An Investigation into the Importance of Propagule Provenance in Restoration Ecology

Barbara Maria Smith

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Abstract

There is increasing concern among restoration ecologists that using non-local propagules in re-vegetation schemes may influence the success of grassland restoration. This research programme investigated the importance of propagule provenance in restoration schemes. In particular the work focused on two areas. Firstly, the study investigated the significance of propagule provenance for plant establishment and persistence using *Lotus corniculatus* as a model species. Secondly, it investigated the practical implications of restricting seed procurement to local sources, using a field trial which assessed the success of limestone grassland re-creation under different treatments.

Propagules were collected from two contrasting habitats within each of six regions in the British Isles and a common garden experiment was used to quantify the genetic component of provenance as estimated by plant morphology and fitness. There were differences in survival, growth habit, pubescence, leaf shape, plant size and fecundity between plants grown from seeds sourced from different regions. In addition these populations differed in the extent to which they were damaged by seed herbivores. Differences between plants grown from seed sourced from contrasting habitats were limited to morphology. These findings suggested that populations would be likely to perform differentially in a restoration environment. Both geographical location and the ecological conditions at the seed source should be considered when procuring seeds for a restoration scheme. A field trial was set up to establish whether local propagules exhibited higher fitness than those collected from non-local sources. Propagules were collected from two contrasting habitats in each of fifteen regions. Two restoration environments at a single site were investigated; one was treated with a dressing of topsoil, the other site was untreated bare clay substrate. Differences between populations were measured in terms of both geographical and ecological distance. Results for both sites demonstrated that although there was no home-site advantage in terms of geographical distance, plants from more distant populations were smaller and less fecund in the restoration environment. An investigation into the relationship between ecological distance and plant performance produced different results on the treated and untreated plots. On the treated plot there was no significant relationship but on the untreated plot, plants from more distant populations were larger and more fecund. The contrast in the results obtained for the effect of geographical and ecological distance on performance in the untreated restoration environment is interesting. The enhanced performance of geographically local populations agrees with findings from previous studies. The findings for ecological distance are unexpected based on other work. However, it is postulated that the initial success of non-local populations maybe misleading, as environmental conditions which are infrequent but typical of the area may lead to high mortalities in the long-term.

The range of what can be considered local is rarely considered in studies that investigate plant provenance, but work from other areas suggests that there is sufficient variation over small distances to warrant seed collection within 100m. Fine scale phenotypic variation over 200m in populations of

L.corniculatus was investigated, however there was no evidence to suggest that seed collection should be restricted to 100m, consequently seeds could be safely collected up to 200m from a restoration site.

If seed procurement is to be restricted to local seed then it is possible that the seed application rate and species mix available for a restoration project may be limited. A field trial investigating the effect of different treatments showed that it is possible to establish an appropriate plant community using a low sowing rate. However, that community will be more vulnerable to changes in the environment during the establishment phase and more open to invasion by colonizing species which are likely to be weedy in the first few years. A comparison of two seed mixes showed that a diverse seed mix resulted in an increased diversity and evenness of vegetation. There was no benefit in adding a nurse grass to compensate for a low application rate of local species. In conclusion, although it is an advantage to use seeds of local provenance in restoration schemes, it will be necessary to balance this with the likelihood of successful re-vegetation given the seed available.

For Jason

Who has been a star

LIST OF CONTENTS

Chapter One

Chapter Two

2.1 Background

2.2 General Methods

Chapter Three

Quantifying the genetic component of provenance as estimated by plant morphology and fitness in a common garden experiment

3.2 Method

Chapter Four

The effect of provenance on differences in plant fitness in a restoration environment

4.2 Method

4.3 Results

Chapter 5

Fine-scale phenotypic variation in *L. corniculatus* with reference to restoration ecology

5.3 Results

Chapter Six

The practical implications of restricting seed procurement to local sources

Chapter Seven

General Discussion

LIST OF FIGURES

Chapter Two

Chapter Three

Chapter Four

Chapter Five

Chapter Six

List of Tables

Chapter Three

Chapter Four

Chapter Five

Chapter Six

Table 6.18 Comparison of the number of individuals that were categorized as 'weedy' or 'calcicolous' on experimental plots in 1997, 1998 and 2000

LIST OF PLATES

Chapter Two

Chapter Six

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Chapter One

Introduction

This research programme investigates the importance of propagule provenance in restoration ecology. In particular the work focuses on two areas. Firstly, the study investigates the significance of propagule provenance for plant establishment and persistence. Secondly, it investigates the practical implications of restricting seed procurement to local sources.

1.1 An introduction to restoration ecology

Restoration ecology is defined by the Society for Ecological Restoration (SER) as '*...the process of assisting the recovery and management of ecological integrity. Ecological integrity includes a critical range of variability in biodiversity, ecological processes and structures, regional and historical context, and sustainable cultural practices*' (http://www.ser.org/definitions.html). This definition incorporates the principles proposed by many of those working in the field; particularly the notion that restoration ecology will assist the recovery of whole ecosystems. Ecological restoration was pioneered in 1934 by Aldo Leopold who instigated the first recorded rehabilitation project that sought to recreate areas of the Wisconsin prairies. Leopold described his work as an '*ecological exercise*' and from the outset aimed to '*reconstruct a sample of what we had to begin with*' (Jordan *et al.*, 1987). Since Leopold's time a general consensus has been reached that the re-constructed community cannot be '*what was there to begin with*' and can only be an approximation; it is not a replacement for established ecosystems (Bradshaw, 1987; Buckley, 1989). More recently, the term 'restoration' has been freely applied to civic improvement and amenity projects. Buckley (1989) drew a distinction between 'political' restoration, which is largely governed by aesthetics, and 'ecological' restoration, which aims to re-create, as closely as possible, a representation of a desired semi-natural community with all its functioning components. It is with this latter definition that this study is concerned. Bradshaw (1987) added an important dimension to the understanding of what restoration ecology is, identifying that natural re-vegetation will inevitably take place without human intervention but that the process will be slow. As a consequence, there is a tacit understanding that a restoration scheme will speed up the process of establishing an appropriate plant community. There are those who debate whether it is necessary to engineer restoration by introducing propagules and suggest that it is preferable to allow sites to colonise naturally (Karel et al., 2001). Whichever method is chosen for the establishment of species at restored sites, propagules must either be available from a local species pool or be introduced (Zobel et al., 1998). Propagule availability will determine whether propagule introduction is required and there is a body of work which suggests that in most cases introduction will be necessary. This evidence is summarized in the next section.

1.2 Propagule availability

The experimental work presented in this thesis was carried out on an area which is being restored to calcareous grassland. There have been several studies that address the potential for natural regeneration in calcareous habitats. In 1988 Graham & Hutchings published work that investigated the prospective regeneration of chalk grassland on former arable land at Castle Hill National Nature Reserve in Sussex. The authors showed that the soil seed bank contained only a small proportion of the regular constituents of chalk grassland (Graham & Hutchings, 1988a). They concluded that the weedy component of the soil seed bank was likely to dominate the site and pre-empt resources before perennial chalk grassland species could become established (Graham & Hutchings, 1988b). In the field, germination of species that were typical of the original vegetation, was low and the authors surmised that colonization would be slow even though there was an area of ancient chalk grassland in close proximity (Graham & Hutchings, 1988b). Castle Hill was revisited 10 years later and the predictions confirmed; although the soil seed bank had increased in species richness there was little contribution from the species characteristic of ancient chalk grassland (Hutchings & Booth, 1996). An examination of the contribution of 'seed rain' (measured as seeds captured on sticky seed traps) showed that there was some patchy and localised contribution on the margins of the experimental area. However, overall it was concluded that there was little potential for the unassisted regeneration of chalk grassland and that to hasten the establishment of a calcicolous community and prevent fast-growing weedy species dominating the site it would be necessary to sow seeds (Hutchings & Booth, 1996). In a comparison of the species composition of seed rain and soil seed bank at an abandoned chalk quarry in

Yorkshire, Jefferson & Usher (1989) concluded that of the two, seed rain is the more likely source of propagules. However, the proximity of a seed source will be an important factor. Hutchings & Booth (1996) found the dispersal of forbs associated with calcareous habitats is restricted to between 0.3 and 3m. In an investigation of natural regeneration, Partel *et al.* (1998) demonstrated that, in ideal conditions, wind dispersal may be sufficient to initiate succession on a site, but that in many cases it will be necessary to augment the species complement with propagules. Jefferson & Usher (1989) do acknowledge the confounding effect of distance and cite previous work in which they showed that the distance of quarry sites from patches of existing chalk grassland profoundly affects the species richness of the site. Even in situations where there is a local source close by, the development of a semi-natural community is a slow process: Gibson & Brown (1991) identify time as the most important factor in the development of '*something resembling ancient grassland*'.

These conclusions are supported by work on other habitats. Bekker *et al.* (1997) examined the soil seed banks in 38 grassland communities in Europe and concluded that none of the grasslands harboured high proportions of species with a long-term persistent seed bank. Both abandonment and cultivation reduced the number of seeds of appropriate species surviving in the soil so that the recolonisation of these sites from the soil seed bank was unlikely. Verhagen *et al.* (2001) looked at dispersal and colonisation in nine heathland sites. In all cases natural establishment was restricted to a few species, despite many of the target species being available within an area which the authors considered nearby. Although natural establishment was limited, when Verhagen and his colleagues spread seed-rich hay over the experimental sites, vegetation established quickly. The authors concluded that given suitable environmental conditions, it is dispersal rather than recruitment which is the limiting factor in vegetation establishment (Verhagen *et al.*, 2001). In summary, work to date indicates that impoverished seed banks coupled with restricted dispersal will limit natural re-vegetation by appropriate species (Bakker & Berendse, 1999). If the purpose of restoration ecology is to speed up and direct the successional process then it appears that it will usually be necessary to introduce propagules.

If appropriate species are to be introduced into a restoration site then their provenance is called into question and there is increasing concern that introduced propagules should be restricted to those of local provenance (Wilkinson 2001; Sackville-Hamilton, 2001; Montalvo & Ellstrand, 2000; Linhart, 1995). This leads to two areas of concern: firstly whether provenance has any effect on the ability of plants to survive and persist in the restored habitat; secondly, whether it is practical to restore habitats if seed collection is restricted to local sources. These areas have been chosen as foci for this thesis.

1.3 Propagule provenance

The need for experimental work to address the issue of propagule provenance has been suggested by many authors, (eg, Wilkinson, 2001; Clewell & Reiger, 1997; Knapp& Rice, 1996; Linhart & Grant, 1996; Fenster & Dudash, 1994; Millar & Libby, 1989). The comprehensive studies conducted by Clausen, Keck & Hiesey (1940; 1947; 1948) examined the geographic variation in morphology within plant species using common garden experiments and developed the idea that plants are locally adapted to their environment. It is this high level of local adaptation, demonstrated by Clausen, and subsequently by other researchers such as Turkington & Harper (1979a), Meyer & Monsen (1991) and Linhart & Grant (1996), and which has raised concerns that using non-local genotypes will result in poor vegetation establishment. (Knapp & Rice, 1994). Furthermore, some authors (such as Keller, Kollmann & Edwards, 2000) are concerned that maladapted individuals will hybridise with local populations and reduce the fitness of the existing vegetation.

Poor performance of non-local genotypes.

Non-local propagules may be maladapted to restoration site conditions and perform poorly (Knapp & Rice, 1996). Knapp & Rice (1996) found differences between populations of *Elymus glaucus,* with fewer differences between closely spaced sub-populations. The occurrence of this pattern was attributed by the authors to self-pollination and a patchy distribution in the species which resulted in a low level of gene flow between populations. From this, the authors concluded that it was likely that translocated populations would perform differently in a restoration environment. Linhart (1995) argues that not only would non-local propagules perform poorly, but that performance would decrease with increasing geographical separation of the sites and that locally sourced individuals would perform best. This has been termed 'home-site advantage' (Montalvo & Ellstrand, 2000). Little work has been done to support this hypothesis, although some recent studies have been published. Jones and Hayes (1999) found some variation in performance between five grassland forbs but found no consistent advantage for the home-site. In more recent work, Jones *et al.*, (2001) showed variation in diverse accessions of *Crataegus monogyna* which were planted in an experimental hedge and monitored for several years. The authors used morphological traits to determine local advantage, arguing that characters such as size and thorniness showed local phenotypes to be better adapted. Unfortunately it was difficult to substantiate the argument, as it was not known how plants appeared at their home location or in a common garden experiment. Although there were differences between populations and the local population was judged superior, there was no attempt to relate performance to the geographical separation of populations. Galloway & Fenster (2000) failed to find home site advantage in *Chamaecrista fasciculata.* In a comprehensive study, Montalvo & Ellstrand (2000) also failed to find a correlation between geographical separation and plant performance in *Lotus scoparius*. However, plants from similar environments showed superior performance, supporting the hypothesis for adaptation to local conditions. There is insufficient experimental evidence to confirm or reject the hypothesis that the geographical separation between the source of propagules and site of establishment will affect propagule performance.

Most work, in common with that described above, defines the separation of populations by geographical distance. However, convincing evidence for the significance of local adaptation for plant fitness when translocated comes from those studies that have taken account of environmental conditions at the sites investigated (Antonovics & Bradshaw, 1970; Turkington & Harper, 1979b; Montalvo & Ellstrand, 2000; Joshi *et al.*, 2001). By comparing the effect of geographical separation and environmental differences between donor and receptor sites, it is possible to establish which factor better describes what can be considered of 'local' provenance. In recent studies, (e.g. Montalvo & Ellstrand, 2000 ; Joshi *et al.* 2001), environmental variation was described by measuring climatic and edaphic variables. However, choosing variables which capture the true extent of variation between sites is fraught with difficulties and it is possible that the selected variables will not include critical factors to which plants are adapted. In addition, measuring soil chemistry and moisture levels at a single point in time may be misleading, as annual fluctuations in response to climatic changes cannot be accounted for. What would be useful is a comprehensive measure that takes into account the differences that can occur between sites. Antonovics *et al.*, (1988) described the '*external environment*' as reflecting properties of the environment that are measurable externally (that is, of the species being investigated). In this paper, Antonovics and his colleagues examined the fitness of *Anthoxanthum odoratum* in a field as affected by the micro-environment. They measured vegetation composition to determine any inferred environmental heterogeneity. This is underpinned by the notion that a plant community is a response to the suite of environmental variables at any given site. Antonovics and his colleagues entered the proportion of cover of 22 species they recorded at their experimental site into a Principal Components Analysis (PCA) and used the first axis generated by the analysis to determine differences in the field. Similarly, in the experimental work presented in this thesis, differences in the vegetation composition between the sites investigated were used to infer differences in the respective environments. The differences in environmental conditions were measured by comparing vegetation composition as determined by Detrended Corespondance Analysis (DECORANA). In this way the difference between the communities was measured and assigned a numerical value that was used for further statistical analyses. The separation of the communities (that is, the difference between them) measured in this way is hereafter referred to as 'ecological distance'. The geographical separation of populations was determined by the Euclidean distance between the sites measured in metres or kilometres and is hereafter referred to as 'geographical distance'. This thesis will compare the effect of ecological and geographical distance on determinants of plant fitness, both in a common garden and at a restoration site.

If propagules are to be collected locally this raises the question of what constitutes a local population. There have been no published attempts to quantify this with the exception of Linhart (1995). Linhart used geographical distance as a determinant and suggested that the limits of seed collection for a restoration scheme are 1km for woody species and 100m for herbaceous species, basing this on what is known about seed dispersal. Linhart proposed that, if there is a preponderance of short distance dispersal, then there will be groups of closely related individuals living in close proximity and that over a distances greater that 100m, individuals of herbaceous species are no longer closely related. Linhart posited that translocating individuals over distances greater than 100m may result in the establishment of plants which are significantly different from the 'local' plants and that these will disrupt the local pattern of adaptation through hybridisation (Linhart, 1995). Fine-scale biotic differentiation was investigated by Turkington & Harper (1979b) who demonstrated that locally differentiated populations occur within small areas. Using *Trifolium repens* in reciprocal transplant experiments in a 1ha field, the authors further showed that plants were not mutually interchangeable and that the differences between the plants affected fitness attributes when translocated (Turkington & Harper, 1979b). In short, it is possible that local variation would lead to poor performance in translocated individuals over short distances. Although some have commented on Linhart's 100m guideline (eg. Knapp & Dyer, 1997), there have been no other studies attempting to quantify what is 'local'. In this thesis, Linhart's 100m guideline will be tested by investigating fine-scale differences in plants over short distances, to establish if 100m is an appropriate guideline to use when selecting herbaceous propagules for restoration projects.

The disruption of local gene pools.

Introducing non-local germplasm may negatively influence the local population (Knapp & Rice, 1996). It has been a concern that hybridization between local and non-local plants which are adapted to different local conditions will lead to a loss of fitness caused by the breakdown of local co-adapted gene-complexes (Storfer, 1999). This is supported by recent experimental work. For example, Keller *et al.*, (2000) showed that introgression of maladapted genes reduced plant performance in weed species. Montalvo & Ellstrand (2001) also found that hybrids performed poorly compared to local plants when put into a restoration environment. Experimental work described in this thesis will not directly address this issue but results will be discussed with reference to existing research.

1.4 Seeding regime

Restricting seed collection to local sites will have practical implications for the restoration ecologist. In particular, using locally sourced seed may mean that a small amount of seed is available to sow at a restoration site. This is partly because there may be small areas of appropriate vegetation available for harvesting and also because it is undesirable to strip existing areas of seed (Stevenson *et al.*, 1995). Reducing the ecological impact on the donor site caused by seed collection can be effected in two ways; the seed application rate can be reduced or seed mixes can be designed to contain fewer of the rare species.

The type of seed mix sown may affect the stability and productivity of the establishing grassland. Mixes with fewer species may lead to a less diverse plant community and diversity has been shown to increase the resilience of communities to perturbation (Tilman & Downing, 1994). Studies by Tilman and his colleagues also suggest a positive relationship between diversity and ecosystem function and also that increased diversity results in a community which is more likely to persist (Tilman, *et al.*, 1996).

Seed application rates will affect the establishment of a plant community. Low seed rates may be expected to produce a more open sward which may be easily invaded by weedy species. The effect of sowing rate was investigated by Stevenson *et al.*, (1995), who found that although species richness increased with sowing rate, after two years there was no significant difference between swards sown at different rates. Unfortunately, seeding between plots in the second year confounded the experiment and the long-term effect of sowing rate requires further investigation.

1.5 Aim and Objectives

This thesis aims to investigate the effect of propagule provenance on the success of the re-vegetation component of restoration schemes. There are four objectives:

- To quantify the genetic component of provenance as estimated by plant morphology and fitness in a common garden experiment (Chapter three)

- To determine the effect of provenance on differences in plant fitness in a restoration environment (Chapter four)

- To investigate fine-scale phenotypic variation in *L. corniculatus* with reference to restoration ecology (Chapter five)

- To investigate the practical implications of restricting seed procurement to local sources (Chapter six)

Propagule provenance was investigated using *Lotus corniculatus* L. as a model species. Propagules were collected from populations in different regions and different habitats within those regions. The separation of populations was determined by geographical and ecological distance and a key component of this study was to evaluate the relative significance of these two variables on differences in plant phenotype and fitness. The results of this study are used to assess the importance of using propagules of local provenance in restoration projects. 'Local' is defined by geographical proximity and ecological similarity. Fine-scale phenotypic variation in populations of *L. corniculatus* was investigated using 200m transects at 10 of the collection sites. The aim is to observe variation in plant phenotype over small distances, using geographical and ecological distance to quantify the separation of plants along the transects. The results are used to test the hypothesis put forward by Linhart (1995) that 100m is a boundary beyond which propagules can no longer be considered 'local'.

The practical implications of using the restricted seed regimes which may be associated with local seed procurement will be a matter of concern for restoration ecologists. The effect of seeding regime on the success of vegetation establishment at a restoration site was investigated using a field trial. Two types of seed mix were applied at three sowing rates and the results are used to assess the practicability of recreating species rich grassland using a limited seed mix at low application rate.

Chapter Two

Background and General Methods

This chapter gives supporting information and describes methodologies that were common to more than one experiment described in this thesis. It contains two sections, as follows:

Background

- Description of Swanworth Quarry (the site for field experiments: Chapters four and six)
- Description of *L. corniculatus* which was the model species used to investigate the effect of provenance on plant establishment and fitness in restoration schemes (Chapters three, four and five);
- Rationale for choosing, and a description of the traits chosen, to assess plant fitness and determine differences between populations of *L. corniculatus.*

General Methods

- Methods used to record character traits of *L. corniculatus.*
- Method for determining geographical and ecological distance (key concepts: Chapters three, four and five).

2.1 Background

2.1.1 Site Description: Swanworth Quarry (SY 968 784) (40 Hectares)

The field trials described in Chapters four and six were carried out at a limestone quarry in Dorset, owned by Tarmac Southern Ltd, and in operation throughout the experimental period. Operation is expected to cease by 2010 when the quarry will be landscaped and re-vegetated to resemble nearby limestone grassland. The field trials were carried out on an area that had been landscaped in preparation for re-vegetation.

Location

Swanworth Quarry is situated on the Isle of Purbeck in Dorset, approximately 6 kilometres to the west of Swanage and 3 kilometres south of Corfe Castle (Figure 2.1). The quarry is located on the Purbeck coastal plateau in a landscape of

Tarmac
Tarmac Quarry Products Limited
Land and Minerals Department
Millfields Road, Ettingshall
Wolverhampton WV4 6JP
Tel. +44(0)1902 353522
Fax. +44(0)1902 494511

Swanworth Quarry

Location Plan FIGURE 2.1

Drawn By N.T/A.C.J	Scale 1:50,000
Date June 2000	Drawing No S46/310

predominantly open farmland consisting of pasture and arable fields. Arable fields lie to the east of the quarry while land to the west supports unimproved pasture used for grazing. The quarry is bounded on three sides by the steep sided valleys of Coombe Bottom and Hill Bottom with farmland to the east extending to Compact Farm and Worth Matravers village. The valleys support a mosaic of grazed limestone grassland and scrub. The southern part of the quarry perimeter abuts the South Dorset Coast SSSI and the coast lies approximately 1.6 kilometres to the south at Chapman's Pool.

Geology

The geology on the Isle of Purbeck consists entirely of Jurassic and Cretaceous sedimentary rocks which are principally composed of shales and mudstones, with some sandstones and limestones. The greatest development of limestone is that of the Portland limestone which is extracted at Swanworth Quarry. The geological succession within Swanworth Quarry and its immediate vicinity consists of Upper Jurassic strata ranging from the Lower Purbeck to the older Portland sands, beneath which are beds of Kimmeridge Clay. The Lower Purbeck series forms the overburden above the extracted Portland limestone and consists of clays and marls: this overburden will be landscaped to form the new contours of the restored landscape. The experimental area was located on a former tip consisting of overburden and quarry waste: this formed the substrate for the field experiments described in this thesis.

2.1.2 The model species: *Lotus corniculatus* L.

Lotus corniculatus was selected as a model species to investigate the effect of propagule provenance plant fitness in re-vegetation programmes. Four factors indicated that it would be suitable as a model species. Firstly, *L. corniculatus* is a common grassland species which is frequently included in seed mixes designed for the re-vegetation of calcicolous grassland. For example, it was sown during the restoration of land surrounding the M3 extension in Hampshire (Ward & Snazell, 1995) and is included in standard mixtures by seed houses such British Seed Houses (BSH) and Emorsgate Seeds. It was therefore considered representative of species likely to be included in seed mixes and sown in places other than where it was grown. Secondly, *L. corniculatus* is found in most parts of the British Isles and tolerates a wide range of conditions (Jones & Turkington, 1986) and so it was possible to collect propagules from sufficient sites to compare the effect of provenance on translocated plants. Thirdly, *L. corniculatus* has been widely studied so that differences between plants and the subsequent effect on fitness can be interpreted with more confidence than would be possible in a less studied species. Finally, it was known that, typically, seeds of *Lotus corniculatus* have over 50% viability (Jones & Turkington, 1986) which indicated that, where seeds were used, there would be enough germination to carry out experimental work.

General description

Lotus corniculatus is a perennial leguminous herb: it has a long woody tap root up to 1m in length with shallow rooting laterals which frequently form a dense root mat. Growth habit can be prostrate, decumbent or erect. Aerial stems are solid and glabrous to pubescent. The leaves have two basal leaflets, which are lanceolate to obvate and three distal leaflets, which are obvate, obtuse or apiculate (shown in Figure 2.3). Inflorescences are borne on an erect peduncle in the axils of the upper leaves. There are 2-6 flowers forming cymose heads. The flower consists of a corolla which is bright yellow and a standard which may be streaked with red. Seeds form in cylindrical pods which are dark brown when ripe and borne at the top of the peduncle. The arrangement of the pods, which are set at a right angle to the peduncle, is said to resemble a bird's foot, hence the common name. The seeds are irregularly rounded and shiny, varying in colour from an olive green through to a dark brown; some phenotypes are speckled and others uniformly coloured. Lotus is a self-incompatible species with a few plants exhibiting a low degree of voluntary self-compatibility (Bader & Anderson, 1962). Pollination is mostly carried out by *Bombus* spp, although *Apis mellifera* has also been found to be an effective pollinator (Jones & Turkington, 1986).

Lotus corniculatus is the most ecologically wide-ranging legume in Britain, being found in maritime to montane communities up to 915m above sea level (Jones & Turkington, 1986). It is found in diverse habitats from shingle beaches, dune systems and cliff-top turf to limestone pastures, chalk heaths, wasteland and road-side verges. While the plant is found on spoil and open habitats as well as more closed grassland, it is absent from tall swards where it is a relatively poor competitor (Grime *et al.*, 1988). *L. corniculatus* survives in moderately fertile to infertile soils and tolerates a wide range of pH, although it is excluded from very acid soil (Jones & Turkington, 1986). Although largely drought intolerant, *L. corniculatus* may grow in areas with seasonal dry periods as the long tap root will access moisture deep in the soil or held in cracks and fissures in limestone (Jones & Turkington, 1986). *Lotus corniculatus* is absent from wetlands where it is replaced by *Lotus uliginosus* (Grime *et al*, 1988).

Determining plant fitness

In experimental work presented in this thesis plant fitness was determined by plant size, seed yield and seed weight; in this study seed weight was used as a surrogate for seed size. Plant size is the dominant influence on individual reproductive success in *L. corniculatus* as larger plants produced more flowers and consequently more seed (Ollerton & Lack, 1998). There is evidence that there are significant differences in seed yield between genotypes of *L. corniculatus* (Long, *et al.*, 1989) so that it can be expected that plants from different areas will perform differently in both common garden and field experiments. Seed size is also a significant factor in plant fitness and is correlated with seedling vigour in *L. corniculatus* (Twamley, 1967; Carlton & Cooper, 1972; Beuslink & McGraw, 1983; Bullard & Crawford, 1996). Although it has not been related to speed of germination or seedling viability, it has been speculated that the detected vigour is related to the ability of large seeds to produce larger seedlings which tend have improved field establishment (McKersie *et al.*, 1981). It is not so in all cases and it may be unwise to use seed size alone to predict vigour (Negri & Falcinelli, 1990). Seed size has also been found to be a predictor of longevity in the soil, with larger seeds having a shorter life-span (Thompson *et al.*, 1993; Bekker *et al.,* 1998; Hodkinson *et al.*, 1998). The ability to persist in the soil prior to germination may increase the fitness of an individual.

Morphological characters

Three morphological characters were used to characterise plants in Chapters three and five. These were leaf shape, growth habit and pubescence; characters which have been shown to be heritable (reviewed in Jones & Turkington, 1986).

Polymorphisms

Lotus corniculatus has been identified as being polymorphic for two traits that have been well studied; keel petal colour and cyanogenesis. These polymorphisms have been identified as being under genetic control and a pattern of distribution has been established, as described below.

Keel petal colour

In experimental work presented in Chapters four and five of this thesis keel colour was recorded; in Chapter four the aim was to investigate a possible link between keel colour and plant fitness and in Chapter five keel colour was recorded to differentiate between and to characterise plants.

European populations of *Lotus corniculatus* are usually polymorphic for keel colour. Plants exhibit a 'light' keel, which is yellow (Plate 2.1), or 'dark' keel, which has a red/brown pigmentation (Plate 2.2). Colour is determined by a pair of alleles at a single locus that shows a tetrasomic pattern of inheritance where the dark trait is dominant (Ramnani & Jones, 1984a). The intensity of the dark pigment varies among individuals from a diffuse red at the tip of the keel, to a dark brown which may cover most of the keel. Crawford & Jones (1988) refer to unpublished observations by A. Ramnani who noted that the number of dark keel alleles in the tetraploid genotype has no influence on the intensity of pigmentation; subsequent studies have not attempted to further sub-divide this class (Crawford & Jones, 1988; Jewell *et al.*, 1994).

Plate 2.1 ***L. corniculatus*** **exhibiting a light keel**

Plate 2.2 ***L. corniculatus*** **exhibiting a dark keel**

A comprehensive study of the distribution of morphs in Great Britain was carried out by Crawford & Jones (1988). The study described a cline for the polymorphism of keel colour and showed a predominance of dark keeled morphs in the north and east and a predominance of light keels in the south and west. Abbott (1981) further demonstrated that in Scotland light keels predominate in the east and that in the west dark keeled plants occur more frequently. There are some local differences maintained, for example throughout the British Isles there is a tendency for coastal sites to have a low frequency of dark-keeled morphs. Crawford & Jones (1986) found there were very few places where populations were monomorphic for keel petal colour and in these cases the populations were generally small with few individuals to test.

It has been suggested that temperature partly accounts for the keel-colour cline observed in Britain (Crawford & Jones, 1986). Abbott (1992) showed that the dark keel colour is highly associated with low mean annual temperature. Work by Jewell *et al*, (1994) supports the idea: they found that the temperature in the apex of the keel was higher in the dark morph and that at most sites studied, the dark morph tended to inhabit colder micro-sites. The authors concluded that if there is an optimum temperature for pollen germination and pollen tube growth it would be reasonable to suggest that in cold micro-sites the dark-keeled plants would be better adapted, but in warmer sites the light-keeled plants would dominate, so maintaining the polymorphism (Jewell *et al*, 1994).

Keel colour has not been found to influence pollinator foraging behaviour or colonization by phytophagous invertebrates nor is there published evidence that morphs differ in terms of maternal reproductive output (Jones *et al*, 1986). However, there has been some unpublished experimental work which suggests that plants with a dark keel produce more seeds that those with light keels (McKee, 1997). No evidence has been found for selective damage by herbivores to petals, buds, pods or leaves and no difference between morphs for infestation by seed herbivores or in the production of good viable seed has been established (Compton, Beesley and Jones, 1988). However, few experiments have been done to support these studies and additional evidence would be useful.

Cyanogenesis

In experimental work presented in Chapters four and five of this thesis the cyanogenic status of plants was recorded; in Chapter four the aim was to investigate a possible link between cyanogenesis and plant fitness, and in Chapter 5 keel colour was recorded to differentiate between and to characterise plants.

It has been shown that *L. corniculatus* is polymorphic for cyanogenesis in the leaves and that cyanogenesis is the dominant trait (Dawson 1941). Production of HCN in *L. corniculatus* depends upon the hydrolisis of a substrate, in this case the cyanoglucosides linmarin and lotaustralin, by the β-glucosidase linmarase, when the leaf tissue is damaged, thereby releasing hydrocyanic acid (Jones 1962; Ramnani & Jones, 1984b). Linamarin and lotaustralin may occur independently but usually occur together (Jones, 1972a). β-glucosidase is determined by a single dominant allele and so are linamarin and lotaustralin but they are not linked (Jones, 1972a). Some phenotypes are stable but others are plastic and change phenotype (Jones, 1962). The cues that control this are not fully understood but it has been shown in some cases there is a relationship between temperature and changes in phenotype (Ellis, Keymer & Jones, 1977c; Jones & Ramnani, 1985). Jones and Ramnani (1985) suggest that overall there is a positive correlation between the frequency of cyanogenic morphs and increases in temperature. This plasticity is significant for experimental work, as an individual plant may change phenotype with the seasons. As Jones (1977) noted, any changes in the recorded frequency of cyanogenesis in a population when testing over a period of time may be due to phenotypic plasticity, not real changes in allele frequency. In experimental work described in Chapters four and five of this thesis plants were tested for cyanogenesis on a single occasion; the number of plants in the study and the need to test within one day (where possible) meant it was not possible to do so more often. In Chapter four, randomly chosen plants growing in previously established grassland at Swanworth Quarry were tested over a period of six months in order to ascertain how common it was for plants to exhibit plasticity in this trait. The results were used to establish a notional level of confidence with which experimental work could be interpreted.

There is no clear cline for the distribution of cyanogenic morphs in Britain (Jones, 1977) but there is evidence that populations tend to be heterogeneous and that there are changes in phenotype over small distances (Jones, 1972b). Genetic polymorphisms are maintained by some selective force and it has been suggested that predation of the acyanogenic form is part of the mechanism in this case (Jones, 1962). It has long been considered that the production of cyanogenic compounds is at least in part a defence mechanism to inhibit grazing by herbivores (Jones, 1962; Jones *et al.*, 1962). In particular, molluscs preferentially graze on acyanogenic plants and it has been suggested that rabbits and voles may also avoid cyanogenic morphs (Jones, 1962). Incidence of cyanogenesis inhibits herbivory in some organisms but may not prevent it completely. The earwig, *Forficula auricularia,* prefers to feed on acyanogenic morphs but will tolerate cyanogenic morphs if necessary. *F. auricularia* has developed a method of chewing leaves a little to induce wilting which reduces the production of HCN, allowing the earwig to return and finish feeding (Compton & Jones, 1985). Preferential grazing may not only be a matter of toxicity. *Agrilomax reticulates* will eat both acyanogenic and cyanogenic morphs of *Trifolium repens* but will exhibit differential eating in *Lotus corniculatus,* something which may be attributed to palatability (Jones, 1972a). Those species which have *L. corniculatus* as a principal part of their diet are likely to be adapted to tolerate a cyanogenic diet and show a general lack of selectivity (Compton & Jones, 1985). Insects which are generalist feeders and have *L. corniculatus* as a part of a varied diet tend to be more selective. In other studies, soil water content (Foulds & Grime, 1972) and temperature changes (Ellis, Keymer & Jones, 1977c) have been found to be linked with incidence of cyanogenic morphs but no single factor has been identified to explain morph distribution.

The effect of cyanogenesis on plant fitness has not been thoroughly investigated. Keymer & Ellis (1978) showed that in a common garden the cyanogenic phenotype appears to be more productive than the acyanogenic phenotype. They applied different treatments to represent selective pressures that might be encountered in the field such as non-selective grazing (by cutting), salt applications and trampling and showed the performance of both phenotypes was reduced when exposed to these pressures. In particular, grazing reduced the performance of the phenotypes differently; reproductive output of the cyanogenic morph was proportionally much greater that that of the acyanogenic morph when grazed (Keymer & Ellis, 1978). There is a need for more research to investigate the effect of cyanogenesis on plant fitness.

2.2 General Methods

2.2.1 Method for measuring plant characters.

A sketch of *L. corniculatus* (Figure 2.2) illustrates its morphology. The number of branches on each plant was counted. Quantitative traits were measured using calipers. On each plant, the third inter-node of the longest branch was measured. Together, branch number and inter-node length were used as an estimate for plant size. The length of the distal leaflet, of the five largest leaves on the longest branch, was measured and mean leaf length was calculated. The width of the whole leaf, of the five largest leaves on the longest branch, was measured and mean leaf width was calculated (recorded as leaf span). Categorical traits were scored as follows; growth habit (scored as prostrate, decumbent or erect), leaf shape (scored as obvate, obtuse or apiculate) and leaf pubescence (scored as glabrous, sparsely pubescent or pubescent).

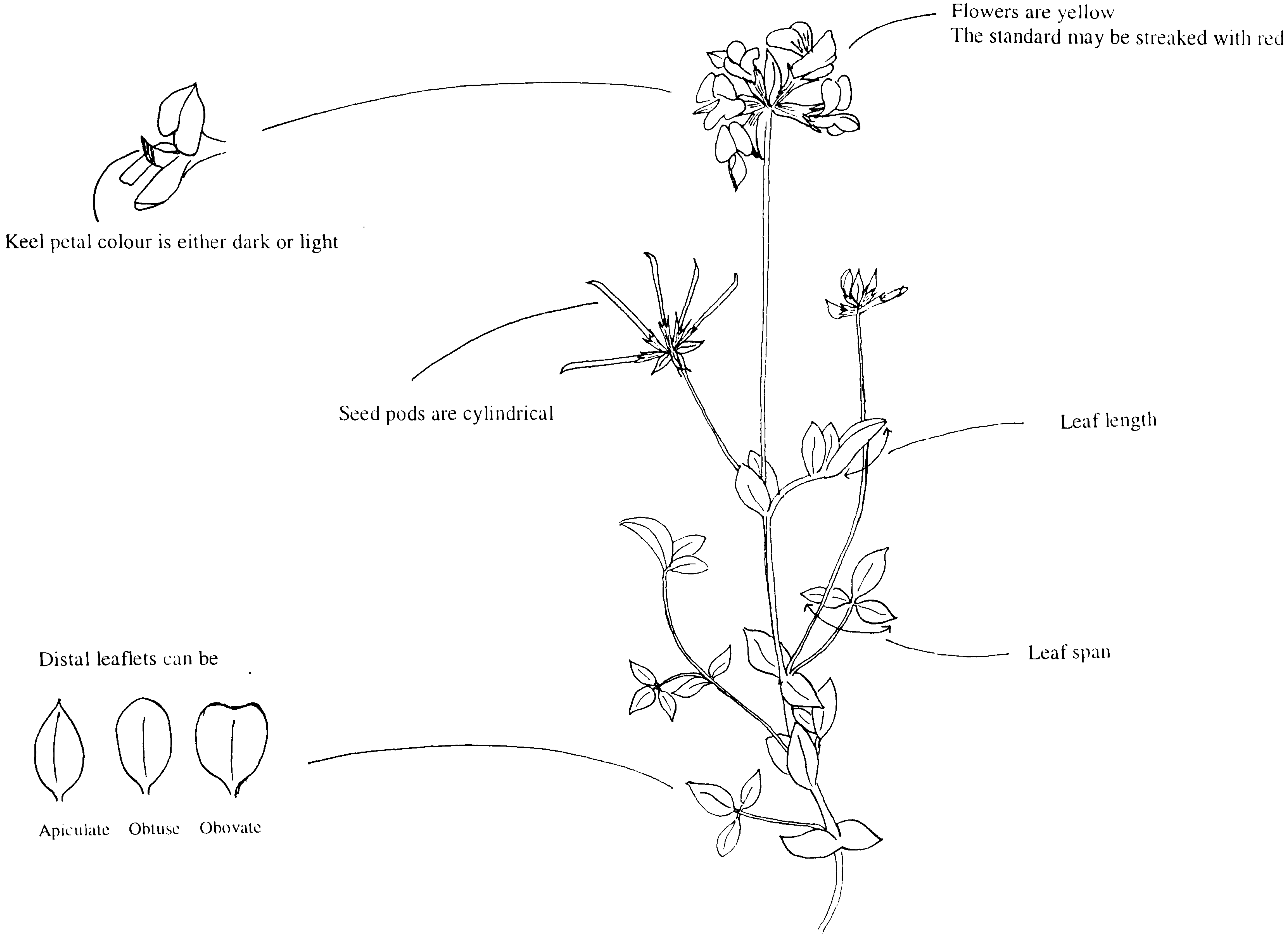

Figure 2.2 Sketch of *Lotus corniculatus* L.

2.2.2 Seed cleaning and weighing.

To obtain data for analysis in Chapters three and four, harvested seeds were cleaned and weighed. To release the seed, the seed pods were gently pressed in a sieve with a pestle which caused them to split. The resulting seed sample contained some remnants of husk and these were removed by placing the sample in a deep glass dish and gently blowing with a hairdryer on a low cool setting. The light husk readily separated from the heavier seeds and a clean seed sample was prepared. The total seed mass was weighed. Three batches of 20 seeds were then weighed separately to calculate mean seed weight. Total seed mass was divided by mean seed weight to derive an estimate for total seed number. These seed yield characteristics have also been shown to be under genetic control (reviewed in Jones & Turkington, 1986). After weighing, the seeds were inspected for damage by invertebrates. Typically, seed damage meant either small holes in the seeds or more extensive damage where a larger part of the seed was completely eaten. If there was any sign that the seeds had been attacked, the sample was recorded as predated. The proportion of plants with damaged seeds from each site was calculated.

2.2.3 The test for cyanogenesis

Plants were tested using Dawson's method (Dawson, 1941). Sodium picrate papers were prepared by dissolving 25g of Na_2CO_3 in a solution of 5g of picric acid (C.P.) in 1L of distilled water. Strips of absorbent chromatography paper were soaked in the solution prior to use. To test a plant for cyanogenesis, three leaves were randomly chosen, removed and placed into a glass tube where they were crushed with a glass rod. Two drops of toluene were added to aid the break down of the cell membrane, thereby facilitating the release of HCN. A prepared sodium picrate paper was then suspended above the crushed leaves in the tube and secured with a stopper. In the presence of HCN the yellow paper turned red.

2.2.4 The calculation of geographical and ecological distance

Geographical distance

The geographical distance between sites referred to in Chapters three and four was the Euclidian distance measured in kilometres. Pair-wise distances were calculated by entering Ordnance Survey co-ordinates into 'Mantmake' (R.T.Clarke, *pers. comm.*) In

Chapter five, geographical distance between the sample points on the transect was measured in metres and pair-wise distances were also calculated using Mantmake.

Ecological distance

At each of the sites referred to in Chapters three and four, the cover of plant species present was recorded (using the Domin scale) in 20 randomly placed 1m x 1m quadrats. The data collected from all sites used in the experiment in question were ordinated in a single Detrended Correspondance Analysis (DECORANA). The mean positions of the twenty quadrats from each site on the first two axes generated by DECORANA were used as co-ordinates to calculate the pair-wise ‘distance’ between the sites using Mantmake. In this way degrees of difference between each site's plant community was quantified and used as a surrogate for ecological distance. In Chapter five, ecological distance between points on the transect was calculated in the same way but used single quadrats of vegetation data at each sampling point on the transect.

Chapter Three

Quantifying the genetic component of provenance as estimated by plant morphology and fitness in a common garden experiment

3.1 Introduction

Intra-specific variation in the morphology of geographically separated populations of plants is well documented (for example: Clausen, Kerk & Hiesey, 1940; Daday 1940; Bonnemaison & Jones, 1986; Meyer & Monsen, 1991). In a series of classic experiments, Clausen *et al.* (1940;1947;1948) grew populations of plants from different locations in common garden trials to examine morphological differences. From these experiments the authors developed a theory which described the adaptation of plants to local environmental conditions through natural selection. Recently, studies demonstrating morphological variation between geographically separated populations have used the local adaptation hypothesis to support arguments for using only locally-sourced material in restoration projects (Sorensen, 1983; Knapp & Dyer, 1997; Knapp & Rice, 1997; Keller & Kollman, 1999). The definition of 'local' in this context is unclear. It has been argued that plants collected in close proximity to the restoration site will be similar to local plants and therefore be better adapted to survive at the site (Knapp & Rice, 1997). However, not all studies demonstrate that distance between populations has an effect on their differentiation. Using seven populations, Galloway & Fenster (2000) showed a lack of differentiation between populations of *Chamaecrista fasiciculata* within 1000km. It has also been suggested that the adaptation of plants to local selection pressures will mean that it is most appropriate to collect seeds from a similar environment rather than using geographical proximity as a 'rule of thumb' (Montalvo & Ellstrand, 2000).

In this study, the effect of geographical and ecological provenance on plant morphology and seed yield traits was estimated. Investigating the effect of provenance on variation in heritable morphological traits will identify whether selection at a regional scale has taken place, or whether differences between plants are a response to local environmental conditions. Measuring seed yield traits will not only demonstrate variation, it will estimate the relative fitness of plants. Fitness is here defined as the contribution that plants will make to the next generation

(Demetrius, 1977). Previous studies investigating the importance of plant provenance tend to focus on one .of these areas, either on morphology or plant fitness. For example, Knapp & Rice (1997) showed that there was a correlation in quantitative trait variation and climatic variation in eight populations of *Nasella pulchra*, but did not carry out an assessment of reproductive output to support their findings. In contrast, Sorensen (1983) found that seeds collected from two populations of Douglas fir on west and east facing slopes of a mountain differed in seed size. These seeds were germinated in laboratory conditions and differences were found in the timing of germination and in the resource allocation in roots and shoots. The variation found between these two populations was attributed to evolution of locally adapted genotypes but no morphometric analysis was carried out. Similarly, Keller & Kollman (1999) described differential germination in eight weed species collected from four sites but did not correlate these findings with morphological variation. Neither Keller & Kollman (1999) nor Sorensen (1983) quantified the geographical separation between the experimental populations or defined the environmental differences that they noted.

The advantage of investigating the effect of geographical and ecological provenance on variation in both morphology and seed yield characteristics in native populations is twofold. Firstly, it would establish if variation in the chosen species was related to provenance and demonstrate the relative importance of geographical location and ecological conditions at the donor site. Secondly, it would be possible to establish if there is a link between morphology and plant fitness. It would aid those procuring seeds for restoration projects if it was known whether provenance affects plant performance and whether observed morphological variation is related to subsequent plant fitness. This study will meet these needs. To establish the effect of provenance on heritable differences between populations it is necessary to raise plants in a common garden. Using a common environment is an attempt to control the experimental conditions, removing, as much as possible, environmental heterogeneity so that genetic variation is investigated (Jones & Wilkins, 1971).

3.1.2 Aim and Objectives

The aim of this study was to quantifying the genetic component of provenance, conferred by the maternal parent, as estimated by plant morphology and fitness in a common garden experiment. The purpose was to investigate heritable traits that may affect plant fitness in a restoration environment.

The objectives were to:

- Determine whether plant survival is affected by propagule provenance

- Investigate the effect of provenance on plant morphology and seed yield characteristics.

- Investigate the effect of geographical and ecological distance between seed collection sites on the magnitude of difference between plant character traits. (This would establish if plants from different populations were increasingly different as separation between the origins of the populations increased).

- Identify any association between morphology and plant fitness.

Seeds were collected from two contrasting habitats in six regions and the plants were raised in a common garden. Data were analysed to investigate regional differences and also differences accounted for by ecological conditions at the seed collection site. Plant phenotype and seed yield characteristics were correlated to investigate the relationship between morphology and plant fitness.

3.2 Method

3.2.1 Seed collection

Seeds of *L. corniculatus* were collected from twelve populations in August 1997, listed in Table 3.1. Seeds were collected from a calcicolous grassland and an alternative habitat in each of six regions. The alternative habitat varied between regions but, in each case, was the nearest contrasting site (which supported a population of *L. corniculatus*) to the calcicolous grassland chosen. In order to sample plants randomly, two 100m tapes were laid out at right angles to form a grid. Seeds were then collected from 10 plants closest to randomly generated co-ordinates within the grid. All the seeds on each of the plants were collected by removing the umbels intact, using scissors to snip the stem. These umbels were stored in brown paper envelopes that effectively prevented sweating and slowed desiccation: this minimized pod shattering.

Table 3.1 Seed collection sites of *Lotus corniculatus* in the British Isles.

Site	OS Reference	Ecology	Description
Dorset			
Swanworth Quarry	SY968782	Calcicolous grassland	Rabbit grazed limestone grassland on the margins of a quarry. Dominated by *Festuca rubra* and *Lotus corniculatus* with characteristic limestone species.
Chapman's Pool	SY956771	Coastal grassland	Dry coastal grassland dominated by *Arrenatherum elatius, Festuca rubra, Holcus lanatus*. Frequent *Carex panicea*. Patches of bare ground with *Tussilago farfara*.
East Sussex			
Mount Cabourn	TQ448090	Calcicolous grassland	Chalk grassland. Dominated by *Festuca ovina* and *Bromus erectus* with many characteristic herbs of chalk grassland. Sheep and rabbit grazed.
Lullington Heath	TQ545017	Chalk Heath	Chalk heath community on neutral loess soil. *Calluna vulgaris* , *Festuca rubra* mosaic with both calcicolous and calcifugous herbs. Short turf, rabbit grazed.
Isle of Wight			
Afton Down	SZ8538998	Calcicolous grassland	Rabbit grazed chalk grassland, dominated by *Festuca rubra*, Abundant *Koeleria macrantha* and typical chalk grassland species.
Newtown meadows	SZ4058998	Ungrazed salt marsh	Wetland meadow dominated by *Holcus lanatus*. With *Succisa pratensis, Blackstonia perfoliata*, *Genista tinctoria* .
Norfolk			
Ringstead Downs	TF691401	Calcicolous grassland	Rabbit grazed chalk grassland. South facing slope in a dry valley. Species rich sward, dominated by *Festuca rubra, Festuca ovina* with abundant *Helianthemum nummularium* and *Sanguisorba minor*.
Hunstanton Dunes	TF690430	Coastal dune	Coastal grassland between stabilized dunes. Patchy with areas of bare sand. Dominated by *Ammophila arenaria* and *Festuca rubra* with abundant *Rhinanthus minor* and *Lotus corniculatus*. Some rare coastal plants such as *Eryngium* sp.
Somerset			
Brean Down	ST290580	Calcicolous grassland	Rabbit grazed limestone grassland. Dominated by *Anthoxanthum odoratum* and *Festuca rubra*. Frequent *Plantago lanceolata* and *Galium verum*. Rich in limestone species.
Berrow Dunes	ST293520	Coastal Dune	Stable dune grassland. Dominated by *Festuca rubra* and *Carex arenaria*. Area included a small dune slack with *Oenanthe lachenalii* and *Dactylorhiza incarnata*.
North Wales			
Great Ormes Head	SH770830	Calcicolous grassland	Rabbit and sheep grazed limestone grassland. Dominated by *Festuca ovina, Festuca rubra* and *Anthoxanthum odoratum*. Abundant *Helianthemum nummularium*.
Conwy Morfa	SH767791	Coastal dune	Patchy coastal grassland on shingle. Abundant *Ononis repens, Elytrigia atherica* and *Ammophila arenaria*.

3.2.2 Seed Sowing and Propagation

Experimental work took place at the Institute of Terrestrial Ecology (now part of the Centre for Ecology and Hydrology), Furzebrook, Dorset. Collected seeds were sown in January 1998. The seeds were removed from the pods and scarified to encourage germination. Scarification was carried out by gently rubbing the seeds between two sheets of fine glass paper. Each maternal source was allocated a 5cm pot filled with peat compost into which all the seeds were sown. The pots were placed into plastic trays in order to retain any excess water and to slow down desiccation and were then placed on benches in a greenhouse maintained at 15^{0}C. The day length was extended to twelve hours with mercury vapour lamps. By 28 days after germination, the seedlings appeared robust and ten were selected randomly from each pot and planted individually into 15cm pots containing peat compost. The potted plants were placed into plastic trays and returned to the greenhouse. Pot position in the greenhouse was fully randomized. Lamps were used until natural day length reached 12 hours.

3.2.3 Characterisation of plants

In March the plants were large enough to enable morphological data collection. The following traits were recorded using the method described in the *General Methods* section of this thesis; branch number, inter-node length (mm), leaf length (mm), leaf span (mm), growth habit (scored as prostrate, decumbent or erect), leaf shape (scored as obovate, obtuse or apiculate) and leaf pubescence (scored as glabrous, sparsely pubescent or pubescent). In April the plants were removed from the greenhouse and placed into a gravel filled plunge bed in a fully randomised array. They were checked daily, watered as necessary and any mortalities were recorded. In July the first seed pods reached maturity. Seed pods were considered ripe when the pod began to dry and had turned brown. To ensure that all seeds were collected before the pods dehisced, the plants were checked every three days and any mature seed pods were collected with the umbels retained intact. The pods were stored in brown paper envelopes. Collection continued until September when harvesting was complete.

Using the methods described in the *General Methods* section of this thesis, four components of seed yield were measured; number of umbels, number of pods, total seed mass and mean seed weight. The proportion of plants with damaged seeds from each site was also recorded.

3.2.4 Analysis

Correlation of geographical and ecological distance between seed collection sites (pair-wise, between all sites) was carried out using a Mantel randomisation test (Mantel, 1967) using a program written by RT Clarke (*pers comm*), following the procedures outlined in Manley (1997). A Mantel test deals with two matrices (X and Y), obtained independently and describing the relationships between the same sampling points (Mantel, 1967). X matrix is held constant while *n* (in this case 10,000) randomisations of Y matrix are computed to derive random columns. The statistical significance of the observed value is the proportion of the derived values which are more than or equal to the observed value.

The effect of provenance on morphology and fitness

To investigate the effect of provenance on the recorded traits, the data were grouped by region and habitat of origin. Regional groups contained the two populations collected from each county combined together. Differences between these groups are hereafter referred to as regional differences. Data were also divided into two further groups, those collected from calcicolous grassland and those collected from the alternative habitat. The aim of these groupings was to distinguish between variation accounted for by geographical separation and that accounted for by the type of site. GLM Analysis of Variance (ANOVA) was used to investigate differences in the quantitative character traits: branch number, inter-node length, leaf length, leaf span, number of umbels, number of pods, seed weight and seed number. The data collected for branch length and leaf length fulfilled the assumptions for ANOVA, but all other quantitative data were square root transformed before analysis to ensure equality of variance between groups. To establish if there were significant differences in plant type between the regions and the habitats of origin, the categorical characters (survival, growth habit, leaf pubescence, leaf shape and seed damage) were analysed using chi-squared analysis. Again, the data were categorised into one of six regions and also as calcicolous grassland or alternative habitat.

A Mantel randomisation test was used to establish if the magnitude of differences in quantitative morphological variables and seed yield characteristics were correlated with geographical or ecological distance.

Association between traits

Association between character traits was investigated using correlation, chi square analysis and GLM ANOVA. The quantitative character traits were correlated using Pearson's correlation co-efficient and the association between categorical data was investigated using chi-square analysis. To establish if there was a relationship between the quantitative traits and the categorical data GLM ANOVA was employed and quantitative traits were grouped using morphological categories.

3.3 Results

The geographical distance (measured in kilometres), and the ecological distance, (measured in separation along axes generated by DECORANA), between all seed collection sites are shown in Table 3.2. There was no correlation between geographical and ecological distance (r =-0.189 *P*=0.807) so it was possible to investigate the effects separately .

Table 3.2 The pairwise geographical and ecological distance between all collection sites. Geographical distance is shown in kilometres.
Ecological distance is estimated by the degree of difference between the vegetation assembage at each site as measured by DECORANA (DCA score).

Geographical distance (Km)

	Afton Down	Berrow Dunes	Brean Down	Chapman's Pool	Conwy Bay	Great Orme	Hunstanton Dunes	Lullington Heath	Mount Cabourn	Newtown Meadows	Ringstead Down
Afton Down											
Berrow dunes	126.1										
Brean Down	130.2	7.0									
Chapman's Pool	41.8	100.0	105.6								
Conwy Bay	334.2	126.1	226.2	324.6							
Great Orme	337.5	236.8	230.0	328.1	3.9						
Hunstanton Dunes	289.5	306.5	302.4	317.4	294.5	294.7					
Lullington Heath	119.1	230.7	232.7	160.8	392.6	395.1	241.7				
Mount Cabourn	110.8	219.7	221.5	152.6	380.6	383.1	235.2	12.1			
Newtown Meadows	8.4	128.4	132.1	49.1	332.0	335.2	281.5	112.5	103.8		
Ringstead Down	287.0	304.8	300.7	315.1	295.0	295.2	2.9	238.8	232.4	279.0	
Swanworth Quarry	40.4	100.0	105.5	1.6	324.0	327.5	313.5	159.4	151.2	47.6	313.5

Ecological distance (DCA)

	Afton Down	Berrow Dunes	Brean Down	Chapman's Pool	Conwy Bay	Great Orme	Hunstanton Dunes	Lullington Heath	Mount Cabourn	Newtown Meadows	Ringstead Down
Afton Down											
Berrow dunes	108.4										
Brean Down	155.2	46.8									
Chapman's Pool	139.0	30.6	16.2								
Conwy Bay	95.0	13.4	60.2	44.0							
Great Orme	2.5	106.0	152.8	136.6	92.6						
Hunstanton Dunes	59.4	49.0	95.8	79.6	35.6	57.0					
Lullington Heath	20.6	87.8	134.6	118.4	74.4	18.2	38.8				
Mount Cabourn	65.5	173.9	220.7	204.5	160.5	68.0	124.9	86.1			
Newtown Meadows	320.0	211.6	164.8	181.0	225.0	317.6	260.6	160.5	385.5		
Ringstead Down	98.2	10.2	57.0	40.8	3.2	95.8	38.8	77.6	163.7	221.8	
Swanworth Quarry	130.2	21.8	25.0	8.8	35.2	127.8	70.8	109.6	195.7	189.9	32.0

3.3.1 Survival

There was variation in the percentage of plants which survived the seedling stage and grew to adult plants (Figure 3.1). In particular, those grown from seed collected at Conwy Morfa in north Wales suffered high mortality; only 9 plants remained for analysis. Chapman's Pool (Dorset), Afton Down (Isle of Wight) and Hunstanton Dunes (Norfolk) all showed high survival rates of 80% and above. There was no significant variation in survival between plants from different regions (*F*= 0.858, *P*= 0.564) and there was no difference in survival of plants between those sourced from calcicolous grassland and those from the alternative habitat (*F*= 0.039, *P*= 0.851). The analysis is summarized in Table 3.3.

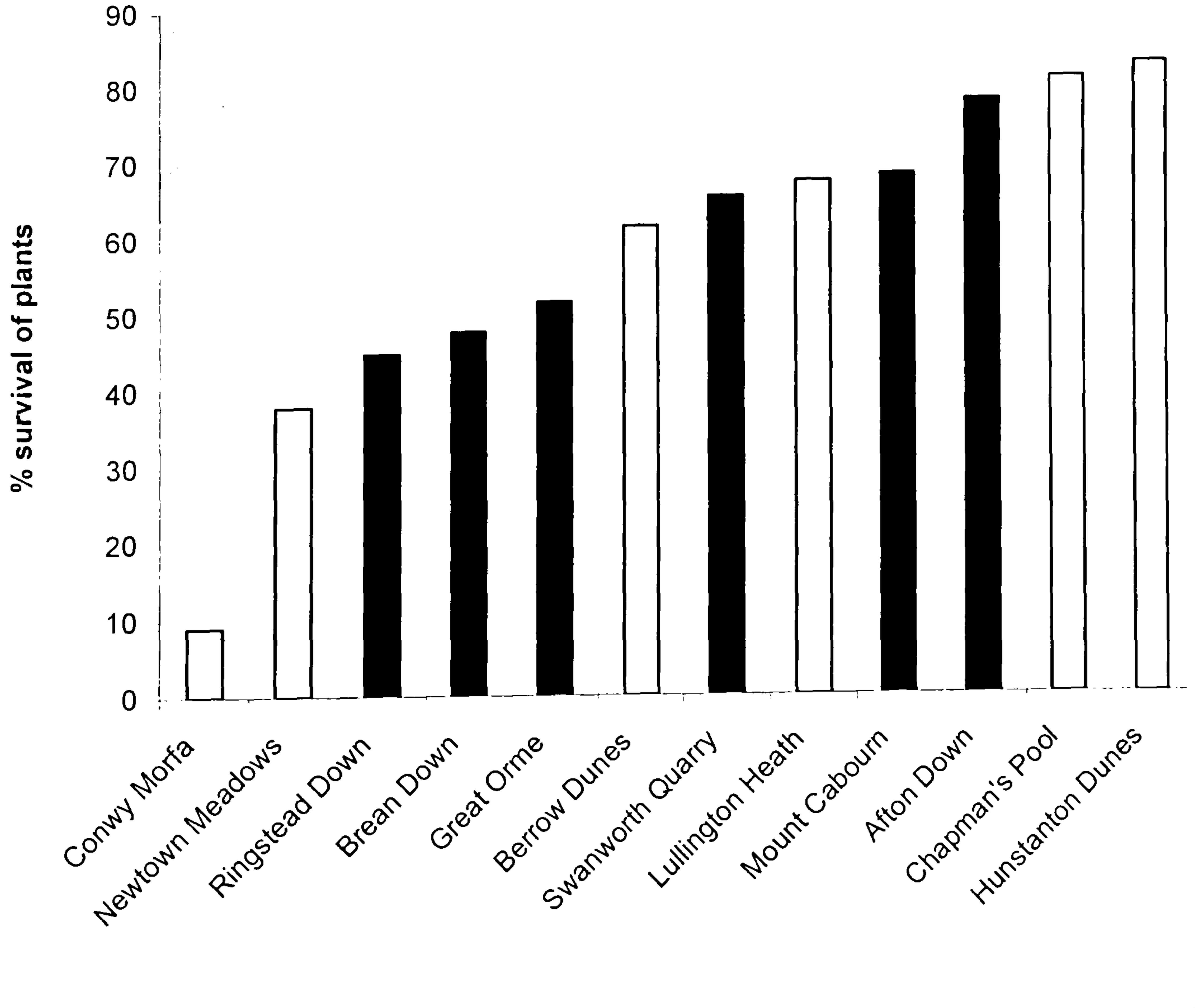

Figure 3.1 Percent survival of seedlings in *L. corniculatus* populations. Calcicolous grassland represented by dark bars, alternative habitat represented by light bars.

Table 3.3 Variation in mean percentage survival of plants grown from seed sourced from calcicolous grassland and an alternative habitat in six regions of the British Isles.

	Mean survival (%) ± SE
Region	
Dorset	74.0 ±11.31
East Sussex	68.5 ±0.50
Isle of Wight	58.5 ±20.50
N. Wales	30.5 ±21.50
Norfolk	64.5 ±19.50
Somerset	55.0 ±7.00
ANOVA	**df = 5, F= 0.858, *P*= 0.564**
Habitat of origin	
Calcicolous grassland	59.833 ±7.25
Alternative habitat	57.167 ±10.42
ANOVA	**df = 1, F= 0.039, *P*= 0.851**

3.3.2 The effect of provenance on morphology and fitness

There were differences in morphological traits and in seed yield characters both regionally and between the calcicolous grassland and the alternative habitat. Not all the plants which were scored for morphological traits survived to yield fruit. Therefore, there is a discrepancy in the number of plants between the analyses for morphology and that for seed yield traits. 703 plants were scored for morphology whilst 682 survived to bear seeds. Within seed yield traits, 20 plants either did not form seed pods or the seed pods were empty which means that the number of individuals scored for total seed number is less than those scored for number of umbels and pods

Morphology

Table 3.4 summarizes the results. Inter-node length (*F*=8.039, *P*=<0.001), leaf length (*F*=12.86, *P*=<0.001) and leaf span (*F*=12.86, *P*=<0.001) differed between regions. Figures 3.2 –3.4 show that plants from east Sussex, and the Isle of Wight tended to have long inter-node lengths and large leaves. Plants from Somerset and Norfolk had short inter-node lengths and smaller leaves; north Wales and Dorset were intermediate in all three characters and similar to each other. Leaf length (F=6.94, *P*=0.009) and leaf span (F=3.676, *P*=<0.056) varied between habitat of origin. The number of branches per plant did not differ between regions, but plants from the

Table 3.4 The morphology of plants grown from *L. corniculatus* seeds collected at twelve locations around the British Isles and raised in a common garden. Data presented as means ±standard error. GLM ANOVA showed variation in the means by region and by habitat of origin. Significance: *= 0.05 ** = 0.01 *** =0.001

	Number of branches.		Inter-node length(mm)		Leaf length (mm)		Leaf span (mm)	
Regional means		N		N		N		N
Dorset	7.13 ±0.311	148	9.38 ±0.303	148	6.67 ±0.13	148	12.15 ±0.238	148
East Sussex	7.27 ±0.322	137	11.32 ±0.314	137	7.80 ±0.134	137	14.12 ±0.245	137
Isle of Wight	6.37 ±0.372	117	10.53 ±0.362	117	7.19 ±0.155	117	12.89 ±0.284	117
North Wales	7.01 ±0.679	61	9.34 ±0.662	61	6.65 ±0.284	61	12.15 ±0.519	61
Norfolk	7.27 ±0.345	130	8.61 ±0.339	130	6.45 ±0.144	130	11.91 ±0.264	130
Somerset	6.37 ±0.362	110	8.99 ±0.352	110	6.65 ±0.151	110	11.49 ±0.278	110
ANOVA	**F=1.43, *P*=0.211, df=5**		**F=8.04, *P*=<0.001***, df=5**		**F=12.86, *P*=<0.001***, df=5**		**F=12.94, *P*=<0.001***, df=5**	
Means by habitat of origin		N						
Calcicolous grassland	6.54 ±0.202	360	9.92 ±0.198	360	7.09 ±0.085	360	12.76 ±0.155	360
Alternative habitat	7.26 ±0.275	343	9.47 ±0.268	343	6.72 ±0.115	343	12.15 ±0.21	343
ANOVA	**F=4.35, *P*=0.037*, df=1**		***F*=1.44, *P*=0.23, df=1**		**F=6.94, *P*=<0.009**, df=1**		**F=3.67, *P*=0.056, df=1**	
Region * Habitat	**F=0.52, *P*=0.765, df=5**		***F*=0.74, *P*=0.592, df=5**		***F*=4.30, *P*=0.001***, df=5**		***F*=3.50, *P*=0.004**, df=5**	

alternative habitat had significantly more branches than those from the calcicolous grassland (*F*=4.355 *P*=0.037) and bore smaller leaves (F= 12.86, *P*=<0.001).

Figure 3.2 Mean inter-node lengths of plants grown from seed collected in six regions and grown in a common garden.

