UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Biological Sciences

***Maculinea arion* as an indicator of rare niches in semi-natural acid grasslands in South West England and the role of *Myrmica* species of ant**

by

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ABSTRACT

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MACULINEA ARION AS AN INDICATOR OF RARE NICHES IN SEMI-NATURAL ACID GRASSLANDS IN SOUTH WEST ENGLAND AND THE ROLE OF *MYRMICA* SPECIES OF ANT.

By Zoë Randle

Anecdotal evidence suggests that habitat management for the Large Blue butterfly, *Maculinea arion*, benefits other rare species. Selected plant and invertebrate species were monitored on a degraded site which was under going restoration management for the future re-introduction or natural recolonisation of *M. arion*. This study measured the shift of the floral and invertebrate community in order to understand the species composition and rate of successional changes in the re-establishment of early seral, semi-natural, acid grassland. Several UK Red Data Book species increased on the restoration site including the Pearl-bordered Fritillary, *Boloria euphrosyne* and the Small Pearl-bordered Fritillary, *Boloria selene*. The increase in these two butterfly species coincided with an increase in *Viola riviniana*, a food plant of these two *Boloria* species, and ants of the genus *Myrmica*.

A detailed study was undertaken to investigate the processes explaining the increases in *Viola* and *Boloria* species, with emphasis on their possible interactions with various *Myrmica* species. A key question was: Does management for *M. arion* merely create a scarce niche shared by a guild of other species, or is there a direct impact due to the increase in *Myrmica sabuleti* or other *Myrmica*?

There was a positive *Myrmica* affect on *Viola* density, due to the elaiosomes on the seeds of both *Viola* species, which were highly attractive to all *Myrmica* species tested but not to the other ants (*Formica* spp.) inhabiting the study sites. *Myrmica scabrinodis* $_{\text{(HOT)}}$ preferred *Viola lactea* seeds, the species of *Viola* that coincides most within its niche in the field. The relative density of *Viola riviniana* to *V. lactea* was primarily determined by microclimate, with *V. lactea* replacing *V. riviniana* in the hottest niches.

Boloria euphrosyne oviposition sites are also influenced by microclimate and coincide with the niche of *M. sabuleti*, straddling the warmest microclimates occupied by *V. riviniana* and the coolest ones occupied by *V. lactea*. *B. selene* inhabits a cooler niche, ovipositing mainly on *V. riviniana* near *M. ruginodis.*

Habitat management for *M. arion* increases the larval food plant and niches of both *Boloria* species, but especially that of *B. euphrosyne*. This is partially due to the creation of a scarce niche that is shared by a guild of species and partly due to the activity of *Myrmica* species ants acting as ecosystem engineers.

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I, ……Zoë Randle…………………………………………….,

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Chapter 1: Introduction

1.1 Introduction to Grasslands

The semi-natural grasslands of the UK and Europe have been created over thousands of years as a result of man's activities (Erhardt & Thomas, 1991; Morris, 2000; Morris et al., 1994). The majority of semi-natural habitats in the UK and Europe have undergone a dramatic decline in extent over the past 100 years (WallisDeVries et al., 2002). Many that still exist are severely degraded and in need of regular conservation management for them to maintain biodiversity value. The demise of these habitats is a direct result of human perturbations; changes in agricultural practices are largely to blame (Thomas, 1995a). Conservationists are faced with the challenge of conserving semi-natural grasslands and the species that they support.

Habitats, particularly the Dorset heathlands (Webb, 1990), have been directly destroyed through urban sprawl and development, cultivation of land and draining of wetlands for agricultural use. The fertilisation of land to increase productivity has also directly contributed to habitat loss. The more indirect degradation of semi-natural habitats is due to abandonment of traditional management practices such as extensive livestock grazing and scrub management. The cessation of these practices has allowed successional processes to take place; vegetation has grown and scrub encroachment has reduced the overall mosaic structure creating unsuitable conditions which ultimately leads to the local extinction of species. Less mobile species are unable to disperse and re-colonise nearby habitats due to isolation and fragmentation of the landscape (Hanski et al., 1996).

Early conservation attempts failed to save habitats and species because intervention methods were not adopted. It was thought that exclusion methods alone would be sufficient at saving a species or habitat. For example, in the late 1920's the Committee for the Protection of British Lepidoptera acquired Chipman Valley in Cornwall as a Large Blue butterfly, *Maculinea arion* reserve. Wardens were employed to deter collectors, the site was fenced to exclude grazing stock and the burning of gorse, *Ulex europaeus* was prohibited. The vegetation grew and the butterflies went extinct within a few years. (Oates, 1995; Thomas, 1995b). Many early conservation schemes failed in this way and lessons have been learnt. Successful species conservation requires an in depth knowledge of the target species ecology and habitat requirements (Thomas, 1995a). Once these have been established the appropriate management methods can be adopted and implemented to re-create and maintain the habitat.

