# Ecology and population genetics of the neophyte alien, Hoary Mustard (Hirschfeldia incana (L.) Lagreze-Fossat).

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# Ecology and population genetics of the neophyte alien, Hoary Mustard (*Hirschfeldia incana* (L.) Lagreze-Fossat)

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A thesis submitted for the degree of Master of Philosophy

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# Summary

The invasion of natural habitats by alien plant species is now recognised as one of the most important factors contributing to the current loss of biodiversity in our planet. In the UK alone there are now approximately equivalent numbers of alien and native plant species (Stace, 1997), and a small number of these are spreading in rural and urban areas.

This study looked at *Hirschfeldia incana* (L.) Lagreze-Fossat belonging to the family Brassicaceae. Commonly known as Hoary Mustard, it is an established wool, grain and bird-seed alien in the UK. *H. incana* is native to southwest Europe, the Mediterranean region and southwest Asia. In the UK, this neophyte alien has shown a significant ($p=0.004$), exponential increase in its spread from 1930 to the year 2000.

In this investigation a number of *H. incana* populations in South Wales were studied, in terms of the communities they were associated with, in semi-natural and natural habitats. The three species most commonly associated with *H. incana* were *Senecio jacobaea*, *Holcus lanatus* and *Medicago lupulina*, all native to the British flora. Cluster analysis and TWINSPAN indicated three major types of habitats, open urban gap habitat, closed habitat (semi-natural) and sand dune habitat (natural). Species indicative of the three habitats were *Mycelis muralis* (open urban gap), *Euphorbia peplus* (closed) and *Ammophila arenaria* (sand dune).

Random amplified polymorphic DNA (RAPD) data was collected for England, Wales and southern European populations. Estimated genetic diversities were calculated using Shannon's Index, and showed that diversity was similar in native and alien populations ($p=0.271$ for $H'_j$, and $p= 0.018$ for $H_j$). The genetic diversities between populations compared well with those for other outcrossing plants. The distinct clusters of populations found in the British Isles together with the evidence obtained from the RAPD data suggests that founding populations probably originated from multiple source populations.

# Chapter 1

# General Introduction

## 1.1 Alien Invasions

An invading species has been described as one that "enters a territory in which it has never before occurred regardless of circumstance" (Mack, 1985). Therefore, an invasive alien plant is one that tends to dominate an area, thus excluding and crowding out other plants. This subsequently leads to a decrease in the biodiversity of the area that it is growing in and, in some cases, native plants become completely obliterated from an area, and all that is left are the invasive alien species. It must be noted, however, that these events take time. In fact, a plant may be alien to an area, yet not be invasive for some time, perhaps even as long as 100 years. Then some factor could change in the local environment or ecosystem, thus enabling the plant to then take on invasive characteristics. Many terms have been used to describe an invading species in the literature. Pysek (1995) described an alien species "as one which reached the area as a consequence of activities of Neolithic and post-Neolithic man or of his domestic animals".

The spread of alien invasive plants has been, and continues to be, recognised as an increasingly serious threat to biodiversity. This has been the case globally and locally, and has consequently led to the widespread study of these alien invasions. In fact, it now seems that there is no nature reserve in the world outside the Antarctica that is without introduced plant species (Usher, 1988). Nowadays, most invasions happen because of human activities, as the flow of commerce is much more widely spread and faster, and species travel in all directions. This human factor has increased over the last 200-500 years (Hodkinson and Thompson, 1997; Vitousek *et al.*, 1997) and this recent increase has been found to be due to the introduction of crop or pasture species (Lonsdale, 1994), horticultural plants (Auld and Tisdell, 1986) and also predators or pathogens used for biological control of weeds or crop predators and pathogens (Simberloff and Stiling, 1996).

One reason for studying invasions is that many invasive species have become serious pests. However, it must be noted that most successful invaders are not pests, and most invaders are not successful (Williamson, 1996). But still, even though most invaders fail and have small effects, the cumulative effects of those that do succeed has been, and will continue to be large.

Darwin (1996) recognised that not all non-indigenous species would successfully colonize their new environment. He theorized that native species are more suitably adapted to their environments than invaders and thus wrote, "we can see when a plant or animal is placed in a new country amongst new competitors, though the climate may be exactly the same as in its former home,...the conditions of its life will generally be changed in an essential manner".

The most well known invasive plant in the British flora is probably Japanese Knotweed (*Fallopia japonica* var. *japonica*), a species that was first introduced into the British Isles in the 1850s (Bailey and Conolly, 2000) and subsequently spread rapidly. It is now considered a pest in the British Isles and the abundance of this species in urban areas and the costs associated with it has given it much bad publicity. It has shoots that are able to push through asphalt and damage pavements and car parks, and it grows to heights that lead to reduced visibility along roadsides and railways. In addition, when growing along water courses, decaying shoots of this plant can cause blockages and increase the risk of flooding (Hollingsworth and Bailey, 2000). So serious are the consequences of the introduction of this pest in the

British Isles, that it is a criminal offence to knowingly introduce *Fallopia japonica* var. *japonica* to the wild, since 1981.

### 1.1.1. Invasions and disturbances

It is often said that invasions happen more readily in disturbed sites than elsewhere (eg, Williamson, 1996). Certainly they are more common there, and it might be thought that that tells us something about the biological nature of invasions. But it only reflects the fact that species are more likely both to be transported from disturbed areas and to arrive in them because of human activities. The role of soil disturbances by either man (Kotenen, 1997) or animals (McIntyre *et al.*, 1995) in aiding plant invasions, has been extensively studied. Soil disturbances create bare ground that directly control the abundance of invading species (Burke and Grime, 1996). Prieur-Richard and Lavorel (2000) found that the reason for this increase in invading species in disturbed areas is because of the increase in resource availability and a decrease in competition from resident species which can result in colonization by ruderal species or species with greater competitive abilities than the natives. In addition, Hobbs and Huenneke (1992) stated that the highest rates of invasion are particularly apparent when there are interactions between several types of disturbances. So not surprisingly, the greatest resistance to invasions was found in highly productive communities with moderate levels of disturbance, which also had the highest number of species (Prieur-Richard and Lavorel, 2000). Lonsdale (1999) found that all habitats could be invaded, including protected nature preserves and natural parks. In a review of plant invasions in US national parks and preserves, Vitousek *et al* (1997) demonstrated that non-natives contribute 50 % to 70% to the flora in Hawaiian reserves. Also, Usher (1988) in his study of 23 nature reserves worldwide, found that each contained at least one exotic vertebrate and several invasive vascular plant species. Interestingly, Usher (1988) and Lonsdale (1999) both demonstrated that the susceptibility of these "protected" environments to plant invasion was positively related to the number of visitors to the park (i.e. humans serve as a mechanism to increase transport of seeds). Similarly, Brothers and Springarn (1992), in their study of alien invasions of central Indiana old-growth forests, found that alien species richness and frequency dropped sharply inward from forest edges, and forest interiors were relatively free of aliens. This was mainly due to the low light availability in the forest interiors, though limited dispersal and low disturbance levels also played a part.

Disturbance has been defined in many ways by different authors. These have varied from Grime's (1979) view of disturbance as "a process removing or damaging biomass", to White and Pickett's (1985) definition of "any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability, or the physical environment". Furthermore, Petriatis *et al.* (1989) described disturbance as any "process that alters birth and death rates of individuals present in the patch". Disturbances are not only caused by physical events, such as fires, storms and floods, but also by other events such as altered grazing regimes or nutrient inputs, which affect resource levels and demographic processes.

The frequency of disturbances is another important aspect that has to be taken into account. In fact, it has been found that the time interval between successive disturbances can have profound effects on plant communities (Hobbs and Hueneke, 1992). The reason for this is that once a disturbance event has taken place, the species in the community take time to adjust to the changes and recover in terms of reproductive maturity. In the meantime, if a second disturbance occurs, then there may not be re-colonization of the patch due to a lack of availability of propagules.

### 1.1.2. Invasions and plant communities

Species richness has also been put forward by investigators as a factor affecting invasiveness. In fact, studies have shown that there are positive (McIntyre *et al.*, 1988); Robinson *et al.*, 1995; Palmer and Maurer, 1997) and negative (Tilman, 1997) relationships between the species richness of a plant community and the number of invading species. McArthur and Wilson proposed the Basic Island Biogeography Theory in 1967 which states that the rate of invasion of a region or community is the net balance between rate of immigration (both intentional and accidental introductions) and the rate of extinction of newly introduced species. The extinction of these newly introduced species is accounted for by two main mechanisms; the competition from native vegetation and mortality from herbivory or pathogens (Mack, 1996). However, it has been shown that the composition of communities might be more important than richness for the functioning of communities (Prieur-Richard *et al.*, 2000). For example, species identity was found to be more important species richness in the productivity of North American grassland (Symstad *et al.*, 1998). This was also found to be the case in the invasion by seed-rain of British grasslands (Crawley *et al.*, 1999). However, Prieur-Richard *et al.* (2000) found a negative relationship between the biomass of *Conyza bonariensis* and species richness, thus following the hypothesis that increased species richness increases the resistance of plant communities to invasion. Also, they found that functional richness had no effect on the invasiveness of *Conyza bonariensis*.

### 1.1.3. Invasions and reproductive strategies

Reproductive strategies have been of interest when considering invasive species. Self-fertilizing and apomictic species, in particular, are the most common strategies found in successful colonizing species (Brown and Marshall, 1981; Price and Jain, 1981). This was demonstrated in the large, perennial species of *Polygonum* and also in species of *Fallopia* which have been introduced into the British Isles from eastern Asia and the Himalayas as decorative horticultural plants, and show varying degrees of ability not only to persist out of cultivation but also to naturalize and compete with natural vegetation by means of vegetative spread (Conolly, 1977). However, it is not unknown for an outcrossing species to be a successful colonizer as was seen in the case of *Echium plantagineum* with high levels of multi-locus genotypes in its introduced range (Burdon and Brown, 1986). Within a self-fertilizing invasive plant species less variation is likely to be present than in an invasive outcrossing species.

Polyploidy is also considered an important factor in plant invasions. In fact, polyploidy is commonly found among successful colonizing plants, and polyploids are believed to have wide environmental tolerance (Stebbins, 1971; Roose and Gottlieb, 1976; Clegg and Brown, 1983; Barrett and Richardson, 1985). Many successful colonizing species are polyploid, such as *Knautia* spp. (Ehrendorfer, 1965), *Claytonia perfoliata* (Stebbins, 1965), *Ageratum* spp. (Baker, 1965), *Deschampsia caespitosa* (Rothera and Davy, 1986), and *Eichornia* (Barrett, 1988b). However, other studies have shown a correlation between diploidy and colonizing ability, such as in the case of *Eupatorium microstemon* aggregate where diploids appeared to be better weeds than polyploids (Baker, 1965). Moreover, diploids and polyploids could also be equal in their ability to colonize (Barrett and Shore, 1989). Therefore, it seems that although there is an association between polyploidy and colonizing ability, it is not the case for all species.

Hybridisation is an important component of plant evolution, and is commonly found in natural populations (Rieseberg and Ellstrand, 1993). Alien species have been known to hybridise with native species or other introduced species. In fact, in the *New Flora of the British Isles,* Stace (1997) has pointed out 770 angiosperm hybrids of which 58 involve at least one non-native taxon, and 12 are hybrids between two introduced species. Colonization events bring together introduced species that would be unlikely to come together otherwise due to their different native environments (Hollingsworth and Bailey, 2000).

It has been found that the genetic structure of an invasive population determines how effective control methods are. Burdon and Marshall (1981) reported that it was easier to control asexually reproducing weeds by biological means than sexually reproducing ones. The reason for this was given as the different population genetic structures associated with the two reproductive methods. Asexual species tend to be genetically more homogenous, which makes it easier to match a biological control agent to their host genotype, making them vulnerable to these control methods (Van Driesche and Bellows, 1996). Sexually reproducing weeds, on the other hand, have greater genetic variation, which might allow them more rapid adaptive evolution and escape from the biological control agent.

### 1.1.4. Enhanced performance and vigorous growth in introduced ranges

Crawley (1987) first quantified data for European plants in Europe and California and found that aliens were larger in California. This has subsequently resulted in numerous studies looking at the size of alien plants in their introduced range as compared to their native range (Memmott and Foresster, 2000; Thebaud and Simberloff, 2001). It has been found that plant species grow taller and have higher reproductive capacity where they are non-indigenous invaders than where they are native. Traditionally, the reason for this increase in size has been accepted as a plastic response to a benign environment, though recently this assumption has been challenged and a genetic basis for increased plant size has been suggested. Memmott and Forrester (2000) tested the hypothesis that the increase in size is genetically, rather than environmentally, based. No significant differences were found in the size of *Carduus nutans*, *Digitalis purpurea*, *Echium vulgare* or *Senecio jacobaea* sampled from alien (Australia and New Zealand) or native (Britain and continental Europe) habitats. They concluded that it is quite rare for the increase in size to be explained genetically, and is probably due to a plastic response to a novel environment. It has been suggested that this increased growth in non-indigenous plants is caused by plants shunting more of their resources into defence against herbivores in their native ranges, so that fewer resources are available for growth and reproduction (Blossey and Notzold, 1995; Blossey and Kamil, 1996). This has led to the proposal of the evolution of increased competitive ability (EICA) hypothesis that predicts that genotypes from a plant's introduced range ('invasive genotypes') grow faster and produce more seeds, but are not as well defended from enemies as genotypes from the native range ('native genotypes') (Blossey and Notzold, 1995). Therefore, there is a reduced allocation of resources to defence and increased allocation to growth and/or reproduction (Blossey and Notzold, 1995; Daehler and Strong, 1997; Willis *et al.*, 1999). This decrease in defence mechanisms has been proven in greenhouse experiments (Daehler and Strong, 1997), but in the field the results have been inconclusive (Blossey and Notzold, 1995; Willis *et al.*, 1999).

Wolfe (2002) proposed the escape-from-enemy hypothesis, and said "successful biological invaders often exhibit enhanced performance following introduction to a new region". The reason for this was that the natural enemies, such as competitors and predators, that were present in the native range, were probably absent from the introduced range. Wolfe demonstrated this using *Silene latifolia*, which was found to be 17 times more likely to be damaged, by generalist enemies, in its native Europe than in its introduced range in North America. Lodge (1993) reinforced this concept by the idea that interactions with other trophic levels (e.g. herbivore pressure) play an important part in determining the resistance of communities to invasion.

### 1.1.5. Genetic structure of invasive species

Genetically depauperate populations are thought to be characteristic of introduced species due to founder effects (Mayr, 1963). This genetic bottleneck effect is maintained due to a lack of recurring gene flow with other populations of the species. Examples of introduced species that have undergone a reduction in genetic diversity include *Xanthium strumarium* (Moran and Marshall, 1978), *Avena barbata* (Clegg and Brown, 1983), *Chondrilla juncea* (Burdon *et al.*, 1980) and *Sorghum halepense* (Warwick *et al.*, 1984). However, low genetic diversity in introduced populations is not always found, as was shown with *Avena barbata* in California (Clegg and Allard, 1972), *Bromus mollis* in Australia (Brown and Marshall, 1981), and *Trifolium hirtum* in California (Jain and Martins, 1979), all of which indicated levels of genetic variation in the introduced range similar to those in the native range. Warwick *et al.* (1987) suggested that this similarity in genetic variation could not be explained as the result of many colonization events alone, but could have been due to evolution subsequent to the initial stage of colonization by a few genotypes. According to theory, introduced species are expected to show lower levels of intrapopulation diversity and higher levels of population differentiation as compared to the native members (Brown and Marshall, 1981). Many studies have suggested that the genetic structure of colonizing populations is determined by the size, composition and dynamics of the founding population(s) (Nei, Maruyama and Chakraborty, 1975; Barrett and Richardson, 1986; Barrett and Shore, 1989). So, if the founder population contain a small number of individuals, is genetically depauperate and remains small in size for generations, then the effects described by theory will be more obvious. On the other hand, if there are multiple founder populations, which are large and diverse, and which expand rapidly following introduction, the effects will be minimal. This reduction in genetic diversity can have two consequences. First, the population growth may be limited due to inbreeding depression and lower the chances of the population persisting (Ellstrand and Elam, 1993; Newman and Pilson, 1997). Secondly, reduced genetic diversity will limit the ability of the species to evolve, as the invading species may be pre-adapted to some features of its new environment but other features will be novel (Nieminen *et al.*, 2001). Therefore, it seems that adaptive evolution following initial colonization is just as important as pre-adaptation for successful invasion (Sakai *et al.*, 2001).

There have been few studies comparing the genetic diversities of native and alien populations, and those have shown conflicting evidence for founder effects (Sun, 1997). Some studies have shown no significant differences in genetic variation between native and alien populations (Brown and Marshall, 1981; Antrobus and Lack, 1993), while others have shown a higher genetic diversity in alien populations (Harding and Barnes, 1977). However, many other studies have reported depauperate genetic diversities both between and within alien populations (Brown and Marshall, 1981; Barrett and Richardson, 1985; Warwick, 1990).

A lag time between initial colonization, and rapid population growth and range expansion is commonly found as a feature of invasions (Mack, 1985). During this lag time evolutionary change can occur such as the evolution of adaptations to the new habitat, the evolution of invasive life history characteristics, or the purging of genetic load responsible for inbreeding depression (Sakai *et al.*, 2001). In addition, it has been suggested that the lag times found in invasions may be the result of the time taken for adaptive evolution to overcome genetic constraints (Ellstrand and Schierenbeck, 2000; Mack *et al.*, 2000). Different rates of colonization rates have been found among three *Echium* species in Australia (Forcella and Harvey, 1983), where *Echium plantagineum* spread faster than *E. vulgare* and *E. italicum*. However, these species had three different times of onset of spread, and it is suggested that the slowly spreading species may have reached their distribution limits.

Multiple introductions have dual effects on introduction, as they will influence the rate of spread as well as the genetic variation (Novak and Mack, 1995). When founders arrive in isolated geographical locations the genetic differentiation is likely to be high among the populations (Brown and Marshall, 1981). In species with a uniparental mating system (self-pollination or apomixis) with low levels of gene flow, this differentiation would be maintained (Hamrick, 1987; Barrett and Husband, 1990). The combination of uniparental mating systems and little gene flow in alien species leads to low levels of genetic variability within populations (Barrett and Richardson, 1986; Barrett and Shore, 1989). In addition, offspring of apomictic species benefit from the maintenance of heterozygous genotypes (Williams, 1975; Barrett and Richardson, 1986).

### 1.1.6. The effects of global change on plant performance and migration

Another reason for studying potential ranges of invasive species is the effects that global change has on plant performance and migration. For instance, fossil fuel combustion and deforestation leads to a rise in temperature that will probably affect the distribution of the vegetation zones and the geographical distribution of individual species (Emanuel *et al.*, 1995).

Therefore, the species most likely to respond to global warming are those that are already spreading, such as invasive species and weeds. This is because such species are adapted to varying environmental conditions caused by disturbance and can tolerate a wide range of climatic conditions. In fact it has been suggested that global warming plays a role in enhancing the spread and establishment of invasive species, and leads to a faster rate of migration in these species than in native species (Mooney and Hofgaard, 1999).

## 1.2 *Hirschfeldia incana*

In the UK alone there are now more alien species than native species (Stace, 1997). A small number of these are spreading in rural and urban areas. This study investigates one such species, *Hirschfeldia incana* (Figure 1.1).

**Figure 1.1. *Hirschfeldia incana* seen in a typical its typical habitat in a waste ground in Swansea**

*Hirschfeldia incana* is an established wool, grain and bird-seed alien in the UK. It belongs to the family Brassicaceae, and is commonly known as Hoary Mustard. *H. incana* is a biennial plant, which is branched from the bottom to the top (Figure 1.1). The leaves are produced in a basal rosette and are arranged close to the ground (Figure 1.2). Flowers are yellow and typical of the family. These are cruciate and tertradynamous (Figure 1.3). The fruit is adpressed to the stem, swollen around the seeds, and narrowing distally (Figure 1.4). There are a few other yellow crucifers with fruits adpressed to the stem, and *H. incana* is distinguished from these by the very small size of its fruits. It is frequently confused with *Brassica nigra*, which however has a seedless beak to the fruit and larger petals and fruits (Rich, 1991). The plants grow to over 1 metre in height.

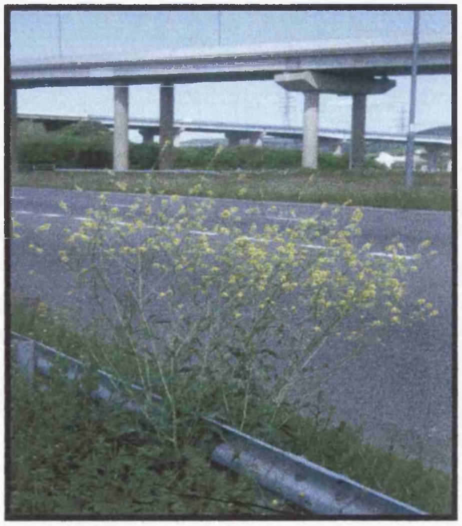
*H. incana* is diploid annual or short-lived perennial herb found growing on lowland. It is locally abundant on waste ground, roadsides (Figure 1.5), shingle beaches and dunes, and by railways (Rich, 1991). It is well-established in many places and probably overlooked as another yellow crucifer.

**Figure 1.2 Rosettes growing flat on the ground**

**Figure 1.3 Yellow, tetradynamous flowers**

Figure 1.4 Fruits adpressed to the stem

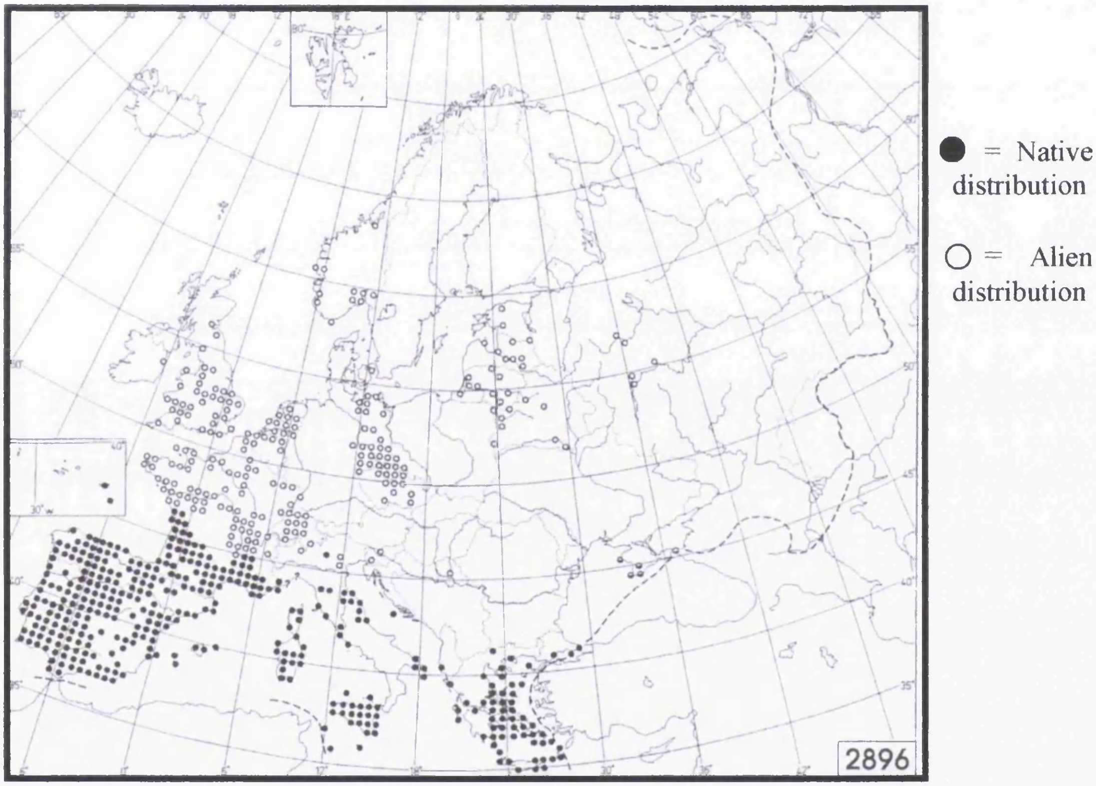
Figure 1.5 *H. incana* on a roadside verge.



### 1.2.1 Native Distribution

*H. incana* is native to southwest Europe, the Mediterranean region, southwest Asia and is widely naturalised further north in Europe (Figure 1.6). Here it is a weed in arable fields and along roadsides, and colonises wild habitats on the Atlantic coast and some inland locations in natural plant communities on sandy riverbeds.

**Figure 1.6 Native distribution of *H. incana* (Atlas Florae Europaeae)**



*H. incana* was cultivated in Britain by 1771, and was recorded from the wild as far back as 1837.

## 1.3. Molecular markers

Molecular and biochemical techniques provide a powerful set of tools for the study of plant population genetics (Chalmers *et al.*, 1992). Many studies have used restriction-site diversity to investigate population genetic structure (Clegg, 1989a,b). One of the most frequently used DNA marker has been the restriction fragment length polymorphism (RFLP) (e.g. Sebastian *et al.*, 2000; Foisset *et al.*, 1996; Zhang *et al.*, 1993). The RFLP assay due to requiring large quantities of relatively pure DNA and species-specific DNA probes, as well as being laborious and requiring considerably more DNA than PCR-based (polymerase chain reaction-based) methods, is impractical for many population-based studies. Allozymes, on the other hand, can be limited by the number of detectable loci, or lack of variable loci when compared to other molecular markers (Liu and Furnier, 1993; Waycott, 1995; Lanner-Herrera *et al.*, 1996; Spooner *et al.*, 1996).

The development of the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) has revolutionized the analysis of nucleotide sequence variability, but a major limitation of this method has been the requirement of DNA-sequence information (Innis *et al.*, 1990). Therefore, Williams *et al.* (1990) and Welsh and McClelland (1990) devised a new method for the identification of polymorphism in plants based on PCR, which is not dependent on prior knowledge of DNA sequence. The random amplified polymorphic DNA (RAPD) is a PCR-based method based on the amplification of unknown DNA sequences using single, short, random oligonucleotide primers. The RAPD method has been used in many fields including studying genetic diversity (Waycott, 1995; Palacios and Gonzalez-Candelas, 1997), genetic fingerprinting (Wilde *et al.*, 1992) and linkage maps (Rafalski *et al.*, 1991).

However, RAPDs, like all other molecular techniques, has its limitations and drawbacks. RAPDs are often found to have poor reproducibility, but this can be overcome by optimisation of the reaction conditions (Weising *et al.*, 1995). In fact, it has been found that since RAPDs are PCR-based slight changes in the reaction conditions may affect the reproducibility of amplification products (Willliams *et al.*, 1990; Arnold *et al.*, 1991). This technique is sensitive to the type of polymerase used, the $Mg^+$ concentration and the temperature profile. The first two can be optimised in the reaction mixture, while the latter is a property of the thermal cycler and must be standardized. The primer size is another factor of the PCR-based RAPD method that determines the specificity of the technique. In fact, studies that have used standard RAPD conditions with fragment separation on agarose gels have found 10-bp primers to be the optimal size (Hadrys *et al*, 1992). Beyond a certain primer size increasing primer length may also increase non-specific primer annealing, consequently increasing the probability random non-reproducible amplification patterns. As with other genetic markers, some RAPD fragments may be ambiguous and not easy to detect (Williams *et al.*, 1990), and as a result are not useful as genetic markers. However, many studies (e.g. Arnold *et al.*, 1991; Hu and Quiros, 1991; Williams *et al.*, 1990) have shown that if the RAPD amplification is repeated two or more times then the majority of the markers are reproducible and scorable. In addition, in some cases the amplification products are found even in the absence of template DNA in the reaction mixture (Innis *et al.*, 1990; Klein-Lankhorst *et al.*, 1991). This appearance of 'ghost' bands can be solved by adding template DNA to the reaction mixture. The most well-known drawback of RAPDs are their dominant nature, limiting their use to studies where the knowledge of heterozygosity is not required (Hadrys *et al.*, 1992). However, RAPDs are competitive with RFLPs even in analyses of genomes with high levels of heterozygosity (Williams *et al.*, 1990; Carlson *et al.*, 1991). Therefore, it seems that RAPD fingerprinting has the potential for a wide range of applications, and has the added advantage of requiring the least in technology, labour and expenses.

### 1.4. Aims of this study

(a) To study the spread of *H. incana* in the UK.

(b) To investigate the different types of communities *H. incana* is found in and species associated with it.

(c) To investigate the different habitats invaded by *H. incana* in South Wales, and, possibly deduce its mode of dispersal.

(d) To attempt to group the habitats in order to deduce whether some habitats are more prone to invasion by *H. incana.*

(e) To study the role of disturbances in the invasion of *H. incana* in South Wales sites.

(f) To use random amplified polymorphic DNA (RAPD) markers and polymerase chain reaction (PCR) to identify polymorphic loci in the native southern European and introduced UK samples.

(g) To compare genetic diversities of native and introduced samples.

(h) To compare the partitions of gene diversities for all geographical groups of locations.

(i) To determine relatedness of different populations of *H. incana.*

# Chapter 2

# Ecology and Biology

## 2.1. Introduction

### 2.1.1. Distinguishing between Native and Non-native

The invasion of natural habitats by alien species is now recognised as one of the most important factors contributing to the current loss of biodiversity on our planet. In the UK alone there are now almost as many alien species as native species (Preston *et al.*, 2002) and some of these are spreading in rural and urban areas. *Hirschfeldia incana* is one such alien species that has spread dramatically in the UK.

In order to determine whether a species is an alien, before anything else, it is useful to distinguish it from a native species. Usher (2000) proposed that there are "shades of nativeness" between the two extremes, native and non-native. A classification was devised by the Scottish Natural Heritage to define the categories that might fall between these two extremes, namely, native, formally native, locally non-native, long-established, recently arrived and non-native. Native species are presumed to be those that occur in Great Britain due to natural means e.g. *Bellis perennis*. Formerly native species, as the name suggests, are those that are no longer present in Great Britain and have become extinct, since the last ice age. Hence, many of the species in this category became extinct because the environment changed. Other species became extinct due to human activities e.g. *Trichophorum alpinum* whose only British site was destroyed by marl digging in the 13 years following the species' discovery in 1791 (Lusby, 1998).