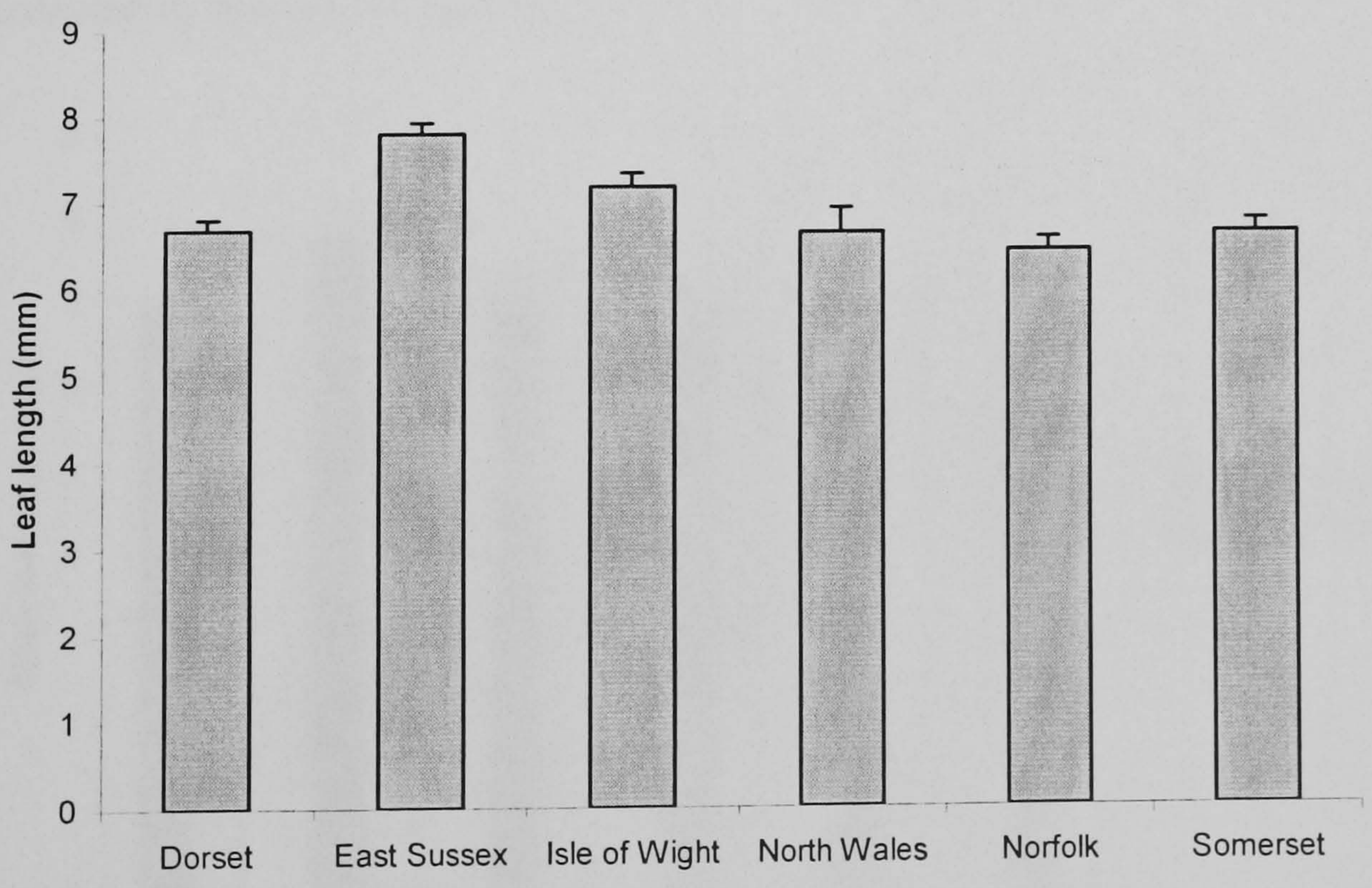

Figure 3.3 Mean leaf length of plants grown from seed collected in six regions and grown in a common garden.

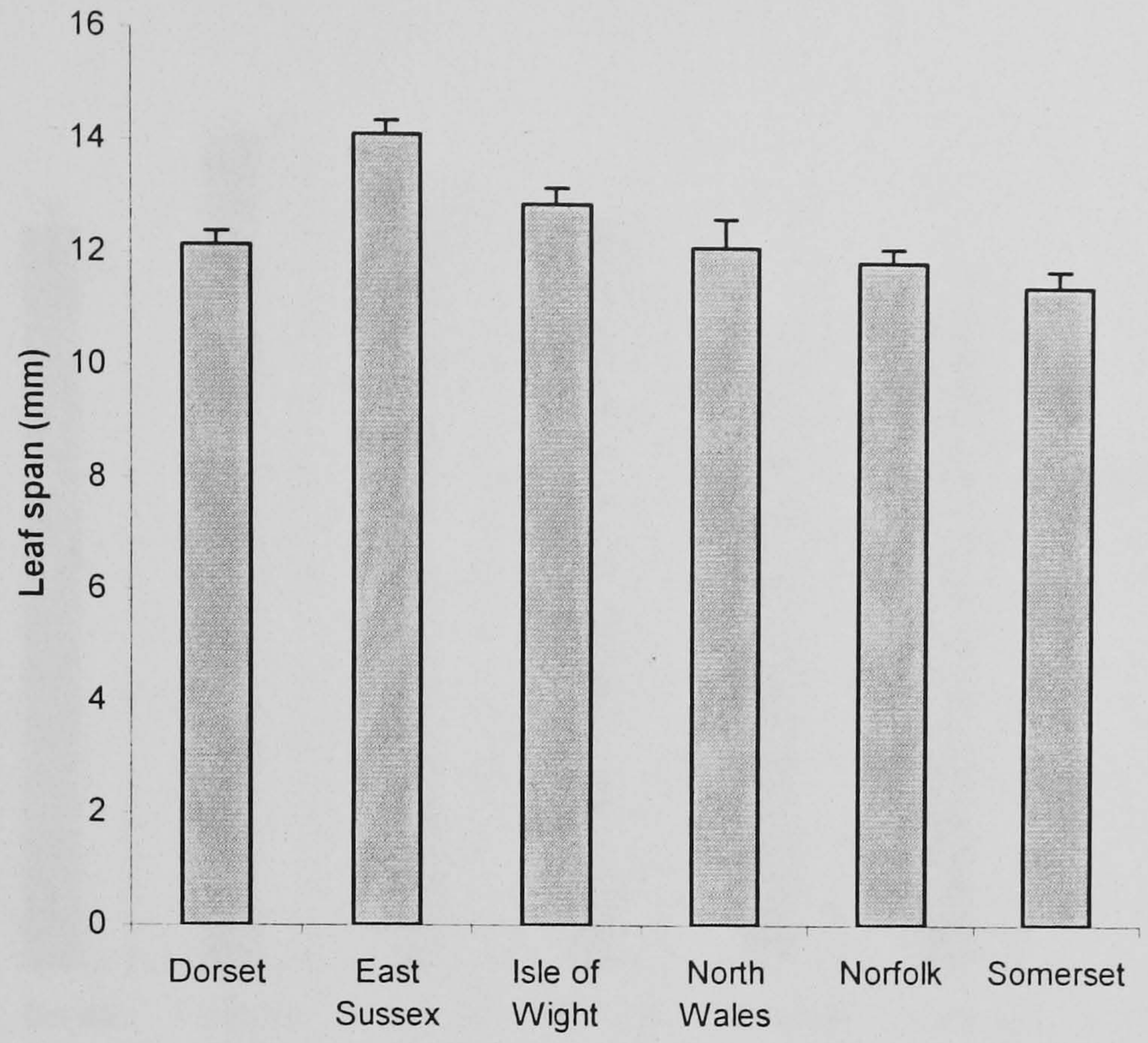

Figure 3.4 **Mean leaf span of plants grown from seed collected in six regions and grown in a common garden.**

There was an interaction between the effects of region and habitat of origin in the two measures of leaf size. This was due to the much smaller leaf length and leaf span of plants sourced from Hunstanton dunes, the alternative habitat, in Norfolk. This is illustrated in figures 3.5 and 3.6.

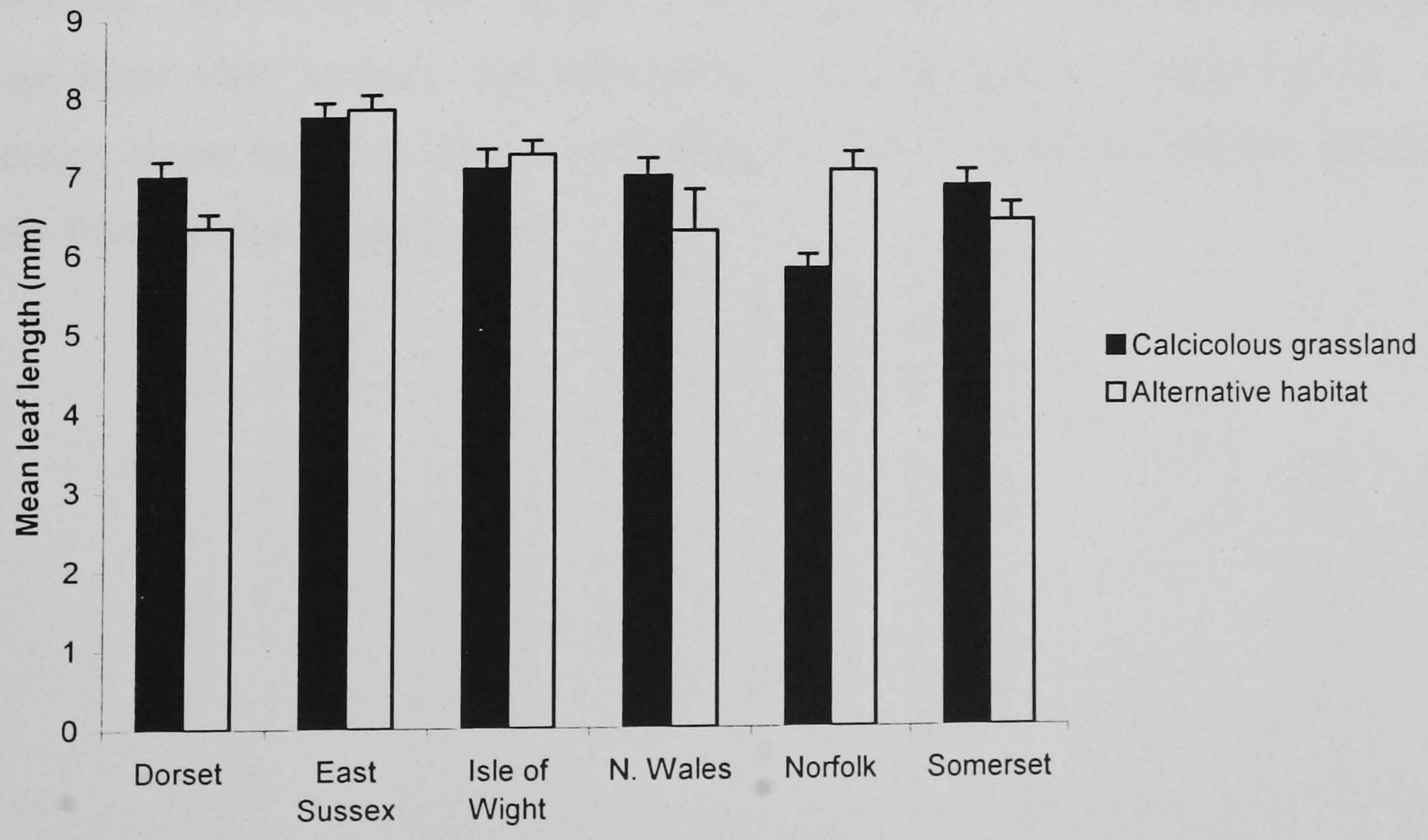

Figure 3.5 **Interaction between region and habitat of origin effects on variation in mean leaf length of plants collected from two habitats in six regions.**

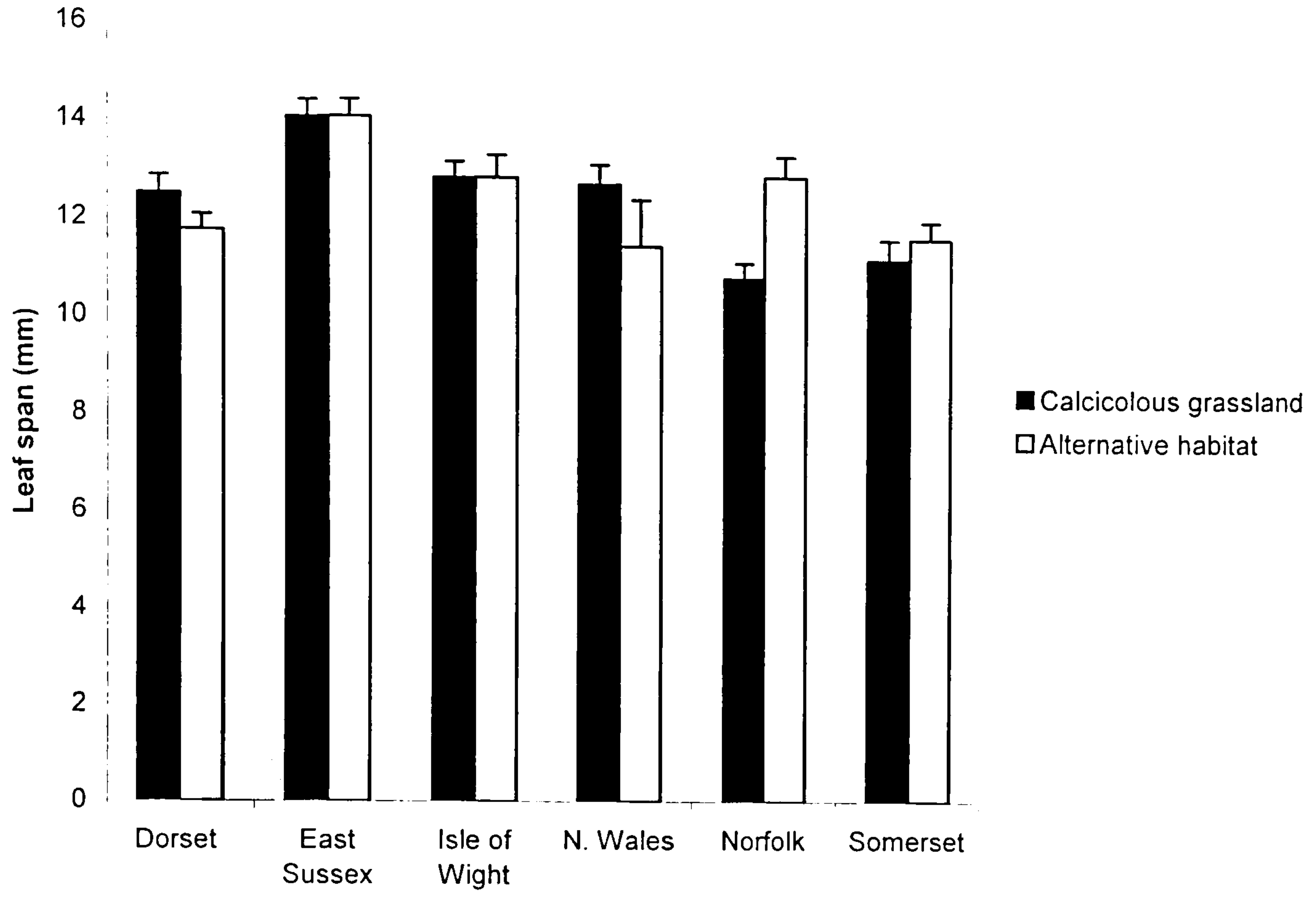

Figure 3.6 **Interaction between region and habitat of origin effects on variation in mean leaf span of plants collected from two habitats in six regions.**

The leaf shape, growth habit and degree of pubescence varied between regions and habitats of origin (Table 3.5). Plants were less hairy in Dorset and East Sussex than elsewhere. Dorset and Norfolk plants were significantly more often prostrate than those from other sources. The differences are illustrated in Figures 3.7-3.9. On average, plants from the alternative habitat of origin were hairier and less erect than those from calcicolous grassland.

Table 3.5 Seed damage, growth habit, pubescence and leaf shape of L. corniculatus plants from seed collected from two habitats in six regions and grown in a common garden. Significance: * = 0.05 **=0.01 ***0.001

	Seed damage		Habit			Pubesence			Leaf Shape		
Region	damaged	undamaged	Prostrate	procumbent	erect	glabrous	sparsely pubescent	pubescent	obovate	obtuse	apiculate
Dorset	95	53	84	58	6	24	75	49	75	27	46
East Sussex	53	62	46	73	18	19	71	47	68	45	24
Isle of Wight	78	39	47	65	5	13	40	64	70	19	28
North Wales	46	15	21	27	13	2	20	39	33	20	8
Norfolk	94	36	69	51	10	16	60	54	18	31	21
Somerset	73	37	49	53	8	5	41	62	45	8	57
	χ^2=12.56, *P*=0.028*		**χ^2 = 40.62, *P*= <0.001*****			**χ^2=40.12, *P*=<0.001*****			**χ^2=72.75, *P*=<0.001*****		
Habitat											
Calcicolous	288	213	152	163	45	48	164	146	205	78	77
Alternative	112	130	164	164	15	31	143	169	164	72	107
	χ^2=3.58, *P*=0.058		**χ^2=15.15, *P*= <0.001*****			**χ^2=6.456, *P*=<0.040****			**χ^2=9.28, *P*=<0.001*****		

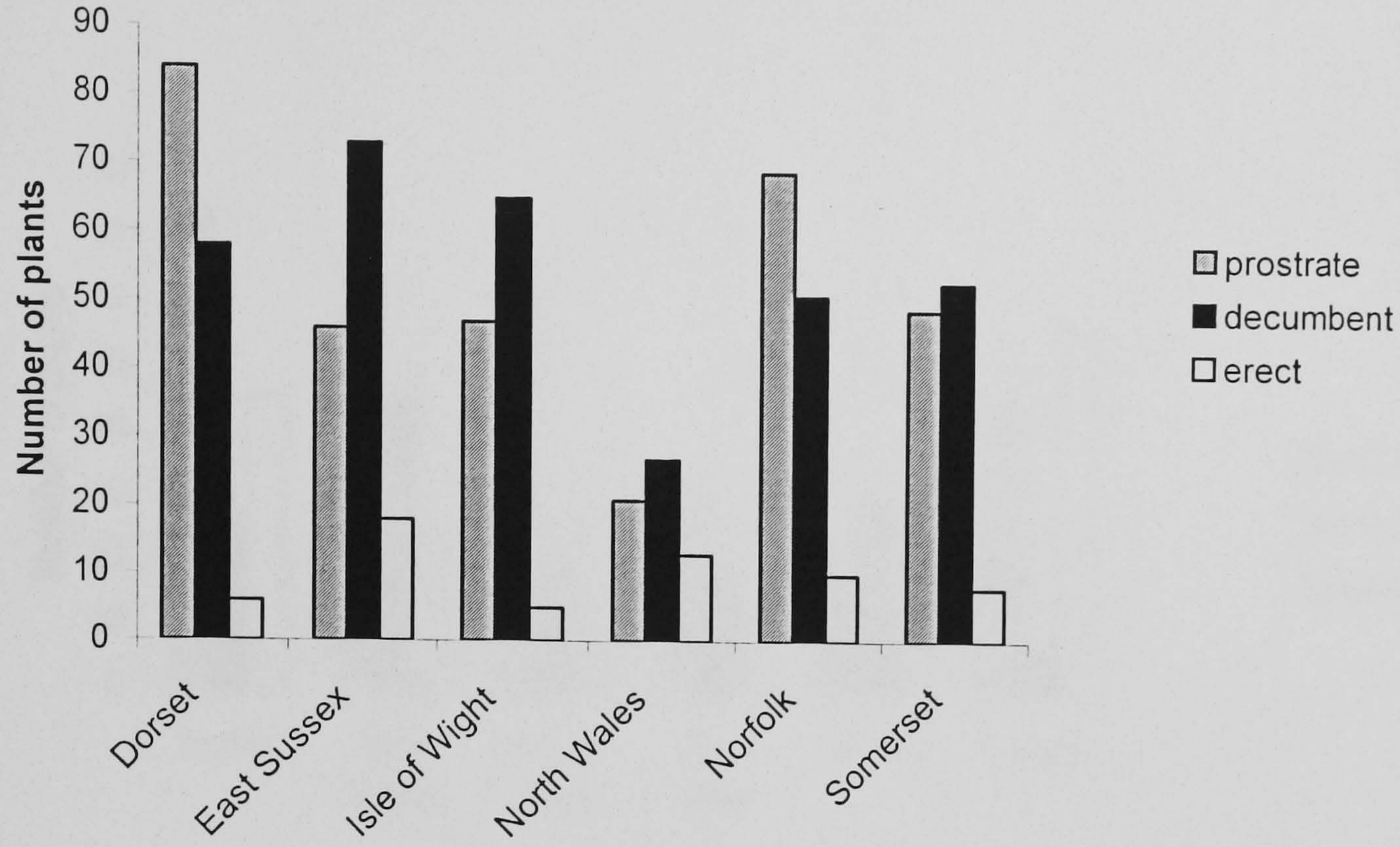

Figure 3.7 Proportion of plants with prostrate, decumbent and erect growth habit grown from seed collected in six regions and grown in a common garden.

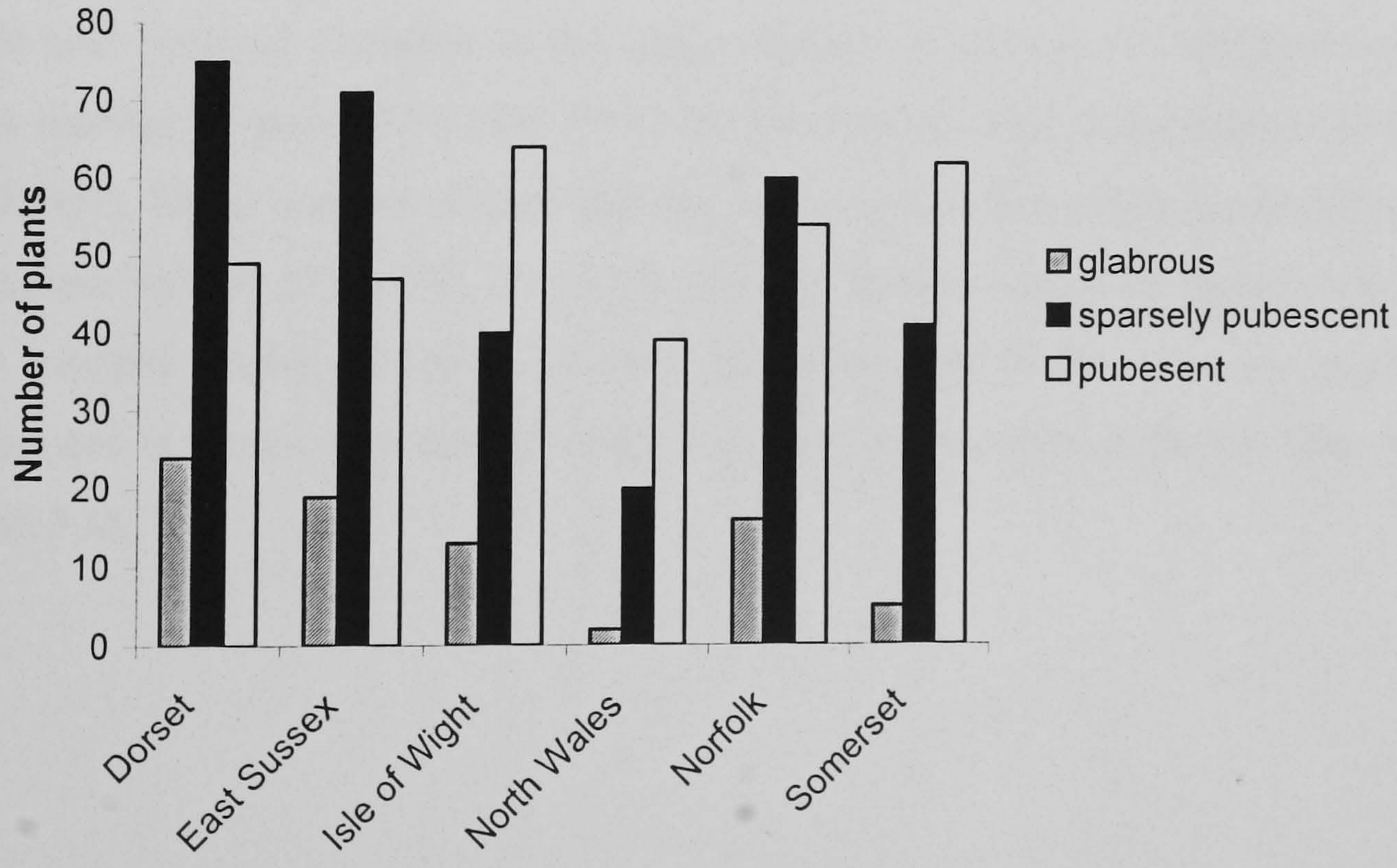

Figure 3.8 Proportion of plants with glabrous, sparsely pubescent and pubescent leaves grown from seed collected in six regions and grown in a common garden.

Figure 3.9 Proportion of plants with obovate, obtuse and apiculate leaves

grown from seed collected in six regions and grown in a common garden.

Reproductive characters

There was regional variation in the mean number of umbels (F=5.952, P=<0.001) mean number of pods (F=5.584, P=<0.001) and mean total seed number (F=5.526, P=<0.001). Mean number of pods differed between the calcicolous grassland and the alternative habitat (F=4.268, P=0.039). Neither mean number of umbels nor mean seed number varied between habitats of origin and there was no significant differences in mean seed weight either regionally or between different types of site (Table 3.6).

Table 3.6 The reproductive output of plants grown from *L. corniculatus* seeds collected at twelve locations around the British Isles and raised in a common garden. Data presented as means ±standard error. GLM ANOVA showed variation in the means by region and by habitat of origin.
Significance: *=0.05 level **= 0.01 ***=0.001

	Number of pods		Number of umbels		Seed no.		Seed weight (mg)	
Regional means		N		N		N		N
Dorset	32.41 ±2.851	137	11.59 ±0.909	137	214 ±18.679	141	1.32 ±0.213	141
East Sussex	48.56 ±2.844	135	14.64 ±0.907	135	206 ±19.764	124	1.18 ±0.227	124
Isle of Wight	43.31 ±3.262	117	16.21 ±1.04	117	274 ±21.873	116	1.64 ±0.249	116
North Wales	31.11 ±5.969	61	12.62 ±1.901	61	197 ±39.974	60	1.78 ±0.455	60
Norfolk	49.45 ±3.118	124	17.53 ±0.994	124	343 ±20.706	121	1.21±0.236	121
Somerset	43.69 ±3.196	108	16.72 ±1.025	108	228 ±22.184	100	1.24 ±0.253	100
ANOVA	**F=5.95, *P*=<0.001***, df=5**		**F=5.58, *P*=<0.001***, df=5**		**F=5.53, *P*=<0.001***, df=5**		**F=1.56, *P*=0.167, df=2**	
Means by habitat of origin								
Calcicolous grassland	43.05 ±0.074	346	15.91 ±0.579	346	272.53 ±12.037	351	1.52 ±0.137	351
Alternative habitat	39.80 ±0.099	336	13.86 ±0.772	336	233.63 ±16.445	311	1.27 ±0.188	311
ANOVA	**F=4.27, *P*=0.039*, df=1**		**F=1.63, *P*=0.202, df=1**		**F=1.72, *P*=0.190, df=1**		**F=1.38, *P*=0.241, df=1**	
Region * Habitat	**F=1.81, *P*=0.108, df=5**		**F=3.70, *P*=0.003**, df=5**		**F=1.25, *P*=0.282, df=5**		**F=0.80, *P*=0.547, df=1**	

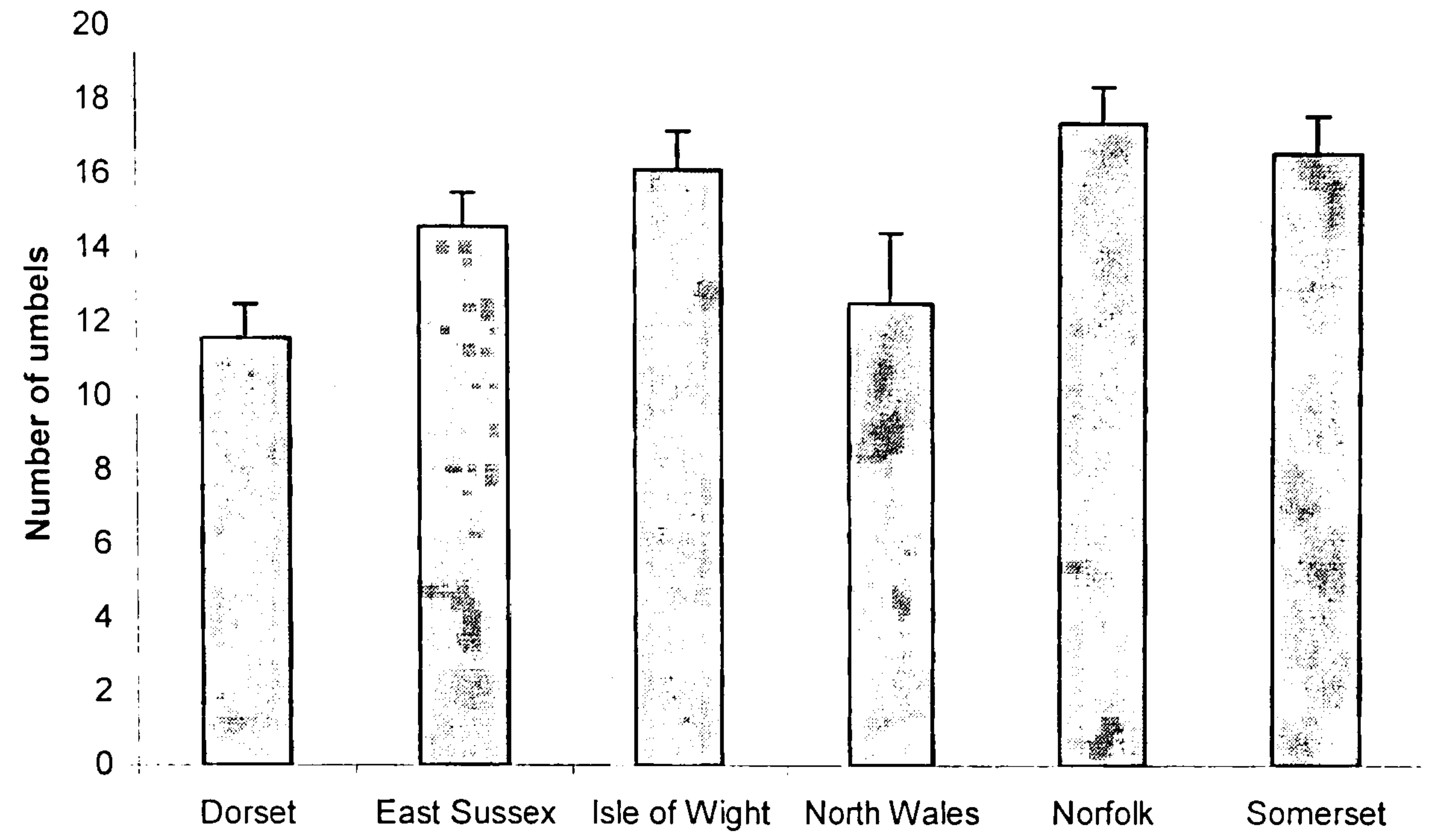

Figure 3.10 Mean number of umbels produced by plants grown from seed collected in six regions and grown in a common garden.

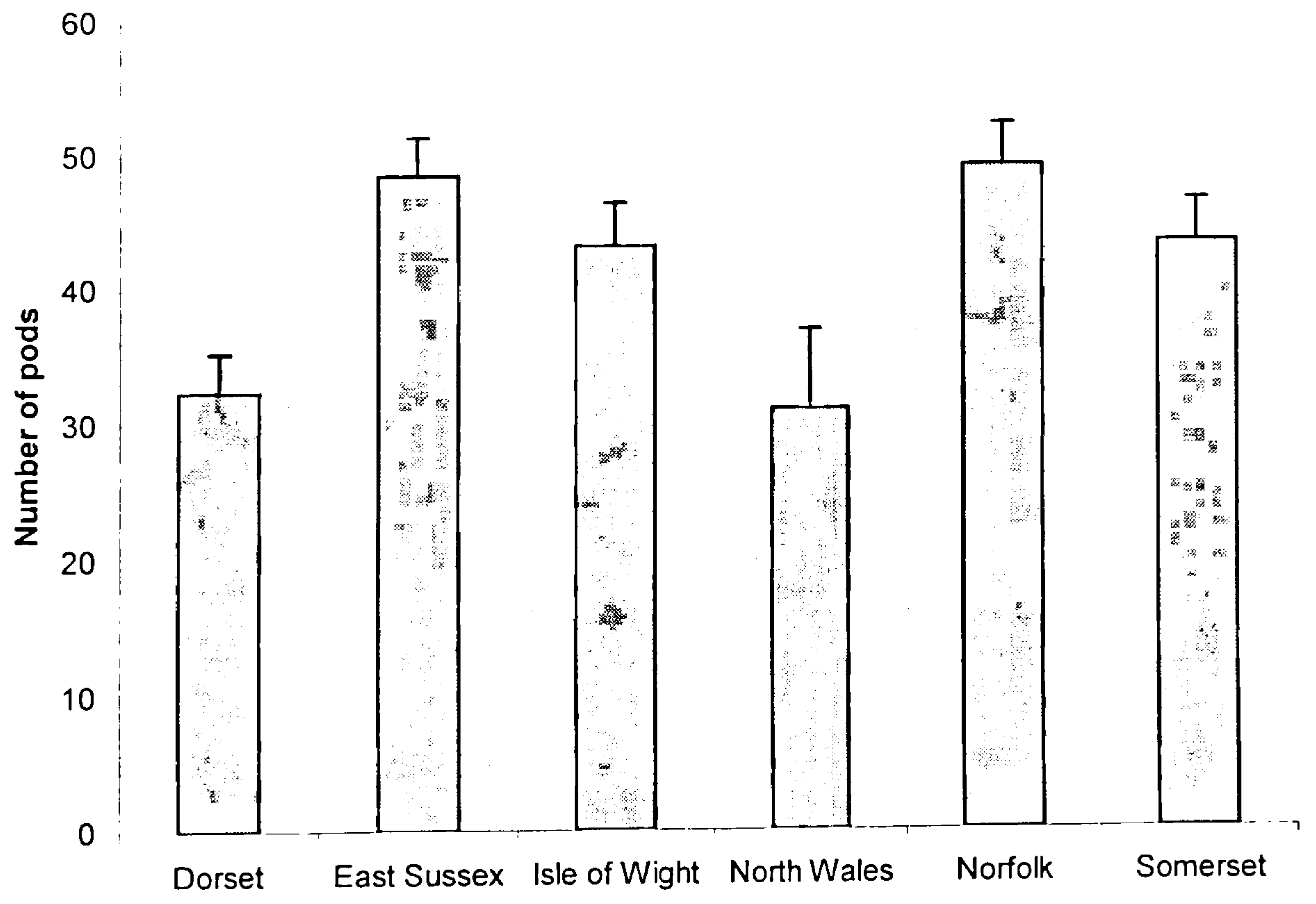

Figure 3.11 Mean number of pods produced by plants grown from seed collected in six regions and grown in a common garden.

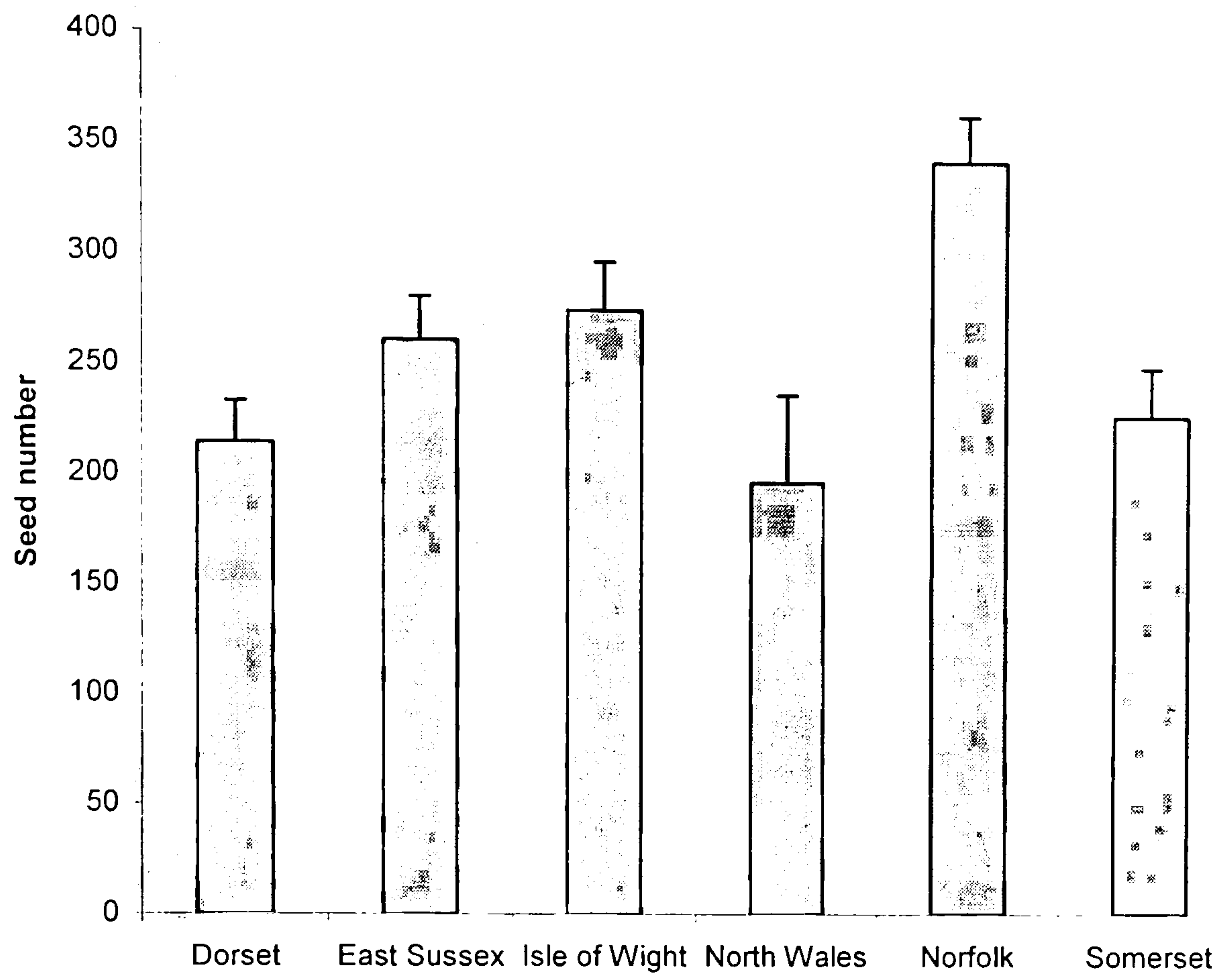

Figure 3.12 Mean seed number produced by plants grown from seed collected in six regions and grown in a common garden.

A significant interaction was identified between the effects of region and habitat of origin on the number of umbels produced per plant (Figure 3.13). This interaction is due to the high number of umbels on the plants from calcicolous grassland compared with those from the alternative habitat in the Norfolk region, as before. This is not reflected in the total number of seeds produced by the site.

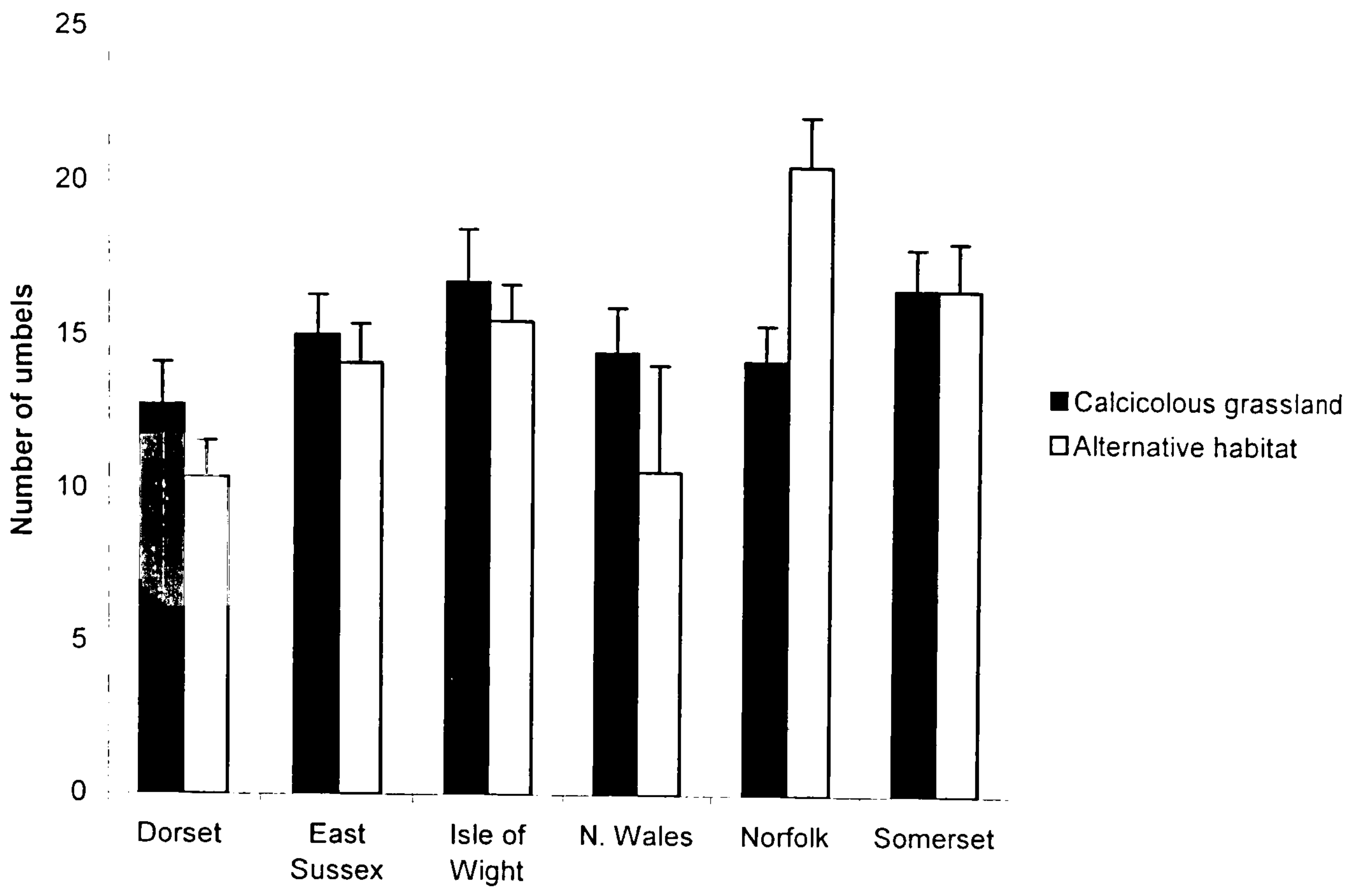

Figure 3.13 Interaction between region and habitat of origin effects on variation in the mean number of umbels produced by plants collected from two habitats in six regions.

At all sites there was seed damage caused by invertebrates but the proportion of plants which had been damaged varied between region (χ^2=12.56, *P* =0.028). In five regions, the number of plants with damaged seeds exceeded those with no damage, however among plants from east Sussex, the majority of individuals were undamaged (F = 12.56, *P*=0.028, df = 5), (Figure 3.14). There was no variation in the proportion of plants with damaged seeds between the calcicolous grassland sites and the alternative habitat.

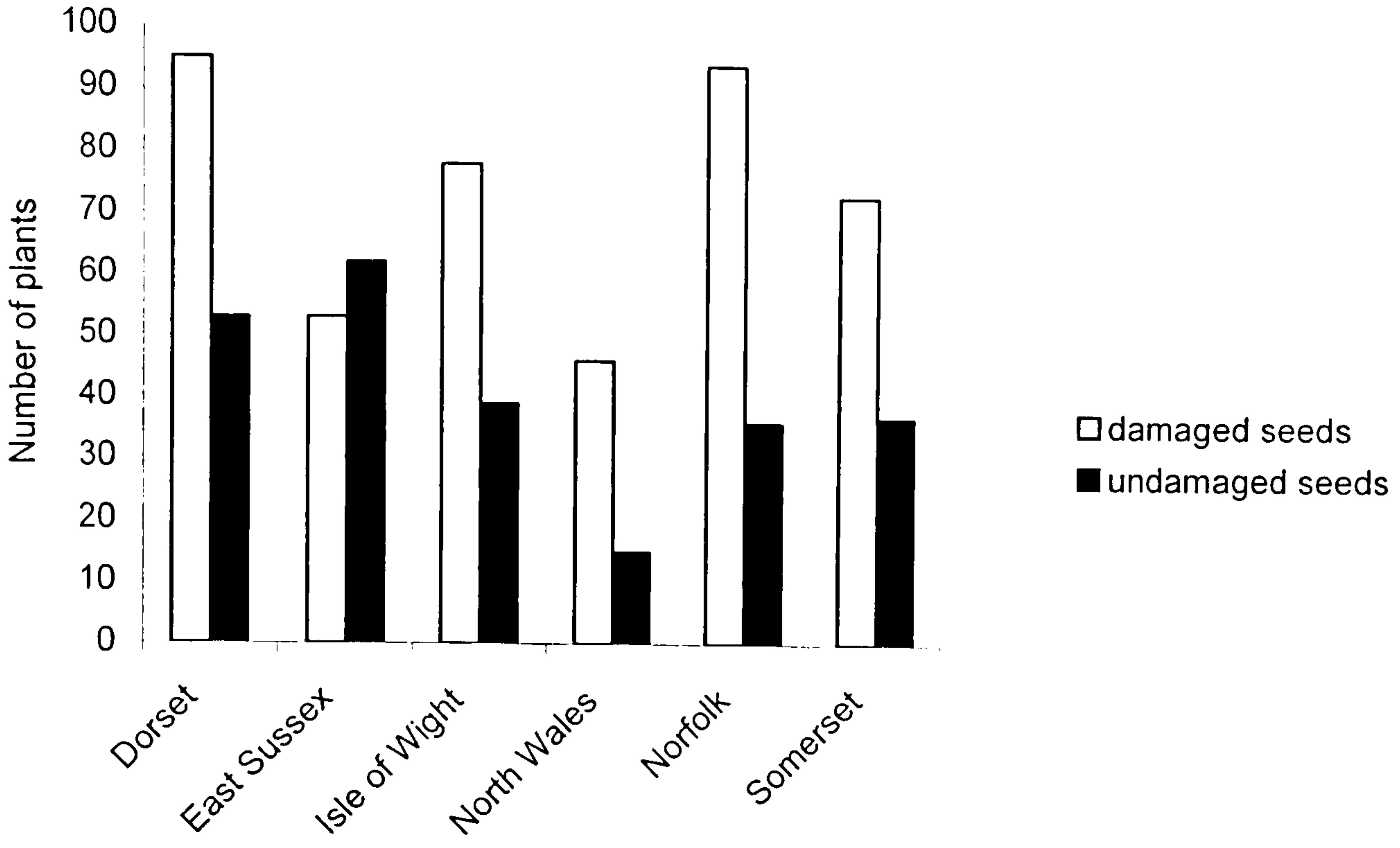

Figure 3.14 Proportion of plants with damaged seeds grown from seed collected in six regions and grown in a common garden.

In summary, there was a significant effect of provenance on morphology and seed yield of plants raised from seeds collected from different populations of *L. corniculatus* and raised in a common garden. Both geographical location and type of site had effects. It was possible to distinguish regional and ecological phenotypes using character traits which varied significantly. Table 3.7 shows a summary of regional phenotypes which have been defined including plants from two contrasting populations in each area.

Table 3.7 **Plant phenotypes of *L. corniculatus* collected from two populations in six regions in the British Isles and grown in a common garden.**

Norfolk	Somerset	Dorset	North Wales	Isle of Wight	East Sussex
Smallest plants of all six regions.	Small plants.	Plants intermediate in size.	Plants intermediate in size.	Large plants.	Largest plants of all the regions.
Tendency to be prostrate.	Most plants were decumbent	Tendency to be prostrate	Variable growth habit.	High proportion of decumbent plants.	Tendency to have erect growth habit.
Mainly sparsely hairy leaves.	Hairy leaves.	Sparsely hairy leaves	Hairy leaves.	Large, hairy leaves.	Largest leaves, which tended to be hairy.
Majority of leaves are obtuse.	Leaves tended to be either obovate or apiculate.	Leaves tended to be either obovate or apiculate	Leaves mostly obovate.	Leaves mostly obovate.	Leaves mostly obovate and obtuse.
Produced the most seeds of all the regions.	Intermediate seed yield.	Low seed yield.	Low seed yield.	Second highest seed yield.	Intermediate seed yield.
Majority of the plants suffered seed damage by invertebrates.	Majority of the plants suffered seed damage by invertebrates.	Majority of the plants suffered seed damage by invertebrates.	Majority of the plants suffered seed damage by invertebrates.	Majority of plants suffered seed damage by invertebrates.	The only region within which a minority of plants suffered seed damage by invertebrates.

There were also significant differences between plants grown from seed collected from calcicolous grassland and those sourced from the alternative habitat. In comparison with calcicolous grassland plants, those from the alternative habitat tended to have smaller leaves, were less erect in growth habit and were more likely to have hairier, apiculate leaves. However, there was no significant difference in plant fitness as represented by seed yield.

3.3.3 The effect of geographical and ecological distance on the magnitude of difference between quantitative character traits .

The data were examined to see if there was a correlation between geographical distance and differences in plant traits, i.e. whether plants became more different as the distance between the collection sites increased. It was shown that there was a significant positive correlation between differences in mean seed number and geographical distance, this indicated sites that the further apart sites were, the larger the difference in their seed yield. There was no correlation between any of the other quantitative traits and geographical distance. Data were also examined to see if there was a similar relationship between differences in plant traits and ecological distance. However no significant correlations were found (Table 3.8).

Table 3.8 Results of Mantel tests between geographical and ecological distance and five plant traits *=significant at the 0.05 level.

Mantel test	r (observed correlation)	*P* (>/=Z)
Geographical Distance x Ecological distance	-0.189	0.807
Branch number x geographical distance	0.204	0.095
Branch number x ecological distance	-0.138	0.481
Inter-node length x geographical distance	0.099	0.211
Inter-node length x ecological distance	0.118	0.266
Leaf length x geographical distance	0.244	0.075
Leaf length x ecological distance	0.749	0.738
Seed number x geographical distance	0.28	0.038*
Seed number x ecological distance	-0.194	0.885
Seed weight x geographical distance	0.235	0.08
Seed weight x ecological distance	-0.189	0.879

3.3.4 Association between traits

Correlation of quantitative traits

As indicated in Table 3.9, internode length was positively correlated with both leaf length and leaf span; similarly leaf span and leaf length were positively correlated. Plants with more umbels produced a larger number of pods and also larger number of seeds. There were positive correlations between these three characters. Seed number was negatively correlated with seed weight, so that as seed number increased, the weight of individual seeds fell. Of the morphological variables leaf size was correlated with the seed yield characteristics: plants with larger leaves produced a larger number of seeds. The number of branches per plant was not correlated with any of the other traits.

Table 3.9 A correlation matrix of quantitative traits measured on *L. corniculatus* plants in a common garden experiment. Significant correlation coefficients are in bold type.

		Branch	Internode	Leaf length	Leaf Span	Umbels	Pods	Seed number
Branch	*Pearson correlation*							
	P							
	N							
Internode	*Pearson correlation*	0.03						
	P	0.433						
	N	702						
Leaf length	*Pearson correlation*	0.045	**0.421**					
	P	0.229	<0.001					
	N	703	702					
Leaf span	*Pearson correlation*	0.015	**0.301**	**0.671**				
	P	0.691	<0.01	<0.001				
	N	703	702	703				
No. umbels	*Pearson correlation*	-0.041	0.003	**0.09**	0.068			
	P	0.289	0.934	0.01	0.075			
	N	683	682	683	683			
No. pods	*Pearson correlation*	-0.052	-0.008	**0.82**	0.058	**0.941**		
	P	0.173	0.826	0.019	0.127	<0.001		
	N	682	681	682	682	682		
Seed number	*Pearson correlation*	0.007	0.003	**0.091**	**0.09**	**0.705**	**0.718**	
	P	0.865	0.937	0.019	0.020	<0.001	<0.001	
	N	662	661	662	662	642	641	
Seed weight	*Pearson correlation*	-0.35	-0.027	-0.044	0.002	**0.161**	**0.161**	**-0.224**
	P	0.362	0.481	0.256	0.962	<0.01	<0.01	<0.01
	N	682	681	682	682	662	661	662

Association between categorical traits

In general plants were more likely to be prostrate or decumbent if they had pubescent and/or obovate leaves. Apiculate leaves were particularly associated with pubescence. Prostrate plants were more likely to be glabrous than were more erect types (Table 3.10).

Table 3.10 Association between categorical traits measured on *L. corniculatus* plants in a common garden experiment.
* = significant at 0.05, **= significant at 0.01, *** significant at 0.001

	Leaf shape		
	Obovate	Obtuse	Apiculate
Pubescence			
Glabrous	45	21	13
Sparsely pubescent	163	76	67
Pubescent	160	51	103
	χ^2 = 17.36, P=0.002**, df=4		

	Leaf shape		
	Obovate	Obtuse	Apiculate
Growth habit			
Prostrate	171	77	67
Decumbent	173	55	97
Erect	24	17	19
	χ^2 = 13.05, *P*= 0.011*, df =4		

	Pubescence		
	Glabrous	Sparsely pubescent	Pubescent
Growth habit			
Prostrate	53	140	122
Decumbent	20	140	165
Erect	6	26	27
	χ^2 = 21.43, *P* = <0.001***, df=4		

GLM ANOVA was employed to establish if there was a relationship between the quantitative traits and the categorical data. Quantitative traits were grouped using morphological categories. Prostrate plants bore the largest number of branches (F=12.96, *p*=<0.001) and had the longest inter-node lengths (F=10.68, *P*=<0.001). Decumbent plants had the longest leaves (F=51.52, *P*=<0.001). There was no effect of growth habit on any of the seed yield characteristics. Sparsely pubescent to pubescent leaves had the longest inter-node lengths (F=12.08, *P*=<0.001) and longest leaves (F=11.66 *P*=<0.001). Plants with glabrous to sparsely pubescent leaves bore the most branches. There was no significant relationship between leaf pubescence and seed yield. Leaf shape had no significant relationship with any other morphological traits but there was significant difference in mean seed number between plants with different leaf shapes. Obtuse leaves were associated with higher seed numbers (F=2.97, *P*= 0.05). None of the plant traits were significantly associated with damaged seeds. The results are presented in Tables 3.11 – 3.14.

Table 3.11 The relationship between pubescence and plant size and fecundity
Data presented as mean ±standard error. Significance: * =0.05 **=0.01 ***=0.001

	Mean number of branches.		Mean inter-node length (mm)		Mean leaf length (mm)		Mean leaf span (mm)	
Pubesence		N		N		N		N
Glabrous	7.12 ±0.257	79	7.86 ±0.428	79	6.01 ±0.185	79	11.20 ±0.338	79
Sparsely pubescent	7.07 ±0.13	309	9.94 ±0.214	309	7.17 ±0.093	309	12.89 ±0.171	309
Pubescent	6.63 ±0.284	315	10.08 ±0.212	315	6.91 ±0.092	315	12.42 ±0.169	315
ANOVA	*F*=0.83 *P*=0.435, df=2		*F*=12.08, *P*=<0.001***, df=2		F=11.66, *P*=<0.001***, df=2		*F*=9.594, *P*=<0.001***, df=2	

	Mean number of pods		Mean number of umbels		Mean seed no.		Mean seed weight (mg)	
Pubesence		N		N		N		N
Glabrous	38.79 ±3.88	76	14.22 ±1.232	76	243 ±25.996	75	1.34 ±0.292	75
Sparsely pubescent	42.27 ±1.963	296	14.63 ±0.632	296	267 ±13.243	288	1.37 ±0.149	288
Pubescent	42.13 ±1.921	310	15.21 ±0.611	310	252 ±13.019	299	1.50 4±0.146	299
ANOVA	*F*=0.39, *P*=0.678, df=2		*F*=0.48, *P*=0.621, df=2		*F*=0.61, *P*=0.544, df=2		*F*=.256, *P*=0.774, df=2	

Table 3.12 The relationship between growth habit and plant size and fecundity
Data presented as means ±standard error. Significance: *=0.05 **= 0.01 ***=0.001

	Mean number of branches.		Mean inter-node length (mm)		Mean leaf length (mm)		Mean leaf span (mm)	
Growth habit		N		N		N		N
Prostrate	7.38 ±0.209	316	8.88 ±0.21	316	6.27 ±0.088	316	11.46 ±0.163	316
Decumbent	6.78 ±0.208	327	10.55 ±0.206	327	7.58 ±0.086	327	13.43 ±0.16	327
Erect	4.72 ±0.479	60	10.12 ±0.494	60	6.81 ±0.201	60	12.74 ±0.377	60
ANOVA	F=12.96, *P*=<0.001***, df=2		F=10.68, *P*=<0.001***, df=1		F=51.52, *P*=<0.001***, df=1		F=33.08, *P*=<0.001***, df=1	

	Mean number of pods		Mean number of umbels		Mean seed no.		Mean seed weight (mg)	
Growth habit		N		N		N		N
Prostrate	41.54 ±1.935	306	14.60 ±0.614	306	265 ±13.101	295	1.354 ±0.147	295
Decumbent	41.86 ±1.898	317	14.99 ±0.603	317	246 ±12.739	311	1.339 ±0.143	311
Erect	43.82 ±4.406	59	15.27 ±1.399	59	278 ±30.07	56	2.307 ±0.336	56
ANOVA	F=0.08, *P*=0.923, df=2		F=0.06, *P*=0.945, df=2		F=0.83, *P*=0.437, df=1		F=1.06, *P*=0.346, df=1	

Table 3.13 The relationship between leaf shape and plant size and fecundity
Data presented as mean ±standard error. Significance: *= 0.05 **=0.01 level ***=0.001

	Mean number of branches.		Mean inter-node length (mm)		Mean leaf length (mm)		Mean leaf span (mm)	
Leaf shape		N		N		N		N
Obovate	6.66 ±0.114	369	9.93 ±0.199	369	6.993±0.088	369	12.65 ±0.158	369
Obtuse	7.40 ±0.567	150	9.53 ±0.312	150	6.718±0.124	150	12.36 ±0.249	150
Apiculate	6.89 ±0.171	184	9.56 ±0.281	184	6.951±0.129	184	12.26 ±0.224	184
ANOVA	F=2.063, *P*=0.128, df=2		*F*=1.29, *P*=0.276, df=2		F=1.09, *P*=0.337, df=2		F=1.82, *P*=0.163, df=2	

	Mean number of pods		Mean number of umbels		Mean seed no.		Mean seed weight (mg)		
Leaf shape		N		N		N	Mean	SE	N
Obovate	43.42 ±1.793	355	15.19 ±0.57	355	278.68 ±12.001	348	3.390	±0.135	348
Obtuse	41.65 ±2.787	147	14.44 ±0.886	147	241.28 ±18.948	139	3.310	±0.215	139
Apiculate	38.80 ±2.511	180	14.48 ±0.80	181	229.84 ±16.996	174	3.470	±0.192	174
ANOVA	F=0.67, *P*=0.514, df=2		F=1.14, *P*=0.320, df=2		F=2.97, *P*=0.052, df=2		F=0.478, *P*=0.620, df=2		

Table 3.14 The relationship between seed damage by invertebrate herbivores and plant size and fecundity
Data presented as mean ±standard error. Significance: *= 0.05 level **= 0.01 *=0.001**

	Mean number of branches.		**Mean inter-node length (mm)**		**Mean leaf length (mm)**		**Mean leaf span (mm)**	
Proportion of plants with seed damage		N		N		N		N
No seed damage	6.71 ±0.185	316	9.89 ±0.182	316	6.87 ±0.08	316	12.42 ±0.145	316
Seed damage	7.10 ±0.256	327	9.45 ±0.251	327	7.00 ±0.11	327	12.65 ±0.201	327
ANOVA	**F=12.96, *P*=<0.001***, df=1**		**F=10.68, *P*=<0.001***, df=1**		**F=51.52, *P*=<0.001***, df=1**		**F=33.08, *P*=<0.001***, df=1**	

	Mean number of pods		**Mean number of umbels**		**Mean seed no**		**Mean seed weight (mg)**	
Proportion of plants with seed damage		N		N		N		N
No seed damage	41.89 ±1.626	306	15.14 ±0.515	306	259 ±10.779	295	1.38 ±0.121	295
Seed damage	44.77 ±2.258	317	15.47 ±0.714	317	256 ±14.971	311	1.51 ±0.168	311
ANOVA	**F=0.08, *P*=0.923, df=1**		**F=0.06, *P*=0.945, df=1**		**F=0.83, *P*=0.437, df=1**		**F=1.06, *P*=0.346, df=1**	

3.4 Discussion

The effect of provenance on morphology, survival and fitness

The detection of differences in phenotype and fitness between the accessions examined in this study supports the findings of other work which shows differences between populations of *L. corniculatus*. McGraw, Beuslink & Smith (1986) found variation in cultivars of *L. corniculatus* in vegetative biomass, seed mass, seed yield, number of umbels, number of pods and seed weight. Bullard & Crawford (1996) found that there was variation in seed number and seed weight between eighteen accessions of *L. corniculatus*. Four of the eighteen accessions were described as UK ecotypes, but no details of seed collection were given by the authors. On a broader scale, Bonnemaison & Jones (1986) were able to distinguish between native and alien genotypes of *L. corniculatus* growing in roadside populations by studying their morphology and seed yield characteristics. Although there is no work describing the morphological variation between diverse populations in the British Isles, Chrtkova-Zertova, (1973), defined fourteen morphological types in central and northern Europe. More recently, in North America, Steiner & Garcia de los Santos (2001) have described variation between populations of *L. corniculatus* in ecologically distinct areas.

The maintenance of variation between populations or sub-populations of plants has been identified as having two sources; spatial separation and adaptation to local conditions (Clausen, Keck & Hiesey, 1947; Levin, 1988). Both these factors were shown to contribute to the variation observed in this study. Spatial isolation is significant as it infers genetic isolation, i.e. an absence of gene flow between the populations. Differences arise between populations because of founder effects and stochastic processes such as genetic drift (Rasmussen & Brødsgaard, 1992) or local selection pressures (Slatkin, 1987; Loveless & Hamrick, 1984). Where there is no exchange of genes between groups of plants, the differences between those groups will be maintained (Loveless & Hamrick, 1984). In plants, potential gene flow is the movement of seed and pollen as a function of distance (Levin & Kerster, 1974). It is unlikely that all potential gene flow will be realized. Actual gene flow will depend on the amount of fertilization that takes place and the number of reproductive individuals which are able to establish (Levin & Kerster, 1974). If gene flow is determined by the dispersal of pollen and seeds, the distance across which there will be an exchange of genes will depend upon the species in question (Ellstrand, 1992). The magnitude of this distance will depend upon the method of dispersal for both pollen and seeds (Wright, 1978).

L. corniculatus is pollinated by the smaller *Bombus* species and in some cases *Apis mellifera* (Jones & Turkington, 1986; Morse, 1958). The seeds, which form in pods, are expelled ballistically as the pods dehisce (Jones & Turkington, 1986). Bees tend to have small foraging areas and in the case of *A. mellifera* most individuals will forage within 500m of their hives (Free, 1970). Despite this tendency, there is evidence that bees will travel some distance. As bees work on failing sources, they become restive and search larger distances (Heinrich, 1979). In particular, in the case of honey bees there may be an exchange of pollen in the hive between bees that have been foraging in different areas. (Free,1970). Janzen (1971) records tropical bees travelling up to 20km on foraging trips. Therefore, although the majority of bees forage locally and within small patches, there may be a small amount of long distance pollen dispersal. This leptokurtic distribution of dispersal has been described (Waddington, 1981; Levin & Kerster, 1969). Rasmussen & Brødsgaard (1992) used pollinator behaviour and seed dispersal to investigate gene flow in *L. corniculatus.* Rasmussen & Brødsgaard investigated an area of dune heath which had been established for twenty years and supported patches of *L. corniculatus.* The authors assessed pollinator behaviour and seed dispersal and compared this with DNA analysis that revealed genetic differentiation between the patches. Although the pair-wise distances between the patches are not described, inter-patch distance ranged between 10 and 40m. Rasmussen & Brødsgaard found that the bees tended to forage within patches, turning back into the patches when reaching the edge. Only 2.6% of pollinator flights were between patches. Seed dispersal was within 2m of the mother plant in 80% of the cases and extended to a maximum of 4.95m. DNA analysis showed high differentiation between patches and it was concluded that limited gene flow contributed to these differences. It is notable, however, that very low amounts of gene carry over are sufficient to prevent significant variation (Levin and Kerster, 1974) and Rasmussen & Brødsgaard (1992) acknowledge that the differences in their study may be partly due to founder effects. With time, the patches may become undifferentiated. The limited dispersal reported in the work by Rasmussen & Brødsgaard suggests that it is possible that there was no, or little, gene-flow between described in this thesis. It is also unlikely that there is gene flow between the two populations sampled within each region, as the sites are separated by a minimum of 1.6 km (in Dorset) and a maximum of 12.1 km (in East Sussex).

Whilst geographical separation plays a major role in maintaining differences, this study has shown that differentiation may also be maintained by adaptation to local conditions. There was phenotypic variation between populations from calcicolous grassland and the alternative habitat. Plants growing in similar conditions, although geographically distant, displayed similar morphology and this suggests that *L. corniculatus* had adapted to local ecological conditions. Previous studies indicate that traits measured, such as leaf pubescence, may have adaptive significance (Ehleringer & Clarke, 1988). For example, the results presented in this thesis show that there was variation in the degree of pubescence between plants from the calcicolous grassland and the alternative habitat and also that hairier leaves were correlated with larger leaf size. Ehleringer & Clarke (1988) report that a change in leaf pubescence from glabrous to pubescent resulted in a 4% change in leaf temperature. It was suggested that the temperature rise would increase the transpiration rate thereby having an impact on the plants metabolic rate and water loss. These results are supported by Zhang *et al.* (1992) who found that, in soybean, hairiness of the female parent was linked with plant size and seed weight. The authors suggested that this was due to amplified leaf surface reflectivity in hairier leaves, which reduced transpiration and reflected light to lower leaves which may otherwise have been in shade. This may have increased photosynthesis and encouraged greater plant growth. The degree of leaf pubescence also affects species which have a close relationship with the plant. Pubescence has been linked to larval food choice in invertebrates. Leaf pubescence reduced feeding in *Melasoma lapponica*, a leaf beetle, on *Salix borealis* (willow) (Zvereva, Kozlov & Niemela, 1998). Conversely, in soybean, *Bemisia argentifolii* (whitefly) preferentially laid on the hirsute leaves. (McAuslane, 1996). This species' specific impact on herbivores could have an adaptive significance for plants in different ecological conditions.