To conserve biodiversity two main approaches to habitat management can be adopted: a species approach or a systems or process orientated approach. (WallisDeVries et al., 2002). Successful ecosystem restoration requires the re-establishment of ecological processes many of which involve plant and animal interactions (Majer, 1989). Habitat restoration is an important priority in conservation and is complementary to existing site management (Morris et al., 1994). As a result of changes to government policy, Environmental Stewardship schemes now pay farmers to undertake effective environmental management, and restoration schemes are becoming more common place. The mean decline of priority butterfly species has been reduced by agri-environment schemes; however, Warren et al. (2005) have found that some schemes actually exacerbate the decline of butterflies that require habitat mosaics and scrub edges.

The basic principle of conservation management is to arrest the process of succession in order to maintain the status quo in semi natural habitats (Fry & Lonsdale, 1991; Morris et al., 1994; Oates, 1995). Grasslands in particular have a long history of intervention by man. Grazing is the most commonly adopted management practice for acid grasslands, calcareous grasslands and lowland heathlands. Cutting and coppicing are the methods used to manage hay meadows and woodlands respectively. Restoration management can be short or long term depending upon the time span since abandonment. Maintenance management is generally continuous (Oates, 1995), the same management being repeated periodically.

My study system is a semi-natural acid grassland mosaic with *Ulex europaeus* and *Pteridium aquilinum* and therefore I am concentrating on the management methods employed for this habitat; livestock grazing, which creates and maintains a mosaic sward structure, and controlled burning of scrub. Grazing animals such as cattle, ponies or sheep have three main effects on vegetation: defoliation, treading and dunging (Morris, 2000). Cattle trample and open up tall coarse vegetation, creating small openings within the turf which are suitable micro-sites for seedling germination; they browse scrub and generally eat longer coarse grasses. Horses and ponies are patchy grazers and are excellent at creating species rich mosaic structures that are favoured by butterflies (Oates, 1995). Native breeds also eat *Rubus fruticosa, Ulex europaeus,* and coarse grasses, which prevents these from becoming problematic. The combined force of cattle and ponies is often favoured as a grazing regime as their grazing habits compliment each other. In contrast, sheep tend to generate a more uniform sward. Grazing intensity and timing is critical for maintaining plant and animal biodiversity (Watkinson and Ormerod, 2001).

Scrub management is an important factor in habitat restoration and maintenance. Rotational winter burning of *Ulex* and *Pteridium aquilinum* is the traditional method of controlling scrub encroachment in Dartmoor. Burning is more effective than cutting for several reasons: scrub regeneration is slower; burning is less labour intensive; burning removes the dense litter layer and allows the emergence of seedlings from the seedbank, some of which are stimulated by fire to germinate. For example, *Viola* species, the larval food plant for Fritillary butterflies thrive after burning events (Oates, 1995).

Conservation management of sites for the myrmecophilous Large Blue butterfly, *Maculinea arion,* has been associated with an increase in the abundance and diversity of other species, including *Myrmica sabuleti* (*M. arion's* obligate larval host); the threatened butterflies, the Pearl-bordered Fritillary *Boloria euphrosyne,* Small Pearl-bordered Fritillary *Boloria selene,* The Grayling *Hipparchia semele*; and two species of Violet, namely *Viola riviniana* and *Viola lactea* (a nationally notable species). The relationships between some of these species are well known; others are less well understood making the relationship between these populations ambiguous. It is has been previously established that *Myrmica* ants create microhabitats and provide enemy free space for certain plants (Elmes, 1999) and that *Viola* species have a seed with an elaiosome, an ant attractant that may aid seed dispersal and or survival.

This thesis attempts to address these ambiguities and to investigate the relationship between ants, plants and other invertebrates to ascertain the ecological function of ants, and of *M. sabuleti* in particular, in determining community structure within their ecosystem.

1.2 The Ecology of *Maculinea arion*

The Large Blue butterfly, *Maculinea arion* (figure 1.1) is the only member of its genus present in the UK. *M. arion* has an interesting and highly specialised life cycle, being a social parasite of the red ant, *M. sabuleti*. The butterflies are univoltine (i.e. one generation per year) and typically occur in closed populations occupying 0.5 – 5 ha of grassland (Thomas, 1995b). The adults are short lived with an average life span of five to seven days and are on the wing from mid June until late July. They lay their eggs on the tight unopened flower buds of Wild Thyme, *Thymus drucei* (Thomas & Elmes, 2001). After a week the egg hatches and the larva (hereafter called caterpillar to distinguish it from ant larvae) feeds on the flower head. Once the caterpillar has undergone its final moult it drops to the ground and awaits adoption by its host ant *M. sabuleti.* The caterpillar has a posterior dorsal nectary organ on its body and many pore cupola (Malicky, 1969), that secrete solutions that any ant in the genus *Myrmica* is attracted to (Frowhawk, 1924; Thomas, 2002). The ant repeatedly taps the caterpillar with its antennae, causing the caterpillar to secrete more solution. Several ants may be involved in the adoption process which may take up to an hour. The final stage in adoption is the rearing up of the caterpillar, which invokes the ant to pick it up and take it to the brood chamber in the nest (Frowhawk, 1924; Thomas, 2002). Normally ants attack intruders to their nest, however, *M. arion* uses chemical mimicry (Akino et al., 1999), causing the ant to treat the caterpillar like its own larvae. Within the nest, the caterpillar eats the ant brood until it undergoes pupation the following June and emerges from the nest. The freshly eclosed butterfly then climbs up the stem of a plant to inflate its wings.

