0.09), fruticose (0.06, SE 0.02), foliose (0.21, SE 0.04), and mosses (0.07, SE 0.02); South and West had the lowest liverwort richness (0.09, SE 0.02 for both aspects). The greatest richness of species (Shannon Index) was found at the North aspect (0.72, SE 0.05), whilst the least was found at the West aspect (0.47, SE 0.04).

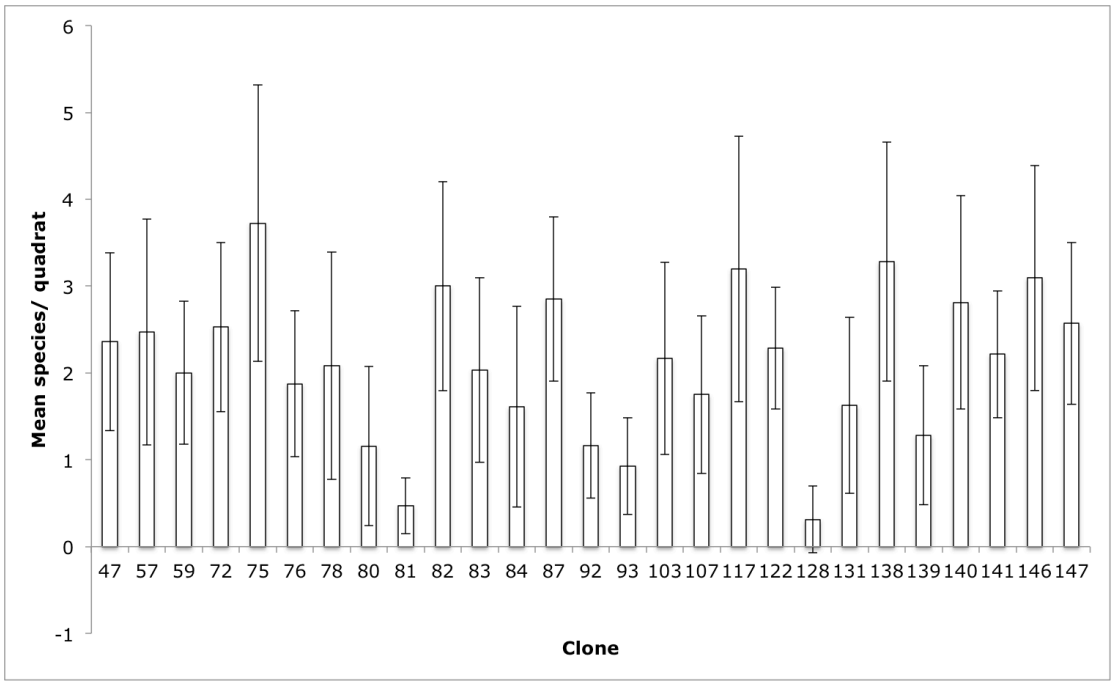

Figure 4.23: Mean number of epiphyte species per quadrat (with SE) for each of the clones

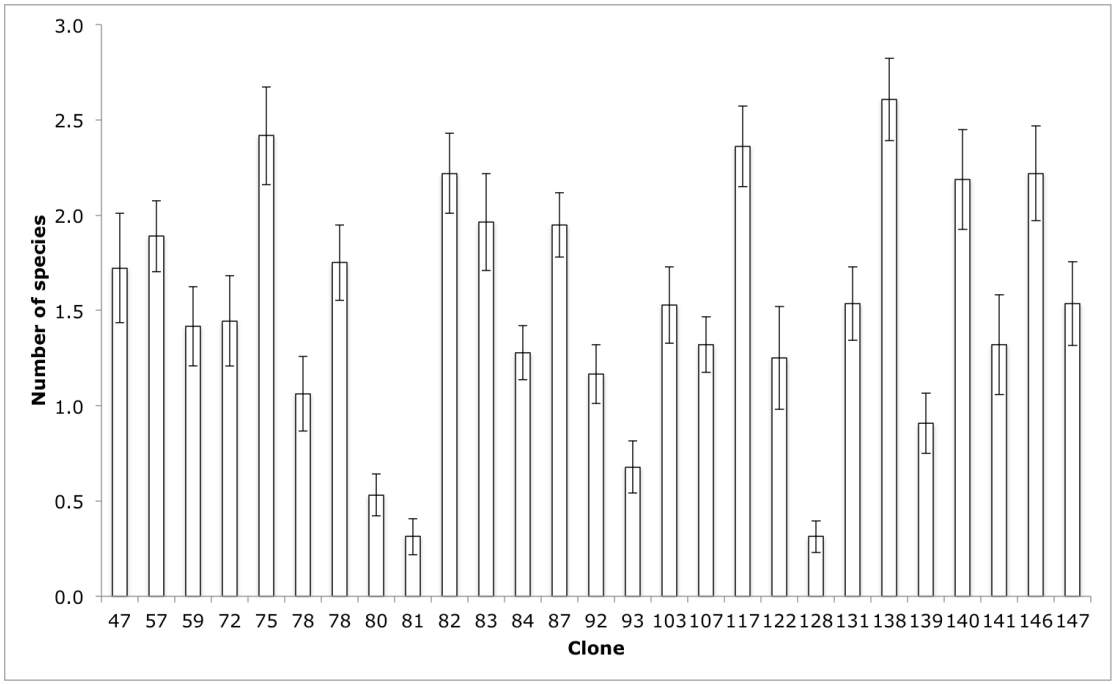

Figure 4.24: Mean number of crustose species per quadrat (with SE) for each of the clones

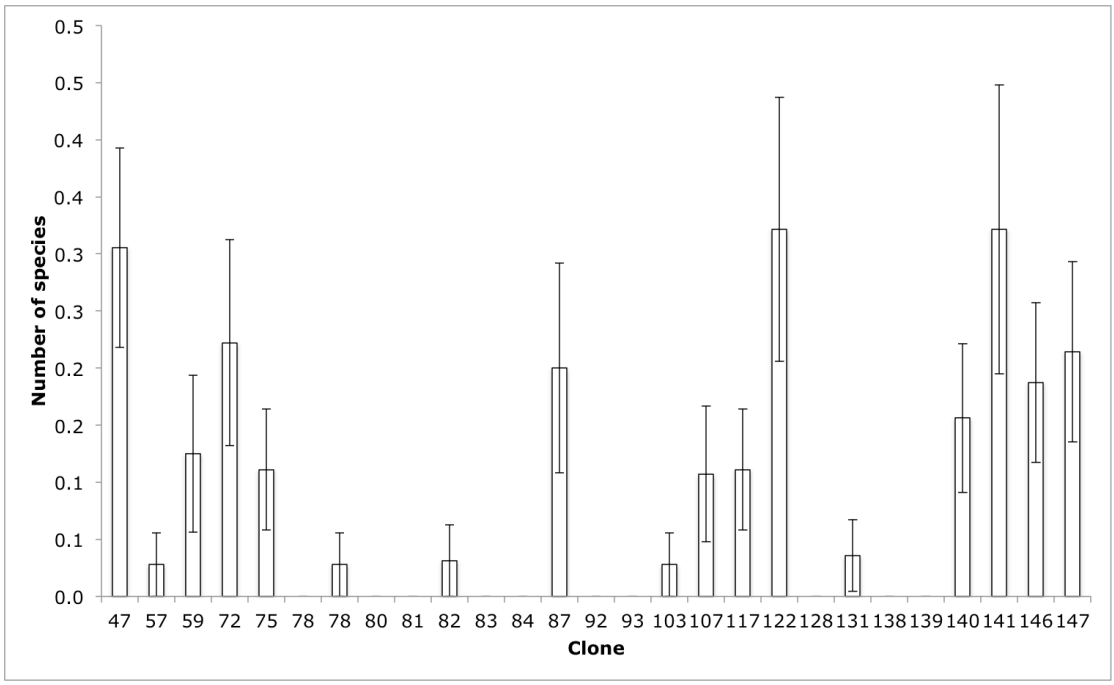

Figure 4.25: Mean number of fruticose species per quadrat (with SE) for each of the clones

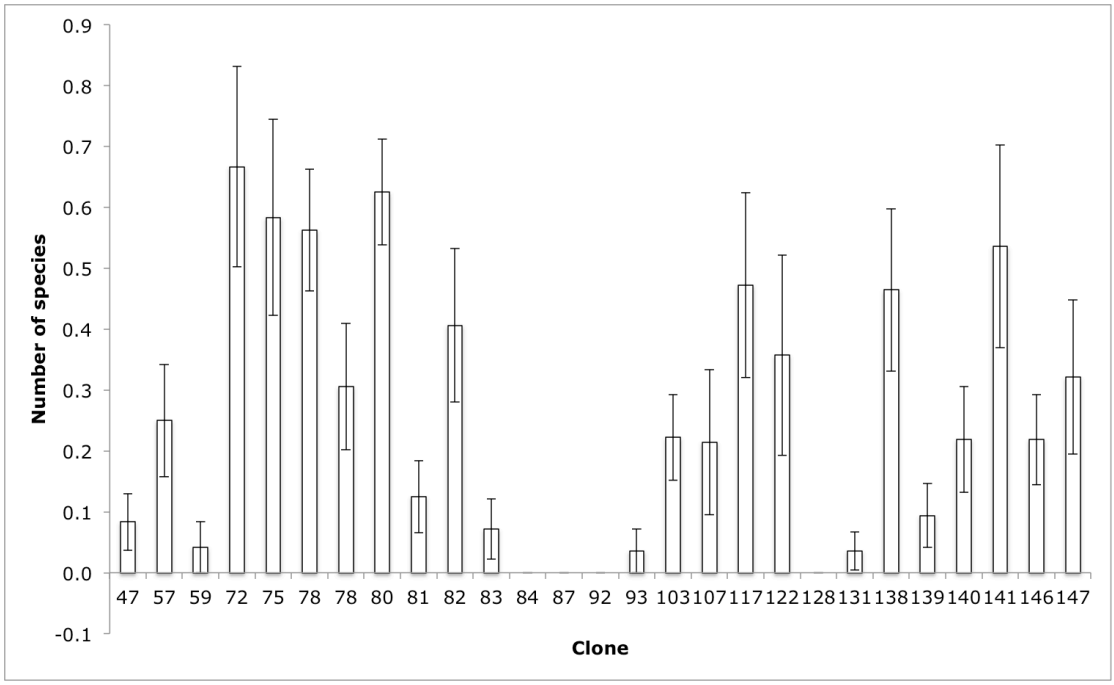

Figure 4.26: Mean number of foliose species per quadrat (with SE) for each of the clones

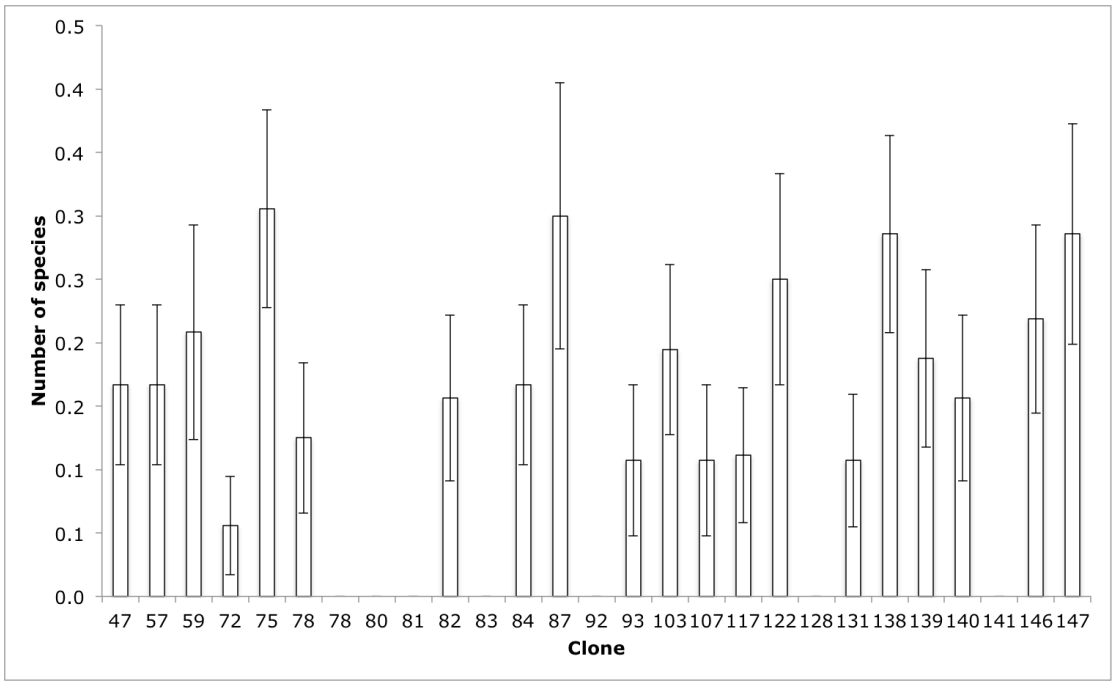

Figure 4.27: Mean number of liverwort species per quadrat (with SE) for each of the clones