Locally non-native species are those that are native in parts of Britain but have been introduced locally in the area being considered e.g. *Luronium natans* is native in

England and Wales but not native in Scotland where it has been introduced in at least three sites (Stewart *et al.*, 1994). Long-established species have been described by the Scottish natural Heritage as "naturalised", as they have become part of the food webs where the majority of the species are native. An example of these is *Acer pseudoplatanus*, which was probably introduced by the Romans two thousand years ago (Salisbury, 1961).

Recently arrived species, on the other hand, are those where there is a lack of understanding about whether they arrived "naturally" or whether they have been introduced through human activity. As a result, this category is considered temporary pending sufficient evidence that would allow them to be classified in either category 1 (native) or category 6 (non-native). An example of this is *Serapius parviflora* found in Cornwall in 1989, which has had contrasting descriptions to its status depending on different researchers. Sell and Murrell (1996) and Stace (1997) believed that this orchid species was deliberately introduced, whereas French *et al.* (1999) considered that it was likely to be natural colonisation because it occurs in dry maritime grassland on islands off the Brittany coast. Finally, non-native species have been described as those that have been bought to Great Britain by people, either intentionally or unintentionally.

Webb (1985) also attempted to devise a series of criteria which could be used to determine whether a species is native or not. His eight criteria were as follows: fossil evidence, historical evidence, habitat, geographical distribution, frequency of known naturalisation, genetic diversity, reproductive pattern and possible means of introduction. Preston (1986) added a ninth criterion, namely, entomological evidence.

Williamson (1996) proposed the tens rule to explain introductions. It basically states that 10% of feral (introduced) invaders become established, and 10% of those established become pests. For plants, it predicts that 10% of those imported escape to become introduced (feral).

Macpherson *et al.* (1996) drew up definitions, which are used by Preston *et al.* (2002) in the *New Atlas of the British and Irish Flora.* There, a native species is defined as "one which arrived in the study area without intervention by man, whether intentional or unintentional, having come from an area in which it is native or one which has arisen *de novo* in the study area", while an introduced species is "one which was brought to the study area by man, intentionally or unintentionally, even if native to the source area or one which has come to the area without man's intervention, but from an area in which it is present as an introduction".

### 2.1.2. Archaeophytes and Neophytes

Recently, non-native species have been classified into archaeophytes and neophytes, denoting when they first became naturalized. Archaeophytes are described as those plants that became naturalized before AD1500, while neophytes are those that were first introduced after AD1500 (Preston *et al.*, 2000; Hipkin, 2003). Neophytes could have been present before AD1500 but only as casuals, and subsequently naturalized as a result of re-introductions (Preston *et al.*, 2000).

Archaeophytes include plants that are not usually as problematic species, although some exceptions lie in this group such as Ground-elder (*Aegopodium podagraria*). Neophytes include several species that have become pests such as the Japanese Knotweed (*Fallopia japonica*).

Hipkin (2003) has further divided the neophytes into three groups depending on their impact on areas of conservation importance. These are high-impact, low-impact and no-impact neophytes. High-impact neophytes are described as "aggressive species that invade and colonise natural and semi-natural habitats", low-impact neophytes are described as those that invade these habitats but merge into the communities, enhancing their biodiversity without actually dominating them, and finally no-impact neophytes are those which, as their name suggests, have no (or negligible) impact on natural and semi-natural communities (Hipkin, 2003). Japanese Knotweed (*Fallopia japonica*) is probably the best example of a high-impact neophyte that has become a nuisance in a number of semi-natural communities, and consequently prompted widespread research into its control. Examples of low-impact neophytes are *Conyza canadensis*, *Epilobium brunnescens* and *Hirschfeldia incana*, all of which are spreading dramatically in the British Isles. Finally, *Veronica filiformis* and *Galinsoga parviflora* are good examples of low-impact neophytes that have almost no effect on the natural and semi-natural communities, and thus do not trigger cause for concern.

### 2.1.3. Members of plant families as invaders

It would be useful if we could understand those characteristics that make a species a successful alien invader. Are members of some families likely to be more invasive than those of other families? It has been found that the influence of Man on the British flora since the middle ages has resulted in introductions which "have been drawn from a wide and largely unpredictable spectrum of families and life forms" (Gray, 1986). They include escaped ornamental shrubs, such as *Rhododendron ponticum*, *Buddleja davidii*, *Fallopia japonica* and *Symphoricarpos albus*, as well as aquatic and wetland species, such as *Impatiens glandulifera* and species of *Elodea*. Pysek (1998) analysed the alien floras of 26 regions distributed over the globe and covering a variety of habitats to assess whether there was a taxonomic pattern to plant invasions. This study recorded alien species in 164 families of which, it was found, that the best invaders belonged to the families Papaveraceae, Chenopodiaceae, Amaranthaceae, Brassicaceae (*Hirschfeldia incana* belongs to this), Polygonaceae and Poaceae. Furthermore, Pysek proposed that the most successful families possess specific features that could be attributed to their invasiveness. However, it was also found that no simple morphological, physiological or ecological character could be generally related to the invasiveness of a family.

There is no doubt about the fact that some species make better invaders than others. Pysek (1997) analysed records of alien species in 26 local floras all over the world. In particular, this study looked at the representation of the Asteraceae in these floras and these were compared with that of other families that contribute most to the alien floras. It was found that members of the *Asteraceae* make up, on average, 13.5% of the alien floras, which makes it the second most represented family after Poaceae. Members of this family are also over-represented among aliens in the world flora. The *Asteraceae* is a remarkably successful family in terms of dispersal and establishment.

Reynolds (1996) studied alien plants at ports in coastal habitats on the east coast of Ireland and found that the well-established aliens found on less disturbed waste ground were *Buddleja davidii*, *Centranthus ruber*, *Epilobium ciliatum*, *Hirschfeldia incana*, *Hordeum murinum*, *Matricaria discoidea*, *Melilotus officinalis*, *Senecio squalidus*, *Sisymbrium orientale* and *Rapistrum rugosum*. These were found amongst a limited range of common native plants. In addition, the crucifers, *Rapistrum rugosum* and *Hirschfeldia incana* were thought to have been introduced through ports with grain. In fact, in Ireland, *H. incana* is found largely confined to Dublin and surroundings.

### 2.1.4. Plant invasions and disturbance

Disturbance seems to be one of the key features contributing to plant invasions. This, together with high fertility and high propagule influxes, is the reason why riparian habitats are considered to be so susceptible to plant invasions (De Ferrari and Naiman, 1994; Tabacchi, 1995). Disturbances act to promote invasions by non-native and weedy plants in plant communities (Hobbs, 1991). Petriatis *et al.* (1989) proposed the intermediate disturbance hypothesis, where it was pointed out that the highest species numbers are found when disturbances occur at intermediate frequencies. The reason behind this hypothesis was that most species can survive in regions with an intermediate frequency of disturbance, while only a few species can survive in areas of frequent, severe disturbances, or even when there is no disturbance at all.

There have been contrasting views explaining the role of disturbances in invasions. One view is that high levels of disturbance may increase invasibility within communities (Horvitz *et al.*, 1998), while another view indicates that the scale of disturbance and local species diversity are as important as the scale of disturbance (Levine, 2000). Vitousek *et al.* (1996) found that human-mediated disturbance into natural communities results in an increase in the range of characteristics that are responsible for successful colonization. This in turn leads to an increase in the frequency of invasion into existing communities.

However, it is essential to look at the effect of disturbances on communities over a longer period of time, in order to determine the true role it plays in invasions. In fact very few investigators have attempted to study invasion by manipulating either the characteristics of the invaded community or the identity of potential invaders (Robinson *et al.*, 1995; Tilman, 1997; Crawley *et al.*, 1999; Levine, 2000), over long periods of time. One such study was carried out by Thompson *et al.* (2001) who studied grasslands in order to investigate plant traits and temporal scale. They found that at the early stages of the experiment, while the individuals were still juveniles, the distribution was mainly dependent on disturbance. As time went on, disturbance became less important and invasion seemed to focus more on tapping resources. These results were found to be consistent with the hypothesis put forward by Davis *et al.* (2000), which stated that invasibility is correlated with the availability of unused resources.

### 2.1.5 Plant invasions and communities

Many studies have found that most communities are susceptible to invasions by exotic species (Usher, 1988; Lodge, 1993a; Gordon, 1998), but the levels of susceptibility differ between communities. Sakai *et al.* (2001) found that the reason for some communities being more likely to be invaded than others could be due to the way species composition, functional groups present in the community, and trophic structure interact with one another. Thus, mechanisms of invasion need to be looked at from the point of view of the community that is subject to invasion. Very few studies have addressed this issue (Prieur-Richard and Lavorel, 2000). Characteristics such as the diversity of the plant community could offer insights into aspects that lead to invasibility. However, both negative (McCloskey *et al.*, 1996) and positive (McIntyre *et al.*, 1988) relationships have been reported between community invasibility and plant species diversity. Stohlgren *et al.* (1999) found that the scale of the study could explain these contrasting results as the opposite was found when going from the local to the regional. For example, the invasibility of a Californian weed grassland by introduced seeds of a grass and a dicotyledon was positively related to the number of resident species (Robinson *et al.*, 1995).

Vacant niches have been considered as an important factor contributing to successful invasions, due to the fact that successful invaders occupy vacant niches in the community, while other species are excluded from the community as their niches are already occupied (e.g. Williamson, 1996). Many studies have found that the reason for a species to be invasive depends on the traits it possesses that are different from or similar to those of the native (Mack, 1996; Levine and D'Antonio, 1999). This would allow the invasive species to occupy "empty niches". However, there have been studies where this view of "vacant niches" is disputed (e.g. Herbold and Moyle, 1986), where it has been suggested that vacant niches are not actually empty but occupied by species that do not interact with the members of the communities. This means that a species, which is not a member of the community, in the "vacant niche" uses resources that are not needed by the species in the community. Hence, the invading species will be able to occupy this niche and take resources that the other species do not use. Taking this view into account, it seems that invasions cannot involve occupying "empty" niches.

### 2.1.6 Biotic homogenisation

Biotic homogenisation is an issue that has recently been considered in relation to invasions. McKinney and Lockwood (1999) investigated biotic homogenisation in relation to a few winners replacing many losers in the next mass extinction. They proposed that human activities are not random in their positive and negative impacts on biotas. Evidence shows that most species are declining as a result of human activities, referred to as 'losers'. The 'winners' are those that are replacing the 'losers' and those that thrive in human-altered environments. However, some species will benefit from disturbances (Morris and Heidinga, 1997). Such species will expand their range and replace those species that cannot survive in the face of persistent disturbance. Therefore, biotic homogenisation has been defined by Baskin (1998) as a phenomenon that occurs when a widespread environmental change promotes the geographic expansion of some species ('winners') and the geographic reduction of others ('losers').

This process of biotic homogenisation is now occurring on a global scale because of reasons that are two-fold, environmental modification and transportation of exotic species (Williamson, 1996). Environmental modification leads to the loss of local endemic species that cannot tolerate human activities, while increased global transport promotes the spread of non-indigenous species (Lockwood, 1999). Many of these non-indigenous species will thrive in disturbed environments, while some will invade. These invading species will in turn homogenize relatively undisturbed natural areas (Williamson, 1996). More interestingly, homogenisation could increase on a continental scale if there is an increase in species extinction (Myers, 1997) and species introductions (Williamson, 1996).

Brown (1989) stated that 'geographically restricted species with sensitive requirements will continue to have high extinction rates while those widespread broadly tolerant forms that can live with humans, and benefit from their activities, will spread and become increasingly dominant'.

### 2.1.7. The success and failure of invasions

Introduced species will succeed or fail due to two processes, boom and bust, and propagule pressure (Williamson, 1996). Boom and bust is described when a species goes to its peak of density and then declines. This has been observed rarely in Britain (Williamson and Brown, 1986) and an example of a boom and bust species is *Elodea canadensis* (Canadian pondweed) (Simpson, 1984). Propagule pressure is another important reason to be considered, as increasing the number of propagules, increases the chances of a species establishing. One reason for this is because if only a few individuals are introduced, they may be dispersed far enough from each other that they do not get the chance to come together in order to be able to reproduce. On the other hand, if many individuals are introduced there is a greater chance of some of the individuals finding a suitable habitat.

The presence of enemies in the introduced range has often been discussed by investigators, as an abundance of enemies prevents invasions, while a lack of enemies allows invasions to happen. Most of the evidence put forward has been anecdotal but studies looking at biological control show that sometimes parasites can severely depress the population of an invasive species. Richardson *et al.* (1992) looked at Australian plants introduced into South Africa and vice versa. These plants were found to be imported with few of their natural enemies. It was found that *Acacia longifolia*, a tree, produces 5.6 viable seeds per $m^2$ in its native Australia, and *Hakea gibbosa*, a shrub, produces four times the number of seeds per plant in South Africa as in Australia. However, Weiss and Milton (1984) found that *Chrysanthemoides monilefera*, found growing on Australian sand dunes, produces 2500 seeds per $m^2$, of which 2000 are viable, a number which is similar to that found in its native South Africa (2300 seeds per $m^2$). This might suggest that a lack of enemies is important in moulding an invader into a pest.

It has been suggested that the size of a species' range could decide how successful an invader could be. In fact, Daehler and Strong (1993) believed range to be one of the best predictors of success in invasion. Many studies have looked at this predictor, one of which found a positive relation of range size and invasion success in passerines in Hawaii (Moulton and Pimm, 1986). Roy, Navas and Sonie (1993), on the other hand, found a positive correlation between the number of native climatic zones and the number of continents invaded in brome grasses.

A species climatic range is another important aspect that requires consideration with respect to invasions. Questions that need to be addressed are whether the limits of climate in the native range predicts the limits of an invasion, and whether a species can invade an area with a different climate from that it is used to. Obviously, all species have a restricted range and so can live in certain climates but not in others.

This however, is not conclusive as species can invade new climates, and can also be successfully introduced to new climates (Williamson, 1996). For example, Griffin and Critchfield (1976) looked at the Monterey pine, *Pinus radiata*, which has a small, restricted range on the coast of California, but is a very successful forest tree in many parts of the world. It is also an invasive species in South Africa (Richardson, Williams and Hobbs, 1994). Wilson *et al.* (1992) compared the climate range of exotic plants in New Zealand with that in Europe. It was found that some species, such as *Echium vulgare*, *Onopordum acanthium* and *Senecio jacobaea*, had closely matched climatic ranges, whereas others such as, *Verbascum virgatum*, showed considerable differences when the climates in their native and introduced ranges were compared. In the UK, Himalayan Balsam (*Impatiens glandulifera*) is a good example of a species with a restrictive native range, where it is endemic to a small area of the western Himalaya. In the UK, on the other hand, it is found along river banks, canal banks and ditches (Wade *et al.*, 1988). This tells us that climatic matching, whilst needing investigation when looking at invasions, is a weak indicator.

### 2.1.8. Are alien plants larger in their introduced range?

Crawley (1987) observed that plants in alien environments tend to be more vigorous and taller, producing more seeds than in their native distribution. For example, seed production of *Chrysanthemoides monilifera* (native to South Africa) in Australia and of *Acacia longifolia* (native to Australia) in South Africa is significantly higher than in their native range (Noble, 1989). This success of invaders in their introduced range is due to two reasons, the environment being more favourable and the plants having escaped their natural phytophagous enemies (Crawley, 1987). Two models have been proposed which explain these two reasons, the optimal defence hypothesis and the environmental constraint hypothesis. The optimal defence hypothesis predicts that plants with limited resources will allocate them among maintenance, growth, storage, reproduction and defense (Coley *et al.*, 1985; Bazzaz *et al.*, 1987; Fagerstrom, 1989; Herms and Mattson, 1994; Lerdau *et al.*, 1995). The environmental constraint theory (Bryant *et al.*, 1988), on the other hand, predicts that the evolution of defence mechanisms will result in only minor reduction in growth. Therefore, it has been suggested by Blossey and Notzold (1995) that "resource availability is the primary force determining whether there is surplus photosynthate available for allocation to secondary metabolism".

Crawley (1987) first quantified height data for European plants in Europe and California and found that the aliens were larger in California. This has subsequently resulted in numerous studies looking at the size of alien plants in their introduced range as compared to their native range (Memmott and Forrester, 2000; Thebaud and

Simberloff, 2001). It has been found that plant species grow taller and have higher reproductive capacity where they are non-indigenous invaders than where they are native. Traditionally, the reason for this increase in size has been accepted as a plastic response to a benign environment, though recently this assumption has been challenged and a genetic basis for increased plant size has been suggested. Memmott and Forrester (2000) tested the hypothesis that the increase in size is genetically, rather than environmentally based. No significant differences were found in the size of *Carduus nutans*, *Digitalis pupurea*, *Echium vulgare* or *Senecio jacobaea* sampled from alien (Australia and New Zealand) or native (Britain and continental Europe) habitats. They concluded that it is quite rare for the increase in size to be explained genetically, and is probably due to a plastic response to a novel environment. It has been suggested that this increased growth in non-indigenous plants is caused by plants shunting more of their resources into defence against herbivores in their native ranges, so that fewer resources are available for growth and reproduction (Blossey and Notzold, 1995; Blossey and Kamil, 1996).

There is no fast rule that species are larger in their introduced range than in their native range, as this is not always the case. Thebaud and Simberloff (2001) demonstrated this in their study, which looked at European species introduced to California or the Carolinas, and vice versa. They found that, on average, individuals of Californian species were taller in California than in Europe, while those native to Europe did not differ between Europe and California. Similarly, individuals of species in the Carolinas were taller, on average, in the Carolinas than in Europe, while European species were the same height in Europe and the Carolinas.

### 2.1.9. Life History

It is important to know the type of life history strategies that are employed by plants, that would in turn allow one to work out the patterns of life history that would prosper in different environments. MacArthur (1972) first proposed the distinctions between *r*-selection and *K*-selection, where *r*-strategists are good dispersers but poor competitors, while *K*-strategists are poor dispersers but good competitors. This r- K-strategy has been described as a two-way strategy model by Grime *et al.* (1988).

A three-strategy model was subsequently proposed (Grime *et al.*, 1988) called the C-S-R model. This model originated following a suggestion made by Grime (1974) that it would be useful to classify external factors which affect vegetation in two categories, namely, stress (restriction of photosynthesis by factors, such as shortages of light, water and nutrients) and disturbance (partial or total destruction of plant biomass due to activities of herbivores, pathogens and human, and factors, such as fire and soil erosion). This led to the identification of three strategies which make up the basis for evolution in plants (Ramenskii, 1938; Grime, 1974). These are the competitors (associated with low stress and low disturbance), the stress-tolerators (associated with high stress and low disturbance), and the ruderals (associated with low stress and high disturbance). The C-S-R model proposes that " the vegetation which develops in a particular place and at a particular time is the result of an equilibrium which is established between the intensities of stress (constraints on production), disturbance (physical damage to the vegetation), and competition (the attempt by neighbours to capture the same unit of resource)" (Grime, 1988). Here, stress and disturbance dictate the extent of the density and vigour of the vegetation by controlling the intensity of competition. Therefore, according to this C-S-R model, the equilibria between stress, disturbance and competition occupy a triangular area, representing the full spectrum of habitat conditions and associated plant strategies.

### 2.1.10. This study

Very little is known about the biology of *Hirschfeldia incana* (Maillet *et al.*, 1996). Therefore, the study presented in this chapter investigates the occurrence of *H. incana* populations in South Wales, and attempts to understand its success at invasion by:

(a) Studying the spread of *H. incana* in the UK from the 1930s to the year 2000;
(b) Investigating the different types of communities that it occurs in and species that are associated with it in South Wales;
(c) Looking at the different habitats that *H. incana* is invading in South Wales, and, possibly, deducing its mode of dispersal;
(d) Attempting to group the different habitats in order to work out whether some habitats, in South Wales, are more prone to invasion by *H. incana*;
(e) Studying the role of disturbances on invasion of *H. incana* in South Wales sites;
(f) Observing plants grown in the greenhouse, and those growing wild in the South Wales sites.

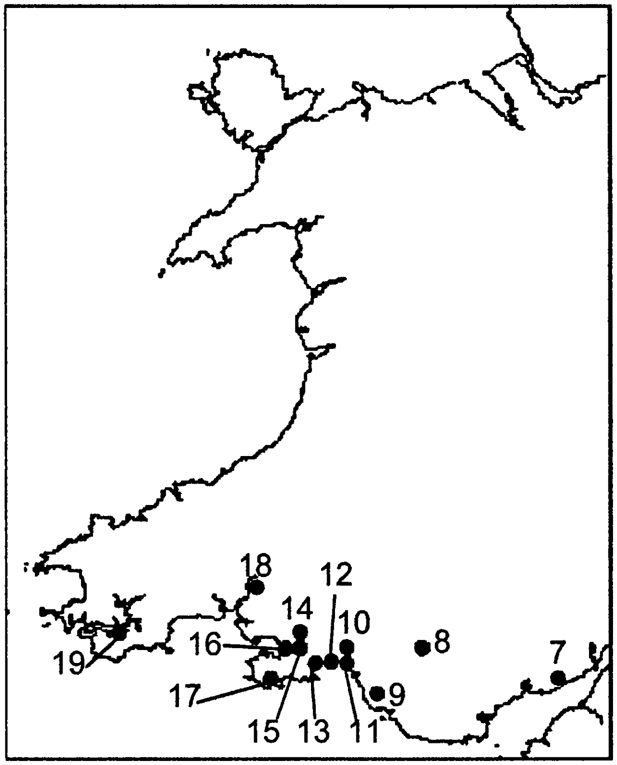
Autecological studies of successful neophytes like *H. incana* should give us a unique opportunity to investigate the way in which aggressive alien species become established and spread.

## 2.2 Materials and Methods

### 2.2.1. Site Selection and Collection of Samples

Samples were obtained from various sites around the United Kingdom and from Europe. The Welsh samples (except for those from East Glamorgan and Monmouthshire) were collected (Figure 2.1), as leaves from natural populations. Also, two sites were sampled form each of Kenfig and Carmarthen Pensarn. Two leaves were collected from each of eight to ten plants (depending on the number of plants present in the population), from each site. The grid references were noted using a Garmin eTrex Summit GPS System. Leaves were stored at -20°C, enabling DNA to be effectively extracted at a later date using the Puregene DNA Isolation Kit (Chapter 3).

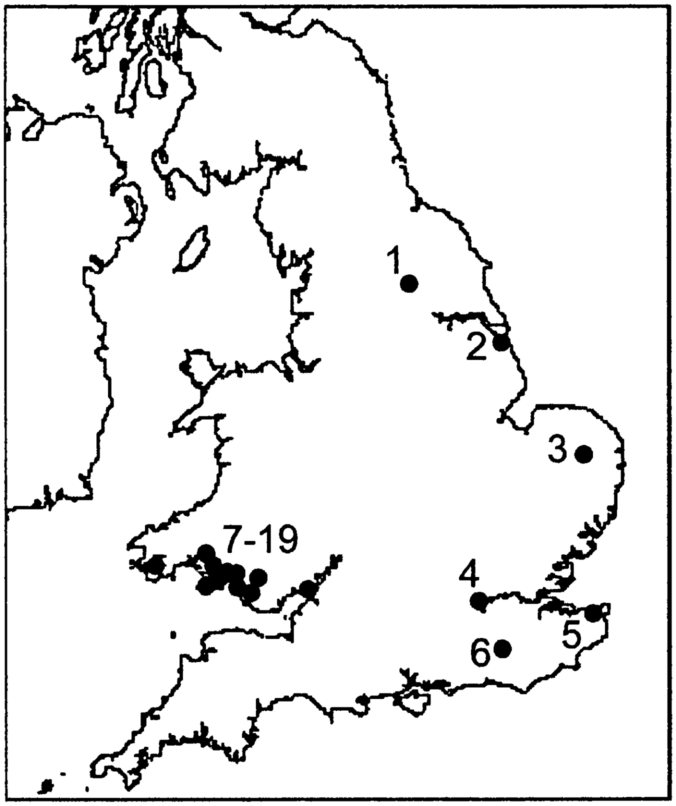
**Figure 2.1. Sampling sites in South Wales**



7 = Monmouthshire; 8 = East Glamorgan; 9 = Kenfig; 10 = Neath Abbey; 11 = Sandfields; 12 = Tawe Bridge; 13 = Fabian Way; 14 = Pontardulais; 15 = Gowerton; 16 = Crofty Industrial Estate; 17 = Horton; 18 = Carmarthen Pensarn; 19 = Pembroke Dock

Samples from the rest of the UK were obtained from BSBI Vice County Recorders (Figure 2.2; Table 2.1). These samples sent as seed, were collected from five plants from each site (grid references provided). The seed was germinated by placing them on multi-purpose potting compost, in the greenhouse. Initially, seeds were germinated in the laboratory, in petri dishes by placing them on filter paper soaked in potassium nitrate ($KNO_3$). Seedlings were then transferred to compost, in the greenhouse. Subsequently, potassium nitrate treatment was found to be unnecessary so in later experiments seed was sown and germinated on potting compost.

**Figure 2.2. Sampling sites in the UK**



1 = Yorkshire; 2 = Lincolnshire; 3 = East Norfolk; 4 = Middlesex; 5 = Kent; 6 = Sussex; 7-19 = Sites in South Wales

**Table 2.1. Vice Counties and Vice County Recorders**

| VICE COUNTY | VICE COUNTY RECORDER |
|---|---|
| West Sussex (13) | Mr. A. G. Hoare |
| East Sussex (14) | Mr. A. G. Hoare |
| East Kent (15) | Mr. E. G. Philp |
| West Kent (16) | Mr E. G. Philp |
| Middlesex (21) | Mr R. M. Burton |
| East Norfolk (27) | Mr. R. W. Ellis |
| Monmouthshire (35) | Mr. T. G. Evans |
| East Glamorgan (41) | Mr. J. Woodman |
| Lincolnshire (54) | Mrs. I. Weston |
| Southwest Yorkshire (63) | Mr. M Wilcox |
| Midwest Yorkshire (64) | Mr. M. Wilcox |

European seed was acquired from Portugal (Botanical Garden, University of Coimbra), Lanarca, Cyprus (received from Botanic Garden, University of Hamburg, Germany), Leipzig (Botanic Garden, University of Leipzig, Germany), and Crete (seeds collected by Dr. Rosemary John, Department of Biological Sciences, University of Wales, Swansea). In addition, leaf samples from Mallorca were collected by Dr. Quentin Kay (Department of Biological Sciences, University of Wales, Swansea) from El Malpas (5km east of Alcudia).

### 2.2.2. Information collected at South Wales sites

Species lists were constructed for each site sampled in South Wales and the abundance of each species was recorded using the DAFOR scale (D – Dominant; A – Abundant; F – Frequent; O – Occasional; R – Rare). Other characteristics were also recorded for each site, namely, the area, elevation, habitat type, grid reference, percentage of bare ground, and number of *H. incana* plants (Table 2.2). Species nomenclature is that used by Stace (1997).

**Table 2.2. Characteristics of each site in South Wales**

| SITE | CHARACTERISTICS |
|---|---|
| Kenfig car park | **Area**: 100 $m^2$<br>**Elevation**: 27 m<br>**Habitat**: gravel path<br>**Grid Reference**: SS 80158/81118<br>**Bare Ground**: 90% |
| Kenfig car park area – rough grassland | **Area**: 100 $m^2$<br>**Elevation**: 27 m<br>**Habitat**: disturbed MG1 grassland<br>**Grid Reference**: SS 80168/81129<br>**Bare Ground**: 5% |
| National Botanic Garden, Carmarthen | **Area**: 10$m^2$<br>**Elevation**: 30 m<br>**Habitat**: end of damp roadside verge near farm gate (disturbed)<br>**Grid Reference**: SN 51807/17387<br>**Bare Ground**: 5% (habitat dominated by graminoid species) |
| Carmarthen Pensarn | **Area**: 200$m^2$<br>**Elevation**: 5 m<br>**Habitat**: rough grassland on stony ground ( → MG1)<br>**Grid Reference**: SN 41569/19563<br>**Bare Ground**: 25% |
| Carmarthen Pensarn | **Area**: 180$m^2$<br>**Elevation**: 14 m<br>**Habitat**: recently seeded verge with bare stony patches<br>**Grid Reference**: SN 41420/19677<br>**Bare Ground**: 20% |
| Crofty Industrial Estate | **Area**: 50 $m^2$<br>**Elevation**: Sea Level<br>**Habitat**: pavement-hedgerow<br>**Grid Reference**: SS 52394/95468<br>**Bare Ground**: 85% |
| Gowerton | **Area**: 90$m^2$<br>**Elevation**: 7 m<br>**Habitat**: stone (hedge) wall<br>**Grid Reference**: SS 57897/96559<br>**Bare Ground** (vertical face): 50% |

| | |
|---|---|
| Horton | **Area**: 100m$^2$<br>**Elevation**: Sea Level<br>**Habitat**: disturbed, partially fixed dunes<br>**Grid Reference**: SS 47393/85559<br>**Bare Ground**: |
| Sandfields | **Area**: 550 m$^2$<br>**Elevation**: 12 m<br>**Habitat**: bare, gravely ground<br>**Grid Reference**: SS 74608/92353<br>**Bare Ground**: 65% |
| Neath Abbey | **Area**: 750 m$^2$<br>**Elevation**:<br>**Habitat**: rough grassland on stony ground (dominated by graminoids)<br>**Grid References**: SS 72990/96780<br>**Bare Ground**: 25% |
| Neath Abbey Industrial Estate | **Area**: 500 m$^2$<br>**Elevation**: Sea Level<br>**Habitat**: gravel-bed border of car park<br>**Grid Reference**: SS 73439/97184<br>**Bare Ground**: 40% |
| Ocean View (Jersey Marine) | **Area**: 1500 m$^2$<br>**Elevation**: 22 m<br>**Habitat**: clinker (PFA) tip<br>**Grid Reference**: SS 71592/93984<br>**Bare Ground**: 70% |
| Pontarddulais | **Area**: 75 m$^2$<br>**Elevation**: 24 m<br>**Habitat**: artificial boulder scree at side of River Dulais<br>**Grid Reference**: SN 58775/03938<br>**Bare Ground** (boulders): 70% |
| Tawe Bridge | **Area**: 250 m$^2$<br>**Elevation**: 10 m<br>**Habitat**: open, gravely waste ground<br>**Grid Reference**: SS 66189/933445<br>**Bare Ground**: 65% |
| MacDonalds – Fabian Way | **Area**: 100 m$^2$<br>**Elevation**: Sea Level<br>**Habitat**: disturbed, sandy loam<br>**Grid Reference**: SS 67825/93060<br>**Bare Ground**: 40% |

| | |
|---|---|
| Swansea Bay | **Area**: 200 m$^2$<br>**Elevation**: Sea Level<br>**Habitat**: disturbed fore dune<br>**Grid Reference**: SS 68095/91660<br>**Bare Ground** (sand): 75% |

### 2.2.3. Weight of native and alien seeds

1000 seeds collected from a native site (Larnaca, Cyprus) and an introduced site (Swansea) were weighed to determine any differences.