In Britain, *M. arion* occurs in the South West of England, on south facing slopes with high densities of *M. sabuleti* nests in close proximity to *Thymus* species. *M. sabuleti* is adapted to a warmer climate than is common in Britain (Thomas et al., 1989) and is abundant only on south facing, tightly grazed swards of less that 3cm (Thomas, 1995b; Thomas et al., 1998). If the sward height increases by just two centimetres the soil surface temperature can be reduced by 5º C - 10ºC (Thomas et al., 1989). This change in microclimate creates unsuitable conditions for *M. sabuleti* during its colony growing phases in spring and autumn, resulting in the exodus of *M. sabuleti* and replacement by *Myrmica scabrinodis* which is adapted to a cooler niche (Thomas, 1980; Elmes and Thomas, et al. 1992).

1.3 Conservation of *Maculinea arion*

M. arion was always a localised species whose population declined steadily during the twentieth century. Despite attempts to conserve *M. arion* from the 1920's onwards, the butterfly continued to decline. This led conservationists to believe that it was impossible to maintain this species in Britain. However, no scientific investigation into *M. arion's* ecology had been undertaken until 1972, when intensive studies were started to identify its exact requirements. The key finding of the 1970s study was that *M. arion* was host specific to a single ant (*M. sabuleti*) rather than all *Myrmica* species, as had previously been believed. This restricted *M. arion* to a much narrower niche within its traditional grasslands than had been realised and showed UK conservationists that sites must be grazed to less than 3cm tall to support a population (Thomas, 1980). Unfortunately this information came too late to save *M. arion* in Britain and by 1979 the butterfly was extinct in the UK. Changes in the suitability of grassland sites were due to modifications in agricultural practices; farmers abandoned traditional management practices such as grazing with livestock and scrub clearance by burning because these methods were no longer financially viable. Rabbits declined from the mid 1950s' onwards, due to myxomatosis, which was itself encouraged by farmers. All these changes affected the *Myrmica* ant population dynamics on *M. arion* sites. The abandonment of grazing changed the vegetation structure and therefore the soil temperature cooled. These successional changes created unsuitable microclimatic conditions for *M. sabuleti* and populations of this ant declined to an extent whereby they could no longer support the populations of *M. arion* butterflies.

In 1983, with the new knowledge of *M. arion's* requirements, optimum management for *M. sabuleti* was imposed on a former site in Devon, and a small trial reintroduction programme was attempted. *M. arion* eggs of a suitable race were collected from three populations that comprised part of a single meta-population from Oland, South East of Sweden. These eggs developed into final instar caterpillars and were released on one of the Devon sites. The following summer seven adults emerged and formed a small population. In 1986 additional individuals were introduced from the same Swedish source, to reduce the chance of extinction through stochasticity and inverse density dependent mortality of the previously introduced small population, and to increase genetic diversity. Despite the weather conditions during the flight period in 1987 being sub-optimal, it was estimated that 2030 eggs were laid (Thomas, 1989). Twenty three generations later, a *M. arion* population exists on the reintroduction site which is referred to, hereafter, as site 'X'. By 2006, there were more than 25 sites in the south west region of the UK that were occupied by *M. arion*. This was achieved by a combination of natural colonisation and re-introduction of adult butterflies.

Figure 1.1 : *Maculinea arion* on larval food plant *Thymus* species.
Photograph courtesy of J.A. Thomas.

1.4 Habitat Restoration and Management on the Dartmoor sites

The *M. arion* sites in Devon are found on the culm measures on the south east edge of Dartmoor National Park. Site 'X' is approximately 15 hectares, site 'Y' 10 hectares and site 'W' has an area of about 2 hectares (figure 1.2).