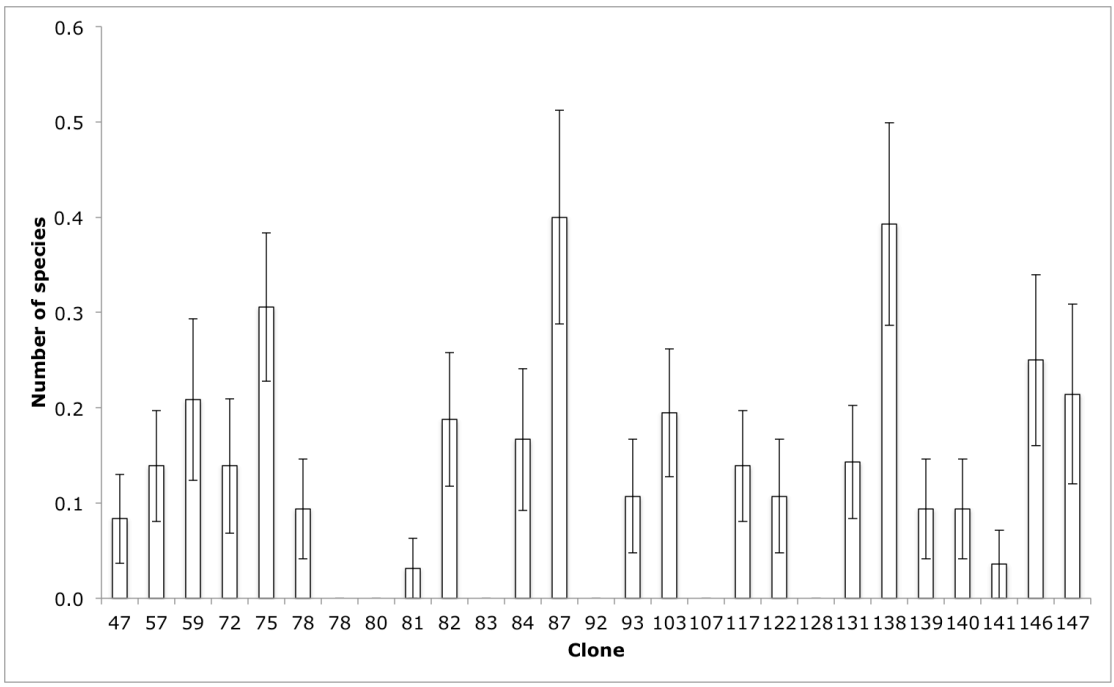

Figure 4.28: Mean number of moss species per quadrat (with SE) for each of the clones

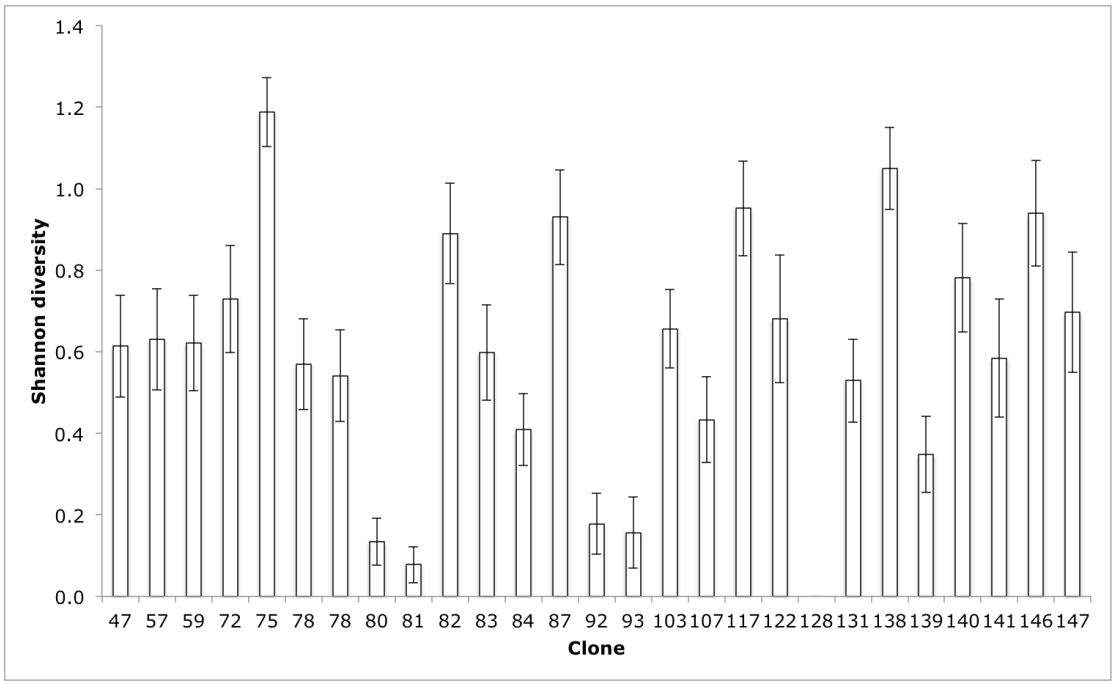

Figure 4.29: Mean Shannon Index per quadrat (with SE) for each of the clones

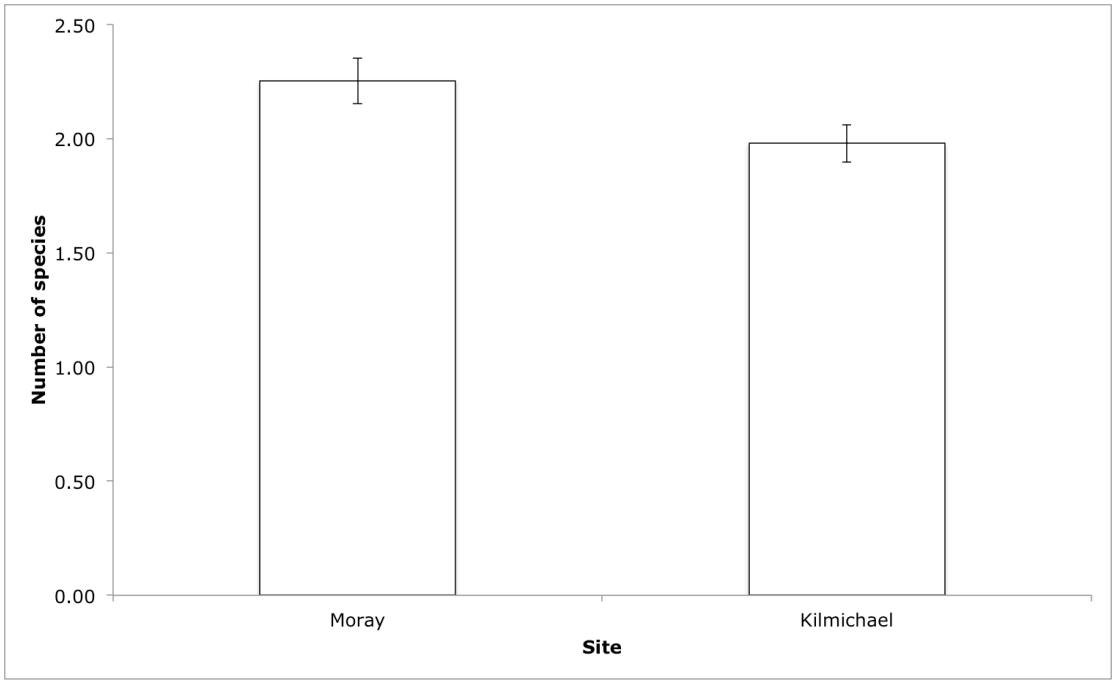

Figure 4.30: Mean number of species per site (with SE)

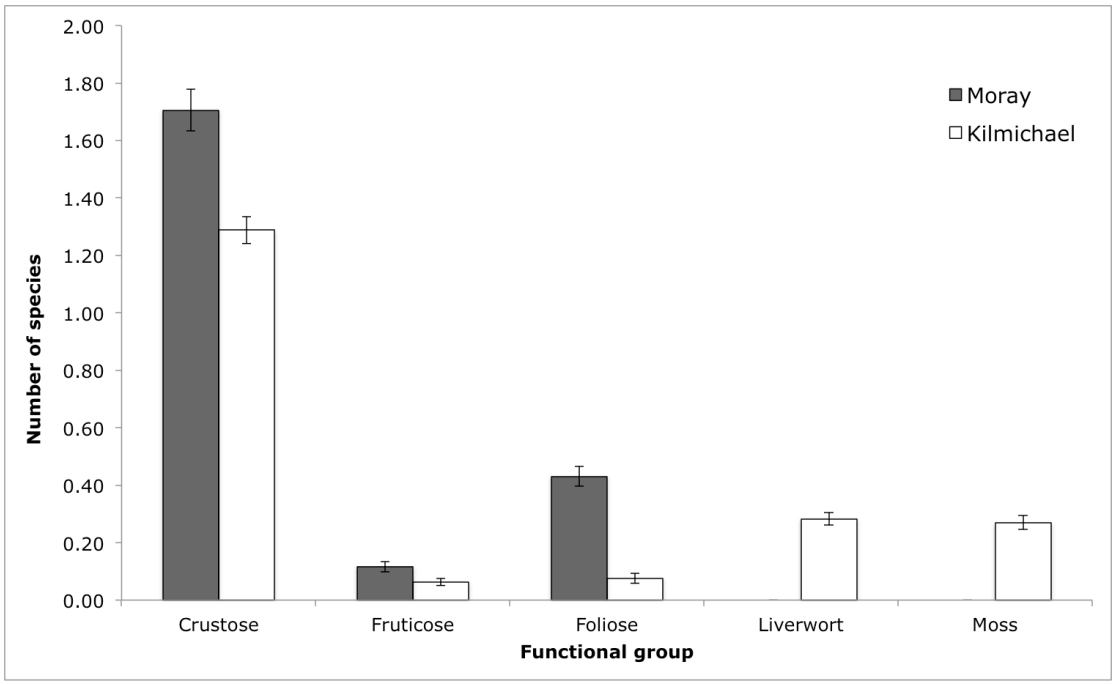

Figure 4.31: Mean number of species within each functional group per site (with SE)

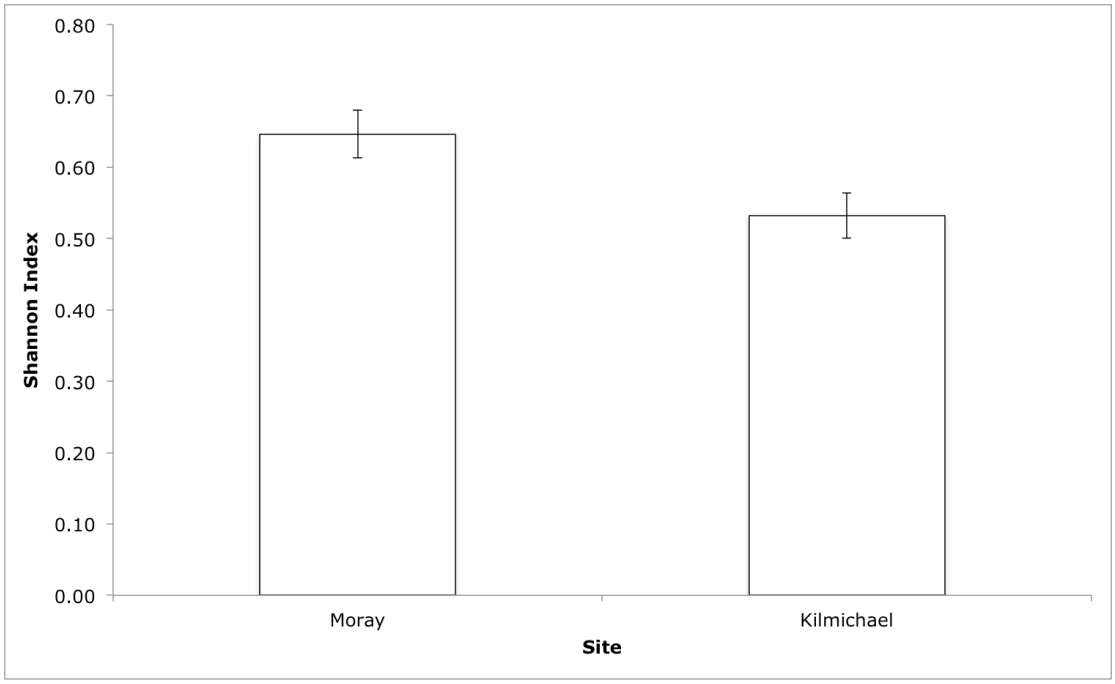

Figure 4.32: Mean Shannon Index of species per site (with SE)