Not all plant traits have been studied as widely as pubescence. However, there is some evidence to suggest that growth habit may be adaptively significant. For example, lucerne grows on grasslands which are traditionally grazed. Under these conditions the prostrate plants produce more seed than those with an erect habit (Piano *et al.*, 1996). Van Tienderen & Van der Toorn (1991a) investigated phenotypic selection in *Plantago lanceolata* and found that plants from a grazed pasture were smaller and less erect than those from a hayfield which was cut. As the plants maintained their growth habit after transplantation to a different environment the authors concluded that the phenotype was selected by grazing. In the work presented in this thesis, plants from grazed grasslands were associated with a more prostrate growth habit. Although there is no conclusive evidence, it is possible that this is an adaptive trait selected under grazing pressure.

The effect of geographical and ecological distance on the magnitude of difference between quantitative character traits

Having established that there were differences between populations, data were analyzed to establish whether there was a linear relationship between the geographical and ecological separation of the sites and the magnitude of difference in the quantitative traits measured. There was no correlation between ecological distance and any of the traits. Geographical distance was also uncorrelated with the majority of the traits, however there was a significant positive correlation between distance and the mean number of seeds produced by each site, i.e., the further apart two sites were, the greater the difference in seed yield was. Plotting mean seed number on a map of the British Isles (Figure 3.15) for the twelve populations showed a tendency for seeds to be larger on the east side of the country, but there are too few populations to define a trend.

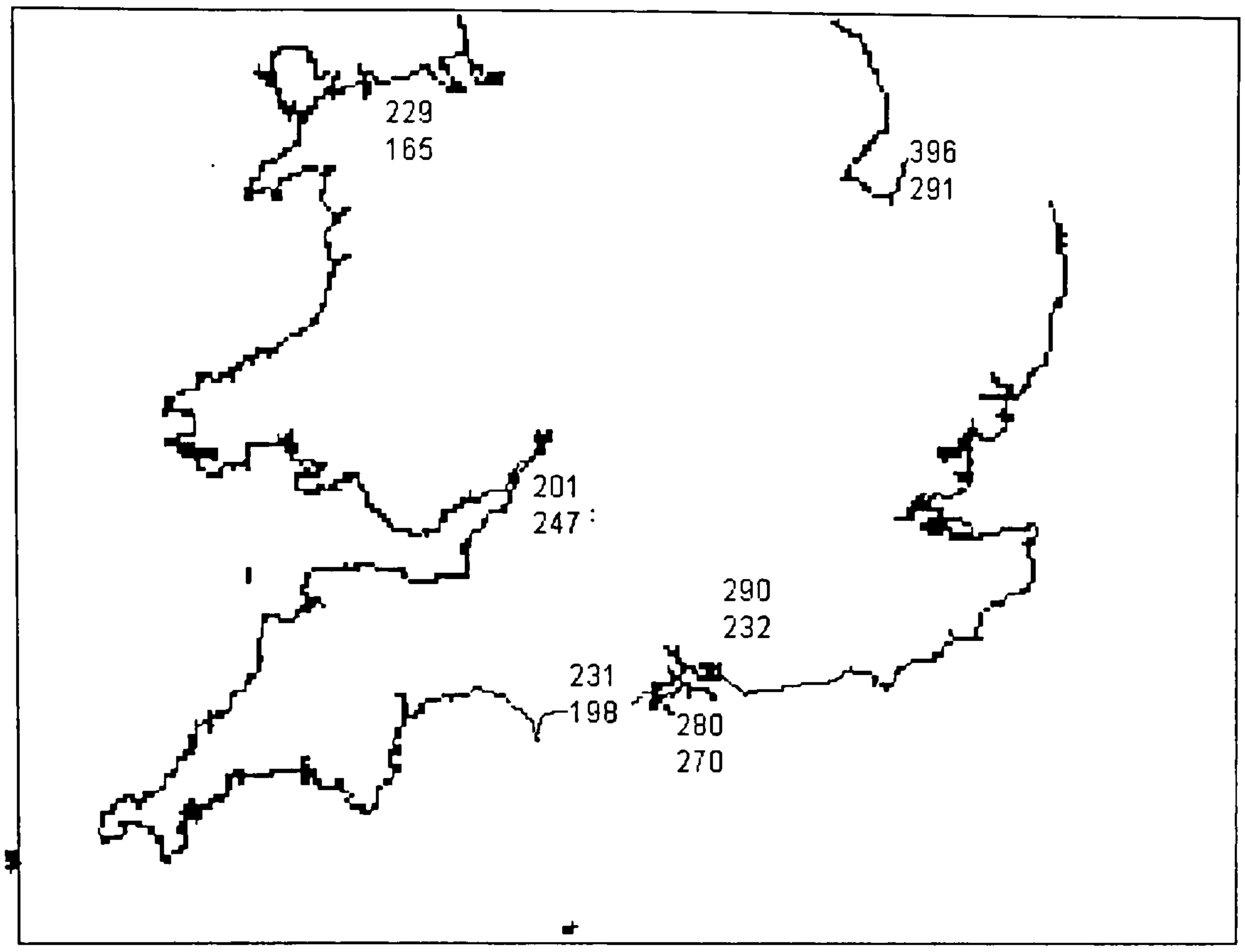

Figure 3.15 Mean seed number per plant for plants collected from twelve locations in the British Isles. The upper number in each pair is for the calcicolous grassland, the lower is for the alternative habitat.

Association between traits

A further objective of this study was to investigate the relationship between morphology and plant fitness. This study has demonstrated a correlation between leaf length and leaf shape on reproductive output. Larger leaved plants produced more seed. This supports the findings of Ollerton & Lack (1998) who investigated flowering phenology and reproductive output in *L. corniculatus* and found that components of plant size were related to seed yield. Mean seed number differed significantly between leaf shapes. Plants with obovate leaves had a lower seed yield. There is no literature that investigates the relationship between leaf shape and seed yield in *L. corniculatus.* Ehleringer & Clarke (1988) suggest that leaf shape may interact with abiotic and biotic factors to affect growth and reproduction, but they present no experiments to support this.

In common with other studies, the work presented here found that seed size was negatively correlated with seed number (e.g. Carlton & Cooper, 1972). Plants tended to produce a large number of small seeds or a small number of large seeds. There are advantages to both strategies, while large seeds will produce larger more vigorous seedlings (Beuselinck & McGraw, 1983; McKersie *et al.*, 1981), small seeds will survive longer in the soil (Thompson *et al.*, 1993; Bekker *et al.*, 1998). There was no evidence of variation in seed weight between populations which was surprising as other workers have found differences in seed weight between accessions of *L. corniculatus* (Negri & Falcinelli, 1990; Bullard & Crawford, 1996). However the use of cultivars in these experiments is misleading and may not reflect seed size variation between natural British populations, as demonstrated here.

Although none of the categorical plant traits was associated with seed damage by invertebrates, plants with more branches and larger leaves were more likely to show signs of seed predation. Differences were also found in the proportion of plants damaged between regions. Plants from five of the regions investigated had a majority of plants with damaged seeds but plants from East Sussex were mostly undamaged. Although there is little research in this area, in a controlled study, Keller *et al.* (1999) found that slugs distinguished between weeds according to provenance and concluded that plants from different sources varied in palatability. Ollerton & Lack (1996) investigated the effect of seed predation by *Apion loti* (a seed weevil) on germination in *L. corniculatus*. The authors showed that partly eaten seeds were able to germinate and suggested that the feeding of *Apion loti* speeded up germination by breaking hard coat seed dormancy. If invertebrates are sensitive to plant provenance then this has implications for restoration ecology: this is an area which needs further work.

In summary, this study suggests that plant fecundity can be predicted by leaf length and leaf shape in the common garden. These characters may be useful for assessing potential plant performance in a restoration environment. Differences in *L. corniculatus* in the British Isles can be identified regionally and also by the type of site. The traits that were identified as variable have adaptive significance and may be crucial for the plant's persistence in the environment. In particular, geographical distance between sites is important, as there is some evidence that increased distance between sites will mean that there is an increased difference in seed yield. It is concluded that there is sufficient variation between sources both regionally and in terms of ecology that when seeds are to be selected for restoration, they are chosen within a local region and from a similar habitat. Work presented here suggests that both these factors may be important for the plant's subsequent success and field experiments are necessary to confirm this.

Chapter Four

The effect of provenance on differences in plant fitness in a restoration environment

4.1 Introduction

There is concern that using non-local propagules in restoration programmes may influence the establishment and long term success of introduced populations (Millar & Libby, 1989, Knapp & Rice, 1994, Linhart, 1995). Over time, plants undergo selection by local environmental conditions and as a consequence adaptive genetic differentiation between populations may be expected. This is supported by a substantial body of work demonstrating local adaptation in plant species (eg. Clausen *et al.*, 1947, Turkington and Harper, 1979a; Turkington & Harper, 1979b; Schmitt & Gamble,1990, Kindell *et al.*, 1996). The effect of local adaptation will be countered by gene flow which can constrain adaptation to a heterogeneous environment by preventing local differentiation (Slatkin, 1987). The degree of environmental differentiation and genetic isolation can be expected to increase with distance (Wright, 1978). It may be hypothesised that in restoration projects plants with the highest fitness will be those whose original site was most similar to the restored site (Schmitt & Levin, 1985) and that there will be a 'home-site' advantage for local plants (Montalvo & Ellstrand, 2000). Furthermore, translocated plants are likely to be less well adapted to sites increasingly distant from their original location (Schmitt & Levin, 1985). The importance of geographical distance as a predictor of propagule establishment and performance has led workers such as Linhart (1995) to emphasise the importance of collecting propagules close to the restoration site, asserting that sites that appear 'ecologically' close but are 'geographically' distant may perform poorly.

There are few studies investigating performance of non-local propagules when used in restoration schemes. Recent studies have begun to address this issue but the results are contradictory. Jones & Hayes (1999) investigated the performance of five species using non-local and local propagules. Their results were inconsistent; in some cases non-locals out performed locals. However, the experiment was confounded by the collection of 'local' propagules within an 8km radius of the study site; this scale may be too large to detect local adaptation in some of the chosen species. Additionally the study did not describe any environmental variables so that the ecological characteristics of the donor sites were uncertain.

A study by Galloway & Fenster (2000), using *Chamaecristata fasciculata* as a model species, found that geographical distance of donor site significantly affected plant performance in a novel environment but only at a large spatial scale (over 1000km). This result was unexpected and was attributed in part to the plastic nature of the species used as *C. fasciculata* appeared to adjust to the environmental variation found within 100km but was less able to survive the environmental extremes represented by long distance transfer (Galloway & Fenster, 2000). It was also possible that the spatial scale used was too large to detect variation that was present, or that there was some gene flow that the experimenters had not accounted for (Galloway & Fenster, 2000). Montalvo & Ellstrand (2000) took account of environmental variables as well as geographical location and showed that in *Lotus scoparius* plant performance in a common garden experiment was related to environmental and genetic distance rather than geographical distance. They concluded that geographical distance was not a useful predictor for propagule success in restoration projects but identified that ecological distance might be an important factor to consider. However, as Galloway & Fenster (2000) suggested, geographical and ecological distance may be closely related. In order to separate geographical and ecological components the experimental work presented in this chapter investigated the effects of geographical and ecological distance simultaneously. This was achieved by sampling propagules over a range of distances and at sites that had different local conditions. Donor sites were selected to ensure that a simple correlation between ecological distance and geographical distance did not confound the study.

4.1.1 Aim and Objectives

The aim of this study was to determine the effect of geographical and ecological provenance on the survival and establishment of propagules in two restoration environments at a single location, using *Lotus corniculatus* as a model species.

A field trial used cuttings from 30 sources. Clones of each population were planted into two experimental plots at a single quarry site in Dorset; an area of untreated clay waste and an area which had been dressed with local topsoil. These sites represented two environments which are typically found in restoration projects. The objectives of this study were to:

- Determine whether there was a specific home-site advantage for plants sourced from Swanworth Quarry in terms of survival and fitness.

- Investigate the general relationship between propagule source and plant fitness in a restoration environment

- Assess whether conditions at the receptor site influenced the effect of provenance on plant survival and fitness

- Investigate whether plant fitness was linked to the expression of keel colour and cyanogenesis polymorphisms in *L. corniculatus*

4.2 Method

4.2.1 Collection sites

L. corniculatus cuttings were taken from two habitats within 15 regions (summarized in Table 4.1). In each region the cuttings were collected from calcicolous grassland and an alternative habitat as described in the *General Methods* section.

Table 4.1 Sites selected for the collection of cuttings of *Lotus corniculatus* to investigate the effect of provenance on establishment in a restoration environment. The table is arranged in order of distance from Dorset.

Site	OS Reference	Site description
Dorset		
Swanworth Quarry	SY968782	Rabbit grazed limestone grassland on the margins of a quarry. Dominated by *Festuca rubra* and *Lotus corniculatus* with characteristic limestone species.
Chapman's Pool	SY956771	Dry coastal grassland dominated by *Arrhenatherum elatius, Festuca rubra, Holcus lanatus*. Frequent *Carex panicea*. Patches of bare ground with *Tussilago farfara*.
Isle of Wight		
Afton Down	SZ8538998	Rabbit grazed chalk grassland, dominated by *Festuca rubra*, Abundant *Koeleria macrantha* and typical chalk grassland forbes.
Newtown Meadows	SZ4058998	Wetland meadow dominated by *Holcus lanatus*. And *Cynosurus cristatus, Avenula pratensis*, with *Succisa pratensis, Blackstonia perfoliata, Genista tinctoria*
Somerset		
Berrow Dunes	ST293520	Rabbit grazed limestone grassland. Dominated by *Anthoxanthum odoratum* and *Festuca rubra* with frequent *Plantago lanceolata* and *Galium verum*. Rich in limestone species.
Brean Down	ST290580	Dune grassland dominated by *Festuca rubra* and *Carex arenaria*. Area included a small dune slack with *Oenanthe lachenalii* and *Dactylorhiza incarnata*.
Oxford		
Warburg Reserve	SU590930	Forb rich calcicolous grassland on a cleared woodland dominated by *Festuca rubra* , *Trisetum flavescens* with abundant *Lotus corniculatus* and *Origanum vulgare*.
Sandford Pit	SU467997	Abandoned sand quarry, dominated by *Festuca rubra* with abundant *Hieracium pilosella* and *Medicago lupulina*.
East Sussex		
Mount Cabourn	TQ448090	Chalk grassland. Dominated by *Festuca ovina* and *Bromus erectus* with many characteristic forbs. Sheep and rabbit grazed.
Lullington Heath	TQ545017	Chalk heath community on neutral loess soil. *Calluna vulgaris* , *Festuca rubra* mosaic with both calcicolous and calcifugous herbs. Short turf, rabbit grazed.
Kent		
Crown Meadows	TR072461	Species rich chalk grassland, with *Festuca rubra, Festuca ovina, Carex flacca* and a wide range of calcicolous species.
Hollybush Farm	TQ9911350	Pasture grazed by cattle and horses. Dominated by *Agrostis stolonifera, Festuca rubra* with abundant *Anthoxanthum odoratum, Potentilla erecta and Lotus corniculatus.*
Cornwall		
St. Ives	SW520411	Rabbit grazed limestone grassland dominated by *Festuca rubra* and *Agrostis stolonifera* with

		abundant *Festuca longifolia*, *Holcus lanatus,*and *Armeria maritima.*
Hayle	SW568392	Patchy grassland in a dune slack. *Ammophila arenaria* and *Festuca rubra* with abundant *Galium verum* and *Echium vulgare.*
Norfolk		
Ringstead Downs	TF691401	Rabbit grazed chalk grassland. South facing slope in a dry valley. Species rich sward, dominated by *Festuca rubra, Festuca ovina* with abundant *Helianthemum nummularium* and *Sanguisorba minor.*
Hunstanton Dunes	TF690430	Coastal grassland between stabilized dunes. Patchy with areas of bare sand. Dominated by *Ammophila arenaria* and *Festuca rubra* with abundant *Rhinanthus minor* and *Lotus corniculatus.* Some rare coastal plants such as *Eryngium* sp.
Lancashire		
Ribble Estuary	SD375240	Species poor damp grassland dominated by *Lolium perenne, Plantago lanceolata* and *Achillea millefolium.* Abundant *Trifolium repens* and *Lotus corniculatus.*
Warston Crag	SD779431	Rabbit grazed limestone grassland dominated by *Festuca ovina* and*Trisetum flavescens* with abundant *Festuca rubra* and *Thymus polytrichus.*
North Wales		
Great Ormes Head	SH770830	Rabbit and sheep grazed limestone grassland. Dominated by *Festuca ovina, Festuca rubra* and *Anthoxanthum odoratum.* Abundant *Helianthemum nummularium.*
Conwy Morfa	SH767791	Patchy coastal grassland on shingle. Abundant *Ononis repens, Agropyron pungens* and *Ammophila arenaria.*
Humberside		
Far Ings nature reserve	TA005230	Short calcareous grassland dominated by *Trisetum flavescens* and *Lotus comiculatus* with abundant *Euphrasia nemorosa.*
Field edge	TF975853	Grassland on a field edge dominated by *Arrhenatherum elatius, Dactylis glomerata* and *Brachypodium pinnatum* with *Lathyrus pratensis* and *Trifolium pratense.*
Northumberland		
Druridge Bay	NZ286943	Coastal dune grassland dominated by *Ammophilia arenaria*, *Festuca rubra* and *Trisetum flavescens*, with abundant *Geranium sanguineum* and *Lotus corniculatus.*
Gunnerton Crag	NY900770	Rabbit grazed limestone grassland, dominated by *Festuca rubra* with abundant *Lotus corniculatus* and typical limestone forbes.
Edinburgh		
Burntisland	NT230860	Cattle grazed limestone grassland dominated by *Festuca rubra* with abundant *Festuca gigantea and Lolium perenne.*
Longriddry	NT440760	Coastal dune grassland dominated by *Festuca repens* and *Potentilla reptans* with abundant *Geranium sanguineum.*
Grampian 1		
Derry Lodge	N00493	Deer and rabbit grazed acid grassland growing along side a track in a valley. Dominated by Ericaceous species, with *Festuca ovina* and *Agrostis capillaris*
Findlater	WJ5467	Forb rich rabbit grazed limestone grassland dominated by *Festuca rubra* with abundant *Anthyllis vulneraria.*
Grampian 2		
Tomintol	NJ172178	Sheep grazed limestone grassland dominated by *Festuca rubra, Holcus lanatus* and *Cynosaurus cristatus* with abundant *Anthyllis vulneraria* and *L. corniculatus.*
Banff	NJ693642	Sand flats dominated by *Festuca rubra, Trifolium repens* and *Lotus corniculatus.*

4.2.2 Collection of cuttings

Cuttings were taken in May 1998. To sample plants randomly, two 100m tapes were laid out at right angles to form a grid and cuttings were taken from 40 plants at the nearest point to randomly generated co-ordinates. Two branches were cut at the base of each plant; it was important to collect more than one cutting to ensure that enough surviving plants were available for the experimental work.

4.2.3 Propagation of cuttings

The cuttings were sent from the donor site by post to minimise the time between taking the cuttings and potting them up. Cuttings were wrapped in damp paper, put into plastic sample bags and sent to the Institute of Terrestrial Ecology (ITE) Furzebrook, Dorset, by first class post. At the ITE cuttings were put into seed trays which were filled with fine vermiculite, placed into water retaining trays and covered with propagator lids to retain moisture. These trays were then placed under benches in an unheated greenhouse out of direct sunlight and the plants were sprayed with water each day using a hand held mister. After five days the propagator lids were removed. By 21 days the plant roots were long enough to emerge from the bottom of the seed trays and the cuttings were planted individually into 20cm plastic pots filled with a 1:4 mixture of vermiculite and John Innes No. 2 compost. These were placed in trays and set on top of the benches in the greenhouse. After five days the trays were put outside with a small amount of water to prevent the pots drying out while the plants acclimatised to a cooler temperature. After a week the pots were transferred to a gravel filled plunge bed, arranged in a fully randomized block and watered regularly. These plants formed a stock population from which cuttings for experimental work were taken. It has been shown that there may be some carryover effect of the parent environment on the growth and performance of clones (Bullock, *et al.*, 1993). To mitigate this effect the stock plants were left to over-winter in the plunge bed at Furzebrook.

4.2.4 The Field experiment

In March 1999, two cuttings were taken from 30 stock plants from each of the sampled populations and propagated in the same way as described above. This created two identical experimental populations with a clone of each of 30 plants from 30 populations of *Lotus corniculatus*. When cuttings had been potted, they were placed randomly in a plunge bed until they were large enough to be transplanted into the field. In the first week of September 1999 the plants were put into two experimental plots (15m x 15m) at Swanworth Quarry in Dorset. The plots were laid out on a tip area where the substrate was a mixture of clays, marls and waste limestone chips. One of these plots received no treatment and plants were put into bare clay; hereafter this plot is referred to as the '<u>untreated</u>' plot. The other plot received an application of approximately 15cm of local topsoil that had been stripped from an adjacent agricultural field; hereafter this plot is referred to as the '<u>treated plot</u>'. Although other work investigating home-site advantage in propagules has used vegetated areas for evaluating local adaptation (Montalvo & Ellstrand, 2000, Galloway & Fenster, 2000) and Kindell *et al.* (1996) found that the expression of local adaptation was affected very little by the presence of surrounding vegetation, both areas were unvegetated at the time of planting. The experiment presented here was designed to replicate the conditions at a typical restoration site where the land is unvegetated and these plots represent two typical scenarios found at restoration sites.

To minimize mortalities from shock, plants were transplanted with the soil from the pots in which they were growing. The plants were positioned at 0.5m intervals so that during harvesting individuals were easily identifiable. This spacing was suitable for *Lotus corniculatus*, which performs best under low density conditions (Bullard & Crawford, 1995). Position in the plot was allocated randomly. Plants from Derry Lodge in the Grampian 1 region of Scotland were excluded as only three plants survived the winter period.

At the same time as the cuttings were taken, the plants were tested for cyanogenesis using the method described in the *General Methods* section. Previous work has shown that the trait of cyanogenesis is flexible in *L. corniculatus* and individuals may switch between phenotype (Ramnani & Jones, 1985). It was a concern in this experiment that the plants were only tested once but collecting data on more occasions was impractical. It would be useful to know if 'switching' is common in established populations of *L. corniculatus* to assess with how much confidence the results of experimental work can be interpreted. To investigate the extent of 'switching' in populations of *L. corniculatus* at Swanworth Quarry, twenty-eight plants were randomly selected in established calcicolous grassland on the quarry margins. The plants were tested on 15th January, 20th February, 16th May and 9th June 2001. Not all plants survived the winter and on the 9th June it was difficult to distinguish many of the plants as other vegetation encroached; sampling was abandoned after this time.

4.2.5 Data collection

The survival of plants was recorded at the onset of flowering (17th May 2000) at which time the keel colour of each plant was noted. It is difficult to time harvesting in a species such as *L. corniculatus* where flowering time can be protracted (Ollerton & Lack, 1996) and it has been acknowledged that the proper stage of maturity is hard to assess (Anderson, 1955). In this case, plants were harvested as soon as the majority of the pods were ripe but before they began to dehisce in order to minimize seed loss. The plants were cut at ground level and stored in brown paper envelopes which prevented sweating and minimized pod shattering. After harvesting the plants were laid out on benches in a laboratory at Bournemouth University and air-dried, they were then returned to the envelopes and stored in a cool dry cupboard until processing.

A sub-sample of ten plants was randomly selected from each population of the harvested plants. In each case the whole plant was weighed, together with pods and any seeds which had fallen into the envelope. Pods were then removed from the plants and all pods and seeds were weighed. From these data vegetative biomass and reproductive biomass were calculated. The seeds were removed from the pods and weighed to obtain total seed mass. Three batches of twenty seeds from each plant were weighed and from these data mean seed weight and total estimated seed number per plant were calculated. Where seed damage by invertebrates was observed, it was recorded as described in the *General Methods* section of this thesis.

4.2.6 Data analysis

Home-site advantage

Home-site advantage was assessed by comparing plants from Swanworth Quarry with those from other populations. Where the magnitude of mean data from Swanworth Quarry exceeded that of all other populations, the plants from Swanworth Quarry were considered to have home-site advantage. This simple comparison has been used by other authors such as Montalvo & Ellstrand (2000) and Jones *et al.* (2001).

Differences in plant survival and fitness between regions and type of site.

Survival

Differences in survival between plants from different regions and habitats of origin were investigated using chi-square analysis.

Plant traits

Differences in fitness between plants from different regions and habitats of origin were investigated using univariate general linear model analysis of variance (GLM ANOVA).

Mean data for each trait were grouped into:

i) mean data for each of fourteen regions

ii) mean data for plants from calcicolous grassland and the alternative habitat.

Data were square root transformed before analysis to ensure equality of variance between groups.

The plants from Derry Lodge (alternative habitat) in Grampian 1 were not planted out due to a large number of mortalities and there was also a low number of survivors from Findlater in Grampian 2 (calcicolous grassland). Tomintoul (the calcareous site in Grampian 1) and Banff (the alternative site in Grampian 2) were combined to form a single Grampian region. The data sets for analysis were constructed from the measurements of 10 individuals which were randomly sub-sampled from the harvested plants from each population. However, plants from Banff (Grampian) and Far Ings (Humberside) survived poorly and only nine individuals were alive to be processed. GLM ANOVA was used to account for the unbalanced data.

The effect of ecological and geographical distance on plant survival and fitness

Survival

Single linear regression was used to investigate whether plant survival decreased as geographical and ecological distance between the donor and receptor site increased.

Plant traits

The data were analysed using single linear regression to investigate the relationship between geographical and ecological distance and the measured plant traits. Two regression analyses were carried out for each indicator. Firstly data collected from each plant were included in the analysis which gave a high level of statistical power. However, the variation between plants within populations meant that the proportion of variation captured by the analysis was low. Using the mean data for each population increased the amount of variation explained by the analysis but reduced the power of the test, so that some variation failed to be detected. It was useful to carry out both tests and draw conclusions from any discrepancies.

Plant performance indicators were entered into a Principal Components Analysis (PCA) to give a combined measure of plant performance. A regression analysis was performed to investigate the relationship between geographical and ecological distance and the PCA scores on axis 1 for each site.

Differences between the treated and untreated plots

To establish if there were differences in plant size and productivity between plants growing on the experimental plots, the data were compared using univariate GLM ANOVA: the data were grouped by plot.

Cyanogenesis

Previous work has demonstrated that the proportion of cyanogenic plants in populations of *L. corniculatus* differs according to the location of the population (Jones, 1977). It has also been suggested that cyanogenesis may confer a fitness advantage on plants by discouraging herbivory (Compton *et al.*, 1983; Compton & Jones, 1985). To investigate the differences in the proportion of cyanogenic plants between regions and type of site, GLM ANOVA was used. To investigate the effect of cyanogenesis on fitness, plant survival and incidence of cyanogenesis were correlated using Pearson's coefficient. The relationship between cyanogenesis and difference in plant fitness was investigated using GLM ANOVA.

Keel colour

Investigation into the keel colour polymorphism in *L. corniculatus* has followed a similar pattern to that carried out on cyanogenesis. It has been established that keel colour varies regionally (Jones & Crawford, 1977; Abbott, 1981) and there is some evidence that dark keeled plants are more productive (McKee, 1997) although other studies have not found this (Jones *et al.*, 1986). The difference in keel colour of plants in different regions and between habitats of origin was investigated using chi-square analysis. The effect of keel colour on the measured traits was investigated using GLM ANOVA, grouping the data by pale or dark keel.

4.3 Results

At each site a number of plants were missing. Some appeared to have been grazed close to ground level whilst others had been dug up and were found close by and other plants had disappeared. It is likely that these plants were damaged by herbivores at the site. There is a large rabbit population at Swanworth quarry and brown hare and deer are regular visitors although there is no conclusive evidence to account for the fate of these plants.

4.3.1 Home-site advantage

Survival on the site treated with topsoil

On the site treated with topsoil there was a home-site advantage for the Swanworth Quarry population where no mortalities were recorded (Figure 4.1)

Survival on the untreated plot

Although the Swanworth Quarry population survived well at the untreated site, Lullington Heath, Burntisland and Brean all had a higher proportion of survivors. There was no home-site advantage (Figure 4.2).

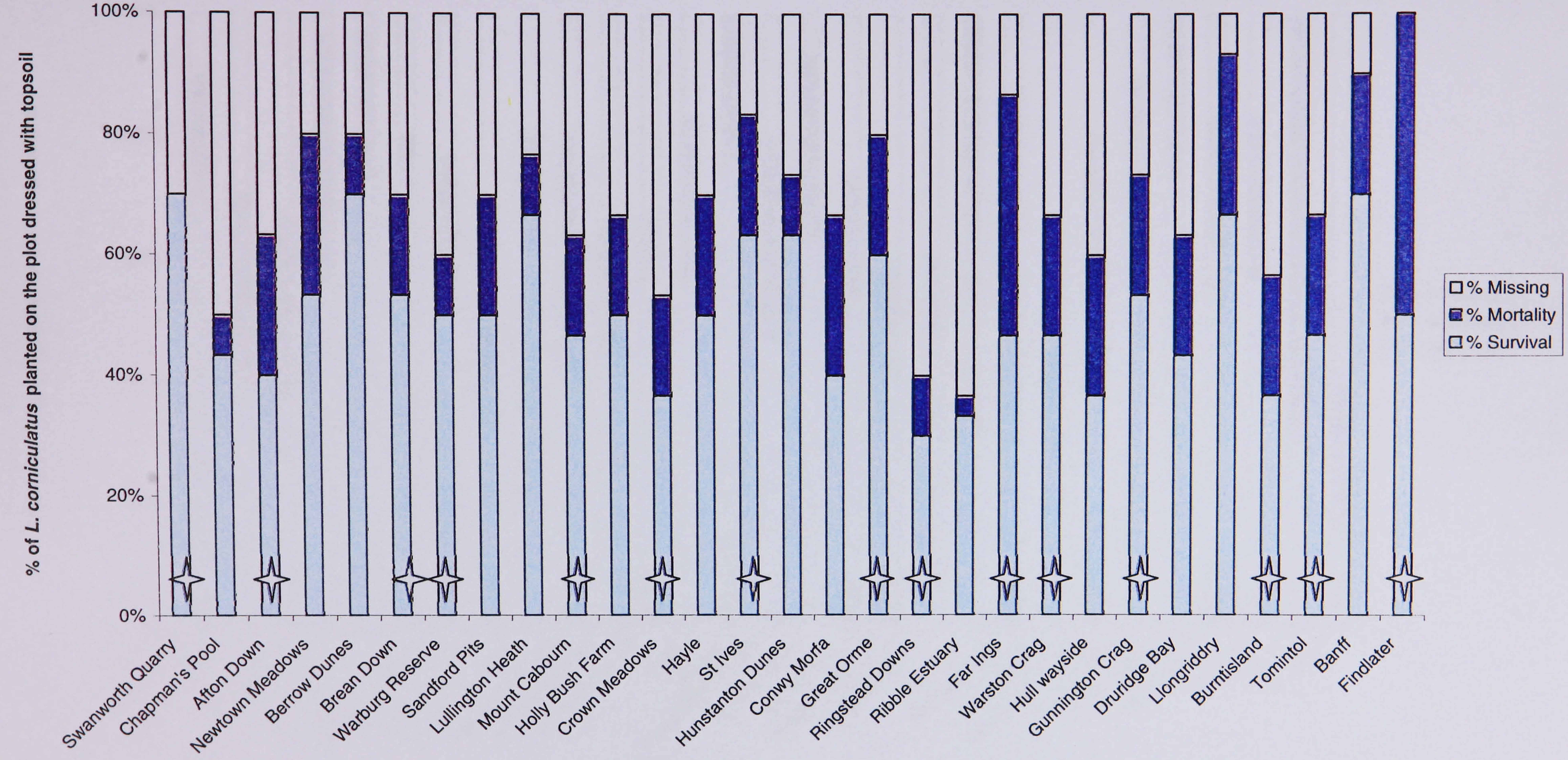

Figure 4.1 Survival of plants growing in an experimental plot treated with top soil at Swanworth Quarry in Dorset. Distance from Swanworth Quarry increases from left to right on the graph and calcicolous sites are marked with a star.

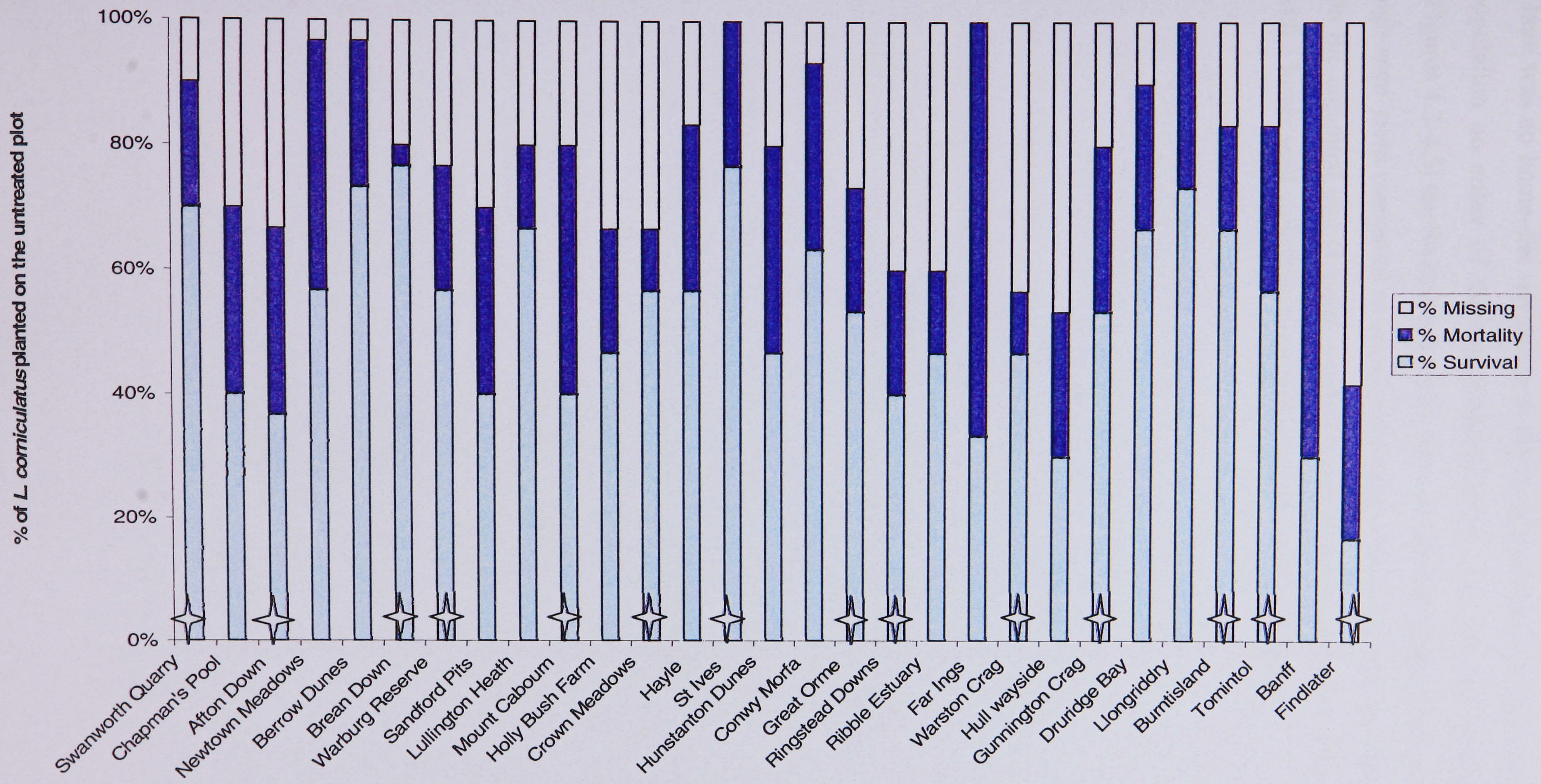

Figure 4.2 **Survival plants growing in an untreated experimental plot at Swanworth Quarry in Dorset. Distance from Swanworth Quarry increases from left to right on the graph and calcicolous sites marked with a star.**

Home-site advantage for determinants of plant fitness

There was no home-site advantage in the measured traits for the Swanworth Quarry population on either of the experimental plots. On the plot treated with topsoil (Figures 4.3-4.5) the Swanworth plants were among the largest plants with a relatively high seed yield compared to plants from many, but not all, of the other populations. On the untreated plot (Figures 4.6-4.8) plants from the local site were much smaller with a lower seed yield than the majority of the other populations.

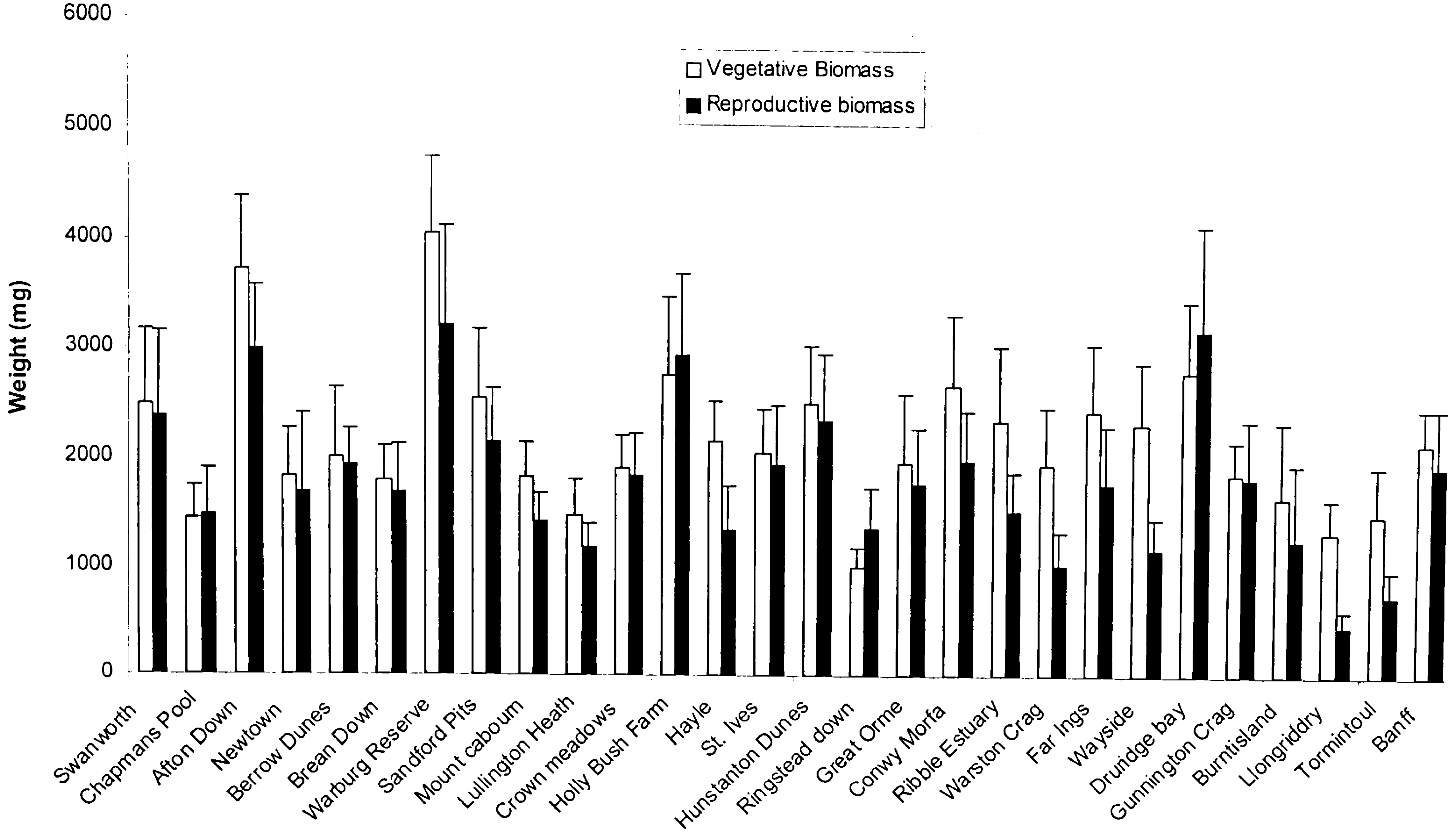

Figure 4.3 Vegetative and reproductive biomass of plants from 28 locations planted on a plot treated with topsoil. Distance from the home-site, Swanworth Quarry, increases left to right on the graph.

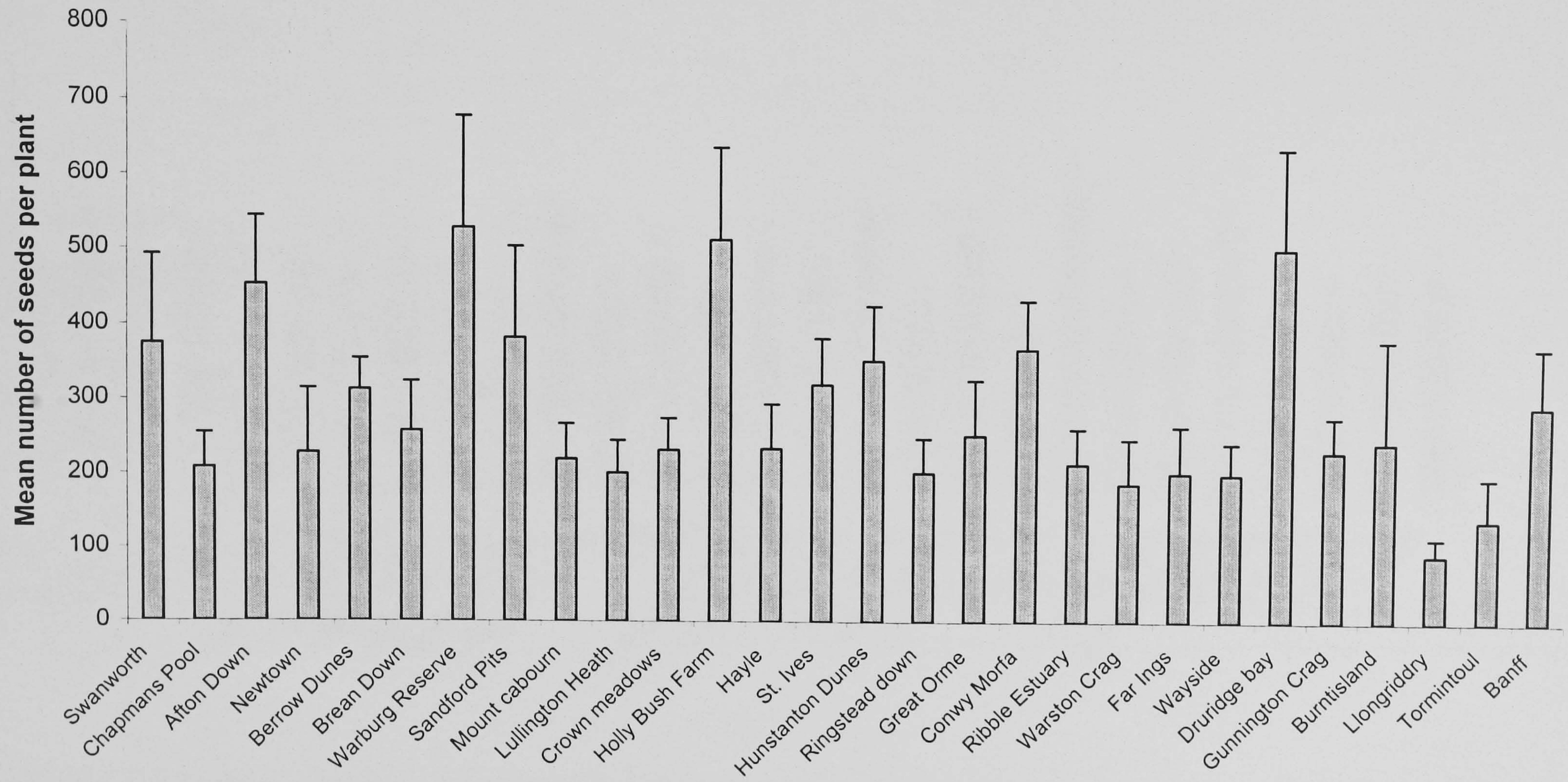

Figure 4.4 Mean seed number of plants from 28 locations planted on a plot treated with topsoil. Distance from the home-site, Swanworth Quarry, increases left to right on the graph.

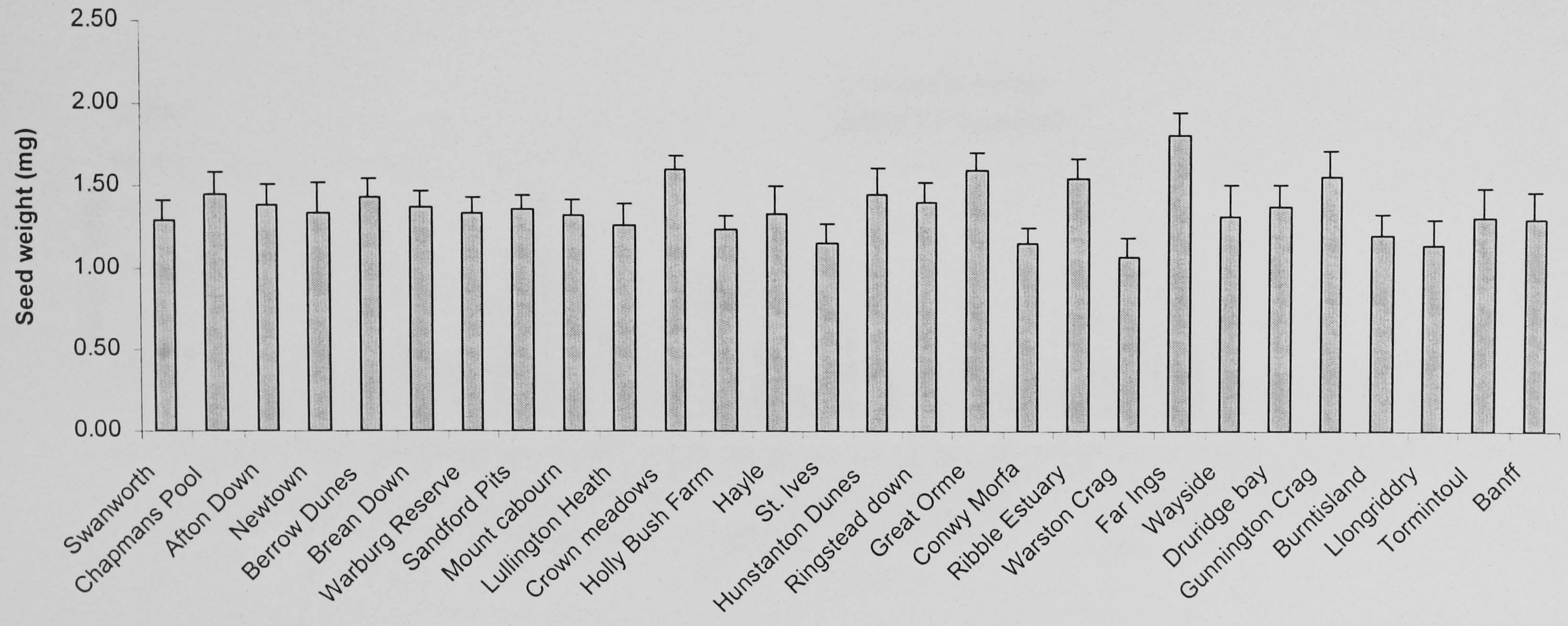

Figure 4.5 Mean seed weight of plants from 28 locations planted on a plot treated with topsoil. Distance from the home-site, Swanworth Quarry, increases left to right on the graph.

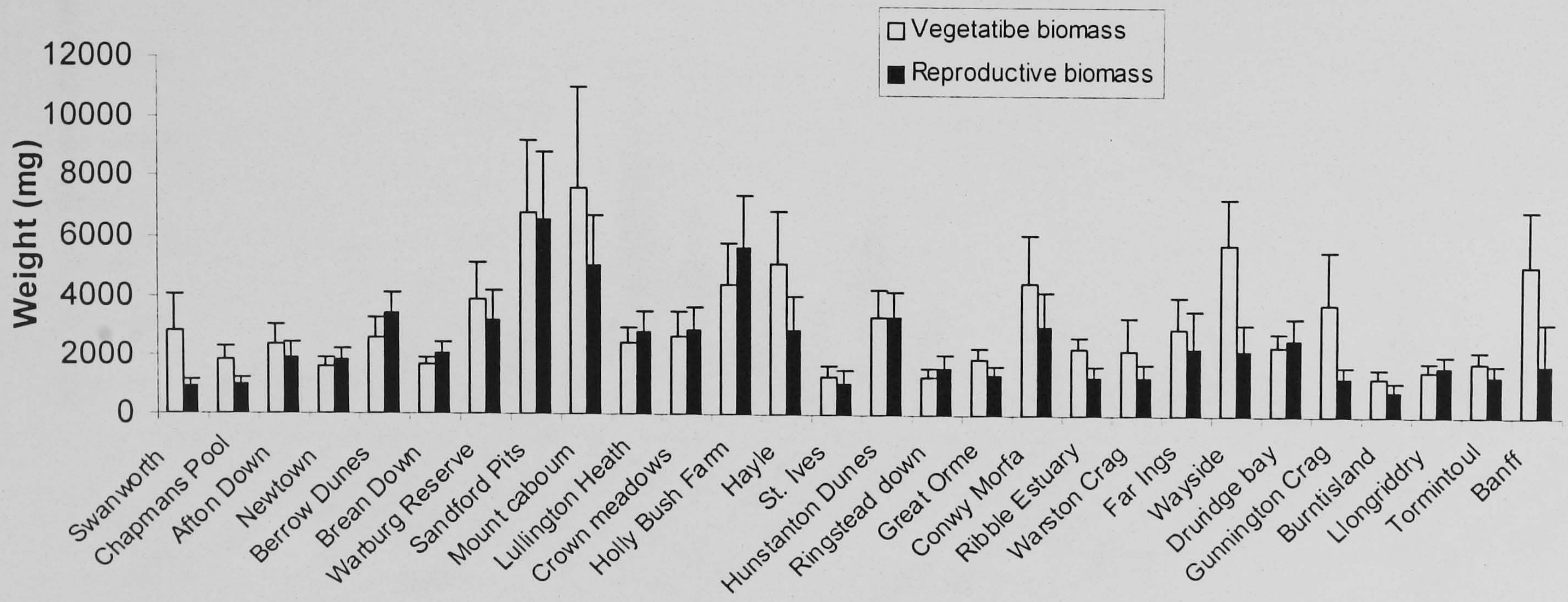

Figure 4.6 Vegetative and reproductive biomass of plants from 28 locations planted on the untreated plot. Distance from the home-site, Swanworth Quarry, increases left to right on the graph

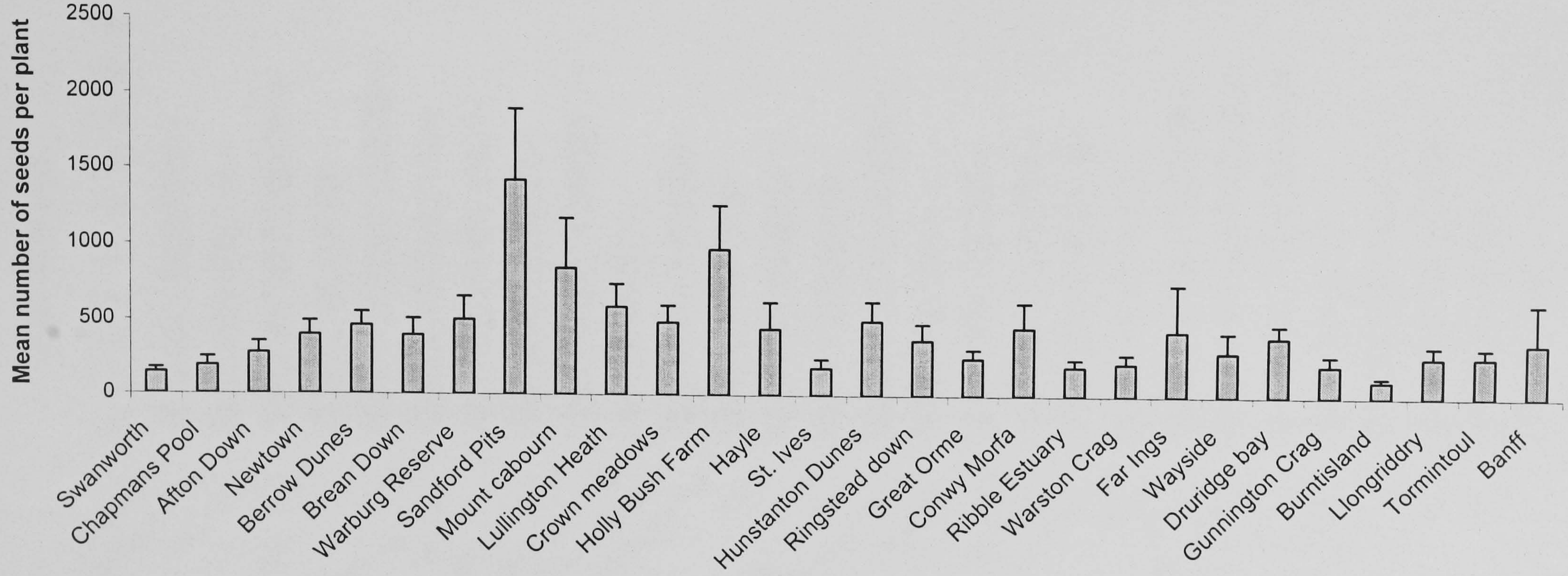

Figure 4.7 Mean seed number of plants from 28 locations planted on the untreated plot. Distance from the home-site, Swanworth Quarry increases from left to right.

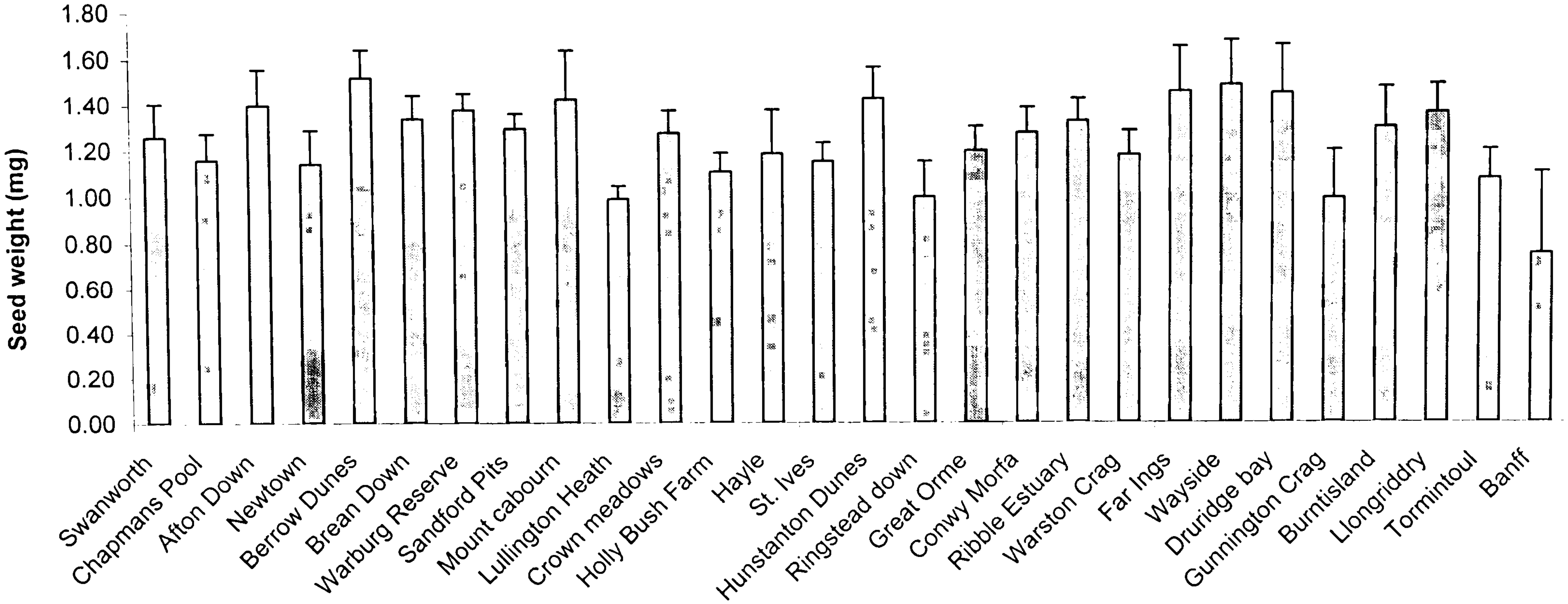

Figure 4.8 Mean seed weight of plants from 28 locations planted on an untreated plot. Distance from the home-site, Swanworth Quarry, increases left to right on the graph.

4.3.2 Differences in plant survival and fitness between regions and type of site.

The Treated Plot

Survival

Data were analysed to investigate whether plant survival was related to regional provenance or type of site (Table 4.2). There was significant variation in survival of plants from different regions (df= 14 χ^2 =26.9 P=0.020) but no difference in plant survival between the calcicolous grassland and the alternative habitat (df= 1 χ^2 =0.263 P=0.430).

Table 4.2 Cross-tabulation of survival and mortality of *L. corniculatus* plants on the treated plot at Swanworth Quarry. *=significant at 0.05, ** = significant at 0.01, *= significant at 0.001.**

Region			
	Mortalities	Survivors	Total
Cornwall	12	34	46
Dorset	0	36	36
E.Sussex	8	34	42
Edinburgh	14	31	45
Grampian1	6	6	12
Grampian2	9	20	29
Humberside	15	16	31
Isle of Wight	15	28	43
Kent	10	26	36
N. Wales	14	30	44
Lancashire	7	24	31
Norfolk	6	27	33
Northumberland	12	29	41
Oxford	9	30	39
Somerset	8	37	45
	χ^2 =26.9, P=.020*, df=14		
Habitat of origin			
Calcicolous grassland	78	200	278
Alternative habitat	69	206	275
	χ^2 =.623, P=.430, df=1		

Plant traits

Differences were found in plant traits between populations collected from different regions and planted on the treated plot at Swanworth Quarry in Dorset. Variation was found in vegetative biomass (df=13, F=1.77, *P*=0.049), reproductive biomass (df=13, F=2.34, *P*=0.006), the ratio of vegetative to reproductive biomass (df=13, F=2.07, *P*=0.017) and seed number (df=13, F=1.03, *P*=0.013). Figures 4.9 and 4.10 show that plants from the Dorset region were not bigger or more fecund than those from other sites. Plants from Oxford and the Isle of Wight were the largest and most productive in this environment. There was no significant difference between the calcicolous grassland sites and the alternative habitats for any of the plant traits. The data are summarized in Table 4.3.

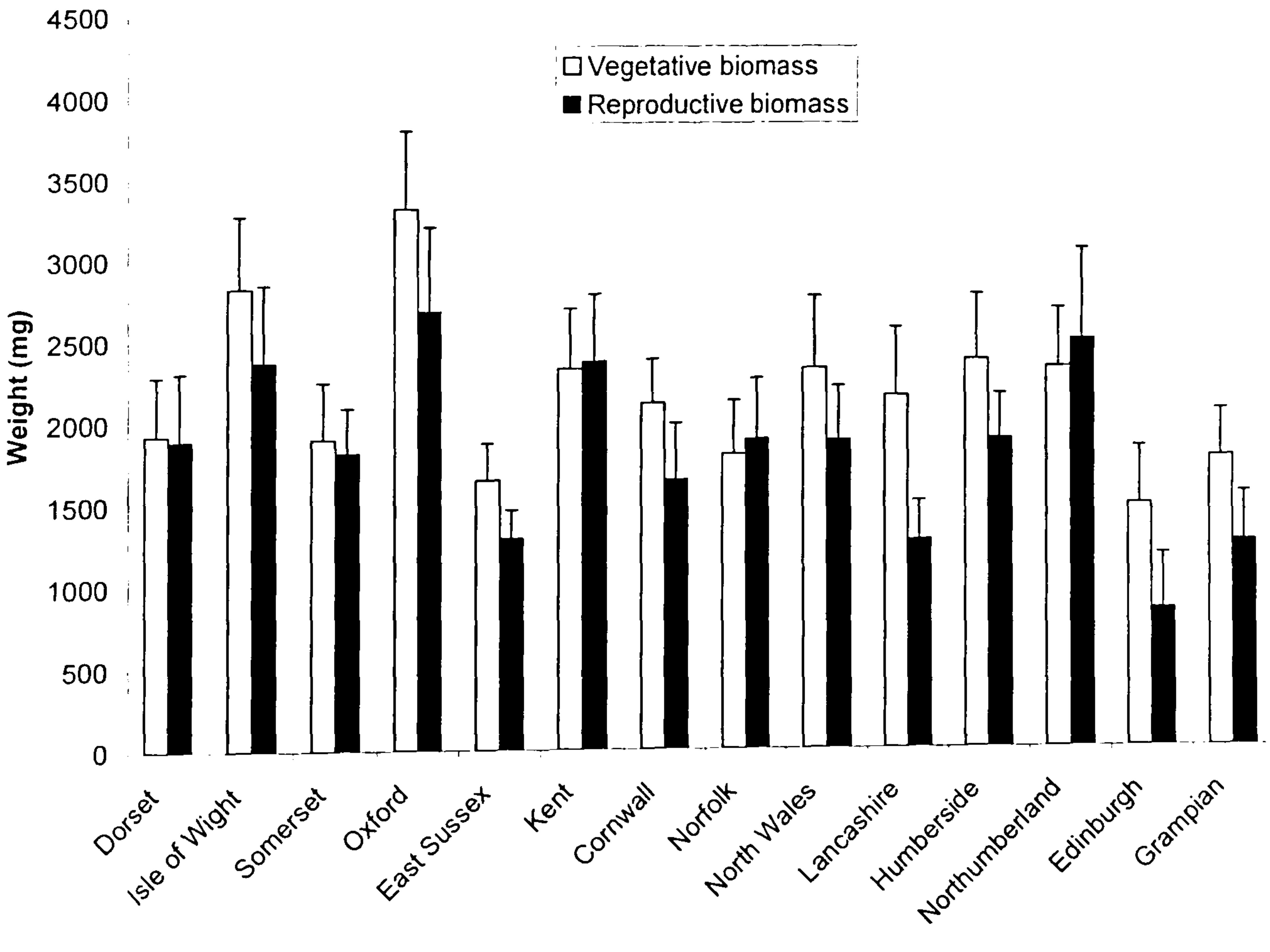

Figure 4.9 Mean vegetative and reproductive biomass of plants from 14 regions in the British Isles on the treated plot. Distance from Dorset increases from left to right.

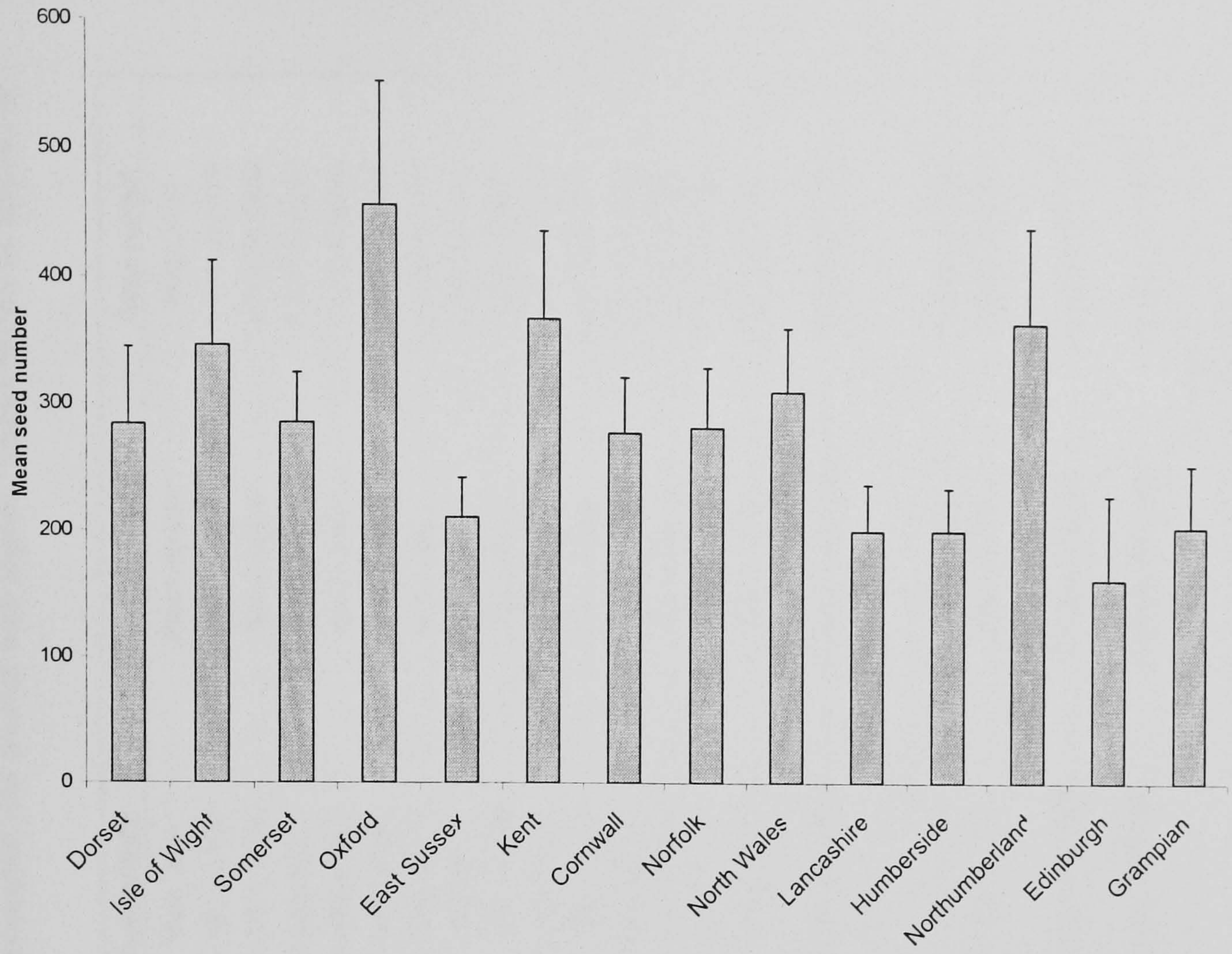

Figure 4.10 Mean seed number produced by plants from 14 regions in the British Isles on the treated plot. Distance from Dorset increases from left to right on the graph.