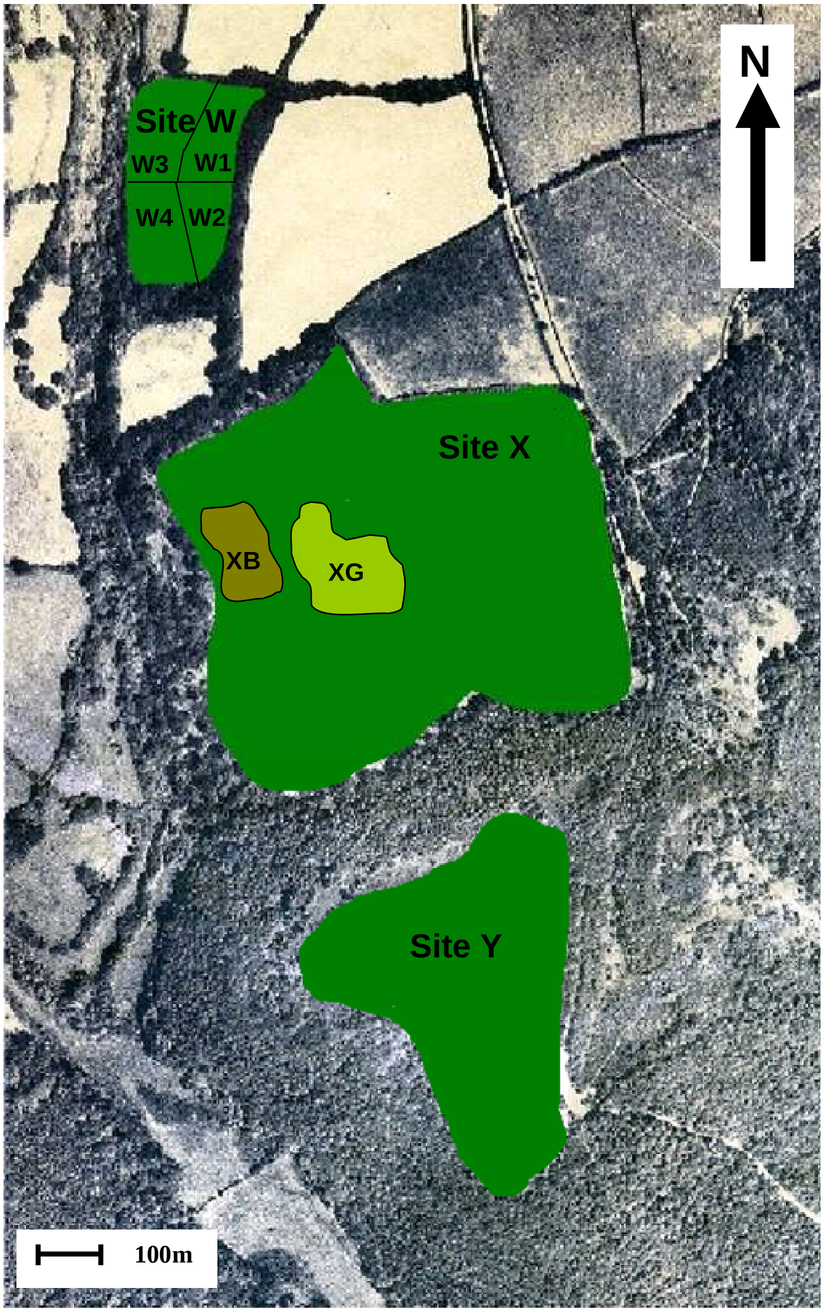


Figure 1.2: *M. arion* site, Devon. Legend: **Site W** – Degraded site undergoing restoration. Site W is divided into 4 areas for this study; **W1** *Pteridium aquilinum* dominant; **W2** dominated by *Agrostis curtisii, Erica cinerea* and associated species. **W3** & **W4** *Pteridium aquilinum* dominant. **Site X** – semi-natural acid grassland mosaic with areas of *Pteridium aquilinum, Rubus fruticosus* and *Ulex europaeus* scrub. Area **XG** is the core semi-natural acid grassland area of the site. Area **XB** is an area of dense *P. aquilinum* that is unmanaged. **Site Y** – restored semi-natural acid grassland site.

The sites are semi-natural acid grassland mosaics with pockets of *Ulex europaeus* and *P. aquilinum* scrub. The species composition includes *Erica cinerea*, *Agrostis curtisii*, *Leontodon hispidus, Pedicularis sylvatica, Poa* species, *Polygala serpyllifolia, Pteridium aquilinum, Teucrium scorodonia, Thymus drucei, Potentilla erecta, Ulex europaeus*, *V. riviniana* and *V. lactea.*

M. arion occupies sites X and Y. Habitat restoration work began on site X in the mid 1970's and this was extended 25 years ago to incorporate a neighbouring hillside, site Y. In addition, for this current study another restoration programme was started in 2003 to the West of site X, on site W, with the aim of encouraging natural colonisation and creating conditions for a meta-population of *M. arion* and its associated wildlife.