### 2.2.4. Observations of plants grown in the greenhouse

Plants grown in greenhouse from European and UK sites were observed over the two-year study period. Information noted included whether the plants flowered or not and if they did flower whether the resulting plants were 'simple' or 'complex'.

### 2.2.5 Multivariate Analysis

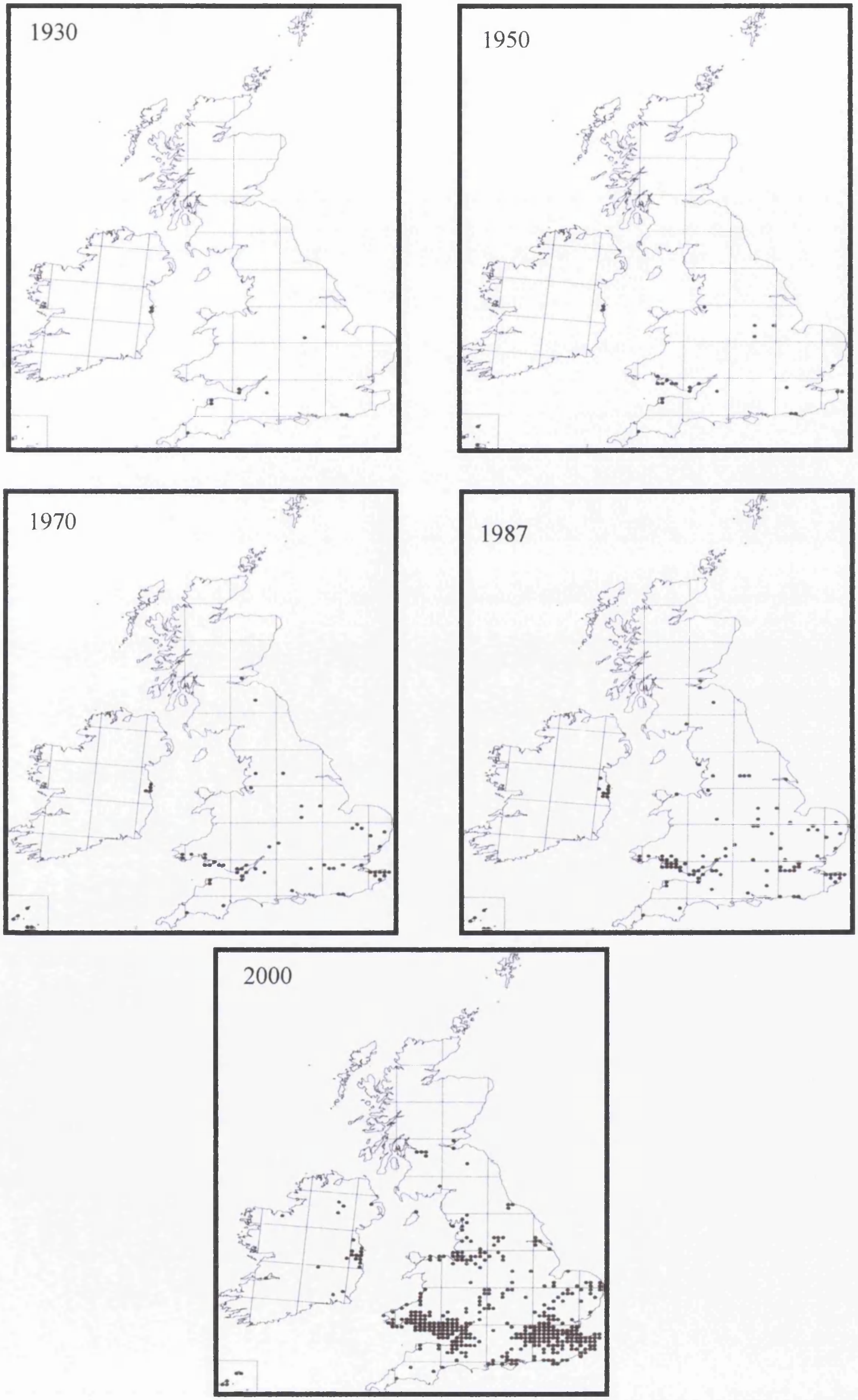
Species data collected from different sites in South Wales were subjected to multivariate analysis. Cluster analysis, by Ward's method, was undertaken using an SPSS program where sites were classified according to their species composition. Two way indicator species analysis (Hill *et al.*, 1975) was undertaken with a Community Analysis software package (Pisces Conservation Ltd.) which incorporates a version of the TWINSPAN software (Hill *et al.*, 1975; Hill, 1977, 1979b).

## 2.3 Results

### 2.3.1. Historical changes in the distribution of *H. incana*

Figure 2.3 shows the spread of *H. incana* from the year 1930 to 2000. Data for these figures were provided by Dr. Chris Preston (Biological Records Centre, Institute of Terrestrial Ecology) and mapped by Dr. Quentin Kay (Swansea University, Biological Sciences Department) using DMAP. Each point represents the presence of the species in a 10 km square of a grid of such squares covering the British Isles.

**Figure 2.3. Spread of *Hirschfeldia incana* from 1930 to 2000 in the UK**



Moreover the spread of *H. incana* over time was plotted (Figure 2.4). An exponential increase was found from the year 1930 to 2000. An $R^2$ value of 0.957 (calculated using SPSS) indicated that the exponential model explained 96% of the variation (Table 2.3).

**Figure 2.4. Spread of *H. incana* over time**

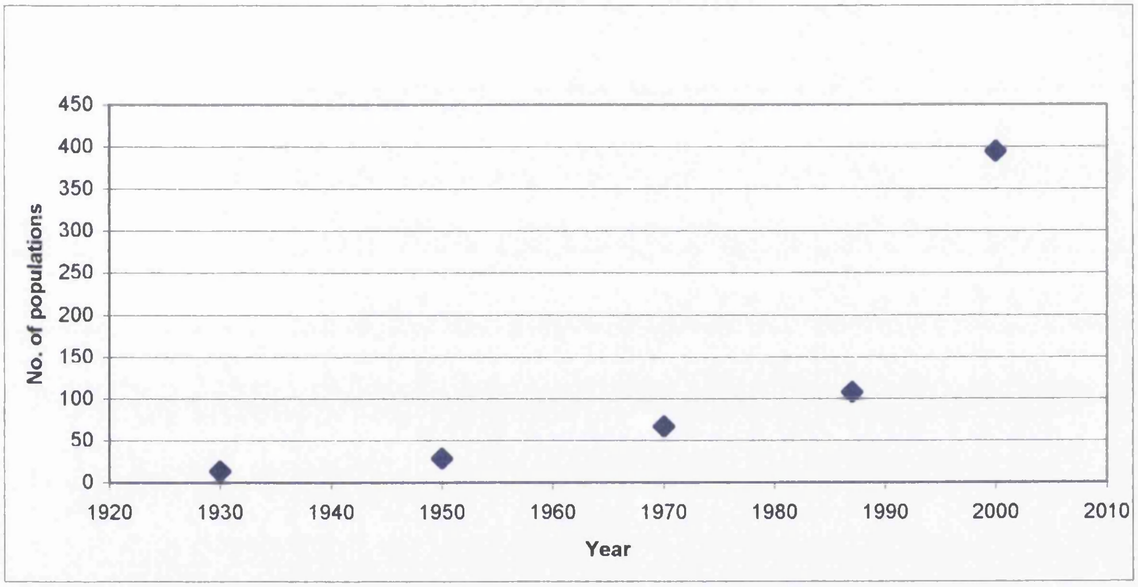


**Table 2.3. $R^2$ explaining the exponential model**

**Model Summary**[b]

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | .978[a] | .957 | .942 | .13339 |

a. Predictors: (Constant), YEAR

b. Dependent Variable: LOGPOP

Figure 2.5 shows the logs of population numbers plotted against time, with a line of best fit. Regression analysis indicated that the regression is very significant (*p*=0.004) (Table 2.4). Furthermore, the residual plot (Figure 2.6) indicated that there was no residual curvature.

**Figure 2.5. Logs of population numbers against time**

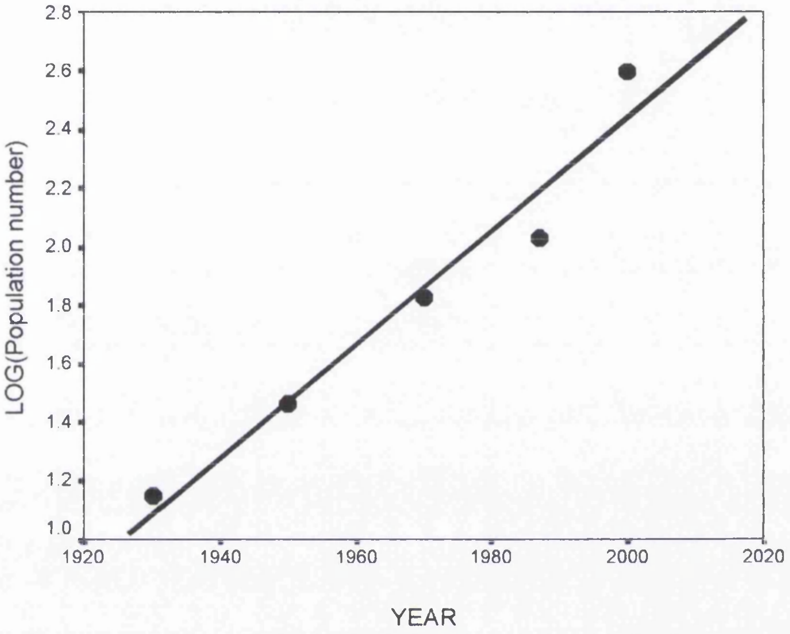


**Table 2.4. Regression analysis**

**ANOVA**$^b$

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 1.177 | 1 | 1.177 | 66.154 | .004$^a$ |
| | Residual | .053 | 3 | .018 | | |
| | Total | 1.230 | 4 | | | |

a. Predictors: (Constant), YEAR

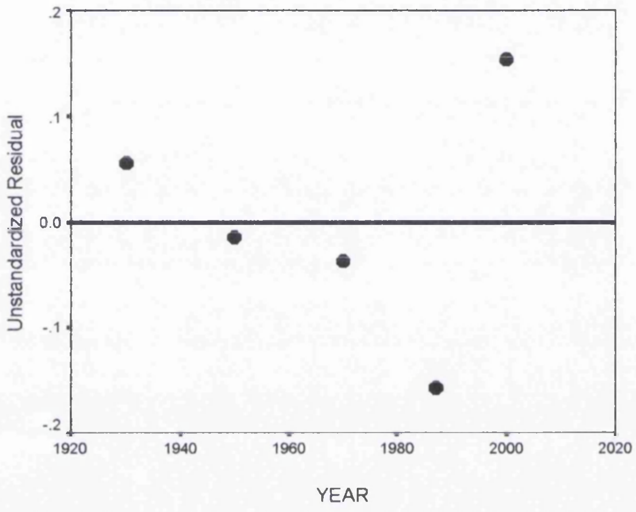
b. Dependent Variable: LOGPOP

**Coefficients**[a]

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | -36.186 | 4.672 | | -7.745 | .004 |
| | YEAR | 1.931E-02 | .002 | .978 | 8.134 | .004 |

a. Dependent Variable: LOGPOP

**Figure 2.6. Residual plot**



### 2.3.2. *H. incana* habitats in South Wales

The images shown in figures 2.7 to 2.9 illustrate the different types of habitat *H. incana* was found in throughout South Wales. Figure 2.7 show open habitats, with (a) rough grassland on stony ground and (b) gravel-bed border of car park. Closed habitats are shown in Figure 2.8, with (a) a stone wall and (b) a hedgerow. Finally, a sand dune habitat is shown in Figure 2.9.

## Figure 2.7. Open habitats: (a) Rough grassland on stony ground, (b) Gavel-bed border of car park

(a)

*Hirschfeldia incana* found at an open habitat in Carmarthen. This is a habitat with rough grassland on stony ground

(b)

*Hirschfeldia incana* found in an open habitat in Kenfig, Bridgend. The site is situated in a gravel-bed border of a carpark with disturbed MG1 grassland.

## Figure 2.8. Closed habitats: (a) a stone wall, (b) a hedgerow

(a)

*Hirschfeldia incana* found in a closed habitat in Gowerton. Here the alien crucifer is growing out of a crevice in a stone wall.

(b)

*Hirschfeldia incana* found in a closed habitat in Gowerton. This habitat is a hedgerow near a farm gate.

## Figure 2.9. Sand dune habitat

*Hirschfeldia incana* found in a sand dune habitat in Swansea Bay. This site is in a disturbed fore dune.

### 2.3.3. Species associated with *H. incana*

From the species lists drawn-up at each site (Table 2.5) it was possible to determine the species most commonly associated with *Hirschfeldia incana*. Percentages were calculated for the number of species found at each site and the top 20 species associated with *H. incana* are shown in Table 2.6. The top two (*Senecio jacobaea* and *Holcus lanatus*) were found in 16 of the 18 sites, while *Medicago lupulina* was found in 10 of the 18 sites.

**Figure 2.5. Species lists for each South Wales site: (a) Kenfig carpark (B1a); (b) Kenfig carpark area B1b); (c) Near National Botanic Gardenn (CM1); (d) Carmarthern Pensarn (CM2); (e) Carmarthen Pensarn (CM3); (f) Crofty Industrial Estate (G1); (g) Gowerton (G2); (h) Horton (G3); (i) Sandfields (NPT1); (j) Neath Abbey (NPT2); (k) Neath Abbey Industrial Estate (NPT3); (l) Ocean View, Jersey Marine (NPT4); (m) Briton Ferry Docks (NPT5); (n) Pontardulais (P1); (o) Tawe Bridge (S1); (p) Fabian Way (S2); (q) Swansea Bay (S3).**

**(a)**

*Anisantha sterilis*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Avenula pubescens*
*Cirsium arvense*
*Cirsium vulgare*
*Crepis capillaris*
*Crepis vesicaria*
*Dactylis glomerata*
*Epilobium hirsutum*
*Epilobium montanum*
*Equisetum arvense*
*Festuca ovina*
*Festuca rubra*
*Geranium molle*
*Geranium pyrenaicum*
*Hirschfeldia incana*
*Holcus lanatus*
*Hordeum murinum*
*Hypochoeris radicata*
*Lolium perenne*
*Malva sylvestris*
*Ononis repens*
*Poa annua*
*Reseda luteola*
*Senecio jacobaea*
*Sonchus asper*
*Sonchus oleraceus*
*Taraxacum officinale*

Kenfig carpark (B1a)

**(b)**

*Cirsium vulgare*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Cirsium vulgare*
*Daucus carota*
*Equisetum arvense*
*Festuca rubra*
*Geranium pyrenaicum*
*Heracleum sphondylium*
*Hirschfeldia incana*
*Holcus lanatus*
*Hordeum murinum*
*Ranunculus repens*
*Rumex crispus*
*Rumex obtusifolius*
*Senecio jacobaea*
*Sisymbrium officinale*
*Stachys palustris*
*Symphytum uplandicum*
*Urtica dioica*

Kenfig carpark area (B1b)

**(c)**

*Anthriscus sylvestris*
*Arrhenatherum elatius*
*Centaurea nigra*
*Cirsium arvense*
*Cirsium palustre*
*Cirsium vulgare*
*Corylus avellana*
*Dactylis glomerata*
*Epilobium hirsutum*
*Fraxinus excelsior*
*Geranium robertianum*
*Heracleum sphondylium*
*Hirschfeldia incana*
*Holcus lanatus*
*Lapsana communis*
*Lathyrus pratensis*
*Lolium perenne*
*Plantago lanceolata*
*Poa trivialis*
*Potentilla anserina*
*Ranunculus repens*
*Rubus fruticosus*
*Taraxacum officinale*
*Trifolium medium*
*Trifolium repens*
*Urtica dioica*

Near National Botanic gardens (CM1)

**(d)**

*Agrostis capillaris*
*Agrostis stolonifera*
*Arrhenatherum elatius*
*Calystegia sepium*
*Cirsium arvense*
*Cirsium vulgare*
*Crepis capillaris*
*Dactylis glomerata*
*Dipsacus fullonum*
*Epilobium ciliatum*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Epilobium tetragonum*
*Equisetum arvense*
*Euphorbia helioscopa*
*Fallopia japonica*
*Galium aparine*
*Geranium molle*
*Geranium robertianum*
*Heracleum sphondylium*
*Hirschfeldia incana*
*Holcus lanatus*
*Impatiens glandulifera*
*Lapsana communis*
*Lolium perenne*
*Lotus corniculatus*
*Matricaria discoidea*
*Medicago lupulina*
*Odontites vernus*
*Plantago major*
*Poa annua*
*Poa trivialis*
*Potentilla anserina*
*Potentilla reptans*
*Ranunculus repens*
*Rubus fruticosus*
*Rumex crispus*
*Rumex obtusifolius*
*Rumex sanguineus*
*Scrophularia auriculata*
*Senecio jacobaea*
*Sonchus asper*
*Stachys sylvatica*
*Taraxacum officinale*
*Trifolium hybridum*
*Trifolium pratense*
*Trifolium repens*
*Tripleurospermum maritimum*
*Urtica dioica*
*Vicia sativa*

Carmarthen Pensarn (CM2)

(e)

*Agrostis capillaris*
*Agrostis stolonifera*
*Arrherantherum elatius*
*Calystegia sepium*
*Conium maculatum*
*Crepis capillaris*
*Dactylis glomerata*
*Epilobium ciliatum*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Fallopia japonica*
*Festuca rubra*
*Geranium dissectum*
*Glechoma hederacea*
*Hirschfeldia incana*
*Holcus lanatus*
*Lolium multiflorum*
*Lolium perenne*
*Phleum pratense*
*Potentilla reptans*
*Ranunculus repens*
*Rorripa palustris*
*Rumex obtusifolius*
*Senecio aquaticus*
*Senecio jacobaea*
*Sonchus asper*
*Sonchus oleraceus*
*Stachys sylvatica*
*Taraxacum officinale*
*Trifolium repens*

Carmarthen Pensarn (CM3)

(f)

*Achillea millefolium*
*Agrostis stolonifera*
*Anisantha sterilis*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Calystegia sylvatica*
*Cirsium arvense*
*Cirsium vulgare*
*Convolvulus arvensis*
*Crepis vesicaria*
*Daucus carota*
*Equisetum arvense*
*Festuca rubra*
*Galium aparine*
*Geranium dissectum*
*Heracleum sphondylium*
*Hirschfeldia incana*
*Hypochoeris radicata*
*Leontodon saxatilis*
*Lolium perenne*
*Matricaria discoidea*
*Medicago lupulina*
*Mentha spicata*
*Pastinaca sativa*
*Plantago lanceolata*
*Plantago major*
*Poa annua*
*Potentilla anserina*
*Rubus fruticosus*
*Rumex crispus*
*Senecio jacobaea*
*Sonchus asper*
*Stachys sylvatica*
*Taraxacum officinale*
*Trifolium repens*
*Tussilago farfara*
*Urtica dioica*
*Verbena officinalis*
*Vicia sativa*

Crofty Industrial Estate (G1)

**(g)**

*Acer campestre*
*Agrostis stolonifera*
*Anisantha sterilis*
*Cirsium vulgare*
*Crataegus monogyna*
*Crepis capillaris*
*Epilobium ciliatum*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Epilobium tetragonum*
*Equisetum arvense*
*Galium aparine*
*Geranium robertianum*
*Glechoma hederacea*
*Hirschfeldia incana*
*Holcus lanatus*
*Lapsana communis*
*Lathyrus pratensis*
*Lotus corniculatus*
*Plantago lanceolata*
*Potentilla anserina*
*Prunus spinosa*
*Ranunculus acris*
*Ranunculus repens*
*Rosa micrantha*
*Rubus fruticosus*
*Rumex obtusifolius*
*Rumex sanguineus*
*Senecio jacobaea*
*Sonchus asper*
*Urtica dioica*
*Viburnum opulus*
*Vicia cracca*

Gowerton (G2)

**(h)**

*Achillea millefolium*
*Ammophila arenaria*
*Anacamptis pyramidalis*
*Anthyllis vulneraria*
*Arrhenatherum elatius*
*Avenula pubescens*
*Calystegia soldanella*
*Carex arenaria*
*Cirsium vulgare*
*Crepis capillaris*
*Dactylis glomerata*
*Erodium cicutarium*
*Festuca rubra*
*Galium verum*
*Geranium sanguineum*
*Hirschfeldia incana*
*Hordeum murinum*
*Hypochoeris radicata*
*Jasione montana*
*Leontodon saxatilis*
*Lobularia maritima*
*Lolium perenne*
*Lycopsis arvensis*
*Matthiola sinuata*
*Medicago lupulina*
*Oenothera cambrica*
*Ononis repens*
*Pimpinella saxifraga*
*Plantago lanceolata*
*Pteridium aquilinum*
*Raphanus maritimus*
*Rosa pimpinellifolia*
*Rubus caesius*
*Rumex crispus*
*Sisymbrium orientale*
*Sonchus oleraceus*
*Taraxacum officinale*
*Trifolium repens*
*Trisetum flavescens*
*Verbascum phlomoides*

Horton (G3)

(i)

*Agrostis capillaris*
*Agrostis stolonifera*
*Anthyllis vulneraria*
*Arenaria serpyllifolia*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Bromus hordeaceus*
*Buddleja davidii*
*Calystegia sepium*
*Carex arenaria*
*Centaurea nigra*
*Cerastium diffusum*
*Cerastium glomeratum*
*Chenopodium album*
*Cirsium arvense*
*Cirsium vulgare*
*Conyza canadensis*
*Coronopus didymus*
*Crepis vesicaria*
*Dactylis glomerata*
*Daucus carota*
*Deschampsia caespitosa*
*Dipsacus fullonum*
*Elymus repens*
*Epilobium ciliatum*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Epilobium tetragonum*
*Equisetum arvense*
*Euphorbia peplus*
*Galium aparine*
*Geranium robertianum*
*Hieracium agg.*
*Hirschfeldia incana*
*Holcus lanatus*
*Hypochoeris radicata*
*Leontodon hispidus*
*Leontodon saxatilis*
*Leucanethemum vulgare*
*Linaria repens*
*Matricaria discoidea*
*Medicago lupulina*
*Melilotus altissimus*
*Melilotus officinalis*
*Myosotis arvensis*
*Odontites vernus*
*Oenothera cambrica*
*Pastinaca sativa*
*Persicaria maculosa*
*Phleum pratense*
*Picris echioides*
*Plantago lanceolata*
*Plantago major*
*Poa annua*
*Polygonum aviculare*
*Pulicaria dysinterica*
*Ranunculus repens*
*Rubus fruticosus*
*Rumex conglomeratus*
*Rumex crispus*
*Rumex obtusifolius*
*Salix cinerea*
*Senecio jacobaea*
*Sonchus asper*
*Taraxacum officinale*
*Trifolium campestre*
*Trifolium pratense*
*Trifolium repens*
*Tripleurospermum maritimum*
*Tussilago farfara*
*Urtica dioica*
*Verbascum thapsus*
*Veronica persica*
*Veronica serpyllifolia*
*Vicia cracca*
*Vicia sativa*
*Vulpia bromoides*
*Vulpia myuros*

Sandfields (NPT1)

(j)

*Agrostis capillaris*
*Agrostis stolonifera*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Buddleja davidii*
*Calystegia sepium*
*Cerastium fontanum*
*Cetaurea nigra*
*Chamerion angustifolium*
*Cirsium arvense*
*Crepis capillaris*
*Crepis vesicaria*
*Cynosurus cristatus*
*Dactylis glomerata*
*Daucus carota*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Equisetum arvense*
*Eupatorium cannabinum*
*Fallopia japonica*
*Festuca rubra*
*Hirschfeldia incana*
*Holcus lanatus*
*Hyperichum perforatum*
*Hypochoeris radicata*
*Juncus inflexus*
*Lolium perenne*
*Lotus corniculatus*
*Medicago lupulina*
*Melilotus altissimus*
*Oenothera cambrica*
*Pastinaca sativa*
*Phleum pratense*
*Plantago lanceolata*
*Plantago major*
*Potentilla anglica*
*Potentilla reptans*
*Prunella vulgaris*
*Pulicaria dysinterica*
*Quercus petraea*
*Ranunculus repens*
*Rubus fruticosus*
*Rumex conglomeratus*
*Rumex crispus*
*Rumex obtusifolius*
*Salix cinerea*
*Senecio jacobaea*
*Stachys sylvatica*
*Taraxacum officinale*
*Trifolium medium*
*Trifolium pratense*
*Trifolium repens*
*Ulex europaeus*
*Urtica dioica*
*Vicia sativa*

Neath Abbey (NPT2)

**(k)**

*Agrostis stolonifera*
*Anisantha sterilis*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Bromus hordeaceus*
*Buddleja davidii*
*Capsella bursa-pastoris*
*Cardamine flexuosa*
*Carex hirta*
*Cerastium fontanum*
*Cerastium glomeratum*
*Cirsium arvense*
*Cirsium vulgare*
*Coronopus didymus*
*Crepis capillaris*
*Crepis vesicaria*
*Cynosurus cristatus*
*Dactylis glomerata*
*Daucus carota*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Epilobium tetragonum*
*Equisetum arvense*
*Eupatorium cannabinum*
*Galium aparine*
*Geranium dissectum*
*Geranium robertianum*
*Geranium rotundifolium*
*Heracleum sphodylium*
*Hirschfeldia incana*
*Holcus lanatus*
*Lolium perenne*
*Lotus corniculatus*
*Medicago lupulina*
*Melilotus sp.*
*Odontites vernus*
*Oenothera cambrica*
*Pastinaca sativa*
*Plantago lanceolata*
*Plantago major*
*Poa annua*
*Poa pratensis*
*Poa trivialis*
*Polygonum aviculare*
*Potentilla reptans*
*Ranunculus acris*
*Ranunculus repens*
*Reseda luteola*
*Rumex crispus*
*Rumex obtusifolius*
*Salix caprea*
*Senecio jacobaea*
*Senecio viscosus*
*Senecio vulgaris*
*Solanum nigrum*
*Sonchus arvensis*
*Sonchus asper*
*Sonchus oleraceus*
*Sorbus aucuparia*
*Taraxacum officinale*
*Trifolium dubium*
*Trifolium hybridum*
*Trifolium repens*
*Tripleurospermum maritimum*
*Tussilago farfara*
*Urtica dioica*
*Vicia sativa*

Neath Abbey Industrial Estate (NPT3)

(l)

*Agrostis capillaris*
*Betula pubescens*
*Buddleja davidii*
*Cerastium fontanum*
*Chamerion angustifolium*
*Cirsium arvense*
*Cirsium vulgare*
*Conyza canadensis*
*Echium vulgare*
*Epilobium ciliatum*
*Epilobium parviflorum*
*Eupatorium cannabinum*
*Filago minima*
*Filago vulgaris*
*Galium aparine*
*Hirschfeldia incana*
*Holcus lanatus*
*Hyperichum perforatum*
*Lotus conrniculatus*
*Medicago lupulina*
*Oenothera cambrica*
*Parentucellia viscosa*
*Pastinaca sativa*
*Persicaria maculosa*
*Plantago lanceolata*
*Poa annua*
*Polygonum aviculare*
*Pulicaria dysenterica*
*Rubus fruticosus*
*Rumex crispus*
*Sagina apetala*
*Salix caprea*
*Scrophularia nodosa*
*Senecio jacobaea*
*Trifolium arvense*
*Trifolium repens*
*Tripleurospermum maritimum*
*Urtica dioica*
*Veronica arvensis*

Ocean View, jersey Marine (NPT4)

**(m)**

*Agrostis stolonifera*
*Anisantha sterilis*
*Anthyllis vulneraria*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Bellis perennis*
*Buddleja davidii*
*Campanula poscharskyana*
*Catapodium rigidum*
*Cerastium fontanum*
*Chamerion angustifolium*
*Cirsium arvense*
*Cirsium vulgare*
*Coincya monensis* ssp. Recurvata
*Conyza canadensis*
*Crepis capillaris*
*Crepis vesicaria*
*Dactylis glomerata*
*Daucus carota*
*Dipsacus fullonum*
*Epilobium ciliatum*
*Epilobium montanum*
*Epilobium parviflorum*
*Epilobium tetragonum*
*Equisetum arvense*
*Eupatorium cannabinum*
*Fallopia japonica*
*Festuca rubra*
*Geranium dissectum*
*Geranium pyrenaicum*
*Geranium robertianum*
*Hieracium* spp.
*Hirschfeldia incana*
*Holcus lanatus*
*Hypericum maculatum*
*Hypericum perforatum*
*Hypochoeris radicata*
*Lapsana communis*
*Lathyrus latifolius*
*Lathyrus pratensis*
*Lavatera arborea*
*Leontodon hispidus*
*Leucanthemum vulgare*
*Linaria repens*
*Lotus corniculatus*
*Medicago lupulina*
*Melilotus albus*
*Melilotus altissimus*
*Odontites vernus*
*Oenothera cambrica*
*Pastinaca sativa*
*Picris echioides*
*Picris hieracioides*
*Plantago lanceolata*
*Plantago major*
*Poa annua*
*Poa trivialis*
*Potentilla reptans*
*Prunella vulgaris*
*Ranunculus repens*
*Reseda lutea*
*Reseda luteola*
*Rubus fruticosus*
*Rumex crispus*
*Rumex obtusifolius*
*Sagina procumbens*
*Salix cinerea*
*Scrophularia nodosa*
*Senecio jacobaea*
*Silene dioica*
*Silene latifolia*
*Sonchus asper*
*Sonchus oleraceus*
*Stachys sylvatica*
*Taraxacum officinalis*
*Trifolium campestre*
*Trifolium dubium*
*Trifolium hybridum*
*Trifolium pratense*
*Trifolium repens*
*Tripleurospermum maritimum*
*Tussilago farfara*
*Urtica dioica*
*Verbascum thapsus*
*Veronica arvensis*
*Vicia sativa*
*Vulpia bromoides*
*Vulpia myuros*