Table 4.3 Differences in plants traits between populations of *L. corniculatus* grown on an experimental plot treated with topsoil. Values shown as means ± SE *= significant at 0.05 level, ** = significant at 0.01 ***= significant at 0.001.

Region		Vegetative biomass (VB)	Reproductive biomass (RB)	Ratio VB/RB	Seed number	Seed weight
	N	*Mean (mg)*	*Mean (mg)*	*Mean ratio*	*Mean number*	*Mean (mg)*
Dorset	20	1928 ±362	1895 ±417	0.982 ±0.122	284.1 ±60.3	1.386 ±0.0896
Isle of Wight	20	2833 ±445	2378 ±478	0.857 ±0.105	346.2 ±66.5	1.376 ±0.0988
Somerset	20	1907 ±350	1822 ±275	2.020 ±0.953	285.7 ±39.1	1.410 ±0.0750
Oxford	20	3329 ±481	2697 ±520	0.855 ±0.090	457.2 ±96.2	1.359 ±0.0633
East Sussex	20	1656 ±226	1300 ±175	0.847 ±0.085	211.3 ±31.3	1.305 ±0.0829
Kent	20	2343 ±370	2386 ±417	1.019 ±0.111	368.2 ±69.3	4.438 ±0.0742
Cornwall	20	2128 ±273	1658 ±346	0.083 ±0.137	278.5 ±43.8	1.386 ±0.1040
Norfolk	19	1813 ±331	1905 ±374	1.147 ±0.158	283.3 ±47.3	1.447 ±0.0941
North Wales	20	2341±446	1896 ±334	0.845 ±0.080	312.0 ±50.7	1.385 ±0.0892
Lancashire	20	2172 ±421	1282 ±240	0.765 ±0.210	202.2 ±36.8	1.325 ±0.0976
Humberside	17	2392 ±404	1905 ±278	0.865 ±0.315	202.5 ±33.8	1.541 ±0.1360
Northumberland	20	2349 ±363	2516 ±562	0.965 ±0.087	367.7 ±76.1	1.490 ±0.1000
Edinburgh	20	1497 ±355	853 ±340	0.586 ±0.113	163.7 ±67.2	1.181 ±0.0994
Grampian	16	1793 ± 291	1270 ±301	0.650 ±0.149	206.2 ±49.5	1.319 ±0.1190
ANOVA	**df=13**	**F=1.77, *P*=0.049***	**F=2.34, *P*=0.006***	**F=2.07, *P*=0.017***	**F=2.12, *P*=0.013***	**F=1.03 *P*=0.426**
Habitat of Origin						
Calcicolous grassland	136	2056 ±139	1753 ±152	0.912 ±0.064	266.2 ±23.2	1.3862 ±0.0358
Alternative habitat	136	2313 ±150	1891 ±146	0.982 ±0.141	306.4 ±22.7	1.3556 ±0.0355
ANOVA	**df=1**	**F=2.67, *P*=0.103**	**F=1.12, *P*=0.209**	**F=0.02, *P*=0.896**	**F=2.91, *P*=0.089**	**F=0.55, *P*=0.460**
Region*Habitat	**df=13**	**F=1.73, *P*=0.056**	**F=0.21, *P*=0.235**	**F=1.26, *P*=0.235**	**F=1.48, *P*=0.125**	**F=1.76, *P*=0.051**

There were no differences in the proportion of damaged plants, either by region (χ^2= 8.641, P=0.799) or habitat of origin (χ^2= 2.692, P=0.101). However incidence of seed damage is not random as bigger plants with more seed were more likely to suffer seed damage (Table 4.4).

Table 4.4 The relationship between plant size and fecundity and seed damage in *L. corniculatus* plants growing on the treated plot. *= significant at 0.05, ** = significant at 0.01, *= significant at the 0.001 level.**

	Plants with damaged seeds (N= 190)	Plants with undamaged seeds (N = 86)	F	*P*	d.f.
Mean vegetative biomass (mg)	2449 ±119	1645 ±189	13.02	<0.001***	1
Mean reproductive biomass (mg)	2172 ±139	1063 ±116	24.65	<0.001***	1
Mean seed number	344 ±21.5	159 ±15.8	29.36	<0.001***	1

The Untreated Plot.

Survival

There were differences in survival between regions at the untreated site (df =14 χ^2 = 28.4 p=0.013) but no significant difference in survival between plants sourced from the calcicolous grassland and the alternative habitats (df =1 χ^2 = 1.25 p=0.265). Table 4.5 shows the cross-tabulated counts for each region and habitat of origin and indicates that, although the Dorset population had a high survival rate, Cornwall, East Sussex and Somerset had a higher proportion of survivors.

Table 4.5 Cross-tabulation of survival and mortality of *L. corniculatus* plants on untreated plot at Swanworth Quarry. *=significant at 0.05, ** = significant at 0.01, *= significant at 0.001.**

Region			
	Mortalities	Survivors	Total
Cornwall	15	40	55
Dorset	15	33	48
E.Sussex	16	32	48
Edinburgh	13	42	55
Grampian1	3	2	5
Grampian2	15	20	35
Humberside	17	14	31
Isle of Wight	21	28	49
Kent	9	31	40
N. Wales	15	35	50
Lancashire	7	28	35
Norfolk	16	26	42
Northumberland	15	36	51
Oxford	15	29	44
Somerset	8	45	53
	χ^2 =28.4, *P*=.013, df=14**		
Habitat of origin			
Calcicolous grassland	93	226	319
Alternative habitat	107	215	322
	χ^2 =1.240, *P*=.265, df=1		

Plant traits

Regional differences were also found on the untreated plot where there was variation in vegetative biomass (df=13, F=1.97, *P*=0.024), reproductive biomass (df=13, F=3.48, *P*=<0001), the ratio between vegetative and reproductive biomass (df=13, F=2.13, *P*=0.013) and seed yield (df=13, F=4.26, *P*=<0.001). There was no advantage for the Dorset population which performed poorly in this environment (Figures 4.11 and 4.12). Data are presented in Table 4.6.

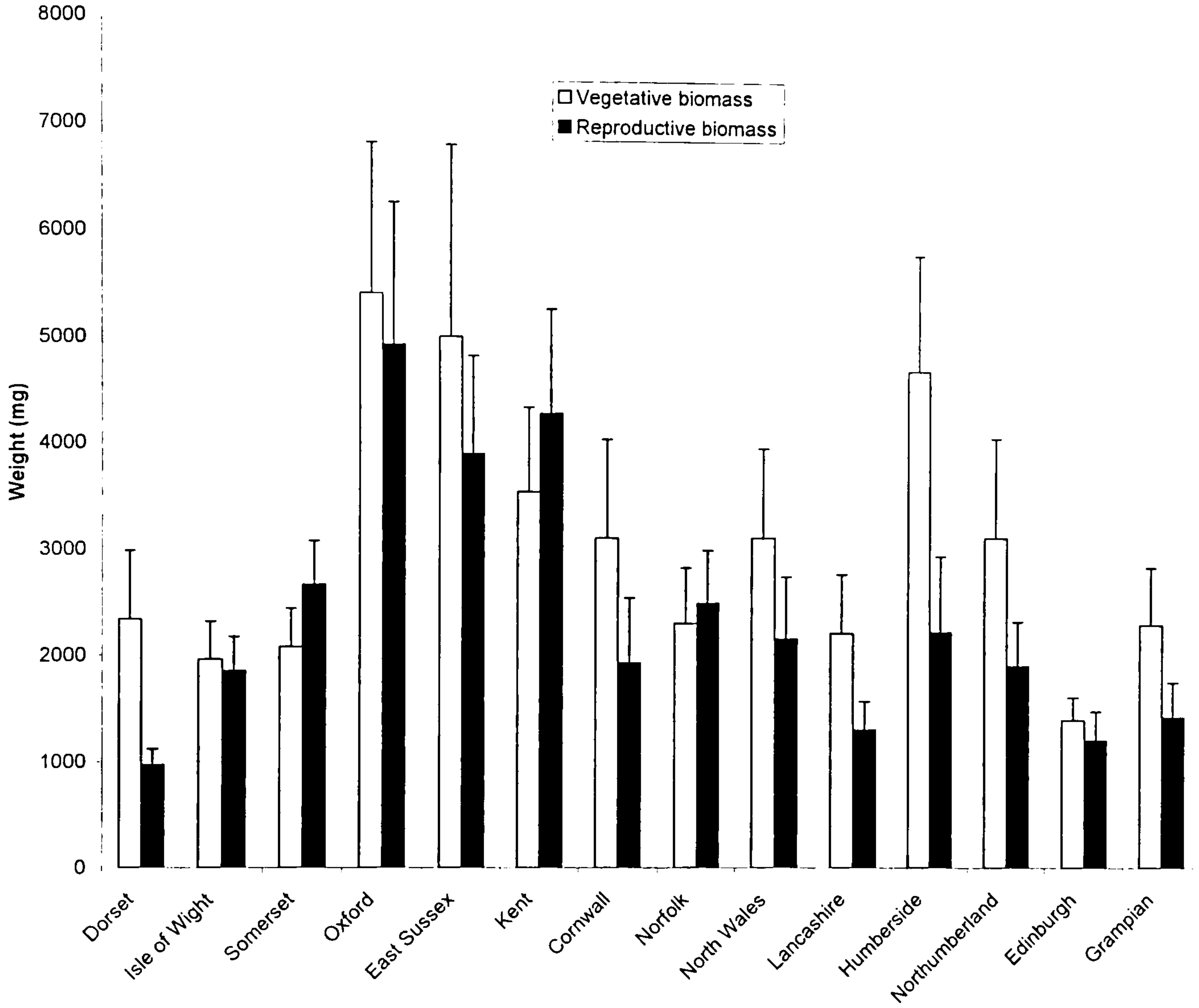

Figure 4.11 Mean vegetative and reproductive biomass of plants from 14 regions in the British Isles on the untreated plot. Distance from Dorset increases from left to right on the graph

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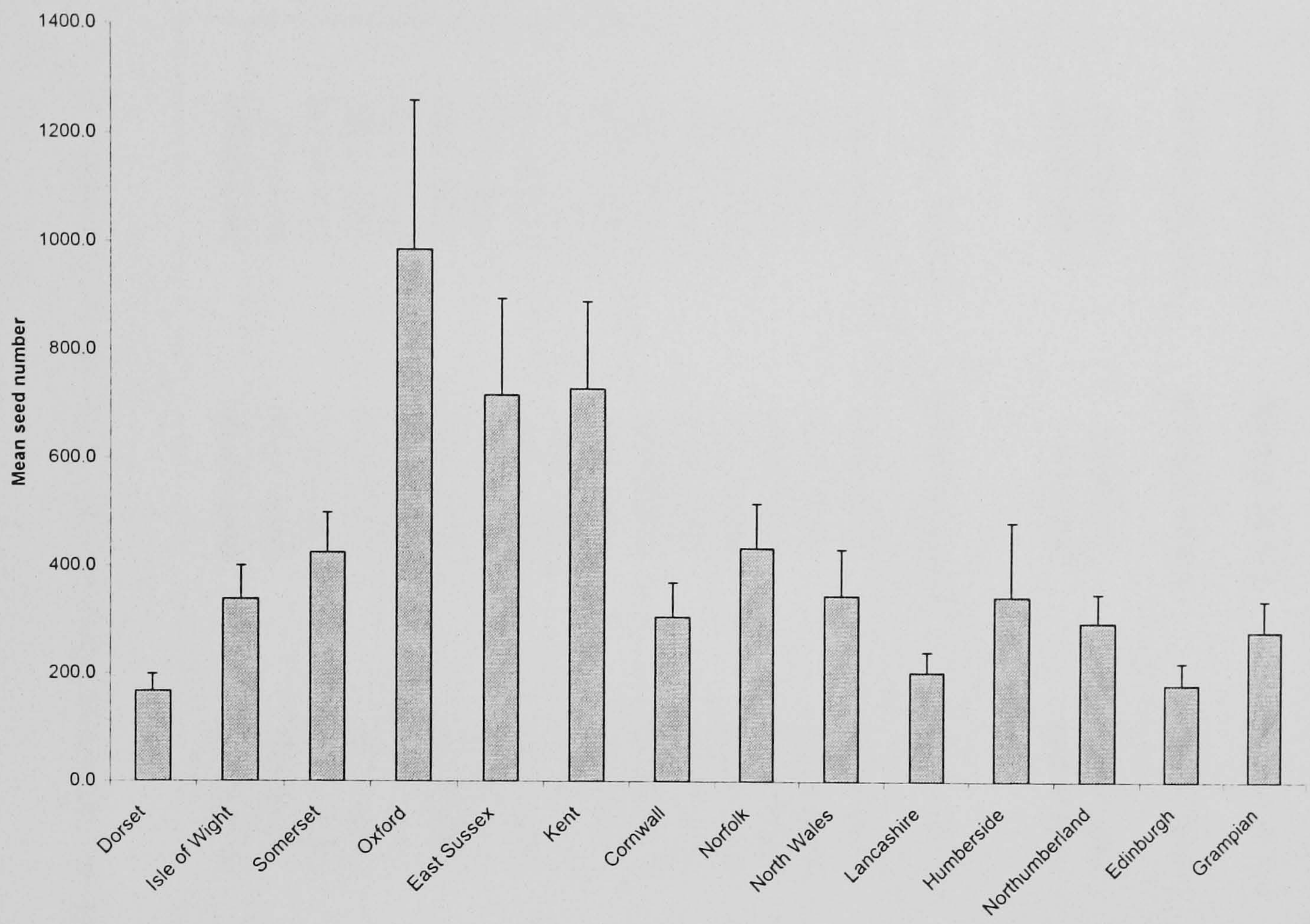


Figure 4.12 Mean seed number produced by plants from 14 regions in the British Isles on the untreated plot. Distance from Dorset increases from left to right on the graph.

In contrast to the treated site, differences were found between vegetative and reproductive biomass of plants from calcicolous grassland and the alternative habitats at the untreated site. The plants from the calcicolous grassland were significantly smaller (df=1, F=14.80, *P*=<0.001) with less reproductive biomass (df=1, F=9.60, *P*=0.002) and a lower seed yield (df=1, F=6.41, *P*=0.012) (Table 4.6). The ratio between the two measures of biomass also varied. This is illustrated in Figure 4.13 which shows that the calcicolous grassland plants tended to produce a proportionately higher reproductive biomass than plants sourced from the alternative habitats.

Table 4.6 Differences in plants traits between populations of *L. corniculatus* grown on an untreated experimental plot. Values shown as means ± SE *= significant at 0.05, **= significant at the 0.01, *** = significant at 0.001.

Region		Vegetative biomass (VB)	Reproductive biomass (RB)	Ratio RB/VB	Seed number	Seed weight
	N	*Mean (mg)*	*Mean (mg)*	*Mean ratio*	*Mean number*	*Mean (mg)*
Dorset	20	2341 ±645	974 ±148	0.685 ±0.102	167.0 ±32.0	1.210 ±1.210
Isle of Wight	20	1965 ±355	1856 ±322	0.986 ±0.122	339.0 ±61.5	1.267 ±1.267
Somerset	20	2084 ±361	2672 ±412	1.532 ±0.230	425.0 ±74.4	1.423 ±1.423
Oxford	20	5419 ±1408	4940 ±1331	0.943 ±0.110	987.0 ±275.0	1.338 ±1.338
East Sussex	22	5015 ±1793	3906 ±930	1.105 ±0.141	719.0 ±178.0	1.209 ±1.209
Kent	20	3552 ±801	4292 ±988	1.220 ±0.081	732.0 ±161.0	1.195 ±1.195
Cornwall	20	3120 ±931	1942 ±615	0.742 ±0.163	306.9 ±64.0	1.171 ± 1.171
Norfolk	20	2316 ±525	2507 ±502	1.310 ±0.228	436.4 ±83.6	1.215 ±1.215
North Wales	20	3126 ±842	2172 ±588	0.743 ±0.100	348.2 ±86.9	1.238 ±1.238
Lancashire	20	2226 ±559	1315 ±266	0.878 ±0.191	204.4 ±37.8	1.265 ±1.265
Humberside	13	4709 ±1090	2240 ±719	0.477 ±0.100	346.0 ±140.0	1.477 ±1.477
Northumberland	20	3138 ±935	1922 ±421	0.819 ±0.156	298.2 ±54.0	1.210 ±1.210
Edinburgh	20	1409 ±216	1220 ±271	0.829 ±0.203	182.4 ±40.0	1.329± 1.329
Grampian	12	2317 ±545	1438 ±331	2.640 ±2.150	281.9 ±58.4	1.023± 1.023
ANOVA	**df=13**	**F=1.97, *P*=0.024***	**F=3.48, *P*=0.001*****	**F=2.13, *P*=0.013***	**F=4.26, *P*=<0.001*****	**F=1.18, *P*=0.296**
Habitat of Origin						
Calcicolous grassland	135	2212 ±228	1762 ±151	1.104 ±0.201	322.6 ±28.7	1.186 ± 0.0357
Alternative habitat	130	3875 ±438	3098 ±337	0.9632 ±0.068	517 ±62.9	1.330 ±0.0397
ANOVA	**df=1**	**F=14.80, *P*=<0.001*****	**F=9.60, *P*=0.002****	**F=0.25, *P*=0.616**	**F=6.41, *P*=0.012***	**F=2.84, *P*=0.093**
Region*Habitat	**df=13**	**F=1.04, *P*=0.416**	**F=0.43, *P*=0.960**	**F=1.27, *P*=0.230**	**F=0.78, *P*=0.682**	**F=1.27, *P*=0.229**

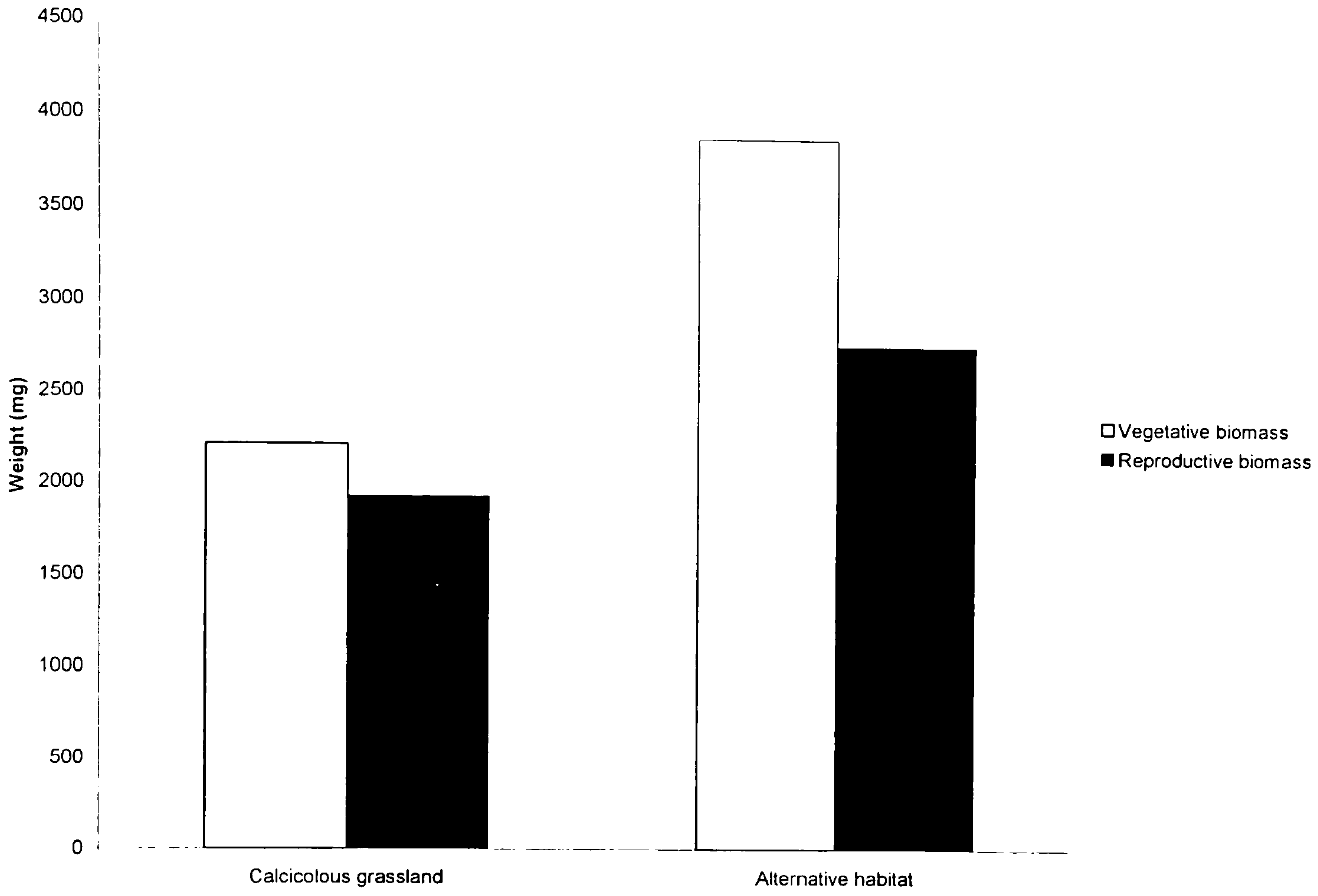


Figure 4.13 The ratio of vegetative biomass to reproductive biomass of *L. corniculatus* on an untreated plot at Swanworth Quarry.

There was no difference in the number of plants with damaged seeds between different regions ($\chi^2$12.616, *P*=0.478) or between calcicolous grassland and the alternative habitat ($\chi^2$2.852, *P*=0.091). However, as on the treated plot, larger plants with less seed were more likely to be damaged (Table 4.7).

Table 4.7 The relationship between plant size and fecundity and seed damage in *L. corniculatus* plants growing on the untreated plot. *= significant at 0.05, **= significant at the 0.01, *** = significant at 0.001.

	Plants with damaged seeds (N= 176)	Plants with undamaged seeds (N = 94)	F	*P*	d.f.
Mean vegetative biomass (mg)	3640 ±355	1767 ±238	24.11	<0.001***	1
Mean reproductive biomass	3032 ±267	1149 ±169	47.05	<0.001***	1
Mean seed number	279 ±44.9	479.1 ±53.5	20.78	<0.001***	1

4.3.3 The effect of geographical and ecological distance

The geographical and ecological distances between sites are shown in Table 4.8 There was no correlation between geographical and ecological distance (Pearson coefficient= –0.198 *P*=<0.313), therefore it was appropriate to analyse the effects of geographical and ecological sites separately.

Table 4.8 The ecological and geographical distance between Swanworth Quarry and all other sites.

Site	Geographical distance (km)	Ecological distance (DCA)
Swanworth Quarry (Dorset)	0	0
Chapman's Pool (Dorset)	1.6	93.9
Afton Down (Isle of Wight)	40.4	130
Newtown Meadows (Isle of Wight)	47.6	111.9
Berrow Dunes (Somerset)	100	59.9
Brean Down (Somerset)	104.7	24.1
Warburg Reserve (Oxford)	130.6	128.3
Sandford Pit (Oxford)	131.3	123.5
Lullington Heath (East Sussex)	149.5	143.1
Mount Cabourn (East Sussex)	151.2	223.5
Hollybush Farm (Kent)	205.4	76.8
Crown Meadows (Kent)	219.7	161.9
Hayle (Cornwall)	243.2	190.9
St. Ives (Cornwall)	247.6	47.1
Hunstanton Dunes (Norfolk)	315.9	175.3
Conwy Morfa (N. Wales)	324	132.9
Great Ormes Head (N. Wales)	327.5	156.5
Ringstead Downs (Norfolk)	329.1	91.6
Ribble Estuary (Lancashire)	350.8	116.7
Far Ings (Humberside)	360.1	16.2
Warston Crag (Lancashire)	365.4	48.2
Hull Wayside (Humberside)	366.9	103.8
Gunnerton Crag (Northumberland)	498.8	44.2
Druridge Bay (Northumberland)	517.1	101.8
Longriddry (Edinburgh)	570.3	89.7
Burntisland (Edinburgh)	612.3	85.3
Derry Lodge (Grampian 1)	720.8	102.1
Tomintoul (Grampian 1)	743.9	20.6
Banff (Grampian 2)	786.5	78.9
Findlater (Grampian 2)	790	45.3

The Treated Plot.

Survival

Regression analyses were performed to compare the percentage of the remaining plants which were alive with i) geographical and ii) ecological distance of the donor sites from Swanworth Quarry (Table 4.9). These analyses discounted the missing plants. A significant negative relationship was found between geographical distance and plant survival (R^2=0.191, F=6.356, *P*=0.018). However, the potential of missing plants to survive (had they not been removed) could not be established. Alternative regression analyses were performed using the proportion of survivors of all those originally planted. No significant relationships were found. Taking both analyses of geographical distance and survival into consideration, it is concluded that a weak negative relationship exists. Ecological distance was not significantly related to plant survival in either analysis.

Table 4.9 Regression analysis of geographical and ecological distance and plant survival on the treated plot. *=significant at 0.05, ** = significant at 0.01, *= significant at 0.001.**

Regression	Correlation coefficient	R^2	F	*P*
Plot treated with topsoil				
Geographical distance/% survival of all planted	-0.041	0.002	0.46	0.832
Ecological distance/% survival of all planted	-0.18	0.032	0.905	0.35
Geographical distance/% survival of remaining plants	-0.437	0.191	6.356	0.018*
Ecological distance/% survival of remaining plants	0.032	0.001	0.028	0.867

Plant traits

Regression analyses were used to investigate the relationship between distance and the fitness measures. There was no relationship between ecological distance and any of the measures on the treated site, though there was a weak negative relationship between geographical distance and reproductive biomass (df=1 F=5.34 *P*=0.022) and seed number (df=1 F=6.42 *P*=0.032). The low R^2 values show that little variation was accounted for. When mean data were entered into the regression analysis, more variation was accounted for but no significant relationship was detected. A principal components analysis (PCA) was carried out: this combined the variables to give an inclusive determinant of plant fitness. The first axis, PCA1 (eigenvalue 2.73), captured 91% of the variation and this is accounted for by the three variables (vegetative biomass –0.557 reproductive biomass –0.588 seed number-0.587). There was no relationship between PCA1 and geographical or ecological distance (Table 4.10).

Table 4.10 Regression analyses of plant fitness indicators and geographical and ecological distance on a site treated with topsoil at Swanworth Quarry. *= significant at 0.05, **= significant at the 0.01, * = significant at 0.001.**

Regression	equation	F	*P*	R^2
All data				
Vegetative biomass/geographical distance	VB = 2422-0.0833G	2.76	0.098	1.0%
Reproductive biomass/geographical distance	RB = 2158-0.119G	5.34	0.022*	1.9%
Seed number/geographical distance	SN = 335-0.0171G	4.64	0.032*	1.6%
Seed weight/geographical distance	SW = 1.39-0.000008G	0.42	0.519	0.1%
Predation/geographical distance	SP =0.622+0.000023G	2.90	0.090	1.0%
Means only				
Vegetative biomass/geographical distance	VB = 2385-0.0722G	1.44	0.241	5.2%
Reproductive biomass/geographical distance	RB = 2127-0.107G	3.26	0.083	11.1%
Seed number/geographical distance	SN= 331-0.0160G	2.63	0.117	9.2%
Seed weight/geographical distance	SW = 1.37-0.000007G	0.21	0.650	0.8%
PCA				
PCA1/geographical distance	PCA1 = -0.695-0.00024G	2.64	0.116	9.2%
All data				
Vegetative biomass/ecological distance	VB = 2031+1.55E	0.67	0.413	0.2%
Reproductive biomass/ecological distance	RB = 1766+0.57E	0.08	0.771	0.0%
Seed number/ecological distance	SN = 278+0.087E	0.08	0.771	0.0%
Seed weight/ ecological distance	SW = 1.34+0.0003E	0.47	0.495	0.2%
Predation/ ecological distance	SP = 0.737-0.0005E	0.86	0.345	0.3%
Means only				
Vegetative biomass/ecological distance	VB = 2032+1.41E	0.36	0.555	1.4%
Reproductive biomass/ecological distance	RB = 1756+0.57E	0.06	0.815	0.2%
Seed number/ecological distance	SN = 272+0.115E	0.08	0.773	0.3%
Seed weight/ ecological distance	SW = 1.33+0.00058E	0.06	0.813	0.2%
PCA				
PCA1/ecological distance	PCA1= 0.225+0.0027E	0.15	0.701	0.6%

The Untreated Plot

Survival

Regression analyses showed that there was no relationship between either the geographical or ecological distance of the donor site and plant survival at Swanworth Quarry (Table 4.11)

Table 4.11 Regression analysis of geographical and ecological distance and plant survival on the untreated plot.

Regression	Correlation coefficient	R^2	F	*P*
Untreated plot				
Geographical distance/% survival of all planted	-0.244	0.06	1.715	0.201
Ecological distance/% survival of all planted	-0.12	0.014	0.395	0.595
Geographical distance/% survival of remaining plants	-0.296	0.088	2.599	0.119
Ecological distance/% survival of remaining plants	-0.046	0.002	0.056	0.814

Plant traits

Using all the data points, reproductive biomass (df=1 F=4.39 *P*=0.021) and seed yield (df=1 F=6.45 *P*=0.012) are significantly related to geographical distance (Table 4.12) shows that the regression equation is negative, indicating that reproductive biomass and number of seeds decreased with geographical distance of the source populations away from the quarry. Little of the variation is explained. A regression analysis using mean fitness data increased the amount of the variation captured, but reproductive biomass (df=1 F=2.69 *P*=0.113) and seed number (df=1 F2.44 *P*=0.131) were no longer significant. However, there is a significant negative correlation with seed weight, suggesting that seeds produced by plants originating at different distances show a decline in weight as the distance to the collection site increases (F=4.55, *P*=0.043, df=1).

Using all data points in the regression analyses, there is a strong correlation between ecological distance and vegetative biomass (df=1 F=10.73 *P*=<0.001), reproductive biomass (df=1 F=12.19 *P*=0.001) and seed number (df=1 F= 10.35 *P*=0.002). Table 4.15 shows that the regression equation in all cases is positive so that in general plants were bigger and produced more seed as the ecological distance of the source site from Swanworth Quarry increased. This analysis captures a limited amount of the variation and a second regression analysis was run for each of the performance indicators using the mean plant fitness value for each site. This increases the R^2 value which ranges between 13.6%-18.8%. The correlations remain significant although confidence is reduced as follows; vegetative biomass (df=1 F=5.32 *P*=0.029), reproductive biomass (df=1 F=6.02 *P*=0.021), seed number (df= 1 F=105.2 *P*=0.054).

The plant traits were combined in a principal components analysis (PCA). Axis 1 of the PCA analysis (eigenvalue 2.497) captured 83% of the variation which was accounted equitably by the three variables (vegetative biomass =-0.513; reproductive biomass= –0.612; seed number= –0.602). A regression analysis of PCA1 against the ecological predictor was negatively correlated as shown in Table 4.12 (df=1 F=6.34 *P*=0.018). Overall plant performance decreased with ecological distance from the home-site. There was no correlation between geographical distance and PCA1 (df=1 F=1.68 *P*=0.206).

Table 4.12 Regression analyses of plant fitness indicators and geographical and ecological distance on an untreated site at Swanworth Quarry. *= significant at 0.05, **= significant at the 0.01, * = significant at 0.001.**

Regression	equation	F	*P*	R^2
All data				
Vegetative biomass/geographical distance	VB = 3493-0.148G	1.36	0.244	0.5%
Reproductive biomass/geographical distance	RB = 3021-0.218G	5.39	0.021*	1.9%
Seed number/geographical distance	SN = 541-0.0442G	6.45	0.012*	2.3%
Seed weight/geographical distance	SW = 1.29-0.00001G	0.91	0.341	0.3%
Predation/geographical distance	SP = 0.707-0.00002G	1.80	0.181	0.7%
Means only				
Vegetative biomass/geographical distance	VB = 3271-0.041G	0.06	0.802	0.2%
Reproductive biomass/geographical distance	RB = 2982-0.207G	2.69	0.113	9.4%
Seed number/geographical distance	SN = 526-0.0389G	2.44	0.131	8.6%
Seed weight/geographical distance	SW = 1.30-0.00003G	4.55	0.043*	14.9%
PCA				
PCA1/geographical distance	PCA1 = 0.582-0.00002G	1.68	0.206	6.1%
All data				
Vegetative biomass/ecological distance	VB = 1550 +14.6E	10.73	0.001***	3.7%
Reproductive biomass/ecological distance	RB = 1238+11.6E	12.9	0.001***	4.2%
Seed number/ecological distance	SN = 218+1.97E	1013	0.002*	3.5%
Seed weight/ ecological distance	SW = 1.20+0.0005E	1.11	0.293	0.4%
Means only				
Vegetative biomass/ecological distance	VB = 1839+13.2E	5.32	0.029*	17%
Reproductive biomass/ecological distance	RB = 1253+11.3E	6.02	0.021*	18.8%
Seed number/ecological distance	SN = 225+1.88E	4.08	0.054	13.6%
Seed weight/ ecological distance	SW = 1.16+0.0005E	0.64	0.430	2.4%
PCA				
PCA1/ecological distance	PCA1 = 1.23=0.0128E	6.34	0.018*	19.6%

Overall differences in plant performance between the experimental plots.

Figure 4.14 shows that there were differences in survival between the two experimental plots. Considering all individuals planted, the untreated plot had a higher number of survivors and also deaths, but fewer missing plants than the plot treated with topsoil. It is possible that the treated plot was more attractive to herbivores as it was invaded by weedy species during the experimental period and this may account for the heavy losses. When missing plants were discounted, observation shows that more plants survived on the plot treated with topsoil.

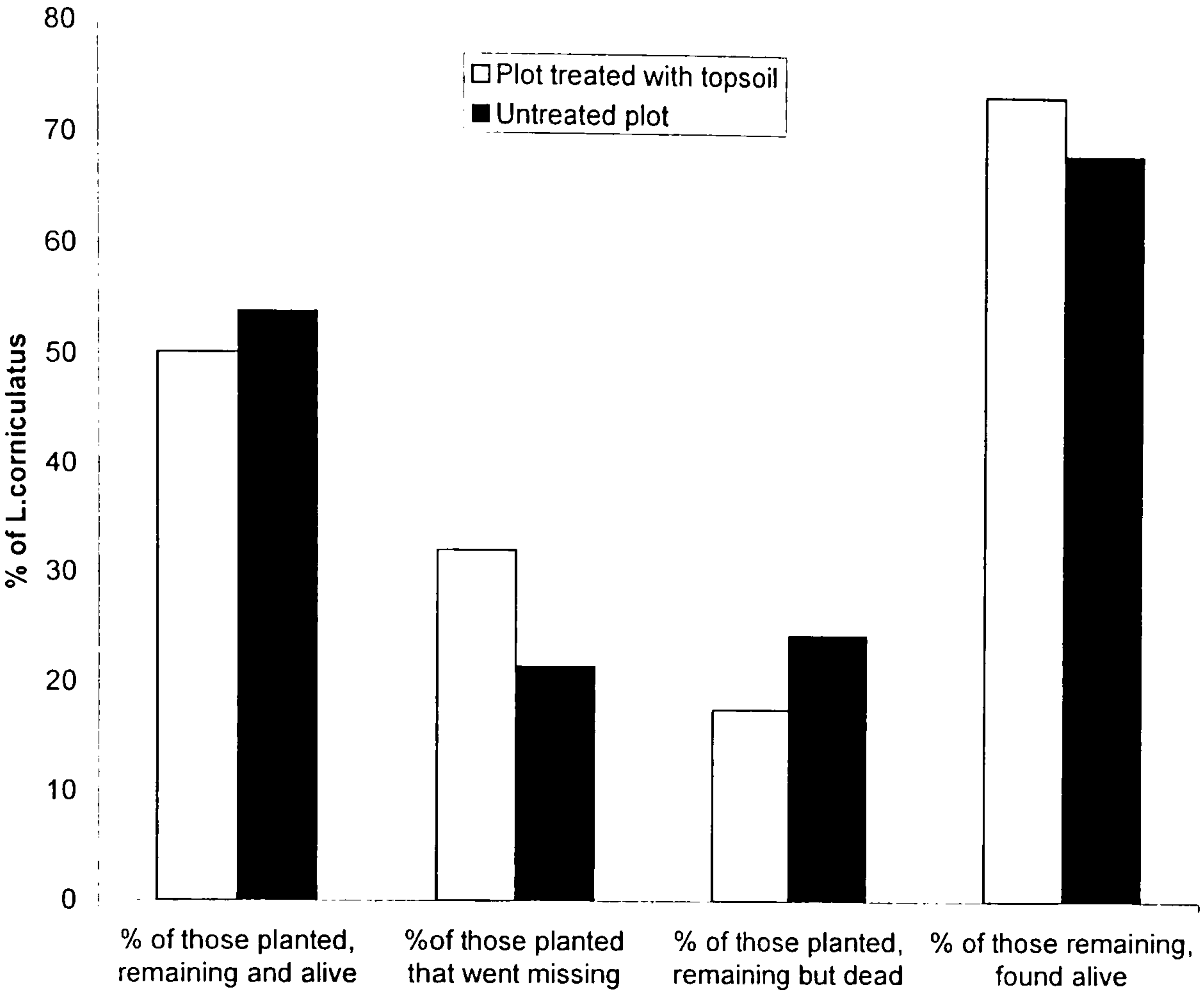


Figure 4.14 The fate of *L. corniculatus* plants in two experimental plots at Swanworth Quarry in Dorset. Showing: i) the proportion of plants which survived on the plots and the proportion that went missing. ii) Of the plants which remained on the plots, the proportion which survived and the proportion which died.

Plants were larger and more productive on the plot treated with topsoil, but seed weight did not vary between the plots (Table 4.13). Correlations between plant fitness measures were similar on the two plots; bigger plants produced more reproductive biomass and had a higher seed yield. Seed yield was negatively correlated with seed weight; plants either produced a high number of small seeds or a lower number of larger seeds (Table 4.14).

Table 4.13 Different fitness of plants growing on two experimental plots at Swanworth Quarry. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

	Treated plot N = 289	Untreated plot (N= 294)	F	*P*	d.f.
Mean vegetative biomass (mg)	2940 ±240	2172 ±99	8.82	0.003**	1
Mean reproductive biomass (mg)	2353 ±180	1841 ±102	6.86	0.009**	1
Mean seed number	406 ±33	283 ±16	11.26	<0.001***	1
Mean seed weight (mg)	1.562 ±0.309	1.376 ±0.024	0.36	0.548	1

Table 4.14 Correlations between fitness components measured on plants growing on two experimental plots at Swanworth Quarry. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

Correlations, treated plot

		Vegetative biomass	Reproductive biomass	Seed number
Reproductive biomass	Pearson Correlation	0.65		
	P	<0.001***		
	N	272		
Seed number	Pearson Correlation	0.57	0.86	
	P	<0.001***	<0.001***	
	N	272	272	
Seed weight	Pearson Correlation	0.11	0.12	-0.20
	P	0.09	0.07	<0.001***
	N	272	272	272

Correlations, untreated plot

		Vegetative biomass	Reproductive biomass	Seed number
Reproductive biomass	Pearson Correlation	0.58		
	P	<0.001***		
	N	265		
Seed number	Pearson Correlation	0.46	0.88	
	P	<0.001***	<0.001***	
	N	264	264	
Seed weight	Pearson Correlation	0.11	0.14	-0.16
	P	0.16	0.09	0.05*
	N	264	264	264

4.3.4 Cyanogenesis

The proportion of cyanogenic plants varied between regions (df =13 F=7.75 *P*= <0.001). Calcicolous grassland sites had a lower proportion of cyanogenic individuals (66%) than the alternative habitats (84%), (df = 1 F=5.53 *P*=<0.001). Cyanogenesis was not correlated with survival on either the plot treated with topsoil (Pearson correlation=0.131 *P*=0.497) or the untreated plot (Pearson correlation = 0.042 *P*=0.830). There were no differences in plant size or fecundity between cyanogenic and acyanogenic morphs and cyanogenic status was not an important factor for the incidence of seed damage.

Table 4.15 Difference in fitness between acyanogenic and cyanogenic *L. corniculatus* plants on a plot treated with topsoil. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

	Acyanogenic (N =84)	**Cyanogenic (N = 195)**	F	*P*	d.f.
Mean vegetative biomass (mg)	2094 ±182	2220 ±123	0.71	0.402	1
Mean reproductive biomass (mg)	2036 ±234	1746 ±116	1.84	0.175	1
Mean seed number	313.9 ±35.2	276.7 ±18.1	1.6	0.208	1
Mean seed weight (mg)	1.31 ±0.050	1.39 ±0.029	0.16	0.069	1

Table 4.16 Difference in fitness between acyanogenic and cyanogenic *L. corniculatus* plants on an untreated plot. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

	Acyanogenic (N = 79)	**Cyanogenic (N = 202)**	F	*P*	d.f.
Mean vegetative biomass (mg)	2953 ±538	3059 ±269	0.31	0.576	1
Mean reproductive biomass (mg)	2013 ±279	2586 ±236	1.5	0.222	1
Mean seed number	343 ±51.7	449.3 ±43.5	1.06	0.305	1
Mean seed weight (mg)	1.28 ±0.055	1.24 ±0.030	1.82	0.179	1

Some *L. corniculatus* plants sampled at Swanworth Quarry were plastic for cyanogenesis. Figure 4.15 shows that some plants changed phenotype more than once over a period of six months. On each sampling date, a number of plants switched phenotype. However, there was no uniform change, some switched from cyanogenic to acyanogenic and others switched *vice versa*. The temperature increased on each sampling date but this did not appear to affect the direction in which phenotypes switched. It was concluded that temperature was not an important effect.

Plant number	15/1/01 Phenotype	temp °C	20/2/01 Phenotype	temp °C	16/5/01 Phenotype	temp °C	9/6/01 Phenotype	temp °C
1		1.2		10.1		15.5		23.5
2		1		9.2		17.2		22
3		1.3		8.7		16.7	XXX	
4		2.2		9.1	XXX		XXX	
5*		2.2		9.4		18.5	XXX	
6*		1.6		8.9		20.6	XXX	
7*		1.4		8.7		19.4	XXX	
8		1.6		9.4		19.6		18.2
9		2.3		8.7		18.4		19
10*		2.5		8.9		18.5		17.5
11		3.7		8.7		18.1		18.8
12		1.9		9.9	XXX		XXX	
13*		1.7		9.9	XXX		XXX	
14		1.8		10.2		14.2	XXX	
15*		2.1		10	XXX		XXX	
16		2.3		9.5		15.9	XXX	
17		2.8		9.3		15.2	XXX	17.9
18		6.7		9.6		18		21.9
19**		4.9		9.9		14	XXX	
20		3.7		10.2		20	XXX	
21*		4.4		11.1	XXX		XXX	
22		3.1		9.9	XXX		XXX	
23**		3.6		9.6		16	XXX	
24		3.2		9.7		14	XXX	
25		5.3		11.7	XXX		XXX	
26		5.8		10.4		13	XXX	
27		3.6		10.9	XXX		XXX	
28		3.7		10.6		14	XXX	

Figure 4.15 Changes in phenotype of 28 plants at Swanworth Quarry. Dark bars represent cyanogenic morphs, white bars represent acyanogenic morphs, XXX represent dead or plants which could not be identified. * indicates that a plant switched phenotype once during the sampling period. ** indicates that a plant switched phenotype more than once.

4.3.5 Keel colour

There were differences between the keel colour of plants from different regions. On the treated plot there were also differences between plants from calcicolous grassland and the alternative habitat although this was not detected on the untreated plot (Table 4.17 and 4.18). The relationship between keel colour and plant fitness varied between plots. On the treated plot light keeled plants produced more seed; on the untreated plot plants with dark keels were significantly larger (Table 4.19 and 4.20).

Table 4.17 Difference in the number of dark and light keeled plants between 14 regions and two habitats of origin growing on the treated plot at Swanworth Quarry. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

Region	Dark keel	Light keel
Cornwall	3	17
Dorset	5	15
East Sussex	10	10
Edinburgh	9	10
Grampian	11	5
Humberside	9	8
Isle of Wight	3	17
Kent	8	13
Lancashire	9	11
Norfolk	8	11
North Wales	3	17
Northumberland	10	10
Oxford	10	10
Somerset	3	17

$\chi^2=32.50$, $P=0.002^{**}$, df=13

Habitat of origin	Dark keel	Light keel
Calcicolous grassland	59	77
Alternative habitat	42	94

$\chi^2=4.55$, $P=0.033^{*}$, df=1

Table 4.18 Difference in the number of dark and light keeled plants between 14 regions and two habitats of origin growing on the untreated plot at Swanworth Quarry. *= significant at 0.05, **= significant at 0.01, *= significant at 0.001**

Region	Dark keel	Light keel
Cornwall	6	14
Dorset	4	16
East Sussex	9	11
Edinburgh	12	8
Grampian	9	3
Humberside	5	8
Isle of Wight	3	17
Kent	3	17
Lancashire	6	14
Norfolk	11	9
North Wales	11	9
Northumberland	14	6
Oxford	9	11
Somerset	5	15

$\chi^2=38.29$, $P=<0.001^{***}$, df=13

Habitat of origin	Dark keel	Light keel
Calcicolous grassland	52	83
Alternative habitat	55	75

$\chi^2=0.395$, $P=0.530$, df=1

Table 4.19 Difference in fitness between light and dark keeled *L. corniculatus* plants on a plot treated with topsoil. *= significant at 0.05, **= significant at 0.01, ***= significant at 0.001.

	Light Keel (N =171)	Dark keel (N = 101)	F	*P*	d.f.
Mean vegetative biomass (mg)	2357 ±139	1921 ±146	0.07	0.402	1
Mean reproductive biomass (mg)	2040 ±138	1507 ±162	0.84	0.175	1
Mean seed number	321.6 ±21.4	233.4 ±24.6	0.59	0.201	1
Mean seed weight (mg)	1.37 ±0.029	0.92 ±0.046	6.13	0.014**	1

Table 4.20 Difference in fitness between light and dark keeled *L. corniculatus* plants on an untreated plot. *= significant at 0.05, **= significant at 0.01, ***= significant at 0.001.

	Light Keel (N =165)	Dark keel (N = 114)	F	*P*	d.f.
Mean vegetative biomass (mg)	2787 ±257	3375 ±479	0.41	<0.001***	1
Mean reproductive biomass (mg)	2312 ±228	2560 ±313	0.07	0.789	1
Mean seed number	413.8 ±45.9	422.5 ±52.0	0.004	0.949	1
Mean seed weight (mg)	1.26 ±0.033	1.25 ±0.045	0.74	0.389	1

4.4 Discussion

Home-site advantage

The home-site advantage described by other authors (Montalvo & Ellstrand, 2000; Jones & Hayes, 2001; Joshi *et al,* 2001) was not consistently demonstrated for all the determinants of fitness measured in this study. Although there was some evidence that local plants were able to survive better than non-local plants, once established plants from non-local populations were more productive. It is notable that on the untreated site local plants were relatively small and unproductive in comparison with others. Despite this, a weak negative correlation with geographical distance was observed, indicating that although local plants may not be significantly superior in performance, plants moved over large distances are also unlikely to perform well.

The effect of provenance on plant survival and fitness

Geographical provenance

Potential differences in the fitness of plants collected from populations which are separated geographically has been cited as an important factor for consideration when introducing non-local populations in restoration schemes (Millar & Libby, 1989; Fenster & Dudash, 1994). In this study, differences in survival and factors likely to affect subsequent fitness were found between plants from fifteen regions in the British Isles. Other studies have identified similar variation in a range of herbaceous species (Van Tienderen & Van Der Toorn, 1991a; Jones & Hayes, 1999; Joshi *et al,* 2001). On a continental scale, Joshi *et al* (2001) have found that local provenance enhanced performance in three test species. However, the distances in this case were grouped into home>away or near>far and this large grouping variable may have enhanced the detection of variation. The importance of geographical distance at a large scale is supported by Galloway & Fenster (2000), who looked at the performance of *Chamaecrista fasciculata* in trials using seeds collected at six intervals between 0.1 – 2000km and found local adaptation only at the largest spatial scales. Montalvo and Ellstrand (2000) investigated the hypothesis of home-site advantage in *Lotus scoparius* using 12 donor populations, but found geographic separation of populations to be unimportant. Both Galloway & Fenster and Montalvo & Ellstrand used a small number of populations and it is possible that the use of 28 populations at a wide range of distances was enough to detect a weak correlation that was undetected in those with fewer populations. The findings of this study suggest that the geographical separation of donor and receptor site is only moderately important for *L. corniculatus* at this scale.

Ecological provenance

An alternative suggestion is that local selection by environmental conditions at the donor site mean that locally sourced material will be better adapted and consequently show enhanced performance (Knapp & Rice, 1994; Linhart, 1995; Gray, *in press*). In this study, the ecological provenance of populations was found to be unimportant at a site which had received an application of topsoil. However, there was a strong effect of the type of donor site on the untreated plot. Unlike work such as that carried out by Montalvo & Ellstrand (2001) and Joshi *et al* (2001) where a strong positive correlation between environmental parameters and plant performance was found, there was no apparent advantage for the Swanworth Quarry population. Quite the reverse, plants were increasingly larger and more productive as the ecological differences between the donor site and Swanworth Quarry increased. There are two possible explanations. It is possible that the local population is poorly adapted. Rapson & Wilson (1988) showed a lack of adaptation in *Agrostis capillaris* and there is evidence of maladaptation in *Bromus tectorum* (Rice & Mack, 1991). Lesica & Allendorf (1999) suggest that local genotypes will not be adapted to highly degraded environments such as a quarry and throw doubt on the usefulness of selecting local propagules for such a site. If this is so, then it would be reasonable to assume that plants from the margins of Swanworth Quarry are poorly adapted to the conditions at the untreated site and that plants from other types of site are more suited to this environment. However there is a strong negative correlation between ecological distance and plant performance which indicated that plants collected from sites with similar ecological conditions responded to the conditions on the untreated plot in a similar way. This suggests that an argument for a simple case of maladaptation may be insufficient to account for differences in plant performance at the site and that other factors may be important.

The untreated plot was an inhospitable environment, with no developed soil and without weedy species to provide the plants with cover. The plants on this site were subjected to periods of flooding, together with a high level of reflected light from the pale clay substrate. There were striking differences in resource allocation between vegetative and reproductive biomass by plants from calcicolous grassland and those from alternative habitats and this suggests different strategies for surviving in the conditions at the untreated site. Knapp & Rice, (1994) concluded that plants may adapt to periodic disturbance which enables the local genotype to respond to the degradation in an appropriate way. This supports work by Cooper (1954) who described 'hidden variation' where variation previously unselected can be 'uncovered' by periods of extreme environmental conditions. Environmental factors which require different adaptations vary in both time and space, so that individuals, generations and populations may experience different environments (Bradshaw & Hardwick, 1989). Cooper (1964) describes an example of leaf area development in *Lolium perenne* and *Dactylis glomerata.* These species grow in both Mediterranean and northern European countries and all populations show normal leaf growth which is at its maximum at 20°C. Mediterranean populations show relatively high growth rate at low temperatures and poor growth rate at high temperatures but in northern areas the converse is true. Cooper attributes these differences to local adaptation to climate. The cool periods in Mediterranean areas are best for leaf growth when there are no extremes of heat or water loss, however in northern Europe, cool periods indicate the onset of winter when leaf growth would not be advantageous. For an individual to be successful, there must be potential to adapt phenotypically in response to a changing environment (Bradshaw & Hardwick, 1989) and there are many examples of species within which only some populations demonstrate the ability to do this. For example, it has been shown that populations of *Ranunculus flammula* from temporary lakes change leaf shape when exposed to air but populations adapted to permanent lakes do not do so (Cook & Johnson, 1968). Bradshaw & Hardwick (1989) cite work by Sorensen who found that while sand dune populations of *Capsella bursa-pastoris* were able to advance their flowering time in response to a depletion of moisture and nutrients, populations from fertile grasslands were not. This 'hidden variation' may affect plant fitness. Millar & Libby (1989) describe an experiment using *Psuedotsuga menziesii* (douglas fir) in a re-forestation programme. In this case, non-local populations initially did well, however an unusually harsh freeze, which was an infrequent but characteristic environmental extreme at the site, later killed the introduced stock. It is possible that the plants from calcicolous grassland grown at Swanworth Quarry on the untreated site that are small and produce low number of large seeds are more likely to persist longer than the large fecund plants from the alternative habitats.

The significance of provenance in a restoration site does not only rest on the ability of the plant to persist. Plant – invertebrate interactions may also be affected. There is evidence, from laboratory trials, to suggest that herbivores may find plants of local provenance more palatable than plants of non-local provenance (Keller *et al*, 1999). If this is repeated in the field, then using non-local genotypes may have implications for other species in the restored community. Although work has found examples of local adaptation of insects to individual plants (Edmunds & Alstad,1978), other studies have failed to find similar relationships (Strauss, 1977). The relationship between provenance and seed damage by invertebrates was investigated on the experimental plots at Swanworth Quarry. There was no difference in the proportion of plants which were damaged between populations. Seed damage was correlated with plant size and fecundity. Plants which were larger and produced more seed were more likely to have damaged seeds. At Wytham, Ollerton & Lack (1998) show that larger plants are likely to suffer a lower proportion of predated seeds although the study was not conclusive as the relationships between plant size, reproductive output and seed predation varied from year to year. The simple measure used here is inadequate to detect differences in the proportion of predation. However the results are in agreement with Biere (1995) who found that fruit predation by caterpillars of *Hadena bicuris* was dependent on plant size.

The influence of the receptor site on plant performance

What is not immediately apparent in the analysis of initial survival and plant performance on a relatively rich site such as the treated plot in this experiment is the resilience of genotypes to infrequent events which occur at the site. This is an important finding. The performance of plants on the untreated site at Swanworth Quarry indicates that were some catastrophic event to occur, the importance of ecological provenance could be significant. Research has shown that environmental conditions at the receptor site will influence the growth of plants; in particular the availability of water has been found to be an important environmental factor (Vasseur & Gagnon, 1994). In the case of *L. corniculatus,* it has been shown that drought will reduce the reproductive capacity, growth rate and above ground biomass (Carter *et al*, 1997). McGraw *et al* (1986) found strong genotype x environment interactions in the seed yield of *L. corniculatus* and concluded that only experiments carried out *in situ* are valid. Little of the work designed to evaluate the importance of provenance for restoration is carried out in environments typical of restoration sites. Galloway & Fenster (2000) and Montalvo & Ellstrand (2000) used sites with established vegetation. Reciprocal transplant experiments such as those carried out by Van Tienderen & Van Der Toorn, (1991a & 1991b) primarily address local adaptation rather than the effect of this in a restoration environment. The work presented in this chapter demonstrates the importance of considering the history of a site that is to be restored and indicates a need for long-term experiments which will provide information beyond initial survival and establishment.

The effect of cyanogenesis and keel colour polymorphisms on survival and plant fitness

Two polymorphisms in *L. corniculatus* were selected for investigation as they have been potentially linked to plant fitness. *L. corniculatus* is polymorphic for leaf cyanogenesis and it has been suggested this ability of some individuals to produce small amounts of hydrogen cyanide is a defence against herbivores (Compton *et al*, 1983; Compton & Jones, 1985) thereby conferring some advantage. This study showed that the proportion of plants in each population that were cyanogenic varied regionally and between habitat of origin. The Dorset region had a very high proportion of cyanogenic plants, second only to those from Hull and this may be considered some local advantage. However, neither adult survival, plant productivity or seed predation were related to cyanogenesis and this suggests that phenotypic expression of this gene in a population is not related to short-term adult survival or establishment. One of the problems associated with quantifying the expression of cyanogenesis in a group of plants is its inherent flexibility, as some individuals switch between phenotypes (Ramnani & Jones, 1985). This has been attributed to low fluctuating temperatures (Ramnani & Jones, 1985) although it is not shown in all experiments (Ellis *et al*, 1977c). The work presented in this thesis demonstrated that switching phenotype was not uncommon in a naturalised population of *L. corniculatus* at Swanworth Quarry. Although there was no evidence to suggest that temperature was correlated with these changes, Jones & Ramnani (1985) suggest that plants may alter their phenotypes in different directions in response to the same environmental change. The implications for assessing the relationship between the expression of cyanogenesis and plant fitness is clear. The results must be interpreted with caution, as the flexibility of the phenotype may confound the results.

L. corniculatus is also polymorphic for flower keel colour. The proportion of light and dark keeled plants varied between populations; plants in the south and west were more likely to be light keeled while those from the north and east were more likely to be dark keeled. This reflects a pattern which has been observed by Crawford & Jones (1988). However, there was conflicting evidence for the effect of keel colour on plant fitness. On the treated plot, light keeled plants produced heavier seeds while on the untreated plot, dark keeled plants were larger. There is insufficient evidence to suggest that, overall, keel colour was a significant effect on the plant's success.

In conclusion this study shows that, although geographical provenance is a factor in the successful establishment of plants in a restoration environment, the conditions at the donor site are more important. Although the importance of ecological provenance may not be apparent on sites where there has been some remediation, in an untreated environment the effects may be significant. Furthermore, initial success of non-local populations may be misleading as environmental conditions which are infrequent but typical of the area may lead to high mortalities in the future.

Chapter Five

Fine-scale phenotypic variation in *L. corniculatus* with reference to restoration ecology.

5.1 Introduction

There has been much discussion over the importance of using local propagules in restoration schemes (Wilkinson, 2001; Sackville Hamilton, 2001; Keller & Kollman, 1999; Linhart & Grant, 1996; Knapp & Dyer, 1997; Linhart, 1995; Fenster & Dudash, 1994) but authors rarely define a precise scale by which they determine 'local'. In the little material that has been published investigating the importance of provenance, what is considered 'local' depends on the scale of the experiment in question and this has differed between authors. For example, Joshi *et al.* (2000) used reciprocal transplant experiments to investigate differences between populations distributed across Europe, whereas Jones *et al.* (2001) investigated differences between British populations and European populations of *Crataegus monogyna*. In each case, 'local' was defined imprecisely. Galloway & Fenster (2000) attempted to quantify locality by investigating a scale between 0.1 – 2000m but the results were inconclusive. Linhart (1995) is the only author to be prescriptive, suggesting that an appropriate guideline for the translocation of herbaceous species is 100m; a fine scale, which has not been successfully investigated by those working in the field of restoration ecology. Linhart's (1995) definition is based on the concept of neighbourhoods as described by Wright (1978) and the 100m guideline was arrived at by considering neighbourhood sizes in herbaceous species. Although neighbourhood size will be different for each species, those that have been determined are approximately within 100m; Linhart (1995) reports neighbourhood sizes which vary between 25m^2 (*Viola rostrata)* and 108m^2 (*Phlox pilosa*). Linhart does not give a source for the neighbourhood sizes he quotes but the data can be found in Levin (1988). Not all authors are prepared to be prescriptive without further research and Keller & Kollman (1999) suggest that there remains investigative work to be done on a range of species before a general rule is developed.

Studies which do not directly consider the effect of provenance on plant fitness in restoration schemes but investigate fine-scale adaptation in plant populations support

Linhart's suggestion, as they demonstrate that adaptation can occur over very small areas; frequently several metres or less. For example, Antonovics & Bradshaw (1970) investigated morphology and tolerance to heavy metals in *Anthoxanthum odoratum*. Using a transect across a mine boundary, they showed that heavy metal tolerance/intolerance and morphological traits were found in close association (within 100m) and were dictated by the substrate. Investigating differences between discrete patches, rather than contiguous patches or continuous transects, will facilitate classification into local and non-local categories but will not necessarily establish principles of change over Euclidean distance. For example, Turkington & Harper (1979b) demonstrated fine-scale biotic adaptation of *Trifolium repens* using reciprocal planting experiments that showed that individuals of *T. repens* were adapted to perform best in their sward of origin. The work was carried out using patches in a single field. However, distance between patches was not defined as the investigators were primarily interested in the evolutionary response of plants to companion species. Although Turkington & Harper (1979b) demonstrated the effect of local conditions on plant variation, it is not possible to use the study to define a scale over which plants changed. Linhart (1995) does not attempt to separate out the influence of environmental variables (which may be determinants of phenotypic expression) and the geographical distance between plants (which may be an auto-correlate of environmental differences). However, there is an implicit assumption that sites over 100m apart will be sufficiently different that translocated plants will be poorly adapted.

The study presented in this chapter investigates fine-scale variation in *L. corniculatus* and relates phenotypic differences to both spatial and ecological distance. This is based on the premise that the phenotypic characters selected are indicative of genetic differences which, in turn, reflect reproductive history combined with selective effects. If there is a neighbourhood effect, it may be expected that plants at the ends of the transects would be the most dissimilar. If local environmental conditions are the dominant selective agents, then it may be expected that there will be phenotypic differences that correspond to environmental differences (which are here represented by ecological distance).

5.1.2 Aim and Objectives

The aim of this study was to investigate fine-scale phenotypic variation in *L. corniculatus* with reference to restoration ecology. There were two objectives:

- To determine the relationship between fine-scale geographical and ecological distance and fine-scale phenotypic differences in *Lotus corniculatus*

- To test Linhart's (1995) hypothesis that 100m constitutes 'local' in herbaceous species.

The study used a 200m transect at ten sites in the British Isles. The relationship between phenotypic differences of *L. corniculatus* plants occurring along the transect and the geographical and ecological distance between them was investigated using partial regression analyses. To test Linhart's hypothesis, spatial structure in the distribution of phenotypic types along the transect was investigated using cluster analysis. ANOVA was performed to establish whether the groups of similar plants identified by cluster analysis could be accounted for by ecological conditions. If Linhart is correct then it is to be expected that there would be structure in the distribution of plant phenotypes along the transect and that groups of similar plants would be found in area of approximately 100m.

5.2 Method

A 200m transect was selected at each of ten sites representing a variety of environments. Where possible a transect was laid over an area that was homogenous in terms of topography and vegetation cover, but in some cases there were unavoidable changes. These are noted in Table 5.1

Table 5.1 A summary of 10 locations at which a 200m transect was laid out to investigate phenotypic differences in *Lotus corniculatus*.

Site	OS Reference	Description
Berrow Dunes	ST293520	Stable dune grassland. Transect crossed a bund and a small dune slack with *Oenanthe lachenalii* and *Dactylorhiza incarnata*.
Brean Down	ST290580	Rabbit grazed limestone grassland. The site was on a slight incline.
Chapman's Pool	SY956771	Coastal grassland with unbroken vegetation cover. The transect was laid on a north-south axis and the latter part of transect was increasingly exposed to the sea.
Conwy Morfa	SH767791	Patchy coastal grassland on shingle.
Great Orme	SH770830	Rabbit and sheep grazed limestone grassland on a gentle incline.
Hunstanton Dunes	TF690430	Coastal grassland between stabilized dunes. Patchy with areas of bare sand.
Lullington Heath	TQ545017	Chalk heath which comprised a *Festuca rubra* mosaic with both calcicolous and calcifugous herbs. Short turf, rabbit grazed.
Mount Cabourn	TQ448090	Short, sheep grazed, species rich, chalk grassland. The area appeared homogenous.
Ringstead Downs	TF691401	Rabbit grazed species rich limestone grassland. South facing slope in a dry valley, appeared homogenous.
Swanworth Quarry	SY968782	Species poor, rabbit grazed limestone grassland; strip of vegetation along side a track used by lorries visiting the quarry. In places, the vegetation cover was broken by boulders

Each transect was laid out using a 100m tape; the point of origin was chosen arbitrarily. A 1m x 1m quadrat was placed at 5m intervals and species cover was recorded using the Domin scale. Within each quadrat the individual of *Lotus corniculatus* closest to the 5m mark was selected and morphological measurements were made. If no individuals of *L. corniculatus* occurred within the quadrat, a score of 'absent' was recorded.

5.2.1 Characterisation of the environment

Ecological distance was determined using the method described in the *General Methods* section of this thesis.

5.2.2 Characterisation of plants

Using the method described in the General Methods section, the inter-node length on the longest branch was measured together with the length of the five largest leaves on the longest branch (to give a mean leaf length). Plants were scored for the following traits: pubescence (scored as glabrous, sparsely pubescent or pubescent); leaf shape (scored as apiculate, obovate or obtuse); keel colour (scored as dark or light); cyanogenesis (scored as cyanogenic or acyanogenic).

5.2.3 Testing plants for cyanogenesis

Plants were tested using the method described in the *General Methods* section. All plants were tested in the field; chemical analysis was started as soon as the leaves were harvested. A field kit comprising of 250ml of toluene, 250ml of distilled water, sodium picrate papers and small glass tubes was constructed and transported in a cool box.

5.2.4 Analysis

To establish whether there was a relationship between geographical and ecological distance on each transect, these two distance measures were correlated by means of a Mantel randomisation test (Mantel, 1967) using a program written by RT Clarke (*pers comm*), following the procedures outlined in Manley (1997). A Mantel test deals with two matrices (X and Y), obtained independently and describing the relationships between the same sampling points (Mantel 1967). The X matrix is held constant while *n* (in this case 10,000) randomisations of Y matrix are computed to derive random columns. The statistical significance of the observed value is the proportion of the derived values which are more than or equal to the observed value.