Habitat management for *M. arion* butterflies is aimed at creating suitable conditions for the host ant *M. sabuleti*. Sward height is the most important factor to consider when promoting *M. sabuleti*. As stated previously, *M. sabuleti* is only abundant under UK climates on swards less than 3 cm. Consequently, *M. arion* sites are intensively grazed in spring and autumn to maintain the high densities of *M. sabuleti* nests. Dartmoor ponies graze the sites from April to December. Between January and March the ponies are replaced by bullocks. Stocking densities vary depending upon climatic conditions; during wet springs and autumns, they are reduced to avoid damage to the turf through poaching. On average, the stocking densities are 0.5 bullocks per hectare per year and 1 pony per hectare per year. For three months spanning the flight period of the butterfly, grazing animals are removed from the sites to avoid trampling of eggs and larvae.

The *Ulex europaeus* on sites undergoes a yearly rotational winter burn to create early successional niches. Burning is a more effective way of controlling *Ulex europaeus* compared to swiping, as it removes the litter layer and growth is slower. *P. aquilinum*, which can also be invasive on these soils, is managed in two ways. Dense stands are managed by controlled burning, which removes the dense mulch layer as well as the dead fronds. Re-generation from the underground rhizomes is then cut during June and July and sprayed with the herbicide, Asulam. Less dense areas of bracken are rolled in June or July to suppress encroachment. During the early stages of the restoration, *T. drucei* was plug planted to aid colonisation, because natural spread was exceedingly slow. No other plant species were sown or planted on the restoration sites.

1.5 Habitat management for *Maculinea arion* benefits other rare species

Preliminary studies suggested that alpha and beta biodiversity increased on sites managed for *M. arion* (Thomas, 1999). The narrow niche created on *Maculinea* sites appears to benefit several noteworthy insect species, for example the green tiger beetle, *Cicindela campestris* and the Lesser Cockroach, *Ectobius panzeri.* Both are ground-dwelling thermophilous insects that have experienced large increases in population density on the study site, site X (New et al., 1995; Thomas, 1999; Elmes & Thomas, 1992). Several declining butterfly species have also increased, such as the Grayling, *H. semele*, High Brown Fritillary, *A. adippe*, Dark Green Fritillary, *A. aglaja*, Pearl-bordered Fritillary, *B. euphrosyne* and the Small Pearl-bordered Fritillary, *B. selene* (Thomas & Elmes, 1989). *A. adippe* and *B. euphrosyne* are category 2 Red Data Book species that have disappeared from c80% of known sites in 1980 – 2000 and declined to low densities in most surviving populations. *B. selene* and *H. semele* are now considered to be priority species and were added to the UK Biodiversity Action Plan in 2007. The increase in these fritillary butterfly species, on both sites X and Y, confounding the national trend, are attributed to a >100 fold increase in the pale dog violet, *V. lactea* (itself a Nationally Notable species) and the common dog violet, *V. riviniana* on *Maculinea* sites (Thomas pers comm.; New et al., 1995). In woodlands all four species of fritillary lay their eggs on or very near to *Viola* plants, with the larvae of each using a different violet growth form corresponding to different ages and successional stages (Thomas et al., in press).

The evidence that habitat management for *M. arion* is benefitting other rare species requires further tests and validation.

1.6 *Maculinea* as an Indicator of Biodiversity and an Umbrella species

In the UK *M. arion* can be considered to be an indicator of early successional, warm, ephemeral niches within the grasslands the species occupies. Insects, especially butterflies are ideal indicator species due their species richness, high diversity, sensitivity to environmental change and their ecological function (Andersen, A. N., et al., 2002; Hodkinson I. D. & Jackson J. K. 2005; Maes, D. & Van Dyck, H. 2005;

McGeoch M.A., 2007). Bioindicators are defined as “*a species or group of species that readily reflects the abiotic or biotic state of an environment; represents the impact of environmental change on a habitat, community or a subset of taxa, or of wholesale diversity, within an area*.” (McGeoch M.A., 2007) The term bioindicator is divided into three categories; environmental indicator; ecological indicator and biodiversity indicator (McGeoch M.A., 2007). Several terms are related to biodiversity indicators including umbrella species and flagship species (McGeoch M.A., 2007).

Umbrella species are “*species whose protection serves to protect many co-occurring species*” (Fleishman et al., 2000). The criteria upon which such species are selected are determined by species rarity, sensitivity to human disturbance and the mean percentage of co-occurring species (Fleishman et al., 2000; Roberge & Angelstam, 2004). There are two types of umbrella species; those that are demanding with respect to habitat area, for example large mammals; or site selection umbrellas such as birds and butterflies (Roberge & Angelstam, 2004). In the case of butterflies large habitat area is not an indicator of habitat quality which is essential for species that have specific habitat requirements. Lambeck et al (1997) suggest a focal species approach; identifying the most sensitive species within the ecosystem to use as an umbrella. This approach would generally identify butterfly species as umbrellas especially since these taxa have specific habitat requirements particularly in their larval stages (Thomas, 2005; Thomas, 1993). Launer & Murphy (1994) found that conservation aimed at the Bay Checker spot butterfly, *Euphydryas editha bayensis* would protect 98% of the flowering plants on occupied sites.