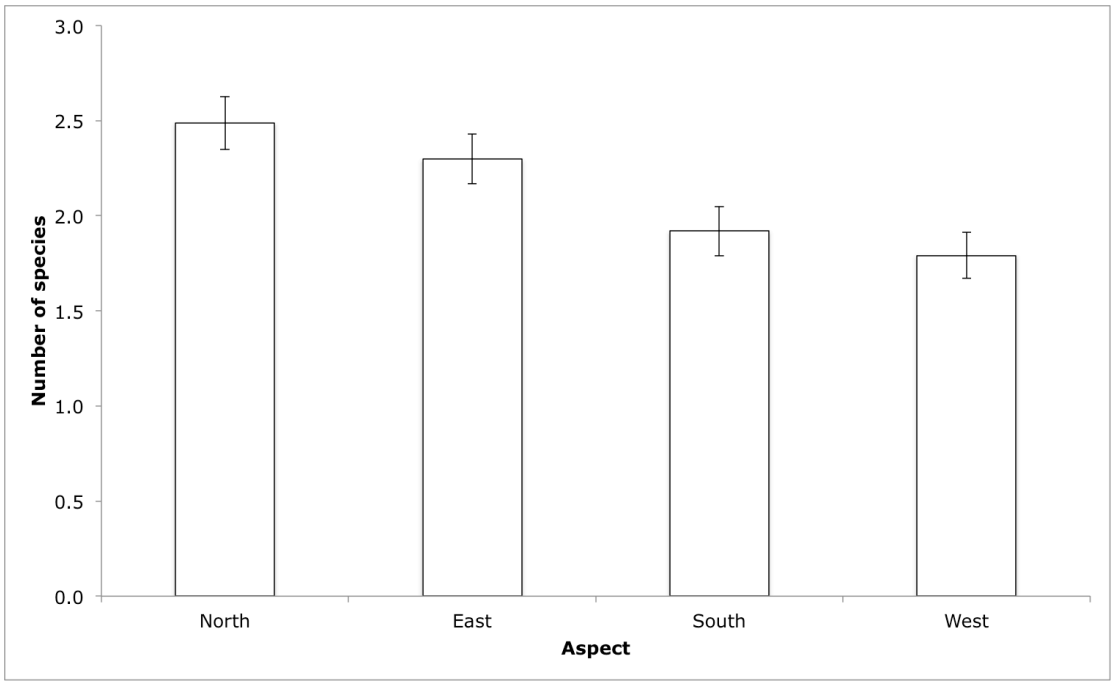

Figure 4.33: Mean number of species per aspect (with SE)

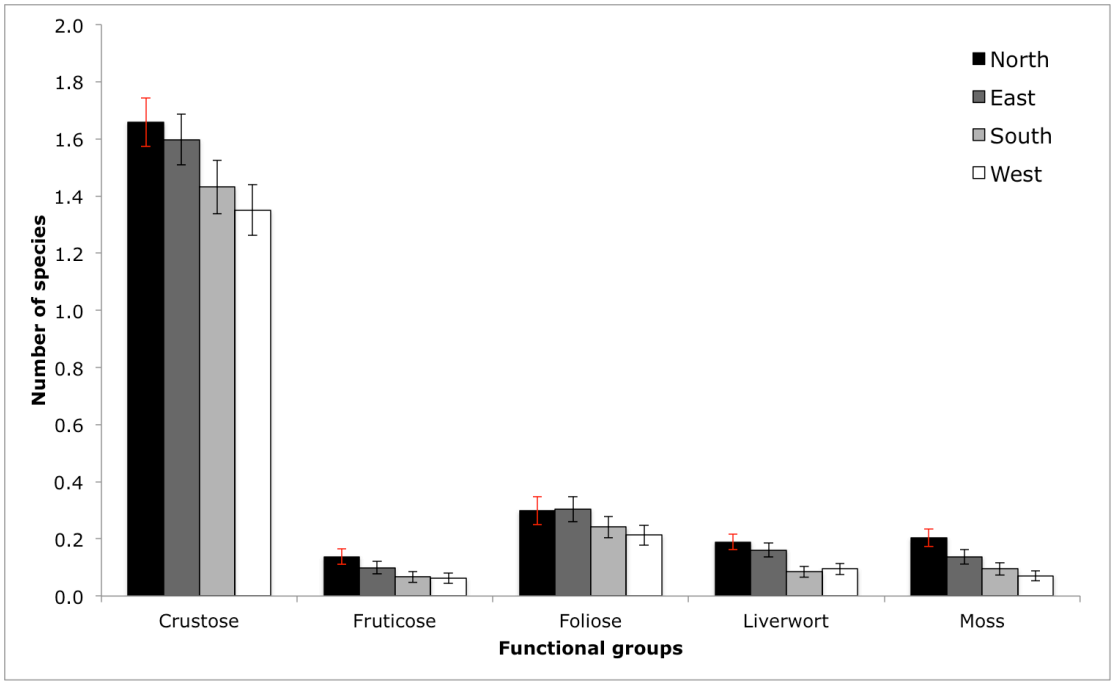

Figure 4.34: Mean number of species per aspect (with SE) for each functional group

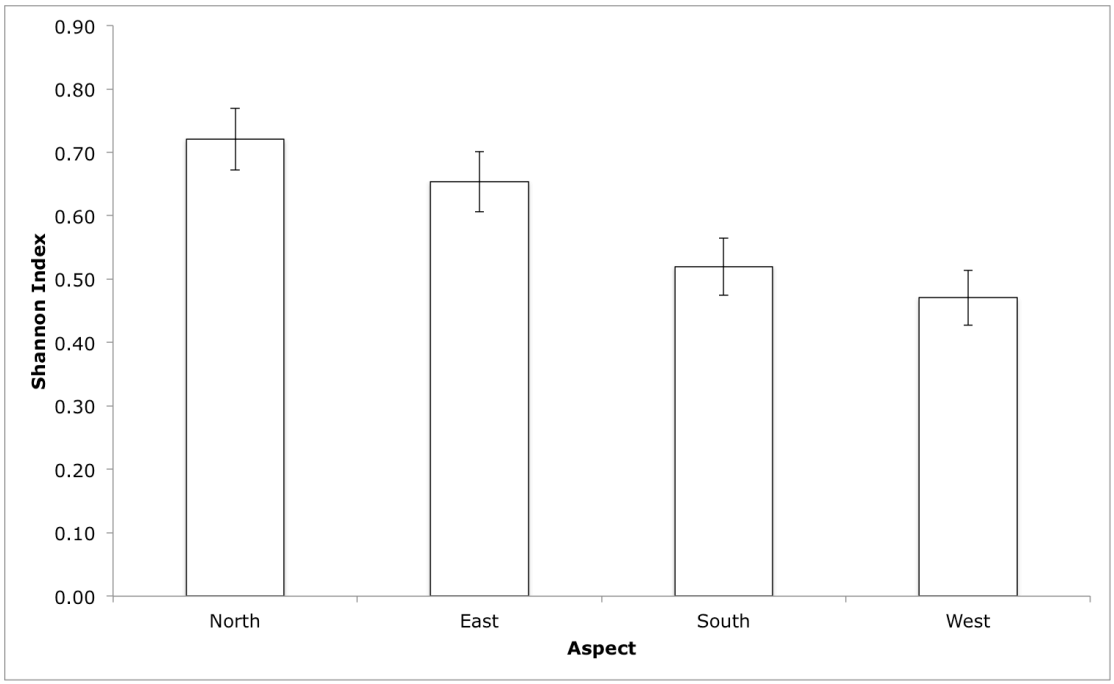

Figure 4.35: Shannon Index of species per aspect (with SE)

Table 4.11: Results of ANOVA testing for effects of clones, sites, blocks and interactions on various parameters of epiphyte community richness and functional groups on experimental aspen clones. *** p <0.001; ** p <0.01; * p <0.05; ns not significant

Source of Variation	df	MS % cover	MS Total species	MS Crustose lichens	MS Fruticose lichens	MS Foliose lichens	MS Liverworts	MS Mosses	MS Shannon Index
Among Clones	26	9265.37	23.334	11.884	0.370	1.464	0.305	0.345	2.924
		***	***	***	***	***	***	***	***
Among Sites	1	1.03	8.551	28.630	6.920	24.267	17.502	15.548	1.729
		ns	.	***	**	***	***	***	*
Among Blocks	4	3094.18	21.501	5.311	0.419	4.918	0.865	0.685	2.769
		**	***	**	***	***	***	***	***
Among Aspects	3	24923.72	22.190	4.311	0.260	0.409	0.543	0.715	2.708
		***	***	*	*	ns	***	***	***
Blocks within Site	7	5059.06	13.424	3.217	0.472	4.180	0.763	0.660	1.923
		***	**	*	**	***	***	***	***
Clone x Site	26	6454.60	13.884	5.703	0.279	1.254	0.399	0.402	1.556
		***	***	***	***	***	***	***	***
Error	702	894.64	2.548	1.207	0.087	0.244	0.072	0.083	0.330

4.6 RDA of epiphyte community structure

The epiphyte community dataset was analysed using DCA. Gradient length for DCA axis one is used as a standard bench mark to select contrasting linear response or unimodal response tools, with gradient lengths < 3-4 indicating low species turnover along the first axis, consistent with a linear response. The observed gradient length in this study was < 3 (2.786), indicating that RDA was an appropriate tool for constrained ordination with only environmental variables. Results of RDA indicate that 12% of the overall variation in epiphyte community structure is attributable to effects of 'Clone' (equivalent to the broad sense heritability for this trait), or 50% of the variation relative to other measured effects of 'Site', 'Aspect' and 'Block' (Table 4.12). Of the remaining variation, Site and Block accounted for 5% overall variation (or 21% relative to other measured effects); the effect of 'Block' is perfectly nested within 'Site' and may be considered a redundant variable. A scatterplot of the RDA on epiphyte community data testing the unique effect of clone and other effects as co-variables, show discrete clusters grouped according to clone (Figures 4.36 & 4.37).

Table 4.12: Sum of canonical eigenvalues from RDA showing the % total variation in epiphyte community structure attributable to each effect with other effects as co-variables, and % relative variation.

	% total variation	% relative variation
Clone	12	50
Site	5	21
Aspect	2	8
Block	5	21
Total	24	100

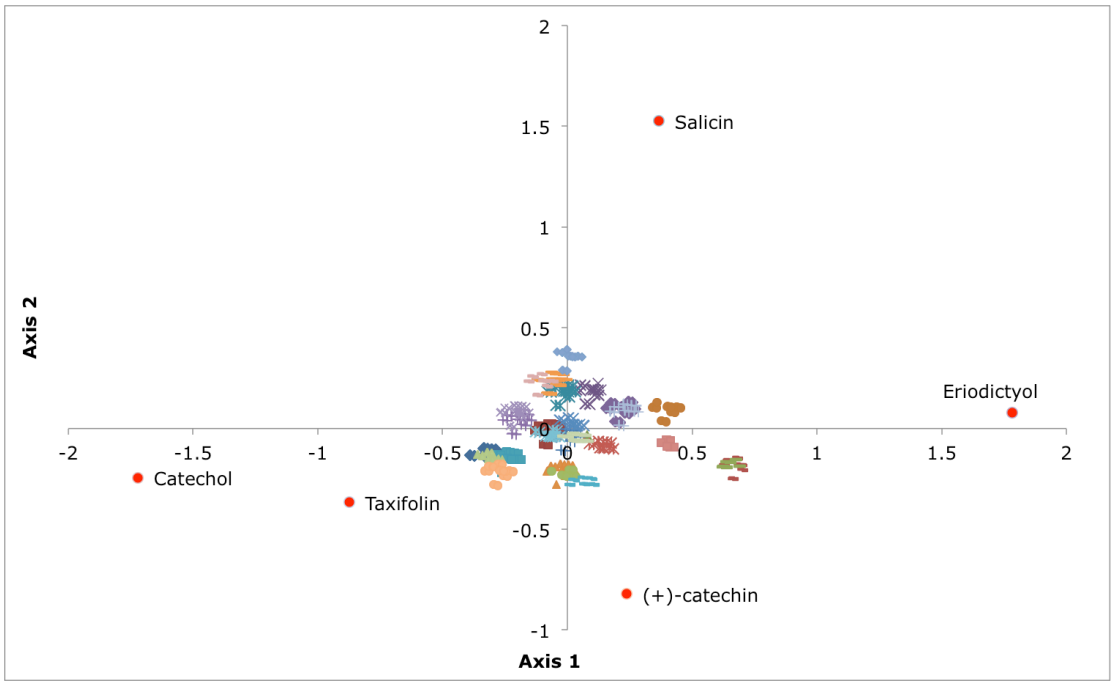

Figure 4.36: RDA of epiphyte community data. Each point represents a quadrat (5cm x 25cm) from which epiphyte count data was collected. Clones are colour-coded. Eigenvalues were 0.055 (axis 1) and 0.026 (axis 2). Phenolic compounds are highlighted with a red point and are 'passive' co-variables.