Randomization tests were used here as it was suspected that there was some degree of autocorrelation between geographical and ecological distance on the transects. Autocorrelation describes a situation where it is possible to predict the values of a variable at some point of space (or time) from the known values of another variable at some other point of space (or time) (Legendre & Fortin, 1989). It implies a spatial structure in the data. Positive autocorrelation liberally distorts classical tests such as correlation and regression so that false significant results are produced by the tests. In classical tests, where data are assumed to be independent, each observation donates one degree of freedom. If the variable is partially known in advance then it contributes only a fraction of a degree of freedom, yet the size of the fraction is indeterminable (Legendre & Fortin, 1989). One solution to this problem is to employ randomisation tests.

To investigate the relationship between similarity of plant phenotype and geographical and ecological distance of plant position on each transect, a partial regression analysis was carried out using a Mantel randomisation test. As it was established that there was a relationship between geographical and ecological distance on each transect, it was not possible to use independent regression analyses for the two distance measures. Analysis was performed using a further programme written by RT Clarke (*pers. comm.*) using procedures from Manley (1997). In order to construct a single variable that represented phenotype, the data were combined using Gower's similarity co-efficient. The phenotypic variables were recorded using mixed data types and Gower (1971) proposed a coefficient that is applicable to quantitative, binary and multistate characters. The similarity matrices based on Gower's co-efficient make it possible to represent the observations as a set of points in Euclidian space (Sneath & Sokal, 1973). This coefficient was applied to the phenotypic data to produce a similarity matrix using CLUSTAN computer programme (Wishart, 1999) and the resulting matrix was regressed against the matrices of geographical and ecological distance.

At all sites, except Mount Cabourn, *L. corniculatus* was absent at some points on the transect. Analysis aimed to investigate whether this absence was a spatial phenomenon explained by geographical distance or ecological conditions (as described by DCA1), where the environment at specific locations was unsuitable for

L. corniculatus. The presence/absence of *L. corniculatus* on the transects was analysed using binary logistic regression which predicts binary data using continuous explanatory variables.

To investigate whether groups of plants with similar phenotypes occurred in close association along the transects, the similarity matrix of phenotypic data on each transect was clustered using the sum of squares procedure in CLUSTAN. As sum of squares requires a dissimilarity matrix, similarities were converted to dissimilarities by subtracting every value from the maximum similarity (Wishart, 1999). The Best Cut function in CLUSTAN was applied to identify the maximum number of significant clusters in each data set. Best Cut uses significance tests on the fusion values to show the proposed cluster partitions in the current tree which are significant at the 5% level (Wishart, 1999). The clusters were then plotted against distance (0-200m) to identify patterns in plant similarity along the transect and to investigate Linhart's (1995) suggestion that 100m is a significant distance when considering plant similarity. The mean DCA score for each cluster was compared using one-way analysis of variance (ANOVA) to investigate whether the clusters were related to differences local conditions.

5.3 Results

5.3.1 Examining potential autocorrelations

The relationship between geographical distance and ecological distance

The geographical (in metres) and ecological distance of quadrats on each transect were correlated using a Mantel randomisation test. In all cases, there was a strong correlation indicating that there were local differences in environmental conditions and that these increased with distance between two points on the transect (Table 5.2).

Table 5.2 The correlation of geographical distance and ecological distance of 41 quadrats along a 200m transect at 10 sites in the British Isles.*=significant at 0.05, **=significant at 0.01, *= siginificant at 0.001.**

Site	Normal deviate test value (Z-Ze)/se(Z)	Correlation coefficient R	p
Berrow Dunes	8.494	0.45	<0.001**
Brean Down	4.521	0.285	<0.001**
Chapman's Pool	3.186	0.174	0.003*
Conwy Morfa	2.488	0.145	0.01*
Great Orme	3.364	0.198	0.002*
Hunstanton Dunes	3.186	0.174	0.002*
Lullington Heath	11.041	0.576	<0.001**
Mount Cabourn	4.148	0.255	<0.001**
Ringstead Downs	6.237	0.379	<0.001**
Swanworth Quarry	3.409	0.207	<0.001**

The relationship between phenotypic differences and geographical and ecological distance

There were differences between the sites (Table 5.3). At Brean Down, Chapman's Pool, Great Orme, Mount Cabourn and Swanworth Quarry there was a significant negative relationship between geographical distance and the similarity of plant phenotype. This indicated that plants in close association along the transect were more likely to be similar and to become increasingly different as the geographical distance between them increased. There was no relationship between ecological distance and phenotype at these sites although at Swanworth Quarry the relationship was close to significance ($P = 0.093$). At the remaining sites, Berrow Dunes, Conwy Morfa, Hunstanton Dunes, Lullington Heath and Ringstead Downs, no significant relationships between phenotypic similarity and either distance measure were found.

Table 5.3 Partial regression analysis for phenotypic similarity of *L. corniculatus* against geographical and ecological distance using a Mantel randomisation test.
***= significance at 0.05 level, **= significance at 0.01 level, ***=significance at 0.001 level.**

Key to symbols:
s=phenotypic similarity (Gower's index)
g= geographical distance (metres)
e= ecological distance (dca scores)
P= probability that the coefficient is not significant‡
(c_g) regression co-efficient of g
(c_e) regression co-efficient of e

	Regression equation for phenotypic similarity (s)	$P\ (c_g)$	$P\ (c_e)$
Berrow	$s=0.64 - 0.0018g + 0.00023e$	0.529	0.926
Brean	$s=0.62 - 0.0065g - 0.0028e$	<0.00005***	0.336
Chapman's Pool	$s=0.64 - 0.0041g - 0.00002e$	0.022*	0.993
Conwy Morfa	$s=0.54 -0.0032g - 0.0011e$	0.117	0.499
Great Orme	$s=0.57 - 0.0044g + 0.00092e$	0.016*	0.510
Hunstanton Dunes	$s=0.50 - 0.0003g + 0.0021e$	0.950	0.316
Lullington Heath	$s=0.69 -0.0029g + 0.0021e$	0.301	0.245
Mount Cabourn	$s=0.55 - 0.0050g + 0.00006e$	0.003**	0.975
Ringstead Downs	$s=0.59 - 0.0049g + 0.0043e$	0.205	0.298
Swanworth Quarry	$s=0.59 - 0.0228g + 0.0023e$	<0.00005***	0.093

‡=significance for the extra sum of squares when the variables are added to the equation in order

The relationship between plant presence/absence and geographical and ecological distance

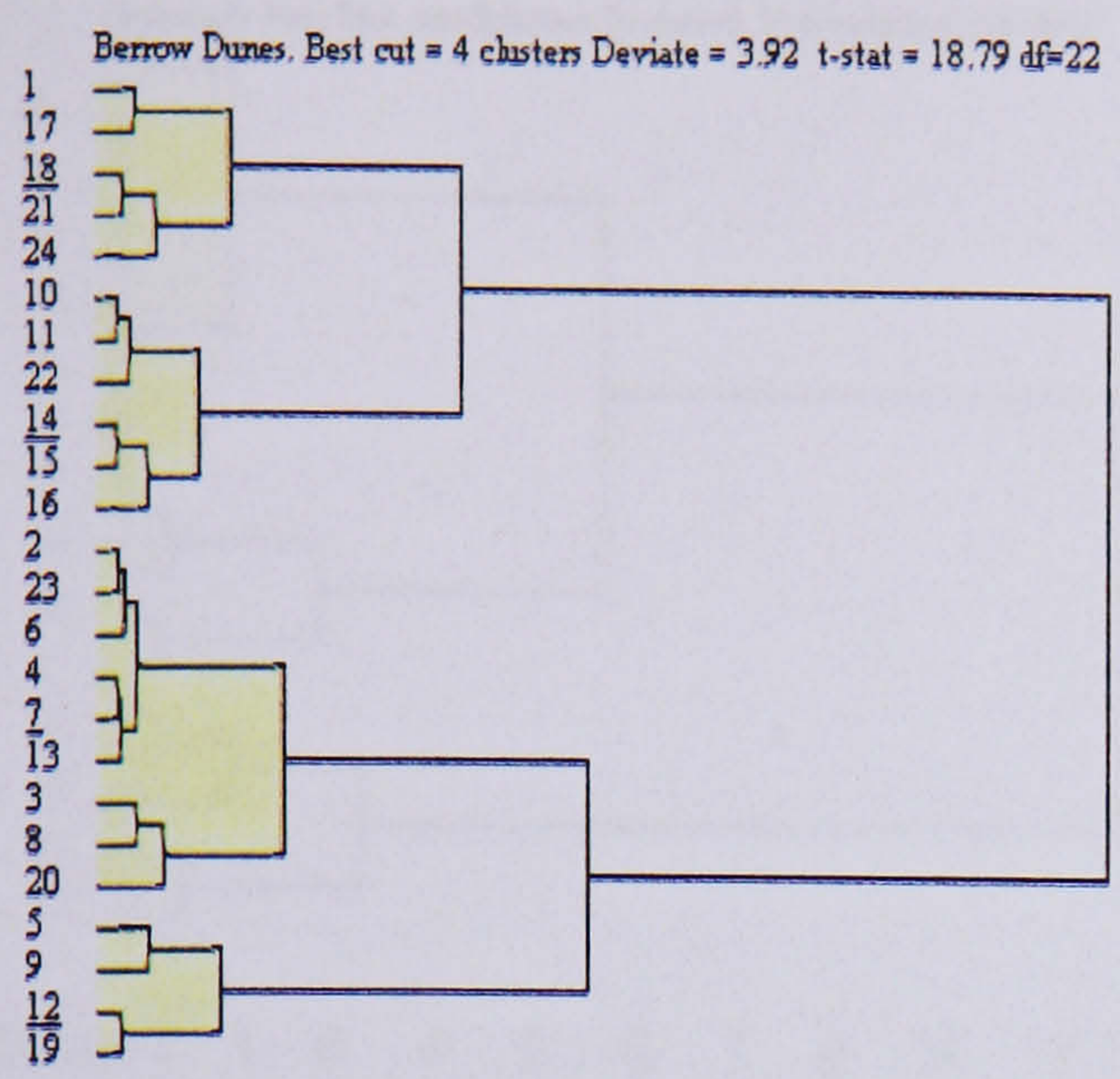
The results differed among transects (Table 5.4). At Conwy Morfa, Great Orme, Hunstanton Dunes, Lullington Heath and Swanworth Quarry there was no association between presence/absence of *L.corniculatus* and geographical or ecological conditions. At Brean Down, Ringstead Downs and Chapman's Pool, geographical location was not significant but the results indicate that the distribution of plants along the transect is significantly related to ecological conditions. Only at Berrow Dunes were both geographical distance and ecological distance significant. Sites where no pattern was identified had a low number of missing plants; this may be because conditions were suitable all along the transect or it may be that the low number of missing plants reduced the power of the statistical test.

Table 5.4 The effect of geographical distance and type of site on the presence/ absence of *L. corniculatus* on a 200m transect located at ten sites in the British Isles
***= significant at 0.05 **=significant at 0.01 ***=significant at 0.001**

Site	Regression equation for presence/absence(p/a)	Variables	*P*	No.of plants sampled	df
Berrow Dunes	p/a = 0.564 + 0.024g – 0.014e	Geo. distance	.011**	24	1
		DCA scores	.055*		1
Brean Down	p/a = 5.118 + 0.005g - 0.028e	Geo. distance	.648	32	1
		DCA scores	.004**		1
Chapman's Pool	p/a = 4.915 + 0.049g - 0.062e	Geo. distance	.260	29	1
		DCA scores	.004**		1
Conwy Morfa	p/a = 1.518 + 0.008g - 0.006e	Geo. distance	.425	34	1
		DCA scores	.534		1
Great Orme	p/a = 2.350 + 0.012g - 0.009e	Geo. distance	.345	35	1
		DCA scores	.431		1
Hunstanton Dunes	p/a= 4.279 - 0.013g - 0.016e	Geo. distance	.139	33	1
		DCA scores	.141		1
Lullington Heath	p/a = 4.420- 0.006g - 0.019e	Geo. distance	.765	35	1
		DCA scores	.301		1
Mount Cabourn	*No absent plants*	Geo. distance		41	
		DCA scores			
Ringstead Downs	p/a = 2.333 - 0.004g - 0.019e	Geo. distance	.562	21	1
		DCA scores	.030*		1
Swanworth Quarry	p/a = 8.620 - 0.030g - 0.007e	Geo. distance	379	36	1
		DCA scores	737		1

5.3.2 Testing Linhart's hypothesis

Plant phenotypes were grouped into "significant clusters" of similar plants on each transect and the number of clusters identified differed between the transects. Data from the majority of transects were clustered into five groups but Conwy Morfa, and Swanworth Quarry had six, Berrow Dunes and Brean Down had four and Ringstead Downs data were grouped into three. Evidence for spatial aggregation of plants with similar phenotypes on the transects was found at four sites where small discrete groups were identified; Berrow Dunes (Figure 5.2), Brean Down (Figure 5.4), Chapman's Pool (Figure 5.6) and Swanworth Quarry (5.18). In particular, Swanworth Quarry shows a clear separation. In the majority of cases, separation of similar groups was not found. The figures below (Figures 5.1-5.20) show two graphs for each transect. The graph in the left column is a dendrogram which displays the clusters as identified by CLUSTAN. The graph in the second column is a scatter plot which shows how similar plants are distributed along the transect, this aids visualisation of what was happening in the field.



Cluster 1	1 17 18 21
Cluster 2	10 11 22 14 15 16
Cluster 3	2 23 6 4 7 13 3 8 20
Cluster 4	5 9 12 19

Figure 5.1 Dendrogram showing maximum number of clusters of similar plants on a transect at Berrow Dunes

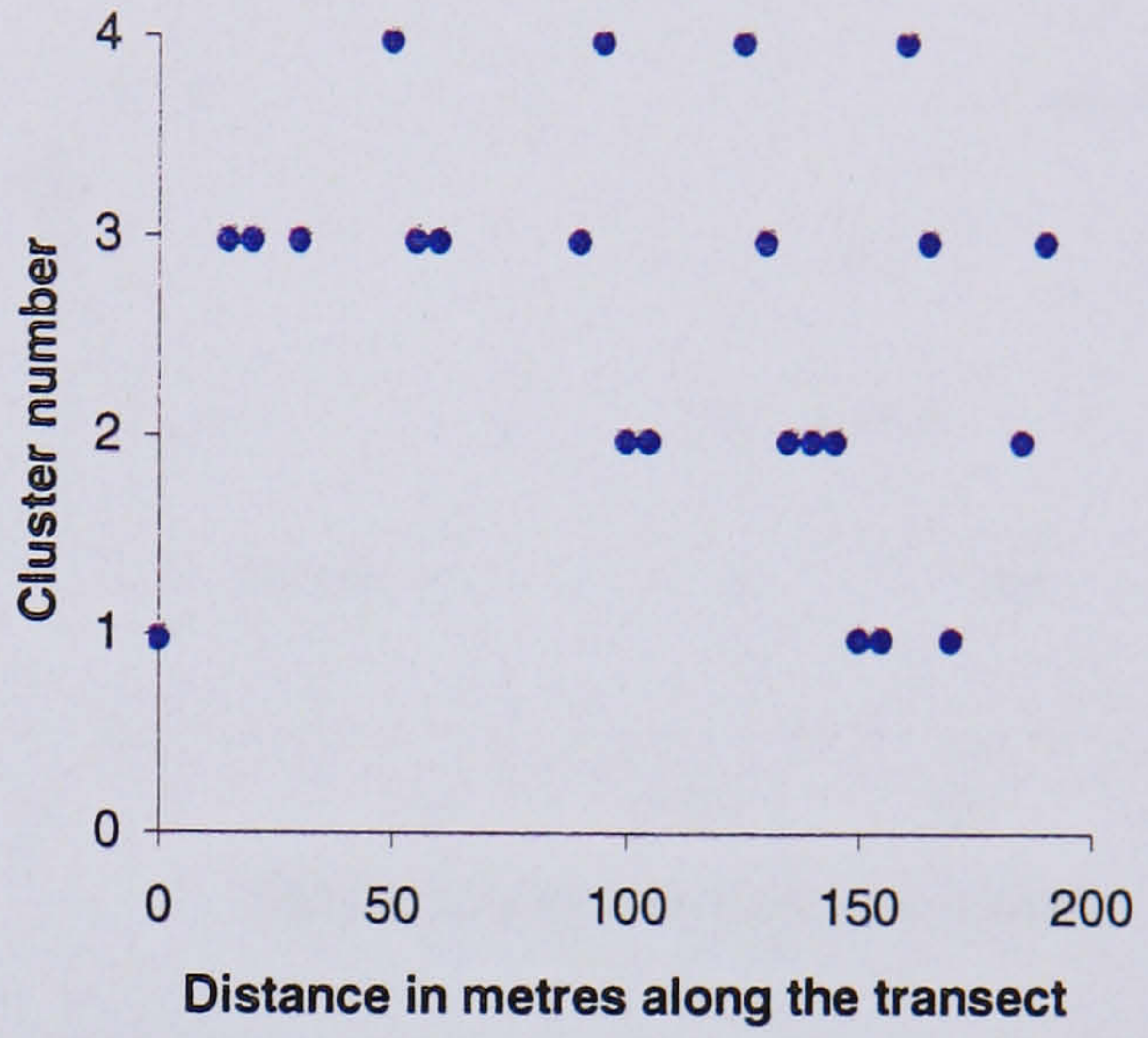
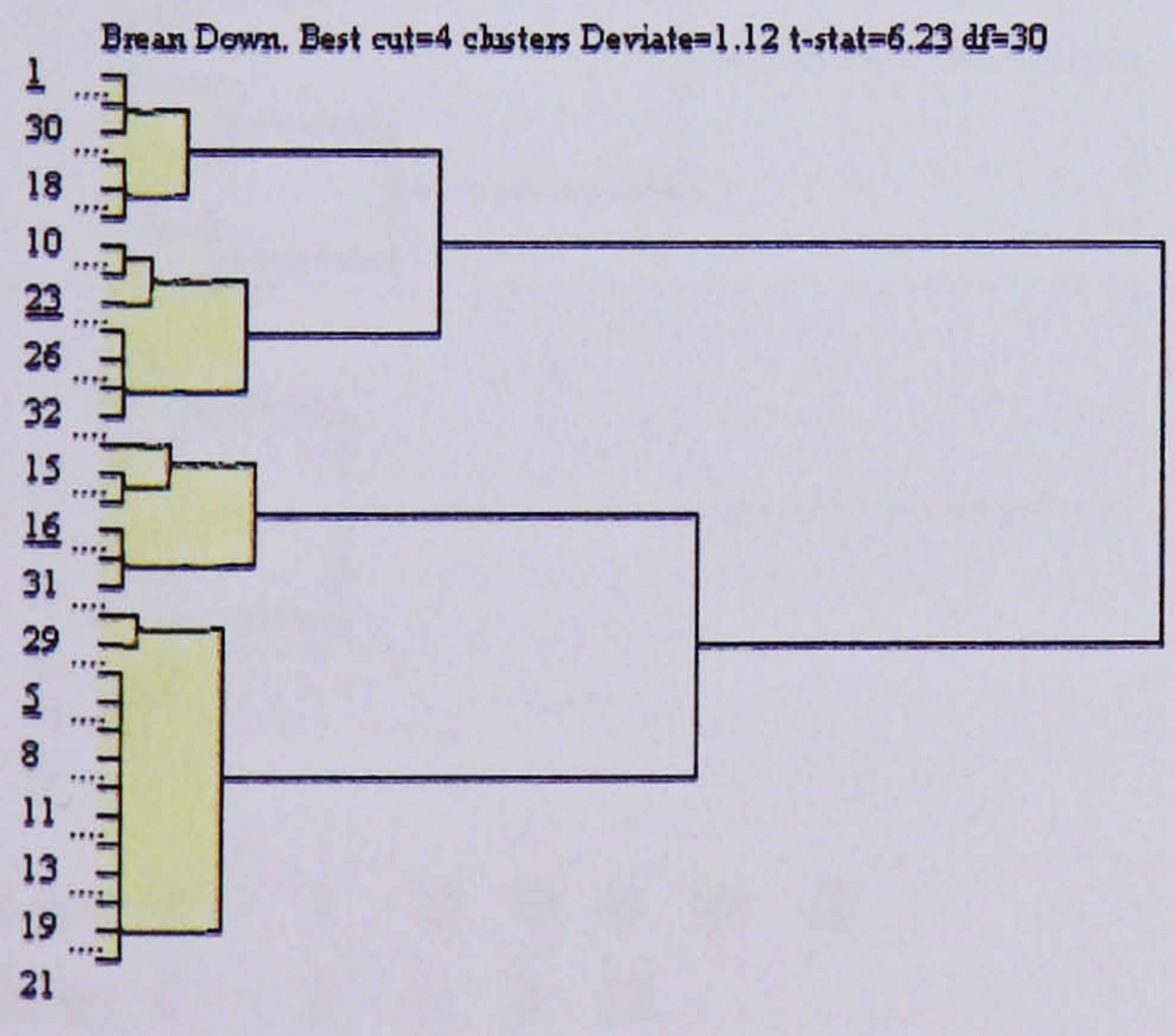


Figure 5.2 Distribution of clusters of similar plants along a transect at Berrow Dunes



Cluster 1	1 22 4 18 20
Cluster 2	10 25 24 23 26 28
Cluster 3	2 15 17 16 27
Cluster 4	3 29 5 6 7 8 9 11 12 13 14 19 21

Figure 5.3 Dendrogram showing maximum number of clusters of similar plants on a transect at Brean Down

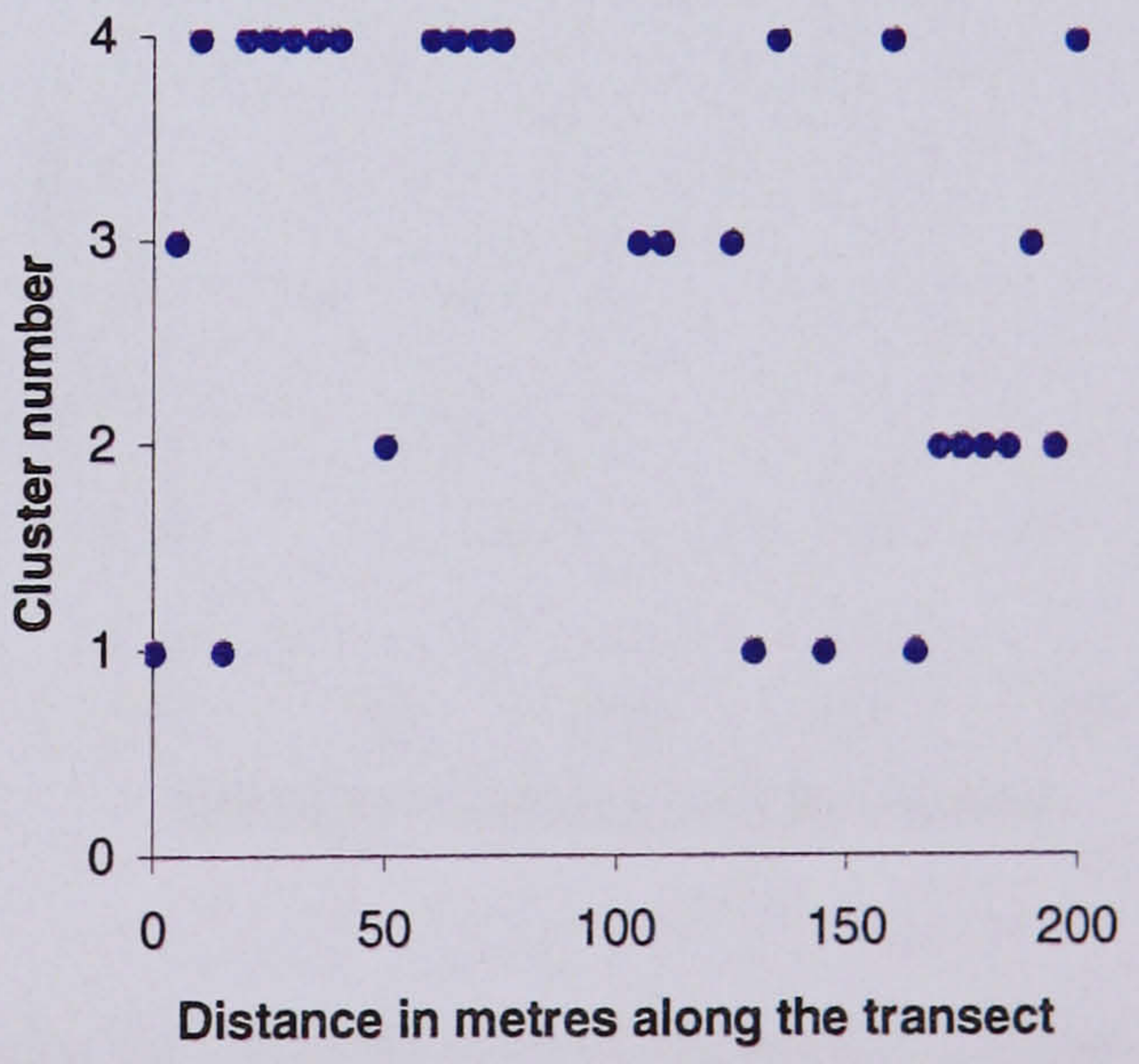
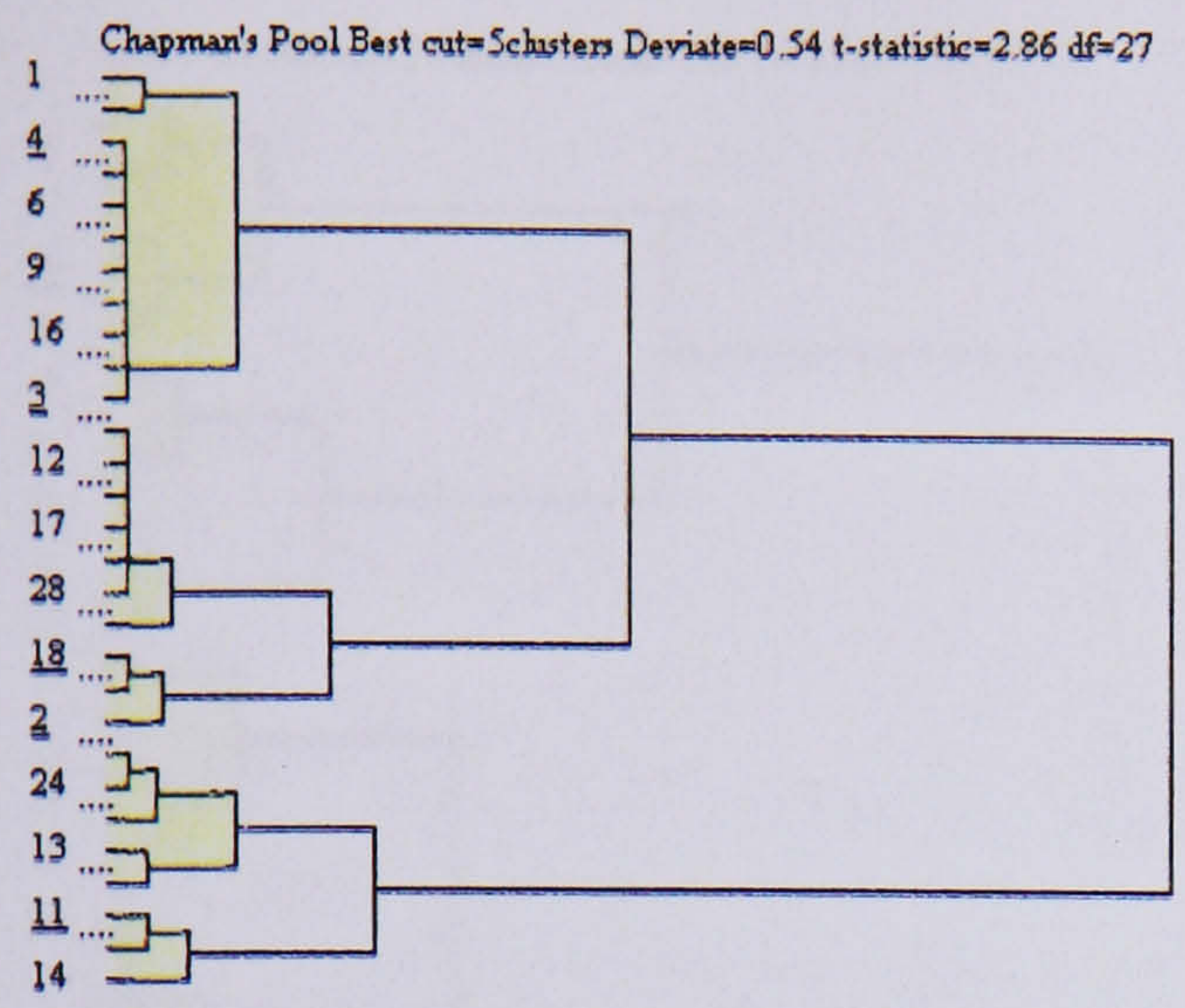


Figure 5.4 Distribution of clusters of similar plants along a transect at Brean Down.



Cluster 1	1	8	4	5	6	7	9	10	16	21	22
Cluster 2	3	12	15	17	19	28	20				
Cluster 3	18	27	29								
Cluster 4	2	24	26	13	23						
Cluster 5	11	25	14								

Figure 5.5 Dendrogram showing maximum number of clusters of similar plants on a transect at Chapman's Pool

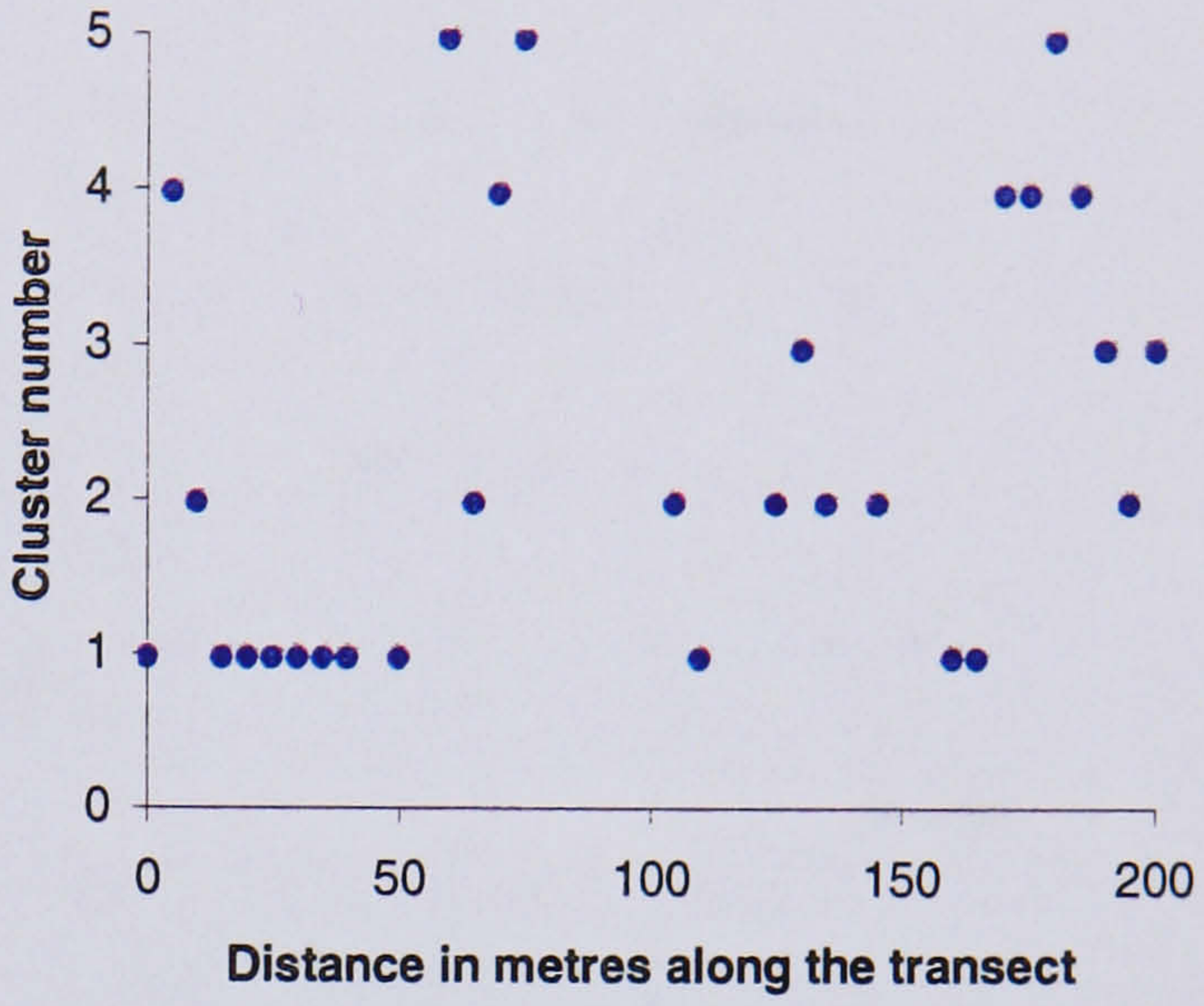
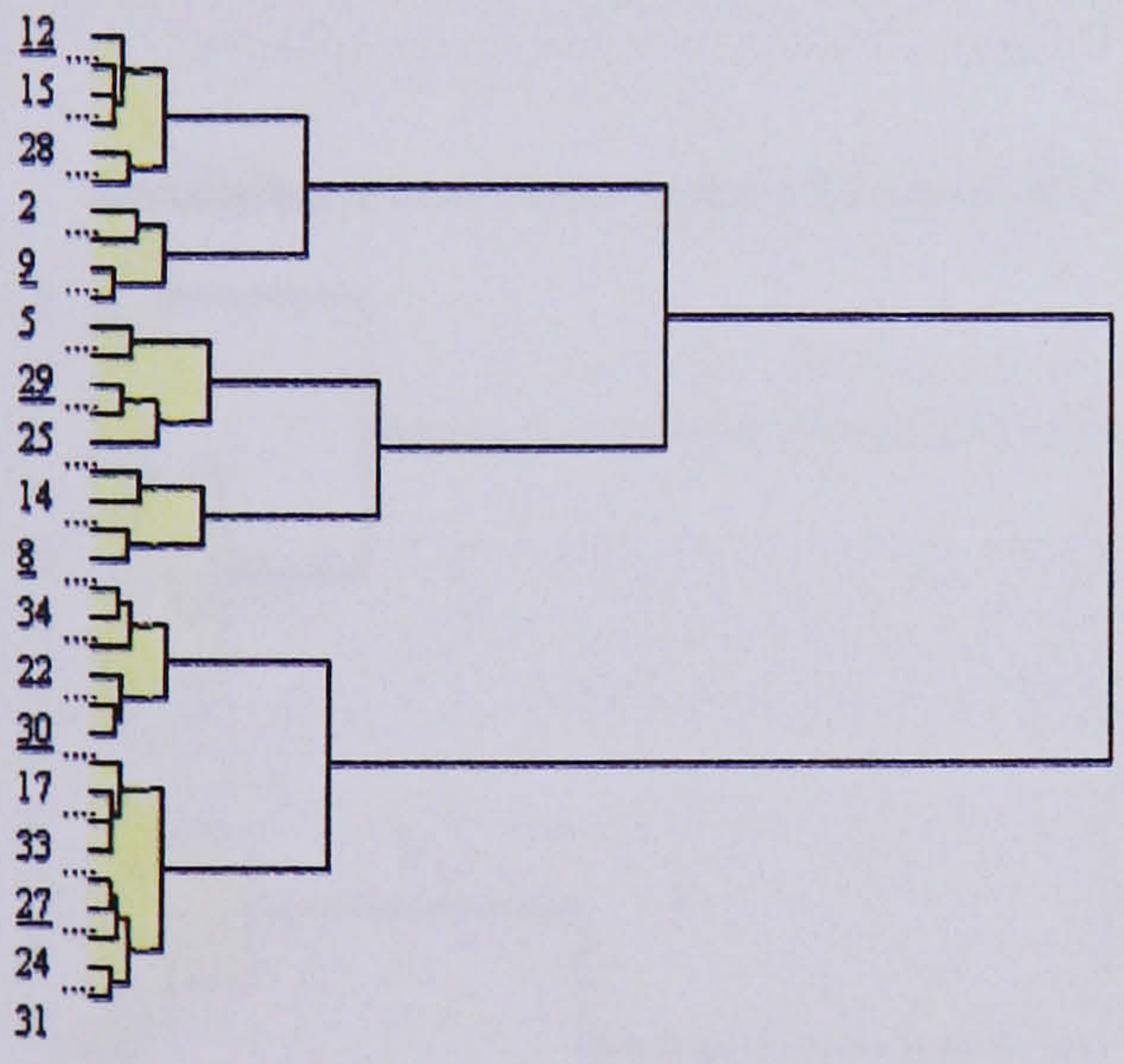


Figure 5.6 Distribution of clusters of similar plants along a transect at Chapman's Pool



Cluster 1	1	12	15	21	28	32			
Cluster 2	2	11	9	13					
Cluster 3	5	16	10	29	25				
Cluster 4	6	14	8	19					
Cluster 5	3	34	7	22	26	30			
Cluster 6	4	17	18	33	20	27	23	24	31

Figure 5.7 Dendrogram showing maximum number of clusters of similar plants on a transect at Conwy Morfa

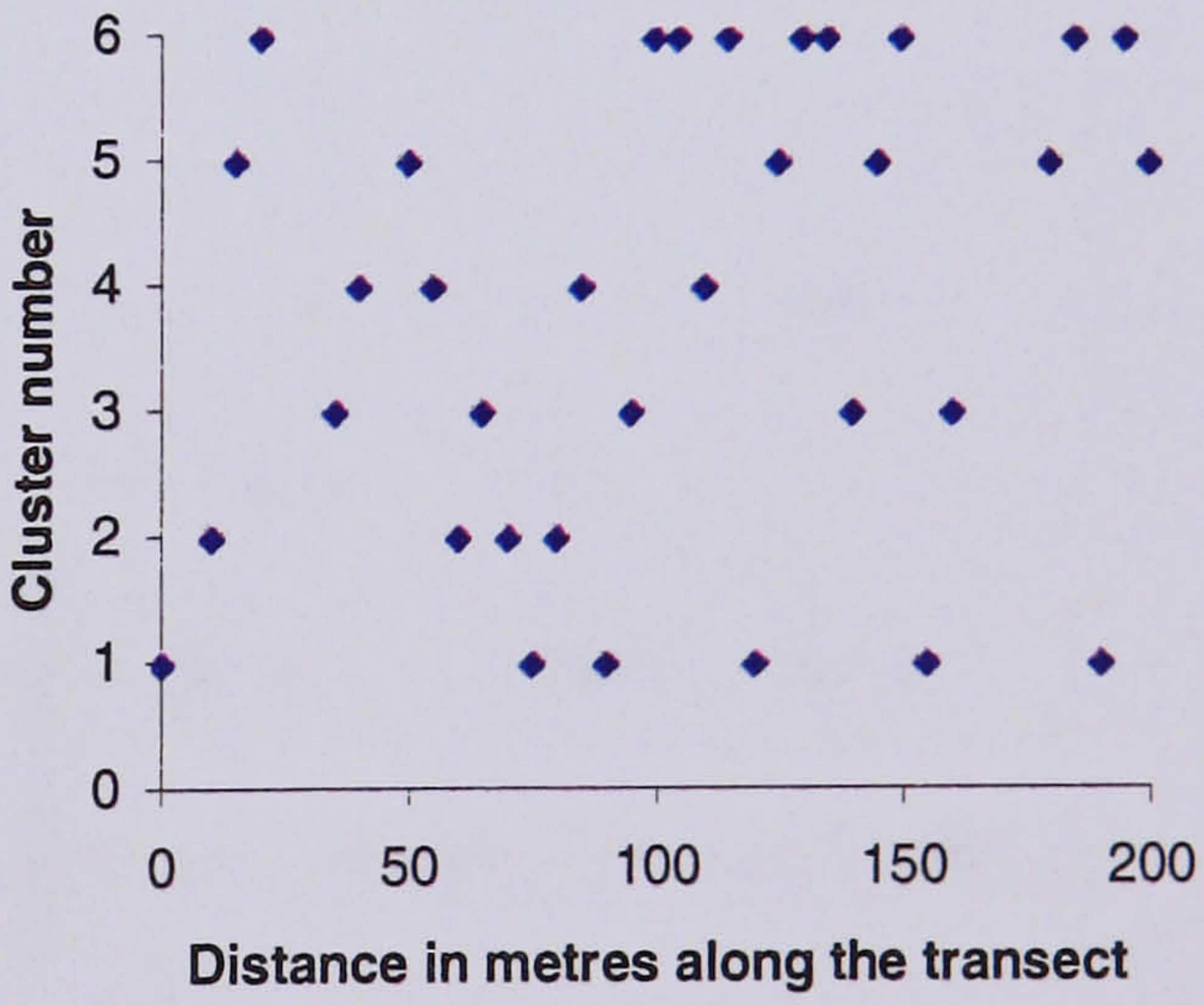
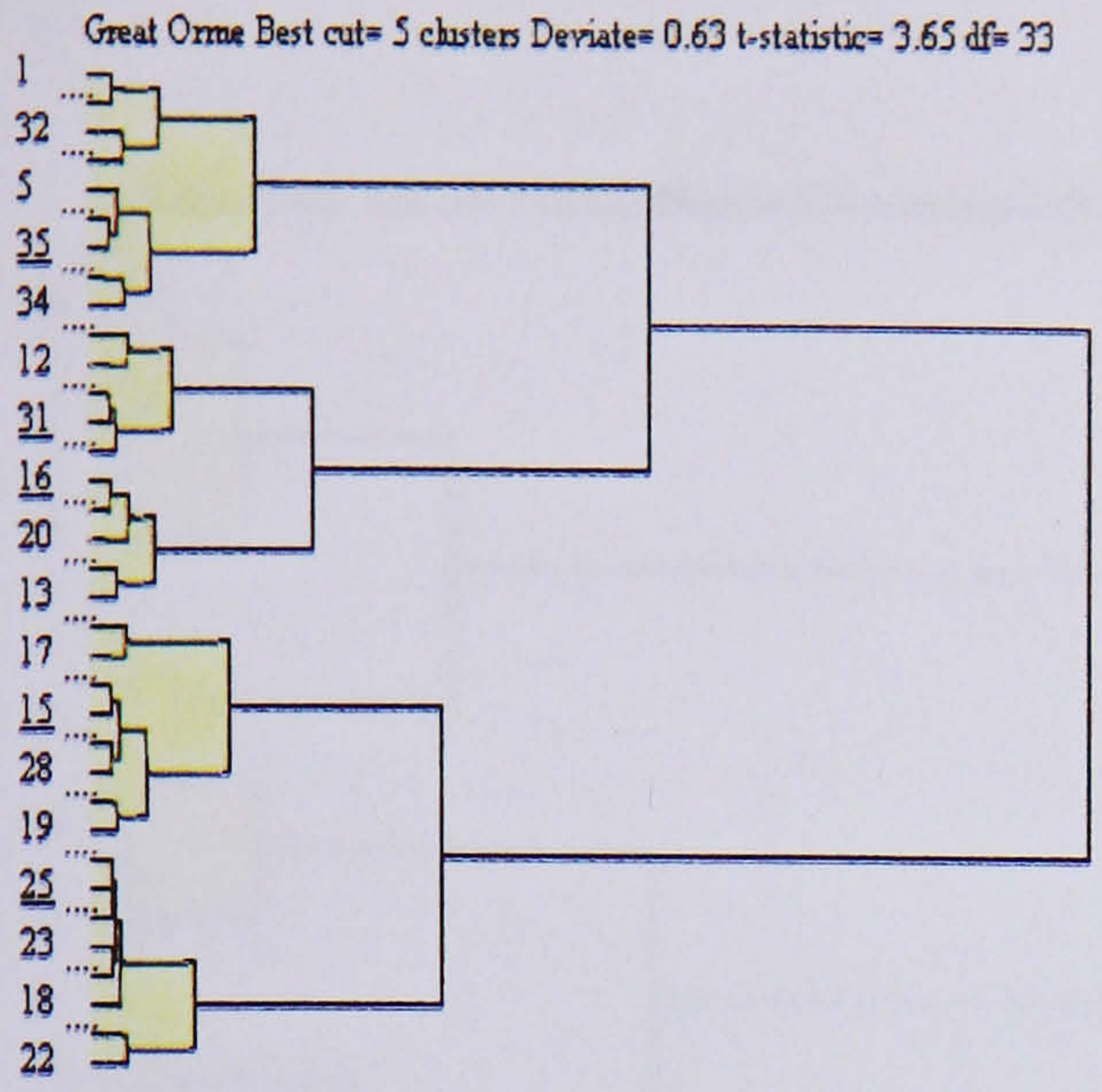


Figure 5.8 Distribution of clusters of similar plants along a transect at Conwy Morfa



Cluster 1	1	4	32	33	5	29	35	30	34
Cluster 2	2	12	21	27	31				
Cluster 3	8	16	20	9	13				
Cluster 4	3	17	7	15	10	28	14	19	
Cluster 5	6	25	26	23	24	18	11	22	

Figure 5.9 Dendrogram showing maximum number of clusters of similar plants on a transect at Great Orme

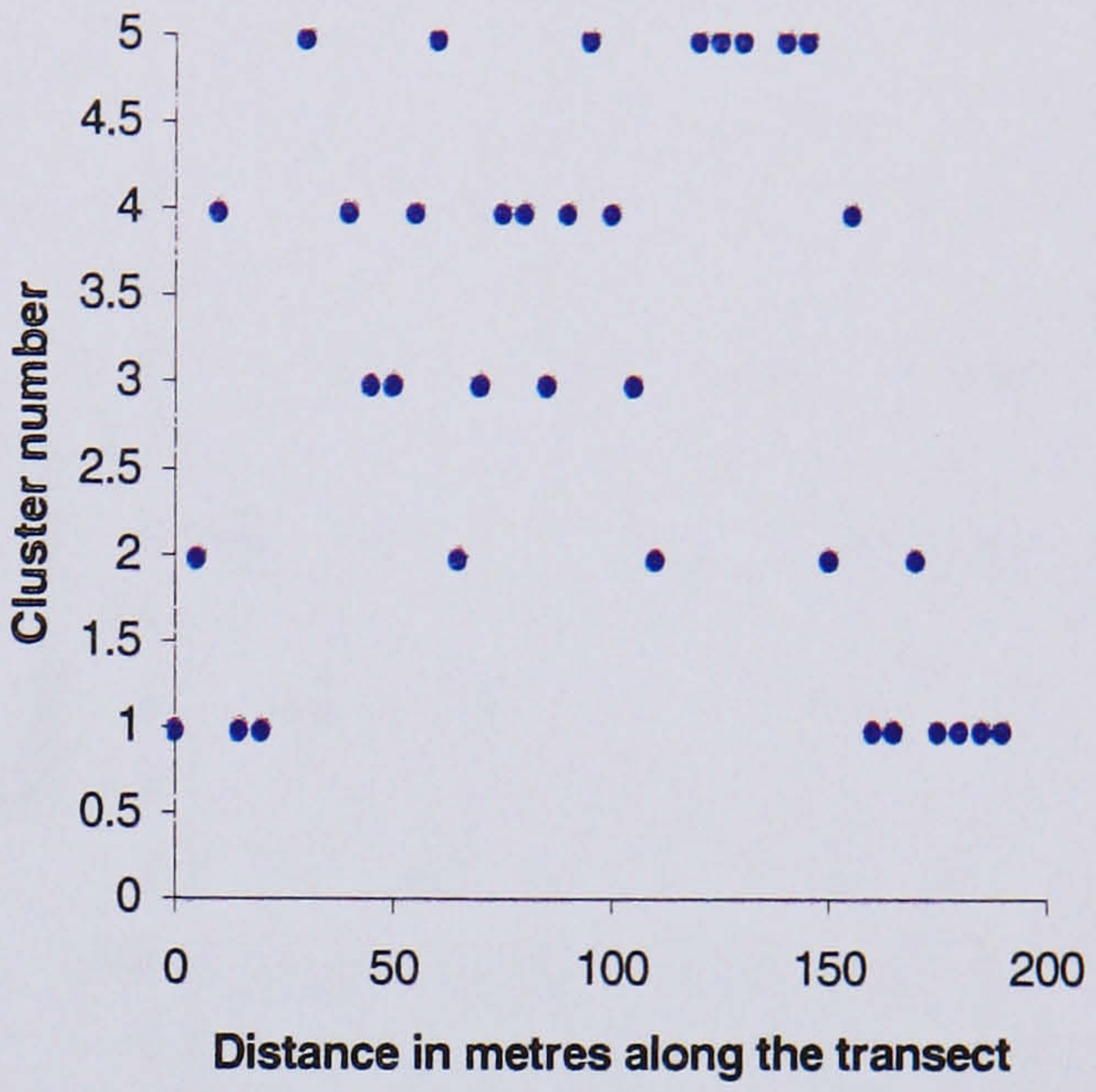
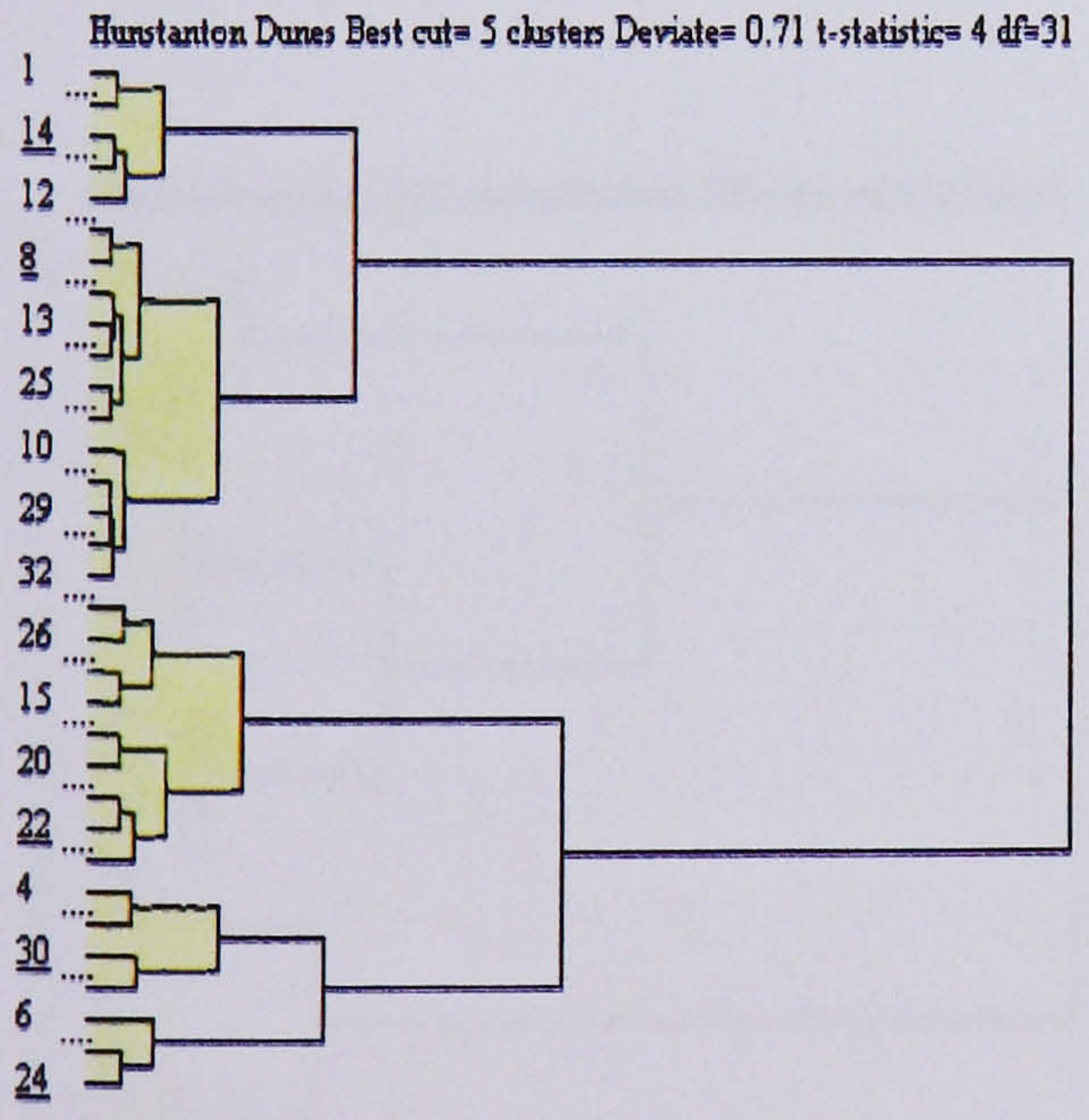


Figure 5.10 Distribution of clusters of similar plants along a transect at Great Orme



Cluster 1	1	9	14	12									
Cluster 2	2	11	8	13	27	25	28	10	19	29	21	2	32
Cluster 3	3	26	5	15	17	20	18	22					
Cluster 4	4	7	16										
Cluster 5	6	23	24										

Figure 5.11 Dendrogram showing maximum number of clusters of similar plants on a transect at Hunstanton Dunes

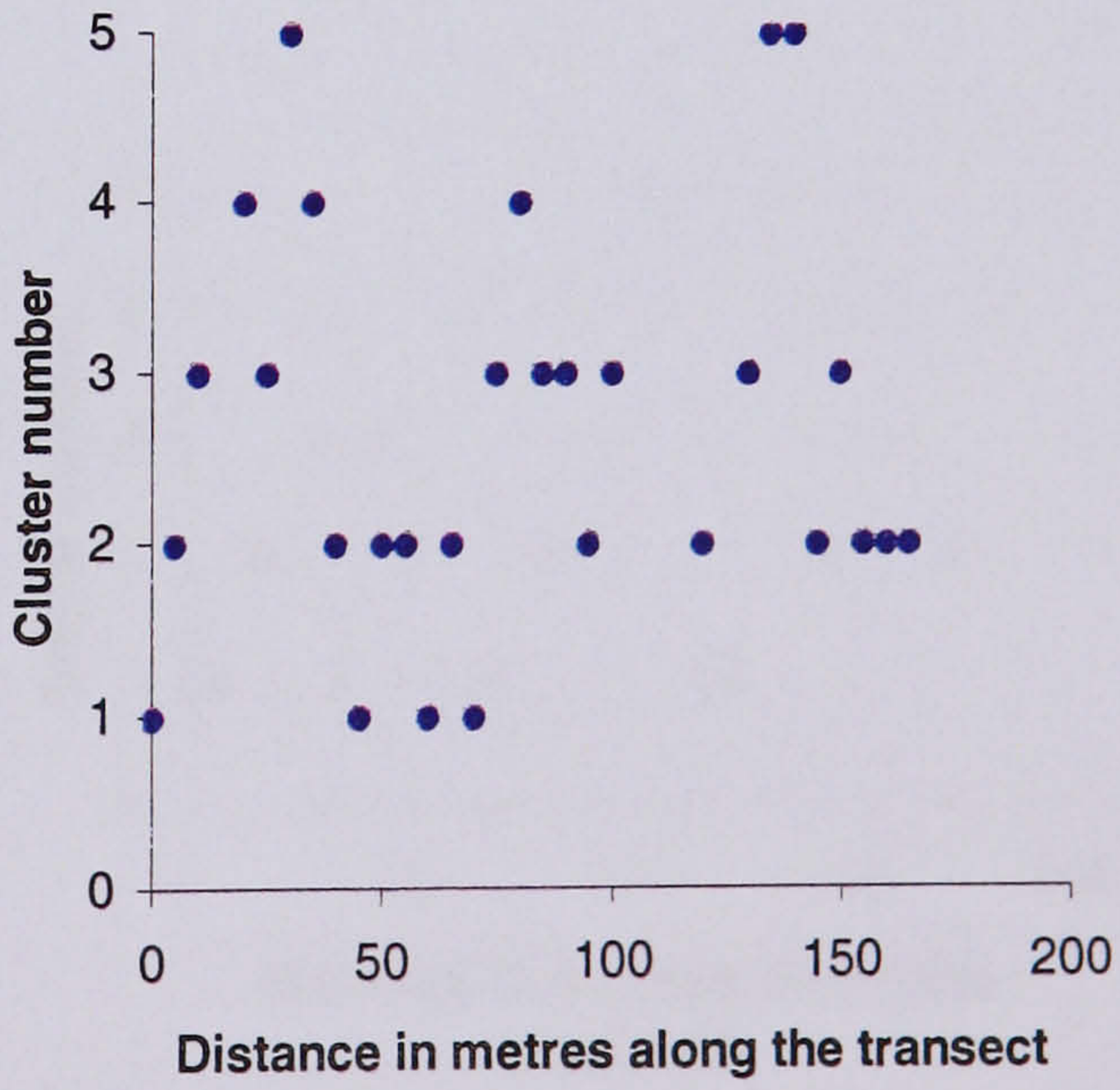
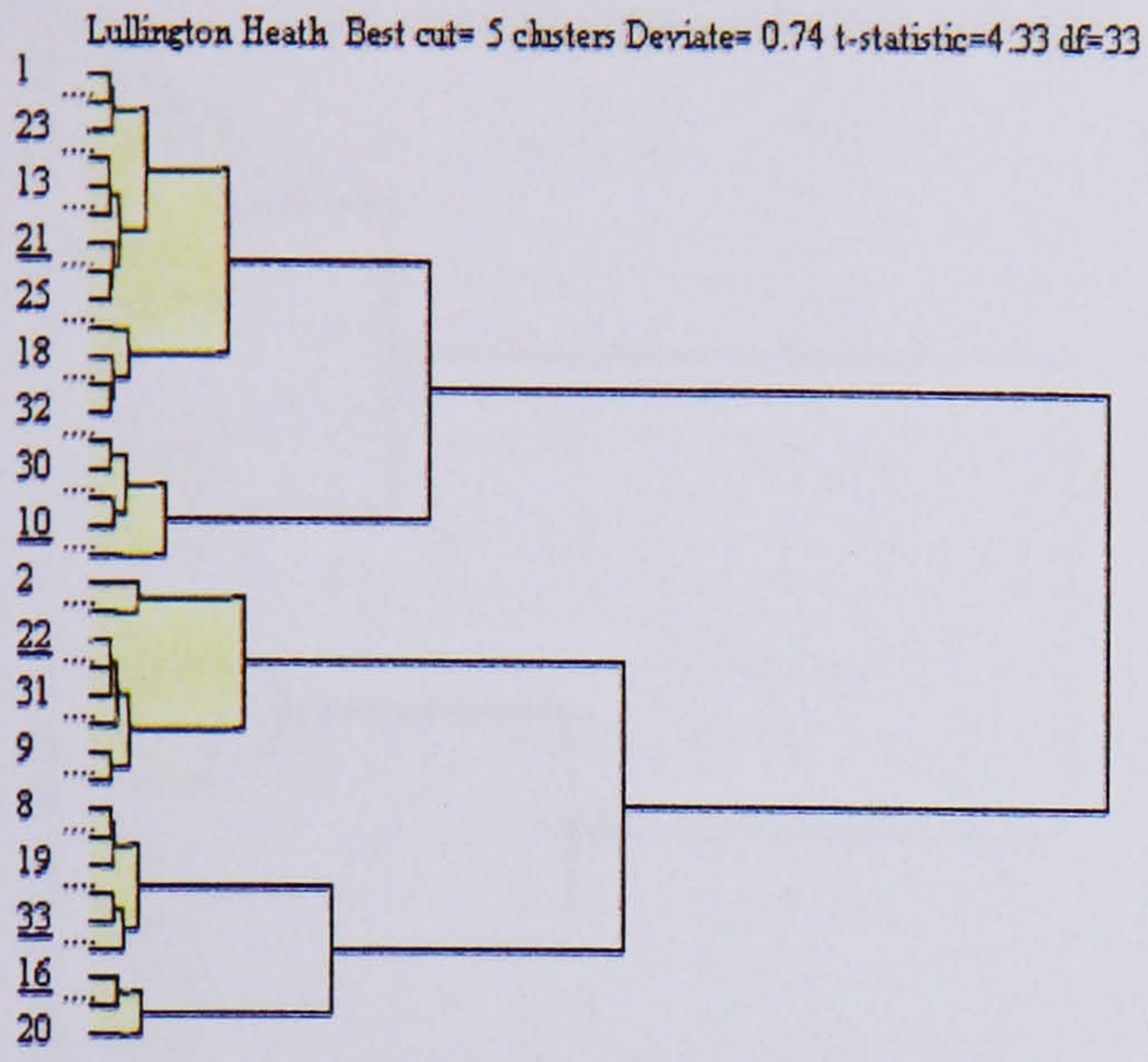


Figure 5.12 Distribution of clusters of similar plants along a transect at Hunstanton Dunes



Cluster 1	1	27	23	11	13	17	12	21	25	6	18	28	32
Cluster 2	5	30	7	10	15								
Cluster 3	2	34	3	22	31	4	9	29					
Cluster 4	8	24	19	26	33	35							
Cluster 5	14	16	20										

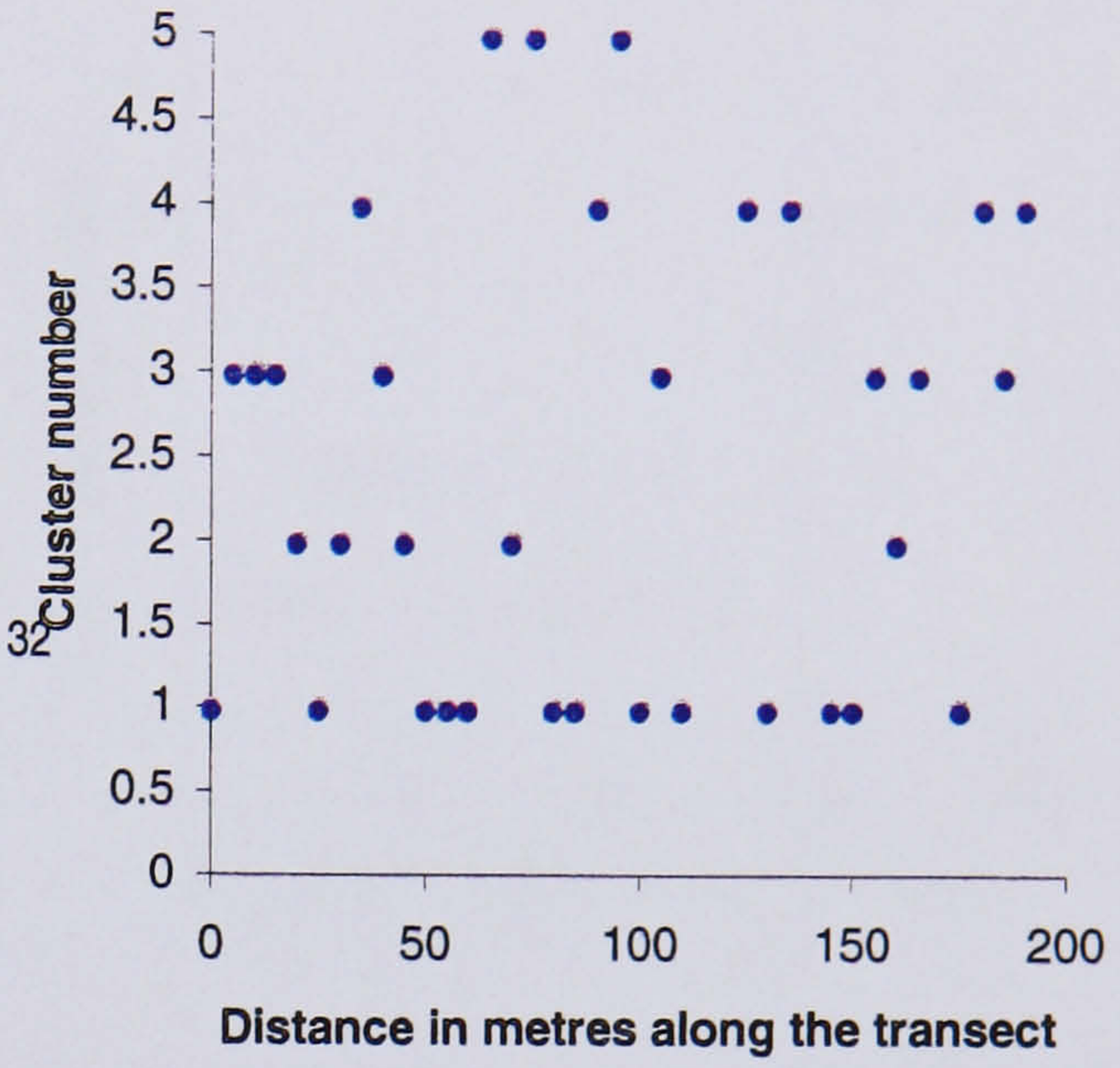
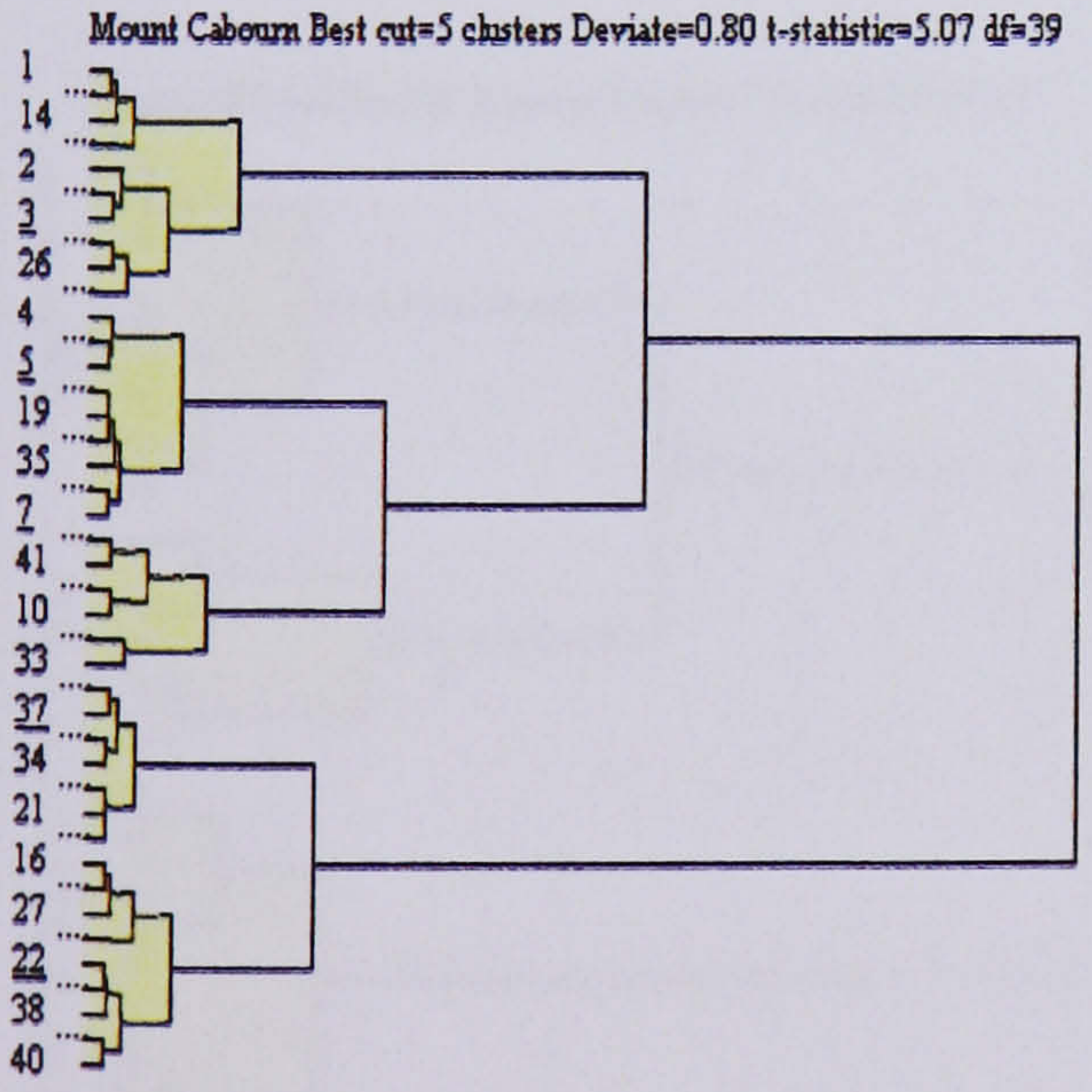