Whilst there has been much debate on the use of “Umbrella” species in conservation planning (see Roberge & Angelstam, 2004), to date there exists a lack of published studies examining how management of selected sites for umbrella species impacts upon the wider community. Lepidopteran species have many more schedules for protection and conservation in the United Kingdom than any other phylogenetic order (New, 1997). Individual species are frequently targeted by conservation management and their habitats therefore receive considerable financial investment. The ecology of several Lepidopteran species is well detailed, making them highly attractive as ‘flagship’ species: “*Popular and usually charismatic, species that provide a symbol or focus for conservation awareness and action*.” (Calow, 1999)

In Europe at least, butterflies have experienced greater declines and more local extinctions than other well studied groups; terrestrial vertebrates or plants (Thomas et al., 2004). Butterflies appear to be representative of other insect groups (Randle et al., 2005; Thomas, 2005; Thomas & Clarke, 2004) whose ecology is much less well known. Minor changes in grassland habitat structure are detected rapidly in rare Lepidopteran species. Studies in the UK of the Large Blue butterfly, *M. arion* showed that when grazing was reduced on sites, the population declined ten times faster than that of the host ant or plant species (Erhardt & Thomas, 1991). Lycaenidae are strong candidates for consideration as Umbrella species due to the complexity of their lifecycles (New 1997).

There are five species of *Maculinea* butterfly found across Europe, all of which are considered to be globally threatened and are listed on the IUCN (World) Red Data Book (Anon., 1990) as well as being included on the European Red List of endangered butterflies (Heath, 1981). Each of the species has experienced one or more national extinctions (Elmes & Thomas, 1992). *Maculinea* butterflies are an important component of European flora and fauna, some of the food plants that they rely upon are protected species and the butterflies themselves are host to extremely rare species-specific parasitoid wasps of the genus *Neotypus* and *Ichneumon*, which have only been recorded on 25 sites across Europe (Thomas & Elmes, 1989). *Maculinea* species form a close knit ‘community module’ (Holt, 1984) of high conservation value.

Maculinea are often considered to be ‘Flagship’ species: beautiful rare, endangered, conspicuous organisms that catch the imagination (Thomas & Settele, 2004). One of the aims of this thesis is to study whether they are also indicators of biodiversity and ‘Umbrella’ species. A second aim is to test the hypothesis that the characteristic assemblages on *M. arion* sites function as a community, being linked by the major impact that *M. sabuleti* has, when at high densities, on other selected invertebrates and plants on these sites. In other words, is *M. sabuleti* a keystone species, sensu Paine (1966)?

1.7 Biology of *Myrmica* ants

Myrmica are red ants with a Holarctic temperate distribution (Elmes et al., 1998). The biology of *Myrmica* ants has been extensively studied in the laboratory because they are very easy to maintain. However, some of their ecological characteristics such as colony fidelity, nest site persistence and community structure have been poorly studied, as field studies of these ants can be difficult, (Elmes et al., 1998). *Myrmica* colonies generally have 200 – 500 workers and can have multiple queens (Elmes, 1991b). Nests are constructed in rotten wood, sphagnum moss, grass tussocks and soil. Soil nests can be under stones or under ground (Elmes et al., 1998). Nests that are constructed in moist dense vegetation have solaria to improve insolation (Elmes, 1991b). Since most *Myrmica* species have overlapping niches there is also strong inter-specific competition in these zones. Most small colonies, especially newly founded ones, are destroyed by different *Myrmica* species or absorbed by conspecifics, (Elmes & Wardlaw, 1982b). On grazed grassland habitats *Myrmica* ant nest densities can sometimes exceed 1 per square meter. Each nest site can be a permanent feature which is fought over and occupied by a succession of different colonies (Elmes, 1991b).

In habitats where *Myrmica* communities exist, most new colonies are formed by fragmentation of existing colonies (budding). When nest sites are scarce, the strong competition results in rapid colonisation of new sites by a bud of an existing colony, which soon becomes a separate entity from the parent colony (Elmes et al., 1998). It is unlikely that other ant species can invade a site once one or more *Myrmica* species have colonised an area, unless there is a change in the physical conditions or new large areas of habitat are created (Elmes et al., 1998; Elmes, 1991b).