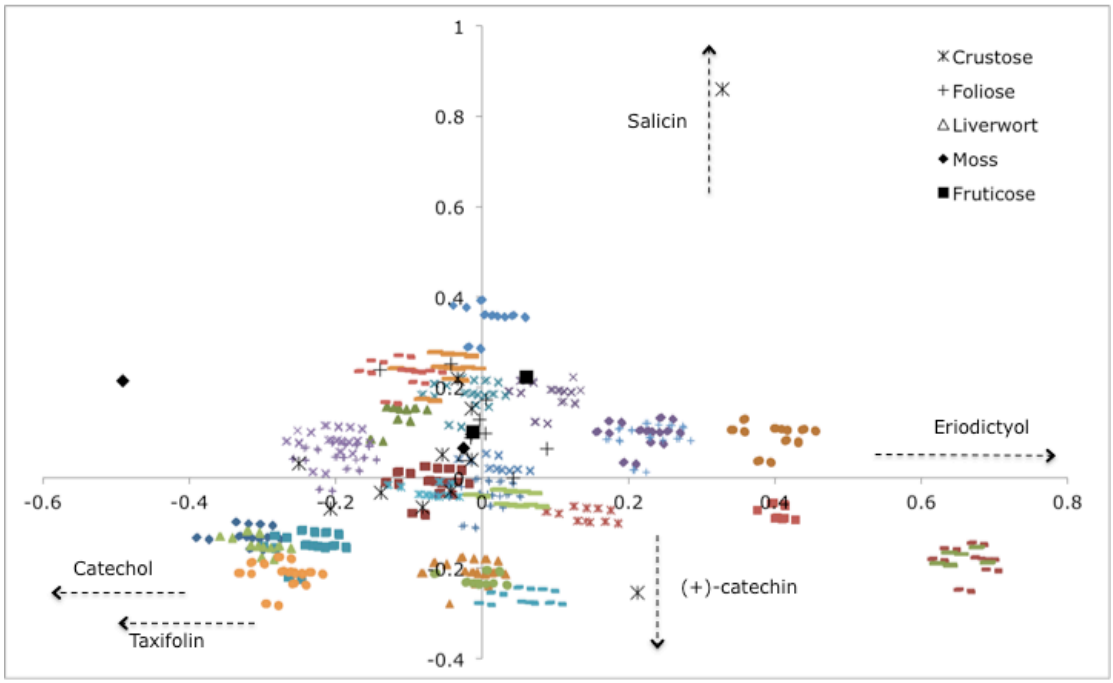

Figure 4.37: RDA of epiphyte community data as per previous figure (4.36), but at a finer scale to highlight the distribution of each functional group in ordination space. Each point represents a quadrat (5cm x 25cm) from which epiphyte count data was collected. Clones are colour-coded. Eigenvalues were 0.055 (axis 1) and 0.026 (axis 2). Direction of arrows indicates approximate location of each phenolic compound in ordination space.

4.6.1 Linking epiphyte community characteristics and clonal attributes

A simple regression analysis was conducted of RDA axis 1 scores for epiphyte community as response to the concentrations of each of the five phenolic compounds, and the scores extracted from the PCA carried out on phenolic chemistry (section 4.4.8). No significant effects of phenolic chemistry were revealed for the epiphyte community structure (Tables 4.13 and 4.14).

Species richness, mean % cover and Shannon diversity were each modelled as a response to PCA axis scores 1 and 2 (from phenolic chemistry analysis), and as a response to mean concentration of each compound, but no significant effects were found (Tables 4.15 and 4.16).

Table 4.13: Results of linear regression for axis 1 scores from RDA, with 187 degrees of freedom. Separation of epiphyte community along axis 1 were tested as a response against each of the five phenolic compounds. r^2 values are shown, with significance: *** p <0.001; ** p <0.01; * p <0.05; ns not significant

	Salicin	(+)-catechin	Eriodictyol	Taxifolin	Catechol	dbh
RDA axis 1	0.03ns	0.00ns	0.00ns	0.04ns	0.01ns	0.00ns

Table 4.14: Results of linear regression for axis 1 scores from RDA against PCA scores for axis 1 and 2 from the analysis of phenolic compounds. R^2 values are shown, with significance: *** p <0.001; ** p <0.01; * p <0.05; ns not significant

	PC1	PC2
RDA axis 1	0.01ns	0.02ns

Table 4.15: Results of linear regression for species richness, mean % cover and Shannon diversity modelled against PCA scores for axis 1 and 2 from the analysis of phenolic compounds. R^2 values are shown, with significance: *** p <0.001; ** p <0.01; * p <0.05; ns not significant

	PC1	PC2
Richness	0.03ns	0.00ns
Cover	0.04ns	0.00ns
Shannon	0.03ns	0.03ns

Table 4.16: Results of linear regression for species richness, mean % cover and Shannon diversity modelled against each phenolic compound. R^2 values are shown, with significance: *** p <0.001; ** p <0.01; * p <0.05; ns not significant

	Salicin	(+)-catechin	Eriodictyol	Taxifolin	Catechol
Richness	0.01ns	0.00ns	0.09ns	0.00ns	0.00ns
Cover	0.01ns	0.00ns	0.07ns	0.02ns	0.00ns
Shannon	0.01ns	0.04ns	0.07ns	0.00ns	0.00ns

4.6.2 GLMM and variance components of epiphyte community composition

A mixed model was applied to scores for each clone extracted from the RDA of epiphyte community (axis 1) to test amount of variation attributable to effects of clone, provenance, site and block. Clone was highly significant for shaping epiphyte community (illustrated in Figures 4.36 and 4.37), accounting for an estimated 98% of the total variation observed (Table 4.17).

Table 4.17: Variance components of GLMM on RDA scores for axis 1, showing % variation attributable to each effect of clone, site, block and provenance

	Clone	Site	Block	Provenance	Residual
RDA axis 1	98	0	1	0	1

4.7 Discussion

Genetic variation for ecologically important characters likely to influence epiphyte communities is very large among aspen clones within Scotland. Clones from within Scotland vary very significantly in growth rate, with little effect of site differences (i.e. environment). Of the total variation in growth rate (i.e. dbh), 44-50% was accounted for by clonal differences in the experimental trial. Clones from within Scotland also differ very significantly for their secondary bark chemistry. On average 15-51% of variation in bark chemistry is accounted for by differences between clones in the experimental trial. These characters are significantly affected by site, but response to site is not consistent across clones.

Apart from their physical and chemical attributes, clones in the provenance trial differ very significantly for the communities of epiphytes that they support. These communities differ in % cover of epiphytes, species richness, both for total and for particular life forms, and in species diversity. Clonal effects for the first component of the RDA, a measure of the epiphyte community composition, are significant. For this 'extended phenotype' character, clone accounts for 12% of total variation compared with only 5% accounted for by large scale (site) and small scale (block) environmental variation and 2% of variation accounted for by aspect. Thus clone identity is likely to be a significant determinant of epiphyte community composition in the field, and is therefore highly likely to be detectable despite other environmental effects (Tack et al., 2010).

4.7.1 Epiphyte community composition not attributable to measured physical traits

Despite finding large differences in RDA1 score for epiphyte community composition among clones, we were not able to account for any of this variation either by differences in dbh or secondary bark chemistry of clones. One possible reason for this is that the phenolic compounds important in determining epiphyte community composition were not effectively assayed by the HPLC analysis that was conducted. Many other compounds were eluted at lower concentration that those

reported here on the chromatographic analysis but could not be identified from their spectra. It is possible that these compounds contained a sugar moiety or some other such structure that made their identification impossible. Without being able to identify and quantify these compounds it cannot be said that phenolic chemistry has no role to play in shaping epiphyte communities on aspen.

A second possible reason for a lack of relationship between phenolic chemistry and lichen community composition is that lichens may themselves metabolise or detoxify the phenolics by producing secondary metabolites that can counteract effects of host plant defensive chemistry, though this is speculative. Nevertheless, epiphytes produce enzymes that can alter their substrate to facilitate their own survival (Hyvärinen et al. 2000). It would be prudent to investigate relationships between each phenolic compound and epiphyte species as specific responses may be diluted to insignificance by general community effects.

Recent research by Lamit et al. (2011) has used a similar clonal trial approach to investigate variation in the abundance of epiphytic lichens on different genotypes of a *Populus* species (*P. angustifolia*), and to investigate whether this could be accounted for by physical and chemical properties of the bark. Their research differed from the study reported here in that only a single species of crustose lichen, *Xanthomendoza galericulata*, was monitored, and chemical analysis was confined to analysis of total condensed tannins. In addition, although the *P. angustifolia* ramets were the same age as the *P. tremula* ramets used in our experiment (16 years), a diversity of smooth and rough bark types had already developed.

Lamit et al. (2011) found similarly large genetically determined clonal variation for growth (bole basal circumference – 25%), secondary chemicals (total condensed tannins – 31%) and cover of single lichen species (32%) as detected in our study. Their results are complementary to those reported here in that they also found no effect of bark phenolics, in their case measured as concentration of condensed tannins on abundance of the epiphytic lichen. However, the abundance of lichen was influenced by bark roughness, the larger diameter trees having both more rough bark and more of the lichen. These results parallel those found in chapter 3 where percentage of smooth bark explained differences in epiphyte community composition

among five *P. tremula* clones in a natural population. Major differences between our own research and that of Lamit et al. (2011) is that we attempted to identify and quantify the full range of abundant individual lower molecular weight non-tannin phenolic compounds present in aspen bark, and to record all epiphyte species present. Thus the response of the entire epiphyte community present on the Scottish aspen clones was tested in our study with a more detailed sampling strategy.

Abundant evidence exists of intra-specific variation in tree species affecting other communities of dependent organisms. Barbour et al. (2009b) examined potential genetic effects on the relative thickness of living bark and characteristics of decorticating bark in common garden experiments of *Eucalyptus globulus*. Twenty trees each from five genotypes were selected at random from a common garden trial established in northern Tasmania. Trees were sampled at 17 years old, and 25m in height. Of the ten bark traits measured, significant variation was found in eight of the traits and attributed to genotype (ranging from p <0.001-0.02). Overall, decorticating bark decreased with height up the trunk. Sampling macro-arthropod communities living within the bark revealed a significant effect of host tree genotype on species richness and abundance, with 60% of the overall variation in community attributable to differences in bark structure associated with *Eucalyptus* genotype.

Experimental studies have found strong direct effects of genetic variation in foliar leaf chemistry between hybridizing tree species and their parents, and arthropod community composition (Hemming & Lindroth, 1995 & 1999; Osier & Lindroth, 2001 & 2004; Bailey et al. 2006; Wimp et al. 2007). These effects are often reproducible in wild systems (Bangert et al. 2006a & 2006b), but may be dependent on spatial scale (Huston, 1999) and degree of environmental variation, often with a limited genetic effect (Maddox & Cappuccino, 1986; Tack et al. 2010). Many experimental studies on arthropods and host plant chemistry isolate the insect herbivore from all other food sources, which is likely to have an impact on development and maturity if the herbivore is not a specialist feeder on that particular plant. Segregating an organism from its environment creates stress that can also hinder maturation and exacerbate potential effects of host plant chemistry. These effects may be diluted in the wild due to natural environmental variation, with

particularly stressful environments overriding host plant genetic effects (Maddox & Cappuccino, 1986). Allocation and partitioning of defence compounds to leaves may work more swiftly than bark tissue as they are the primary photosynthetic organs and will require a greater level of protection. It is also possible that rhizines of lichenized fungi may be able to penetrate outer layers of non-conducting periderm tissue without disrupting the suberized Casparian strip beneath, thus cellular damage, and therefore the triggering of defence mechanisms, is avoided.

4.7.2 Implications for conservation

Evolution by natural selection requires genetically based intra-specific variation of significant physical traits and here it has been shown that epiphyte communities vary between different genotypes of *Populus tremula*. Thus epiphyte community composition can be seen as a variable and genetically determined trait of aspen, which has consequences for epiphyte populations if the frequency of aspen genotypes is altered within populations. The clonal composition of aspen in woodlands will have important effects on epiphyte communities, specifically on the proportion of functional types, species diversity and species richness. Restricting or reducing clonal diversity will reduce these aspects of epiphyte diversity. Currently, challenges to increasing aspen clone diversity exist in obtaining diverse planting stock due to the paucity of flowering events and lack of seed; propagation from root cuttings is the optimal method, yet laborious and the results are slow and unpredictable. Even so it is imperative to include as much diversity of aspen genotypes as possible to ensure diversity in associated epiphyte communities.

As yet there have been no studies addressing the question of *Populus* genetic variation and extended consequences for epiphyte evolution. Some of the lichen epiphyte species, such as *Xanthoria parietina*, are found in abundance on other tree species and substrates (e.g. rocks), whereas *Lecanora populicola* favours aspen as a niche habitat. Certain other rare species, such as *Caloplaca flavorubescens* tend to be found in woodlands containing aspen as a primary component (C. Ellis, pers. comm.). Other *Populus* species are also regularly colonised by unique and rare lichens not commonly found on neighbouring trees of other species (Uliczka &

Angelstam, 1999; Hedenås & Ericson 2000). It may not be true of all lichen species that selective pressures on physical traits of aspen will have extended consequences, though the possibility exists for rare, endangered or specialist species.

Here we have demonstrated the profound physical and chemical variation that exists between twenty-seven native Scottish aspen clones. Profound variation in epiphyte community structure also exists between these same clones, but with no significant link between the observed variation in tree growth and bark phenolic chemistry and epiphyte community diversity. However there appears to be an extended phenotype affect of aspen and its epiphytes and great potential exists for native aspen clones to provide a significant contribution to enhancing Scotland's ecological integrity and supporting ecosystem processes. This study demonstrates the necessity of increasing genetic diversity of aspen across the landscape because of the important role they play in enhancing epiphyte flora.

5 Genetic and environmental influences on the epiphyte extended phenotype of aspen: a synthesis

5.1 Introduction

The phenotype of an individual is determined by its genetic constitution and/ or the environment in which it lives, therefore it is important to know the relative contribution of these effects when planning to manipulate or manage the species in any way, for example for agricultural or conservation purposes. These factors not only determine the phenotype of the individual but this in turn can become a driver of its interactions with other individuals of the same or other species (Whitham et al. 2003; Wolf et al. 2011). In the case of foundation species on which other organisms depend, the extended phenotype of an individual can determine a range of associated communities and assemblages (Whitham et al 2006). When studying 'community genetics' the relative importance of the environmentally and genetically determined traits of the foundation species on associated communities might be studied via calculation of the coefficient of determination (r^2) (Hersch-Green et al. 2011). The r^2 value can be obtained by applying restricted maximum likelihood methods (such as those implemented in Generalized Linear Mixed Models) to measure the variance components, specifying each of the physical traits and ecological variables as random factors. This method would indicate the relative contribution of measured factors to response variables that could consist of community measurements such as standard diversity and species richness indices. These basic methods suggested by Hersch-Green et al. (2011) have been employed in a similar fashion to the research described here, to address the genotypic and environmental determination of the very species-rich epiphyte communities growing on aspen in Scotland. They were applied to both an inductive field-sampling approach using five naturally occurring aspen genotypes and a common environment (or 'common garden') experimental manipulation, in which plants of known genetic and environmental origin were transferred into contrasting characterized environments, and the responses of their epiphyte communities measured.