Briton Ferry Docks (NPT5)

**(n)**

*Acer pseudoplatinus*
*Agrostis stolonifera*
*Arrhenatherum elatius*
*Buddleja davidii*
*Deschampsia caespitosa*
*Epilobium hirsutum*
*Epilobium parviflorum*
*Equisetum arvense*
*Fallopia japonica*
*Festuca rubra*
*Galium aparine*
*Geranium robertianum*
*Hirschfeldia incana*
*Holcus lanatus*
*Imaptiens glandulifera*
*Medicago sativa*
*Mycelis muralis*
*Poa trivialis*
*Rubus fruticosus*
*Scrophularia auriculata*
*Senecio jacobaea*
*Sonchus asper*
*Sonchus oleraceus*
*Taraxacum officinale*
*Trifolium dubium*
*Trifolium repens*
*Tussilago farfara*
*Urtica dioica*
*Veronica chamaedrys*

Pontardulais (P1)

**(o)**

*Achillea millefolium*
*Agrostis capillaris*
*Agrostis stolonifera*
*Arrhenatherum elatius*
*Buddleja davidii*
*Carex hirta*
*Centaurea nigra*
*Centaurium erythraea*
*Cerastium glomeratum*
*Chaenorhinum minus*
*Cirsium arvense*
*Coincya monensis* ssp. Recurvata
*Convolvulus arvensis*
*Conyza canadensis*
*Crepis capillaris*
*Crepis vesicaria*
*Dactylis glomerata*
*Daucus carota*
*Dipsacus fullonum*
*Epilobium ciliatum*
*Epilobium parviflorum*
*Equisetum arvense*
*Foeniculum vulgare*
*Galium aparine*
*Geranium dissectum*
*Heracleum sphondylium*
*Hirschfeldia incana*
*Holcus lanatus*
*Hyperichum perforatum*
*Hypochoeris radicata*
*Lolium perenne*
*Lotus corniculatus*
*Matricaria discoidea*
*Medicago lupulina*
*Melilotus altissimus*
*Oenothera cambrica*
*Oenothera glazioviana*
*Papaver somniferum*
*Phleum bertelonii*
*Phleum pratense*
*Picris echioides*
*Plantago lanceolata*
*Plantago major*
*Potentilla anserina*
*Potentilla reptans*
*Prunella vulgaris*
*Ranunculus repens*
*Reseda lutea*
*Reseda luteola*
*Rubus fruticosus*
*Rumex crispus*
*Rumex obtusifolius*
*Sagina apetala*
*Senecio jacobaea*
*Senecio squalidus*
*Sonchus asper*
*Sonchus oleraceus*
*Taraxacum officinale*
*Tragopogon pratense*
*Trifolium pratense*
*Trifolium repens*
*Tripleurospermum maritimum*
*Tussilago farfara*
*Vicia cracca*
*Vicia sativa*

Tawe Bridge (S1)

**(p)**

*Achillea millefolium*
*Agrostis stolonifera*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Brassica rapa*
*Calystegia sepium*
*Cerastium glomeratum*
*Cirsium arvense*
*Cirsium vulgare*
*Conyza canadensis*
*Crepis vesicaria*
*Dactylis glomerata*
*Daucus carota*
*Elymus repens*
*Epilobium ciliatum*
*Hirschfeldia incana*
*Holcus lanatus*
*Leontodon saxatilis*
*Leucanthemum vulgare*
*Linaria repens*
*Lobularia maritima*
*Lolium perenne*
*Lotus corniculatus*
*Malva sylvestris*
*Medicago lupulina*
*Melilotus officinalis*
*Mercurialis annua*
*Oenothera cambrica*
*Phleum pratense*
*Picris echioides*
*Plantago lanceolata*
*Plantago major*
*Poa annua*
*Poa trivialis*
*Prunella vulgaris*
*Ranunculus repens*
*Reseda luteola*
*Rumex crispus*
*Rumex obtusifolius*
*Senecio jacobaea*
*Sonchus asper*
*Sonchus oleraceus*
*Taraxacum officinale*
*Trifolium arvense*
*Trifolium hybridum*
*Trifolium repens*
*Tripleurospermum maritimum*
*Ulex europaeus*
*Urtica dioica*

Fabian Way (S2)

**(q)**

*Achillea millefolium*
*Agrostis capillaris*
*Ammophila arenaria*
*Anisantha sterilis*
*Arrhenatherum elatius*
*Artemesia vulgaris*
*Buddleja davidii*
*Cakile maritima*
*Calystegia sepium*
*Calystegia soldanella*
*Carex arenaria*
*Cirsium arvense*
*Cirsium vulgare*
*Convolvulus arvensis*
*Crepis capillaris*
*Cynodon dactylon*
*Daucus carota*
*Elytrigia juncea*
*Equisetum arvense*
*Euphorbia paralias*
*Fallopia japonica*
*Festuca rubra*
*Heracleum sphondylium*
*Hippophae rhamnoides*
*Holcus lanatus*
*Honckenya peploides*
*Hypochoeris radicata*
*Lolium perenne*
*Matthiola sinuata*
*Oenothera cambrica*
*Ononis repens*
*Phleum arenarium*
*Picris hieracoides*
*Plantago lanceolata*
*Populus alba*
*Raphanus maritimus*
*Rubus caesius*
*Rubus fruticosus*
*Rumex crispus*
*Saponaria officinalis*
*Senecio jacobaea*
*Sonchus oleraceus*
*Taraxacum officinale*
*Tripleurospermum maritimum*
*Vicia cracca*
*Vulpia fasciculata*

Swansea Bay (S3)

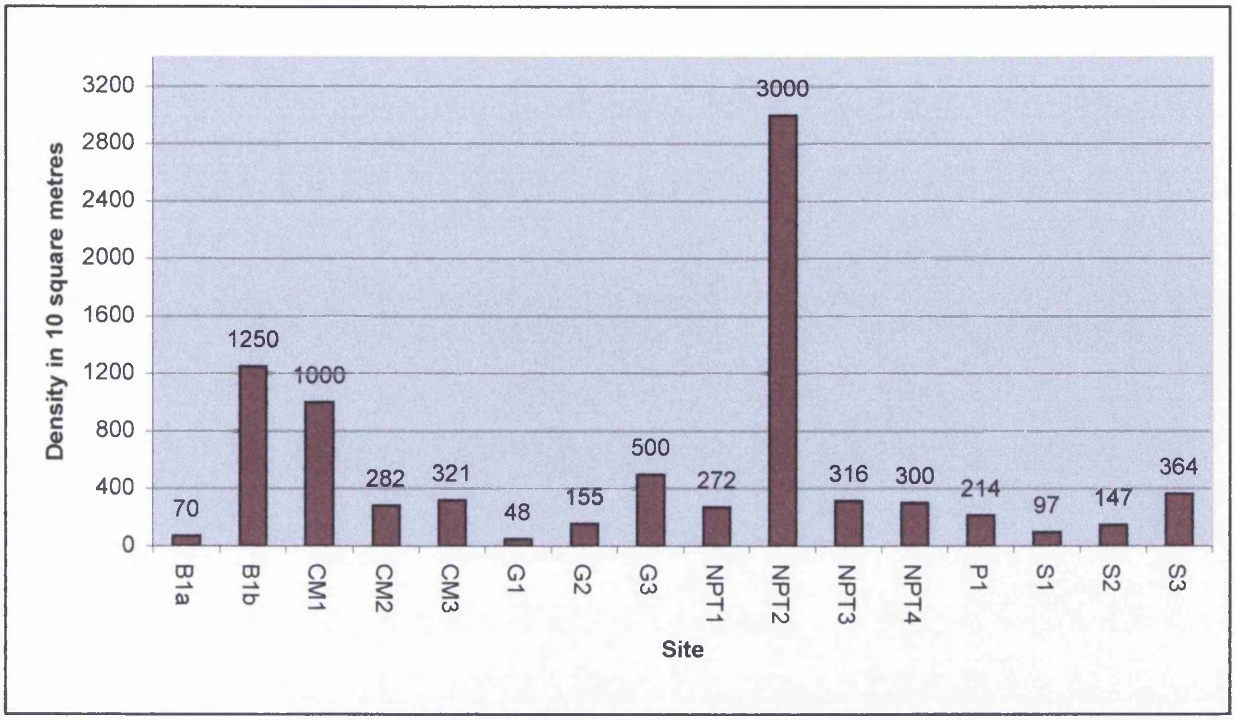
**Table 2.6. Top 20 species associated with *Hirschfeldia incana*.**

| Species | Number of sites species represented in |
|---|---|
| *Senecio jacobaea* | 16 |
| *Holcus lanatus* | 16 |
| *Taraxacum officinale* agg. | 15 |
| *Arrhenatherum elatius* | 15 |
| *Trifolium repens* | 14 |
| *Rumex crispus* | 13 |
| *Plantago lanceolata* | 13 |
| *Cirsium vulgare* | 13 |
| *Urtica dioica* | 12 |
| *Sonchus asper* | 12 |
| *Ranunculus repens* | 12 |
| *Lolium perenne* | 12 |
| *Equisetum arvense* | 12 |
| *Cirsium arvense* | 12 |
| *Agrostis stolonifera* | 12 |
| *Rubus fruticosus* | 11 |
| *Dactylis glomerata* | 11 |
| *Sonchus olaraceus* | 10 |
| *Rumex obtusifolius* | 10 |
| *Medicago lupulina* | 10 |

### 2.3.4. Density of *H. incana* plants

At each site, the number of *H. incana* plants and the area of the site were estimated. Subsequently, the density of plants was calculated and expressed in plants per 10 square metre (Figure 2.10). The highest density was estimated at Neath Abbey (NPT2) with 3000 plants per 10 square metres, followed by Kenfig car park area (rough grassland) (B1b) with 1250 plants per 10 square metres. The lowest densities, on the other hand, were found at Crofty Industrial Estate (G1; 48 plants per 10 $m^2$), Kenfig car park (B1a; 70 plants per 10 $m^2$), and Tawe Bridge (S1; 97 plants per 10 $m^2$).

Figure 2.10. Density of *H. incana* plants found at each site



[B1a – Kenfig car park; B1b – Kenfig car park area, rough grassland; CM1 – National Botanic Gardens; CM2 – Carmarthen Pensarn; CM3 – Carmarthen Pensarn; G1 – Crofty Industrial Estate; G2 – Gowerton; G3 – Horton; NPT1 – Sandfields; NPT2 – Neath Abbey; NPT3 – Neath Abbey Industrial Estate; NPT4 – Ocean View (Jersey Marine); P1 – Pontardulais; S1 – Tawe Bridge; S2 – MacDonald's Fabian Way; S3 – Swansea Bay.

### 2.3.5. Bare ground Vs Number of *H. incana* plants

The proportion of bare ground at each of the sites was also estimated (Table 2.7), and its relationship with the number of *H. incana* plants is graphically shown in Figure 2.11. The higher the percentage of bare ground at a particular site, the greater the abundance of *H. incana* plants was found, and vice versa. For example, at site B1a (Kenfig car park) 142 plants were found with 90% bare ground, while at site CM1 (National Botanic Garden) 1 plant was found with 5% bare ground. However, this association was not always evident, for example, the highest and lowest number of plants were recorded at sites with the same percentage of bare ground (70% at NPT4 and P1). In addition, the lowest number plants (8 plants and 1 plant at B1b and CM1, respectively) were found at the lowest proportion of bare ground (5%).

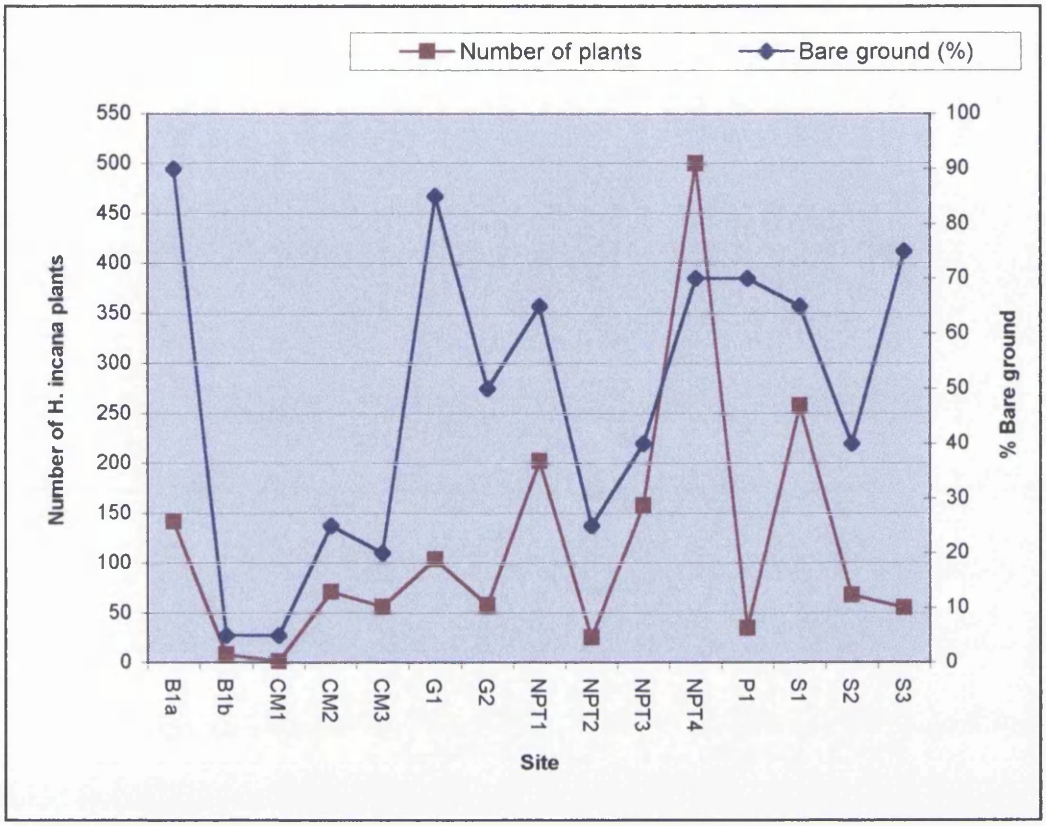
The Spearman's rho non-parametric test was employed to test the correlation of number of plants with bare ground (Figure 2.12). A correlation coefficient of 0.529 was obtained with a 2-tailed probability of 0.043, which is significant (Table 2.8). In addition, Kendall's tau parametric test showed a correlation coefficient of 0.429 and a significant probability of 0.028 (Table 2.8).

**Table 2.7. Proportion of bare ground and number of *H. incana* plants estimated at each site.**

| Site | Bare ground (%) | Number of plants |
|---|---|---|
| B1a | 90 | 142 |
| B1b | 5 | 8 |
| CM1 | 5 | 1 |
| CM2 | 25 | 71 |
| CM3 | 20 | 56 |
| G1 | 85 | 104 |
| G2 | 50 | 58 |
| NPT1 | 65 | 202 |
| NPT2 | 25 | 25 |
| NPT3 | 40 | 158 |
| NPT4 | 70 | 500 |
| P1 | 70 | 35 |
| S1 | 65 | 258 |
| S2 | 40 | 68 |
| S3 | 75 | 55 |

[B1a – Kenfig car park; B1b – Kenfig car park area, rough grassland; CM1 – National Botanic Gardens; CM2 – Carmarthen Pensarn; CM3 – Carmarthen Pensarn; G1 – Crofty Industrial Estate; G2 – Gowerton; G3 – Horton; NPT1 – Sandfields; NPT2 – Neath Abbey; NPT3 – Neath Abbey Industrial Estate; NPT4 – Ocean View (Jersey Marine); P1 – Pontardulais; S1 – Tawe Bridge; S2 – MacDonald's Fabian Way; S3 – Swansea Bay.

## Figure 2.11. Relationship of percentage of bare ground with number of *H. incana* plants



[B1a – Kenfig car park; B1b – Kenfig car park area, rough grassland; CM1 – National Botanic Gardens; CM2 – Carmarthen Pensarn; CM3 – Carmarthen Pensarn; G1 – Crofty Industrial Estate; G2 – Gowerton; G3 – Horton; NPT1 – Sandfields; NPT2 – Neath Abbey; NPT3 – Neath Abbey Industrial Estate; NPT4 – Ocean View (Jersey Marine); P1 – Pontardulais; S1 – Tawe Bridge; S2 – MacDonald's Fabian Way; S3 – Swansea Bay

## Figure 2.12. Number of plants plotted against the percentage of bare ground

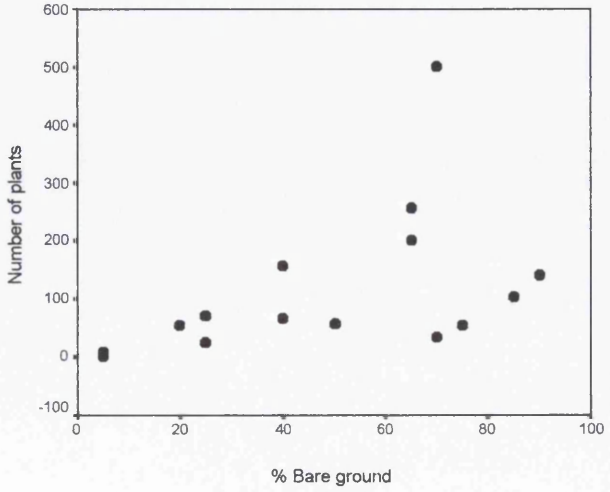


**Table 2.8. Spearman's and Kendall's Correlation Coefficients**

| | | | Number of plants | % bare ground |
|---|---|---|---|---|
| Kendall's tau_b | Number of plants | Correlation Coefficient | 1.000 | .429* |
| | | Sig. (2-tailed) | . | .028 |
| | | N | 15 | 15 |
| | % bare ground | Correlation Coefficient | .429* | 1.000 |
| | | Sig. (2-tailed) | .028 | . |
| | | N | 15 | 15 |
| Spearman's rho | Number of plants | Correlation Coefficient | 1.000 | .529* |
| | | Sig. (2-tailed) | . | .043 |
| | | N | 15 | 15 |
| | % bare ground | Correlation Coefficient | .529* | 1.000 |
| | | Sig. (2-tailed) | .043 | . |
| | | N | 15 | 15 |

*. Correlation is significant at the .05 level (2-tailed).

### 2.3.6. Cluster analysis

Cluster analysis using Ward's method was applied to the data. The results clearly divided the locations into two clusters, 1 and 2 (Figure 2.13; Table 2.9). In addition, the mean Shannon indices of diversity for the two clusters are significantly different [3.4499 (cluster 1), 4.0299 (cluster 2); ($p$=0.001)] (Table 2.10).

Cluster 1 consists of two types of habitats, namely natural habitats and semi-natural habitats. The natural habitats were sand dunes, in this case, found at both Swansea Bay and Horton. The semi-natural habitats included roadside verges, grasslands, hedges and a river bank, found at Crofty Industrial Estate, Fabian Way, Carmarthen Pensarn, Gowerton, Pontardulais and National Botanic gardens.

Cluster 2, on the other hand, consisted of open, urban gap sites. These sites can be described as typical ruderal habitats.

**Figure 2.13. Dendogram using Ward's Method**

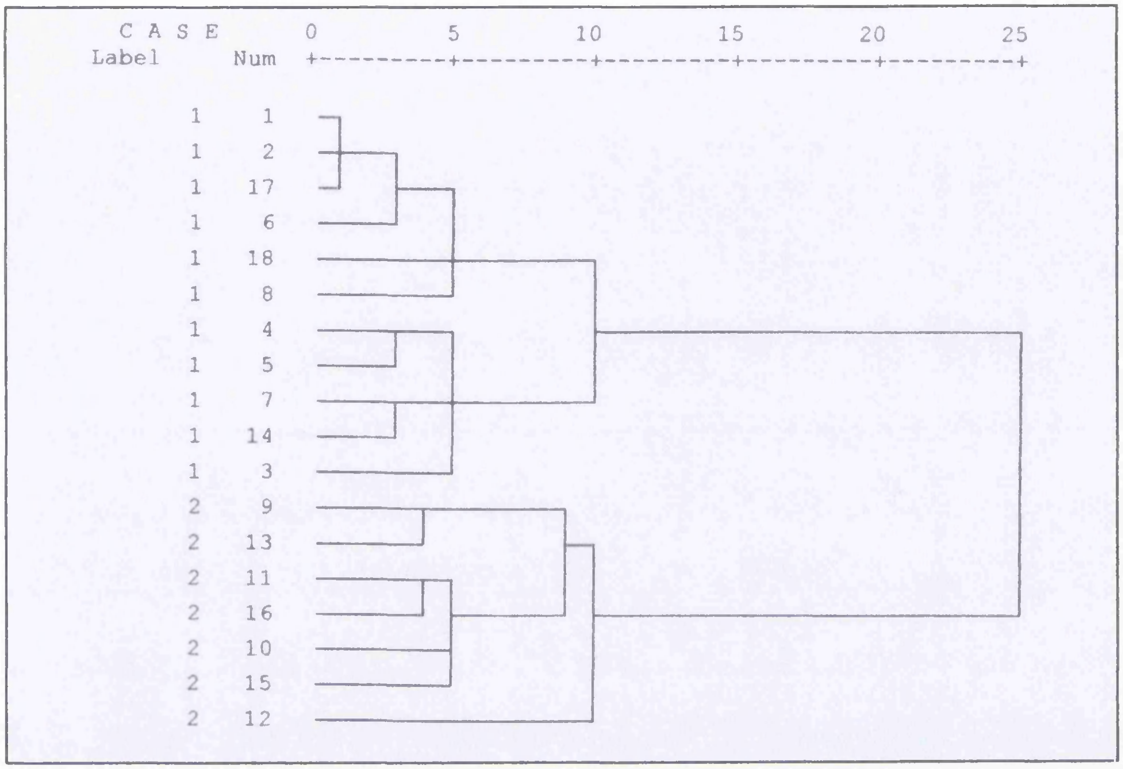


**Table 2.9. Sites found in clusters 1 and 2**

| Cluster 1 | Cluster 2 |
|---|---|
| Kenfig car park (B1a) | Sandfields (NPT1) |
| Kenfig car park area, Rough grassland (B1b) | Briton Ferry Docks (NPT5) |
| Swansea Bay (S3) | Neath Abbey Industrial Estate (NPT3) |
| Crofty Industrial Estate (G1) | Fabian Way, MacDonalds (S2) |
| Horton (G3) | Neath Abbey (NPT2) |
| Carmarthen Pensarn (CM2) | Tawe Bridge (S1) |
| Carmarthen Pensarn (CM3) | Ocean View, Jersey Marine (NPT4) |
| Gowerton (G2) | |
| Pontardulais (P1) | |
| National Botanic gardens (CM1) | |

# Table 2.10. Shannon's indices of diversity

## Oneway

### Descriptives

SHANNON

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| 1 | 11 | 3.4499 | .28607 | .08625 | 3.2578 | 3.6421 | 2.90 | 3.87 |
| 2 | 7 | 4.0299 | .26796 | .10128 | 3.7821 | 4.2778 | 3.61 | 4.39 |
| Total | 18 | 3.6755 | .39766 | .09373 | 3.4777 | 3.8732 | 2.90 | 4.39 |

### Test of Homogeneity of Variances

SHANNON

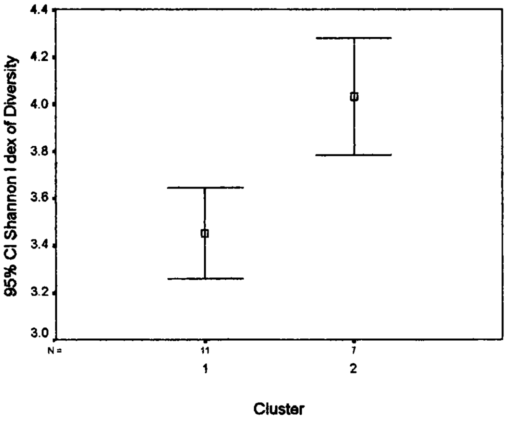
| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .101 | 1 | 16 | .755 |

### ANOVA

SHANNON

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.439 | 1 | 1.439 | 18.433 | .001 |
| Within Groups | 1.249 | 16 | .078 | | |
| Total | 2.688 | 17 | | | |

## Graph



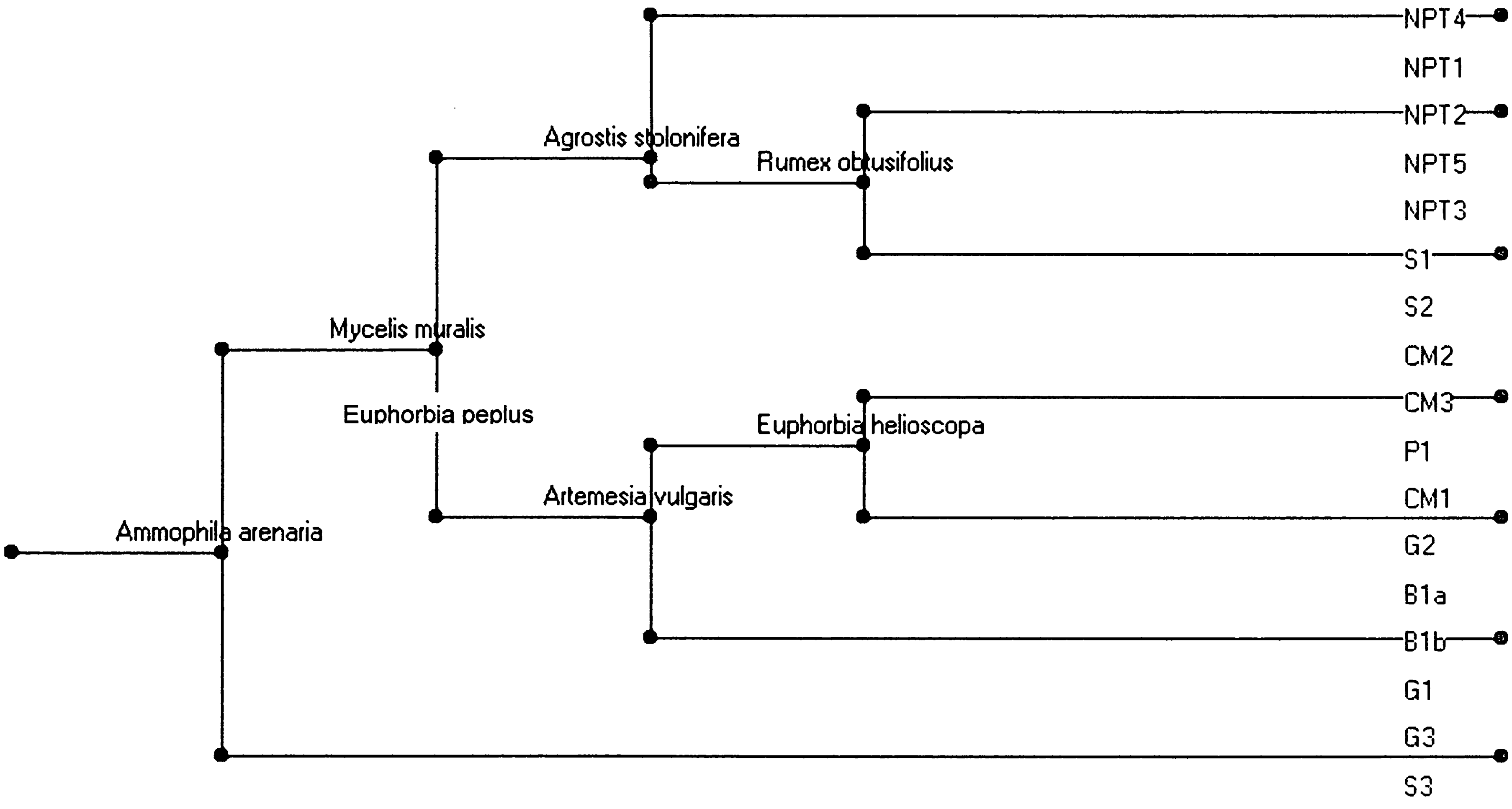
### 2.3.7. Two Way Indicator Species Analysis (TWINSPAN)

The application of TWINSPAN on the data collected at each of the South Wales sites (using presence or absence (i.e. 1 or 0), yielded a dendogram which is shown in Figure 2.14.

Here there are clearly three distinct groups separated initially by the tree. The first of these is the lower arm, indicated by *Ammophila arenaria,* which has grouped the two sand dune communities [Horton (G3) and Swansea Bay (S3)]. The second group is the top arm of the tree indicated by *Mycelis muralis* [Sandfields (NPT1); Neath Abbey (NPT2); Neath Abbey Industrial Estate (NPT3); Ocean View, Jersey Marine (NPT4); Briton Ferry Docks (NPT5); Tawe Bridge (S1); Fabian Way (S2)], which are all open, urban gap sites. Finally, the third group is in the middle of the tree indicated by *Euphorbia peplus* [Kenfig car park and car park area (B1a, B1b); near National Botanic Garden (CM1); the two Carmarthen Pensarn sites (CM2, CM3); Crofty Industrial Estate (G1); Gowerton (G2); Pontardulais (P1)]. These are the closed, semi-natural habitats.

Interestingly, the open habitats had the highest number of aliens (27), followed by the closed habitats with 13 aliens and then the sand dunes habitat (9 aliens).