The primary consumers of insects and small vertebrates in most terrestrial habitats are ants (Holldobler & Wilson, 1990). Springtails, spiders, aphids, adult and larval flies, beetle and moth larvae are the main constituents of *Myrmica* ant diet (Brian, 1977, Skinner and Allen, 1996). Kajak et al (1972) investigated the role of *Myrmica* ants on meadow invertebrates. It was found that ants can eliminate up to 40% of hatching leafhoppers, 30% emerging dipterans and 49% of young spiders. Ants are therefore one of the most effective invertebrate predators (Kajak et al., 1972) and are no doubt responsible for the regulation of invertebrate populations (Brian, 1977). Generally, foraging ants will concentrate on a resource that is momentarily in great abundance

(Brian, 1977). Specialist aphid colonies are maintained by *Myrmica*, whose secretions probably form a large part of the diet.

1.8 *Myrmica* ants and Myrmecochory

Myrmica ants also consume large quantities of elaiosomes (figure 1.3); these are fleshy appendages on seeds that are adapted for ant dispersal. Seed dispersal by ants is called Myrmecochory, and was first described by Sernander in 1906.

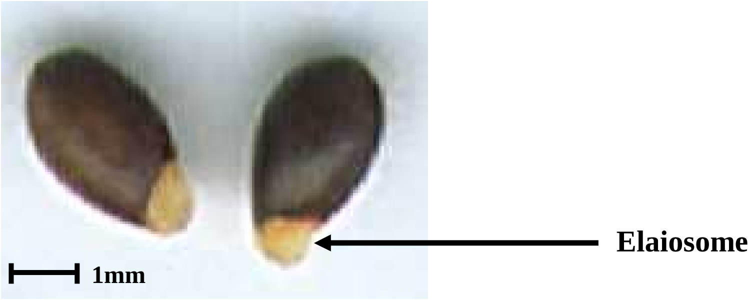


Figure 1.3: *Viola lactea* seed

The elaiosome is a food source that consists of fatty acids, sugars and proteins. Elaiosomes also have a chemical constituent which attracts ants and invokes carrying behaviour. The fatty acid composition of elaiosomes is more similar to insect fatty acids than to the seeds of the same species (Hughes et al., 1994), suggesting that the chemistry of elaiosomes has evolved to attract a particular suite of ant species. The common chemical attractant found in elaiosomes is diglyceride 1, 2 – diolein. Interestingly, 1, 2 – diolein is also found in insect haemoglobin (Hughes et al., 1994). Free fatty acids such as oleic acid may also promote seed gathering behaviour (Marshall et al., 1979; Brew et al., 1989; Skidmore & Heithaus, 1988). It is well documented that oleic acid induces corpse carrying behaviour in some ant species, (Hughes et al., 1994). When ants encounter attractive seeds, they take them back to their nests and remove the elaiosomes which are eaten by both the ants and the ant brood. The remaining seed is discarded within or around the ant nest. Ant nests are considered to be suitable micro-sites for seed germination (Fenner, 1985) due to the accumulation of organic matter and detritus around them, which are higher in nutrients than in non-ant nest soils (Beattie & Culver, 1983).

Myrmecochory is thought to benefit plants in several additional ways; 1) many myrmecochores are found in habitats that are subject to frequent natural burning events. The presence of an elaiosome enables seeds to avoid burning by being taken down into the ants nest. 2) Seed predation avoidance occurs through myrmecochory as shown by Turnbull & Culver (1983). In that study, ants immediately removed *Viola nuttallii* seeds shed from capsules, taking them below ground into the nest, which is highly protected, thereby enabling the seeds to avoid predation by rodents and invertebrates above ground. 3) Myrmecochorous seeds of *Ajuga* species are dispersed by ants further than their parental clones, thereby eliminating intraspecific competition from parent plants (Holldobler & Wilson, 1990). 4) Interspecific competition may be avoided - ants may carry seeds to sites where other competing species grow less well (Holldobler & Wilson, 1990). Culver & Beattie, (1980) also looked at the survival rates of 2 *Viola* species in Southern England. Some seeds were planted on ant nests, others in randomly selected micro-sites. The seedlings that survived best were on ant nests, they were also larger and more numerous.

1.9 *Myrmica* ants influencing soil properties

Studies have found that the presence of ant nests can influence soil nutrient and organic matter content (Jakubczyk et al., 1972). Gammans (2005) found that nests of *Myrmica ruginodis* on heathland were significantly more nutrient rich than non-ant nest sites one meter away. Ants are important soil modifiers, not only due to the accumulation of nutrients. The building of galleries results in soil movement, thereby altering soil structure, which in turn alters soil porosity resulting in improved aeration and drainage, (Petal, 1978). The increase in organic matter, and hence water retention capacity of the soil, creates conditions that are ideal for plant root penetration (Petal, 1978; Elmes, 1991a). The chemical defences of ant nests, including fungicides and bactericides, almost certainly alter their immediate soil communities.