5.1.1 Comparing the two aspen systems: similarities and differences

No previous studies have investigated the effects of intra-specific variation of a foundation tree species on its epiphyte communities at the local, site, and landscape scales. The most significant finding of the research presented here is that epiphyte communities vary significantly according to aspen genotype, even overriding the effects of spatial location and environmental variation. Assessment of epiphyte communities growing on five mature (approximately 60-80 years old) wild clones from the RSPB Insh Marshes Nature Reserve (Invertromie) indicate that approximately 35% of the variation is explained by genotype effects. In a controlled, common garden experiment, containing twenty-seven aspen clones propagated from natural stands collected from various locations throughout Scotland and planted at two very different sites, approximately 12% of the total variation in epiphyte community is explained by aspen genotype. Important parallels exist between the results for the wild and experimental clones studied: the species richness and diversity of all lichen functional types (crustose, fruticose and foliose) and liverworts, varied significantly. Crustose lichens contributed the greatest species richness and diversity to both systems. However, no significant clone effects were found for mosses at Invertromie, but were found for the experimental clones. This may be related to environmental differences between the two sites of the common garden experiment at Moray and Kilmichael; the latter site generally receives more rainfall, is frequently waterlogged and is in close proximity to the River Add, which will enhance conditions important for rapid colonization of mosses.

Although the two studies reinforce one another, nonetheless some differences exist. The genotype of the clones in the experimental trial explained less variation in epiphyte community than in the wild system. This was unexpected firstly because we anticipated greater genetic variation among the clones in the experimental trial than among the clones at Invertromie. Five ramets from each of five clones were sampled from the wild population, across an area of approximately 200m^2, whereas from the experimental aspen twenty-seven clones were sampled from across Scotland. Thus the amount of genetic variation among aspen should have been higher in the experimental populations than at Invertromie. Moreover in the common garden trial

we expected the genetic effects to be greater than the environmental effects because environmental variation is minimised by the experimental design. Thus genetic effects should be more apparent in the trial than in the natural population at Invertromie.

Two observations may help to explain the unexpected results described. Firstly, it was not possible to control for environmental effects at Invertromie, and clones may have occupied quite different micro-environments. For example, the area in which BD11 was growing was more exposed than the other clones on the same site, and experienced very strong winds during sampling of epiphyte communities. Therefore the differences seen in epiphytic communities between clones at Invertromie may be attributable both to environmental as well as to genetic effects. Thus environmental variation could actually enhance the apparent differences among clones in epiphyte communities at Invertromie. Secondly the trees at Invertromie were 40-70 years older than the experimental clones, and would have had more time to be colonized by a greater variety of epiphytes, enhancing the opportunity for differences in community composition to be seen. Finally in the older trees at Invertromie a greater diversity of bark types would have had the chance to develop, giving greater opportunity for epiphyte communities to differentiate. There is thus more potential for clonal differences to translate into differences in epiphyte communities at Invertromie than in the experimental plots where bark texture differences between clones have yet to develop.

Significant effects of aspect were found on wild clones for species richness and diversity, and all functional groups except fruticose lichens. The amount of variation attributable to aspect was 19%. For experimental clones there were significant aspect effects for species richness and diversity and all functional groups except foliose lichens. The amount of variation attributable to aspect was 2% (relative to other effects of clone, block and site). As the Invertromie site is natural, the ramets grow randomly and will be subject to differences in light levels, particularly across the eastern, southern and western aspects. Aspect effects at the experimental sites are likely to be weaker primarily due to the standard spacing between clones creating more uniform shading of the aspen trunks. Any effects of aspect are likely to occur

where replicates have died, where certain clones are not as vigorous as others and are still quite comparatively small, or on the periphery of the sites where light levels are generally higher. This could also be attributed to the effects of growth over time.

Effects of height were quantified on wild clones by sampling communities in quadrats placed 40cm apart from ground level to 200cm. Height accounted for 19% of the overall variation in epiphyte community with significant differences between all functional groups (except mosses), species richness and diversity. An increase in height up the trunk has been positively associated with an increase in smooth bark, and therefore a greater diversity of bark textures for epiphyte communities. The lack of significant height effects for mosses may be accounted for by the distribution of *Orthotrichum* sp., a common moss species recorded across all heights. Sampling effects of height on the experimental aspen clones was not feasible due to there being lots of branches making placement of quadrats difficult, and the gradual thinning of the stems with height.

5.1.2 Variation in aspen physical traits and effects on variation in epiphyte community composition

From the Invertromie clones it was found that bark texture was under genetic control (up to 40%) and explained 21% of the variation in epiphyte community across all five clones, and 10% variation for intermingled clones. Bark texture was significantly related to epiphyte richness even when controlling for spatial location of individual ramets. The proportion of smooth bark relative to rough bark increased with height and aspect (south and west: 40-45%; north and east: 7.5-10%). Both the effects of aspect and height could be indirect effects of differences in bark smoothness, which was significantly different at each aspect/ height measured. It was not possible to measure bark roughness on the experimental clones as they had not developed physical variation in this trait and were all classified as ‘smooth’.

Tree girth was not significantly different between the ramets of the Invertromie aspen clones, but varied significantly between the experimental clones; approximately 44% of the variation in dbh was attributable to genotype. However this trait did not account for any of the observed variation in epiphyte community.

Previous studies have found variation in this trait to have significant effects, though the absolute size differences between the trees sampled was much greater (Riiali et al. 2001; Benson & Coxson, 2002; Friedel et al. 2006).

Finally, significant differences were detected in phenolic chemistry among the genotypes within both the wild and the experimental clones. Bark samples were collected from wild clones from within all the different sample quadrat on each individual ramet, whereas a single bark sample was collected from the experimental trees' southern aspect, just above the epiphyte sampling quadrat. Additional bark samples could not be collected from the other three aspects because the trees were small enough to risk being seriously damaged by the removal of bark. Three major compounds were quantified from the bark samples of the natural aspen stands at Invertromie: eriodictyol, taxifolin and p-coumaric acid. Five major compounds were quantified from the experimental clones: salicin, (+)-catechin, eriodictyol, taxifolin and catechol. Mean concentrations of eriodictyol were much higher in the Invertromie aspen (range 3-8.5µg/ mg) than the experimental aspen (<3.75µg/ mg).

A number of processes may account for the differences in bark phenolic chemistry between the two studies. Compounds allocated to active periderm stem cells that then become thickened may remain 'trapped' within non-conducting tissues and over time accumulate with additional thickening. Many compounds found in younger trees may degrade or diminish with age, and this may account for differences in the number and concentrations of compounds quantified in the Invertromie and clonal trial studies. Site had a significant effect on the mean concentrations of salicin, (+)-catechin, eriodictyol and taxifolin at the two sites in the experimental aspen. Thus differences in site could account for some of the chemical variation found between Invertromie and the clonal trials. Despite the significant variation between clones in both the wild and experimental systems, phenolic chemistry extracted from the bark was not related to epiphyte community structure.

5.2 Limitations of alternative approaches to the study of genetic versus environmental variation

Many studies have been conducted to investigate genetic variation in plant physical traits, biodiversity and community structure (Pregitzer et al. 1990; Whitham et al. 1999, 2003, 2006; Rytter & Stener, 2003; Novaes et al. 2009). Most frequently employed to elucidate these effects are common garden experiments using F_1 hybrids and backcrosses to parental species. These experimental systems have demonstrated highly significant correlations between variable physical and chemical traits under significant genetic control in a structural, or foundation species, and a dependent species or community (Whitham et al. 1999, 2003, 2006; Donaldson & Lindroth, 2007; Tack et al. 2010). Research presented by Whitham et al. (1999, 2003, 2006) positively affirms the influence of plant genetic variation for phenolic chemistry (including condensed tannins) on associated arthropod communities. Similar effects of plant genetic variation on phenolic chemistry and interactions with soil function and fungal epiphytes have also been observed (Bailey et al. 2005; Schweitzer et al. 2008).

These results have been used to promote the use of hybrid systems to assess the evolutionary consequences of genotypic variation at the community and ecosystem level.

One issue with focusing solely on hybrids in community genetics studies is that F_1 and backcrossed progeny will display a large range of variation, which will reflect segregation for differences found between parent species, not variation within the parent species. Thus these experiments do not test the effects of genetic variation *within* species on communities of dependent organisms. Rather they test the effects of genetic differences *between* species, by using the propensity of those species to hybridise. In addition, any differences between the parental species will tend to be inherited together in the hybrids and backcrosses. This means that differences in community structure between hybrids cannot be attributed to any single attribute by which they differ, e.g., chemistry, because any chemical differences will be highly correlated with a range of other physical/ physiological characteristics found to differ

between hybridizing species. Thus the trials that use hybrids to demonstrate a link between secondary chemistry and insect communities do not prove that it is the chemical differences between clones that cause differences in insect communities. Rather, these differences could be due to any one of a number of other correlated differences between the hybrid clones that were included in the trial.

A characteristic of common garden experiments is that they are designed to eliminate spatial and environmental effects, thus genetic effects will be inflated relative to their importance in natural populations. Hybrid vigour may also exaggerate physical traits important for shaping dependent species; therefore it is important to investigate natural variation within a species, both in an experimental trial and a wild environment to test whether the effects seen for hybrids in experimental trials are reproducible in non-hybrids and under conditions where environmental variation is much higher than in controlled trials.

Clonal trials carried out at single sites and using genotypes from restricted areas also fail to thoroughly deal with genetic effects expressed at varying spatial scales. Increasing the distances at which the effects of genetic variation in foundation species are studied, from common garden, to local, site and landscape level, can show a significant decrease in host genetic effects on associated communities (Maddox & Cappuccino, 1986; Huston 1999; Tack et al. 2010). In certain cases, genetic effects on dependent communities can be reduced by environmental variation, as seen in the secondary effects of block and site on epiphyte communities in the experimental aspen trial described in chapter 4, each contributing 5% to the overall observed variation in epiphyte community.

5.2.1 The relationship between trees and their epiphytes and its consequences for the aspen extended phenotype

Whilst phenolic chemistry was not found to be important for structuring epiphyte communities on wild or experimental aspen clones, bark texture was not only under significant genetic control, but also important for structuring the epiphyte flora. In the natural system of aspen clones at Invertromie, 40% of the variation in bark texture was attributable to clone (log-likelihood ratio test: χ^2_6=18.761, p <0.001).

These results are comparable to findings of Lamit et al. (2011), who quantified differences in bark texture between 25 different genotypes (replicated two to nine times) of *Populus angustifolia* grown in a common garden environment at the Ogden Nature Center in Ogden, Utah, USA. Trees were grown from cuttings collected from wild riparian stands close to the site and cultivated in randomized blocks. Bark texture was classified as 'rough' or 'smooth' and recorded from vertical quadrats, 10cm by 1m tall, placed at ground level on the north side of each tree. Broad-sense heritability (H^2) estimates indicated that 35% of the variance in rough bark was explained by genotype. Sampling of the Invertromie study incorporated bark texture up to a height of 2m, whereas the study by Lamit et al. (2011) only reached a height of 1m; smooth bark tends to increase vertically in other tree species (Hamilton et al. 2007; Barbour et al. 2009a), with the thickest, roughest bark usually found nearer ground-level such as was found by their study. The strongest gradient for explaining separation of epiphyte communities on the Invertromie aspen is bark texture (NMDS: $r^2 = 0.218$ $p < 0.001$). Certain lichens are strongly allied with smooth bark, a finding confirmed by studies of other tree species harbouring a diverse epiphyte flora (Moxham, 1981; Morley & Gibson, 2010; Armstrong & Bradwell, 2010, 2011).

Ellis (2008) surveyed stands of Scottish aspen (*Populus tremula*) in Strathspey to determine the composition and diversity of epiphyte communities with the purpose of assessing aspen's role in supporting and maintaining lichen species. It was suggested that changes in bark texture of aspen might have an influence on the changes in epiphyte community composition as trees age, and this appears to be borne out by the results reported here. This phenomenon has also been recorded on *Fagus sylvatica* (Fritz et al. 2009). Sexually reproductive crustose lichens (e.g. *Lecania naegelii*, *Lecanora populicola*, *Lecidella elaeochroma*) tend to be pioneer species and early colonisers of smooth aspen bark, gradually being taken over by asexual crustose lichens, foliose lichens and bryophytes. Species interactions could also account for changes over time (Kiss, 1982).