**Figure 2.14. Tree constructed using TWINSPAN**

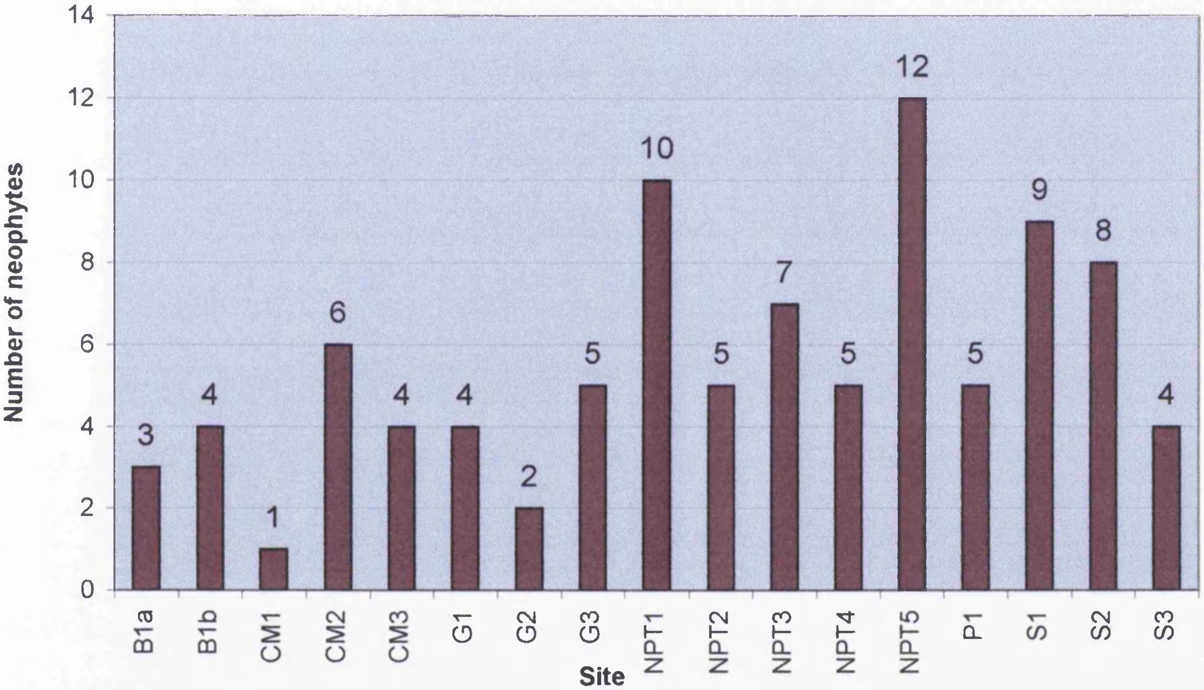


### 2.3.8. Neophytes

The number of neophyte species present at each of the sites is represented in Figure 2.15.

Interestingly, all the sites contained neophytes. Also, the number of neophytes in cluster 1 was found to be 38 and that in cluster 2 were 56.

**Figure 2.15. Number of neophytes found at each site**



[B1a – Kenfig car park; B1b – Kenfig car park area, rough grassland; CM1 – National Botanic Gardens; CM2 – Carmarthen Pensarn; CM3 – Carmarthen Pensarn; G1 – Crofty Industrial Estate; G2 – Gowerton; G3 – Horton; NPT1 – Sandfields; NPT2 – Neath Abbey; NPT3 – Neath Abbey Industrial Estate; NPT4 – Ocean View (Jersey Marine); P1 – Pontardulais; S1 – Tawe Bridge; S2 – MacDonald's Fabian Way; S3 – Swansea Bay.

The highest number of neophytes were found at Briton Ferry Docks (NPT5; 12 neophytes), followed by Sandfields (NPT1; 10 neophytes). The lowest numbers of neophytes, on the other hand, were found at the site near the National Botanic Gardens (CM1; 1 neophyte), and at Gowerton (G2; 2 neophytes).

### 2.3.9. Weight of native and alien seed

Seeds from Larnaca (Cyprus), representing the native site, and seeds from Swansea, representing the introduced site, were weighed. 1000 seeds from each site were weighed to ensure a large enough sample to produce a significant result [Table 2.11 (a) and (b)].

The native (Cyprus) seed was found to be significantly (*p*=0.02) (Table 2.12; Figure 2.16) heavier than the alien (Swansea) seed.

**Table 2.11. Weight of (a) native (Cyprus) and (b) alien (Swansea) seed**

**(a)**

| No. of seeds | Weight of seeds (g) |
|---|---|
| 100 | 0.01480 |
| 100 | 0.14540 |
| 100 | 0.10100 |
| 100 | 0.13400 |
| 100 | 0.03950 |
| 100 | 0.15000 |
| 100 | 0.14700 |
| 100 | 0.12400 |
| 100 | 0.03800 |
| 100 | 0.10000 |
| **weight of 1 seed =** | **0.0009937** |

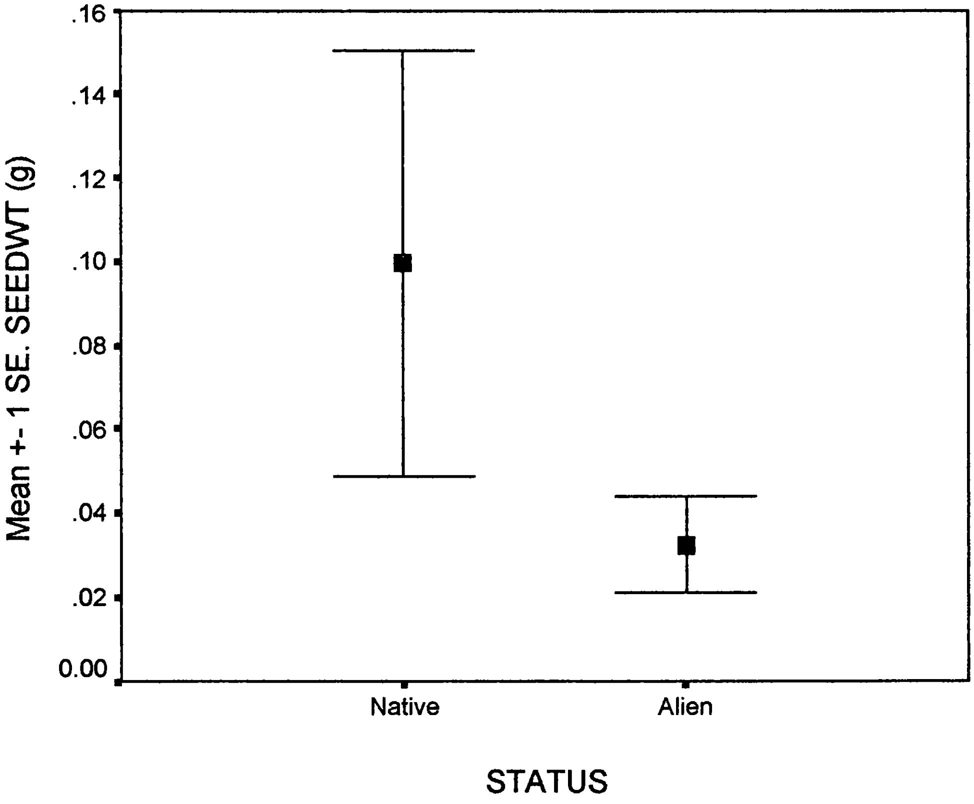
**(b)**

| No. of seeds | Weight of seeds (g) |
|---|---|
| 100 | 0.03790 |
| 100 | 0.04480 |
| 100 | 0.02940 |
| 100 | 0.03910 |
| 100 | 0.01340 |
| 100 | 0.02790 |
| 100 | 0.04500 |
| 100 | 0.01310 |
| 100 | 0.03890 |
| 100 | 0.03540 |
| **weight of 1 seed =** | **0.0003249** |

## Table 2.12. Independent samples t-test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | |
|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) |
| SEEDWT | Equal variances assumed | 13.759 | 0.02 | 4.057 | 18 | .001 |
| | Equal variances not assumed | | | 4.057 | 9.927 | .002 |

## Figure 2.16. Mean SE of seed weight of native and alien seed



### 2.3.10. Observations of *H. incana* plants

Plants grown in the greenhouse were observed over a two-year period. Most notable was the difference between the Swansea plants and the Larnaca (Cyprus) plants, whereby the former remained as rosettes in the first year, while the latter flowered and did not flower until the second year (Figure 2.17). In contrast, Larnaca plants flowered within 6 weeks of germination.

In the field (South Wales), plants were observed as being of the 'complex' type or the 'simple' type. 'Simple' plants produced a single flowering stem from a small rosette (e.g. the Larnaca plants as seen in Figure 2.17). 'Complex' plants were very large with a highly branched stem arising from a large rosette (Figure 2.18).

**Figure 2.17. Swansea (front row) and Larnaca (back row) plants in the greenhouse**

**Figure 2.18. ‘Complex’ plant as observed in Swansea**

## 2.3. Discussion

*Hirschfeldia incana* has dramatically expanded its range in the British Isles, with an exponential increase in its distribution from the 1930s to the year 2000 (Figure 2.3). The significance ($p$=0.004) of this exponential increase further suggests the probability of a continuing range expansion in the UK. Thus, it is important to look at this in terms of the habitats being invaded and the plant communities associated with this species.

*Hirschfeldia incana* is a known birdseed alien that has been introduced in seed mixtures imported for the consumption of domesticated birds, presumably included during the preparation stage. Obviously, the kind of foreign seed that will arrive with the birdseed will depend on the country of origin, and as a result there seems to be a connection to changes in the world market (Hanson and Mason, 1985), which, in turn, will result in changes in aliens occurring in Britain. However, many aliens are not introduced into Britain solely with birdseed, but also introduced with wool, esparto grass and soya beans (Hanson and Manson, 1985). *H. incana* was also formerly introduced with wool shoddy, and likely that it has also been introduced into parts in ballast.

*Hirschfeldia incana* is a neophyte that has been introduced in the last 500 years. It has been classified as a low-impact neophyte (Hipkin, 2003) since it does not bring about aggressive physiognomic changes in natural and semi-natural habitats of conservation importance. One reason for this seems to be its inability to compete in late-successional communities, unlike species, such as Japanese Knotweed (*Fallopia japonica*). *F. japonica* is a known aggressive invader in the UK, and has caused concern about its control as it is resistant to most forms of biological control. This pest, unlike *H. incana*, has invaded communities and dominated them, and even displaced native species in the process. *H. incana* is, however, an aggressive colonist of open, urban gap habitats.

Nowadays most invasions happen because of human activities, as the flow of commerce is much more widely spread and faster, and species travel in all directions. In fact, *H.* incana was cultivated in Britain by 1771 and recorded in the wild by 1837 (Preston *et al.*, 2002), which means that it took this alien crucifer about three decades to become established. As is shown in this study, the highest concentrations of *Hirschfeldia incana* populations are in the areas where there are major towns and cities, and where there is likely to be considerable movement in terms of people travelling from one place to another, hence, carrying seed on their person or their mode of transport.

This study looked at *H. incana* habitats in South Wales (Figures 2.7 to 2.9) and found it growing in a number of communities and particularly in man-made areas where there is a high level of human activity. These included sites next to ports (Swansea, Briton Ferry and Pembroke Docks), areas of tourist attractions (Kenfig and National Botanic Gardens), industrial areas (Crofty, Gowerton, Sandfields, Pontardulais and Neath Abbey Industrial Estates), areas next to busy roads (Fabian Way, Carmarthen Pensarn, Neath Abbey, Tawe Bridge and Ocean View, Jersey Marine), and on sand dunes (Swansea Bay and Horton). All of these sites have one thing in common in that there is a high level of traffic through them, by people or vehicles, which in turn facilitates the spread of *H. incana* seed to other suitable sites.

Disturbance seems to be a key factor determining invasions. In fact, many plant communities and species are dependent on disturbance, especially for regeneration (Pickett and White, 1985). Considering *H. incana*, disturbance seems to play a very important role in its spread (Figure 2.11). This is suggested in the positive correlation of number of plants with the percentage of bare ground ($p$=0.043; $p$=0.028). In fact, it has been known that invasive spread in a patchy environment is dependent on the size and distribution of suitable patches and the distance between them (Mooney and Drake, 1989). In *H. incana* habitats disturbance has usually occurred as a result of human activities, for example in urban gaps. With the exception of sand dunes, the urban gaps and closed habitats that this crucifer is found in are in areas where either land has been cleared of vegetation or bare ground has been exposed due to visitors trampling the existing vegetation, etc. Such disturbance may directly affect the viability of native species and in turn open these communities to invasions (With, 2002), which seems to be the path that *H. incana* has been following. In addition, Prieur-Richard and Lavorel (2000) have pointed out that disturbance enhances diversity by freeing bare ground and other resources that can be exploited by species with a ruderal strategy. This strategy is common among exotic weeds, and it seems *H. incana.* However, this enhancement of establishment by ruderal species is not clear, as it could be due to a temporary increase in the availability of nutrients and other resources or reduced competition from neighbouring plants.

On investigation of the species associated with *H. incana* populations in South Wales (Table 2.6), it was found that the three most commonly found species were *Senecio jacobaea* (Common Ragwort), *Holcus lanatus* (Yorkshire-fog) and *Taraxacum officinale* agg. (Dandelion), all native to the UK. In fact, the top 20 species found associated with *H. incana* are also all native (Figure 2.6). This suggests that once *H. incana* has established itself in a suitable, disturbed site, it lives with the native species, and enhances their biodiversity.

Rejmanek and Randall (1994) looked at the ratio of species present to the global number of species to see which families are over-represented in a flora. They found that *Brassicaceae*, *Leguminosae*, *Labiatae* and *Ranunculaceae* are better represented among the naturalized European species than among the exotics. This suggests that the size of a family does not necessarily indicate the number of alien species in a region.

*H. incana* is found in various communities around South Wales as shown in this study. However, it tends to colonize habitats early in the successional process. Such habitats may have a high alpha diversity (see species lists in Figure 2.5), which is often greater than that seen in late successional communities where competition is fierce. This is not consistent with Elton's (1958) proposal that communities with high species diversity should be resistant to invasion. In fact, models and some experimental studies have also found a negative relationship between invasion success and species diversity (Naeem *et al.*, 2000; Levine and D'Antonio, 1999). Other studies have tried to prove that there might be a positive relationship, such as in the case of seed-rain invading British grasslands, where species identity was found to be more important than species richness (Crawley *et al.*, 1999). On the other hand, Prieur-Richard *et al.* (2000) found a negative relationship between the biomass of *Conyza bonariensis* and species richness, thus following the hypothesis that increased species richness increases the resistance of plant communities to invasion. In the case of *H. incana*, the species richness does not appear to have an effect on its spread, but this species seems to occupy gaps caused by disturbance within vegetation in both the natural and semi-natural habitats it colonizes in South Wales.

Both cluster analysis and TWINSPAN analysis showed that *H. incana* occurs in distinct habitats in South Wales (Figure 2.13 and Figure 2.14, respectively). The cluster analysis divided the habitats in which the species occurs into two distinct types, that is, firstly, semi-natural or natural habitats, and secondly open urban gap sites. The former included sand dunes (natural habitat) and hedges or grasslands (semi-natural habitat), while the latter included a gravely habitat along a disused railway line, gravely waste ground and rough grassland on stony ground. The open urban gap sites are typical ruderal habitats for a ruderal species such as *H. incana*. *H. incana* typically colonizes disturbed areas before natives can re-colonize and from there can possibly start spreading into undisturbed habitats.

TWINSPAN divided sites in which the species is found into three distinct habitat types, two of which were the same as those identified by cluster analysis, with an extra group containing the sand dune communities. TWINSPAN is useful in that it allows identification of the indicator species for each of the habitat types. The three habitat types classified with this method were open habitats, closed habitats and sand dunes. The open habitats, indicated by *Mycelis muralis*, are ruderal habitats, while the closed habitats, indicated by *Euphorbia peplus*, are habitats where there are gaps among the existing vegetation, which *H. incana* is able to occupy. The sand dune communities are important here, as they are part of the UK Biodiversity Action Plan. However, *H. incana* is not a nuisance species on local sand dunes and therefore does not represent a threat to biodiversity.

It has been suggested that all communities are prone to invasions, and the question addressed most is: Are there species that will only invade certain communities? *H. incana* appears to invade certain communities, namely open urban gap, closed habitats (semi-natural) and natural (sand dunes) habitats, which has been demonstrated by this study of populations in South Wales. In fact, when looking at this question from a global point, it has been shown by Usher (1988) that nature reserves in North and South America, Europe, Africa, Australia, Indonesia and on oceanic islands were invasible, with habitats including temperate islands, tropical islands, mainland Mediterranean-type ecosystems, tropical savannahs and dry woodlands, and arid lands. On a more local scale, Crawley (1987) studied invasions into different British plant communities and found a high proportion of invasions in man-made habitats, such as waste sites, walls, fields and hedgerows. In contrast, no invaders were found in habitats, such as upland summits, rock ledges and screes, the sea and brackish water, and oligotrophic lakes. These findings are consistent with those found for *H. incana*, which is also found in habitats such as hedgerows, waste grounds and walls (Figures 2.7 to 2.9).

A higher number of aliens were found in open habitats (27), followed by the closed habitat (13) and then the sand dunes habitat (9). This was expected as open habitats in this study were urban gap habitats lending an opportunity for all prospective invaders and not just *H. incana*. *H. incana*, although found in closed habitats too, prefers urban gap habitats where it develops large populations. This could be explained by the fact that in an open space there are more opportunities available for *H. incana* seed to find a suitable place to germinate. Weber (1998) found that the number of established plants is proportional to the probability of propagules finding safe sites and the number of founding populations. Furthermore, Auld and Tisdell (1986) have shown that the increase in total area occupied by a spreading species is greater when several small populations are expanding compared with a single large population expanding.

Baker (1974) proposed two types of spread: the first being when a single population spreads steadily, while the second is when many satellite populations are scattered from a centre of origin, and followed by filling in of gaps. *H. incana* appears to have adopted the second type of spread whereby it scatters its seed and begins many satellite populations.

Dispersal plays an important role in determining the success of *H. incana* invasions, as once it arrives in a novel environment and establishes itself in a suitable place, it needs to increase its population by dispersing its seed to suitable habitat. These sites have to be accessible in order for seed to be dispersed to it. Therefore, it seems that landscapes where colonization sites are close together are favourable to those invaders that prefer short-range dispersal. This would ensure that most of the propagules would fall within the same area where other suitable habitat sites or mates are available, which would lead to spread over the whole of that habitat (Lavorel *et al.*, 1995). In addition, the invading species with the shorter dispersal distance will more than likely displace the native species, when competing for space in a certain habitat (Lavorel *et al.*, 1994). This is what *H. incana* seems to do, once it has arrived, in the habitats investigated here as it carries out short-range dispersal by starting with one plant and spreading its seed in the near vicinity, thus increasing its population size. However, *H. incana* is also known to be able to disperse over longer distances due to the method it uses for its dispersal, that is, activities of man. Found on roadsides, *H. incana* seed can be carried via vehicles over great distances.

*Hirschfeldia incana* seems to fit into the mechanism often proposed (Elton, 1958; Thompson, 1991) that "invasions can be favoured by the existence of empty ecological niches which are open to colonization by non-native species in the absence of suitable competitors". *Hirschfeldia incana* initially invades habitats, may they be closed or open, with low species diversities. This is consistent with the predictions of the empty niche hypothesis (Simberloff, 1995), where lower species diversity means the presence of situations where resources are not being exploited efficiently because species with suitable niches are not available. Therefore, in these circumstances *H. incana*, who can benefit from these resources, will occupy the empty niches.

After this initial, successful colonization, the next stage for *H. incana* is to establish a viable self-sustaining population. Establishment may require a different set of traits than those required for initial colonization. In fact, Horvitz *et al.* (1998) suggested that establishment in a natural community may require different traits than those required on entering a human-disturbed habitat, and furthermore, these traits may not be consistent across taxa. *H. incana* is required to compete for resources once having colonized a novel environment, which it seems to be doing quite efficiently in the South Wales sites investigated here. In fact, many studies have shown that invaders possess an above average ability to exploit local resources than native residents (Melgoza *et al.*, 1990; Petren and Case, 1996; Kupferberg, 1997; Holway, 1999; Byers, 2000). For example, *Centaurea diffusum* (a noxious invasive weed in North America) has been found to have stronger negative effect on biomass production for North American grasses than for grasses from its native Eurasian communities (Callaway and Aschenhoug, 2000). The reason for this was found to be due to alelopathy, that is, the plants from the native range were found to be better than those in the introduced range at competing with *Centaurea* in the presence of root exudates.

Disturbance provides opportunities for invaders by releasing resources (Sher and Hyatt, 1999), and that coupled with the escape from naturalist enemies in their distant native range (Mack *et al.*, 2000), allows invaders to reallocate resources to reproduction. This is a strategy that appears to have been adopted by *H. incana* as it has significantly ($p$=0.02) lowered the mass of its seed in its South Wales (introduced) range and presumably escaped from its naturalist enemies in its native range.

However, the small sample size used in this study (Swansea and Cyprus) to determine seed weight does not seem sufficient to provide a confident result.

Rejmanek and Richardson (1996) attempted to identify attributes that make plant species more invasive. In their study they stated three variables that seem to be indicative of invasive plant species. A short juvenile period and short interval between large seed crops are two variables that ensure rapid population growth of invasive plants, through early and consistent reproduction. The third variable important for predicting invasiveness is small seed mass, a criterion that has been met by *H. incana* with a reduced seed mass from its native range to its introduced range. The reason for this is that a small seed mass means a larger number of seeds are produced (Werner and Platt, 1976; Greene and Johnson, 1994), there is better dispersal (Harper *et al.*, 1970; Rydin and Borgegard, 1991) a shorter initial germinability (Grime *et al.*, 1988), a shorter chilling period needed to overcome dormancy (Tombock and Linhart, 1990), and the seedlings have a higher growth rate (Walters *et al.*, 1993). Considering *H. incana* the initial germinability was short (three days on average), but this aspect requires further quantitative investigation. One method put forward by Rejmanek and Richardson (1996) was discriminant analysis which allowed them to find out that invasive pines can be accurately detected from non-invasive pines due to a minimum juvenile period, mean seed mass, and mean interval between seed crops.

Life history strategies also play an important role in invasions. *H. incana* is a r-strategist as it settles in a newly-formed habitat, with favourable conditions, and rapidly colonizes it. This strategy is advantageous for *H. incana*, which is an opportunist, and can cope with rapidly changing environments as in the early stages of a succession. It does this by producing numerous seeds of low weight that can be dispersed quickly and efficiently to increase its population size and spread to other suitable habitats. In fact, the r-selected life history is a strategy that has been found in successful colonists due to their use of a novel environment, having a short generation time, high fecundity and high growth rates. In contrast, there is the K-selected strategy where the species exhibits low birth rates, high survival rates among offspring, and prolonged development. Therefore, the most successful species would be those that possess the ability to shift between r- and K-selected strategies (Kolar and Lodge, 2001). In addition, according to the C-S-R model (Grime *et al.*, 1988), *H. incana* follows the ruderal strategy where there is low stress and high disturbance in the habitats that it colonizes.

The success of an invader to become established has been linked with the size of introduction effort (Kolar and Lodge, 2001). That is, species releasing greater number of individuals and with a number of introduction attempts, have a higher probability of becoming established.

However, one specific trait cannot be held responsible for the success of invaders. With *H. incana*, the high seed production, low seed mass, and the ability to disperse efficiently and to great distances are all responsible for its success. Baker (1965, 1974) found that there are many traits that are associated with weedy species, such as the ability to reproduced sexually and asexually, the rapid growth from seedling to sexual maturity, and tolerance to environmental heterogeneity. Baker (1965, 1974) subsequently proposed that species that possess many of these characteristics would be more likely to be highly weedy than those that only have some of these traits. However, the r-selected life history theory and Baker's characteristics of the ideal weed have not been investigated quantitatively.

Colonization rate is described by Weber (1998) as the combination of the species' ability for dispersal and establishment, and of human activities that promote these. For example, Lacey (1957) found that *Galinsoga ciliata* (Rafin.) Blake spread much faster in Britain than did *G. parviflora* (Cav.). The reason for this was explained by the creation of favourable establishment sites during World War II. This argument has also been used to explain the success of *Solidago altissima* in Europe.

*H. incana* populations rapidly increase in size when it is introduced into a new locality where it finds unexploited resources of suitable nutrients, and a lack of negative controls such as predators or parasites. Wolfe (2002) proposed the escape-from-enemy hypothesis, and said "successful biological invaders often exhibit enhanced performance following introduction to a new region". The reason for this was that the natural enemies, such as competitors and predators, that were present in the native range, were probably absent from the introduced range. *H. incana* is native in southwest Europe, the Mediterranean region and southwest Asia, where the natural enemies are probably different to those found in the communities that this crucifer invades in the UK. Therefore, it is at an advantage in these introduced sites, and reduces it's defence mechanisms. Wolfe demonstrated this using *Silene latifolia*, which was found to be 17 times more likely to be damaged, by generalist enemies, in its native Europe than in its introduced range in North America. Lodge (1993) reinforced this concept by the idea that interactions with other trophic levels (e.g. herbivore pressure) play an important part in determining the resistance of communities to invasion.

The reduced defence mechanisms in the introduced range has been explained by the reallocation of biomass used for defence into both reproduction and growth (Blossey and Notzold, 1995), as the invasive species have been released from the pressure of pests in their native range. This was demonstrated by the study of the biomass of *Lythrum salicaria* whose biomass was greater in the non-native habitat than in the native habitat (Blossey and Notzold, 1995). Therefore, it might prove to be very useful to study and compare the pests found in the native and introduced ranges for particular invaders.

Although there is no general tendency for species to be taller in their introduced ranges, many species are, in fact, taller in some regions where they are introduced than in their native ranges (Thebaud and Simberloff, 2001). Considering *H. incana*, this needs to be investigated further as there seems to be an indication that the plants that grow in the South Wales area are bigger and prone to develop into the 'complex' type (Figure 2.18) as opposed to the 'simple' type of plant that seems characteristic of their native range (Figure 2.17).

It has been suggested that the expansion of a species' range, such as that shown here with *H. incana*, is due to genetic changes as well as ecological changes, especially so when the species has moved from one part of the world to another (Baker and Stebbins, 1965; Parson, 1983; Mooney and Drake, 1986). Therefore, the next logical step in this study is to investigate the genetic relationship between the native and alien populations, and this is what has been done in the next chapter.

# Chapter 3
# Genetic diversities in native and alien populations of *Hirschfeldia incana* (Hoary Mustard)

## 3.1. Introduction

### 3.1.1. Genetic variation and invasions

The study of genetic diversities of invasive species and their potential for rapid evolution has been put forward as a useful tool in determining what causes a species to become invasive (Allendorf and Lundquist, 2003). When a species arrives in a novel environment it is likely that this will involve only a few individuals that will subsequently be established. In addition, these small numbers of individuals are unlikely to contain all the variation present in the source population, and as a result a loss in genetic variation would be accompanied with the colonization event. Nei *et al.* (1975) proposed that there is a reduction in genetic diversity from the native to the alien range, but this depends on the number of immigrants involved and the frequency of bottlenecks that follow establishment. Small population sizes is a problem in conservation genetics, due to the rate of extinction, which is increased by the loss of genetic variation, caused by genetic drift and the inbreeding effect (Frankham and Ralls, 1998). Invasive species tend to exhibit this problem, as they tend to have reduced genetic diversity, due to their small populations sizes during colonization of a new area. In fact, this population bottleneck ensures that the newly established population is less genetically diverse than the source population (Barrett and Kohn, 1991). However, comparative studies of genetic diversity between native and alien species have yielded conflicting results. Many studies have shown no difference in genetic variation between the native and alien populations (Antrobus and Lack, 1993; Brown and Marshall, 1981), while other studies have shown higher variation in the alien populations (Harding and Barnes, 1977). Yet, some studies do show alien populations being depauperate in genetic diversity (Barrett and Richardson, 1985; Warwick, 1990). Therefore, it seems that high levels of genetic variation are not a prerequisite for colonizing success.

Wadsworth *et al.* (2000) suggested three factors that are important when considering management of biological invasions. The three factors are the frequency of introductions of a species into a specific region, the size of the introduction, and the resulting pattern of spread of the species across a landscape. The number of introduction events is likely to determine the amount of genetic variation in a population. This is because if there is only one source population then the amount of genetic variation is going to be limited, as opposed to multiple introductions from different regions of the native range, in which case the multiple foci will ensure a more varied genetic make-up (Parker *et al.*, 2003). Multiple introductions also have an effect on the age structure of the colonizing populations, as the founder populations arrive at different times and from different source populations. This means that when subpopulations within a metapopulation are made extinct and recolonized, there will be an age structure as each subpopulation is formed at different times (Whitlock and McCauley, 1990). Therefore, this difference in ages of subpopulations will result in genetic differentiation in the whole population, and genetic variance among subpopulations (Wright, 1940).

If invasive species that spread across a landscape are likely to undergo repeated bottlenecks, this could lead to populations with significant differences among them and little genetic variation within them, due to founder effects and genetic drift (Wang *et al.*, 1995; Pascual *et al.*, 2001). However, this cannot be deemed as conclusive as other factors, such as method of propagule dispersal, would determine the number of individuals that find new populations and how fast a population increases its size (Nei *et al.*, 1975). In fact, as far as establishment of an introduced species is concerned, it seems that propagule pressure (e.g. number of individuals introduced, and number of release events) is the most important predicting factor (Kolar and Lodge, 2001), and it includes both the number of individuals and the number of release events. Propagule pressure will, thus, affect the genetics of an introduced species. It could do this, firstly, by increasing the genetic variation of the newly established population as there are a larger number of founding individuals reducing the effects of any population bottlenecks. And secondly, it could also increase genetic variation if there are many source populations, as there is hybridisation between individuals from different source populations (Allendorf and Lundquist, 2003).