Figure 5.13 Dendrogram showing maximum number of clusters of similar plants on a transect at Lullington Heath

Figure 5.14 Distribution of clusters of similar plants along a transect at Lullington Heath



Cluster 1	1	15	14	8	2	24	25	3	26	12
Cluster 2	4	11	18	5	19	36	35	17	30	
Cluster 3	7	41	9	10	20	33				
Cluster 4	6	37	28	34	13	21	32			
Cluster 5	16	31	27	23	22	29	38	39	40	

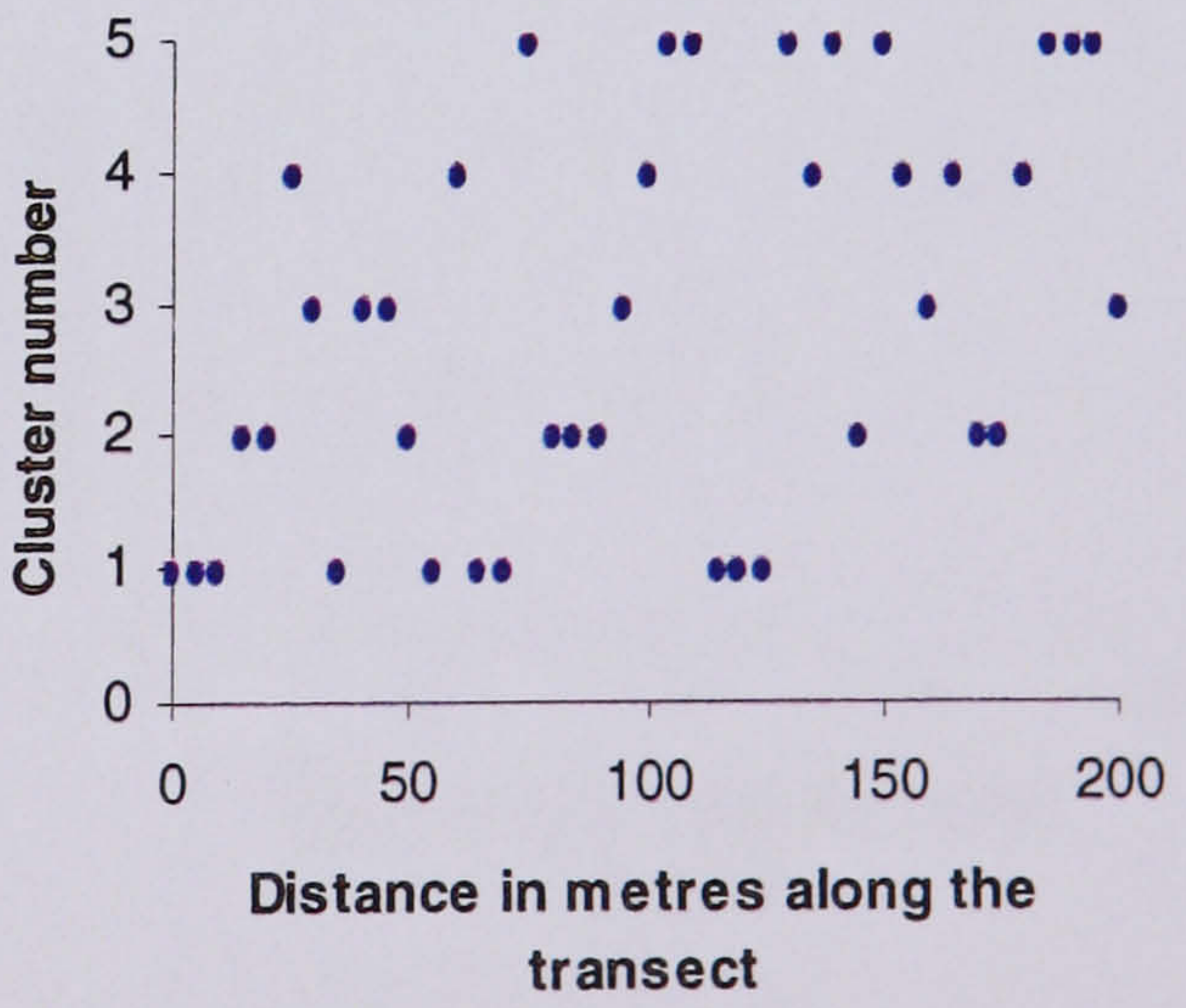
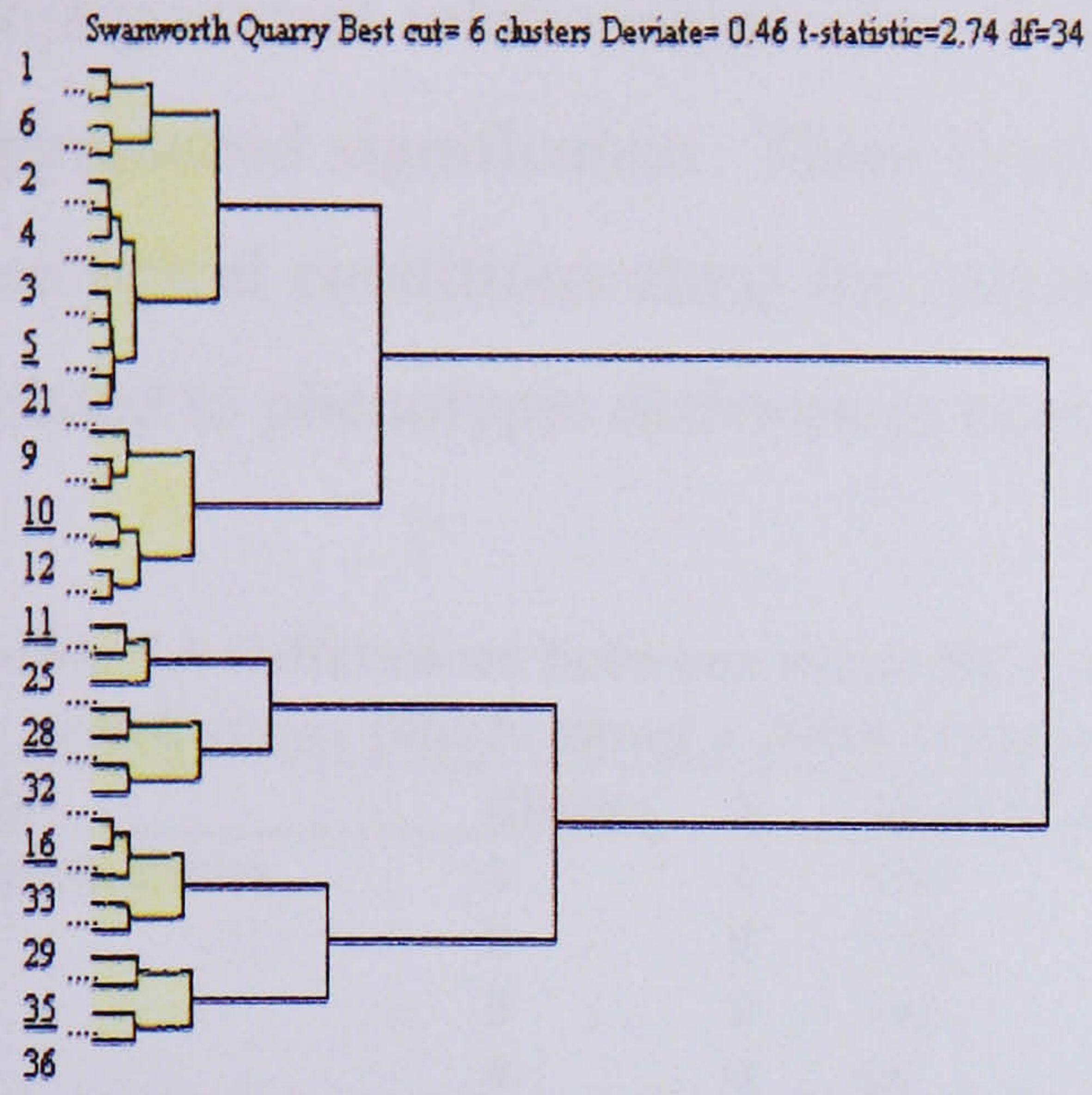


Figure 5.15 Dendrogram showing maximum number of clusters of similar plants on a transect at Mount Cabourn

Figure 5.16 Distribution of clusters of similar plants along a transect at Mount Cabourn



Cluster 1	1	7	6	13	2	19	4	24	3	17	5	15	21
Cluster 2	8	9	23	10	14	12	18						
Cluster 3	11	27	25										
Cluster 4	26	28	30	32									
Cluster 5	16	20	22	33	34								
Cluster 6	29	31	35	36									

Figure 5.17 Dendrogram showing maximum number of clusters of similar plants on a transect at Swanworth Quarry

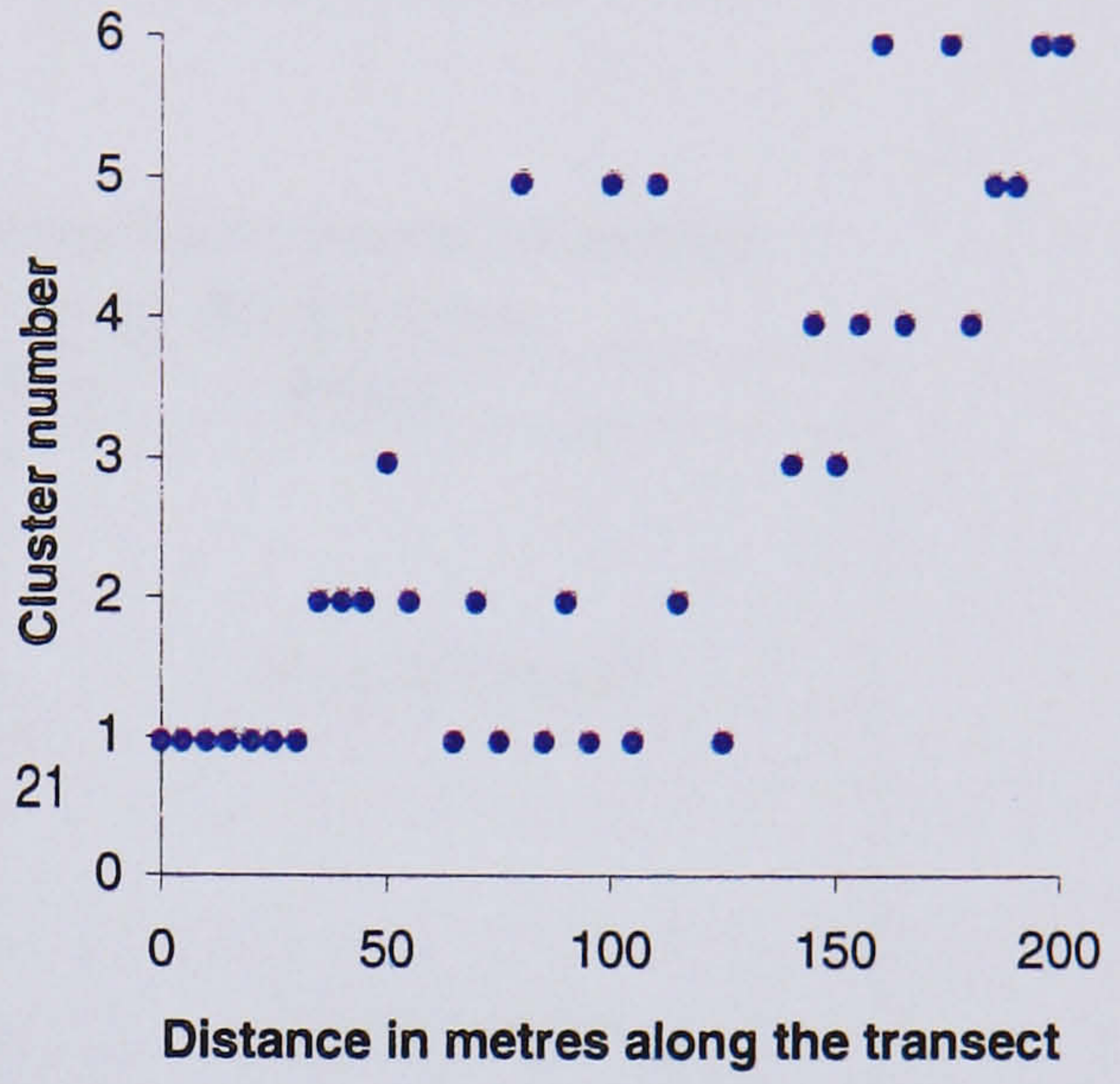
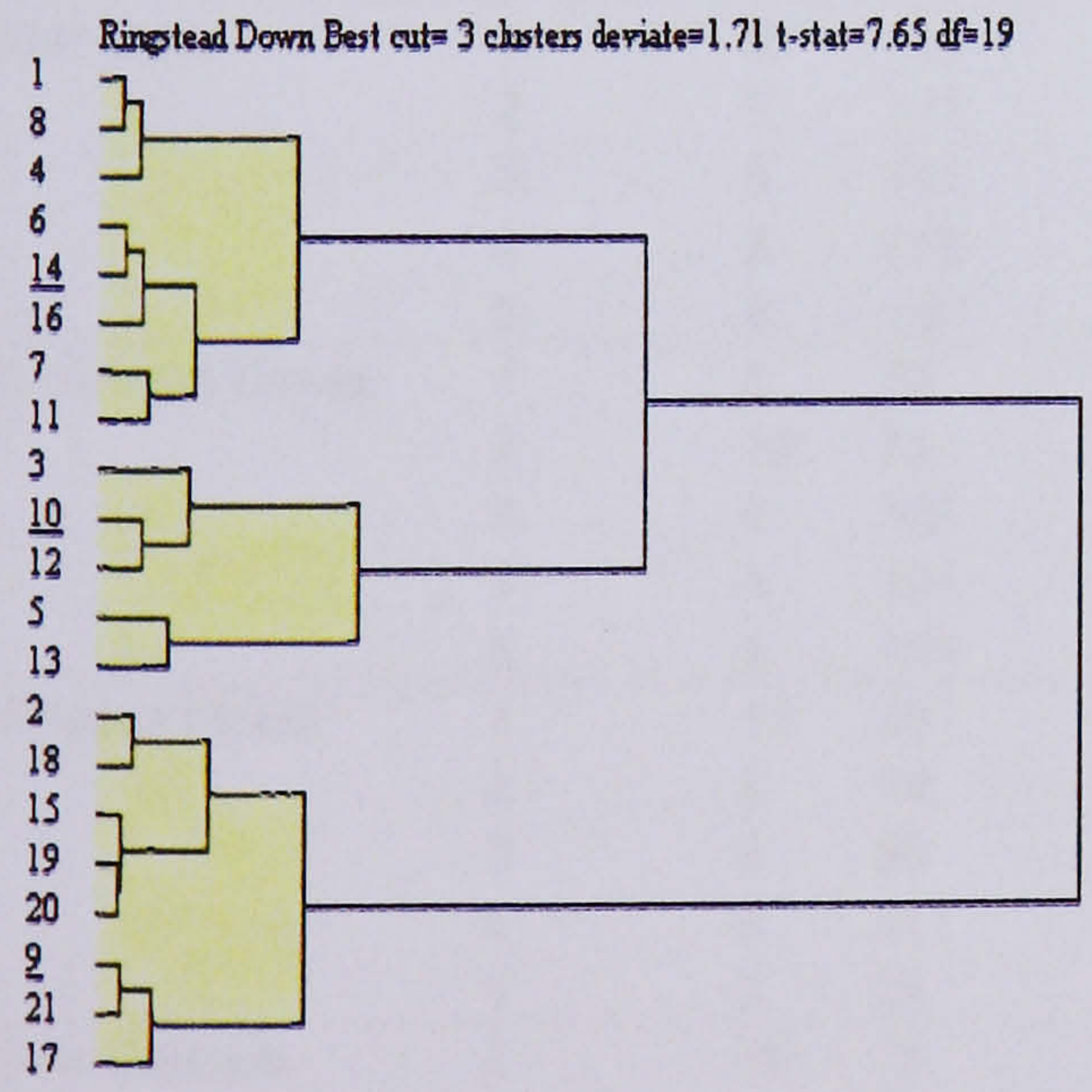


Figure 5.18 Distribution of clusters of similar plants along a transect at Swanworth Quarry



Cluster 1	1	8	4	6	14	11	16	7
Cluster 2	2	18	9	21	15	19	20	
Cluster 3	3	17	10	12	5	13		

Figure 5.19 Dendrogram showing maximum number of clusters of similar plants on a transect at Ringstead Downs

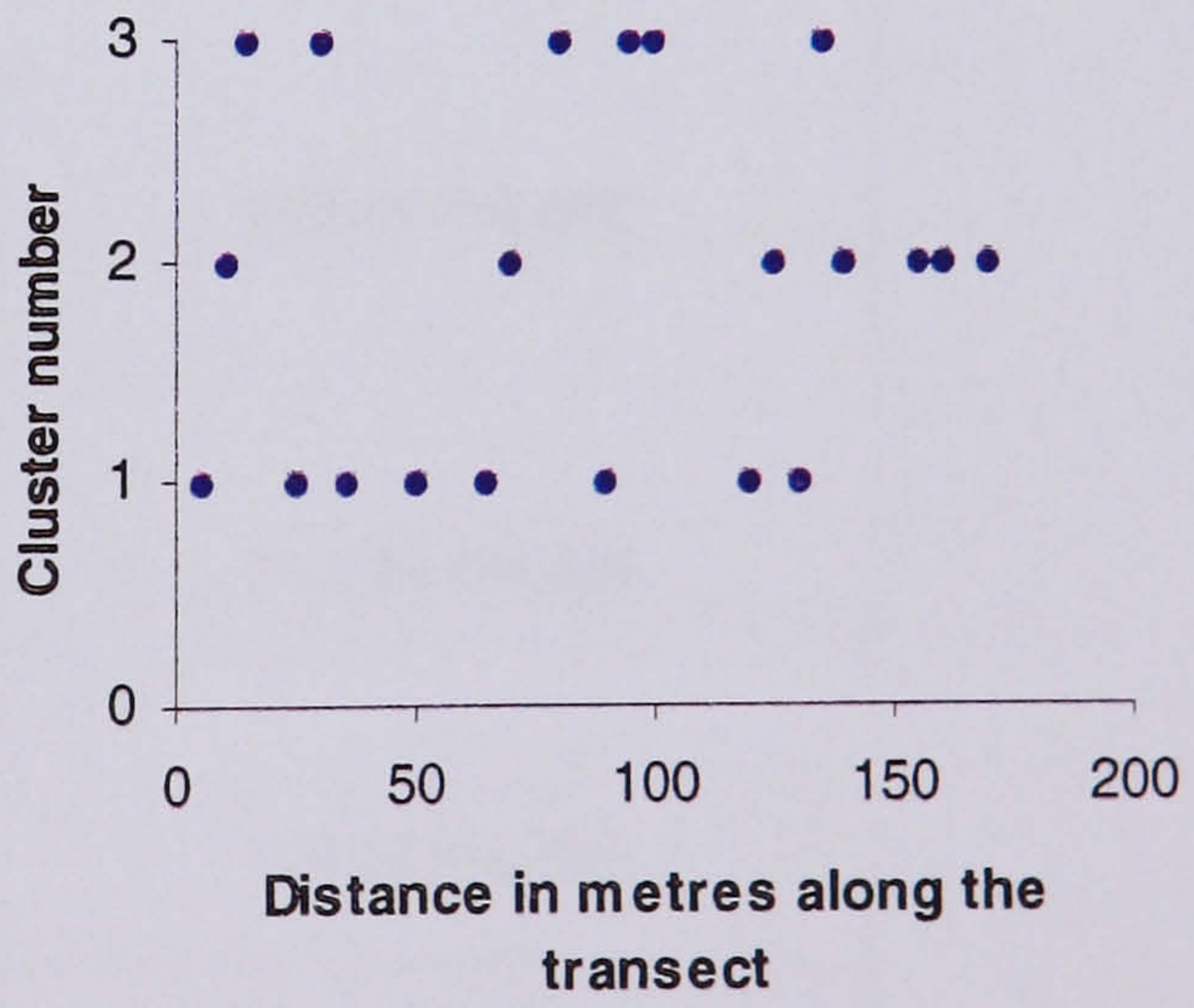


Figure 5.20 Distribution of clusters of similar plants along a transect at Ringstead Downs

When the ecological conditions of sites associated with the clusters were compared, no significant relationships were found, although at four of the locations, the results approached significance. There is no significant evidence to suggest that variation in ecological conditions along the transect, as estimated by changes in the vegetation, is related to phenotypic differences expressed by *L.corniculatus,*(Table 5.5).

Table 5.5 Differences between mean DCA scores associated with clusters of similar *L. corniculatus* plants along a 200m transect at ten sites in the British Isles.

Site	Cluster	N	Mean DCA score	StDev	Anova
Berrow Dunes	1	4	158	89	
	2	6	113	26	
	3	9	142	87	
	4	4	66	18	F= 1.58 *P*=0.227
Brean Down	1	4	77	100	
	2	3	130	30	
	3	12	91	42	
	4	4	180	74	
	5	5	125	23	F=2.44 *P*=0.076
Chapman's Pool	1	11	93	79	
	2	7	108	52	
	3	3	134	47	
	4	5	87	32	
	5	3	124	69	F=0.42 *P*=0.790
Conwy Bay	1	6	183	52	
	2	4	153	36	
	3	5	203	60	
	4	4	143	11	
	5	6	170	59	
	6	9	173	25	F=1.06 *P*=0.402
Great Orme	1	9	150	70	
	2	5	171	112	
	3	5	101	66	
	4	8	150	59	
	5	8	150	37	F=0.75 *P*=0.565
Hunstanton Dunes	1	5	59	28	
	2	12	73	47	
	3	9	104	62	
	4	4	101	47	
	5	3	151	20	F=2.44 *P*=0.070
Lullington Heath	1	13	39	45	
	2	5	74	69	
	3	8	80	67	
	4	6	111	63	
	5	3	24	16	F= 2.24 *P*=0.089
Mount Cabourn	1	10	76	64	
	2	9	58	38	
	3	6	49	30	
	4	7	65	40	F=0.48 *P*=0.747
	5	9	56	17	
Ringstead Downs	1	8	49	36	
	2	7	78	33	
	3	6	52	27	F=1.76 *P*=0.201
Swanworth Quarry	1	13	120	110	
	2	7	115	44	
	3	3	231	105	
	4	4	186	22	
	5	5	193	40	
	6	4	196	49	F=1.95 *P*=0.115

5.4 Discussion

The relationship between geographical distance and phenotypic similarity

At four of the locations where transects were selected and investigated in this study, there was a negative relationship between geographical distance and phenotypic similarity. This suggests that, at these sites, gene-flow was restricted in some way. That spatial structure in plant populations may reflect restricted gene-flow is not unknown (Levin, 1988) and, whilst this effect may be strong in populations of inbreeding species (Levin & Kerster, 1974; Schmitt & Gamble, 1990), it may also be evident in insect pollinated out-crossing species (such as *L. corniculatus*) (Schaal, 1980). Seed dispersal is localised in *L. corniculatus*, as the seeds are expelled ballistically and tend to fall within 5m of the mother plant (Jones & Turkington, 1986). Rasmussen and Brødsgaard (1992) have shown that gene-flow by seed dispersal is limited in *L. corniculatus* and that this reinforces any differentiation in populations. Longer distance gene-flow is likely to be carried out by pollinators. *L. corniculatus* is pollinated by *Bombus* species and, to a lesser extent, *Apis mellifera*. The foraging patterns of *Bombus* have been investigated by several authors (Heinrich, 1979; Waddington, 1981, Zimmerman, 1982; Sih & Baltus, 1987; Rasmussen & Brodsgaard, 1992 and Goverde *et al.*, 2002). The consensus of these authors is that *Bombus* forage locally; where the plant population is fragmented there will be restricted long distance pollen transfer between patches. While plants in a patch still contain pollen, the bees will remain in that area and this tends to reinforce differences between plants in different patches. Goverde *at al* (2002) support this, suggesting that fine-scale habitat fragmentation will affect pollinator behaviour and lead to structure in plants populations. Although structure was observed at four of the locations investigated in this chapter, the data collected from the majority of transects did not suggest any significant relationship between geographical distance and phenotypic similarity.

The relationship between ecological distance and phenotypic similarity

Differences in local conditions appeared to have little effect in determining phenotypic distribution. This was unexpected: large-scale heterogeneity in plant populations is frequently attributed to disruptive selection where different phenotypes are favoured by different environments (Levin, 1988) and studies investigating small scale differences suggest a similar pattern. An early study by Watson (1964) found that populations of *Potentilla erecta* growing in a single piece of grassland with a mosaic of *Molinia* and *Agrostis* were differentiated morphologically and that these differences were maintained when the populations were grown from seed. It was not certain whether this selection was due to the selective pressure of dominant grasses in the sward or to underlying abiotic conditions which also determined the vegetation assemblage. In separate studies, both these factors have been found to be important on a fine-scale. Antonovics and Bradshaw (1970) showed localized selection of plant phenotypes at a mine boundary, where patchy heavy metal contamination selected for tolerance to heavy metals in *Anthoxanthum odoratum.* These plants were also distinguishable morphologically. Turkington & Harper (1979b) investigated fine-scale variation and showed a strong effect of companion species on the fitness of *Trifolium repens.* In the experimental work presented here, some results suggest (although they are only significant at P=0.09) that at Berrow Dunes, Hunstanton Dunes and Lullington Heath groups of similar plants identified by cluster analysis might be associated with particular ecological conditions. These three sites were particularly heterogenous; Chalk heaths, such as the one at Lullington, are mosaics of calcicolous grassland and localised acidic grassland dominated by ericaceous species and calicfugous herbs which may encourage local differentiation. Similarly, at Berrow Dunes the transect crossed a dune slack in which vegetation was different from the rest of the transect and at Hunstanton Dunes, vegetation was interspersed by patches of bare sand. However, the results are not strongly significant and at the majority of locations there was no evidence that ecological conditions affected the distribution of phenotypes.

Presence/absence of L. corniculatus *on the transects*

Ecological conditions were important for the overall distribution of plants at four of the sites. Several authors have investigated fine-scale structure in the distribution of species. Kershaw (1958 & 1959) investigated spatial structure of *Agrostis tenuis, Dactylis glomerata, Lolium perenne* and *Trifolium repens* in a grassland community. He found species distribution to be affected by different factors at various scales. On a scale of a few centimetres, he found the size of neighbouring plants to be important.

Any variation at this scale would be missed at the sites investigated in this chapter. Kershaw (1959) attributed variation at 0.8-1.6m to intra-specific competition and differences at 3.2m and above to soil depth variation. The effect of intra-specfic competition on the structure of populations has been suggested by other authors (Greig-Smith, 1961; Turkington & Harper, 1979a; Franco & Harper, 1988) and a recent study by Purves & Law (2002) demonstrated fine-scale response to competition on a scale of a few centimetres. The results presented here suggest that the pattern of absence at some of the sites was attributable to local conditions which could be due to underlying edaphic conditions or localized competition between species. At the majority of the sites, absences were not explained by analysis. It is possible that there were too few 'absent' data points to be significant or that the absence can be accounted for by the failure of a seed to arrive at the site and become established. Only at Berrow Dunes was geographical distance a useful predictor for plant presence. Berrow was particularly heterogenous with physical features which may have acted as a barrier to seed dispersal and significantly influenced the distribution of plants along the transect.

Testing Linhart's hypothesis

There was some evidence that plants with similar phenotypes aggregate spatially at the scale investigated. At four locations, groups of similar plants were found within 100m which appears to support Linhart's (1995) hypothesis that 100m is a significant distance to consider when translocating plants in restoration schemes. However, the pattern was not identified on seven of the sites and the evidence to support such a conservative limit on seed collection for *L. corniculatus* is not strong.

In conclusion, there was no consistent evidence that phenotypes of *L. corniculatus* are structured within 200m. Although at some locations similar plants were found in close association, when phenotypes were clustered there was little evidence to suggest that the 100m guideline suggested by Linhart (1995) was useful for those selecting propagules for restoration schemes. Notably, structure identified in the populations investigated differed between locations. None of the patterns detected were found at all sites and this emphasises the importance of site-specific research and the limitation of general guidelines.

Chapter Six

The practical implications of restricting seed procurement to local sources

6.1 Introduction

Local seed collection is widely recommended for restoration schemes (Gray, *in press*), but there are practical implications which are rarely discussed. Firstly, it may not be possible to collect sufficient seed to achieve the seed application rates frequently used in re-vegetation projects without depleting local seed sources. Secondly, the number of species available for viable establishment may be limited, either because certain species are rare or because they are known to germinate poorly. This suggests that, if seed is sourced locally, the seed application rate and species mix available for a restoration project may be limited.

There has been little work that directly addresses the effect of low seed rates on grassland development in restoration schemes. Stevenson *et al.* (1995) investigated the effect of three seed application rates on the development of chalk grassland on ex-arable land. They found that seed rate had little effect on the richness of sown chalk species but that a higher sowing rate significantly increased their cover. At the same time increased sowing rate decreased both the number and cover of weed species (weed species were defined as species not commonly associated with chalk grassland). The results of this study (Stevenson *et al.*, 1995) suggested that even a low application of appropriate species was sufficient to allow for the re-vegetation of large areas using local seed. Unfortunately the experiment was terminated after two years as the authors considered that cross contamination between experimental plots confounded the results in the third year and long-term assessment was impossible. Other authors have suggested that using a nurse grass might enhance the success of re-vegetation projects (Wells *et al.*, 1981, Kershaw *et al.* 1995). Nurse grasses provide an early establishment of plant cover which encourages the germination and establishment of sown species, and controls weeds through competitive suppression (Mitchley *et* al., 1996). This method has been found to be effective at a restoration site (Mitchley *et* al., 1996) and the technique may be useful where only a small amount of seed is available. The work presented in this chapter investigates the effect of seed application rate and seed mix on the development of vegetation at a restoration site. It also tests whether the application of a nurse grass influenced the effect of those treatments.

One of the problems with assessing the success of a restoration project is that success is relative and will depend on the goals which were defined at the outset of a project. Confusion frequently arises in the field of restoration ecology as there has been a lack of precision in determining 'goals' (Ehrenfeld, 2000). In addition there is debate about the level of organization (individual species level or community level) which gives the best estimate of the successful establishment of a community (e.g. Parker, 1997; Goldstein, 1999; Risser, 1999). In a review, Ehrenfeld (2000) suggested that for those engaged in restoration it is necessary to recognize that a rigid definition of success is not achievable; in most cases the goals will be site specific and it is impractical to try to replicate a system exactly. One way of evaluating restoration projects is to select a 'target vegetation' as an 'ecological yardstick' by which to test the success in re-vegetating an area (Aronson, Dhillon & Le Floc'h, 1995). This method was used by Stevenson *et al.* (1995) who chose an area of established chalk grassland that was close to their experimental site. The value of comparing vegetation communities, which are dynamic, is debatable, as each site is unique in terms of context and influences (Pickett & Parker, 1994). Comparing the restored grassland with target vegetation is an approximate way of assessing success but it does not indicate whether the restoration is likely to continue to be successful in the long-term. To evaluate the success of the treatments in the experiment described in this chapter, three determinants of successful vegetation establishment were selected for investigation; species diversity, functional diversity and invasibility by unsown species.

The debate surrounding the influence of plant diversity on ecosystem processes has received much attention (e.g. Tilman *et* al., 1996; Tilman, *et al.*, 1997; Grime, 1997; Hooper & Vitousek, 1997; Grime, 1998; Crawley, *et al.*, 1999; Tilman *et al.*, 2001). Despite some differences of opinion amongst authors, there are important principles to be drawn from this body of research, which are useful in assessing the relative success of treatments in experimental work. The importance of species richness in ecosystem processes has been emphasized and linked to more than one process. For example, Tilman has suggested that increased species richness leads to increased primary productivity (Tilman *et al.*,1996). Tilman's experimental work supports what he refers to as the 'diversity – productivity hypothesis'. This is based on the assumption that inter-specific differences in the use of resources by plants allow more diverse plant communities to use limiting resources more fully, and so attain greater productivity (Tilman *et* al., 1996). It has also been shown that species richness limits nutrient loss from the system (Tilman & Downing, 1994; Symstad *et al.*, 1998). The importance of productivity for restoration sites is inferred from work by Wilsey & Potvin (2000) who suggest it is positively associated with the productivity of higher levels such as herbivore consumption and herbivore biomass. Both productivity and nutrient retention have been cited as important factors in the long- term stability of a plant community (Loreau, 2000). However, the definition of 'stability' is contentious (Loreau, 2000). In this chapter stability is defined as "*the capacity of a system to persist in the same state in the face of perturbation*" (Diaz & Cabido, 2001) and includes the notion of resilience (defined as the "*ability* [of a system] *to return to its former state following a perturbation*" (Diaz & Cabido, 2001)). Experimental work has suggested that species richness increases the resilience of grassland communities (Tilman & Downing,1994; Tilman, 1997a, Naeem & Li, 1997). Using controlled field experiments, Tilman & Downing (1994) found that increased species richness increased the ability of grassland to recover after a period of drought. From the work described above, it can be concluded that species richness will indicate the potential of grassland to develop into a stable community on a restoration site.

The functional properties of particular species, or groups of species, may also control productivity and nutrient use (Hooper & Vitousek, 1997, Symstad *et al,* 1998). The loss or addition of plants with certain functional traits may have a great impact on particular ecosystem processes, and each process is likely to be affected by different species and functional groups (Tilman *et al.*, 1997). This work is in its infancy and so far has been focused on the ecological processes of productivity and nutrient retention (Hooper & Vitousek, 1997; Symstad *et al.,* 1998; for reviews: Loreau 2000; Diaz and Cabido, 2001). Grime (1997) supports the hypothesis that plant function will be a determinant of ecosystem processes; he emphasizes that even in species-rich vegetation most of the plant biomass is likely to reside in a small number of dominant species, the characteristics of which are likely to become system controllers. Grime stresses the importance of considering function of species and of presenting the relative abundance of component species in functional groups. This is borne out by experimental work in which Symstad *et al.* (1998) found that the functional group is a good predictor for the type of impact a species will have on biomass but that differences in nutrient retention are related to species identity. Diaz & Cabido (2001) describe '*functional insurance*' where functional richness increases the chances that a community will be able to deal with perturbations. Work in this field suggests that functional diversity, the relative abundance of species within each functional group and identity of particular species will each affect grassland development.

Grassland development will not only be influenced by sown species, but also by those that colonize, because seeds arrive from surrounding vegetation. Ideally, at restoration sites, weedy species will be excluded and species which are part of the target vegetation will be recruited. Areas that are more species-rich are assumed to have a more complete use of limiting resources and thus be less invasible (Robinson *et al.*, 1995). In experimental work, Tilman (1997b) found that the greater the initial plant species richness of a site, the more difficult it was for new species to invade. He also found that some functional groups are better able to invade than others and cited legumes as an example. Tilman (1997b) added seeds to an area of species-poor grassland and discovered that species which were best adapted to local conditions at the site colonized readily. In his experiment, a biased subset of available species became established that was based partly on the character of the colonizing species and partly on the number of species already present in the plots. It is reasonable to expect a similar effect at a restoration site, which may also recruit a biased subset of species dispersed from local sources. Crawley *et al.*, (1999) found that the identity of particular species rather than general species-richness controlled the invasibility of experimental plots and concluded that inter-specific competition limited plant invasion. This work indicates that seed rate and species composition of the seed mix will affect the potential of both calcicolous and weed species to invade at a restoration site.

6.1.2 Aim and Objectives

The aim of this study was to determine the effect of seed application rate, type of seed mix and nurse grass application on the successful development of a grassland sward in a restoration environment. There were two objectives:

- To use a descriptive measure of community structure to assess the effect of seed application rate, type of seed mix and nurse grass application.

- To use three ecological measures as determinants of success: i) species diversity, ii) functional diversity, iii) invasibility. The effect of the treatments on these determinants was investigated.

6.2 Method

6.2.1 The experiment

Site description

The experimental work was carried out at a limestone quarry in Dorset (National Grid Reference SY 970 784; Plate 6.1). Swanworth Quarry is fully described in the General Method section of this thesis. The experimental area was laid out on an area prepared for restoration which consisted of clays and marls incorporating limestone chips. The substrate was prepared by harrowing and raking but was otherwise untreated.

Plate 6.1 The experimental plots at Swanworth Quarry (January, 2000).

The Target Vegetation

The aim of the restoration scheme at Swanworth Quarry was to increase the area of species rich limestone grassland which already existed in the locality and the target vegetation was chosen with this in mind. An area of vegetation, (part of the South Dorset Coast SSSI), was identified as typical of local limestone grassland and chosen as the 'target'. The area (100m x 100m) was located on a south-east facing slope which supported a mosaic of CG2 and CG4 grassland as defined by Rodwell (1992) (Plate 6.2). The higher part of the slope was dominated by *Festuca ovina* with a variety of calcicolous grasses including *Anthoxanthum odoratum, Briza media, Cynosurus cristatus,* and *Festuca rubra,* with abundant calcicolous forbs including *Campanula rotundiflora, Galium verum, Helianthemum nummularium, Thymus drucei* and *Viola hirta.* The lower part of the slope, which was damp and shaded by trees, was less grazed and in these areas the vegetation was taller with an abundance of *Brachypodium pinnatum.*

Plate 6.2 View of the target vegetation selected for the restoration programme at Swanworth Quarry. The photograph shows the quarry boundary, with the target vegetation beyond.

Seed mixes

Two seed mixes were designed and hereafter are referred to the 'basic mix' and the 'preferred mix' (Table 6.1). The basic seed mix of twenty species was an off-the-shelf mixture sold by British Seed Houses (BSH) as suitable for CG2 grassland creation. The preferred mix contained an additional fifteen species that frequently occur on limestone grasslands and were present in the target vegetation. Six of these were either known to germinate poorly or were unavailable as seed and were introduced as plant plugs. In retrospect, it was realised that the inclusion of *Medicago sativa* was inappropriate as this species is more commonly associated with cultivated land. Wells *et al.* (1981) recommended that the annual grass *Lolium multiflorum* is useful as a nurse grass as it is unlikely to persist; *Lolium multiflorum* "Lemnos" was added to half of all the treatments in a split plot design.

Table 6.1 Species composition of seed mixes introduced in experimental work at Swanworth Quarry to investigate the effect of seed mix and application rate on grassland establishment.

Basic mix		**Additional species in preferred mix**	
Achillea millefolium	Yarrow	*Fragaria vesca*	Wild strawberry
Anthyllis vulneraria	Kidney vetch	*Alliaria petiolata*	Garlic mustard
Briza media	Quaking grass	*Echium vulgare*	Viper's-bugloss
Centaurea nigra	Common knapweed	*Origanum vulgare*	Marjoram
Daucus carota	Wild carrot	*Medicago sativa*	Lucerne
Galium verum	Lady's bedstraw	*Rhinanthus minor*	Yellow rattle
Leodonton hispidus	Rough hawkbit	*Campanula rotundiflora*	Harebell
Leucanthemum vulgare	Ox-eye daisy	*Ononis repens*	Restharrow
Lotus corniculatus	Birdsfoot trefoil	*Helianthemum nummularium*	Common Rock-rose
Medicago lupulina	Black medick		
Onobrychis vicifolia	Sainfoin	Plant plugs	
Plantago lanceolata	Ribwort plantain	*Hypochoeris radicata*	Cat's ear
Plantago media	Hoary plantain	*Viola riviniana*	Common dog violet
Prunella vulgaris	Self-heal	*Primula veris*	Cowslip
Sanguisorba minor	Salad burnet	*Centaurea scabiosa*	Greater knapweed
Scabiosa columbaria	Small scabious	*Thymus drucei(praecox)*	Wild thyme
Agrostis stolonifera	Creeping bent	*Knautia arvensis*	Field scabious
Anthoxanthum odoratum	Sweet vernal grass		
Arrenatherum elatius	Tall oat grass		
Cynosurus cristatus	Crested dogstail		
Dactylis glomerata	Cocksfoot		
Festuca ovina	Sheep's fescue		
Festuca rubra ssp litoralis	Slender creeping red fescue		
Poa pratensis	Smooth-stalked meadow grass		
Trisetum flavescens	Yellow oat grass		

Seed Rates

Seed was applied at three rates; 0.4g, 1g, and 4g per m^2. These rates were those used at the M3 extension at Twyford Down (Stevenson, Bullock & Ward, 1996). Plant plugs were added at 0.12 per m^2, 0.2 per m^2 and 4 per m^2. The nurse grass was applied at 4g/m^2 (Table 6.2).

Table 6.2 Application rates of propagules

Seed rate (of each mix)	Application rates m^{-2}	Total applied per plot
Low	0.4g	10g
Medium	1.0g	25g
High	4.0g	100g
Plant plug application rates (of each species)		
Low	0.12	3 plants
Medium	0.2	5 plants
High	4.0	15 plants
Nurse crop		
Lolium mulitflorum 'Lemnos"	4.0g m^{-2}	to half of each plot (in a split plot design)

Experimental design

The seeds were sown into a fully randomised block experiment in March 1997. Each plot measured 5 x 5m and the plots were separated by 2m, which was the maximum distance possible within the allocated experimental area. The treatments were allocated randomly within blocks; the experimental design is shown in Figure 6.1 and the treatments are summarized in Table 6.3. The control plots received no application of the basic or preferred mix but the nurse grass was added to half of each control in the same way as on the treated plots.

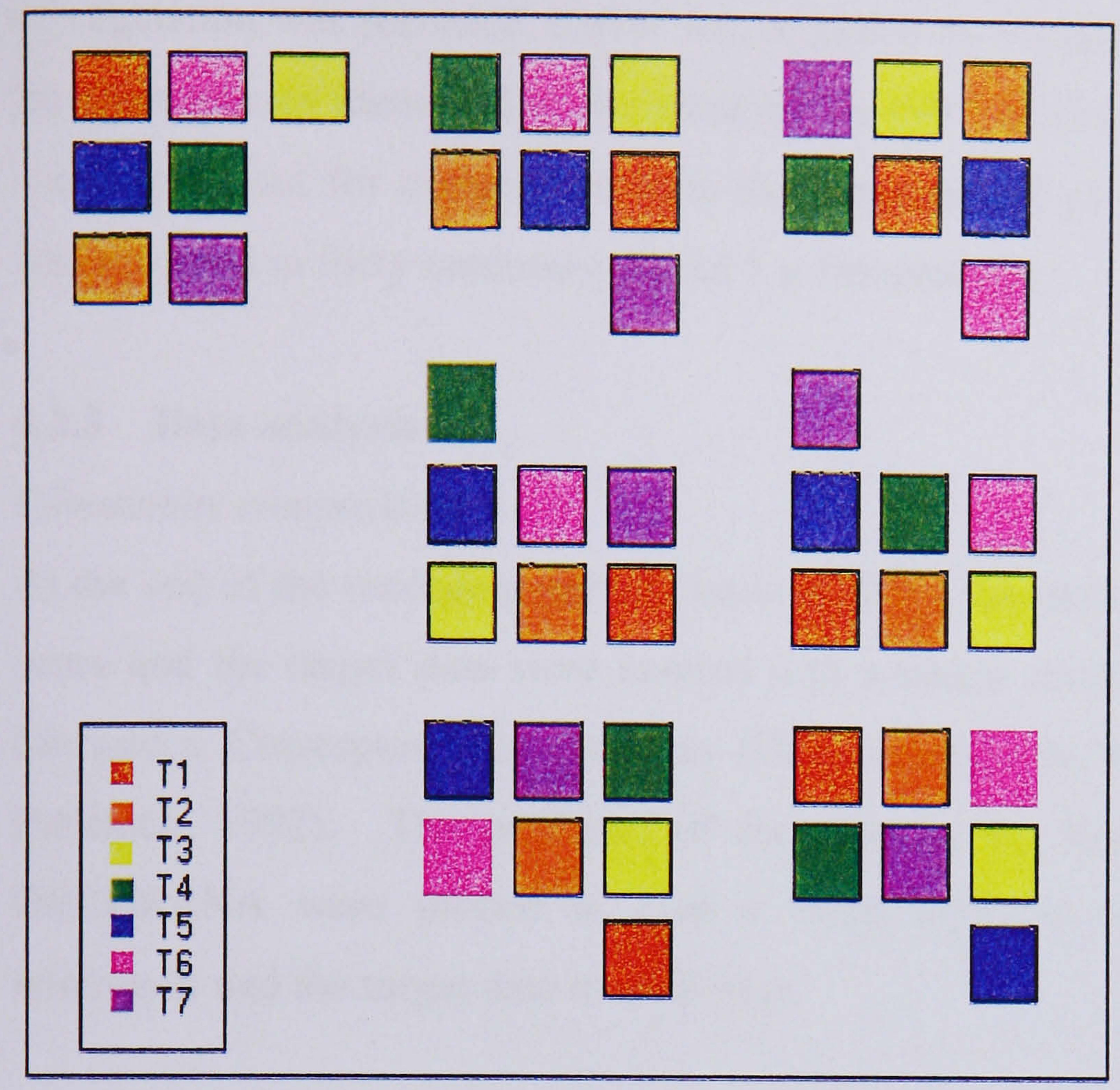


Figure 6.1. Plan of the field experiment at Swanworth Quarry in Dorset. *Lolium multiflorum* was applied to half of each plot in a split plot design.

Table 6.3 Treatments applied to the experimental plots

Label	Treatment
T1+	Preferred mix at low rate with nurse grass
T1-	Preferred mix at low rate without nurse grass
T2+	Preferred mix at medium rate with nurse grass
T2-	Preferred mix at medium rate without nurse grass
T3+	Preferred mix at high rate with nurse grass
T3-	Preferred mix at high rate without nurse grass
T4+	Basic mix at low rate with nurse grass
T4-	Basic mix at low rate without nurse grass
T5+	Basic mix at medium rate with nurse grass
T5-	Basic mix at medium rate without nurse grass
T6+	Basic mix at high rate with nurse grass
T6-	Basic mix at high rate without nurse grass
T7+	Control with nurse grass
T7-	Control without nurse grass

6.2.2 Recording

The experiment was recorded in the first two weeks of June in 1997, 1998 and 2000. The species cover was recorded within a 1m x 1m quadrat which was randomly placed in each half of each split plot leaving 0.5m at the margins to avoid edge effects. The quadrat position was marked with pegs so that in each year the same area of vegetation was recorded. Cover was recorded by visually estimating percent cover for each species identified in the quadrat. In 1997, a survey of the target vegetation was carried out for comparison with the experimental plots. Percent species cover was recorded in forty randomly placed 1 x 1m quadrats.

6.2.3 Data analysis

Community composition

At the end of the recording period (autumn 2000), the experimental data for the three years and the target data were entered into a single analysis and ordinated using a Detrended Correspondance Analysis (DECORANA) in Vespan computer package (Malloch, 1992). The positions of the data on the first two axes generated by DECORANA were plotted to give a visual estimate of similarity between the treatments and the target data in each year.

To investigate the effect of the treatments on the ordination of the experimental data, the values on each of the first four axes generated by DECORANA were grouped by seed rate (three groups), type of seed mix (two groups) and application of nurse grass (two groups). Data from each year were analysed separately. Differences in the mean value on each axis between groups were compared using General Linear Model Analysis of Variance (GLM ANOVA) using Minitab (version 13). The aim of this analysis was to investigate whether the treatments significantly influenced the composition of the vegetative assemblage. A preliminary analysis showed that the control plots were clearly separated from the treated plots. As it was likely that their inclusion in the analysis would generate false significant results, the control data were excluded.

Species diversity

The mean number of species (species richness) in each treatment was calculated. The effect of seed application rate, type of seed mix and the application of nurse grass on the mean number of both sown and unsown species was investigated using GLM ANOVA. Over-all diversity and the evenness of the abundance of species within the treatments was calculated using the Shannon- Weiner index. The Shannon-Weiner index combines species richness with the proportion of cover for each species to give a single score for diversity (H) that incorporates evenness. H was calculated for each treatment in each year using the formula described in Kent & Coker (1992).

$$\text{Diversity } H = -\sum_{i-l}^{s} p_i \log_{10} p_i$$

where s = the number of species
P_i = the proportion of individuals or the abundance of the ith species expressed as a proportion of the total cover

H values usually lie between 1.5 and 3.5, where 1.5 is low diversity. Using the H score, it was then possible to calculate an evenness index of the form:

$$\text{Evenness } J = \frac{H}{H_{\max}} = \frac{\sum_{i-l}^{s} p_i \log_{10} p_i}{\log_{10} s}$$

where s = the number of species
P_i = the proportion of individuals or the abundance of the ith species expressed as a proportion of the total cover

J values lie between 0 and 1, the higher the value of J, the more evenly spread the species were in their distribution within the sample.

Functional groups

The species identified on the experimental plots were categorized into four functional groups: woody species, legumes, forbs and grasses. These have been distinguished as groups of plants which have a similar response to the environment (Diaz & Cabido, 2001). In an analysis of functional types on limestone grassland Thompson *et al.* (1996) also distinguished between forbs and grasses. The effect of seed rate, type of seed mix and application of nurse grass on the number of species in each category was investigated using chi-square analysis. Evenness in the number and cover of functional groups within each treatment was investigated using a Kruskal-Wallis test.

Invasibility

The mean number of unsown species in each treatment was calculated. The higher the number of unsown species, the more 'invasible' the community was considered to be. The unsown species were divided into calcicolous species and weedy species, that is, species which are not normally found in CG2, CG3 and CG4 grassland communities as defined by Rodwell (1992). The effect seed rate, type of seed mix and the application of nurse grass on the number of species in each category was investigated using chi-squared analysis.

6.3 Results

6.3.1 Using a descriptive measure of community structure to assess the effect of seed application rate, type of seed mix and nurse grass application

Variation in the treatment and target vegetation data was well accounted for by the first four axes generated by DECORANA (Eigenvalues: Axis 1, 0.4517; Axis 2, 0.3458; Axis 3, 0.2598; Axis 4, 0.1916). The first axis explained 45% of the variation and axis 2 accounted for a further 36%. Ordination of species on axis 1 showed a gradient from weedy species on the left of the axis, through to species typical of calcicolous grassland on the right. *Brachypodium pinnatum*, not recorded on the experimental treatments, was ordinated at the right of axis 1. The spread of the target data may be a reflection of the mosaic of CG2-CG4 grassland types. On axis 2 the top of the axis reflected woody coarse species such as *Clematis vitalba* and *Rubus fruticosus*, through to leguminous species such *as Anthyllis vulneraria*, *Lotus corniculatus* and *Ononis repens*. The spread of the target data along this axis may represent the presence of woody seedlings on parts of the target vegetation which was overhung by trees. Figure 6.2.-6.8 show the changes that occurred within each treatment and the control plots over the three sampling seasons. In all cases, there was a separation of the data recorded in 2000, as all treatments were more similar to the target data by the end of the recording period. Some of the control plots were also close to the target data in 2000, but the ordination was less consistent (and so had a wider spread) than those of the treatment plots. The effect of seed rate, type of seed mix and the addition of nursery grass on the distribution of data along the first four axes is presented year by year in the next section.

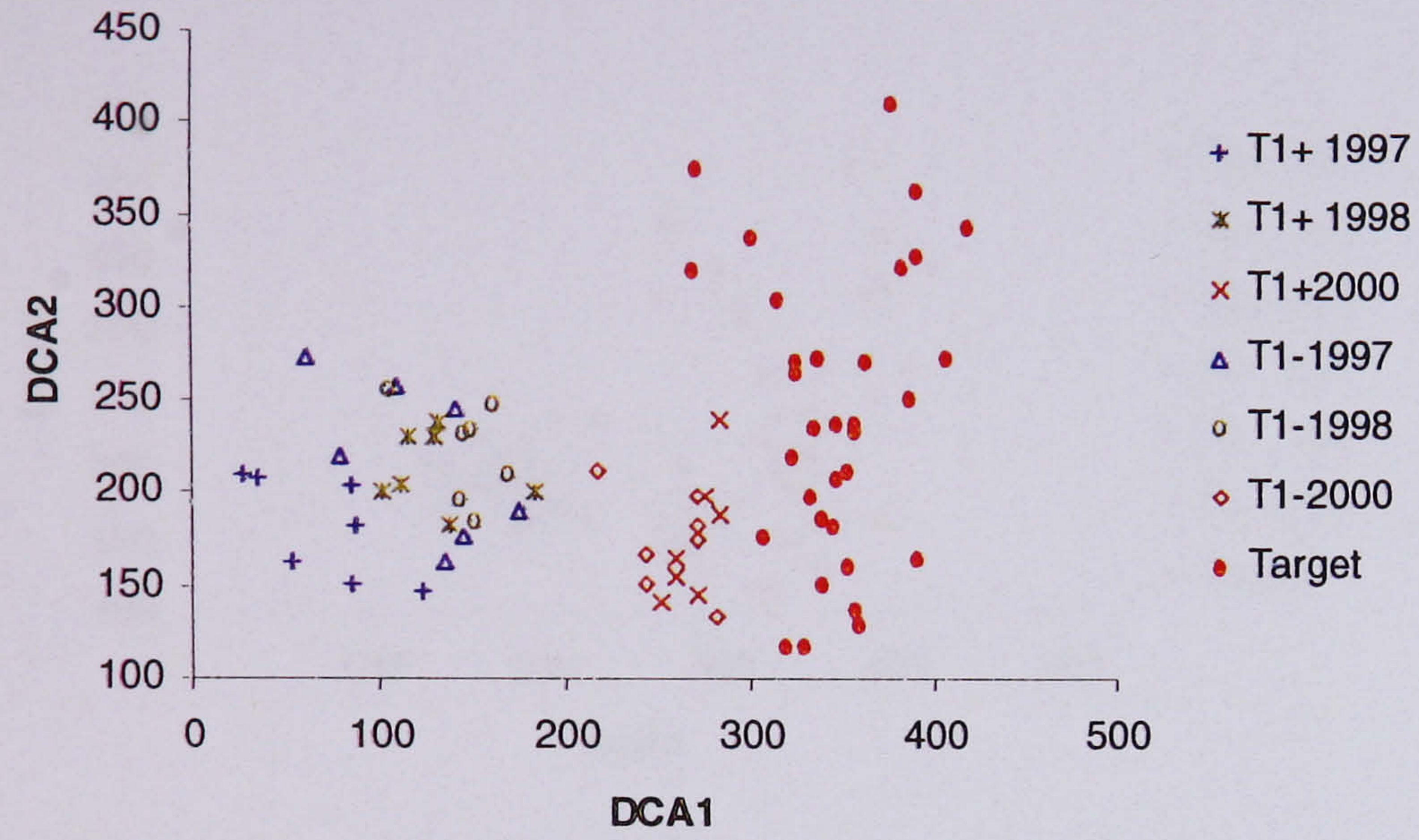


Figure 6.2 Ordination of the preferred mix at 0.4g/m2

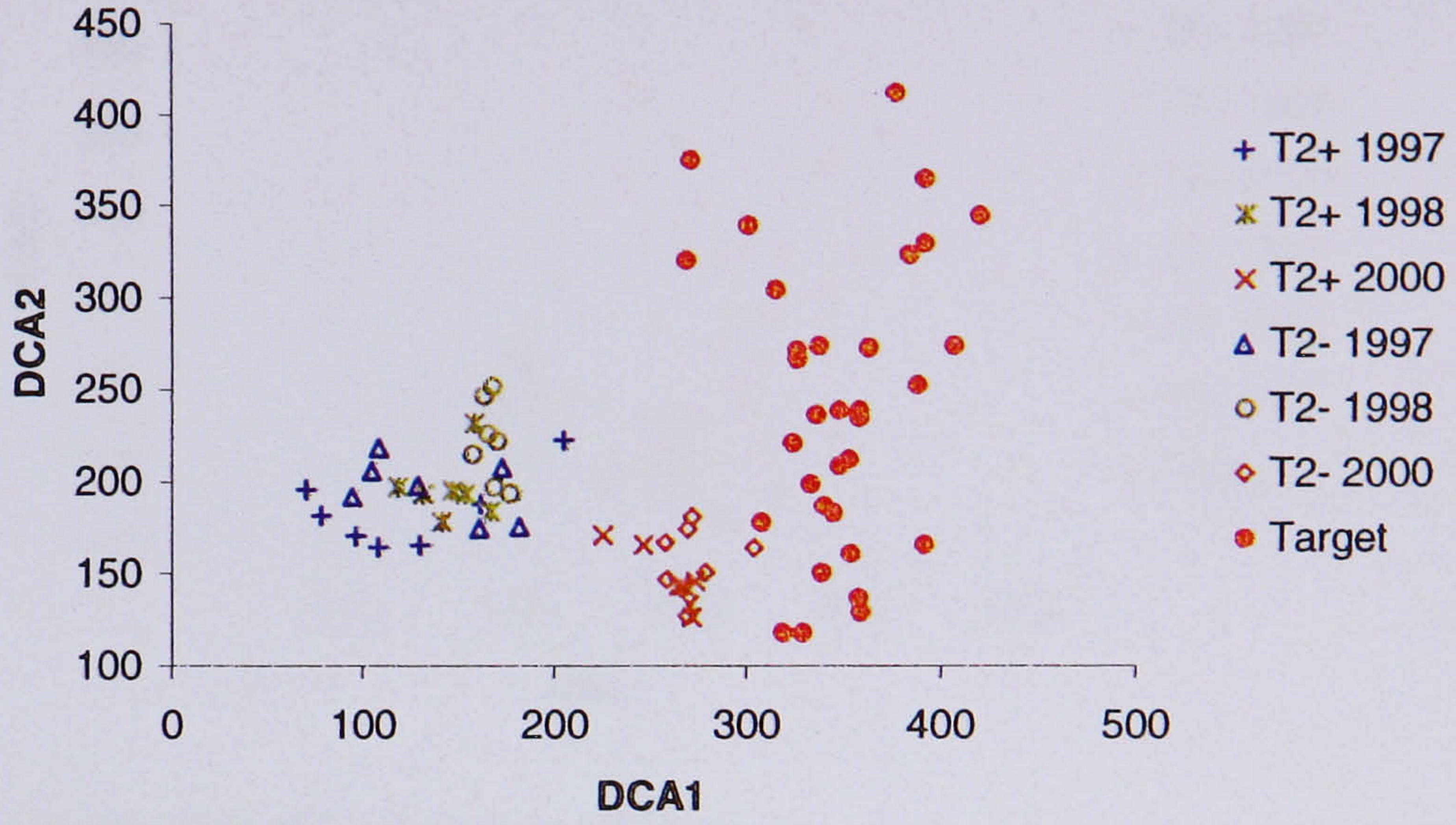


Figure 6.4 Ordination of preferred mix at 1g/m2

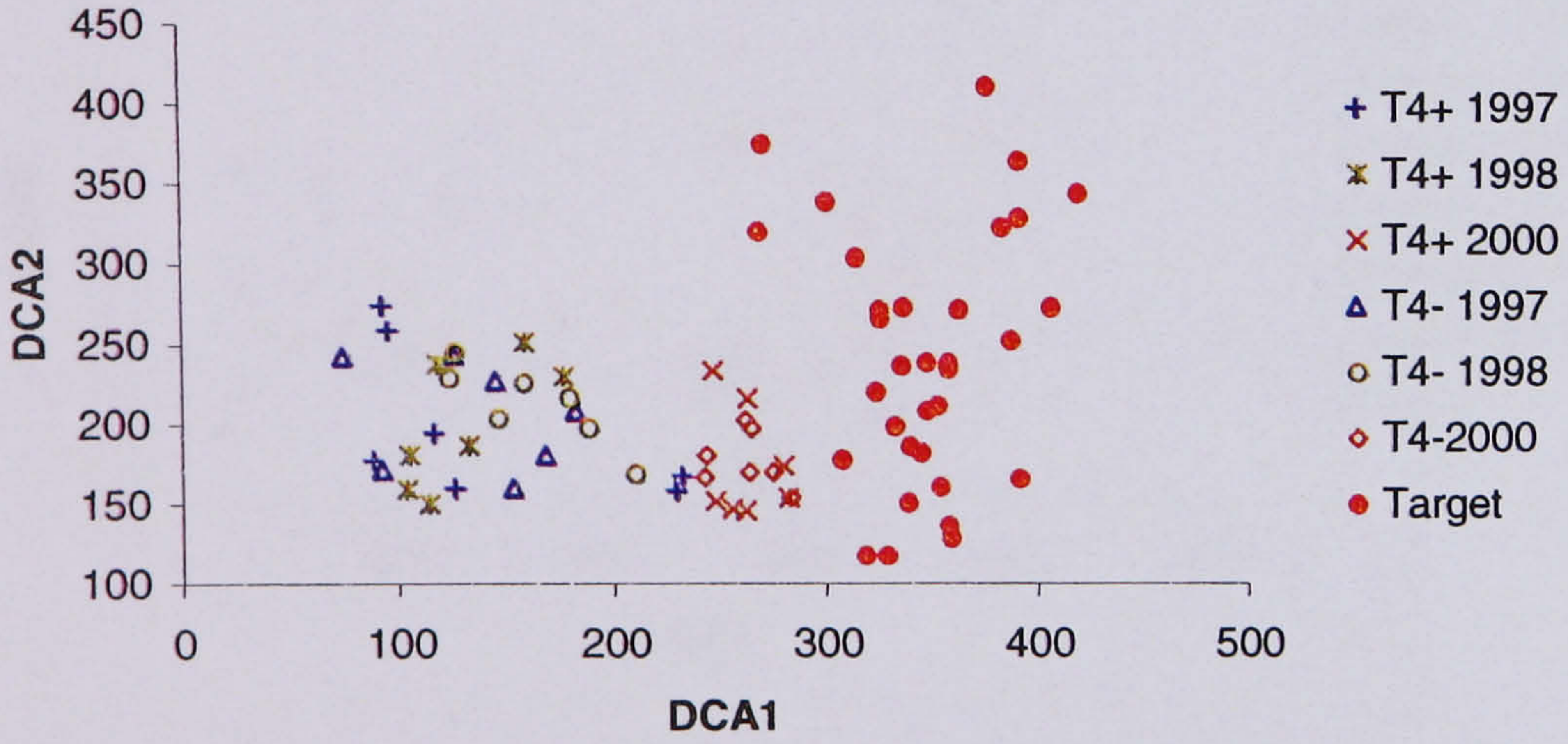


Figure 6.3 Ordination of basic mix applied at 0.4g/m2

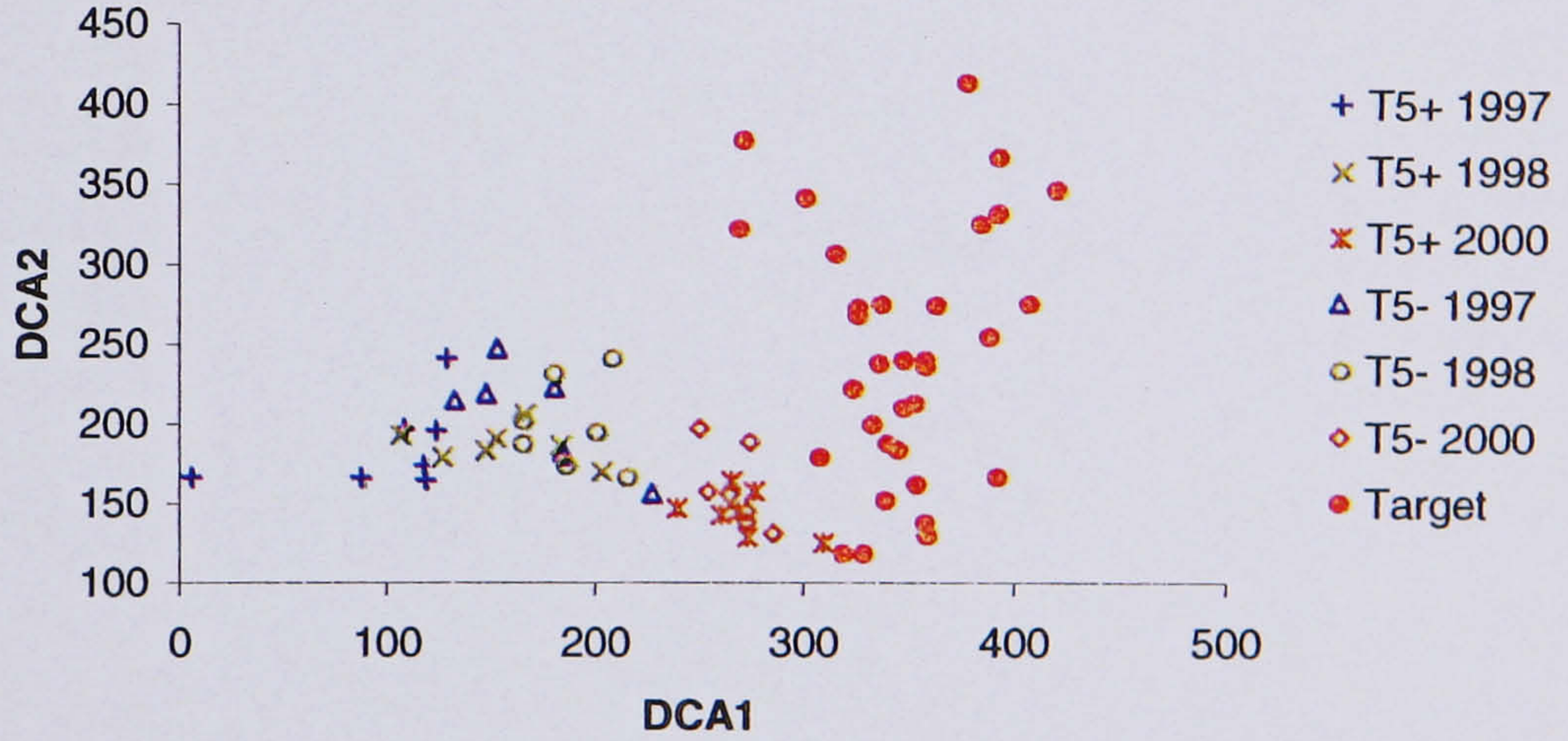


Figure 6.5 Ordination of basic mix at 1g/m2

In all cases, + = with nurse grass and - = without nurse grass

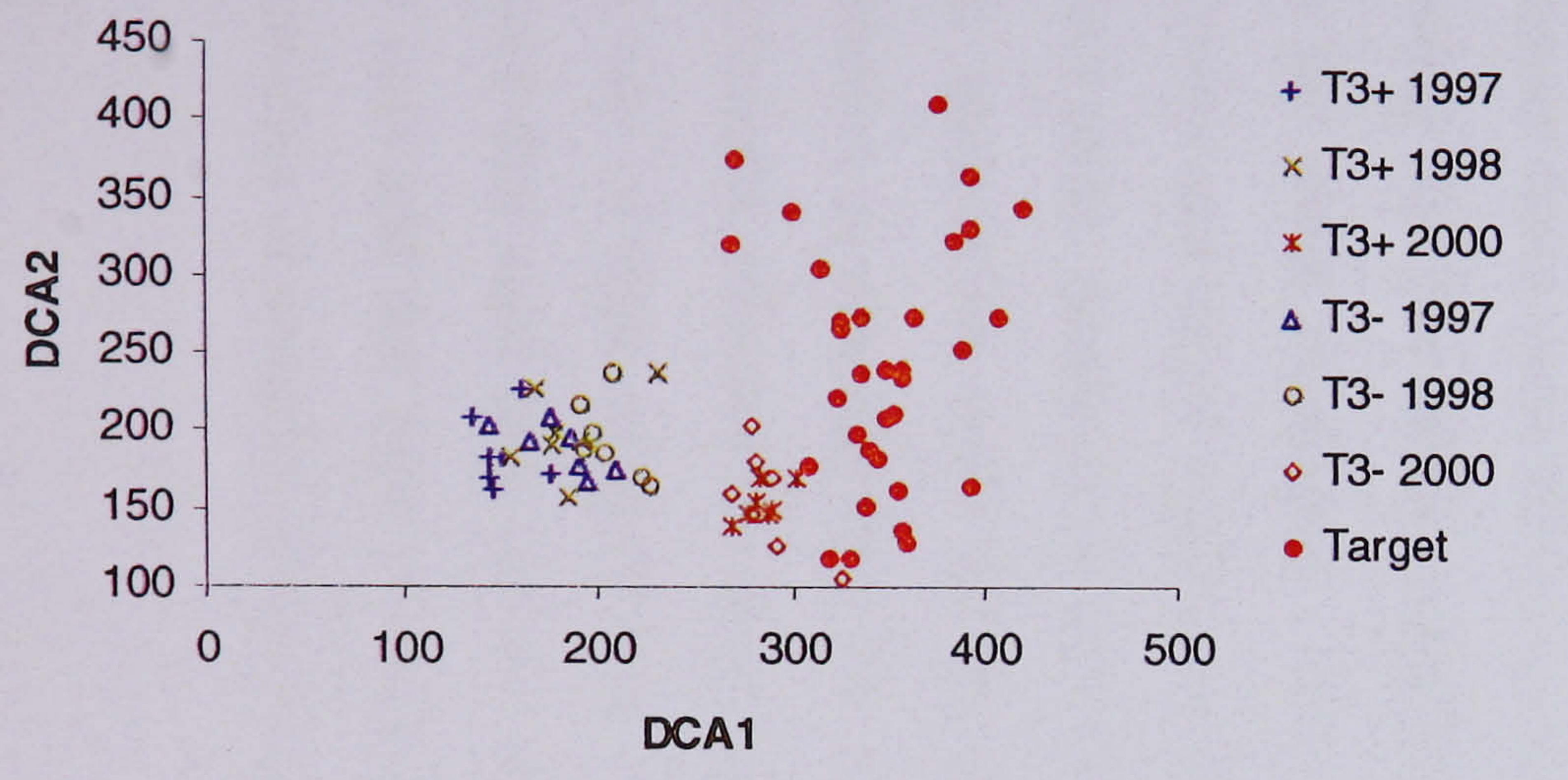
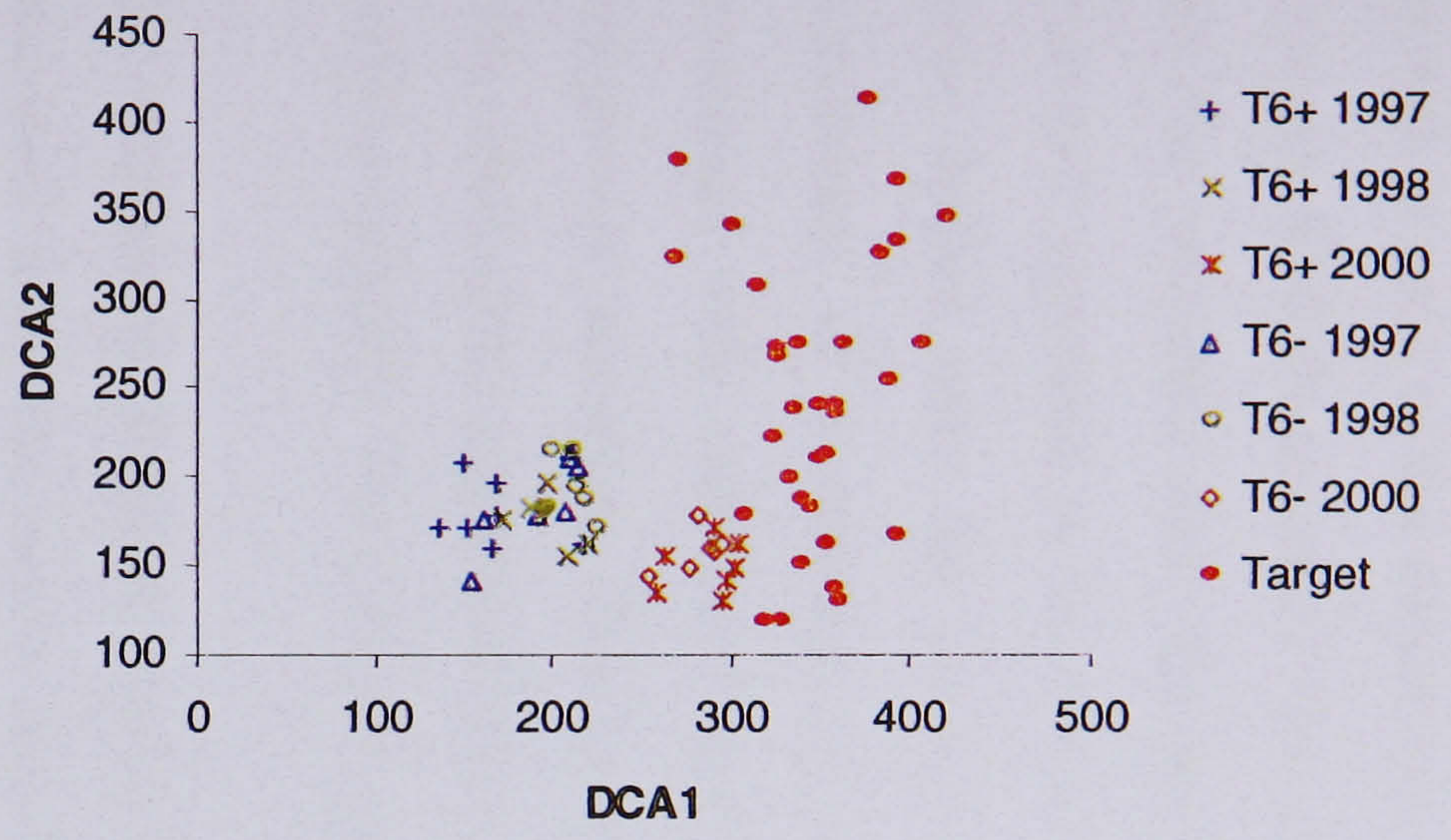