Questions remain as to why epiphyte communities varied between aspen clones in the Moray/ Kilmichael experimental trial, in the absence differentiation of rough and smooth bark. The most obvious place to begin investigation is with the physical

properties of the bark itself, as this is in direct contact with colonizing epiphytes. Casual field and lab observations showed differences between clones in the thickness and consistency of the bark, with potential site effects. When removing bark for chemical analysis it was noticed that some clones possessed a very thin, green periderm, whilst others were thicker, more fibrous and with a whitish bloom. This bloom has been attributed to periderm cells that are being actively sloughed off, possibly as a defensive measure (Covington, 1975). Although tree girth and hence rate of growth did show large genetic variation it did not account for any variation in epiphyte community. However it may be possible that certain clones produce newer periderm cells much faster than others, reducing the potential for colonization by algal and fungal cells. Thalli of *Xanthoria parietina* appeared to rapidly establish on Moray clones with very smooth, green bark, but some colonies had fallen away to reveal pale, fissuring bark underneath. Perhaps initial colonization events of epiphytes on different clones create a cascade effect of bark thickening that facilitates the establishment of certain lichen species. Hypotheses for how bark roughness is initiated have been proposed but not investigated sufficiently to draw firm conclusions. The extent of any protective or defensive traits manifested by a tree will be determined genetically, i.e., the degree of susceptibility to pathogenic invasion and the rate and depth of cell division in the cortical layers, will vary according to the specific genotype of that species. Whether these defensive traits are initiated or not will depend on the individual's degree of exposure to 'attack', and the availability of resources in the environment to supply the tree with sufficient energy to produce extra cellular layers in the periderm.

5.2.2 Relationships between epiphytes and their hosts

In order to explain why different communities of epiphytes are found in the absence of differences in bark texture we should also consider the assumed benign relationship between trees and epiphytes. Different lichen species are capable of producing secondary metabolites that have been consistently useful for taxonomic identification (Bennett 2008) and are implicated in stress-survival, defence against high levels of UV, competition from other epiphytes and pollution (Rao & LeBlanc, 1965; BeGora & Fahselt, 2001; Edwards et al. 2003a; Edwards et al. 2003b; Favero-

Longo & Piervittori, 2010) and competition with other organisms (Fahselt 1994). Three probable pathways for the production of lichen metabolic compounds have been identified: shikimic acid pathway (polysaccharides and pulvinic acid); mevalonic acid pathway (terpenes and carotenoids); acetyl-polymalonyl pathway (anthraquinones, usnic acid and orsellinic acid) (Edwards et al. 2003b). Lichen acids such as evernic acid, usnic acid, barbatic acid, atranorin and chloroatranorin have been found to have deleterious effects on chloroplasts when epiphytic lichens are growing on the leaves, branches, or trunks of vascular plants (Ascaso et al. 1980; Ascaso et al. 1983; Ascaso & Rapsch, 1985; Inoue et al. 1987; Legaz et al. 1988; Bouaid & Vicente, 1998; Legaz et al. 2004), and have been demonstrated to inhibit foliar bud formation (Legaz et al. 1988, Legaz et al. 2004); induce leaf abscission (Bouaid & Vicente, 1998); accelerate degradation of free auxin and reduce chloroplast manganese responsible for oxygen production via the Hill Reaction in photosynthesis (Legaz et al. 2004). These compounds also have the ability to increase the pH of thallus tissue and facilitate protection against pathogens (Hauck & Jürgens, 2008). Lichens are in direct contact with bark via their rhizines, and can penetrate periderm tissues causing cellular damage. As the host plant responds via the formation of wound tissue, thickened outer periderm and the production of secondary metabolites, the lichens may produce metabolites of their own as a counter measure, or possibly serve as a defensive function against pathogens in the form of a physical barrier to their ingress. Lichenized fungi are also capable of producing a gelatinous compound around their tissues that further protects against 'attack'. Lichen acids create a crystalline layer, combined with chitin and polysaccharides to create a defensive matrix around the algal layer, something algal species alone cannot do (Honegger & Bartnicki-Garcia, 1991). These considerations indicate that there is ample opportunity for lichens to inflict damage on the trees that they colonise through production and release of toxic chemicals, and for the trees to mount defences against this.

As well as inflicting damage on trees, lichens may also interfere with valuable photosynthesis carried out in the tree bark, especially in species such as aspen. Solhaug et al. (1995) studied the effects of crustose lichen (i.e. *Lecanora*)

colonization on chlorophyll concentration and rate of photosynthesis in aspen (*Populus tremula*) bark. They reported 'bark without lichen cover had as much chlorophyll per unit area as leaves', with a significant negative relationship between concentrations of bark chlorophyll and *Lecanora* chlorophyll ($r = -0.452$, $p < 0.001$). Light transmission to bark chlorophyll was impeded by the presence of lichen thalli, but aspen chloroplasts and photosynthetic activity was not affected by lichen secondary metabolites. Inhibition of photosynthesis may be sufficient to trigger a defence reaction from a tree, laying down additional layers of cork cambium and production of secondary metabolites to free the 'infected' area. Lichens may also have developed various structural and chemical adaptations to combat, neutralize, or disable tree defences.

These studies suggest that the colonization of trees by lichens is likely to be damaging to the tree, although there is no evidence for this. It is also possible that even if lichens do not normally release secondary metabolites during establishment, environmental stresses, such as pollution, may induce a defence response from the lichen. This may result in the production of compounds from the lichen that are released onto the bark of the host and absorbed. Lichens are also capable of absorbing trace elements and pollutants from the atmosphere and retaining them in their thallus tissues from which they may exude and affect the trees on which they reside. Thus there are many good biological reasons why trees should defend themselves against lichen colonization, and if intra-specific variation in defences exist, then it is reasonable to expect that both overall efficacy of these defences will vary, as well as defences against particular lichen species. In these circumstances the associated epiphyte communities would be hypothesised to vary in cover and composition as a consequence of genetically determined variation in the defensive attributes of aspen genotypes. A possible avenue for further work is to investigate the defensive responses of aspen to artificial inoculation by lichens, and to determine whether there is substantial genetic variation in tree response that could account for observed differences in lichen community composition in young trees. Analogous to this system is the response of conifers to bark damage by boring insects and fungal pathogens, where the host, beetle and fungi have each developed a reciprocal

response and defence mechanism though tends to be a swifter process (Nebeker et al. 1993). As beetles bore though outer bark layers and into phloem, conifers release large quantities of resin rich in secondary metabolites around the area of damage. Beetles will systematically remove this resin until no more is left, which may take several days. Successful colonization of the host by beetle and associated fungus depends on the species attacked, and the inherent ability of the host to mount a defence. Epiphytes are probably not able to create such a fast response from a host and may persist for some time before being 'noticed'.

When Richard Dawkins introduced the extended phenotype concept, he described it as 'the effects of genes on the community and ecosystem' or 'the effects of genes at levels higher than the population' (Dawkins 1982; Whitham et al. 2003; Whitham 2005). The underlying principle is that a phenotype is the biological expression of a gene's attempt to ensure its survival into the next generation by modifying the environment it lives in. Resulting generations will inherit genes favoured by that environment. Applying this definition to the aspen-epiphyte relationships described here raises the question of whether the genetically determined modification of epiphyte communities by aspen results in benefits to the aspen or vice versa. Of course, both aspects could be true and this remains to be investigated.

5.3 Implications for conservation

In a recent report from the Forestry Commission (Harrison, 2009), aspen was highlighted as being particularly valuable for the environment and general biodiversity of woodlands. A prime recommendation was made for aspen to be a major component of British woodlands due to its associations with rare or specialist species. There also exists opportunity for aspen to be included in forestry rotations for biomass and fibre production. Unfortunately Scottish aspen does not flower and set seed reliably, and current efforts at vegetative propagation via root sections is unpredictable and its success varies according to genotype. Various groups have conducted research into methods of enhancing successful propagation from root cuttings (Hollingsworth & Mason, 1991; E. Emmet, pers. comm.). Some progress has been made investigating the efficacy of gibberellins and auxins on root and shoot

development on aspen cuttings obtained through micro-propagation techniques (Žiauka & Kuusiene, 2009) but there is still much work to be done to enable cost-effective production of the full range of aspen genotypes present in Scotland. Members of the Highland Aspen Group, based in Kingussie (Scotland), are making great progress to this end. Two large polytunnels have already been established, containing a large selection of native aspen propagated from root cuttings. Of equal import is the progress made by Scottish Native Woodlands to induce flowering in young aspen clones propagated from root cuttings. It is anticipated that these clones will be used to produce much-needed seed, and provide necessary information about the gender of each clone.

The importance and diversity of lichens on aspen, especially in Scotland, cannot be overstated. However there are serious issues to advancing the much-needed conservation strategies, such as: lack of funding for cryptogam-related research; lack of specific knowledge of epiphyte species and their ecology. Habitat conservation, or indirect conserving of appropriate foundation species like aspen, can conserve the large number of lichen species already identified in Scotland, especially those found only on aspen. This would provide a cost effective means of providing protection for rare and endangered species, whilst bolstering the status of the UK as a biodiverse region. The Scottish Government's target is to increase the cover of woodlands from 17% of land area to 25% of land area by 2050, and the SRPD grants scheme is one of the levers by which this is being implemented (Scotland's Climate Change Adaptation Framework, 2009). This is primarily for climate change reasons, but the requirement for a large proportion of this increased woodland to be of native species also provides the opportunity to increase biodiversity. The research presented here points to how these targets can be achieved to maximum effect with respect to lichens, a diverse and important component of Scotland's overall biodiversity.

This research has demonstrated that epiphyte communities vary between different genotypes of *Populus tremula*, both in a multi-site experimental system and a natural system. The most important conclusion for conservation is that clonal composition of aspen in woodlands has important effects on epiphyte communities, specifically the proportion of functional types, species diversity and species richness, all of which

generally increase with age. Variation in bark texture within and between clones is particularly important for maximizing species richness and encouraging rare or endangered species. Thus it is imperative that despite the problems with producing a genetically variable planting stock of aspen, this must be achieved to ensure long term conservation of the very diverse epiphytic lichen flora of aspen. Restricting or reducing clonal diversity will reduce diversity in associated lichen communities, many of which contain rare and nationally important taxa. Therefore it is imperative to include as many diverse aspen genotypes in planting stock for new native woodlands, or for restocking existing forests, to ensure diversity of the associated epiphyte communities into the coming centuries.

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Appendix A

Table 1: Model selection results for Model 1 of GLMM analysis testing fixed effect of 'Clone' against a null model. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that the model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model 1	Df	AIC	ΔAIC	*w*AIC
Clone	6	718.66	0.00	0.995
Null	2	729.42	10.76	0.005

Table 2: Model selection results for Model 2 of GLMM analysis testing random effects of 'Ramet', 'Aspect', and 'Height' using 'Clone' as a fixed effect (based on the results of Model 1). ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	*w*AIC
Ramet+Aspect+Height	9	666.32	0.00	0.914894
Ramet/Aspect+Height	9	671.07	4.75	0.085098
Ramet/Height+Aspect	9	689.73	23.41	7.55E-06
Ramet+Height	8	712.79	46.47	7.42E-11
Ramet/Aspect	8	718.87	52.55	3.55E-12
Ramet+Aspect	8	720.38	54.06	1.67E-12
Ramet	7	720.66	54.34	1.45E-12
Ramet/Aspect/Height	9	720.87	54.55	1.31E-12
Ramet/Height	8	722.38	56.06	6.14E-13

Table 3: Model selection results for Model 3 of GLMM analysis testing fixed effects of 'Aspect', and 'Height' using a null random effect. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	*w*AIC
Aspect+Height	8	725.78	0.00	0.6588
Height	5	728.91	3.13	0.1377
Null	2	729.42	3.64	0.1067
Aspect	5	729.62	3.84	0.0966
Aspect*Height	17	742.24	16.46	0.0002

Table 4: Model selection results for Model 3 of GLMM analysis testing fixed effects of 'Aspect', and 'Height' using 'Clone' and 'Ramet' as random effects. ΔAIC = AIC - min AIC; wAIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	wAIC
Clone/Ramet	10	653.57	0.00	0.5
Clone+Ramet	10	653.57	0.00	0.5
Clone	9	702.05	48.48	1.48E-11
Null	8	725.78	72.21	1.04E-16

Table 5: Model selection results for Model 1 of GLMM analysis testing fixed effects of 'Clone' against a null model. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	*w*AIC
Clone	6	578.69	0.00	1
Null	2	626.88	48.19	3.43E-11

Table 6: Model selection results for Model 1 of GLMM analysis testing fixed effects of 'Clone' against a null model. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	wAIC
Clone	6	405.15	0.00	1
Null	2	457.99	52.84	3.36E-12

Table 7: Model selection results for Model 1 of GLMM analysis testing fixed effects of 'Clone' against a null model. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	wAIC
Clone	7	622.79	0.00	0.9973
Null	3	634.61	11.82	0.0027

Table 8: Model selection results for Model 3 of GLMM analysis testing fixed effects of 'Aspect', and 'Height' using a null random effect. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	Df	AIC	ΔAIC	*w*AIC
Aspect	5	625.06	0.00	0.6589
Null	2	626.88	1.82	0.2652
Aspect+Height	9	630.02	4.96	0.0552
Height	6	631.98	6.92	0.0207
Aspect*Height	21	649.44	24.38	3.35E-06

Table 9: Model selection results for Model 3 of GLMM analysis testing random effects of 'Clone' and 'Ramet', using a fixed effect of 'Aspect'. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	**Df**	**AIC**	**ΔAIC**	**wAIC**
Clone	6	588.62	0.00	0.5205
Clone+Ramet	7	590.17	1.55	0.2398
Clone/Ramet	7	590.17	1.55	0.2398
Null	5	625.06	36.44	6.36E-09

Table 10: Model selection results for Model 3 of GLMM analysis testing random effects of 'Clone' and 'Ramet', using a randomly estimated fixed effect. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	**Df**	**AIC**	**ΔAIC**	***w*AIC**
Clone	3	420.34	0.00	0.5358
Clone+Ramet	4	421.85	1.51	0.2518
Clone/Ramet	7	422.19	1.85	0.2124
Null	2	457.99	37.65	3.58E-09

Table 11: Model selection results for Model 3 of GLMM analysis testing random effects of 'Clone' and 'Ramet', using a randomly estimated fixed effect. ΔAIC = AIC - min AIC; *w*AIC = weight of evidence that model with the lowest AIC value is the best approximating model, given the data and set of candidate models.