When an invading species arrives in a novel environment, it will compete with native species and may eventually replace them. Some species may be naturally better at competing as they might have evolved in a competitive environment (Callaway and Aschehoug, 2000). Also, the absence of natural enemies in the introduced range means that the introduced species can allocate more resources for reproduction and growth, and thus out-compete native species. This effect can be explained by the Evolution of Increased Competitive Ability (EICA) (Blossey and Notzold, 1995), a hypothesis that proposes that invasive plants increase allocation of their resources to growth and reproduction, and reduce the contribution of resources to defence because of absence of natural enemies in their introduced range. Therefore, according to this hypothesis, the genotypes from the plant's introduced range are predicted to grow faster and produce more seeds, and be less defended as compared to the genotypes from its native range. Siemann and Rogers (2001) investigated genetic differences in growth of an invasive tree species and found that the invasive genotypes were larger than the native genotypes, and they were more likely to produce seeds, while their leaves were poorly defended. However, this hypothesis has not been proven successfully, as experiments have sometimes shown genetic differences in the growth of native and invasive genotypes (Blossey and Notzold, 1995), while other studies have not (Willis *et al.*, 2000; Thebaud and Simberloff, 2001).

It is commonly known that invasive species undergo a lag phase, in their initial period of arrival, when they are present but not invasive (Cousens and Mortimer, 1995; Mack *et al.*, 2000). These time lags are important in evolutionary terms as natural selection acts on the organisms and leads to population growth that is self-sustaining (Parker *et al.*, 2003). Then local adaptation plays an important role in ensuring the expansion of the range of successful invaders into a wide spectrum of sites, through conditions, such as high outcrossing rates, high numbers of founders in new populations, and the formation of new genotypes through gene flow between different introduction foci (Parker *et al.*, 2003). However, Baker (1965) pointed out that not all invaders capable of expanding their range owe it to local adaptation, and proposed the 'general-purpose genotype'. The 'general-purpose genotype' was used by Baker to describe 'colonizing species that thrive in a wide range of environmental conditions through phenotypic or developmental plasticity'. Furthermore, this genotype was found to allow the success of invaders through reproductive strategies that do not involve genetic exchange, such as selfing and clonal reproduction, but at the same time allowing them to reproduce effectively.

### 3.1.2. Polyploidy and invasiveness

The successful colonization of invasive species has been thought to be the result of genetic factors as well as environmental factors. It would, thus, be useful to consider all these factors of successful invasive species globally in order to determine the common factors in these species that may play a role in their success. Brown and Marshall (1981) did just that by looking at the world's worst crop weeds (Table 3.1; Holm *et al.*, 1977), and found four common features, namely, reproduction either by clonal means or self-fertilization, a world-wide distribution which implies adaptation to a wide range of environments, ecotypic differentiation, and polyploidy. In fact, all of the species in Holm's list are polyploid. Polyploidy has been thought to be present in successful colonizing species because of its ability to allow environmental tolerance (Stebbins, 1971; Roose and Gottlieb, 1976; Clegg and Brown, 1983). This has been put forward as the main reason explaining the abundance of polyploids in north temperate floras, which have been thought to have colonized new habitats created by the repeated advances and retreats of the Pleistocene sheets (Gray, 1986). In contrast, Crawley (1987) constructed a list of the 20 most successful British aliens (Table 3.2). This list contained nine diploids, such as *Senecio squalidus* and *Veronica filiformis*, which belong to genera that have undergone extensive polyploidization.

Manton (1934) stated that polyploid plants have a greater colonizing potential than their diploid progenitors. Later, Stebbins (1971) further stressed the importance of polyploidy in colonizing species by stating that 'if related diploids and polyploids exist in the same group of annuals, the polyploids have a greater chance of becoming widespread as weeds than their diploid relatives'. However, it is not to say that diploid species do not invade successfully at all, in fact, observations of weedy species that were introduced into eastern North America from Europe found that 10 diploid species out of the 74 genera (containing both diploid and polyploid species) invaded while the polyploid relatives did not (Stebbins, 1970).

**Table 3.1. The world's worst weeds of crops (after Holm *et al.*, 1977)**

| | |
|---|---|
| *Cyperus rotundus* | *Chenopodium oleracea* |
| *Cynodon dactylon* | *Digitaria sanguinalis* |
| *Echinochloa crusgalli* | *Convolvulus arvensis* |
| *Echinochloa colonum* | *Avena fatua* |
| *Eleusine indica* | *Amaranthus hybridus* |
| *Sorghum halepense* | *Amaranthus spinosus* |
| *Imperata cylindrica* | *Cyperus esculentus* |
| *Eichhornia crassipes* | *Paspalum conjugatum* |
| *Portulaca oleracea* | *Rottboellia exaltata* |

**Table 3.2. Britain's 20 most successful aliens (after Crawley, 1987)**

| | |
|---|---|
| *Acer pseudoplatanus* | *Impatiens glandulifera* |
| *Aegopodium podagraria* | *Matricaria suaveolens* |
| *Avena fatua* | *Mimulus guttatus* |
| *Buddleja davidii* | *Fallopia japonica* |
| *Centrathus ruber* | *Rhododendron ponticum* |
| *Crepis vesicaria* | *Senecio squalidus* |
| *Elodea canadensis* | *Smyrnium olusatrum* |
| *Epilobium brunnescens* | *Symphoricarpos albus* |
| *Epilobium ciliatum* | *Veronica filiformis* |
| *Erigeron canadensis* | *Veronica persica* |

### 3.1.3. Mating systems and Hybridization

The mating systems of invasive plants have been investigated by many studies (Allard, 1965; Brown and Burdon, 1987) in the hope that these systems would help to determine the most appropriate reproductive strategy allowing a species to successfully colonize a novel environment. No single reproductive strategy seems to have been adopted by colonizing species. However, self-fertilization and apomixis (a reproductive process found in plants that on the surface resembles normal sexual reproduction but in which there is no fusion of gametes) have predominantly been found in many colonizing species (Brown and Marshall, 1981; Price and Jain, 1981). In addition, most of the outcrossing species have been found to be self-compatible. These uniparental mating systems are advantageous as they allow the most successful genotypes to be maintained in order for the population to be able to exploit a new habitat (Brown and Burdon, 1987). Furthermore, the reduction in genetic variation associated with colonizing species can be avoided by the mode of reproduction in the introduced species (Barrett and Husband, 1990), such as those invasive plant species that reproduce asexually by apomixis or vegetative reproduction (Baker, 1995; Calzada *et al.*, 1996). Some alien invasive species have been found to have a different reproductive strategy from that exhibited in their native range. This has been demonstrated with *Rubus alceifolius*, which was found as an apomictic species in its introduced Madagascan range as compared to its native Asian range where it reproduces sexually (Amsellum *et al.*, 2001). The switch in reproductive system to apomixis was found to be a consequence of hybridisation of *R. alceifolius* and native *R. roridus*.

Hybridisation is considered as a phenomenon that plays a very important role in the establishment of many species, especially in the part it plays in evolution

(Rieseberg, 1997). The low genetic diversity found in introduced species has been put forward as a reason contributing to limiting the species ability to expand its range (Milne and Abbott, 2000). However, it has also been suggested that if these species were to hybridise with other species, they might receive the necessary genetic variation allowing them to adapt to a novel environment and thereafter spread in the introduced range (Milne and Abbott, 2000). Emms and Arnold (1997) pointed out that the outcome of hybridisation for the purpose of speciation is dependent on the fitness of hybrids, which can vary with environmental conditions. In addition, disturbance brings together species that are capable of hybridising (O'Hanlon *et al.*, 1999). This has been demonstrated with *Carduus* thistles, where two species (*C. nutans* and *C. acanthoides*) native to Europe hybridised when introduced into Canada, and resulted in the local abundance of hybrid and backcrossed plants (Warwick *et al.*, 1989). Furthermore, Milne and Abbott (2000), in their study of *Rhododendron ponticum*, found that this invasive species, which is naturalized in the British Isles, has hybridised with three other *Rhododendron* species (*R. catawbiense*, *R. maximum* and an unidentified species). In arriving at their results Milne and Abbott used three determining criteria, namely, locating the native populations which might have acted as source populations, determining if the introduced species contained only a sample of the genetic variation from the source population, and whether the hybridisation of these species with other species have resulted in them acquiring genes from these other species.

### 3.1.4. Models of colonization

Natural populations of plants are dynamic systems and are subject to variation (Bascompte and Sole, 1995). Consequently, population genetic studies have to deal with non-equilibrium states. In fact, founding events can create persistent non-equilibrium structures (Boileau *et al.*, 1992), resulting from events, such as recolonization of a habitat left unoccupied after the extinction of a previous population, by the introduction of a species in a novel environment, or during the expansion of species' range following disturbances. Therefore, some colonization models are considered below with their effects on genetic structure of invasive populations.

#### 3.1.4.1. Continent-island model

The continent-island model is the simplest model of colonization (Barrett and Husband, 1990), which assumes that migration is unidirectional from a large source (fixed allele frequency) to small isolated colonies or islands. Therefore, at equilibrium, the allele frequencies in these small colonies will be different to those in their source, and as a result of genetic drift will also differ from the genetic composition of the initial migrants (Nei *et al.*, 1975). This model can be applied to plant colonization events that involve long-distance dispersal.

#### 3.1.4.2. Island model

The island model, in contrast to the continent-island model, assumes that migration is multidirectional among subpopulations, which are assumed to be of the same size (Barrett and Husband, 1990). Here two patterns of population differentiation have been identified: the random pattern and the stepping stone pattern (Kimura and Weiss, 1964). In the former pattern, the migration among populations is random, while in the latter the migration occurs only between adjacent subpopulations.

#### 3.1.4.3. Central-marginal model

The central-marginal model assumes that central habitats are less isolated than marginal ones, and as a result the central populations are more genetically differentiated and more variable than the marginal ones (Barrett and Husband, 1990). The reason for this difference in genetic composition between the marginal and central populations could be due to there being colonization of marginal populations by migrants from a central source, making the genetic differentiation in the marginal populations low maybe due to genetic drift.

#### 3.1.4.4. Metapopulation models

The metapopulation model was developed by Slatkin (1977) to explain the effects of extinction and recolonization on genetic variation. Two forms of colonization are explained that have different consequences for population differentiation (Wade and McCauley, 1988). Firstly, there is the migrant pool model which describes the variation to be due to the individual colonists being chosen randomly from the metapopulation. The second model, the propagule model, on the other hand, is described when the colonizing population is chosen randomly from a single source population. The propagule model has a clear advantage over the migrant pool model in that extinction never decreases differentiation but enhances it. Therefore, these two models have different effects on the genetic variation among populations.

### 3.1.5. Random Amplified Polymorphic DNA (RAPD)

Molecular genetic markers have been developed into powerful tools to analyse genetic relationships. One such genetic marker, frequently used in studies of molecular ecology, are Random Amplified Polymorphic DNA (RAPD) markers. In order to use this technique, no previous knowledge of the organism is required. The RAPDs are therefore suitable for genetic mapping, taxonomic and population studies (Howland and Arnau, 1992). The main advantages of the RAPD technique include its suitability for work on anonymous genomes, applicability to problems where only limited quantities of DNA are available, and efficiency and low expense (Hadrys *et al.*, 1992). In addition, RAPDs are resolved using the polymerase chain reaction (PCR), a technique first devised by Kary Mullis in the mid-1980s, which allows the production of enormous numbers of copies of a specified DNA sequence. The PCR amplifies a set of fragments, which can vary in size when different individuals are analysed (Williams *et al.*, 1990). Regardless of its uses, RAPD fingerprinting has its difficulties and limitations, which were described in chapter 1.

### 3.1.6. This study

Comparison of native and alien species would allow further understanding of the factors that make a species a successful invader. However, few studies have addressed the issue of genetic variation, comparing it in the native and alien ranges (Brown and Marshall, 1981; Clegg and Brown, 1983; Barrett and Husband, 1990). Therefore, this chapter investigates the genetic structure of *Hirschfeldia incana* in its native southern European range and its introduced range in the UK. This it attempts to do by:

(a) Using Random Amplified Polymorphic DNA (RAPD) markers and Polymerase Chain Reaction (PCR) to identify polymorphic loci that are informative and repeatable, in both the native southern European and introduced UK populations.

(b) Using the RAPD data to compare the genetic diversities between the native southern European and introduced UK populations.

(c) Using the RAPD data to compare the partitions of gene diversities for all geographical groups of populations, i.e. southern Europe (native), England (introduced), and South Wales (introduced).

(d) Constructing a consensus tree to determine relatedness of the different populations of *H. incana*.

(e) Comparing the results of this study with those of other studies investigating genetic diversities between native and alien plant populations.

## 3.2. Materials and Methods

### 3.2.1. Site selection and collection of samples

Sites were selected as described in Chapter 2. The leaves were stored frozen at –20°C, and then Puregene DNA Isolation Kit was used to extract DNA from these leaves (see later).

### 3.2.2. DNA extraction

DNA was extracted from up to 10 leaves from each population. The Puregene DNA isolation kit was used with a few modifications made to the manufacturer's protocol.

A 10-30 mg frozen leaf tissue disc was used, prepared by placing the leaf between a 1.5ml microfuge tube and it's cap and then snapping the cap closed. 300µl of cell lysis solution was then added to this leaf tissue and ground with a pipette tip. A fresh tip was used for each sample. The cell lysate was then incubated at 65°C for 60 minutes (with the lysate inverted 10 times at 30 minute intervals). At the end of this period the lysate was centrifuged for a few seconds and 1.5µl of RNAse A solution was added. The sample was then mixed by inverting the tube 25 times and incubated at 37°C for 15 to 60 minutes.

Following the RNAse treatment, the sample was cooled to room temperature, and 100µl of Protein Precipitation solution was added to the cell lysate. The Protein Precipitation solution was then mixed uniformly with the cell lysate by vortexing each tube at high speed for 20 seconds. Due to the high polysaccharide content, the sample was placed on ice for 30 minutes. At the end of this period the sample was centrifuged at 13000 x g for 5 minutes, at the end of which the precipitated proteins had formed a tight, green pellet.

Next the supernatant containing the DNA was poured into a clean 1.5µl microfuge tube containing 300µl of 100% isopropanol (2-propanol). The sample was then mixed by inverting gently 50 times, followed by centrifuging at 13000 x g for 3 minutes. The DNA was visible as a pellet that ranged in colour from off-white to light green. The supernatant was then poured off and the tube drained briefly on clean, absorbent paper. 300µl of 70% ethanol were added to the sample and the tube inverted several times to wash the DNA pellet. Then the centrifuge treatment was repeated for 3 minutes and the ethanol poured off carefully to leave the DNA pellet behind. The tube then inverted and drained on clean, absorbent paper that was placed on a heating block at 65°C for a few minutes to aid drying. It was important to ensure that all the ethanol had evaporated from the tube, as ethanol has been known to inhibit the PCR

Finally, the DNA was hydrated by adding 50µl of DNA hydration solution to the tube, and placing it in a rotator at room temperature, overnight.

### 3.2.3. DNA quantification

DNA was quantified using the Gene Quantification Machine (Pharmacia – Gene Quant). 4µl of each sample were taken and mixed well with 76µl of water. For calibration, a 'blank' was prepared with 4µl of DNA hydration solution from the Puregene kit and 76µl of water. The machine was calibrated with the cuvette loaded with 70µl of the 'blank', and then samples were quantified by loading the same volume into the cuvette. Care was taken to ensure that the cuvette was washed out between each sample to prevent any errors.

### 3.2.4. Optimisation of DNA for PCR (Polymerase Chain Reaction)

The DNA was required to be diluted to a concentration that was standard in all the PCRs carried out subsequently. This concentration was found to be at $20ng\mu l^{-1}$, as standardised by optimisation reactions of PCR. The DNA concentrations that were measured using the GeneQuant Machine were used to calculate preparations of DNA solutions at $20ng\mu l^{-1}$. The general formula that was used is shown below:

$$\frac{\text{Total volume wanted}}{\frac{(X\ ng\mu l^{-1})}{(20\ ng\mu\ l^{-1})}} = \text{Volume of DNA extract}$$

As the total volume required was 50 µl:

$$\frac{50\ \mu l}{\frac{(X\ ng\ \mu l^{-1})}{(20\ ng\ \mu l^{-1})}} = \text{Amount of DNA extract} + \text{------}\ H_2O = 50\ \mu l\ \text{total}$$

### 3.2.5. PCR DNA Amplification

Polymerase Chain Reaction (PCR) was performed in a volume of 25µl containing 2.5µl of 10 x reaction buffer (750mM Tris HCL, 200mM $(NH_4)_2SO_4$, 0.1% v/v Tween 20, pH 8.8), 2.5µl of dNTP (Promega), containing each dNTP in equal amounts, 2.0µl of 2.5mM $MgCl_2$, 0.2µl of Red Hot Taq polymerase (Abgene), 2.5µl of DNA extract (or 2.5µl $H_2O$ in the blank), 0.63µl of primer (Operon) and 14.68µl of nuclease-free $H_2O$. A total of 5 primers from the kits A (08, 16), C(11), and E(01, 14) of OPERON Technologies (Alameda, CA, USA) were used for PCR amplification to produce reproducible and informative marker patterns.

DNA amplifications were performed in a DNA Thermal Cycler (MJ Research PTC-225 Peltier Thermal Cycler) programmed for an initial denaturing step of 2 minutes at 94°C followed by 1 minute at 36°C (primer annealing step), 2 minutes at 72°C (primer extension step) and then 44 cycles of 30s at 94°C, 1 minute at 36°C and 2 minutes at 72°C.

### 3.2.6. Data analyses

Shannon's Index of Diversity

The Shannon's index of diversity (Bussell, 1999) was calculated for each RAPD locus, for each population as

$$H'_j = -\sum p_i \log_2 p_i$$

Where $p_i$ is the frequency of the presence or absence of a RAPD in that population. The average diversity over all populations was calculated for each locus as

$$H'_{pop} = \frac{1}{n}\sum H'_j$$

where n is the number of populations. The species diversity was calculated for each locus as

$$H'_{sp} = -\sum p_s \log_2 p_s$$

where $p_s$ is the frequency of presence or absence of the RAPD in the whole sample (233 individuals in this case). Therefore, for each locus, the component of diversity within populations is $H'_{\mathrm{pop}}/H'_{\mathrm{sp}}$ and the component between populations is $(H'_{\mathrm{sp}}-H'_{\mathrm{pop}})/H'_{\mathrm{sp}}$.

$G'$st, the average of $(H'_{\mathrm{sp}}-H'_{\mathrm{pop}})/H'_{\mathrm{sp}}$ values for each polymorphic locus, describes the overall partition of diversity for all populations.

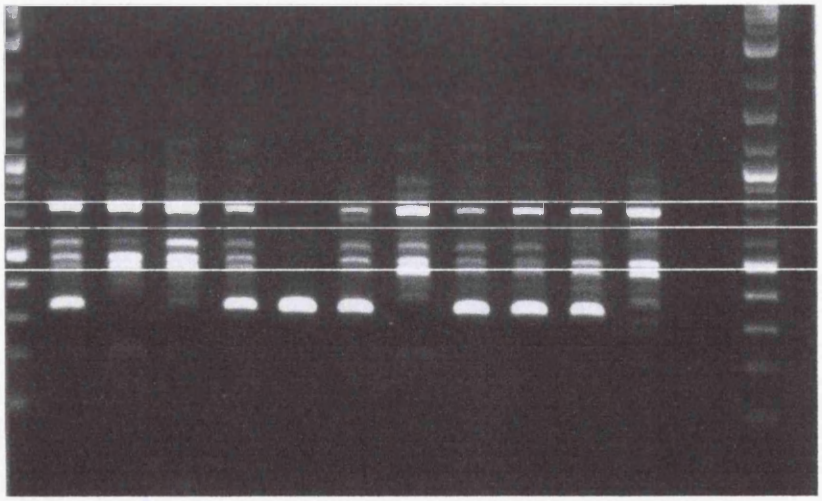
Pairwise genetic distances, Nei's D (Nei, 1972), calculated with the Bayesian-estimated allele frequencies in AFLP-SURV (Vekemans *et al.*, 2002) were also used in tests of spatial autocorrelation. Bootstrapped pairwise genetic distances were further used in neighbour-joining cluster analyses as implemented in PHYLIP (Felsenstein, 1993). This Bayesian approach gives nearly unbiased estimates of genetic distances (as well as heterozygosity and *F*-statistics) following estimates of allelic frequencies at each locus in each population by assuming that they are dominant, and have only two alleles (i.e. presence and absence of a band) (Zhivotovsky, 1999). This method assumes Hardy-Weinberg genotypic proportions. $H_j$, the expected heterozygosity or Nei's gene diversity was also calculated using this method. A simpler, more biased method was proposed by Lynch and Milligan (1994), which estimated the frequency of the null allele by taking the square root of the fraction of individuals with no band. Therefore, the Bayesian approach was deemed a better choice in this study. The neighbour-joining cluster analysis method was subsequently used to construct a tree by successive clustering of lineages.

## 3.3 Results

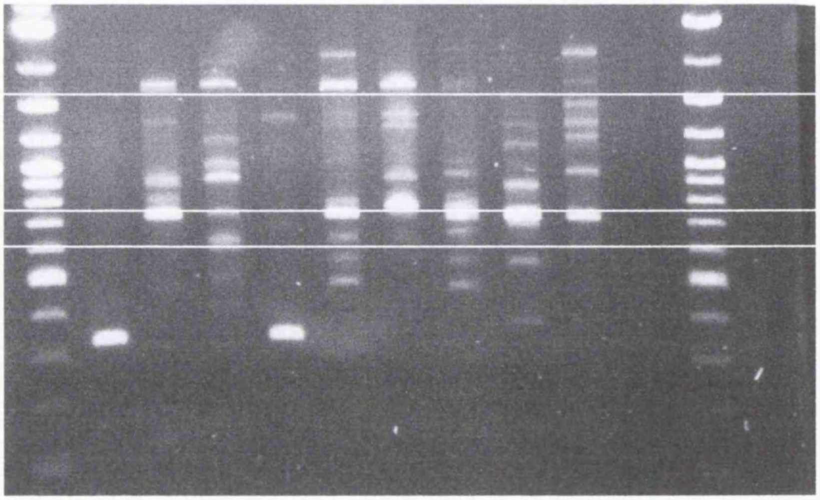
### 3.3.1. RAPD profiles

In all, 30 informative, repeatable and clear loci were identified. Screening with primers OPA-08, OPA-16, OPC-11, OPE01 and OPE-14 yielded 5, 6, 8, 4 and 7 loci, respectively. Some of these are shown in Figure 3.1. The white lines indicate the positions of the chosen loci, which are specified in the legend, with names according to the primer used to screen them and the fragment length, in base pairs. The far right and far left lanes contain the marker enabling the bands to be scored according to size, and each of the lanes in the middle contain DNA solutions with extractions from each of 10 plants in each population.

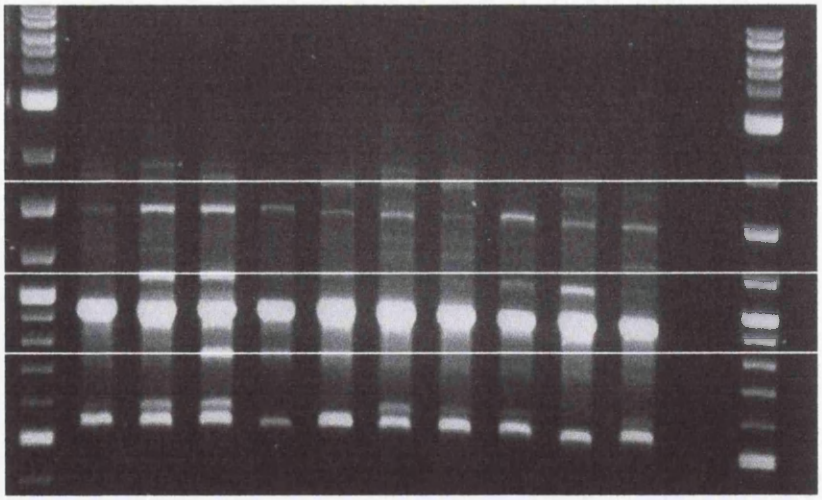
**Figure 3.3.1. RAPD profiles of some South Wales, England and Southern Europe populations.**



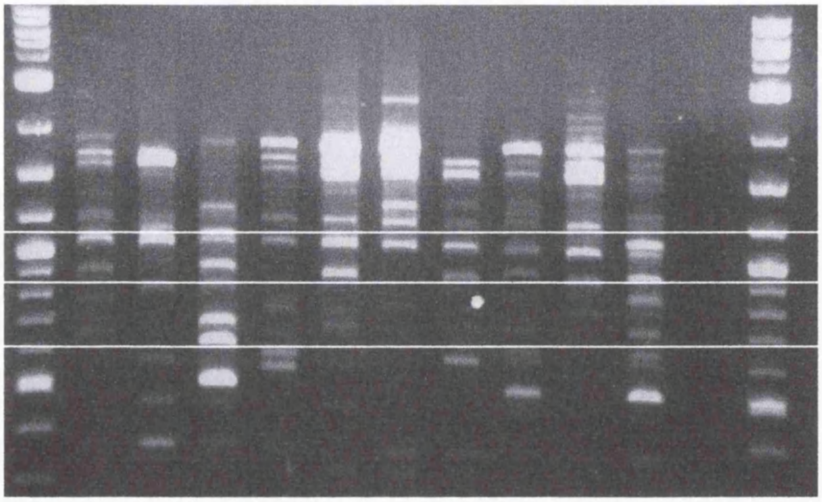
RAPD profile of Bridgend plants screened with primer OPA-08. Lines indicate loci OPA-08/550, OPA-08/750 and OPA-08/900 (bottom to top).



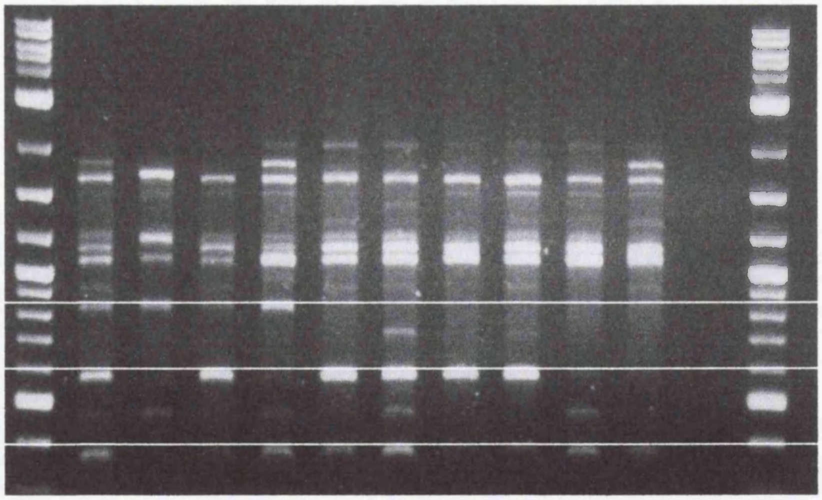
RAPD profile of Kent plants screened with primer OPA-08. Lines indicate loci OPA-08/750, OPA-08/900 and OPA-08/1700 (bottom to top).



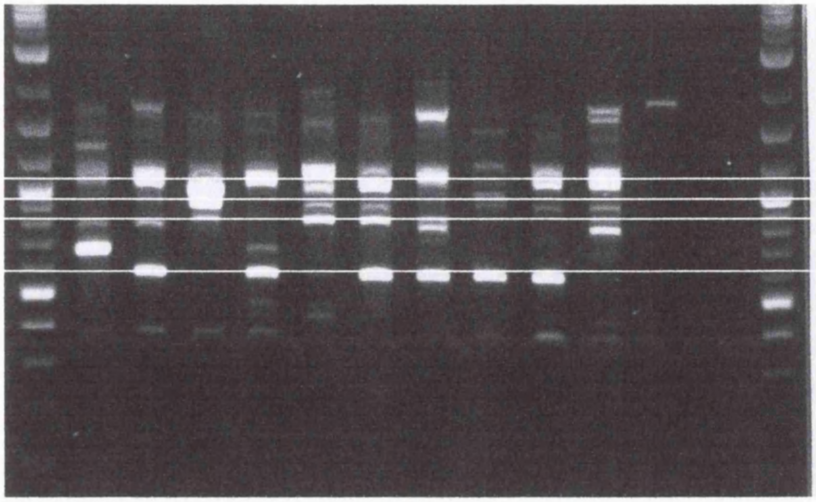
RAPD profile of Mallorca plants screened with primer OPA-08. Lines indicate loci OPA-08/750, OPA-08/1150 and OPA-08/1700 (bottom to top).



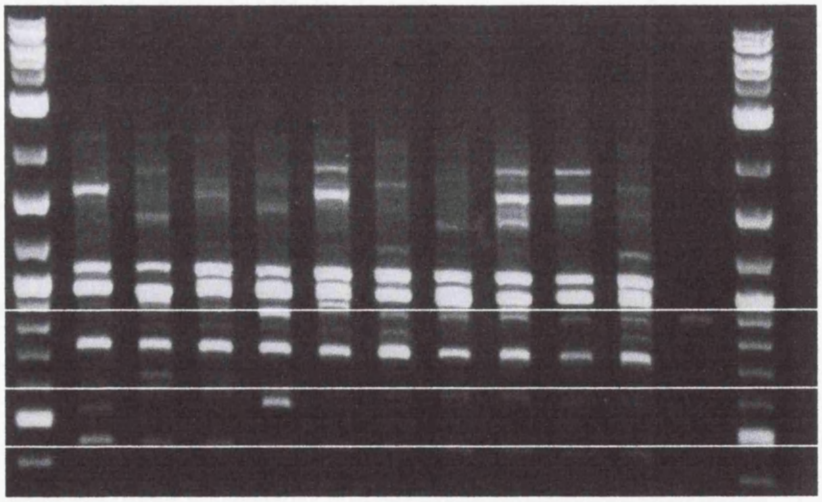
RAPD profile of Crete plants screened with primer OPA-16. Lines indicate loci OPA-16/600, OPA-16/850 and OPA-16/1100 (bottom to top).