Figure 6.6 Ordination of preferred mix at 4g/m2



6.7 Ordination of basic mix at 4g/m2

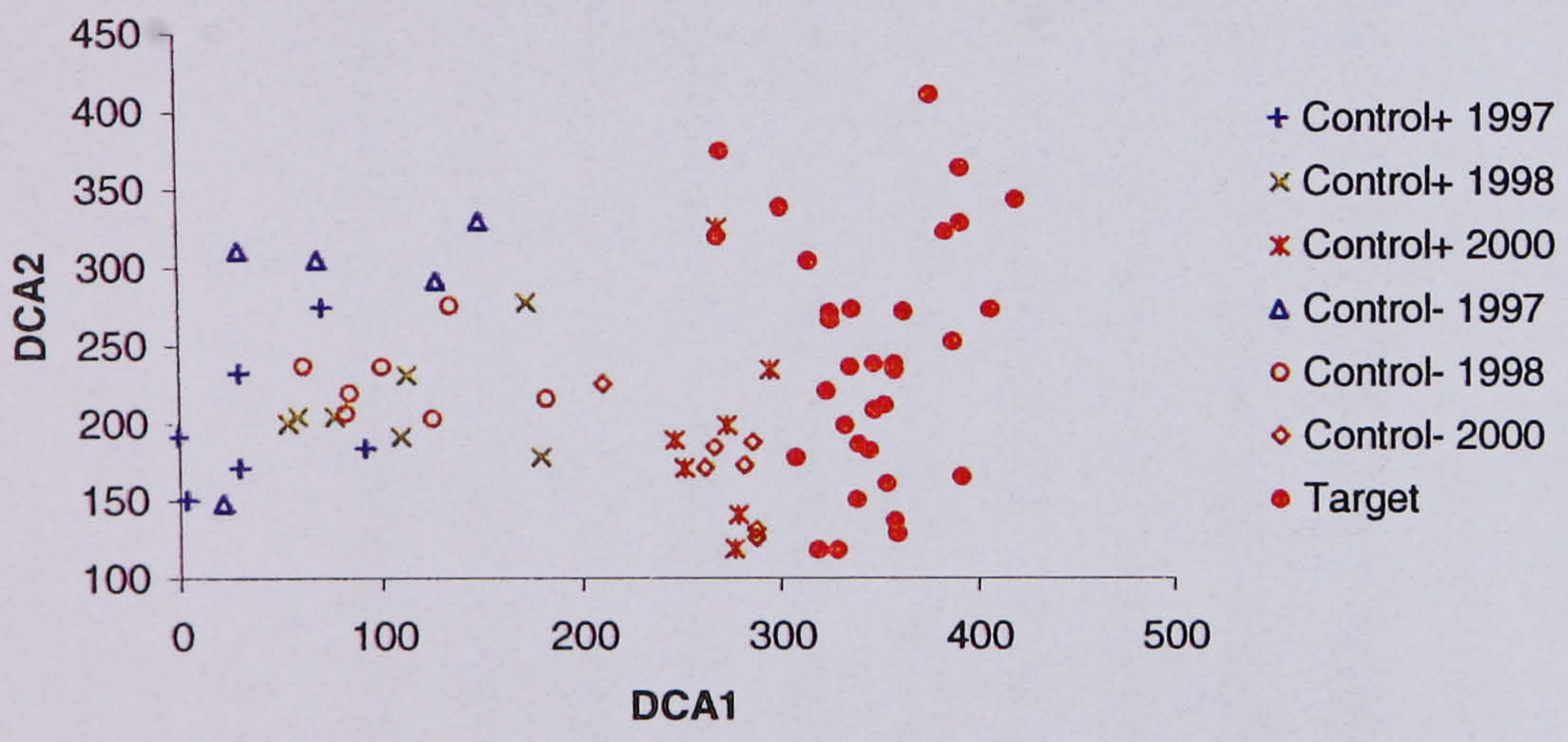


Figure 6.8 Ordination of the control

In all cases, + = with nurse grass and - = without nurse grass

1997

In 1997 the plots were sparsely vegetated, particularly under low sowing regimes. *Dactylis glomerata*, *Festuca ovina* and *Festuca rubra* germinated well and were present in each of the treatments. Both seed mixes sown at the high rate of $4g/m^2$ had between 40-60% cover of *Festuca rubra* and *Festuca ovina,* while *Dactylis glomerata* and *Poa pratensis* were also present on all of the plots. Both *Festuca rubra* and *Poa pratensis* appeared in the control plots (<1% total cover) and it is possible that these seeds drifted onto the control plots when the experiment was sown. There was recruitment of a number of unsown species with a high incidence of species associated with cultivated land, such as *Anagallis arvensis*, *Papaver somniferum*, *Polygonum aviculare, Rumex crispus* and *Sinapis arvensis.* Some species associated with calcicolous grassland also colonised the plots including *Asperula cynanchica* and *Carlina vulgaris.* The data are summarized in Table 6.4.

The ordination diagram shows that the treatment plots were clustered and clearly separated from the target data (Figure 6.9). Although there was some overlap in species composition between the treatments, in general, plots sown with a lower seed rate were positioned lower on axis 1 than the higher seed rates. Within each treatment there were differences in the position of the individual plots on axis 1 and 2, showing that the initial establishment of species was different between plots sown with the same treatment. The control plots were clearly separated from both the treatment and target vegetation and species composition varied considerably between control plots. Analysis by ANOVA showed that type of seed mix, seed rate and nurse grass application were all important factors in the composition of the developing vegetation as described by the first axis (Table 6.5). The application of a nurse crop significantly affected the distribution of samples on axis 2, but there was no effect of seed rate or type of seed mix. Although axes 2 and 3 were not plotted, ANOVA showed that seed rate was an important determinant of species composition on the third axis and that type of seed mix and application rate significantly influenced the distribution of samples along axis 4.

Table 6.4 Mean species cover (%) for each treatment in 1997 += with nursery grass, - = without nursery grass

Sown species	t1+	t1-	t2+	t2-	t3+	t3-	t4+	t4-	t5+	t5-	t6+	t6-	Control+	Control-
Achillea millefolium	*	*	*	*	0.43	0.86	*	*	*	*	1.14	1.29	*	*
Anthyllis vulneraria	0.57	0.28	0.43	1.57	4.57	6.43	*	*	0.57	1.43	8.29	5.57	*	*
Briza media	0.21	0.42	*	0.57	0.71	2.43	*	*	0.43	0.57	3.00	7.43	*	*
Centaurea nigra	*	*	*	*	0.14	0.43	*	*	0.29	0.29	*	*	*	*
Daucus carota	*	0.14	*	0.28	1.00	1.00	*	*	0.29	0.29	1.43	0.86	*	*
Galium verum	*	*	*	*	*	*	*	*	*	0.57	*	*	*	*
Leondonton hispidus	*	*	*	*	*	0.28	*	*	*	0.29	0.71	1.57	0.14	*
Leucanthemum vul	0.14	*	*	0.14	*	1.00	*	*	*	0.14	0.14	0.29	*	*
Lotus corniculatus	0.14	*	0.14	0.14	1.00	1.57	*	*	*	*	0.57	*	*	*
Medicago lupulina	*	*	0.14	0.14	*	*	*	*	*	0.29	0.86	1.57	*	*
Onobrychis viciifolia	0.14	*	0.43	0.28	1.85	2.71	*	*	0.86	0.71	2.43	2.29	*	*
Plantago lanceolata	*	*	*	1.86	8.28	8.57	*	*	0.86	1.14	4.86	3.29	*	*
Plantago media	*	*	*	0.28	0.14	0.14	*	*	*	*	*	*	*	*
Prunella vulgaris	*	*	*	*	0.14	0.14	*	*	*	*	*	*	*	*
Sanguisorba minor	*	*	*	0.28	1.57	2.86	*	*	0.57	0.43	2.71	3.43	*	*
Scabiosa columbaria	*	*	0.14	*	*	*	*	*	*	*	*	*	*	*
Agrostis stolonifera	*	*	0.14	*	0.14	0.14	*	*	*	0.43	*	*	0.71	1.29
Anthoxanthum odoratum	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Arrenatherum elatius	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cynosurus cristatus	*	*	*	*	0.29	*	*	*	*	*	*	*	*	*
Dactylis glomerata	1.14	3.71	6.28	16.00	16.14	30.14	1.14	8.00	3.71	12.00	25.00	27.86	*	*
Festuca ovina	0.14	1.71	1.14	3.14	4.57	4.57	0.43	2.29	1.00	3.71	5.57	2.43	*	*
Festuca rubra	3.28	4.86	9.28	13.85	45.86	47.20	3.00	10.00	10.86	13.14	49.00	59.14	0.86	0.71
Poa pratensis	0.57	3.71	2.71	1.14	3.43	3.57	0.57	0.86	0.43	1.00	2.71	2.71	0.29	0.14
Trisetum flavescens	*	*	*	*	*	*	*	*	*	*	0.14	*	*	*
Fragaria vesca	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Alliaria petiolata	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Echium vulgare	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Origanum vulgare	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Medicago sativa	*	*	*	*	*	0.14	*	*	*	*	*	*	*	*
Rhinanthus minor	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Campanula rotundifolia	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ononis repens	*	*	*	*	0.14	0.57	*	*	*	*	*	*	*	*
Helianthemum numm.	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Plant plugs											*	*	*	*
Hypochoeris radicata	0.14	0.28	0.57	0.28	*	0.29	*	*	*	*	*	*	*	*
Viola riviniana	0.14	*	0.28	0.14	1.14	0.29	*	*	*	*	*	*	*	*
Primula veris	0.14	0.28	*	*	0.86	1.14	*	*	*	*	*	*	*	*
Centaurea scabiosa	*	0.14	*	0.14	*	*	*	*	*	*	*	*	*	*
Thymus drucei(praecox)	0.21	0.14	0.14	0.14	*	1.29	*	*	*	*	*	*	*	*
Knautia arvensis	*	0.28	0.28	0.14	0.86	0.71	*	*	*	*	*	*	*	*
Unsown species														
Acer seedling	*	*	*	*	*	*	0.14	*	*	*	*	*	0.14	*
Anagallis arvensis	0.43	0.86	0.28	0.43	0.14	2.71	0.57	0.57	0.57	0.43	0.71	0.71	0.29	0.29
Asperula cynanchica	*	*	*	*	*	0.14	*	*	*	*	*	*	*	*
Carlina vulgaris	*	*	*	*	*	*	0.14	*	*	0.14	*	*	*	*
Catapodium rigidum	0.14	0.14	0.14	*	*	0.71	*	*	0.14	*	0.14	0.14	*	*
Cerastium fontanum	*	0.28	*	1.14	*	*	*	0.43	0.14	*	*	0.14	*	*
Cirsium sp.	0.71	*	*	0.14	*	*	0.14	0.14	*	*	0.14	*	*	*
Coronopus didymus	*	*	*	*	0.14	*	*	*	*	*	*	*	*	*
Epilobium sp	*	*	*	*	*	0.14	*	0.14	*	*	*	*	0.29	*
Galium sp (seedling)	*	*	*	*	0.29	*	*	*	*	*	*	*	*	*
Galium aparine	*	*	*	*	0.29	0.29	*	*	*	*	*	*	*	*
Geranium molle	*	*	*	*	1.14	1.57	*	*	*	0.14	0.14	*	*	*
Holcus lanatus	*	0.57	*	*	0.14	0.43	*	*	*	0.14	*	1.14	0.14	*
Inula sp.	*	*	*	*	*	0.29	*	*	*	*	0.14	*	*	*
Kickxsia spuria	0.14	0.28	0.14	0.14	0.29	*	*	*	*	*		*	*	*
Myosotis sp.	*	*	*	*	0.14	*	*	*	*	*		*	*	0.14
Atriplex patula	0.14	0.28	*	*	0.14	*	*	*	*	*	0.43	*	*	*
Papaver somniferum	0.14	0.57	0.28	*	0.14	0.57	0.28	0.14	*	0.29	0.29	0.43	0.14	0.29
Pastanica sativa											*	0.14	*	*
Picris echoides	0.43	0.57	0.28	0.14	6.43	1.43	0.43	0.29	*	*	1.43	0.47	*	0.14
Pimpinella saxifraga	*	*	0.14	0.28	*	*	0.14	*	0.14	*	*	0.14	0.14	*
Plantago major	0.28	0.28	*	*	*	0.29	0.29	0.29	*	*	0.14	*	0.29	0.29
Poa annua											*	0.29	*	*
Poa compressa											0.14	*	*	*
Polygonum aviculare	1.42	1.14	0.86	*	0.43	0.01	0.57	0.57	0.14	0.57	1.29	0.43	0.71	0.29
Ranunculus repens	0.28	0.43	0.14	0.57	0.43	0.71	1.86	0.57	0.43	1.00				
Rumex crispus	0.28	0.28	0.14	*	0.86	1.29	0.43	0.14	0.29	*	0.71	0.14	0.85	0.57
Sagina apetala	0.28	*	*	0.14	0.14	0.29	*	0.57	0.57	0.71	0.43	0.29	0.57	0.43
Sagina procumbens	0.07	0.14	0.57	1.00	0.14	*	0.43	*	*	0.29	0.14	0.14	0.14	0.29
Sinapis arvensis	4.86	5.28	2.42	4.28	11.43	6.86	3.57	4.43	0.71	1.43	1.14	0.71	2.14	0.71

Succisa pratensis	*	*	*	*	0.14	*	*	*	*	*	*	*	*	*
Trifolium repens	*	*	*	*	0.29	0.29	0.14	*	0.14	*	0.43	0.29	*	*
Trifolium campestre	*	*	*	*	*	0.14	*	*	*	*	*	*	*	*
Trifolium pratense	*	*	*	0.14	0.57	0.29	0.43	0.29	*	*	*	0.14	*	*
Trifolium sp (seedling)	*	*	*	*	*	0.14	0.14	*	0.43	0.28	0.43	0.29	0.14	*
Trip maritium	*	*	*	*	0.29	0.14	0.28	*	0.14	*	*	*	*	*
Veronica arvensis	*	*	*	*	*	0.28	*	*	*	*	0.43	0.29	*	*
Veronica hederifolia	*	*	*	0.43	*	*	*	0.14	*	*	*	*	*	*
Veronica persica	0.57	0.86	*	0.28	0.71	0.29	*	0.29	0.14	*	*	*	*	*
Senecio jacobaea	*	*	*	*	*	*	*	*	*	*	*	*	*	0.14
Total percentage cover	**17.13**	**27.91**	**27.49**	**49.62**	**117.97**	**137.77**	**15.12**	**30.15**	**23.85**	**41.85**	**117.26**	**126.05**	**7.98**	**5.72**
Sown species cover	6.96	15.95	22.10	40.51	93.26	118.47	5.14	21.15	19.87	36.43	108.56	119.73	2.00	2.14
Unsown species cover	10.17	11.96	5.39	9.11	24.71	19.30	9.98	9.00	3.98	5.42	8.70	6.32	5.98	3.58
Total species richness	**28.00**	**27.00**	**25.00**	**32.00**	**43.00**	**48.00**	**21.00**	**19.00**	**24.00**	**27.00**	**34.00**	**32.00**	**17.00**	**14.00**
Sown species richness	13.00	12.00	14.00	19.00	21.00	25.00	4.00	4.00	11.00	16.00	16.00	14.00	4.00	3.00
Unsown species richness	15.00	15.00	11.00	13.00	22.00	23.00	17.00	15.00	13.00	11.00	18.00	18.00	13.00	11.00

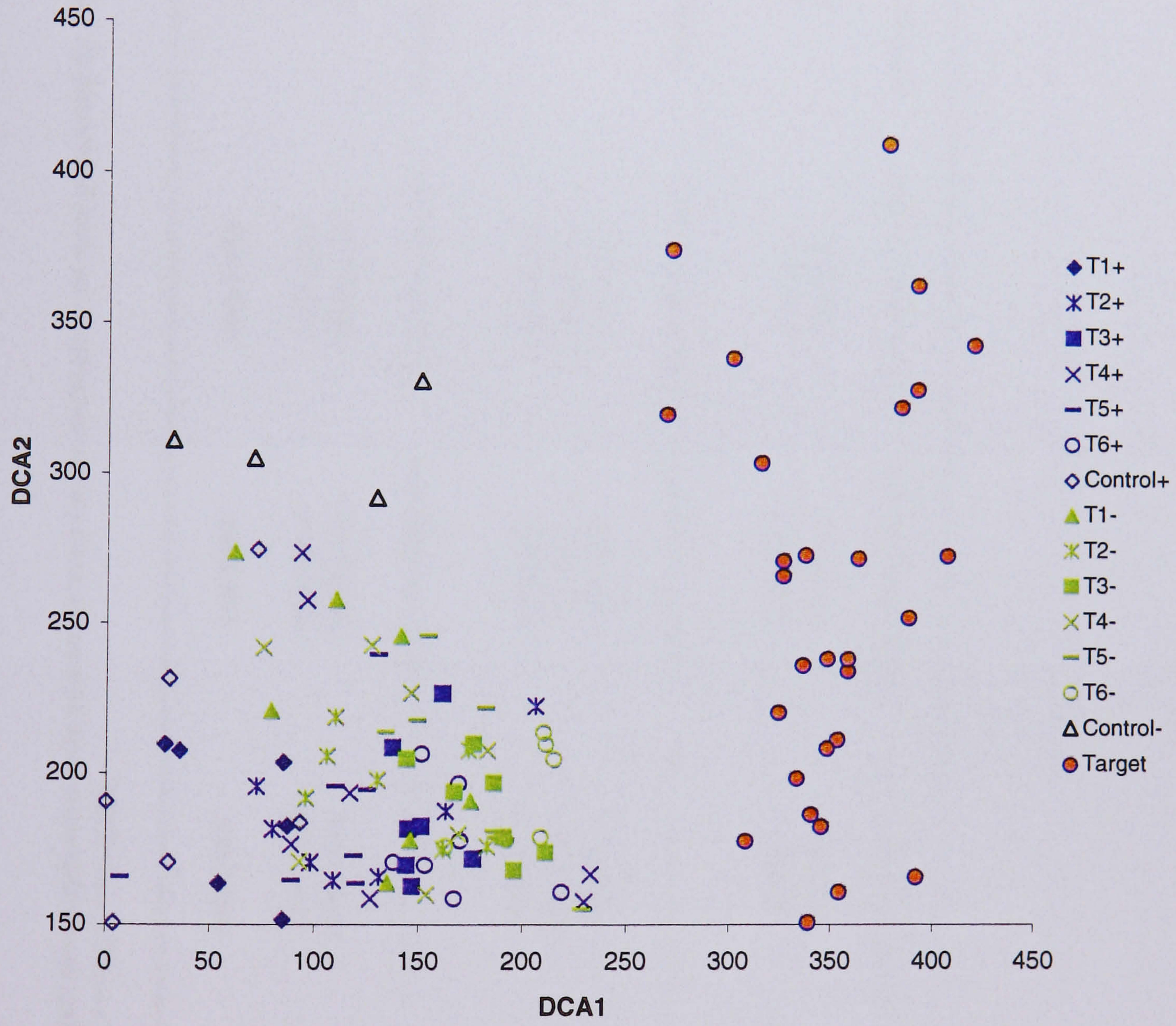


Figure 6.9 Ordination of treatments in 1997, the position of experimental plots on the first two axes generated by DECORANA compared with the target vegetation.

Table 6.5 Effect of seed mix, application rate and nurse crop on the ordination on axes generated by DECORANA in 1997 Significance: *=0.05, **=0.01, ***=0.001.

	Axis 1 ±SE	**Axis 2 ±SE**	**Axis 3 ±SE**	**Axis 4 ±SE**
Mix				
Preferred mix	130.86 ±5.96	192.81 ±4.41	81.52 ±2.03	142.64 ±6.68
Basic mix	151.83 ±5.96	192.36 ±4.41	79.81 ±2.03	184.43 ±6.68
Anova	**F=6.18, P=0.015*, df=1**	**F=0.01, P=0.942, df=1**	**F=0.35, P=0.553, df=1**	**F=19.56, P=<0.001***. df=1**
Rate				
$0.4g/m^2$	117.10 ±7.31	200.64 ±5.4	85.61 ±2.49	160.1 ±8.18
$1g/m^2$	133.61 ±7.31	192.1 ±5.4	79.14 ±2.49	159.8 ±8.18
$4g/m^2$	173.43 ±7.31	185.0 ±5.4	77.25 ±2.49	170.54 ±8.18
Anova	**F=15.74, P=<0.001***, df=2**	**F=2.10, P=0.129, df=2**	**F=3.09, P=0.051*, df=2**	**F=0.05, P=0.580, df=2**
Nurse grass				
With *L. multiflorum*	124.9 ±6.7	184.9 ±4.9	83.1 ±2.3	181.3 ±7.5
Without *L. multiflorum*	156.9 ±6.3	100.0 ±4.6	79.2.±2.2	148.7 ±7.1
Anova	**F=12.0, P=0.001***, df=1**	**F=4.93, P=0.030*, df=1**	**F=1.57, P=0.214, df=1**	**F=10.28, P=0.002**, df=1**

1998

Species richness increased in 1998 as more of the sown species germinated. Notably there was a reduction in the cover of *Festuca rubra (*Table 6.6). There was also an increase in the number of unsown species colonising the site, with a high occurrence of *Sinapis arvensis. Sinapis arvensis* is a weed species which occurs in pastures in low numbers but has years of great abundance and in 1998 this species was evident in many of the arable fields and pastures surrounding Swanworth Quarry.

The distribution of the treatment data on the first two axes of the ordination diagram were more tightly clustered in 1998 (Figure 6.10). In particular, the distance between plots sown with the same treatment had reduced, suggesting that within treatment the plots were more similar to each other than they had been in the previous year. The separation between treatments was clearer; plots sown with a low seed rate were positioned at the low end of axis 1. The control plots were also increasingly similar to each other but remained separated from the treatment data. There was no convergence of the treatment plots with the target data in 1998. ANOVA (Table 6.7) confirms the visual estimation of differences and showed that the position of plots on axis 1 was significantly influenced by seed rate and nurse crop application. Plots sown with low seed rates and an application of nurse grass were positioned lower on axis 1. Species composition as described by axis 2 was significantly influenced by type of seed mix, seed rate and the application of nurse grass. Data from plots sown with the preferred mix, sown at the highest seed rate with no application of nurse grass, were positioned higher on axis 2 than the other treatments. On the unplotted axes, seed rate significantly affected the third and fourth axes while type of seed mix and nurse grass application were significant effects on axis four. This suggests that although type of seed mix and nurse grass application is important in the initial community composition of treatment plots, seed rate emerged as the most consistently influential variable, being significant for the distribution of data on all four axes.

Table 6.6 Mean species cover (%) for each treatment in 1998 += with nursery grass, - = without nursery grass

Sown species	Mean T1+	Mean T1-	Mean T2+	Mean T2-	Mean T3+	Mean T3-	Mean T4+	Mean T4-	Mean T5+	Mean T5-	Mean T6+	Mean T6-	Control+	Control-
Achillea millefolium	0.29	*	0.43	0.29	2.00	1.86	*	0.14	0.29	0.43	1.29	2.57	*	*
Anthyllis vulneraria	0.43	0.14	0.29	0.43	1.86	2.29	0.57	1.14	0.71	1.29	8.57	4.43	*	*
Briza media	*	*	*	0.14	*	*	*	*	*	*	*	*	*	*
Centaurea nigra	*	*	*	*	0.14	0.14	*	*	0.29	0.14	*	*	*	*
Daucus carota	*	0.71	0.86	*	0.43	1.00	0.43	0.14	0.14	0.14	1.57	0.71	0.43	0.14
Galium verum	*	0.14	0.14	0.14	0.86	0.86	*	0.14	*	*	0.14	0.29	*	*
Leondonton hispidus	*	0.14	0.14	0.14	0.14	*	*	0.14	*	0.14	0.14	1.00	*	*
Leucanthemum vul	0.86	0.71	0.14	0.29	1.00	0.29	*	*	0.14	0.14	0.57	0.14	*	*
Lotus corniculatus	*	0.43	0.43	0.14	2.71	3.86	*	0.14	0.29	0.14	1.86	0.29	*	*
Medicago lupulina	*	0.14	*	*	0.43	0.14	0.14	2.00	0.29	0.43	4.57	3.14	0.14	*
Onobrychis viciifolia	*	0.14	*	*	*	0.14	*	*	0.14	*	0.43	0.57	*	*
Plantago lanceolata	1.00	1.29	1.71	3.86	11.43	7.00	1.00	*	3.00	1.71	8.00	8.29	*	*
Plantago media	0.86	0.14	0.29	0.43	0.71	1.14	0.29	0.57	0.43	*	0.14	0.57	*	*
Prunella vulgaris	0.43	0.14	*	*	0.29	0.57	0.14	*	0.43	0.29	0.14	0.14	0.14	*
Sanguisorba minor	*	0.14	0.43	0.57	1.86	2.29	0.14	1.00	0.71	1.00	3.14	1.00	*	*
Scabiosa columbaria	*	*	*	*	0.14	*	*	*	*	*	0.14	0.14	*	*
Agrostis stolonifera	*	*	0.14	0.14	1.57	0.71	0.57	*	*	0.29	0.14	0.43	*	*
Anthoxanthum odoratum	*	0.29	0.29	*	*	*	*	1.29	*	0.14	0.57	0.71	*	*
Arrenatherum elatius	*	*	*	*	*	*	*	*	*	0.29	*		*	*
Cynosurus cristatus	*	*	*	0.71	*	*	0.14	0.14	*	0.57	0.71	1.29	*	*
Dactylis glomerata	6.14	11.57	10.43	22.57	22.57	20.71	5.71	13.86	9.29	20.14	25.43	23.71	*	*
Festuca ovina	6.71	11.86	15.43	16.00	29.43	23.14	11.14	12.71	14.29	15.00	27.00	21.00	0.43	1.29
Festuca rubra	5.43	3.43	3.00	3.86	10.71	7.71	2.00	3.57	5.43	5.14	8.43	12.86	1.00	0.71
Poa pratensis	0.43	1.57	0.86	2.00	2.43	3.29	0.71	1.43	1.86	0.57	3.00	1.43	0.14	*
Trisetum flavescens	*	*	1.57	*	*	0.86	*	*	0.29	*	*	*	*	*
Fragaria vesca	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Alliaria petiolata	0.14	*	*	*	*	*	*	*	*	*	*	*	*	*
Echium vulgare	*	*	*	*	*	0.29	*	*	*	*	*	*	*	*
Origanum vulgare	*	*	*	*	*	0.29	*	*	*	*	*	*	*	*
Medicago sativa	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Rhinanthus minor	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Campanula rotundifolia	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ononis repens	*	*	*	*	0.29	1.00	*	*	*	*	*	*	*	*
Helianthemum numm.	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Plant plugs	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Hypochoeris radicata	0.14	0.43	0.71	*	*	0.43	*	*	*	*	*	*	*	*
Viola riviniana	0.14	0.14	0.29	0.14	1.00	0.29	*	*	*	*	*	*	*	*
Primula veris	0.14	0.14	0.14	*	0.71	0.86	*	*	*	*	*	*	*	*
Centaurea scabiosa	0.14	0.29	0.29	0.14	0.43	0.29	*	*	*	*	*	*	*	*
Thymus drucei(praecox)	0.14	*	*	0.29	0.14	0.71	*	*	*	*	*	*	*	*
Knautia arvensis	0.14	0.14	*	0.14	*	*	*	*	*	*	*	*	*	*
Unsown species	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Acer seedling	*	*	*	*	*	*	0.14	0.14	*	*	*	*	0.14	*
Algae	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Anagallis arvensis	0.57	0.43	0.29	0.29	0.43	0.14	0.14	0.71	*	0.43	0.14	*	0.14	0.86
Arenaria serp	3.43	0.29	*	*	*	*	*	*	*	*	*	*	*	*
Asperula cynanchica	*	*	*	*	0.43	*	0.71	0.43	0.57	1.57	*	*	*	0.29
Bellis perenis	0.29	*	*	*	0.29	*	0.14	*	0.71	*	0.14	*	*	*
Buddlia davidii	*	*	*	*	*	*	*	*	*	0.14	*	*	*	0.14
Carlina vulgaris	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Catapodium rigidum	0.14	0.57	0.43	*	0.43	*	0.71	0.29	*	*	*	*	0.86	0.29
Cerastium fontanum	*	0.43	*	0.43	*	*	0.29	*	0.14	*	*	*	0.14	0.43
Cirsium sp.	2.43	0.57	0.57	0.14	*	*	1.14	1.57	0.29	0.43	*	*	0.43	1.71
Coronopus didymus	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Crepis vesicaria	*	0.14	*	*	*	*	*	*	*	*	*	*	*	*
Elymus glaucus	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Epilobium sp.	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Festuca gigantica	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Galium sp (seedling)	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Galium aparine	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Geranium dissectum	0.29	*	0.14	0.14	*	0.57	0.14	0.14	0.43	0.14	0.14	*	0.57	0.14
Geranium molle	*	*	*	*	*	*	0.14	*	*	0.14	*	*	0.57	*
Holcus lanatus	0.14	0.14	*	*	0.29	0.86	*	0.43	*	0.29	*	1.00	0.57	*
Inula sp.	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Kickxia spuria	*	*	0.14	*	*	*	*	*	*	*	*	*	*	*
Leondonton autumnalis	*	*	*	*	*	*	0.43	0.14	0.71	0.71	1.71	2.00	*	0.14
Linum catharticum	*	*	*	*	*	*	0.14	*	0.71	0.14	1.14		0.14	0.14
Moss	*	*	*	*	0.29	*	*	*	*	*	*	*	*	*
Myosotis sp.	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Atriplex patula	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Papaver somniferum	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pastinaca sativa	*	*	*	*	*	0.14	*	0.14	*	*	0.43	*	0.29	0.14

Picris echoides	*	*	0.71	0.29	2.29	3.14	2.00	1.57	0.14	0.86	0.43	0.29	1.14	1.43
Pimpinella saxifraga	*	*	*	*	0.14	0.14	*	0.14	*	*	*	*	*	*
Plantago major	1.00	1.00	0.43	0.43	*	0.71	*	0.71	*	0.29	*	1.14	0.29	0.29
Poa annua	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Poa compressa	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Polygonum aviculare	2.43	3.57	1.29	1.00	0.86	0.29	0.14	0.14	0.29	0.29	0.14	0.71	1.57	0.71
Rumex acetosa	*	*	*	*	1.00	1.00	0.29	*	*	*	*	*	1.00	*
Ranunculus repens	3.29	2.43	0.71	6.71	0.14	0.14	*	*	1.14	1.14	0.14	1.57	0.86	0.86
Rumex crispus	0.14	1.14	*	0.14	1.43	0.71	0.43	0.14	0.14	0.29	0.29	0.14	0.29	0.29
Sagina apetala	*	*	0.14	*	*	*	2.14	*	0.29	*	*	0.43	0.57	0.14
Sagina procumbens	*	*	*	*	0.43	1.29	*	*	*	*	*	*	*	0.14
Senecio jacobaea	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sinapis arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Succisa pratensis	*	0.14	0.14	*	0.29	0.14	*	*	*	*	0.14	*	*	*
Taraxacum sp.	*	*	*	0.14	*	*	*	*	*	*	*	*	0.29	*
Trifolium repens	*	*	0.14	*	0.14	*	*	0.14	0.29	*	0.43	0.29	*	*
Trifolium campestre	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Trifolium fragaria	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Trifolium pratense	*	*	*	*	0.57	0.43	*	*	0.14	*	*	*	*	0.14
Trifolium sp (seedling)	*	*	*	*	*	*	0.29	0.14	0.14	*	0.57	*	0.14	*
Trip. maritium	17.29	15.29	6.57	3.71	9.43	8.86	8.57	9.57	2.71	3.71	2.29	2.71	2.14	6.43
Tussilago farfara	*	*	*	0.14	0.43	0.14	*	*	*	*	*	0.14	0.14	0.14
Veronica arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Veronica hederifolia	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Veronica officinalis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Veronica persica	*	*	*	*	*	0.14	*	*	*	*	*	*	*	0.14
Total species cover	**55.00**	**60.29**	**49.71**	**66.00**	**112.57**	**101.00**	**41.00**	**55.00**	**46.86**	**58.57**	**104.14**	**95.14**	**14.57**	**17.14**
Sown species cover	23.57	34.14	38.00	52.43	93.29	82.14	23.00	38.43	38.00	48.00	96.00	84.71	2.29	2.14
Unsown species cover	31.43	26.14	11.71	13.57	19.29	18.86	18.00	16.57	8.86	10.57	8.14	10.43	12.29	15.00
Total species richness	**29.00**	**35.00**	**34.00**	**32.00**	**42.00**	**44.00**	**31.00**	**32.00**	**33.00**	**34.00**	**35.00**	**32.00**	**27.00**	**24.00**
Sown species richness	17.00	22.00	21.00	20.00	24.00	27.00	13.00	15.00	17.00	19.00	21.00	21.00	6.00	3.00
Unsown species richness	12.00	13.00	13.00	12.00	18.00	17.00	18.00	17.00	16.00	15.00	14.00	11.00	21.00	21.00

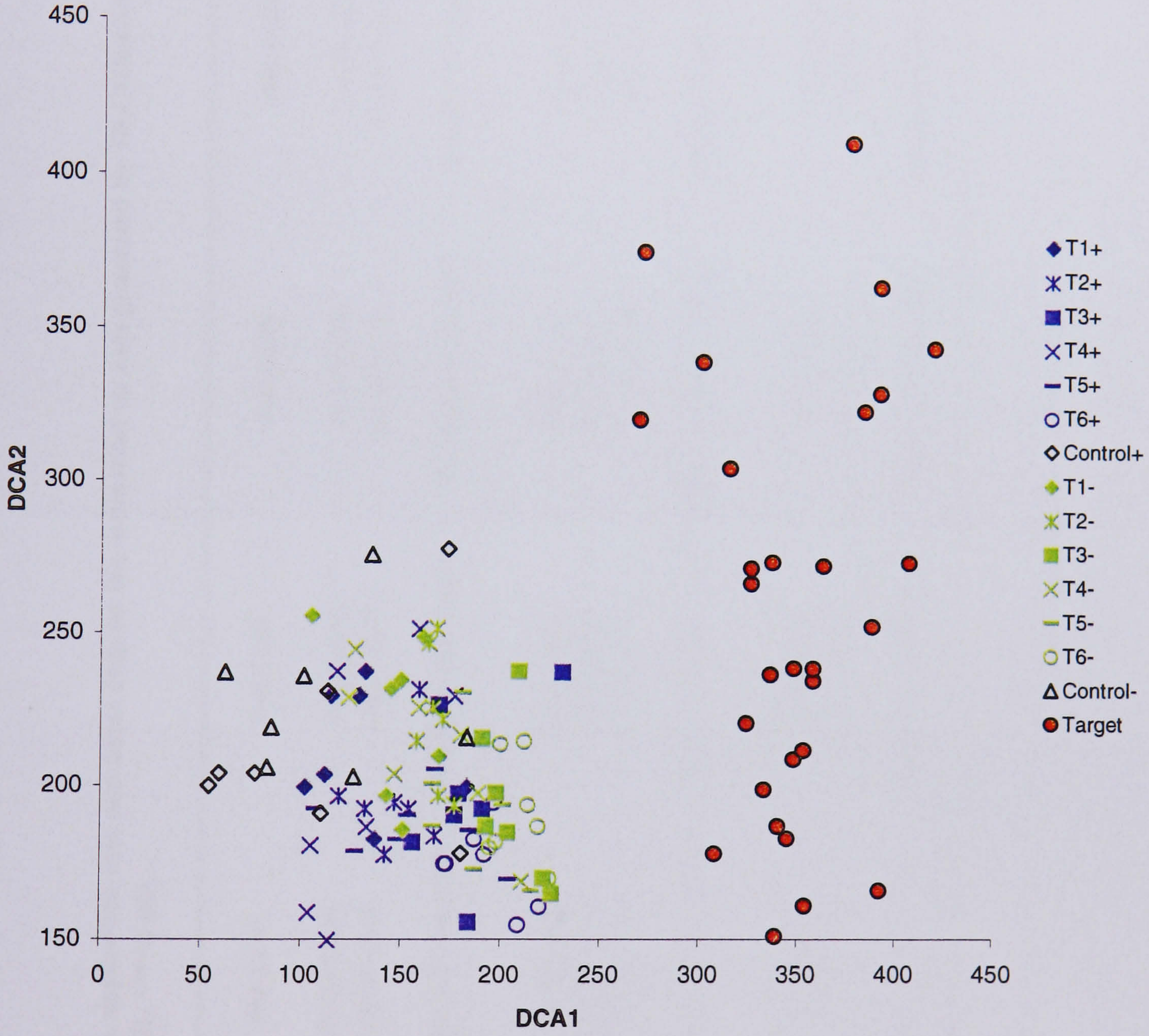


Figure 6.10 Ordination of treatments in 1998, the position of experimental plots on the first two axes generated by DECORANA compared with the target vegetation.

Table 6.7 Effect of seed mix, application rate and nurse crop on the ordination on axes generated by DECORANA in 1998
Significance: *=0.05, **=0.01, *=0.001.**

	Axis 1 ±SE	Axis 2 ±SE	Axis 3 ±SE	Axis 4 ±SE
Mix				
Preferred mix	173.24 ±3.96	194.02 ±3.75	95.74 ±2.09	114.19 ±2.96
Basic mix	165.74 ±3.92	206.02 ±3.79	91.23 ±2.11	105.18 ±2.96
Anova	**F=1.81, *P*=0.183, df=1**	**F=5.45, *P*=0.022*, df=1**	**F=2.28, *P*=0.135, df=1**	**F=4.66, *P*=0.034*, df=1**
Rate				
0.4g/m^2	142.17 ±4.85	189.57 ±4.65	89.96 ±2.59	118.64 ±3.63
1g/m^2	168.33 ±4.77	199.23 ±4.57	89.53 ±2.55	105.95 ±3.57
4g/m^2	197.96 ±4.85	211.96 ±4.65	100.96 ±2.59	104.64 ±3.63
Anova	**F=33.09, *P*=<0.001***, df=2**	**F=5.83, *P*=0.004**, df=2**	**F=6.27, *P*=0.003**, df=2**	**F=4.62, *P*=0.013**, df=2**
Nurse crop				
With *L. multiflorum*	158.84 ±3.92	193.39 ±3.75	94.82 ±2.09	116.28 ±2.93
Without *L. multiflorum*	180.14 ±3.96	207.11 ±3.79	92.14 ±2.11	103.09 ±2.966
Anova	**F=14.60, *P*=<0.001***, df=1**	**F=6.60, *P*=0.012**, df=1**	**F=0.81, *P*=0.371, df=1**	**F=9.98, *P*=0.002**, df=1**

2000

In 2000, species richness had increased, with only 6 of the 40 species introduced as propagules unrepresented in the sampled quadrats; *Fragaria vesca, Alliaria petiolata, Origanum vulgare, Rhinanthus minor, Campanula rotundifolia* and *Viola riviana* were unrecorded. It is possible that these species were present but were not recorded in the sampling procedure, certainly some individuals of *Viola riviniana* survived on the site. The most remarkable increase of a single species in this year was that of *Anthyllis vulneraria* which dominated many plots, especially those sown with the basic mix where some plots had 90% cover (Table 6.8). The *Anthyllis* grew very tall (up to 1m) although other species survived in the understory.

By 2000, the data had converged and visual estimation of differences between treatments was more difficult (Figure 6.11). Overall, the treatment plots were more similar to the target data and some plots, notably those sown with a higher seed rate but also one control plot, were very close to the target vegetation with lower scores on axis 1. The similarity of the control plot may be partly be attributed to seed drift from the treatment plots. By 2000, the effect of seed mix and the application of the nurse crop were not significant but seed rate remained a significant factor in the distribution of the experimental plots on the first three axes. Plots which received a higher application of seed were more similar to the target vegetation (Table 6.9).

Table 6.8 Mean species cover (%) for each treatment in 2000 += with nursery grass, - = without nursery grass

Sown species	t1+	t1-	t2+	t2-	t3+	t3-	Mean T4+	Mean T4-	Mean T5+	Mean T5-	Mean T6+	Mean T6-	Control+	Control -
Achillea millefolium	*	*	0.43	0.29	5.71	1.71	0.43	1.57	0.43	0.86	2.14	3.57	0.14	1.71
Anthyllis vulneraria	23.86	13.43	19.00	19.14	22.57	31.43	54.86	45.71	69.43	55.14	37.57	42.29	4.00	9.43
Briza media	0.29	0.43	0.57	1.00	2.14	1.00	0.43	0.86	*	0.86	0.86	1.57	*	*
Centaurea nigra	*	*	0.29	*	0.57	0.71	*	0.86	0.43	*	0.29	1.29	*	*
Daucus carota	0.57	0.14	0.71	0.14	2.43	0.86	0.29	*	0.14	0.86	0.29	0.86	*	*
Galium verum	*	0.43	0.14	*	1.43	0.86	0.43	0.29	0.29	0.43	*	0.29	*	*
Leondonton hispidus	0.57	*	0.43	*	*	0.28	0.29	0.29	0.14	*	0.14	0.14	0.14	0.14
Leucanthemum vul	*	0.71	*	0.86	1.43	1.14	*	0.14	0.14	*	0.57	0.86	*	*
Lotus corniculatus	8.43	3.29	11.14	9.71	32.00	21.43	3.57	1.86	2.86	5.14	20.14	17.71	2.14	1.14
Medicago lupulina	0.57	4.29	0.57	0.57	8.14	1.29	12.14	17.00	14.29	0.01	21.00	17.29	4.71	3.00
Onobrychis viciifolia	*	*	*	0.43	0.14	0.14	*	*	*	17.00	0.14	*	*	*
Plantago lanceolata	2.14	4.00	3.29	2.43	4.14	3.86	2.14	3.00	1.86	2.14	3.86	2.43	0.14	0.14
Plantago media	0.14	0.14	0.29	0.14	0.29	0.28	*	*	0.14	*	0.43	*	*	*
Prunella vulgaris	1.14	0.14	0.57	1.00	1.14	1.43	0.71	*	0.29	0.29	0.29	1.29	*	*
Sanguisorba minor	0.14	*	0.29	1.14	0.28	0.86	0.29	0.14	1.57	0.57	0.14	3.00	*	*
Scabiosa columbaria	*	*	*	*	*	0.14	*	0.29	*	*	*	*	*	*
Agrostis stolonifera	3.00	4.86	6.14	3.86	8.43	8.86	1.43	2.71	0.29	2.29	2.86	2.86	0.71	*
Anthoxanthum odoratum	0.71	0.86	0.71	1.71	3.57	3.14	2.29	2.71	2.00	2.71	6.71	6.00	*	*
Arrenatherum elatius	0.57	0.57	0.14	0.14	0.71	1.43	1.14	0.14	1.14	*	1.00	0.14	*	*
Cynosurus cristatus	0.14	0.57	1.00	1.43	3.71	2.86	0.43	0.86	0.71	1.86	3.29	5.57	*	*
Dactylis glomerata	2.86	5.57	5.14	5.29	8.71	7.29	3.71	9.57	0.72	3.57	1.71	3.71	0.57	*
Festuca ovina	0.57	1.00	3.29	4.29	1.29	0.71	1.86	0.86	3.29	1.29	1.57	3.71	*	*
Festuca rubra	19.71	7.14	9.29	25.71	23.00	40.00	18.57	16.48	12.43	14.60	39.29	35.43	4.86	4.00
Poa pratensis	1.29	2.14	1.43	0.86	2.14	1.86	1.29	1.00	1.43	1.29	1.00	1.86	0.14	1.72
Trisetum flavescens	1.43	2.43	1.43	3.00	2.57	6.14	0.86	0.14	1.00	0.43	2.29	1.00	*	*
Fragaria vesca	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Alliaria petiolata	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Echium vulgare	*	*	0.14	0.14	*	*	*	*	*	*	*	*	*	*
Origanum vulgare	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Medicago sativa	*	0.86	*	*	*	*	*	*	*	*	*	*	*	*
Rhinanthus minor	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Campanula rotundifolia	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ononis repens	*	0.86	4.86	1.71	9.71	22.29	*	*	*	*	*	*	*	*
Helianthemum numm.	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Plant plugs	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Hypochoeris radicata	0.14	0.43	0.29	0.29	*	*	*	*	*	*	*	*	*	*
Viola riviniana	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Primula veris	*	0.43	0.29	*	0.28.	0.14	*	*	*	*	*	*	*	*
Centaurea scabiosa	*	0.14	0.14	*	0.14	0.43	*	*	*	*	*	*	*	*
Thymus drucei(praecox)	0.29	*	*	0.14	*	*	*	*	*	*	*	*	*	*
Knautia arvensis	*	*	0.29	*	0.28	0.57	*	*	*	*	*	*	*	*
Unsown species	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Acer seedling	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Algae	*	*	*	4.14	*	*	*	*	*	*	*	*	*	*
Anagallis arvensis	*	*	*	*	*	*	0.14	*	*	*	*	*	0.14	*
Asperula cynanchica	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Buddleja davidii	*	*	*	*	*	*	*	0.14	*	0.14	*	*	*	*
Carlina vulgaris	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Catapodium rigidum	3.14	2.86	1.86	1.00	0.14	1.43	1.43	0.71	1.71	0.72	0.29	*	8.86	4.71
Cerastium fontanum	0.14	0.86	0.14	0.14	*	*	0.29	0.71	*	*	0.14	*	*	*
Cirsium sp	0.14	*	0.14	*	*	*	2.29	*	*	*	0.14	*	0.29	1.00
Coronopus didymus	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Elymus glaucus	0.86	*	0.14	*	0.28	*	0.14	*	0.43	0.14	0.71	0.57	*	*
Epilobium sp.	0.57	0.14	0.43	*	*	*	1.14	1.00	*	*	0.14	*	1.57	1.43
Festuca gigantea	0.29	*	*	*	0.14	*	*	*	0.29	*	*	*	*	*
Galium sp (seedling)	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Galium aparine	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Geranium dissectum	0.71	0.14	*	*	*	*	0.29	0.57	*	0.14	*	0.29	0.86	0.71
Geranium molle	*	*	*	*	*	*	*	*	*	*	*	*	*	0.14
Holcus lanatus	0.86	0.14	*	*	0.43	2.00	0.86	0.57	*	1.14	0.43	1.00	1.43	*
Inula sp.	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Kickxia spuria	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Leondonton autumnalis	9.71	0.29	0.57	0.14	*	*	0.43	0.43	0.14	0.29	1.00	0.71	3.43	2.29
Linum catharticum	2.86	1.86	0.29	1.14	*	*	0.43	0.71	1.14	0.71	*	*	8.00	11.00
Moss	1.71	3.57	*	1.71	*	*	*	*	0.71	0.57	*	*	2.14	1.29
Myosotis sp.	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Atriplex patula	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Papaver somniferum	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pastanica sativa	0.29	0.14	*	0.29	*	*	0.43	*	*	*	0.14	0.14	0.57	0.43
Picris echoides	2.00	4.00	1.43	1.29	1.14	1.57	2.14	3.29	0.29	0.57	0.57	0.43	4.71	2.57
Pimpinella saxifraga	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Plantago major	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Poa annua	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Poa compressa	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Polygonum aviculare	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ranunculus repens	0.71	0.14	*	*	*	*	2.43	0.57	*	*	0.43	0.29	*	*
Rumex crispus	0.29	0.43	0.29	*	*	*	*	0.14	*	0.86	*	*	*	*
Sagina apetala	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sagina procumbens	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Senecio jacobaea	1.00	0.57	*	*	*	*	*	0.43	0.14	0.14	*	*	0.43	0.57
Sinapis arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Succisa pratensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Taraxacum sp.	0.43	1.57	0.57	0.43	*	*	0.29	*	0.14	0.29	*	*	1.14	0.86
Trifoilum repens	12.57	2.57	0.86	3.71	1.71	7.14	6.57	3.14	1.00	0.71	8.14	3.29	*	*
Trifolium campestre	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Trifolium fragaria	0.71	0.29	*	*	*	*	*	*	*	*	0.14	*	0.14	*
Trifolium pratense	*	*	1.71	*	0.14	5.71	*	*	*	*	*	*	*	*
Trifolium sp (seedling)	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Trip. maritium	0.29	0.86	0.71	*	*	*	0.57	0.14	*	0.14	*	*	0.57	0.57
Tussilago farfara	*	*	*	*	*	*	0.14	0.29	*	0.14	*	0.57	*	1.71
Veronica arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Veronica hederifolia	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Veronica officinalis	*	0.57	*	*	*	*	*	*	*	*	*	*	*	0.29
Veronica persica	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Total percentage cover	**107.84**	**75.86**	**81.44**	**99.41**	**150.65**	**180.99**	**127.14**	**119.34**	**121.00**	**118.04**	**159.86**	**160.15**	**51.86**	**50.86**
Sown species cover	68.56	54.86	72.30	85.42	146.67	163.14	107.14	106.48	115.00	111.32	147.57	152.86	17.57	21.29
Unsown species cover	39.28	21.00	9.14	13.99	3.98	17.85	20.00	12.86	6.00	6.72	12.29	7.29	34.29	29.57
Total species richness	**41.00**	**42.00**	**41.00**	**35.00**	**33.00**	**34.00**	**37.00**	**36.00**	**32.00**	**34.00**	**35.00**	**31.00**	**25.00**	**23.00**
Sown species richness	21.00	24.00	28.00	25.00	26.00	29.00	20.00	21.00	22.00	19.00	23.00	22.00	10.00	8.00
Unsown species richness	20.00	18.00	13.00	10.00	7.00	5.00	17.00	15.00	10.00	15.00	12.00	9.00	15.00	15.00

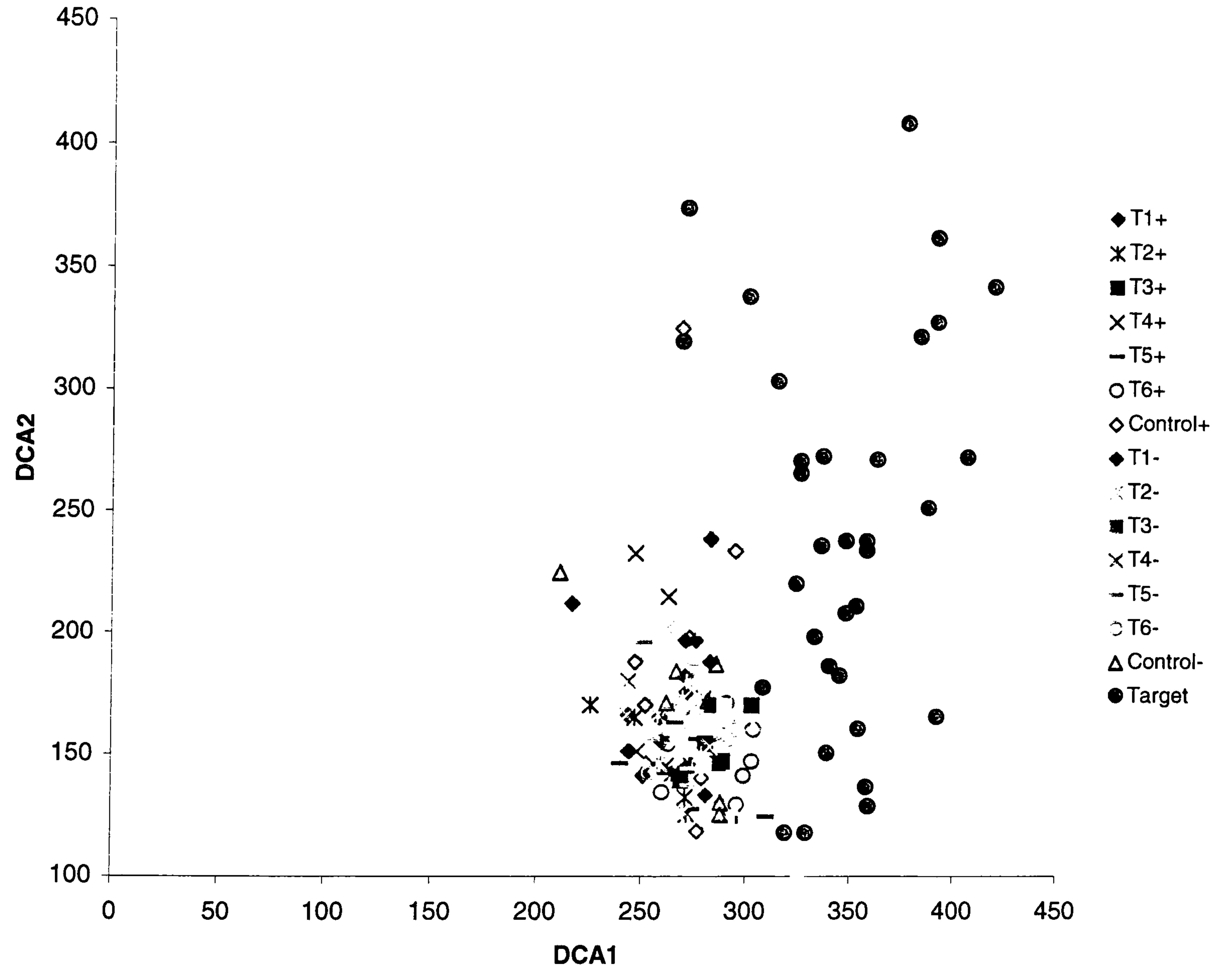


Figure 6.11 Ordination of treatments in 2000, the position of experimental plots on the first two axes generated by DECORANA compared with the target vegetation.

Table 6.9 Effect of seed mix, application rate and nurse crop on the ordination on axes generated by DECORANA in 2000 Significance: *=0.05, **=0.01, ***=0.001.

	Axis 1 ±SE	Axis 2 ±SE	Axis 3 ±SE	Axis 4 ±SE
Mix				
Preferred mix	271.56 ±3.39	161.03 ±4.71	098.40 ±3.42	126.09 ±3.38
Basic mix	272.70 ±2.28	160.57 ±3.18	956.86 ±2.31	131.66 ±2.77
Anova	**F=0.07, *P*=0.793, df=1**	**F=0.01, *P*=0.939, df=1**	**F=0.34, *P*=0.564, df=1**	**F=1.66, *P*=0.201, df=1**
Rate				
0.4g/m^2	262.71 ±3.06	175.89 ±4.26	108.42 ±3.09	134.35 ±3.05
1g/m^2	268.21 ±3.06	152.07 ±4.26	100.03 ±3.09	126.85 ±3.05
4g/m^2	285.46 ±3.75	154.44 ±5.22	082.94 ±3.78	125.43 ±3.74
Anova	**F=11.47, *P*=<0.001***, df=2**	**F=9.06, *P*=<0.001***, df=2**	**F=13.68, *P*=<0.001***, df=2**	**F=2.23, *P*=0.115, df=2**
Nurse crop				
With *L. multiflorum*	272.36 ±2.60	157.60 ±3.62	098.56 ±2.62	127.64 ±2.59
Without *L. multiflorum*	271.90 ±2.60	164.01 ±3.62	095.71 ±2.62	130.12 ±2.59
Anova	**F=0.02, *P*=0.899, df=1**	**F=0.1.69, *P*=197, df=1**	**F=0.64, *P*=0.426, df=1**	**F=0.49, *P*=0.485, df=1**

6.3.2 Ecological measures as determinants of success

Species diversity

The effect of the treatments on plant species diversity on the plots was investigated using ANOVA and the Shannon- Weiner diversity index. The results are presented separately for 1997, 1998 and 2000.

1997

Sown species richness was higher on plots sown with the preferred seed mix (F=4.2, *P*=0.044, df=1), at a higher rate (F=73.81, *P*=<0.001, df=2) and no nurse grass (F=6.95, *P*=0.010, df=1). Unsown species richness was unaffected by treatment (Table 6.10)

Table 6.10 Effect of seed mix, application rate and nurse crop on the number of sown and unsown species recorded in each treatment in 1997. Significance: *=0.05, **=0.01, *=0.001.**

	Sown species richness ± SE	**Unsown species richness ±SE**
Mix		
Preferred mix	6.3 ±0.362	3.9 ±0.452
Basic mix	7.5 ±0.338	4.2 ±0.422
Anova	**F=4.20, *P*=0.044*, df=1**	**F=0.80, *P*=0.373, df=1**
Rate		
0.4g/m^2	3.4 ±0.43	3.9 ±0.55
1g/m^2	4.5 ±0.43	2.6 ±0.55
4g/m^2	10.3 ±0.43	5.4 ±0.55
Anova	**F=73.81, *P*=<0.001***, df=2**	**F=2.59, *P*=0.082, df=2**
Nurse		
With *L. multiflorum*	5.6 ±0.3	3.5 ±0.5
Without *L. multiflorum*	6.6 ±0.3	4.3 ±0.5
Anova	**F=6.95, *P*=0.010**, df=1**	**F=0.33, *P*=0.570, df=1**

The Shannon-Weiner diversity index indicated that there was a difference in the diversity of the vegetation on the experimental plots according to the treatment applied (Table 6.11). Species richness was much greater in the preferred mix sown at 4g/m^2, and these plots also had relatively high H and J scores reflecting the diversity and evenness in the distribution of plant cover between those species. However, high species richness did not predict evenness in all cases; on other plots (for example, the basic mix applied at 4g/m^2) high species richness was associated with uneven distribution of cover between species whilst the control plots with low species richness scored highly for evenness.