Model	**Df**	**AIC**	**ΔAIC**	***w*AIC**
Clone+Ramet	4	630.02	0.00	0.4760
Clone/Ramet	4	630.02	0.00	0.4760
Null	3	634.61	4.59	0.0480

Appendix B

Table 1: Mean (SE) frequency of occurrence for each species across each clone. Species are separated according to functional group (lichens: crustose, fruticose, foliose; mosses; and liverwort

	BD11	BD12	BD13	BD14	BD5
Crustose	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**
Arthonia didyma	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)
Arthonia radiata	0.01 (0.00)	0.02 (0.00)	0.05 (0.00)	0.00 (0.00)	0.00 (0.00)
Buellia disciformis	0.00 (0.00)	0.04 (0.00)	1.05 (0.04)	0.76 (0.04)	0.01 (0.00)
Buellia griseovirens	0.44 (0.02)	0.62 (0.05)	0.05 (0.00)	0.41 (0.02)	0.06 (0.00)
Caloplaca flavorubescens	0.33 (0.02)	0.56 (0.02)	0.00 (0.00)	0.00 (0.00)	0.02 (0.00)
Caloplaca holocarpa	0.08 (0.01)	0.04 (0.00)	0.55 (0.03)	0.00 (0.00)	0.00 (0.00)
Caloplaca sericea	0.00 (0.00)	0.00 (0.00)	0.57 (0.03)	0.51 (0.03)	0.00 (0.00)
Candelariella cf. reflexa	0.92 (0.04)	1.74 (0.05)	1.02 (0.04)	0.55 (0.03)	0.35 (0.01)
Catillaria nigroclavata	0.19 (0.01)	4.96 (0.11)	0.97 (0.04)	0.07 (0.00)	0.30 (0.01)
Cliostomum griffithii	0.00 (0.00)	2.10 (0.06)	0.75 (0.02)	0.30 (0.01)	0.77 (0.03)
Lecania naegelii	0.90 (0.03)	0.86 (0.03)	0.49 (0.03)	0.25 (0.02)	0.13 (0.01)
Lecanora carpinea	0.00 (0.00)	0.06 (0.01)	0.79 (0.04)	0.47 (0.02)	0.07 (0.01)
Lecanora chlarotera	0.28 (0.01)	3.78 (0.06)	3.02 (0.06)	1.13 (0.04)	3.87 (0.07)
Lecanora expallens	0.06 (0.00)	1.18 (0.05)	2.63 (0.08)	5.32 (0.11)	0.54 (0.03)
Lecanora intumescens	0.03 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Lecanora persimilis	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	0.15 (0.01)	0.00 (0.00)
Lecanora populicola	7.66 (0.15)	7.84 (0.15)	2.33 (0.11)	0.20 (0.01)	0.35 (0.02)
Lecanora pulicaris	0.00 (0.00)	0.00 (0.00)	0.18 (0.01)	0.00 (0.00)	0.00 (0.00)
Lecidella elaeochroma	2.14 (0.06)	4.37 (0.13)	0.53 (0.01)	0.94 (0.03)	2.30 (0.05)

	BD11	BD12	BD13	BD14	BD5
Crustose	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**
Leptorhapis atomaria	0.02 (0.00)	0.03 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Megalaria grossa	5.76 (0.11)	7.32 (0.13)	0.07 (0.00)	0.05 (0.00)	1.66 (0.05)
Mycoblastus fucatus	0.00 (0.00)	0.00 (0.00)	0.05 (0.01)	0.13 (0.01)	0.00 (0.00)
Ochrolechia androgyna	0.00 (0.00)	0.06 (0.00)	9.63 (0.24)	15.37 (0.26)	0.00 (0.00)
Ochrolechia microstictoides	0.05 (0.00)	0.00 (0.00)	0.02 (0.00)	0.00 (0.00)	0.00 (0.00)
Pertusaria armara	0.17 (0.01)	0.96 (0.05)	1.13 (0.03)	1.30 (0.03)	7.50 (0.19)
Pertusaria pertusa	0.02 (0.00)	0.28 (0.01)	0.69 (0.03)	0.30 (0.01)	0.02 (0.00)
Phlyctis argena	0.96 (0.03)	4.93 (0.11)	0.05 (0.01)	0.73 (0.02)	0.01 (0.00)
Sclerophora pallida	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.29 (0.02)	0.00 (0.00)
Sterile crust	0.00 (0.00)	3.54 (0.12)	4.17 (0.14)	0.45 (0.02)	3.23 (0.08)
Tephromela atra	7.76 (0.14)	13.15 (0.19)	8.54 (0.15)	4.11 (0.11)	4.93 (0.10)
Fruticose					
Bryoria fuscescens	0.03 (0.00)	0.00 (0.00)	0.03 (0.00)	0.00 (0.00)	0.40 (0.02)
Cladonia squamules	0.00 (0.00)	0.00 (0.00)	0.18 (0.01)	0.32 (0.02)	0.03 (0.00)
Ramalina farinacea	7.10 (0.13)	14.88 (0.18)	20.60 (0.22)	20.93 (0.23)	24.62 (0.21)
Ramalina fraxinea	0.00 (0.00)	0.02 (0.00)	0.18 (0.02)	0.22 (0.02)	1.18 (0.05)
Usnea subfloridana	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.02 (0.00)
Foliose					
Evernia prunastri	0.01 (0.00)	0.00 (0.00)	0.21 (0.01)	1.54 (0.06)	2.74 (0.06)
Hypogymnia physodes	0.71 (0.04)	7.25 (0.17)	7.32 (0.17)	7.28 (0.11)	9.52 (0.17)
Hypogymnia tubulosa	1.34 (0.07)	4.77 (0.12)	1.74 (0.07)	4.68 (0.12)	5.18 (0.15)

	BD11	BD12	BD13	BD14	BD5
Foliose	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**
Melanelixia fuliginosa subsp. glabratula	0.94 (0.07)	0.02 (0.00)	6.12 (0.10)	4.85 (0.09)	0.01 (0.00)
Melanelixia subaurifera	0.00 (0.00)	0.03 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Parmelia saxatilis	2.72 (0.10)	3.57 (0.13)	10.59 (0.21)	13.35 (0.21)	28.22 (0.29)
Parmelia sulcata	2.93 (0.10)	22.16 (0.22)	18.49 (0.25)	15.91 (0.22)	14.44 (0.18)
Phaeophyscia orbicularis	1.70 (0.05)	8.34 (0.15)	0.51 (0.03)	0.00 (0.00)	19.75 (0.23)
Physcia aipolia	1.82 (0.05)	1.11 (0.05)	0.36 (0.02)	0.02 (0.00)	0.66 (0.03)
Physconia distorta	0.69 (0.03)	0.48 (0.03)	0.10 (0.01)	0.17 (0.01)	0.79 (0.08)
Physcia leptalia	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Physcia tenella/ adscendens	26.87 (0.27)	26.11 (0.29)	14.68 (0.26)	3.45 (0.10)	11.93 (0.19)
Platismatia glauca	0.00 (0.00)	0.93 (0.06)	1.66 (0.11)	0.97 (0.06)	0.46 (0.03)
Pseudevernia furfuracea	0.78 (0.05)	2.90 (0.07)	0.48 (0.03)	0.28 (0.02)	2.81 (0.08)
Xanthoria parietina	1.70 (0.05)	0.92 (0.02)	0.75 (0.03)	0.52 (0.04)	0.45 (0.02)
Liverwort					
Frullania dilatata	47.27 (0.39)	5.99 (0.15)	12.55 (0.23)	0.70 (0.03)	2.39 (0.09)
Moss					
Dicranum scoparium	0.47 (0.03)	0.00 (0.00)	0.00 (0.00)	0.09 (0.01)	0.00 (0.00)
Hypnum andoi	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Hypnum cupressiforme	0.00 (0.00)	0.14 (0.01)	4.81 (0.18)	5.11 (0.18)	2.66 (0.13)
Leucodon sciuroides	0.69 (0.04)	0.03 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Orthotrichum speciosum	1.19 (0.03)	1.20 (0.03)	3.94 (0.11)	1.30 (0.04)	1.56 (0.08)

Table 2: Eigenvalues and variances of RDA

Axes	**1**	**2**	**3**	**4**	**Total variance**
Eigenvalues	0.234	0.139	0.067	0.024	1.000
Species-environment correlations	0.801	0.869	0.842	0.714	
Cumulative percentage variance:					
of species data	23.4	37.3	44.0	46.4	
of species-environment relation	50.4	80.4	94.9	100.0	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.464

Table 3: Eigenvalues and variances of RDA

Axes	**1**	**2**	**3**	**4**	**Total variance**
Eigenvalues	0.101	0.136	0.073	0.050	1.000
Species-environment correlations	0.800	0.000	0.000	0.000	
Cumulative percentage variance:					
of species data	17.0	40.0	52.3	60.7	
of species-environment relation	100.0	0.0	0.0	0.0	
Sum of all eigenvalues					0.591
Sum of all canonical eigenvalues					0.101

Table 4: Eigenvalues and variances of RDA

Axes	**1**	**2**	**3**	**4**	**Total variance**
Eigenvalues	0.280	0.223	0.129	0.074	1.000
Species-environment correlations	0.856	0.000	0.000	0.000	
Cumulative percentage variance					
of species data	28.0	50.3	63.3	70.6	
of species-environment relation	100.0	0.0	0.0	0.0	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.280

Appendix C

Table 1: Mean dbh (SE) in cm for each sampled clone. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Clone	Mean dbh (SE) in cm
47	10.72 (0.55)
57	8.60 (1.48)
59	6.85 (1.18)
72	9.10 (1.45)
75	10.93 (0.92)
76	10.99 (0.76)
78	7.96 (1.06)
80	9.27 (1.29)
81	13.88 (0.61)
82	9.20 (0.93)
83	8.77 (0.98)
84	11.10 (1.13)
87	8.92 (0.37)
92	11.07 (1.51)
93	10.67 (0.73)
103	14.47 (1.09)
107	17.39 (1.79)
117	11.85 (0.96)
122	9.90 (0.70)
128	6.31 (0.90)
131	13.26 (2.20)
138	11.36 (1.62)
139	8.38 (2.32)
140	11.13 (1.04)
141	10.19 (1.11)
146	8.71 (1.51)
147	11.16 (0.72)
Significance	***

Table 2: Mean (with SE) concentrations of main phenolic compounds isolated from bark of 27 clones

Clone	Salicin	(+)-Catechin	Taxifolin	Eriodictyol	Catechol
47	2.78 (0.39)	1.08 (0.25)	0.00 (0.00)	1.28 (0.36)	0.00 (0.00)
57	1.35 (0.30)	1.46 (0.36)	0.00 (0.00)	0.22 (0.08)	0.00 (0.00)
59	1.94 (0.11)	0.75 (0.11)	0.00 (0.00)	0.65 (0.26)	0.00 (0.00)
72	1.43 (0.23)	1.36 (0.20)	0.00 (0.00)	1.28 (0.37)	0.00 (0.00)
75	2.33 (0.33)	1.37 (0.21)	0.33 (0.33)	1.22 (0.33)	0.00 (0.00)
76	2.09 (0.17)	1.47 (0.31)	0.00 (0.00)	0.17 (0.14)	0.17 (0.08)
78	1.79 (0.16)	1.22 (0.39)	0.00 (0.00)	0.53 (0.25)	0.00 (0.00)
80	2.09 (0.11)	1.37 (0.16)	0.09 (0.09)	0.20 (0.06)	0.06 (0.02)
81	0.59 (0.19)	1.47 (0.43)	0.50 (0.50)	2.24 (1.56)	0.08 (0.05)
82	1.13 (0.14)	1.40 (0.23)	0.00 (0.00)	1.80 (0.54)	0.02 (0.02)
83	2.10 (0.11)	1.52 (0.27)	0.00 (0.00)	0.11 (0.07)	0.00 (0.00)
84	2.24 (0.24)	1.24 (0.37)	0.36 (0.36)	0.29 (0.22)	0.00 (0.00)
87	1.69 (0.20)	1.51 (0.10)	2.14 (0.88)	2.50 (0.76)	0.00 (0.00)
92	2.44 (0.50)	1.88 (0.50)	1.03 (0.42)	0.89 (0.42)	0.00 (0.00)
93	2.09 (0.32)	1.50 (0.28)	0.00 (0.00)	0.01 (0.01)	0.00 (0.00)
103	3.49 (0.73)	2.67 (0.17)	0.65 (0.08)	0.42 (0.07)	0.00 (0.00)
107	1.64 (0.44)	2.32 (0.51)	0.77 (0.71)	2.30 (1.74)	0.00 (0.00)
117	4.31 (0.85)	4.53 (0.78)	3.80 (1.50)	3.57 (1.13)	0.80 (0.15)
122	4.53 (0.34)	2.87 (0.35)	0.00 (0.00)	0.82 (0.37)	0.23 (0.12)
128	4.66 (0.50)	3.80 (0.82)	0.62 (0.40)	0.42 (0.18)	0.22 (0.15)
131	2.80 (0.20)	2.80 (0.34)	1.22 (0.74)	0.17 (0.11)	0.36 (0.18)
138	4.84 (0.75)	2.07 (0.48)	0.19 (0.14)	1.12 (0.38)	0.13 (0.09)
139	2.10 (0.05)	2.10 (0.03)	1.03 (0.39)	1.23 (0.36)	0.93 (0.35)
140	1.88 (0.28)	2.69 (0.17)	2.35 (0.59)	2.17 (0.57)	0.27 (0.11)
141	1.29 (0.23)	1.06 (0.19)	0.51 (0.33)	0.40 (0.13)	0.01 (0.01)
146	1.18 (0.17)	0.79 (0.17)	0.39 (0.27)	1.06 (0.67)	0.00 (0.00)
147	0.78 (0.11)	0.60 (0.09)	1.23 (0.44)	2.03 (0.68)	0.00 (0.00)
Significance	***	***	***	***	***