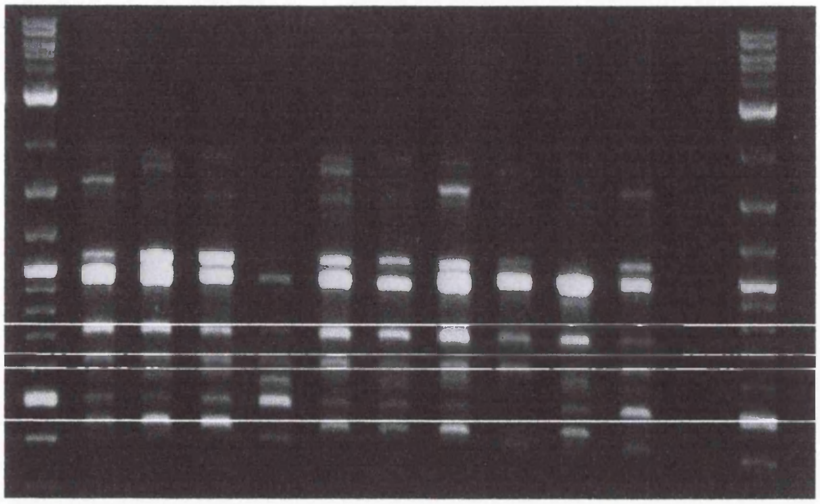
RAPD profile of Middlesex plants screened with primer OPA-16. Lines indicate loci OPA-16/400, OPA-16/600 and OPA-16/850 (bottom to top).



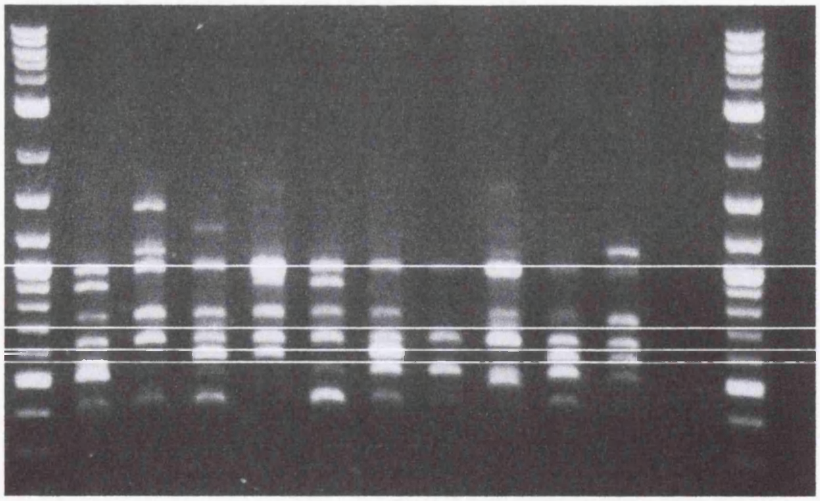
RAPD profile of Middlesex plants screened with primer OPA-16. Lines indicate loci OPA-16/600, OPA-16/850, OPA-16/1000 and OPA-16/1100 (bottom to top).



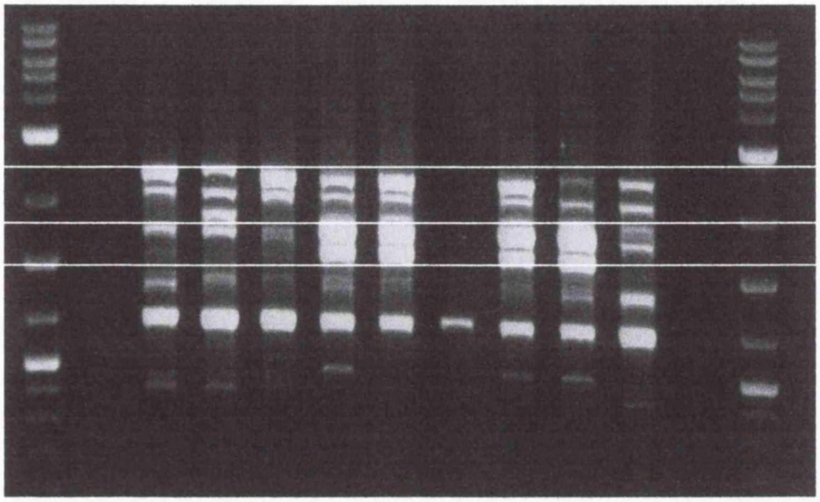
RAPD profile of Kent plants screened with primer OPC-11. Lines indicate loci OPC-11/450, OPC-11/600 and OPC-11/900 (bottom to top).



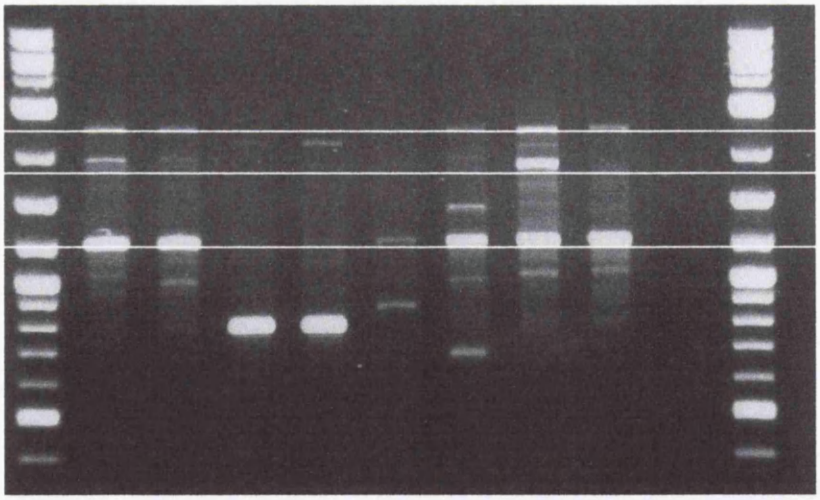
RAPD profile of Mallorca plants screened with primer OPC-11. Lines indicate loci OPC-11/450, OPC-11/600, OPC-11/650 and OPC-11/730 (bottom to top).



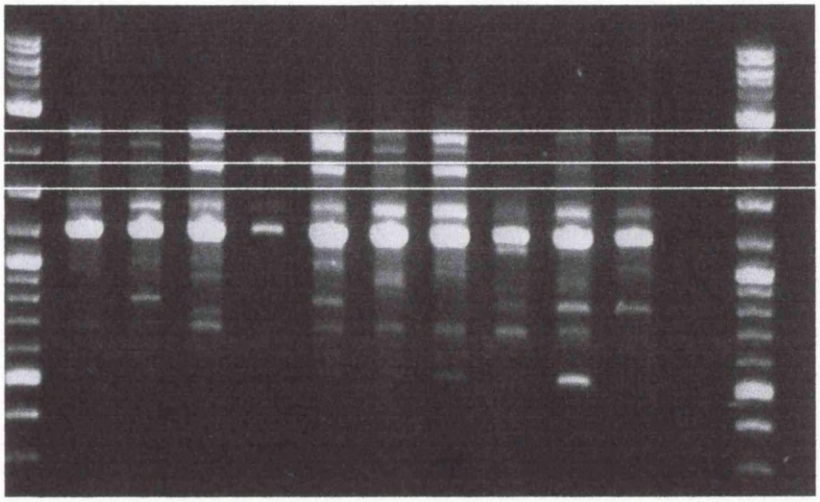
RAPD profile of Swansea plants screened with primer OPC-11. Lines indicate loci OPC-11/580, OPC-11/600, OPC-11/700 and OPC-11/1000 (bottom to top).



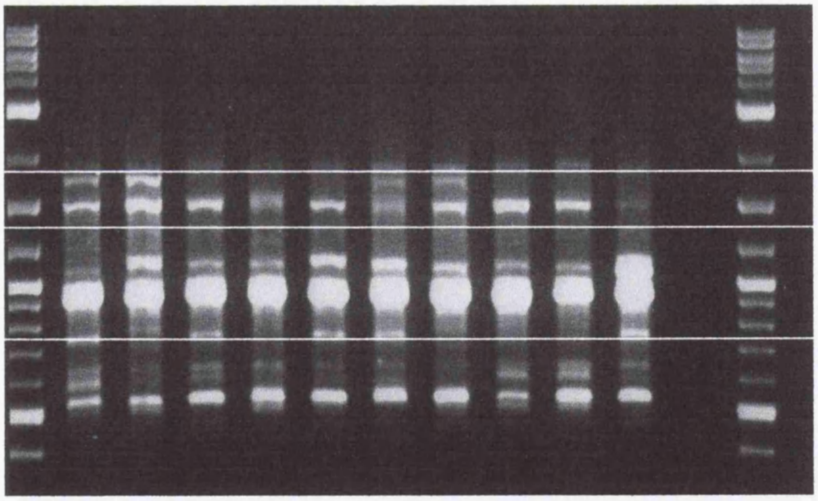
RAPD profile of East Norfolk plants screened with primer OPE-01. Lines indicate loci OPE-01/1500, OPE-01/1700 and OPE-01/2500 (bottom to top).



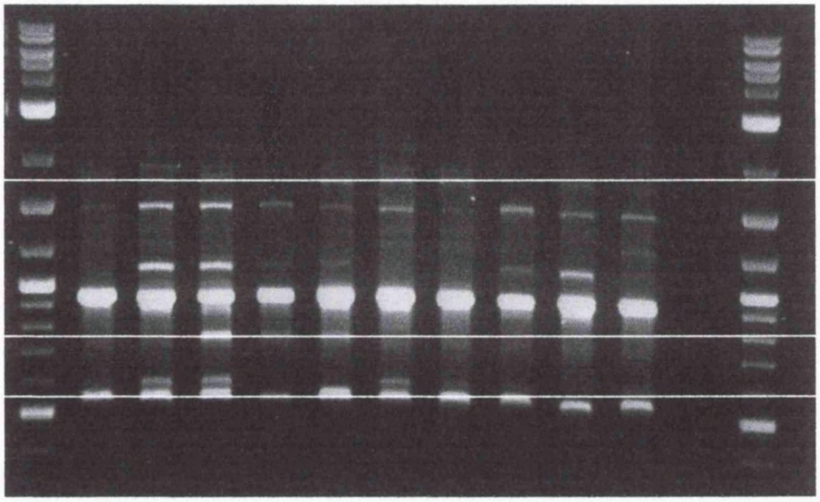
RAPD profile of Gowerton plants screened with primer OPE-01. Lines indicate loci OPE-01/1200, OPE-01/1700 and OPE-01/2500 (bottom to top).



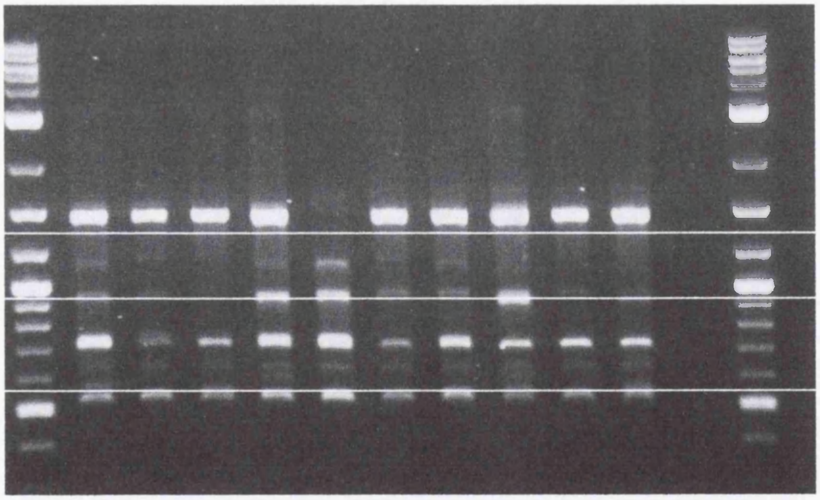
RAPD profile of Mallorca plants screened with primer OPE-01. Lines indicate loci OPE-01/1500, OPE-01/1700 and OPE-01/2500 (bottom to top).



RAPD profile of Kent plants screened with primer OPE-14. Lines indicate loci OPE-14/750, OPE-14/1400 and OPE-14/1700 (bottom to top).



RAPD profile of Mallorca plants screened with primer OPE-14. Lines indicate loci OPE-14/540, OPE-14/750 and OPE-14/1700 (bottom to top).



RAPD profile of Pontardulais plants screened with primer OPE-14. Lines indicate loci OPE-14/540, OPE-14/950 and OPE-14/1300 (bottom to top).

### 3.3.2. Genetic diversities

Table 3.3 shows the genetic diversities averaged over all the loci for *H. incana* samples collected from locations in southern Europe and the UK. Lower diversities were found in the South Wales populations relative to those in the English populations. This was proven by the Mann-Whitney U test that showed a significant value of $p = 0.023$ for $H'_j$, and $p = 0.018$ for $H_j$. However, a wide range of diversities were noted among the southern European native populations, where the Crete and Portugal populations showed higher diversities (0.818 and 0.799, respectively) than the Mallorca populations (0.492). Therefore on further investigation there were no significant differences found between the genetic diversities of the British and southern European populations ($p$=0.271 for $H'_j$ and $p$=0.599 for $H_j$). The genetic diversities in the British samples were looked at further in terms of when the populations appeared in the UK (Table 3.4). All the South Wales populations appeared in 1950 with the exceptions of East Glamorgan (wEGL), which appeared in 1930 and Pembroke Dock (wPBD) that appeared in 1970. The Mann-Whitney U test showed a significant difference between the younger and older populations ($p$=0.007 for $H'_j$ and $p$=0.011 for $H_j$).

**Table 3.3. Gene diversities averaged over all loci (with SE) for *H. incana* samples collected from locations in southern Europe and the UK.**

| Code | Location | N | $H_j$(SE) | $H'_j$(SE) |
|---|---|---|---|---|
| | **South Wales:** | | | |
| wBR1 | Bridgend | 10 | 0.276 (0.029) | 0.398 (0.074) |
| wCM3 | Carmarthen | 10 | 0.299 (0.031) | 0.420 (0.081) |
| wEGL | East Glamorgan | 9 | 0.377 (0.021) | 0.554 (0.074) |
| wGO1 | Gower 1 | 9 | 0.288 (0.032) | 0.470 (0.068) |
| wGO2 | Gower 2 | 9 | 0.354 (0.028) | 0.558 (0.073) |
| wGO3 | Gower 3 | 8 | 0.467 (0.019) | 0.682 (0.062) |
| wMON | Monmouthshire | 10 | 0.438 (0.018) | 0.778 (0.050) |
| wNP1 | Neath Port Talbot 1 | 10 | 0.352 (0.031) | 0.552 (0.083) |
| wNP2 | Neath Port Talbot 2 | 10 | 0.319 (0.034) | 0.493 (0.078) |
| wPON | Pontarddulais | 10 | 0.315 (0.032) | 0.450 (0.079) |
| wPBD | Pembroke Dock | 10 | 0.394 (0.027) | 0.598 (0.069) |
| wSW1 | Swansea 1 | 10 | 0.352 (0.023) | 0.606 (0.072) |
| wSW2 | Swansea 2 | 10 | 0.395 (0.024) | 0.593 (0.077) |
| | Means | | 0.355 (0.026) | 0.550 (0.072) |
| | **England:** | | | |
| eENK | East Norfolk | 10 | 0.485 (0.011) | 0.787 (0.033) |
| eKEN | Kent | 10 | 0.365 (0.025) | 0.537 (0.076) |
| eLIN | Lincolnshire | 10 | 0.412 (0.023) | 0.612 (0.072) |
| eMDX | Middlesex | 10 | 0.439 (0.016) | 0.651 (0.071) |
| eSUX | Sussex | 9 | 0.408 (0.022) | 0.725 (0.058) |
| eYOR | York | 10 | 0.425 (0.011) | 0.908 (0.022) |
| | Means | | 0.422 (0.018) | 0.703 (0.055) |
| | **Southern Europe** | | | |
| cCRE | Crete | 10 | 0.435 (0.016) | 0.818 (0.035) |
| cPOR | Portugal | 10 | 0.416 (0.022) | 0.799 (0.031) |
| cMCA | Mallorca | 10 | 0.350 (0.034) | 0.492 (0.080) |
| | Means | | 0.400 (0.024) | 0.703 (0.049) |

**Table 3.4. Estimates of the year of arrival of *H. incana* populations in the UK**

| SITE | APPROXIMATE YEAR APPEARED |
|---|---|
| East Glamorgan (wEGL) | 1930 |
| Bridgend (wBR1)<br>Carmarthen Pensarn (wCM2)<br>National Botanic Gardens (wCM1)<br>Crofty (wGO1)<br>Horton (wGO3)<br>Gowerton (wGO2)<br>Monmouthshire (wMON)<br>Sandfields (Port Talbot) (wNP1)<br>Neath Abbey (wNP3)<br>Pontardulais (wPON)<br>Tawe Bridge (wSW1)<br>Swansea Bay (wSW3)<br>Kent (eKEN) | 1950 |
| Pembroke Dock (wPBD)<br>Middlesex (eMDX)<br>Yorkshire (eYOR) | 1970 |
| East Norfolk (eENK)<br>Lincolnshire (eLIN)<br>Sussex (eSUX) | 1987 |

### 3.3.3. Genetic differentiation

The $G_{ST}$ (proportion of genetic diversity between populations) and $G'_{ST}$ (overall partitioning of genetic diversities between populations) values were found to be higher in the South Wales populations than in the England and southern Europe populations (Table 3.5). In fact, both the $G_{ST}$ and $G'_{ST}$ values for England and native southern Europe populations were found to be similar. However, the diversities within populations ($H'_{pop}/H'_{sp}$) were higher in the England (0.766) and southern Europe (0.733) populations than in the South Wales populations (0.586).

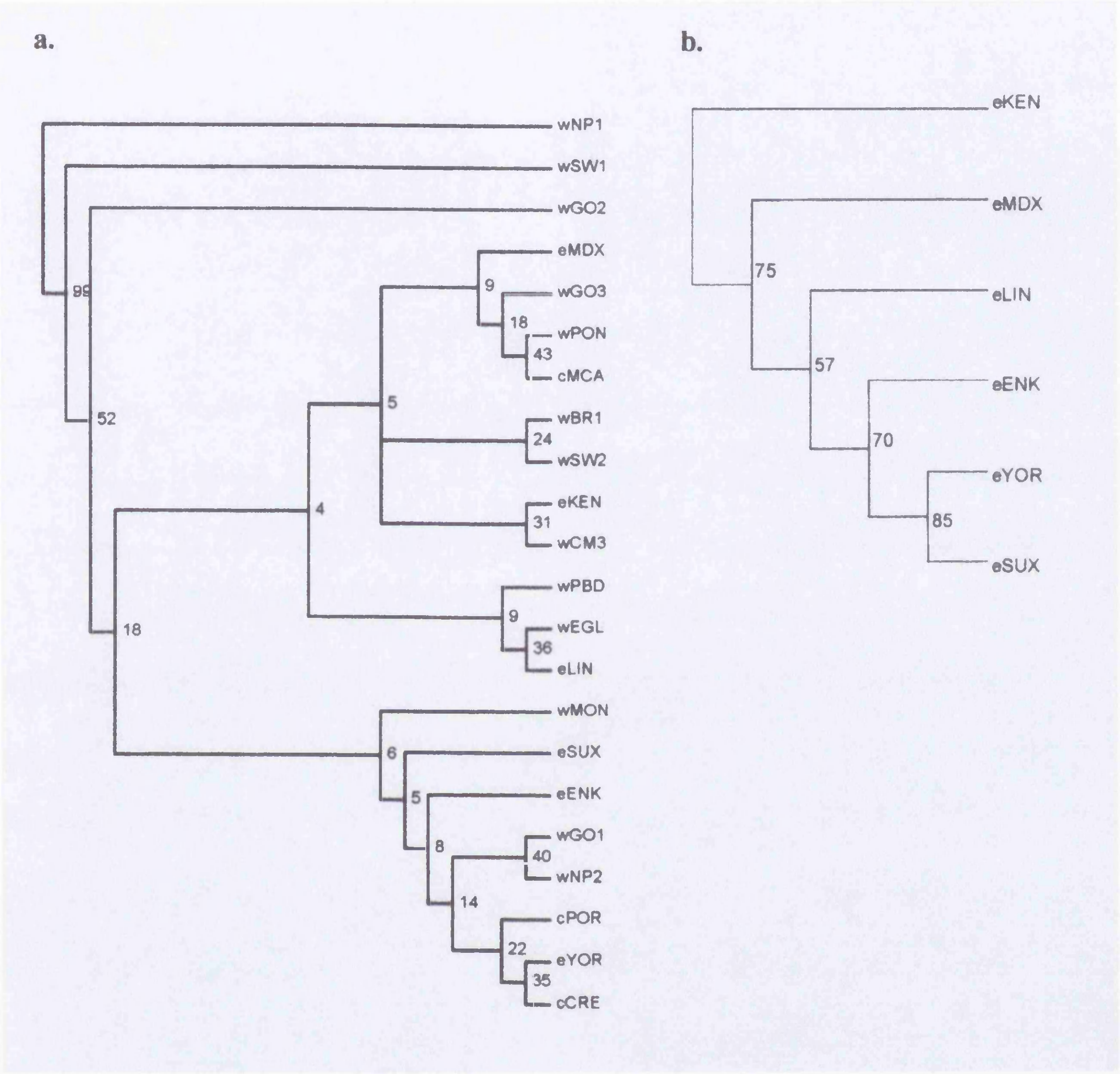
**Table 3.5. Partitions of gene diversities for all geographical groups of populations**

| | $H_w$ | | $G_{ST}$ | | | |
|---|---|---|---|---|---|---|
| Groups | $F_{IS} = 0$ | $F_{IS} = 0.5$ | $F_{IS} = 0$ | $F_{IS} = 0.5$ | $H'_{pop}/H'_{sp}$ | $G'_{ST}$ |
| All | 0.380 | 0.369 | 0.149 | 0.189 | 0.598 | 0.402 |
| S Wales | 0.356 | 0.336 | 0.188 | 0.242 | 0.586 | 0.413 |
| England | 0.422 | 0.422 | 0.080 | 0.098 | 0.766 | 0.234 |
| S Europe | 0.400 | 0.409 | 0.112 | 0.135 | 0.733 | 0.267 |

### 3.3.4. Cluster analysis

Cluster analysis was used to produce consensus trees with bootstrap values for all the locations [Figure 3.2 (a)] and for only the England locations [Figure 3.2 (b)]. No distinct pattern of genetic differentiation was observed from in trees as low bootstrap values (<50) were calculated for all the locations apart from wNP1, wSW1 and wGO2. In addition, the clusters did not form according to geographical positions of the locations. For example, cCRE (native location in Crete) was grouped with eYOR (introduced England location in York). Stronger bootstrap values for all clusters were calculated when only the England locations were used, but again the pattern of clusters was not related to the geographical distances between the locations.

**Figure 3.2. Consensus trees constructed by cluster analysis. (a) All samples; (b) English samples only (key to samples in Table 3.3)**



### 3.3.5. Comparison with other studies

A literature review of other studies investigating genetic diversities in native and alien plant populations is shown in Table 3.6. The table compares the genetic diversities and/or genetic differentiation between native and alien populations according to their reproductive strategy. The plants with an inbreeding (or selfing or asexual) breeding system show a reduction in the genetic diversity from the native to the alien range. *Bromus tectorum* also shows a decrease in the genetic differentiation from the native to the alien range.

The next section shows plants that have switched their reproductive strategy from outbreeding in their native range to inbreeding or asexual in the alien range. Here, on the whole, all species exhibit a decrease in the genetic diversity from the native to the alien range.

The final section shows outbreeding species where *H. incana* has been included. In contrast to the other two sections, here the genetic diversities in the native range were found to be either equal to or very similar to those in the alien range. The genetic differentiation, on the other hand, showed a decrease from the native to the alien range, with the exception of *H. incana* that showed an increase in its introduced range in South Wales.

**Table 3.6. Studies comparing genetic diversities in native and alien populations for various plants of different breeding systems. Symbols indicat the qualitative direction and extent of the difference between genetic diversity measures or estimates of genetic differentiation between native an alien populations: = indicates similar degrees of genetic diversity/differentiation; < or > indicate that genetic diversity/differentiation is smaller o larger (respectively) in the native as compared to introduced populations; double symbols (<< or>>) indicate that the magnitude of the difference i twice or more.**

| | | GENETIC DIVERSITY | | | GENETIC DIFFERENTIATION | | | |
|---|---|---|---|---|---|---|---|---|
| ORGANISM | METHOD | NATIVE | Difference (<,>,=) | ALIEN | NATIVE | Difference (<,>,=) | ALIEN | REFERENCES |
| **a) Inbreeding/Selfing/Asexual breeding system:** | | | | | | | | |
| *Avena barbata* | Isozyme | W Mediterannean | | California | | | | (Clegg and Allard,1972) |
| | | A = 2.4 | > | | | | | |
| | | $H_T$ = 0.435 | >> | 0.001-0.180 | | | | |
| | Isozyme | Spain | | California | | | | (Garcia *et al.*, 1989) |
| | | $G_T$ = 45 | >> | 3 | | | | |
| *Bromus tectorum* | Isozyme | Eurasia & N Africa | | N America | | | | (Novak and Mack, 1993) |
| | | A = 1.01 | = | 1.05 | $G_{ST}$ = 0.754 | > | 0.478 | |
| | | P = 52 | > | 28 | | | | |
| | | $H_j$ = 0.009 | << | 0.046 | | | | |
| *Capsella bursa-pastoris* | Isozyme | Europe | | N America | | | | (Neuffer and Hurka, 1999) |
| | | $G_T$ = 83 | >> | 41 | | | | |
| *Chondrilla juncea* | RAPD | Turkey | | Australia | | | | (Chaboudez, 1994) |
| | | $G_T$ = 91 | >> | 3 | | | | |

**b) Outbreeding system in natives but shift to inbreeding/asexual in aliens:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Eichhornia paniculata* | Isozyme | Brazil | | Jamaica | | | | |
| | | $A = 1.27$ | > | 1.07 | $F_{ST} = 0.324$ | << | 0.633 | (Glover and Barrett, 1987) |
| | | $P = 23.8$ | >> | 7.6 | | | | |
| | | $H_o = 7.8$ | >> | 2 | | | | |
| | | $H_j = 0.09$ | >> | 0.03 | | | | |
| | | $H_T = 0.15$ | >> | 0.06 | | | | |
| *Fallopia japonica* var. *japonica* | RAPD | Asia | | Europe and N America | | | | (Hollingsworth and Bailey, 2000) |
| | | $G_T = 12$ | >> | 1 | | | | |
| *Rubus alceifolius* | AFLP | SE Asia | | Indian Ocean Islands, Australia | | | | (Amsellum *et al.*, 2000) |
| | | SI = 0.73-0.99 | < | 0.83-0.99 | | | | |

**c) Outbreeding:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Allaria petiolata* | ISSR | Europe | | N America | | | | (Meekins, Ballard and McCathy, 2 |
| | | $H_T = 0.940$ | = | 0.91 | | | | |
| *Apera spicaventi* | Isozyme | Europe | = | Canada | | | | |
| | | $P = 62\%$ | = | 57% | $G_{ST} = 0.024$ | >> | 0.01 | |
| | | $A = 2.53$ | = | 2.54 | | | | |
| | | $H_o = 0.228$ | = | 0.23 | | | | |
| | | $H_j = 0.203$ | = | 0.209 | | | | |
| | | $H_T = 0.208$ | = | 0.211 | | | | |
| *Echium plantagineum* | Isozyme | Europe | | Australia | | | | (Brown and Burdon, 1986) |
| | | $A_P = 2.6$ | = | 2.7 | | | | |
| | | $P = 82$ | < | 94 | | | | |
| | | $H_o = 0.29$ | = | 0.32 | | | | |
| | | $H_j = 0.35$ | = | 0.34 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $H_T = 0.38$ | = | 0.39 | | | | |
| *Epipactis helleborine* | Isozyme | Europe | | N America | | | | (Squirrell *et al.*, 2001) |
| | | $A = 1.77$ | = | 1.9 | | | | |
| | | $A_P = 2.4$ | = | 2.54 | | | | |
| | | $P = 55$ | = | 58 | | | | |
| | | $H_j = 0.230$ | = | 0.232 | | | | |
| | cpDNA | $PP = 24$ | << | 92 | $F_{ST} = 0.506$ | > | 0.367 | |
| *Hirschfeldia incana* | RAPD | Southern Europe | | England | | | | This study |
| | | $H_j = 0.400$ | = | 0.112 | $G_{ST} = 0.112$ | = | 0.08 | |
| | | | | South Wales | | | | |
| | | | = | 0.356 | | < | 0.188 | |
| *Ligustrum robustum* ssp. *Walkeri* | RAPD | Sri Lanka | | La Reunion | | | | (Milne and Abbott, in press) |
| | | $H_j = 12.084$ | = | 10.47 | | | | |
| *Lolium perenne* | Isozyme | Italy | | Corsica | | | | (Balfourier and Charmet, 1994) |
| | | $A = 2.84$ | = | 2.79 | | | | |
| | | $P = 87$ | = | 85 | | | | |
| | | $H_j = 0.326$ | = | 0.347 | | | | |
| *Raphanus raphanistrum* | Isozyme | England* | | N America** | | | | |
| | | $P = 13.3$ | << | 75-100 | | | | |
| | | $H_j = 0.133$ | << | 0.27-0.58 | | | $F_{ST} = 0.14$ | (Lewis-Jones, Thorpe and Wallis,<br>(Kercher and Conner, 1996)** |
| *Trifolium hirtum* | Isozyme | Turkey | | California | | | | (Molina-Freaner and Jain, 1992) |
| | | $A = 1.07$ | = | 1.18 | $G_{ST} = 0.82$ | >> | 0.3 | |
| | | $A_P = 1.19$ | < | 1.97 | | | | |
| | | $H_j = 0.0144$ | << | 0.0549 | | | | |
| | | $H_T = 0.0820$ | = | 0.0784 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Turnera ulmifolia* | Isozyme | Latin America | | Caribbean | (Barrett and Shore, 1987) |
| | | A = 2.1 | = | 2 | |
| | | P = 46 | >> | 20 | |
| | | $H_o$ = 0.11 | > | 0.07 | |
| | | $H_j$ = 0.12 | >> | 0.04 | |

Estimators of genetic diversities: A = Mean number of alleles per locus; $A_p$ = Mean number of alleles per polymorphic locus; $G_{mean}$ = Average number of genotypes per population; $G_T$ = total number of genotypes; $H_o$ = Mean observed heterozygosity, $H_j$ = Mean genetic diversity within populations (also known as $H_s$ or average expected heterozygosity); $H_T$ = Mean total genetic diversity; P = Percentage of loci polymorphic; PP = Percentage of polymorphic populations; SI = Similarity index. See respective references for definitions of the estimators used in each study.
Estmators of genetic differentiation between populations: $F_{ST}$ = $F$-statistics; $G_{ST}$ = proportion of genetic diversity between populations.