Table 6.11 Shannon-Weiner diversity scores in 1997. H= Shannon-Weiner index score, S= species richness, J = evenness

	Preferred mix						Basic mix							
	0.4g +N	*0.4g -N*	*1.0g +N*	*1.0g - N*	*4g +N*	*4g -N*	*0.4g +N*	*0.4g -N*	*1.0g +N*	*1.0g -N*	*4g +N*	*4g -N*	*Control+*	*Control -*
H	1.95	2.58	2.18	2.19	2.32	2.42	2.48	1.96	2.14	2.21	2.1	1.91	2.43	2.4
S	28	27	25	32	43	48	21	19	24	27	34	32	17	14
J	0.57	0.78	0.67	0.63	0.61	0.62	0.81	0.66	0.67	0.67	0.59	0.55	0.86	0.91

1998

The data recorded in this year indicated that the type of seed mix that was sown on the plots did not influence sown or unsown species richness. The application of a nurse crop significantly suppressed the number of sown species that established ($F=9.98$, $P=0.002$, df=1) (table 6.12). Seed rate was also influential; increased seed rate encouraged a higher number of sown species ($F= 4.62$, $P=0.013$, df=2) and reduced colonization by unsown species ($F=4.52$, $P=0.014$, df=2).

Table 6.12 Effect of seed mix, application rate and nurse crop on the number of sown and unsown species recorded in each treatment in 1998. Significance: *=0.05, **=0.01, *=0.001.**

	Sown species richness ± SE	Unsown species richness ±SE
Mix		
Preferred mix	8.5 ±0.38	4.97 ±0.43
Basic mix	7.9 ±0.38	5.13 ±0.43
Anova	**F=1.34, *P*=0.251, df=1**	**F=0.06, *P*=0.799, df=1**
Rate		
0.4g/m^2	6.179 ±0.47	6.250 ±0.53
1g/m^2	7.558 ±0.46	3.987 ±0.53
4g/m^2	11.036 ±0.47	4.929 ±0.53
Anova	**F=4.62, *P*=0.013**, df=2**	**F=4.52, *P*=0.014**, df=2**
Nurse		
With *L. multiflorum*	8.110 ±0.38	5.158 ±0.43
Without *L. multiflorum*	8.405 ±0.38	4.952 ±0.43
Anova	**F=9.98, *P*=0.002**, df=1**	**F=0.11, *P*=0.741, df=1**

Diversity, as defined by Shannon-Weiner index (Table 6.13), showed that the plots which were sown had a more uneven distribution of cover between species than the control plots, although the preferred mix applied at 4g/m^2 was relatively even. In general plots with less species were more even.

Table 6.13 Shannon – Weiner diversity scores in 1998. H= Shannon-Weiner index score, S= species richness, J = evenness

	Preferred mix						**Basic mix**							
	0.4g +N	*0.4g - N*	*1.0g +N*	*1.0g - N*	*4g +N*	*4g - N*	*0.4g +N*	*0.4g - N*	*1.0g +N*	*1.0g - N*	*4g +N*	*4g -N*	*Control+*	*Control -*
H	2.38	2.38	2.37	2.12	2.52	2.69	2.35	2.32	2.41	2.21	2.37	2.33	2.96	2.37
S	29	35	34	32	42	44	30	32	33	34	35	31	27	24
J	0.71	0.67	0.67	0.61	0.67	0.71	0.69	0.67	0.69	0.62	0.66	0.67	0.89	0.74

2000

The effect of the nurse grass application was no longer detectable in 2000 but the preferred mix encouraged a higher number of both sown (F= 5.25, *P*=0.025, df=1) and unsown species (F=3.95, *P*=0.050, df=1). Sowing seed at a higher rate successfully established a higher number of sown species (F= 27.06, *P*=<0.001, df=2) but reduced the colonization of unsown species (F= 21.21, *P*=<0.001, df=2), (Table 6.14).

Table 6.14 Effect of seed mix, application rate and nurse crop on the number of sown and unsown species recorded in each treatment in 2000. Significance: *=0.05, **=0.01, *=0.001.**

	Sown species richness ±SE	**Unsown species richness ±SE**
Mix		
Preferred mix	12.2 ±0.49	5.04 ±0.42
Basic mix	10.7 ±0.34	3.96 ±0.28
Anova	**F=5.25, *P*=0.025*, df=1**	**F=3.95, *P*=0.050*, df=1**
Rate		
0.4g/m^2	9.1 ±0.45	6.6 ±0.38
1g/m^2	10.8 ±0.45	3.6 ±0.38
4g/m^2	14.4 ±0.55	3.3 ±0.46
Anova	**F=27.06, *P*=<0.001***, df=2**	**F=21.21, *P*=<0.001***, df=2**
Nurse		
With *L. multiflorum*	11.3 ±0.38	4.6 ±0.32
Without *L. multiflorum*	11.6 ±0.38	4.3 ±0.32
Anova	**F=0.0.35, *P*=0.555, df=1**	**F=0.35, *P*=0. 553, df=1**

The evenness of plots sown with the preferred mix had increased in 2000. In comparison, the plots sown with the basic mix were less even and this is partly attributed to the predominance of *Anthyllis vulneraria* on the 'basic' plots. The control plots had increased in species richness although there were fewer species present on the control plots compared with the plots which had been sown. The vegetation on the control was the most even and this was reflected in the high diversity scores of H and J. (Table 6.15)

Table 6.15 Shannon-Weiner diversity scores in 2000. H= Shannon-Weiner index score, S= species richness, J = evenness

	Preferred mix						Basic mix							
	0.4g +N	*0.4g -N*	*1.0g +N*	*1.0g -N*	*4g +N*	*4g -N*	*0.4g +N*	*0.4g -N*	*1.0g +N*	*1.0g -N*	*4g +N*	*4g -N*	*Control+*	*Control -*
H	2.67	3.02	2.75	2.6	2.62	2.58	2.34	2.25	1.74	2.21	2.28	2.38	2.61	2.57
S	41	42	41	35	33	34	37	36	32	34	35	31	25	23
J	0.72	0.82	0.74	0.73	0.75	0.73	0.62	0.63	0.5	0.63	0.64	0.69	0.81	0.82

Functional type

The species were categorized into four functional types; woody species, legumes, forbs and grasses. The abundance of individuals occurring within each functional type was compared to establish whether type of seed mix, application of nurse grass or seed rate affected the proportion of plants in each category. In 1997, there was a significant effect of the type of seed mix (χ^2=80.65, *P*=<0.001, df=1) where the plots sown with the basic mix had a larger proportion of forbs and fewer leguminous species than the preferred mix (6.16). In all other years the number of individuals in the four functional groups was unaffected by treatment.

Table 6.16 The abundance of individuls in each functional group recorded on the experimental plots.

	1997				1998				2000			
	Woody	Legumes	Forbs	Grasses	Woody	Legumes	Forbs	Grasses	Woody	Legumes	Forbs	Grasses
Mix												
Preferred		97	74	19		18	153	44		27	123	72
Basic		12	107	37	3	20	130	44	2	20	112	69
	χ^2=80.65, *P*=<0.001***, df=1				χ^2=0.10, *P*=0.241, df=1				χ^2=2.77, *P*= 0.427, df=1			
Nurse grass												
With		44	96	30	2	17	144	41	2	22	123	72
Without		65	85	26	1	21	139	47		25	112	69
	χ^2=5.22, *P*=0.156, df=2				χ^2=1.21, *P*=0.749, df=2				χ^2=2.37, *P*=0.498, df=2			
Seed rate												
0.4g		4	69	21	1	10	85	30		14	90	49
1.0g		32	53	19	1	11	92	29	1	16	77	44
4.0g		73	59	16	1	17	106	29	1	17	68	48
	χ^2=63.55, *P*=<0.001***, df=1				χ^2=1.78, *P*=0.938, df=1				χ^2=3.25, *P*= 0.777, df=1			

Table 6.17 shows that although forbs are well represented in terms of species richness on all the plots, legumes and grasses, which are less species rich, dominate the plots in terms of cover. The Kruskal-Wallis test showed that by the final year there was a significant difference in the proportion of cover by each functional group in all treatments except the preferred mix sown at 0.4g/m^2 with a nurse grass application.

Table 6.17 The species richness of functional types within treatments and the abundance of individuals recorded within functional type.
* = significant at 0.05 ** = significant at 0.01 *** = significant at 0.001

Number of species (Mean Rank of % plant cover)

1997

Treatment	Pref. mix at 0.4g +N	Pref. mix at 0.4g -N	Pref. mix at 1.0g +N	Pref. mix at 1.0g -N	Pref. mix at 4.0g +N	Pref. mix 4.0g -N	Basic mix at 0.4g +N	Basic mix at 0.4g -N	Basic mix at 1.0g +N	Basic mix at 1.0g -N	Basic mix at 4.0g +N	Basic mix 4.0g -N	Control +N	Control -N
Woody species	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (4.0)
Legumes	3 (12.0)	1 (10.32)	4 (11.0)	4 (14.0)	4 (27.63)	5 (30.10)	-	1 (3.50)	2 (18.50)	3 (16.83)	4 (23.50)	3 (25.17)	-	-
Forbs	19 (14.13)	19 (12.32)	15 (11.80)	23 (14.43)	31 (19.97)	35 (21.49)	16 (10.09)	15 (8.13)	16 (10.31)	17 (11.62)	22 (15.07)	21 (13.05)	12 (8.83)	11 (6.91)
Grasses	6 (16.92)	7 (19.14)	6 (17.33)	5 (28.0)	8 (27.06)	8 (34.19)	4 (16.50)	4 (16.50)	6 (16.33)	7 (18.57)	8 (21.19)	8 (22.31)	4 (10.75)	3 (9.67)
Kruskal Wallis test	χ^2 = 0.89, *P*=0.64, df=2	χ^2 = 4.24, *P*=0.12, df=2	χ^2 = 2.99, *P*=0.22, df=2	χ^2 = 0.9.51, *P*=0.<0.01**, df=2	χ^2 = 3.03, *P*=0.22, df=2	χ^2 = 6.34, *P*=0.04*, df=2	χ^2 = 5.15, *P*=0.08*, df=2	χ^2 = 8.13, *P*=<0.001***, df=2	χ^2 = 4.91, *P*=0.09*, df=2	χ^2 = 4.31, *P*=0.12, df=2	χ^2 = 3.95, *P*=0.14, df=2	χ^2 = 8.62, *P*=0.01**, df=2	χ^2 = 1.61, *P*=0.45, df=2	χ^2 = 1.10, *P*=0.29, df=2

1998

Treatment	Pref. mix at 0.4g +N	Pref. mix at 0.4g -N	Pref. mix at 1.0g +N	Pref. mix at 1.0g -N	Pref. mix at 4.0g +N	Pref. mix 4.0g -N	Basic mix at 0.4g +N	Basic mix at 0.4g -N	Basic mix at 1.0g +N	Basic mix at 1.0g -N	Basic mix at 4.0g +N	Basic mix 4.0g -N	Control +N	Control -N
Woody species	-	-	-	-	-	-	-	1 (6.0)	1 (8.0)	-	1 (5.50)	-	-	1 (5.0)
Legumes	1 (15.0)	4 (10.50)	2 (16.25)	2 (13.75)	4 (23.50)	5 (23.10)	2 (12.75)	3 (19.67)	4 (13.63)	3 (17.67)	4 (26.13)	4 (18.75)	1 (5.0)	-
Forbs	21 (13.71)	23 (17.35)	24 (15.46)	23 (15.0)	30 (18.55)	32 (19.78)	21 (14.69)	20 (13.93)	24 (15.40)	21 (16.43)	24 (14.58)	20 (13.68)	20 (14.23)	20 (12.10)
Grasses	7 (18.86)	8 (23.63)	8 (23.94)	7 (22.21)	7 (30.07)	7 (34.50)	7 (22.29)	8 (22.81)	5 (27.40)	9 (21.28)	7 (25.07)	8 (22.44)	5 (16.70)	3 (17.33)
Kruskal Wallis test	χ^2 = 2.00, *P*=0.37, df=2	χ^2 = 4.97, *P*=0.08*, df=2	χ^2 = 4.54, *P*=0.10 df=2	χ^2 = 3.62, *P*=0.16, df=2	χ^2 = 5.52, *P*=0.06*, df=2	χ^2 = 7.68, *P*=0.02*, df=2	χ^2 = 5.51, *P*=0.14, df=2	χ^2 = 7.02, *P*=0.07*, df=2	χ^2 = 7.21, *P*=0.03*, df=2	χ^2 = 3.10, *P*=0.38, df=2	χ^2 = 8.89, *P*=0.01**, df=2	χ^2 = 5.29, *P*=0.07*, df=2	χ^2 = 3.31, *P*=0.35, df=2	χ^2 = 2.57, *P*=0.28, df=2

2000

Treatment	Pref. mix at 0.4g +N	Pref.mix at 0.4g -N	Pref. mix at 1.0g +N	Pref. mix at 1.0g -N	Pref. mix at 4.0g +N	Pref. mix 4.0g -N	Basic mix at 0.4g +N	Basic mix at 0.4g -N	Basic mix at 1.0g +N	Basic mix at 1.0g -N	Basic mix at 4.0g +N	Basic mix 4.0g -N	Control +N	Control -N
Woody species									1 (4.0)		1 (4.40)			
Legumes	3 (30.83)	5 (32.0)	4 (34.50)	5 (22.20)	5 (24.80)	5 (22.60)	3 (34.67)	3 (32.67)	3 (29.33)	4 (24.0)	4 (25.88)	3 (29.33)	3 (18.83)	3 (16.67)
Forbs	24 (17.85)	24 (15.96)	26 (15.52)	17 (12.12)	15 (13.13)	17 (12.74)	22 (14.36)	20 (15.50)	17 (11.0)	17.0 (11.97)	19 (13.05)	17 (11.29)	15 (10.47)	16 (9.31)
Grasses	13 (23.0)	12 (26.50)	11 (22.18)	11 (22.18)	13 (18.46)	12 (22.13)	12 23.58)	12 (21.17)	11 (20.09)	11 (23.36)	12 (23.21)	11 (19.64)	6 (14.42)	3 (18.0)
Kruskal Wallis test	χ^2 = 4.21, *P*=0.12, df=2	χ^2 = 11.16, *P*=<0.001*** df=2	χ^2 = 15.72, *P*=<0.001***, df=2	χ^2 = 9.04, *P*=0.01** df=2	χ^2 = 5.98, *P*=0.05* df=2	χ^2 = 7.81, *P*=0.02*, df=2	χ^2 = 12.62, *P*=<0.001***, df=2	χ^2 = 9.82, *P*=0.02*, df=2	χ^2 = 14.05, *P*=<0.001***d f=2	χ^2 = 13.25, *P*=<0.001***, df=2	χ^2 = 10.04, *P*=0.01** df=2	χ^2 = 12.80, *P*=<0.001*** df=2	χ^2 = 4.16, *P*=0.12, df=2	χ^2 = 6.74, *P*=0.03*, df=2

Invasibility

Plots sown with a high seed rate were less easily invaded than those with a lower application of seed and in the first two years an application of nurse crop suppressed the colonization of sown and unsown species. However, the preferred seed mix encouraged colonization by unsown species in 2000. When unsown species were categorized as 'weedy' or 'calcicolous' there was no effect of type of seed mix, application of nurse grass or seed rate on the proportion of plants in each category (Table 6.18). However, in the first two years it was evident that weedy species predominated but by 2000 species categorized as calcicolous were much more frequent than weedy species in all of the plots where seed was applied.

Table 6.18 Comparison of the number of individuals that were categorized as 'weedy' or 'calcicolous' on experimental plots in 1997, 1998 and 2000.

	1977		**1998**		**2000**	
	Weedy sp.	Calcicolous sp.	Weedy sp.	Calcicolous sp.	Weedy sp.	Calcicolous sp.
Mix						
Preferred	84	18	52	32	30	39
Basic	67	25	47	44	37	39
	χ^2= 2.54, P= 0.110, df=1		**χ^2= 1.87, P=0.171, df=1**		**χ^2= 0.39, P=0.530, df=1**	
Nurse grass						
With	76	20	50	40	36	41
Without	75	23	49	36	31	37
	χ^2= 0.19, P=0.658, df=1		**χ^2= 0.07, P=0.780, df=1**		**χ^2= 0.02, P= 0.888, df=1**	
Seed rate						
0.4g	48	14	31	29	32	36
1.0g	42	9	33	23	20	24
4.0g	61	20	35	24	15	18
	χ^2= 0.91, P= 0.634, df=2		**χ^2= 0.89,P=0.638, df=2**		**χ^2= 0.04, P= 0.98, df=2**	

6.4 Discussion

Using a descriptive measure of community structure to assess the effect of seed application rate, type of seed mix and nurse grass application

A comparison of the plant assemblages on experimental plots at Swanworth Quarry with a target vegetation indicated that the rate at which seed is applied in a restoration scheme is an important factor which influences the character of developing grassland. Ordination of the data indicated that all treatments, including the control, became increasingly similar in community composition to the target vegetation over time but that by 2000, the plots sown at 4g/m^2 (the highest rate) were the closest in composition. This simple descriptive analysis is sufficient to detect the effect of seed rate and suggests a high seed rate will enhance the restoration of grassland within the first three years. However, an investigation of the effects of the treatments on species diversity, functional diversity and invasibility, indicate that, together, these determinants are a more accurate indicator of success.

Using three ecological measures as determinants of success: i) species diversity, ii) functional diversity, iii) invasibility

An investigation of species-richness suggests that at Swanworth Quarry, sowing the preferred mix at a low rate would be at least as effective as sowing the high rate of the basic mix. Increased seed rate led to an increase in species richness on plots sown with both mixes, but those sown with the preferred mix contained more species at the end of the recording period. This, in itself, is unsurprising as the preferred mix itself consisted of more species. More importantly, by 2000 the plots sown with 0.4g/m^2 (lowest rate) of the preferred mix were colonized by 24 of the sown species, in comparison with the basic mix sown at 4g/m^2 (highest rate), which were colonized by 23. This suggests that if sown species diversity is a desired outcome, applying the preferred seed mix at a low rate was as effective as a high application of the basic mix. The effect of seed rate on vegetation establishment was investigated by Stevenson, *et al.* (1995), who worked on the restoration of chalk grassland at an extension to the M3 in Hampshire. The experiment at Swanworth Quarry, described in this chapter, used the same sowing rates as Stevenson and his colleagues and the two experiments can be compared. At the M3 extension, differences in sown species richness established by the three application rates were not detectable after two years. The failure to find the same results within seed mix treatment at Swanworth Quarry may be due to particular site conditions. Stevenson *et al.* (1995) applied seed to ex-arable land which retained a thin layer of topsoil, whereas at Swanworth Quarry, the seeds were sown on to bare clay waste. The additional nutrients in the topsoil at the M3 extension may have encouraged germination of the species sown in the first year and seeds which were dispersed locally in the second year. It is possible that in time the experimental plots at Swanworth Quarry will also become increasingly similar, but as time progresses, it will be increasingly difficult to distinguish temporal from treatment effects due to seed drift between plots.

Classical measures of diversity such as the Shannon-Weiner index incorporate a measure of evenness. By 2000, there was a difference in the evenness of the vegetation established on the experimental plots at Swanworth Quarry. Although it is expected that all plant communities will be dominated by particular species (Grubb *et al.*, 1982), increased evenness has been linked with enhanced stability (Wilsey & Potvin, 2000). Evenness increased over time on all the plots at Swanworth Quarry and, although this is a short-term experiment, initial results suggest a trend which supports the 'Three-phase' model described by Gitay & Wilson (1995) and confirmed by Wilson *et al.* (1996). The 'Three –phase' model proposes that in the colonizing phase a community will display small-scale heterogeneity due to dispersal limitation, chance and low competition. In the building phase, the spread of species will reduce the initial effect of dispersal, and increased competition will force out poorer competitors leading to increased evenness. In the mature phase, inter-specific competition and differentiation between microhabitats will again lead to a more uneven community.

The difference in evenness between the vegetation established by the two seed mixes can partly be attributed to the dominance of the legume *Anthyllis vulneraria* on plots sown with the basic mix. It is possible that this dominance was a function of the experimental design, as the basic mix contained fewer species and therefore the proportion of *Anthyllis* sown in each gramme of seed was greater than in the preferred mix. A further difference between the mixes was the addition of plug plants on plots sown with the preferred mix. Although the plants remained small and it seems unlikely that they had any effect, it was not possible to rule out the possibility that their presence prevented *Anthyllis* colonizing so effectively. By 2000, few plant plugs had survived and this meant that it was not possible to investigate the effect of plant plugs on sward development at Swanworth Quarry. Although plant plugs established well at the M3 extension (Ward & Snazell, 1995), Hopkins *et al.* (1999) also found that plant plugs failed to persist at a grassland restoration site. Another possible explanation for the failure of *Anthyllis* to dominate to the same extent on the plots sown with the preferred mix is that greater species-richness prevented the spread of *Anthyllis* through competitive exclusion. Alternatively, the reduced species-richness on the plots sown with the basic mix may be a function of the dense cover of *Anthyllis*: certainly it is to be expected that the density of the vegetation (and associated dead plant material) is likely to exclude species whose regeneration is gap dependent, such as *Helianthemum nummularium, Thymus drucei* and *Cerastium fontanum* (Thompson *et al.*, 1996). The high species cover of *Anthyllis* on the basic plots is likely to exert a controlling effect on the grassland development (Grime, 1998). Aarssen (1997) suggests that the effect a species will have on the functioning (meaning productivity and nutrient retention) of the ecosystem can be predicted from its contribution to plant biomass. What these differences in productivity and nutrient retention will mean for the development of grassland on the basic plots is unclear, but these results do suggest that the two seed mixes have established grassland communities that will develop in different ways.

The initial seed mixes used at Swanworth both contained three functional groups (legumes, forbs and grasses), in addition, some plots sown with the basic mix were colonized by a small number of woody species (a fourth functional group). Tilman *et al.* (1997) have suggested that functional diversity may be important in maintaining ecosystem diversity. The use of broad groupings to describe function was not expected to reveal detailed information about overall functional diversity (Semanova & van der Maarel, 2000). However, it does indicate that by 2000, all three functional groups which were sown were represented on all of the treatment plots and that plots sown with the basic mix were invaded by a fourth group. The literature suggests that as the woody group is species-poor it is unlikely to persist; the probability of a functional group becoming extinct increases with species paucity within the group

(Fonseca & Ganade, 2001). This is supported by the loss of the woody group within two treatments between 1998 and 2000. Were woody species to persist, it can be expected that there would be an effect on the development of the grassland and it is further evidence that grassland development is likely to be different between the seed mixes. Seed rate and type of seed mix were only related to the abundance of individuals in different functional groups in the first year, when the preferred mix and the high seed rate had a more even distribution of species between groups; by the end of the recording period the effect was not significant. In 2000, it was clear that there was a difference in species richness and vegetation cover between functional groups on plots sown with different treatments, although the number of species present on the plots did not predict the proportion of vegetation cover (and therefore) the influence of a functional group on community development, (Aarssen, 1997). For example, although the legume group was represented by the fewest species, legumes covered the greatest proportion of all the plots. The proportion of cover between functional groups was similar in all the treatments.

It has been suggested that it is not only functional diversity which is important, but also functional composition, which is related to the identity of species within the functional groups (Hooper & Vitouseck 1997). This helped to interpret data from Swanworth Quarry where the disproportionate abundance of *Anthyllis* may have been suppressed on the preferred plots by the presence of *Ononis repens*, another leguminous species. This suggests that to prevent the dominance of competitive species such as legumes, it might be useful to ensure that there are several legume species included in the seed mix. However, one cannot discount the possibility that it was species richness *per se* that suppressed *Anthyllis* on the plots sown with the preferred mix. The recommendation by authors to break functional groups down to species level does suggest that broad functional groupings are more useful on a coarse scale.

Stability of the vegetation can be further influenced by how well it resists invasion (Tilman, 1997a). Between 1997 and 2000, higher seed rates reduced the number of unsown species on the experimental plots at Swanworth quarry and this is in accordance with Stevenson *et al.* (1995) who found similar results and concluded that $4g/m^2$ is the optimum rate for eradicating weeds. However, as calcicolous communities support many early successional species which require an open sward, the authors concluded that the dense sward resulting from higher seed rates may exclude calcicolous species. In the first four years at Swanworth Quarry a higher number of sown species established on the plots sown with 4g/m^2, but as overall species richness (that is sown and unsown species combined) was greater on the plots which were sown with 0.4 and 1g/m^2, it may be that in the long term the vegetative cover on the plots sown at a higher rate will suppress a further increase in species richness.

The predominant type of unsown species recorded at Swanworth changed over time. In 1997 weedy species were a major component of the unsown species recorded but by 2000 calcicolous species outnumbered weed species in all the treatments. Experiments in other ecosystems have found a similar trend; in long-term experiments which investigated prairie grassland restoration in Wisconsin, Cottam (1987) found that weeds were out-competed by prairie species. Although a high seed rate application suppressed the invasion of weedy species at Swanworth Quarry, it did not prevent colonization completely. This is important as in some restoration projects recruitment from the local species pool may be a pre-requisite for the establishment of species-rich grassland (Partel *et al.*, 1998). The priority given to excluding unsown species will depend partly on the location of the restoration project. In areas surrounded by agricultural land it may be desirable to prevent invasion, whereas in an area surrounded by an appropriate plant community it may be preferable to leave the sward open to encourage recruitment. Although many calcicolous species are noted for their poor dispersal ability (Hutchings & Booth, 1996), there has been some dispersal from areas up to 50m away at Swanworth, where species growing on the boundary bank have been recorded on the experimental plots.

It is possible that the low seed rates of 0.4 and 1.0g / m^2 would, in time, establish an appropriate vegetation at Swanworth Quarry. However there are two considerations. Firstly, it is not known how much plots sown with a lower rate have been colonized by seeds from neighbouring plots by the year 2000. The control plots recruited 11 of the sown species and it is likely that there has been a similar level of transfer between other plots which had been sown. Secondly, although the vegetation may recruit species and increase in species richness over time, low species richness on these plots during their development means that they are vulnerable to invasion by weedy species and nutrient loss. Diversity provides 'insurance' against environmental changes as different species respond differently and this ensures the survival of the community (Loreau *et al.*, 2001). Vegetation with low diversity has been shown to recover less well from periods of perturbation such as drought, so that species richness is considered to confer resilience in a community (Tilman & Downing, 1994).

The vegetation on plots that had received an application of *Lolium multiflorum* were less similar to the target vegetation than those without and, although the nurse grass did reduce colonization by unsown species, sown species richness was also reduced. By 2000 no effect of the application of *L. multiflorum* was detected. Mitchley *et al.* (1996) successfully used *Lolium perenne* to encourage species diversity at a restoration site in low nutrient conditions, although in higher nutrient conditions the addition of a nurse grass led to competitive exclusion of sown wildflower species. From their results it was thought that an application of nurse grass may have encouraged diversity in plots sown with a low seed rate, but it was not so at Swanworth.

The results of this experiment suggest that, if possible, it is desirable to use a relatively high seed application. At Swanworth, high seed rates resulted in a sward which was more similar to the target vegetation than that established on plots sown a lower rate. Vegetation on plots with a higher application rate was also more diverse and was resistant to invasion by unsown species, without completely excluding colonization by plants from local sources. If only small amounts of seed are available, as is likely if all seeds are to be collected locally, it is still possible to establish an appropriate community. However, that community will be more vulnerable to changes in the environment during the establishment phase and more open to invasion by colonizing species which are likely to be weedy in the first few years. The increased diversity and evenness of the vegetation on plots sown with the preferred mix suggest that it is important to use a mix that has as many species as possible and preferably with a number of species within each functional group. Unfortunately it was not possible to separate the effects of plant plugs from seeds in this mix. The experimental work presented here suggests that success will be greatly enhanced if a wide range of species can be successfully harvested. It would be an advantage if these were collected in sufficient quantities to apply the seeds at $4g/m^2$. There is no benefit in adding a nurse grass to compensate for a low application rate of local species.

Chapter Seven

General Discussion

Summary of Aim and Objectives

This thesis investigated the effect of propagule provenance on the success of the re-vegetation component of restoration schemes. The objectives of the study were to:

- Quantify the genetic component of provenance as estimated by plant morphology and fitness in a common garden experiment
- Determine the effect of provenance on differences in plant fitness in a restoration environment
- Investigate fine-scale phenotypic variation in *L. corniculatus* with reference to restoration ecology
- Investigate the practical implications of restricting seed procurement to local sources

The method for investigating the first three objectives employed experiments which used *Lotus corniculatus* as a model species. The method for investigating the fourth objective was a field trial using different seed regimes. The experimental work was discussed in detail in each chapter. This general discussion will present an overview of the principal findings of this thesis, discuss the implications of this research in context with contemporary studies and identify key areas for future research.

7.1 Introduction

A successful restoration scheme is one which fulfills the objectives that were decided upon at the outset of the project (Erenfeld, 2000). Typically, this means assisting the recovery of an ecosystem so that the following criteria are met:

The restored site will support an appropriate community with similar species composition to that of a reference community. It will fit in with the larger matrix of communities in the surrounding landscape. The community will be stable (but not in stasis) and sustainable, i.e. the established ecosystem will require no more than the traditional management associated with that system. For example, it is reasonable to expect to maintain a chalk grassland community by grazing but not by annual weeding. The system will be resilient, that is, the established community will be able to recover, unaided, from disturbance. These criteria are embodied in a recently published Primer for Ecological Restoration by the Society for Ecological Restoration (SER, April 2002).

In this context, the success of the re-vegetation component can be measured by its contribution to the above criteria. If the re-vegetation process is to be engineered by introducing propagules, then the source of propagules will be an important issue. Increasing concern over the introduction of non-local propagules (Millar & Libby, 1989; Fenster & Dudash, 1994; Knapp & Dyer, 1997) has focused on maladaption of non-local plants to conditions at restoration sites and (potentially) the reduced ability of non-local plants to establish and persist when compared with local plants (Knapp & Rice, 1996; Jones & Hayes, 1999; Keller *et al.*, 2000; Jones *et al.* 2001). The home-site advantage hypothesis suggests that plants sourced from the site to be restored will show enhanced fitness when compared with non-local plants and that the fitness of plants will decrease as the distance between donor and receptor site increases (Montalvo & Ellstrand, 2000). Both geographical separation of populations and local adaptation to ecological conditions are expected to lead to genetic and phenotypic differences between populations (Galloway & Fenster, 2000). This is based on well-established theory which shows that communities which are isolated by distance (and therefore do not exchange genes) will be dissimilar due to initial differences in the founder populations and subsequent genetic drift at each site (Levin & Kerster, 1974). Futhermore, differences will be maintained and enhanced by local adaptation to ecological conditions (Loveless & Hamrick, 1984). Where there is insufficient isolation to prevent gene-flow between populations, the effects of selection are modified by gene-flow, leading to increased similarity between populations (Levin, 1984). If populations, although isolated by distance, undergo selection by similar ecological conditions they may also show phenotypic similarities and be able to persist in similar conditions (Montalvo & Ellstrand, 2000). The relative importance of geographical and ecological distance on the fitness of translocated populations in restoration projects, due to the effect of isolation by distance and local adaptation is currently a subject for debate (Montalvo & Ellstrand, 2000; Wilkinson 2001: Sackville-Hamilton, 2001).

The effect of geographical and ecological distance on heritable differences in morphology and fitness between populations of *L. corniculatus* (Chapter three) and their fitness at a restoration site (Chapter four) will be discussed below. The scale at which propagules can be considered local will be species specific, depending partly on the breeding system of the species (Wright, 1978) and the strength of selection due to local ecological variation. A quantitative scale upon which plants can be considered 'local' is rarely defined in the literature. Only Linhart (1995) has suggested that in order ensure that plants are collected from what is effectively a single, local, population (and consequently unlikely to affect the fitness of translocated individuals), herbaceous species should be collected within 100m. Using the scale defined by Linhart (1995) as a reference, observed phenotypic differences between *L. corniculatus* plants in the field were used to test the hypothesis that there is sufficient structure in the distribution of plant phenotypes to suggest Linhart's conservative guideline for propagule selection is necessary when considering the case of *L. corniculatus* (Chapter five).

Due to the lack of experimental work confirming the importance of using local propagules, many involved in restoration ecology adhere to the precautionary principal and recommend that only locally collected seeds and plants are used in re-vegetation programmes. The possible advantage (in terms of plant fitness) of using locally collected material must be balanced with the possibility that restricting seed procurement to a local source may negatively affect the successful establishment of sown grassland (Stevenson *et al*, 1995). This was considered in Chapter six, where it was suggested that using a local seed source may reduce the volume of seed available for collection and/or restrict the number of species that can be collected without depleting the source of species which are nationally or locally rare. The effect of seed mix and application rate on the successful establishment of an appropriate plant community, balanced with the importance of using locally collected seed, is evaluated in the following discussion.

7.2 The choice of *L. corniculatus* as a model species

L. corniculatus is a common calcicolous grassland plant that is frequently used in restoration schemes, not least because it is included in most commercial mixes for chalk and limestone grasslands. The information gathered in this thesis adds to the body of knowledge already determined for *L. corniculatus* and advises those practically involved in restoration ecology.

As *L. corniculatus* is a well-studied species, it was possible to gather information on which to base experimental work. A literature search revealed that there is sufficient dissimilarity between populations to suggest that translocated plants may respond differently when introduced into a novel environment (Jones & Crawford, 1977; Ramnani & Jones, 1985; Jones & Turkington, 1986; Jones, 1989; Steiner & Garcia de los Santos, 2001). Research has shown that *L. corniculatus* has adapted to a wide range of conditions and is associated with different habitat types (Steiner, *Pers. Com.*). As the experiments in this thesis aimed, in part, to evaluate the effect of local adaptation on plant fitness in a restoration environment, this was an important characteristic. The heritability of the traits measured in this study has been previously established (Jones & Turkington, 1986; Ramnani & Jones, 1984a; Ramnani & Jones, 1984b; Jones, 1989; Steiner, 1997) and the morphological traits have been used successfully to separate native and non-native varieties of *L. corniculatus* (Bonnemaison & Jones, 1986). Experimental work in Chapter 3 supports the findings of these studies and suggests that there are also heritable differences in traits which estimate plant size and fitness. This understanding provided a firm base from which to investigate two areas: 1) the effect of geographical and ecological distance on heritable differences between populations of *L. corniculatus* 2) the effect of geographical and ecological distance on differences in fitness between populations of *L. corniculatus* in a restoration environment. This was enhanced by the widespread distribution of *L. corniculatus* which enabled the collection of plants from a number of locations in the UK.

Constraints

L. corniculatus is a tetraploid and thus inherits traits tetrasomically as demonstrated for the polymorphisms of keel colour (Ramnani & Jones, 1984a) and cyanogenesis (Dawson 1941; Ramnani & Jones, 1884b). The inheritance patterns of the above two polymorphisms are relatively well understood. For example, it has been shown that in populations of *L.corniculatus* from Porthdafarch, Anglesey, Wales, there is a single locus involved for keel colour with alleles B and b inherited tetrasomically (Ramnani & Jones, 1984a). In contrast, the inheritance of cyanogenesis depends upon two loci for a glucoside and an enzyme (which are not linked) (Ramnani & Jones, 1884b). Due to the complicated inheritance patterns associated with tetrasomic inheritance, only detailed experimental work can determine the pattern of inheritance (Fisher, 1949). The results from simple observations in common garden trials show only the different frequencies of morphs expressed within the populations in the generation studied and indicate that it is likely that the populations differ genetically. As a large amount of the variation may not be expressed phenotypically, the extent of variation within the population is not completely revealed. The interpretation of results must take this into account. Using a common garden experiment minimizes the confounding effects of environmental variation frequently found in field conditions. By reducing the effect of environment in this way, the remaining variation observed is considered to have a genetic basis (Jones & Wilkins, 1971). This approach has been used to investigate heritable traits in many studies including the classic work on ecologically isolated 'races' carried out by Clausen, Keck & Hiersy at the Carnegie institute (Clausen *et al.*, 1940; 1947; 1948).

7.3 Investigating the heritability of traits in *L. corniculatus*

In Chapter 3, eleven traits were scored on plants grown from seed collected from 12 populations in the British Isles. These were morphological traits (leaf pubescence, growth habit and leaf shape), traits which indicate plant size (branch number, internode length, leaf length and leaf span) and direct measures of plant fitness in terms of reproductive output (number of umbels, pods, seed number and seed weight). Seeds were collected from two habitats in each of 6 regions, to compare the effects of regional separation and local adaptation on plant morphology and fitness. The data collected from plants grown from different populations in a common garden were pooled for analysis so that plants were compared in two ways. Firstly plant data were pooled by region in order to investigate whether there were any traits which were associated with region of origin within the British Isles; this would suggest that particular phenotypes have developed in areas isolated by distance. Secondly data were pooled by the two categories of habitat, defined as 'calcicolous grassland' and 'alternative habitat'; if significant differences were found, it would show that chalk grassland type plants are phenotypically distinct from other habitat types, independent of genetic isolation.

One finding was unexpected; seed weight was not found to be significantly different between regions or between habitats of origin. The lack of variation in seed size between populations was surprising as Bullard & Crawford (1996) established that there was variation between 6 British populations. The lack of difference in seed size was also demonstrated in Chapter four where plants were grown in two field plots at the restoration site. It is possible that the method for estimating seed weight has lead to the discrepancy in findings. Bullard & Crawford used 100 seed-weight to estimate mean seed weight but in the experimental work presented in this thesis, seed weight was estimated by weighing 3 batches of 20 seeds and calculating a mean seed weight.

7.4 The effect of geographical and ecological distance on the expression of heritable traits in the common garden and on plant fitness in a restoration environment.

The common garden experiment

From the evidence presented in Chapter three, it was suggested that when plants are translocated in restoration schemes, geographical distance should be the primary consideration and subsequent choice of populations should be guided by habitat type. Differences between regions were found for all characters except number of branches. Furthermore, when absolute geographical distance between the sites was investigated it was shown that there was a correlation between reproductive output (seed number) and distance, suggesting that there are heritable differences in seed number maintained by genetic isolation. This is in contrast to Montalvo and Ellstrand (2001) who found no such link in *Lotus scoparius*. Although Montalvo & Ellstrand used field sites to examine differences in populations, the lack of correlation between genetic differences and geographical distance was confirmed by molecular assays.

There was some evidence that plant morphology was related to the ecological conditions at the site of origin. Differences were found for growth habit, pubescence, leaf shape, number of branches and leaf length. Although plants from the two habitats of origin could be distinguished morphologically, there was no difference in fitness (as measured by reproductive output). The morphological traits do not have any immediate effect on plant fitness but these traits may affect long-term persistence. It is not possible to assess this effect in a short-term experiment but other work has shown that morphology may affect long-term plant fitness. For example, pubescence may affect temperatures at the leaf surface which may, in turn, influence the plants transpiration rate and have an impact on the plants metabolic rate and water loss (Ehleringer & Clarke, 1988). There is also evidence to suggest that leaf pubescence may influence herbivore feeding (Zvereva *et al.*, 1998) and egg laying (McAuslane, 1996). Keller *et al.* (1999) showed that slugs distinguished between grassland plants sourced from various locations in Europe and appeared to find some more palatable than others. Their main concern was that differences in palatability would affect the ability of different genotypes to survive in the presence of slugs. The long-term effects of invertebrate herbivory are unknown in *L. corniculatus* but cannot be discounted.

In the experiment described here it was shown that plants from East Sussex were less likely to suffer seed damage than those at the other sites. These plants had the highest number of branches and the largest leaves. This surprising as analysis in Chapters three and four showed that larger plants with more branches and larger leaves were more likely to have predated seeds (Tables 3.14 & 4.4). Ollerton & Lack (1998) also found this to be the case. The low proportion of plants with predated seeds sourced from East Sussex suggests that there was another factor, not recorded in the experiment, which discouraged seed predation. This requires further investigation as invertebrate herbivory of seeds may be linked to plant fitness. For example, Ollerton & Lack (1996) found that partial pre-dispersal seed predation can enhance the germination of seeds and may be part of a mechanism for breaking hard coat seed dormancy associated with *L. corniculatus*. Although plant size traits differed between the calcicolous and alternative habitats, no difference in seed damage was observed.

A further possibility is that provenance will affect larval growth rates in some species. Hoare (1999) showed that larval growth rate of commercially acquired *Polyommatus icarus* was greater on a European variety of *L. corniculatus* than on native British plants. It is not known if larval growth rate would be affected by differences between British populations. During an early phase of work for this thesis, an experiment was devised to investigate the effect of six provenances of plant, including a local source, on the growth rates of larvae produced by *P. icarus* which were collected close to Swanworth Quarry. Unfortunately, the eggs laid by captured butterflies desiccated and the experiment was abandoned. Overall, there is some evidence that herbivores may be selective but as yet no strong evidence for local adaptation of herbivores to common grassland British species has been published. The implication of introducing non-local plants for associated herbivores must be considered and the encouraging results from this study and those by Hoare (1999) and Keller *et al.* (1999) indicate that more research is needed in this area (see *7.9 Suggestions for further research).*

In summary, the results from Chapter three suggested that geographical distance is an important factor determining differences in morphology and fitness between populations. Considering within population differences, Sackville-Hamilton (2001) stated that in terms of non-neutral genes, that is genes that are affected by natural selection, geographical distance is unimportant except as a secondary consequence of ecological distance increasing with geographical distance. The work in Chapter three shows that this was not so between populations of *L. corniculatus*, as an effect of geographical distance was recorded for heritable differences in seed number (a non-neutral character) while ecological distance was not correlated with mean seed number. The effect of ecological conditions was limited to differences in morphology. Although, at present, there is no evidence to suggest that these characters are linked to selection, Sackville-Hamilton (2001) points out that a functional gene that has no effect in some genetic backgrounds for some selection pressures may not be selectively neutral in other situations. He concluded that loss of currently neutral genes may reduce the potential to adapt to future changes where the same genes are no longer neutral. It is possible that there is latent significance in differences observed in the morphological characters associated with different habitat types, which could be linked to long-term plant fitness.

The field experiment

As it was possible to distinguish between plants from different regions using both morphological characters and fitness indicators in a common garden; plants grown from non-local propagules at a restoration site were expected to perform differently when compared with those grown from local stock. This was confirmed in field experiments at Swanworth Quarry. Cuttings were collected from two habitats within each of fifteen regions; so that there were thirty sites of origin, twenty-nine of which were non-local. Two experimental sites were used; one was a site improved by topsoil addition and the second was an untreated site. The results showed that clones from plants responded differently to the conditions at each site.

Consistent home-site advantage for plants grown from local propagules, as described by Montalvo & Ellstrand (2000) was not shown in this study. Survival varied between populations (an effect which was not observed in Chapter three) indicating that there is a selective effect on the survival populations at the restoration site. At the site treated with top-soil, there was a home–site advantage for local plants but at the untreated site, plants from four other sites survived better than the local plants, including plants from Llongriddry in Scotland, a coastal site and one of the furthest translocation distances. All four sites which showed superior survivial were collected from exposed, coastal locations and this may show some tendency for the plants to tolerate the exposed conditions at Swanworth Quarry.

At the restoration area improved by topsoil addition (described in Chapter four), local plants had the highest survival rate, were among the largest plants and produced a relatively high number of seeds but plants collected from four of the twenty-nine non-local populations were larger and more fecund. On the untreated site the local population did not perform well, particularly in terms of reproductive output. These results are comparable to Galloway & Fenster (2000) who also found that local populations did not consistently have an immediate fitness advantage over non-local populations and that plants from an intermediate distance out-performed locals. At Swanworth Quarry plants from many of the distant populations performed poorly (Figures 4.4 - 4.8), something which was also found by Galloway & Fenster (2000) who showed that plants translocated 1000km were less fit than those translocated shorter distances. It is highly probable that there will be a species specific response to the translocation of plants to a novel environment that will depend upon the life history of the species in question. Galloway & Fenster (2000) posited that the ability of *C. fasciculata* to tolerate a wide range of conditions is due to the short time that colonizing species (such as *C. fasciculata*) inhabit an area so that these species are unlikely to adapt to particular environments but to broad range of conditions. *L. corniculatus* is similarly adapted, although much of the wide range of adaptation is due to its highly variable genetic diversity. The range of phenotypes found is believed to have developed as a result of adaptation to the environments in which it is found through continual intraspecific hybridization (Steiner, *Pers.Com.*). The difficulty of drawing up general guidelines governing all species is illustrated by Jones & Hayes (1988). Using seven common grassland species, the authors found that performance (as measured by germination and survival) differed between species; in some cases the non-local varieties performed better. However there are two major problems with drawing firm conclusion from this study. Firstly, the results were obscured by the lack of specific provenance details; 'local' was defined as seeds sourced within 8km and 'non-local' was defined as commercially bought seeds which may have been collected up to 220km away. Secondly, the experiment also incorporated a range of treatments manipulating grazing and nutrient addition. The authors acknowledge that the differences in establishment and survival between alternative provenances of each species may represent differences in niche requirements among those resulting from the range of management treatments imposed (Jones & Hayes, 1988). A study by *Joshi et al.* (2001) showed clearer results. Using 3 species in a reciprocal transplant experiment the authors showed that local plants showed enhanced performance. However, the species varied in the fitness components that contributed to overall increase in performance. This experiment was conducted at eight sites in Europe and the scale makes it more likely that a significant effect would be found. None of the experiments described above were carried out for more than two years and in these, and in the short-term experiments described in this thesis, it is not possible to ascertain whether the advantage shown by particular provenances would be maintained over time.

Further analysis of the data collected for Chapter four indicated that despite the poor performance of local plants, there was evidence of a general trend for plant size and fecundity to decline as the distance between the donor and receptor site increased. However the relationship between geographical plant performance and distance was weak. This was confirmed when data from each population was pooled by region as in Chapter 3. As expected, there were differences in plant size and fecundity but the local group (Dorset) did not out perform other populations. However, the analysis demonstrated the tendency for distant populations to perform poorly on average. These results seem to suggest that the local plants are not optimally adapted for the local site. This is not a new idea; Fisher (1930) was able to demonstrate conditions under which natural selection did not improve fitness. Harper (1982) considered reasons why plants may not be optimally adapted, including, founder effects, available genetic resources and compensation. Harper warns against searching for interpretations of any form or behaviour of an organism and to give it an explanation in its own right as an optimal system. This is something which all the recent studies on local provenance have done, using plant size and fecundity as a demonstration of 'superiority' and ability to persist, without using long-term studies to confirm this.

The evidence for local adaptation demonstrated in Chapter 3 was absent at the treated restoration site described in Chapter four. This contrasts with other authors who have demonstrated that plants translocated between sites with similar conditions show enhanced performance when compared with plants that were sourced from ecologically dissimilar sites (Jones *et al.*, 2001; Joshi *et al.*, 2001; Montalvo & Ellstrand, 2000). However, there was some evidence of local adaptation on the untreated site (Chapter four) where plant size and fecundity decreased as the ecological distance between donor and receptor site increased and plants sourced from calcicolous habitats were, on average, smaller and less fecund than those from the alternative habitats. Using Harper's (1982) concept of compensation, it may be these differences in size and fecundity represented an appropriate response to specific environmental conditions which may, in turn, indicate that these plants, typical of those found in limestone grassland, would be able to survive better than the larger plants more fecund plants over time. This is not to say that the reduction in fitness is an advantage but rather that resources are allocated into another, unidentified, system that will enhance long-term persistence. In other words, there is some compensation for small size and low fecundity, which may be an advantage in the long term. It is tempting to look at simple measures of short-term success and give undue weight to the results. Jones *et al.* (2001) is widely quoted as having shown the advantage of local individuals of *Crataegous monogyna* in a British hedge. The measures of success were thorniness and resistance to mildew. As Harper (1982) warned, it is dangerous to use characters that seem appropriate and use them as an example of an optimal system without evidence that it is so. It tempts the author to make unsafe conclusions. When one British population performed in a similar way to a foreign variety, Jones, *et al.* (2001) surmised that the plants, which were commercially acquired, had been confused with foreign stock by the supplier. There was no evidence to confirm this.

The results from Chapter four do not present a clear picture. Although plants from different regions were dissimilar, there was no consistent evidence that collecting close to the receptor site would enhance size or fecundity. The effect of adaptation to local conditions was not in evidence at the treated site, although there was a positive relationship between ecological distance and plant size and fecundity at the untreated site. Comparing the findings of experimental work in this thesis and those of other studies demonstrates that it may not be possible to draw up simplistic guidelines that cover all species. It is also important to recognize that the characters frequently measured to describe local advantage may not best estimate long term survival and fitness. Furthermore, cloned populations performed differently at the two restoration sites, indicating that conditions at a restoration site will significantly influence fitness of plants in a way that is not predictable from off-site common garden experiments. The wide range of adaptation in *L. corniculatus* reviewed by Steiner & Garcia de los Santos (2001) may be at the root of the variable response at the two restoration sites described in Chapter four. It is possible that the range of genetic variation within particular populations is such that non-local populations are able to out-perform local, perhaps less variable, genotypes. The absence of comprehensive data for a wide range of species would suggest that those working in restoration ecology are not in the position to draw up concrete guidelines at the present time.

Only one author has attempted to prescribe a quantitative guideline. Linhart (1995) suggested that, in order to be considered local, forbs should be collected within a 100m radius of the receptor site. This was tested in Chapter five using *L. corniculatus*. It was demonstrated that there was no consistent pattern of phenotypic differences within 200m and it was concluded that it is not necessary to restrict the collection of *L. corniculatus* to a 100m radius; the limit can be extended to at least 200m. This gives weight to the conclusion that a larger limit may be appropriate for a species which is geographically wide-ranging and perhaps well adapted to a range of conditions. Work in this thesis did not investigate the scale between 200m and 1600m (the smallest distance between sites in Chapters three & four). As the limits of what can be considered local may lie within these distances, it would be informative to extend this experiment (see *7.9 Suggestions for further research).*

When considering short-term fitness, the results of the experimental work in this thesis suggests that geographical distance is an important consideration for the restoration ecologist. As non-local populations perform well, it may be possible to describe seed zones within which it is 'safe' to collect seeds. However, although it was shown that Linhart's (1995) guideline can be extended to 200m, further quantitative guidance cannot be determined from the experimental work presented here. Although, overall, a weak relationship was found between distance and plant fitness, anomalies prevent the development of a general rule. For example, in the common garden experiment at Furzebrook, and at the two field sites, plants sourced from Dorset were similar in both size and fecundity when compared to plants sourced from North Wales. These regions are separated by a distance of approximately 320km. However, plants sourced from East Sussex, although separated by approximately 150km, consistently performed very differently when compared with Dorset populations. Furthermore, plants from sourced from different habitats within each of the regions consistently showed differences in morphology and, in some circumstances, fitness when compared. This suggests that if seed zones were determined, it would remain important that seeds are collected from ecologically similar sites to enhance long-term fitness.

7.5 Defining 'local'

The work presented in this thesis demonstrates that both geographical and ecological distance partly determine what can be considered to be of 'local' provenance when selecting propagules for restoration projects. There was some evidence that geographical distance was correlated with plant fecundity. At the same time there were clear differences in both morphology and plant fitness characters between plants sourced from different regions of the British Isles. Joshi *et al.* (2001) used a large-scale experiment spanning Europe to investigate the performance of three grassland species when translocated; they found that geographical distance between donor and receptor sites predicted plant fitness. However, Joshi and his colleagues (2001) did not investigate geographical distance separately from ecological distance, rather geographical distance was used as a surrogate for environmental differences at their sites. The design of experiments in this thesis separated the effects of geographical and ecological distance by selecting pairs of sites which were geographically close together but ecologically quite different. This enhanced the sensitivity of subsequent analysis so that the effect of geographical distance is clearly distinguished. Other authors (Galloway & Fenster, 2000; Montalvo & Ellstrand, 2000), used smaller scales than Joshi *et al.* (2001) which were similar to those used in this thesis, but failed to find any evidence that geographical distance is a useful determinant of locality. Using *L. corniculatus* as model species, work in this thesis reaffirms that geographical distance is a determinant of what can be defined as 'local' and should be considered when selecting propagules for a restoration scheme.

Ecological distance was used in this study to describe the difference in environmental conditions between the propagule collection sites. Plants from sites defined as 'calcicolous grasslands' and 'alternative habitats' differed in both morphology and fitness. This supports work by Joshi *et al.* (2001); Jones *et al.* (2001) and Montalvo & Ellstrand, (2000), who also found that 'local' can be defined by the similarity of a potential donor site compared with a site which is to be restored.

7.6 Wider implications of introducing non-local plants

The implications of introducing non-local genotypes are not limited to the immediate effect of translocation on the survival and fitness of introduced individuals. Introduced plants may hybridize with those in the local population. Recent experimental work has confirmed that, in the long term, hybrids can be less fit than local plants (Fenster & Galloway, 2000; Keller, Kollman & Edwards, 2000; Montalvo & Ellstrand, 2001). Montalvo & Ellstrand (2001) suggested that fitness differences would accumulate over time, basing this expectation on work by Conkle (1973) who investigated the effect of provenance on transplanted Poderosa pine over 29 years. Montalvo & Ellstrand (2001) found that the most important factors for consideration were the genetic and environmental similarities of source populations relative to the receptor site and that geographical separation was not a good surrogate for either of these. However, they also suggested that the detection of heterosis in the F_1 generation could have been swamped by ecological out-breeding depression and that (under the epistatic model) the consequences of geographical distance matings would be revealed in the F_2 generation. Overall the conclusion was that more severe out-breeding may be observed in subsequent generations and they are carrying out investigations to confirm their hypothesis. It can be speculated that if the plants translocated to Swanworth Quarry (the study site for this thesis) were to hybridise with local plants a similar loss of fitness may occur. If Montalvo & Ellstrand (2001) are correct then any genetic introgression in the local population adjacent to Swanworth due to the introduction of non-local genotypes within the quarry would have negative effects on the established plant community. This may raise particular concerns as it is designated SSSI. Unsurprisingly, the effect of hybridization on plant fitness has been shown to vary between species, moreover, most experiments only consider the F1 generation so that epistatic out-breeding is ignored (Keller *et al.* 2000). If a general guideline for seed collection is to be defined, it will be necessary to use a distance which may be needlessly conservative for some species. To avoid this, more information is necessary so that species specific guidelines can be developed, although it is debatable whether those implementing restoration schemes will have the resources to construct seed mixes based on them.

As suggested above, genetic mixtures of populations which are adapted to different local conditions may result in out breeding depression which will lead to a loss of fitness caused by the breakdown of co-adapted gene-complexes (Storfer, 1999). Choosing local seeds to avoid this problem is a conservative approach advocated by several authors (e.g. Keller *et al.*, 2000; Montalvo & Ellstrand, 2000; Jones & Hayes, 2001). However, others have taken an alternative approach. Fenster & Dudash (1994) suggest that using populations with a high level of genetic diversity would restore the heterozygosity (and, implicitly, the fitness of the receiving populations). In this scenario, the level of genetic diversity, would be the appropriate factor to consider when assessing populations for their potential as donor sites. Lesica & Allendorf (1999) suggested that at highly degraded sites, with conditions to which local plants are unlikely to be adapted, it is useful to sow a mixture of genotypes to maximize the possibility that a successful strain will be introduced. In an area that is isolated by distance from natural populations this may be acceptable but it is important to consider the potential for hybridization between introduced and established populations. This is especially true when the restoration site is close to an area supporting a plant community that is valued, such as a SSSI. Wilkinson (2001), in a critique of current thinking on the importance of seed provenance, supports Janzen's (1985) theory of *ecological fitting* being a more important concept to consider, rather than local adaptation. That, as the environment is constantly changing, the plants that survive in a particular location are those which happened to have the genetic requirements to survive in the year that they established (Wilson, 2001). This is a view of plant communities constantly in flux, where local adaptation is snap shot of a stage in community development and not something to attempt to preserve. Wilson (2001) suggests that natural selection will remove unfit plants and so the problem of reduced fitness is negligible. However, as described in Chapter six, the removal or reduction in the abundance of a single species may effect community composition. If the success of the restoration project is judged by the criteria at the outset of this chapter, then the maintenance of a community which resembles the reference community must be a consideration.

In his answer to Wilson (2001), Sackville-Hamilton (2001) cited further reasons for conserving local genotypes *insitu*. Concerns about the introduction of non-local genotypes are not only focused on the establishment and persistence of transplants or their effect on the fitness of neighbouring populations. It may also be important to conserve the local pattern of genetic diversity. This is a purist stance, where authors are concerned with genetic 'contamination' or 'pollution' (Millar & Libby, 1994; Jones & Hayes, 1999; Gray, 2002). Partly, this perspective has a practical root; disrupting local gene pools reduces the overall genetic diversity of species and reduces the potential for continued evolution (Jones *et al.* 2001). It also incorporates the notion that local populations form part of the national heritage in the same way that monuments do and that they contain evidence of their history within their genotypes (Daniels & Sheail, 1999). The need of mankind to pass on valuable items to his successor cannot be discounted (Daniels & Sheail, 1999) and it is greatly encouraged by organizations such as Common Ground and Flora Locale and has been celebrated in Richard Mabey's *Flora Brittanica* (1999). As many restoration schemes are politically motivated (Buckley, 1989), it may be necessary to pay respect to this idea when considering seed procurement, whatever the outcome of scientific investigation.

7.7 Is using local seed practical?

If it decided that seeds of local provenance are to be used in a restoration scheme then there remains the question of whether this will be practical. In Chapter six it was suggested that restricting seed collection to local sources may mean that it is only possible to sow a narrow range of species or that little seed will available so that application rates are necessarily low. Unsurprisingly, the experimental work in this chapter indicated that vegetation establishment will be maximized by using a relatively high seed rate and a wide selection of species, preferably including representatives from the three functional types typically found on calcicolous grassland. However, it was shown that it is possible to initiate a plant community using relatively low seed rates although the community will be slow to develop. In the early stages of the experimental work at Swanworth Quarry described in Chapter six, areas sown with low seed rates were more vulnerable to invasion by weedy species. By the fourth year these areas were dominated by desired calcicolous species. As the field site was untreated and exposed, the substrate was nutrient poor and this may have restricted the growth of weedy species. It is uncertain if the same effect would be seen in a nutrient rich environment such as an arable restoration site. The work presented in Chapter six suggests that in low nutrient conditions it is practical to use a low seed rate although the process of colonization will be slow. In many situations this will be preferable to introducing non-local stock.

The number of species included in a seed mix will also affect the development of vegetation at a restoration site. In Chapter six, two mixes were sown, a basic mix containing 20 species and a preferred mix containing 35 species. The preferred mix resulted in a more diverse sward while the basic mix was not only less species rich but was also dominated by the legume *Anthyllis vulneraria*. The dominance of *A. vulneraria* was noted at the recreation of chalk grassland at Twyford Down in Hampshire (L. Ward, *Pers. Com.*) On plots sown with the preferred mix the dominance of *A. vulneraria* may have been prevented by intraspecific competition. Overall, the eveness of the sward was greater when the preferred mix was sown. Increased species richness is considered to enhance the stability of a vegetative community and has been cited as 'insurance' against environmental changes (Tilman & Downing, 1994; Loreau *et.al.*, 2001). Similarly, eveness has been linked to increased stability in ecosystems (Wilsey & Potvin, 2000). A species rich seed mix is, therefore, an advantage when carrying out a restoration project. If cost is an issue, then it is preferable to use a diverse mix applied at a low rate rather than a poorer mix applied at a high rate. This is restricted to low nutrient sites where weedy species are unlikely to persist. The effect of seed mix and rate on the development of vegetation on treated substrate was not investigated here.

Natural regeneration at Swanworth Quarry was investigated by using control plots in the experiment. By 2000 there was some vegetative cover on the plots and calicolous species were present. However, as these species may have been recruited from plants which were grown from sown seed, it is not possible to draw firm conclusions on the potential for natural regeneration at this site.

In situations where only a small amount of seed is available, the use of local seed should be considered carefully. Not only will local seed collection deplete the seed source for the area as a whole, it is possible that the restoration scheme may fail (if the conditions favour weedy species) due to invasion by weeds or dominance of particularly competitive species. Authors have suggested that the importance of implementing strategies such as local seed collection will depend upon the goals of the restoration project (Erenfeld, 2000; van Diggelen, 2001). Priorities may vary: Van Diggelen (2001) suggests that in some areas where the purpose is to stablise seriously degraded land then establishing vegetation cover of any kind should be a priority. Others consider that any introduction of non-local propagules should be avoided (A. Jones, *Pers. Comm.*). Ultimately decisions must be site specific. Certainly, if the goal of a project is the restoration of a plant community that is perceived to have high conservation value every effort should be made to use locally collected seed. But this must be balanced with the need to ensure the success of a restoration scheme. Success is important, not just for ecological reasons, but because it will have what van Diggelen (2001) has called 'societal implications'. The failure of a scheme reduces public confidence and, as funding to continue restoring areas of land frequently requires public support, success is an important outcome of any restoration scheme.

7.8 Summary of conclusions

The use of local propagules in restoration projects has been widely recommended by academics and conservation bodies: however little experimental work has been carried out to corroborate this. This thesis aimed to investigate the significance of provenance in restoration ecology. Using *L. corniculatus* as a model species, the following conclusions have been reached.

1. There were heritable differences in the morphology and fitness of propagules collected from different regions and raised in a common garden. Differences between propagules collected from two contrasting habitats and raised in a common garden were limited to morphology. This difference between the effects of region and habitat of origin on heritable plant fitness components was confirmed by an investigation into the relationship between geographical and ecological distance and the measured traits. Geographical distance was shown to be correlated with plant fecundity, i.e. difference in seed number produced by plants in the common garden increased as the distance between the origin of the plants increased. There was no relationship between ecological distance and any of the traits. This suggests that it is

important to consider the geographical distance between donor and restoration site. when selecting propagules for a restoration project. It will also be important to collect propagules form an ecologically similar site. Although there is no heritable difference in fecundity between plants sourced from contrasting habitats, the differences in morphology may affect long-term persistence in the field.

2. There were differences in the fitness of propagules sourced from different regions when grown in two contrasting environments (treated and untreated) at a restoration site. However, there was no consistent home-site advantage for local plants at either site although local plants survived better and were among the larger plants when grown at the treated site. There was also a weak negative relationship between geographical distance and plant fitness at the treated site. Local plants showed no evidence of higher survival or fitness at the untreated site. It is suggested that seed zones may be developed within which it is 'safe' to collect seeds, however it was not possible to determine a quantitative guideline from the work presented in this thesis.

3. An investigation into fine-scale phenotypic differences between *L.corniculatus* plants at 10 locations in the British Isles demonstrated that Linhart's (1995) suggestion that herbaceous species are collected within 100m of a restoration site is not necessary for this species; the limit can be safely extended to 200m.

4. There was no relationship between the ecological provenance of propagules and fitness on the treated site. However, on the untreated site ecological distance and plant size and fecundity were positively correlated. The resulting picture is unclear. It is possible that local plants growing on the untreated site are responding to the untreated conditions in a way that was not measured but was compensation for small stature and low seed number. Clearly, the ecological conditions of the donor site must be considered as any site may undergo periods of regular peturbation to which local may be adapted. Although seed zones could be developed to account for geographical distance, the importance of selecting an ecologically suitable site within those zones must be considered.

5 *L. corniculatus* is a variable species adapted to a wide range of conditions. The recommendations above may not apply to species with a narrow genotypic base or geographic range. There is a need for more research on a range of species and it is unlikely that a general guideline will be developed for all species.

6. If the central concern of a restoration project is to conserve local genotypes then local propagules should be collected.

7. An investigation into the practical implications of using local seeds demonstrated that the low seed application rates associated with local seed collection may not prevent succesful revegetation. Low seed application rates of 0.4 and 1.0 g/m^2 are sufficient to initiate restoration in low nutrient conditions. A higher application rate of 4g/m^2 will reduce invasion by other species, both weedy and desirable. It is important to include a wide range of species if possible as this leads to a more even, stable sward. Furthermore, it is important to include a range of functional species. In conclusion, it is possible to use locally collected seed if cautious in ground preparation and subsequent management practices.

7.9 Suggestions for further research

The effect of provenance on associated herbivores.

Results presented in this thesis and those from other studies (Keller *et al.*, 2001 and Hoare, 1999) suggest that plant provenance may affect the food choice and growth rate of associated herbivores. It would be useful to extend this research to investigate a suite of herbivores on more than one species of plant in both laboratory and field trials. This would indicate whether plant provenance is likely to affect the next trophic level at a grassland restoration site and would enable ecologists to further determine the success of re-vegetation.

The effect of provenance on the fitness of plants at a restored site over several years.

Data from a long-term experiment would indicate whether the effect of provenance persists or whether plants would perform similarly once established. There are constraints to recording long-term experiments. Cropping, although not always fatal in

L. corniculatus, may influence subsequent development, as will removing seed pods. Allowing plants to grow for a number of seasons before monitoring begins will allow natural development but it will be increasingly difficult to distinguish original plants as their progeny germinate and grow. Despite these constraints, long-term evaluation would further increase our understanding of the significance of propagule provenance.

Reciprocal transplant experiments between all sites.

Using reciprocal transplant experiments between all 30 sites used in this thesis was not possible. However, reciprocal experiments better distinguish between the general superiority of particular genotypes and the enhanced performance of plants from particular areas due to local adaptation. Using reciprocal transplanting Joshi *et al.* (2001) and Montalvo & Ellstrand (2000) were able to average data across sites. This resulted in the detection of local advantage even when some local genotypes were not superior. In general it was a more robust analysis and it would be useful to repeat this method using the sites in this study.

The limit of 'local' for L. corniculatus

There was no evidence to suggest that 100m constitutes 'local' for *L. corniculatus* and it was shown that plants appeared to be similar within 200m. It would be useful to extend the transect to 1.6km to see if the limits of what can be considered local can be identified at a larger scale.

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