Table 3: Mean (SE) data for concentrations of each phenolic compound at each aspen clone experimental site

Site	Salicin	(+)-Catechin	Taxifolin	Eriodictyol	Catechol
Moray	2.51 (0.16)	1.42 (0.13)	0.25 (0.10)	0.62 (0.14)	0.11 (0.03)
Kilmichael	2.10 (0.13)	2.23 (0.11)	0.95 (0.16)	1.49 (0.19)	0.13 (0.04)

Table 4: Mean % cover (SE) of total epiphytes per clone. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Clone	Mean % cover (SE)
47	65.49 (1.32)
57	45.80 (1.38)
59	48.77 (2.08)
72	54.32 (1.38)
75	68.09 (1.29)
76	50.43 (1.79)
78	44.98 (1.38)
80	38.46 (1.74)
81	13.02 (1.05)
82	62.28 (1.51)
83	55.94 (1.38)
84	33.98 (1.32)
87	48.79 (2.50)
92	36.87 (2.01)
93	21.68 (1.29)
103	63.73 (1.34)
107	48.51 (1.78)
117	62.88 (1.51)
122	51.61 (1.39)
128	2.66 (0.50)
131	50.88 (1.56)
138	71.93 (1.25)
139	32.23 (1.46)
140	65.38 (1.49)
141	38.92 (1.52)
146	61.81 (1.35)
147	51.50 (1.78)
Significance	***

Table 5: Mean % cover (SE) of epiphytes across sites (Moray and Kilmichael). Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Site	Mean % cover (SE)
Moray	48.91 (0.74)
Kilmichael	47.71 (0.52)
Significance	n.s.

Table 6: Mean % cover (SE) of epiphytes across aspects (north, south, east and west). Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Aspect	Mean % cover (SE)
North	59.89 (3.33)
East	54.98 (3.39)
South	40.39 (3.34)
West	38.13 (3.30)
Significance	***

Table 7: Mean (SE) percent cover for each functional group across each clone. Species are separated according to functional group (lichens: crustose, fruticose, foliose; mosses; and liverwort)

Clone	Crustose lichens	Fruticose lichens	Foliose lichens	Moss	Liverwort
47	0.53 (0.50)	0.09 (0.29)	0.00 (0.03)	0.01 (0.10)	0.05 (0.22)
57	0.46 (0.50)	0.00 (0.01)	0.01 (0.09)	0.01 (0.09)	0.02 (0.13)
59	0.34 (0.47)	0.00 (0.04)	0.00 (0.05)	0.01 (0.10)	0.11 (0.32)
72	0.41 (0.49)	0.03 (0.18)	0.16 (0.37)	0.01 (0.09)	0.03 (0.17)
75	0.45 (0.50)	0.00 (0.06)	0.09 (0.28)	0.04 (0.19)	0.17 (0.37)
76	0.24 (0.43)	0.00 (0.00)	0.25 (0.44)	0.01 (0.10)	0.03 (0.16)
78	0.43 (0.50)	0.00 (0.01)	0.07 (0.26)	0.00 (0.00)	0.00 (0.00)
80	0.08 (0.27)	0.00 (0.00)	0.35 (0.48)	0.00 (0.00)	0.00 (0.00)
81	0.03 (0.16)	0.00 (0.00)	0.11 (0.31)	0.00 (0.04)	0.00 (0.00)
82	0.45 (0.50)	0.00 (0.02)	0.20 (0.40)	0.01 (0.10)	0.02 (0.15)
83	0.54 (0.50)	0.00 (0.00)	0.00 (0.03)	0.00 (0.00)	0.00 (0.00)
84	0.28 (0.45)	0.00 (0.00)	0.00 (0.00)	0.01 (0.09)	0.05 (0.22)
87	0.38 (0.48)	0.00 (0.06)	0.00 (0.00)	0.04 (0.19)	0.11 (0.31)
92	0.39 (0.49)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
93	0.17 (0.38)	0.00 (0.00)	0.00 (0.02)	0.01 (0.07)	0.05 (0.21)
103	0.50 (0.50)	0.00 (0.01)	0.03 (0.17)	0.04 (0.19)	0.11 (0.31)
107	0.38 (0.48)	0.01 (0.11)	0.10 (0.31)	0.00 (0.00)	0.01 (0.12)
117	0.55 (0.50)	0.01 (0.09)	0.06 (0.24)	0.01 (0.12)	0.03 (0.16)
122	0.26 (0.44)	0.05 (0.22)	0.05 (0.22)	0.00 (0.06)	0.11 (0.32)
128	0.03 (0.16)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
131	0.48 (0.50)	0.00 (0.02)	0.00 (0.07)	0.01 (0.11)	0.04 (0.19)
138	0.55 (0.50)	0.00 (0.00)	0.04 (0.20)	0.03 (0.18)	0.14 (0.35)
139	0.28 (0.45)	0.00 (0.00)	0.00 (0.07)	0.01 (0.08)	0.06 (0.23)
140	0.64 (0.48)	0.02 (0.14)	0.02 (0.12)	0.01 (0.08)	0.04 (0.20)
141	0.35 (0.48)	0.08 (0.27)	0.11 (0.32)	0.01 (0.07)	0.00 (0.00)
146	0.54 (0.50)	0.03 (0.17)	0.03 (0.17)	0.03 (0.17)	0.10 (0.30)
147	0.40 (0.49)	0.05 (0.22)	0.04 (0.19)	0.01 (0.12)	0.08 (0.27)

Table 8: Epiphyte community diversity for each clone based on mean total species per quadrat/ clone; mean species per functional group/ clone; and Shannon Index based on mean species per quadrat. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Clone	Total	Crustose lichens	Fruticose lichens	Foliose lichens	Liverwort	Moss	Shannon
47	2.36 (1.03)	1.72 (0.29)	0.31 (0.09)	0.08 (0.05)	0.17 (0.06)	0.08 (0.05)	0.61 (0.12)
57	2.47 (1.30)	1.89 (0.19)	0.03 (0.03)	0.25 (0.09)	0.17 (0.06)	0.14 (0.06)	0.63 (0.12)
59	2.00 (0.82)	1.42 (0.21)	0.13 (0.07)	0.04 (0.04)	0.21 (0.08)	0.21 (0.08)	0.62 (0.12)
72	2.53 (0.97)	1.44 (0.24)	0.22 (0.09)	0.67 (0.16)	0.06 (0.04)	0.14 (0.07)	0.73 (0.13)
75	3.72 (1.59)	2.42 (0.26)	0.11 (0.05)	0.58 (0.16)	0.31 (0.08)	0.31 (0.08)	1.19 (0.08)
76	1.88 (0.84)	1.06 (0.20)	0.00 (0.00)	0.56 (0.10)	0.13 (0.06)	0.09 (0.05)	0.57 (0.11)
78	2.08 (1.31)	1.75 (0.20)	0.03 (0.03)	0.31 (0.10)	0.00 (0.00)	0.00 (0.00)	0.54 (0.11)
80	1.16 (0.92)	0.53 (0.11)	0.00 (0.00)	0.63 (0.09)	0.00 (0.00)	0.00 (0.00)	0.13 (0.06)
81	0.47 (0.32)	0.31 (0.09)	0.00 (0.00)	0.13 (0.06)	0.00 (0.00)	0.03 (0.03)	0.08 (0.04)
82	3.00 (1.21)	2.22 (0.21)	0.03 (0.03)	0.41 (0.13)	0.16 (0.07)	0.19 (0.07)	0.89 (0.12)
83	2.04 (1.06)	1.96 (0.25)	0.00 (0.00)	0.07 (0.05)	0.00 (0.00)	0.00 (0.00)	0.60 (0.12)
84	1.61 (1.15)	1.28 (0.14)	0.00 (0.00)	0.00 (0.00)	0.17 (0.06)	0.17 (0.07)	0.41 (0.09)
87	2.85 (0.95)	1.95 (0.17)	0.20 (0.09)	0.00 (0.00)	0.30 (0.11)	0.40 (0.11)	0.93 (0.12)
92	1.17 (0.61)	1.17 (0.16)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.18 (0.07)
93	0.93 (0.56)	0.68 (0.14)	0.00 (0.00)	0.04 (0.04)	0.11 (0.06)	0.11 (0.06)	0.16 (0.09)
103	2.17 (1.11)	1.53 (0.20)	0.03 (0.03)	0.22 (0.07)	0.19 (0.07)	0.19 (0.07)	0.66 (0.10)
107	1.75 (0.91)	1.32 (0.15)	0.11 (0.06)	0.21 (0.12)	0.11 (0.06)	0.00 (0.00)	0.43 (0.10)
117	3.19 (1.53)	2.36 (0.21)	0.11 (0.05)	0.47 (0.15)	0.11 (0.05)	0.14 (0.06)	0.95 (0.12)
122	2.29 (0.70)	1.25 (0.27)	0.32 (0.12)	0.36 (0.16)	0.25 (0.08)	0.11 (0.06)	0.68 (0.16)
128	0.31 (0.38)	0.31 (0.08)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
131	1.63 (1.01)	1.54 (0.19)	0.04 (0.03)	0.04 (0.03)	0.11 (0.05)	0.14 (0.06)	0.53 (0.10)
138	3.28 (1.38)	2.61 (0.22)	0.00 (0.00)	0.46 (0.13)	0.29 (0.08)	0.39 (0.11)	1.05 (0.10)
139	1.28 (0.80)	0.91 (0.16)	0.00 (0.00)	0.09 (0.05)	0.19 (0.07)	0.09 (0.05)	0.35 (0.09)
140	2.81 (1.23)	2.19 (0.26)	0.16 (0.07)	0.22 (0.09)	0.16 (0.07)	0.09 (0.05)	0.78 (0.13)

Clone	Total	Crustose lichens	Fruticose lichens	Foliose lichens	Liverwort	Moss	Shannon
141	2.21 (0.73)	1.32 (0.26)	0.32 (0.13)	0.54 (0.17)	0.00 (0.00)	0.04 (0.04)	0.58 (0.14)
146	3.09 (1.30)	2.22 (0.25)	0.19 (0.07)	0.22 (0.07)	0.22 (0.07)	0.25 (0.09)	0.94 (0.13)
147	2.57 (0.93)	1.54 (0.22)	0.21 (0.08)	0.32 (0.13)	0.29 (0.09)	0.21 (0.09)	0.70 (0.15)
Sign.	***	***	***	***	***	***	***

Table 9: Epiphyte community diversity for each site based on mean total species per quadrat/ site; mean species per functional group/ site; and Shannon Index based on mean species per quadrat/ site. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Site	Total	Crustose lichens	Fruticose lichens	Foliose lichens	Liverwort	Moss	Shannon
Moray	2.25 (0.10)	1.71 (0.07)	0.12 (0.02)	0.43 (0.03)	0.00 (0.00)	0.00 (0.00)	0.65 (0.03)
Kilmichael	1.98 (0.08)	1.29 (0.05)	0.06 (0.01)	0.08 (0.02)	0.28 (0.02)	0.27 (0.02)	0.53 (0.03)
Significance	.	***	**	***	***	***	*

Table 10: Epiphyte community diversity for each aspect based on mean total species per quadrat/ aspect; mean species per functional group/ aspect; and Shannon Index based on mean species per quadrat/ aspect. Significance: *** p <0.001; ** p <0.01; * p <0.05; n.s. not significant

Aspect	Total	Crustose lichens	Fruticose lichens	Foliose lichens	Liverwort	Moss	Shannon
North	2.49 (0.14)	1.66 (0.08)	0.14 (0.03)	0.30 (0.05)	0.19 (0.03)	0.20 (0.03)	0.72 (0.05)
East	2.30 (0.13)	1.60 (0.09)	0.10 (0.02)	0.30 (0.04)	0.16 (0.03)	0.14 (0.03)	0.65 (0.05)
South	1.92 (0.13)	1.43 (0.09)	0.07 (0.02)	0.24 (0.04)	0.09 (0.02)	0.09 (0.02)	0.52 (0.05)
West	1.79 (0.12)	1.35 (0.09)	0.06 (0.02)	0.21 (0.04)	0.09 (0.02)	0.07 (0.02)	0.47 (0.04)
Significance	***	*	*	ns	***	***	***