## 3.4 Discussion

Invasion processes are often associated with a reduction in genetic diversity in the populations in the introduced range as compared to the native range (Barrett and Richardson, 1986; Frankham, 1997). This reduction in genetic variation has been explained to be a result of founder effects. If a single founder population was responsible for the populations of *H. incana* in the UK then all the populations would have originated from that single founder, and the small numbers of initial individuals passing through a genetic bottleneck would be expected to yield low levels of genetic diversity. The England and South Wales populations in the UK exhibit different patterns of genetic diversity. The South Wales populations were found to be significantly less diverse than the England and native southern European populations, and no significant differences were found between the England and native southern European populations. However, even though diversities varied within Britain, the overall range of genetic diversities were in fact comparable to that observed for native populations. Therefore, it seems that the alien populations are a result of multiple founding groups that have originated from different source populations leading to the significant levels of *H. incana* genetic structure in Britain. Furthermore, the history of the colonization of England and South Wales populations may explain the difference in genetic diversities between the two. In fact, the South Wales populations were estimated to have appeared in the UK in 1950, two decades before the England populations, which appeared in 1970 (Table 3.4).

Genetic diversities were also found to be significantly different in younger and older populations of *H. incana* in the UK. Therefore, the metapopulation model (Slatkin, 1977) can be considered here as extinction and recolonization may result in the establishment of an age-structure that could have greatly affected the level of genetic differentiation among populations. Factors associated with this model are whether the individual colonists are chosen randomly from the entire metapopulation (migrant pool model) or whether the individual colonists are chosen from a single parental population (propagule model). The results of this study suggest consistency with the migrant pool model as the genetic diversity is similar in both the introduced UK populations and the native southern Europe populations. However, it can be argued that the English and South Wales populations arose via two different mechanisms due to their different genetic diversities (lower in South Wales). So, it could be that the South Wales populations exhibit a pattern consistent with the propagule model, while the English populations were founded according to the migrant pool model.

If *H. incana* had been introduced into the UK only in birdseed and wool shoddy, it would be unlikely that a large number of individuals would have been introduced at any one time. The genetic bottleneck associated with small populations sizes should therefore ensure a reduction in genetic diversity which is not apparent with *H. incana*. Therefore, this lack of reduction in genetic variability following a bottleneck can be explained by the maintenance of a large effective population size during the bottleneck and a fast population growth directly after the passage through the bottleneck (Merila *et al.*, 1996). In fact, it has been found that not all long-distance colonizing events result in reduced levels of genetic diversity in alien populations as compared to native populations (Barrett and Shore, 1989). Also, differences in population genetic structure between the native and introduced range may be less marked in outcrossing weeds that expand rapidly following establishment, as was found in the self-incompatible, annual *Apera spica-venti* where there was little difference in the levels and patterns of genetic diversity among native and introduced

Canadian populations (Warwick *et al.*, 1987a). *H. incana* appears to be a species that has arrived in the UK in large numbers and subsequently increased its population size rapidly. In addition, *H. incana* was first cultivated in Britain by 1771 and was recorded from the wild in 1837 (Preston *et al.*, 2002), so has been around for a while. The modes of arrival of *H. incana* into the UK have been diverse, ranging from birdseed, wool shoddy, and ship ballast leading to the assumption that there have been multiple introductions of this crucifer into the UK. This in turn, may explain the different genetic diversities found in the UK populations and the similar genetic diversities between the English and native southern Europe populations. Hence, these trends suggest, in accordance with theory (Nei *et al.*, 1975) that only the most severe bottlenecks may result in loss of genetic variability.

Many of the populations of *H. incana* found in the UK are on roadsides, probably due to their seed being dispersed by traffic and people. Jain and Martins (1979) found some general features associated with roadside colonies, which included an increased outcrossing rate, high genetic variability within populations, and more stable plant densities in successful colonies. But in addition to good dispersal methods, *H. incana* seed would require a suitable habitat to be able to germinate and grow. In the introduced populations investigated in this study , disturbance is likely to have played a vital part in the establishment of this alien crucifer. High levels of disturbance result in periodic germination of genetically diverse seeds from the seed bank, while in less disturbed sites strong biotic pressures operate on individuals and lead to populations with less genetic diversity (Barrett and Shore, 1989).

Moreover, the consensus trees (Figure 3.2) showed no pattern as far as clustering populations according to their geographical distribution. This is not unusual as other studies have reported similar results. *Lathyrus latifolius* showed no association between the genetic identity and geographical distance between population (Godt and Hamrick, 1991).

*H. incana* is diploid, and an annual or short-lived perennial herb (Preston *et al.*, 2002). Darmency and Fleury (1999) in their investigation showed self-incompatibility to be the mating system of most individuals within a population of *H. incana*, although some plants had some ability to self-fertilize. Perennial species display various reproductive systems, including selfing, outcrossing and apomixis (Barrett and Shore, 1989). Although, polyploidy has been put forward as a common feature in most invasive plants, in other cases there has been a correlation between diploidy and weediness, or both diploidy and polyploidy have been found to be equally weedy (Barrett and Shore, 1989). In fact, reproductive strategies have been held responsible for the success of invasive plants as links between colonizing success and uniparental modes of reproduction, such as self-fertilization or apomixis or clonal propagation (Brown and Marshall 1981; Brown and Burdon, 1987). This led Baker (1967) to devise a rule that states that 'weedy annuals will be either self-fertilizing or agomospermous, or if outcrossing they will tend to be wind-pollinated, and that weedy perennials, which may be self-compatible, will display extensive vegetative reproduction'. The genetic diversities and partitions of genetic diversities between populations of *H. incana* compared well with other outcrossing plants (Brown and Marshall, 1981; Muluvi *et al.*, 1999), and were dissimilar to those of self-fertilizing plants (Novak and Mack, 1993). Also, the similar levels of genetic diversity between the native and alien populations of *H. incana* suggest that the degree of outcrossing has not decreased in alien populations.

Many studies have supported the association between selfing and weediness (e.g. Mulligan and Findlay, 1970; Price and Jain, 1981). The genetic variations in studies of self-fertilizing plants have demonstrated a reduction from the native to the introduced range (Table 3.6). This reduction is particularly the case when invading species have reproductive systems involving predominantly selfing and apomixis (Husband and Barrett, 1991). In fact, species have been found to switch reproductive systems from being sexual in their native range to asexual or apomictic in their introduced range (e.g. Glover and Barrett, 1987; Hollingsworth and Bailey, 2000). A change to apomixis from the native to the introduced range has been demonstrated by *Rubus alceifolius*, and thought to have allowed a particularly well-adapted and aggressive genotype of this species to spread and invade native plant communities on Indian Ocean Islands (Pickett, 1976; Mueller-Dombois, 1981).

The genetic diversities found for *H. incana* are similar to those found in other outbreeding species, where there does not appear to be a marked reduction in genetic diversity from the native to the introduced range (Table 3.6). In fact, not unlike *H. incana*, similar levels of genetic diversity were found in native and introduced populations of *Trifolium hirtum* (Molina-Freaner and Jain, 1992), and *Alleria petiolata* (Meekins *et al.*, 2001). Also, in contrast to self-fertilizing species, *Apera spica-venti* (Warwick *et al.*, 1987) and *Echium plantagineum* (Burdon and Brown, 1986) showed no reduction in genetic diversity, and values in the introduced and native ranges were found to be somewhat similar (Table 3.6). Furthermore, genetic differentiation has been found to follow an extreme pattern with very low within- and high between-population variation in colonizing species (Hamrick, 1989). The reasons for this pattern could be a result of a number of phenomena, such as founding of populations by a few individuals, low levels of repeated migration due to geographic isolation, novel selection in new habitats, and a propensity to self-fertilize (Barrett and Shore, 1989). *H. incana* does not follow this pattern as it exhibits high within-population and low between-population differentiation (Table 3.5), a pattern also shown in *Raphanus raphanistrum* (Kercher and Conner, 1996). Therefore, it seems that many individuals with multiple introductions founded *H. incana* populations in the UK.

A common theme in studies of the ability of plant species to colonize is whether the process is based on genetic variation or phenotypic plasticity. So, it can be argued that these two facets are responsible for determining whether a species can successfully colonize a novel environment or not. Scheiner and Goodnight (1984) found no relation between genetic variation and phenotypic plasticity in their investigation of the grass *Danthonia spicata.* However, this is not conclusive as it cannot be assumed that the success of the colonizing species is due to phenotypic plasticity in the absence of genetic variation (Gould and Lewontin, 1979). Therefore, it might be assumed that both these factors work together and contribute to the success of the colonizing species.

The following chapter discusses the ecological and genetic factors that have contributed to the success of colonization of *Hirschfeldia incana* in the British Isles.

# Chapter 4

## Main Discussion and conclusions

When a species moves from one part of the world to another, the expansion of the species' range might be accompanied by ecological and genetic changes of evolutionary importance (Baker and Stebbins, 1965; Parsons, 1983; Mooney and Drake, 1986). This study has investigated one such species, *Hirschfeldia incana*, that has spread from southern Europe to sites in the British Isles. Therefore, hypotheses were formulated as follows:

(a) Alien populations of *Hirschfeldia incana* have spread in the UK dramatically.

(b) Alien populations of *H. incana* in South Wales are found in communities containing common species.

(c) The habitats colonized by *H. incana* in South Wales can be grouped into specific types.

(d) The colonization of *H. incana* in South Wales is prevalent in man-made habitats, which may explain its mode of dispersal.

(e) Environmental disturbance plays an important role in the colonization of *H. incana* populations in South Wales.

(f) The genetic diversity of native southern European populations is considerably higher than that of alien UK populations.

(g) Genetic differentiation in the *H. incana* populations in the UK should be partitioned as low within- and high between-populations, as is expected with colonizing species.

(h) Colonizing *H. incana* populations in the UK should be genetically more closely related, the closer they are geographically.

*H. incana* has spread dramatically in the British Isles over the last 70 years (Figure 2.3.1). This increase in its distribution has been exponential ($p$=0.004). Successful plant invaders often demonstrate a linear or exponential increase in area (Weber, 1998). However, this is only seen on a large geographic scale, and the pattern of spread may be influenced by the size of the area considered. In the case of *H. incana* the UK seems to be a large enough area to be able to see the exponential increase in its spread.

The sites around South Wales were studied more closely and it was found that the top three species most commonly found growing with *H. incana* were *Senecio jacobaea* (Common Ragwort), *Holcus lanatus* (Yorkshire-fog) and *Taraxacum officinale* agg. (Dandelion). These are all native to the UK. In fact all of the top 20 species found associated with *H. incana* are native (Figure 2.3.11). This suggests that *H. incana* arrives in a suitable habitat and colonizes it eventually establishing itself in communities containing native species. However, all the sites investigated in South Wales (Figure 2.3.23) also contained neophytes (introduced after AD1500). Therefore, it seems from this that factors associated with the successful colonization of *H. incana* can be, to a degree, related to other invasive species.

Plant invasions follow a sequence of events, which begin from the arrival of propagules in a novel area, establishment of populations (with a lag phase when there is a slow increase), and expansion of population size (Bazzaz, 1986; Weber and Schmid, 1998; Hobbs and Humphries, 1995; Wade, 1997). So, dispersal and subsequent establishment in new sites are important processes in plant invasions. On investigation of the habitats *H. incana* is found growing in around South Wales, three distinct types of habitats were classified (using cluster analysis and TWINSPAN). These are open urban gap habitats, closed habitats (semi-natural) habitats and natural (sand dune) habitats. Presumably, the open urban gap habitats provide a habitat that is free of any other vegetation and allows *H. incana*, and indeed any other invasive species, to arrive and colonize it. In fact, the highest number of neophytes were found in the open habitats (27), followed by the closed habitat (13) and then the sand dune habitat (9). So, *H. incana* seeds seems to arrive in the open habitats and establish themselves in the absence of other vegetation, while in the closed habitats seeds are likely to land in gaps in the existing vegetation. This is further suggested when the density of the plants per 10 square metre are considered (Figure 2.3.13). Here as expected the open urban gap habitats had higher densities of *H. incana* plants than the closed habitats. For instance, the highest number of plants were found in site NPT4, an open habitat, (Ocean View, Jersey Marine) with 500 plants in an area of $1500m^2$, and the lowest number of plants were found in site B1b, a closed habitat, (Kenfig car park area) with 8 plants in an area of $100m^2$. However, in the open habitat NPT2 (Neath Abbey) only 25 plants were found in a fairly large area of $750m^2$, while in a closed habitat at G1 (Crofty Industrial Estate) there were as many as 104 plants in a fairly small area of $50m^2$. Therefore, it seems that the availability of a suitable habitat is not the only factor that plays a role in the successful colonization of *H. incana* in habitats around South Wales. Analyses of different floras have suggested that certain plant-growth features and habitat characteristics can be used to predict the success of invasions (Sakai *et al.*, 2001). For instance, it was found that species introduced into the Czech Republic since 1492 showed invasion success related to plant height, life form and competitiveness, and in addition, the sunflower family (Asteraceae) was found to be over-represented in the introduced flora as compared to the native one (Pysek *et al.*, 1995a). Although no single predictor was found for invasion success, some traits were found to be more common in the alien flora, and more prevalent for aliens in particular habitats (Sakai *et al.*, 2001).

Biogeographic theory predicts that the number of species should increase with area (Preston, 1962; MacArthur and Wilson, 1967). However, in the study of the alien flora of Europe, Weber (1997) found no relationship between species and area, and concluded that factors other than area, such as land use change and propagule import, also play an important role in determining the number of alien species in a country. Most of the *H. incana* sites around South Wales were found to be close to roadsides or areas where there was a considerable amount of movement in the form of people and transport. This suggests that the dispersal of *H. incana* seeds is mostly aided by activities of man as it is carried on vehicles or on persons to suitable habitats around South Wales. In fact, it has been reported that most long-distance introductions of non-native species are the direct or indirect result of human activities, but social and economic factors often play as important a role in invasions as biological factors (Sakai *et al.*, 2001). Alien plants have been introduced deliberately as forage, fibre, medicines or ornamentals, for erosion control, and for timber plantations (Baker, 1974, 1986). And sources of introductions have ranged from ballast in ships (Ruiz *et al.*, 2000), impure crop seeds, stuck on animals, and soil surrounding roots of nursery stock (Baker, 1986). In addition, activities such as agriculture, logging and grazing further enhance the establishment of alien species by creating disturbed sites for colonization (Sakai *et al.*, 2001). *H. incana* is introduced into the British Isles with grain imports and bird-seed, and was formerly introduced with wool shoddy (Preston *et al.*, 2002). Nowadays most invasions happen because of human acitivities, as the flow of commerce is much more widely spread and faster, and species travel in all directions. As is seen in figure 2.3.1, the concentrations of *H. incana* populations are in areas where there are major towns and cities, and where there is likely to be a lot of movement in terms of people travelling from one place to another. Hence, carrying seed on their person or the mode of transport. Also, it is likely that *H. incana* has also been introduced into parts of the UK in ballast, as it is found in areas near docks, such as Swansea and Pembroke Dock.

Disturbance is an important factor in invasions and seems to be playing a vital role in the colonization of *H. incana* in the South Wales sites (Figure 2.3.15), where a significant relationship was found between the bare ground and number of *H. incana* plants (Spearman's rho, $p$ = 0.043; Kendall's tau, $p$ = 0.028). More recently it has been suggested that invaders are capable of altering disturbance regimes and can cause drastic changes, such as species replacement and changes to ecosystem processes (Mack and D'Antonio, 1998). In addition, invasions have been reported to result in a positive feedback between disturbance and the abundance of non-native species, such as that observed between some introduced grasses and fire (Vitousek, 1986). On the other hand, the feedbacks may result in depression or removal of the invader from the system (Dudley and Grimm, 1994). This study shows a clear advantage of disturbances to the invasion of *H. incana* in the South Wales sites as it tends to colonize open urban gap habitats where there is a large proportion of disturbance, and closed habitats where disturbances have produced gaps in the vegetation open to colonization by *H. incana*.

For an invader to be successful, it needs to initiate a new population following arrival to the new habitat, and as a result the reproductive strategies adopted by the invasive species play an important role in their success. It has been suggested that many of the traits that are historically associated with invasive species may be involved in initial colonization (Sakai *et al.*, 2001). For example, Baker (1965) found that species in which isolated individuals can self-fertilize are generally good colonists. Furthermore, species that have multiple reproductive strategies, such as both vegetative reproduction and seeds, and also plants that have multi-seeded fruits are thought to be good colonists (Hueneke and Vitousek, 1990). Phenotypic plasticity is another important life-history trait that is required by an invader to be able to colonize new areas because they must be able to cope with a range of environmental conditions (Baker, 1965, 1974; Gray, 1986). *H. incana* is an outcrossing species that is on the whole self-incompatible, although some individuals have been observed to be able to self-fertilize (Darmency and Fleury, 1999). This ability to be able to self-fertilize when needed, together with the production of multi-seeded fruits might contribute to its success as an invader. Alien *H. incana* seed was found to be significantly lighter ($p$=0.02) than native seed. This smaller seed mass has been correlated with higher seed production, faster individual growth rate, and the absence of special requirements for germination (Baker, 1965). This was demonstrated with invasive pine species, which were found to have small seed mass, shorter juvenile period, and shorter intervals between seed crops (Rejmanek and Richardson, 1996). However, Forcella (1985) found the opposite with agricultural weeds where heavier seeds led to faster germination rates, which would enhance invasion rate relative to plants with smaller seeds. Because the sample size of the *H. incana* seeds weighed in this study was small, the results must be treated with caution. However, these preliminary results suggest that further study is required.

*H. incana* plants in South Wales were categorized as having a 'complex' type growth form (Figure 2.3.28), that is, a larger and vigorous plant form compared to the native 'simple' type (Figure 2.3.27). Numerous studies have investigated factors that lead to an increase in the abundance and vigour of invasive plants in their introduced range (Elton, 1958; Blossey and Notzold, 1995; Crawley, 1987; Mack *et al.*, 2000; Thebaud and Simberloff, 2001). Two factors have been recognised of which the first is that some species might simply be innately better competitors as they have evolved in a competitive environment (Darwin, 1859; Crawley, 1987; Vitousek and Walker, 1989). Secondly, it has been proposed that in their introduced range invasive plants have low losses to enemies (Elton, 1958; Lodge, 1993; Yela and Lawton, 1997), and the resources that would be normally lost to enemies are probably allocated to growth and/or reproduction by a plastic phenotypic response (Bazzaz *et al.*, 1987; Tilman, 1999; Thebaud and Simberloff, 2001). However, there is no specific rule that species are larger in their introduced range than in their native range, as this is not always the case. For instance, Thebaud and Simberloff (2001) demonstrated in a study which looked at European species introduced to California or the Carolinas, and vice versa, that, on average, individuals of Californian species were taller in California than in Europe, while those native to Europe did not differ between Europe and California. Similarly, individuals of species in the Carolinas (North America) were taller, on average, in the Carolinas than in Europe, while European species were the same height in Europe and the Carolinas. The increase in size of the alien plants of *H. incana* suggests that it might have increased its allocation of resources to growth and/or reproduction and decreased resources for defence, thus being consistent with the evolution of increased competitive ability (EICA) hypothesis. This could be possible as the natural enemies of *H. incana* in its native southern European would be expected to be absent from its introduced range in the UK. Life-history theory also predicts a trade-off between fast reproductive rates and competitive ability (Pianka, 1970; MacArthur and Wilson, 1967). However, this trade-off is not the case for all invasive species. For example, Keddy *et al.* (1994) found that *Lythrum salicaria* (an invasive wetland species) has very high fecundity but is also capable of suppressing the biomass of three indicator species when grown in competition. Therefore, this observation of an increase in size of *H. incana* plants to the 'complex' type in its introduced range as compared to the 'simple' type in its native site needs investigating further.

The genetic structure of *H. incana* was investigated using random amplified polymorphic DNA (RAPD) markers. Lower genetic diversities were found in South Wales populations than in English populations ($p$=0.023), although no significant differences were found between the diversities of the introduced British samples and the native southern European samples ($p$=0.271). Often, new populations of invaders are formed by just a few individuals from the native habitat (Elton, 1958), and this leads to a reduction in genetic diversity due to genetic bottlenecks and founder effects (Nei *et al.*, 1975). The genetic diversities calculated in this study for *H. incana* are not consistent with this theory, even though a reduction in genetic diversity is commonly found with weedy species introduced by man to new areas. An important factor influencing the genetic diversity of invasive species has been suggested to be the number of introduction events, as species that have entered a new area and spread quickly as a result of a small number of introduction events show a reduction in genetic diversity (Schierenbeck *et al.*, 1995). This was demonstrated in *Bromus hordeaceus* where the native populations had a higher genetic differentiation than alien Australian populations (Brown and Marshall, 1981). However, other studies have shown similar levels of genetic variation present in the native and introduced populations, as was found for *H. incana.* For example, *Echium plantagineum* has similar levels of genetic variation in its introduced Australian range and its native Mediterranean range (Burdon and Brown, 1987). This is an outbreeding annual and the similar levels of genetic variation in the native and introduced range have been explained by the hybridisation among individuals from populations that have resulted from multiple introductions. This study has not provided evidence for any hybridisation events between the *H. incana* populations introduced into the UK. However, the lack of reduction in genetic diversities in the introduced populations suggests that there might have been multiple introductions of this alien into the UK. In addition, low levels of genetic differentiation were found between populations of *H. incana* in their introduced range in the UK (Table 3.3.4). This could be due to a high number of founders as a result of long-distance dispersal by humans. In fact, it has been reported that differentiation is reduced when the number of individuals founding a population is large (Wade and McCauley, 1988). *H. incana* has been introduced into the UK via various methods ranging from bird-seed, wool shoddy and ship ballast, which can lead to the assumption that this alien has had multiple founders.

The mating system of colonizing species is considered to be a fundamental parameter determining its success (Baker, 1955; Allard, 1965; Brown and Burdon, 1987). Although, no single mating strategy has been attributed to the success of colonizing species, many are found to be self-fertilizing or apomictic species (Brown and Marshall, 1981; Price and Jain, 1981), and even the outbreeders are found to be self-compatible. There are advantages associated with uniparental mating, which include reproductive assurance, and the suppression of genotypic flux so that the most successful genotypes can be maintained for exploiting new habitats (Brown and Burdon, 1987). *H. incana* is an outbreeding species that is capable of self-fertilization, as well as being an annual or short-lived perennial. Regardless of being an outbreeder, *H. incana* has demonstrated successful colonization in the UK. This is not unusual as the genetic diversities of *H. incana* are comparable with other outbreeding species

(Table 3.3.6), where similar levels of genetic variation were found in both the native and alien populations. Therefore, not all colonizing events over long distances involve a reduction in genetic diversity. This is particularly apparent in outcrossing weeds that expand rapidly following establishment, where differences in the genetic structure between the native and alien populations are not significant. Rapid expansion of the range of *H. incana* in the UK has been demonstrated to be exponential, and it seems that this has led to a lack of reduction in the genetic diversity of this species in its introduced range. This has also been seen in the self-incompatible, annual species *Apera spica-venti* where little difference was found in the genetic diversity between the alien (Canadian) and native (European) populations (Warwick *et al.*, 1987a). However, outbreeders are also likely to show a reduction in genetic diversity initially following introduction because breeding systems are thought to evolve in introduced populations (Barrett, 1996). *H. incana* is known to be able to self-fertilize (Darmency and Fleury, 1999), and it seems that this alien crucifer might have favoured this reproductive system in the plants that colonized the new areas in the UK initially during the foundation events and expansion of population size.

Annual and perennial species have also been studied with respect to colonization. Annual weeds have been found to be predominantly self-fertilizing and incapable of clonal propagation, while perennial species display a variety of reproductive systems, such as selfing, outcrossing and apomixis (Barrett and Shore, 1989). *H. incana* appears to be at an advantage as it displays both annual and perennial strategies. The perennial nature of invasive species, in particular, has been found to be an advantage as the failure to generate successful sexual or asexual offspring one year does not prevent future opportunities (Hancock and Bringhurst, 1978). In fact, the success of any organism depends on the efficient allocation of energy for growth, maintenance and reproduction (Gadgil and Bossert, 1970). So, if established plants maintain themselves until favourable conditions occur and then reproduce successfully, their contribution to the gene pool is increased (Hancock and Bringhurst, 1978).

Novel environments are also, often, accompanied with a change in climate, and this new climate will not be favourable to aliens. However, species that are able to shift their ranges quickly would be at an advantage. *H. incana* is one such species that appears to be able to shift range quickly, due to its short generation time and small, light seeds. In fact, Rejmanek and Richardson (1996) found this to be the case with *Pinus* species, which has also got the ability to rapidly shift range due to its short juvenile period and low seed mass. Also, in South Wales, *H. incana* is mostly found along roadsides, making it possible for its seed to be dispersed over long distances by vehicles to disturbed sites. Therefore, it can be deduced from this that *H. incana* (and other roadside weeds) are good contenders for species that would be some of the first to shift their range when climate changes.

Many studies have shown that successful plant invaders, often extend their new ranges within a short time, following an initial lag phase (Elton, 1958; Hengeveld, 1989; Weber, 1998). *Hirschfeldia incana* has demonstrated an exponential increase in it's population numbers from the 1930's to 2000, which has also been seen in many other cases where the rate of spread has been exponential (or linear) from the start of the spread (Forcella, 1985; Pysech and Prach, 1995; Weber, 1998). These observations suggest that many exotic species have not reached a state of equilibrium and are still in the process of extending their range. Therefore, *H. incana* is also one such invading species that seems to still be on the increase.

According to the tens rule (Williamson, 1996) only 10% of introductions will become established or naturalized, and only 10% of those will become pests. Therefore, most invaders that are established do not have a serious nuisance effect on the biodiversity. However, as has been demonstrated in this study with *H. incana*, there is an exponential increase in the range which does not signify that it will stop spreading at any time soon, and there is also no indication whether this species or another with similar effects would not become a noxious weed down the line. Hence, it is essential that these invasive weeds receive as much attention in relation to research as the more serious weeds that have become pests. This point has been very well demonstrated by the history of *Impatiens glandulifera*, which was declared a weed in 1890, 35 years after it was first recorded in the wild, and 51 years after it was first imported (Perrins, Fitter and Williamson, 1993). This species is a pest in woodlands, but not considered a pest in other areas where it lends a somewhat aesthetic property to the flora with its colourful appearance. Another example of an invasive species, which has become a notorious pest in time *Mimosa pigra* a South American shrub introduced to Australia between 1870 and 1890. This was considered a minor weed in Australia for about a century, but in the late 1970s there was a major increase in its range in the Northern Territory (Braithwaite *et al.*, 1989), and it is now known to have invaded all of the sedgelands, most of the billabong and a great deal of the paperbark forest, creating a new, impoverished ecosystem.

The conclusions deduced from this study are outlined below:

1) *Hirschfeldia incana* has spread exponentially in the British Isles, and has successfully established itself as a neophyte alien. *H. incana* populations are concentrated in areas where there are major towns and cities, and where there is likely to be considerable movement in terms of people travelling from one place to another, hence, carrying seed on their person or the mode of transport.
2) *H. incana* has preferences to the type of habitats it will grow in and the species it will grow with. The species most commonly associated with *H. incana* were found to be *Senecio jacobaea*, *Holcus lanatus* and *Medicago lupulina*, all native to the UK. In fact, all the top 20 species found associated with *H. incana* in the South Wales sites were all native. This suggests that *H. incana* is capable of competing with the native flora following its establishment, and enhances biodiversity.
3) *H. incana* grows in three types of habitats in South Wales as shown by cluster analysis and TWINSPAN. These habitats are open, urban gap habitats, closed habitats (semi-natural), and sand dune habitats (natural). The sand dune habitats are important in that they are a part of the UK Biodiversity Action Plan. However, *H. incana* populations that are found on sand dunes are not thought to be serious as they are not pests. In fact, *H. incana* is not considered to be aggressive like other aliens, such as Japanese Knotweed, and therefore, does not represent a significant threat to biodiversity. In closed habitats *H. incana* forms small populations and prefers urban gap habitats where it builds large populations.
4) Disturbance plays an important role in the colonization of *H. incana* in South Wales. This neophyte typically colonizes disturbed areas before natives can re-

colonize and from there can possibly start spreading into undisturbed habitats. In closed habitats, *H. incana* colonizes gaps in the vegetation, thus doing tapping available resources.

5) Some evidence of larger growth in introduced *H. incana* plants was found as plants with a 'complex' growth form in South Wales sites, as compared to the 'simple' type of plant form that occurred commonly in the native southern European range. Also, lower mass was found in the introduced seed, which might explain the high number of seeds produced and the low initial germinability. However, this requires further study as the number of samples used to determine seed weight was not representative for the whole range of *H. incana* locations.
6) English and South Wales populations exhibited different patterns of genetic variation. The South Wales populations were found to be significantly less diverse than those in England and in the native range in southern Europe. However, no significant differences in diversity were found between the English and native southern European populations. Overall genetic diversities were similar in native southern European and alien UK populations. Therefore, alien populations of *H. incana* populations in the UK seem to have resulted from multiple founding groups that have originated from different source populations leading to reasonably high levels of diversity in British populations. Genetic diversities found for *H. incana* are similar to those found in other outbreeding species, where there does not seem to be a marked reduction in genetic diversity from the native to the introduced range.
7) Genetic diversities were found to be significantly different in younger and older populations of *H. incana* in the UK, suggesting the establishment of an

age-structure that has probably affected the level of genetic differentiation among populations.

*Hirschfeldia incana* continues to increase its range in the UK and this study has investigated the reasons for its current success, and attempted to provide useful information on the spread and control of other alien, invading species.

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