1.10 Ants and above nest vegetation

The influence of ants on soil properties at nest sites will intuitively affect the vegetation structure above ant nests. The gathering of myrmecochorous seeds is very likely to bias above nest vegetation towards these species. King (1977) found that flora on the mounds of *Lasius flavus* were significantly different from that of non mound vegetation.

On the other hand, Elmes & Wardlaw (1982a) found no evidence that plants above *M. sabuleti* nests were responding to the physical changes to soils and vegetation that has been generated by the ants. They hypothesized that the microhabitat features of the nest spot would determine the vegetation assemblage and the suitability for *M. sabuleti* nesting. I now question that hypothesis since *Myrmica* nests have been shown to increase local soil nutrients and organic matter, and I suggest that in the longer term this will influence above nest vegetation.

Also, as described above, the gathering of myrmecochorous seeds by ants is likely to alter the above nest flora. To test this hypothesis I will assess the above nest vegetation of *Myrmica* and *F. fusca* ants and compare it with non-ant nest areas, with particular reference to *Viola lactea* and *V. riviniana* (Chapter 3). If *Viola* species are more abundant near to ant nests compared to non-nest spots, I hypothesis that this is due to the presence of an elaiosome on *Viola* seeds which is attractive to the ants. This idea is tested in Chapter 4, which investigates the attractiveness of different myrmecochorous seeds found on the study site to different ant species that co-occur there. *Myrmica* ants may be responsible for the dispersal of *Viola* seeds and may influence *Viola* distributions, activities which may have knock on effects for violet feeding fritillary butterflies such as *B. euphrosyne* and *B. selene*. The increase in violet feeding fritillary butterflies on sites X and Y can be attributed to a >100 fold increase in *Viola* densities which may, ultimately be due to the role of *M. sabuleti* as a seed disperser. If confirmed, this would qualify *M. sabuleti* as a keystone species for *M. arion, B. euphrosyne* and *B. selene* on these sites.

1.11 *Myrmica sabuleti* as a Keystone Species

The concept of keystone species was introduced in 1966 by Paine, who applied the theory to predators in marine communities. He stated that: "*Local species diversity is directly related to the efficiency with which predators prevent the monopolization of the major environmental requisites by one species.*" Mills et al (1993) broadened the scope of keystone species to include any species whose removal has strong effects on community diversity. There has been much debate as to the validity of the keystone species concept since its introduction (Simberloff, 1998; Paine, 1995; Mills et al., 1993; de Maynadier & Hunter, 1994; Power et al., 1996). Calow (1999) defines keystone species as: "*…one upon which many other species in an ecosystem depend and the loss* *of which could result in a cascade of local extinctions. The keystone species may supply a vital food resource…it may be a predatory species that holds in check the populations of certain herbivores which would otherwise overgraze, reduce primary production potential and lead to the loss of other herbivorous animals, together perhaps with certain specific parasites and predators.*"

The extinction of sea otters from the Pacific coasts of North America is perhaps one of the most famous examples used to discuss the keystone species concept. The extinction of the sea otters led to an increase in sea urchins (the favoured prey for sea otters). The urchins overgrazed the giant kelp, the kelp became locally extinct, and the fish and invertebrate species that inhabited the kelp forest were lost (Ebenman & Jonsson, 2005). Many other examples of keystone species exist (see Power et al., 1996). The problems in identifying keystone species have been widely argued, for example by Ebenman & Jonsson (2005), Power et al. (1996), Simberloff (1998), de Maynadier and Hunter (1994), Mills et al. (1993) & Paine (1995). However, what is accepted is that a keystone species is not determined by its position within a food chain; keystone status is not confined to top predators (e.g. sea otters) within a system (Power et al., 1996). Keystone species that affect their physical environment through burrowing in soil (kangaroo rats), or building dams (beavers), for example can also be considered as 'Ecosystem Engineers' (Jones et al., 1994).

It is evident that the presence of ants in many ecosystems is fundamental to the existence of communities. Ants are host to myrmecophiles and social parasites. *M. arion* is absolutely dependant upon the presence on high densities of *M. sabuleti* ants to ensure its survival. They are a key predator in their communities, often regulating the population densities of other invertebrate fauna (Brian, 1977). Ants are eaten and form a substantial constituent of many other organisms' diet, both at larval stage and as adults (Skinner 1996). There is also no doubt that ants play a vital role in the dispersal of elaiosome bearing seeds, and aid germination and survival of seedlings by creating enemy free space and nutrient rich micro-sites. Any impact on the composition of plant communities will inevitably have knock on effects on their specialist herbivores and mychorizzae. It is therefore reasonable to consider that *M. sabuleti* may be a keystone species and an ecosystem engineer within my study system.

1.12 Aims of the Project

This thesis describes ecological experiments made on *M. arion* sites in the UK. The purpose of this project is to address the following questions: (1) what is the impact of targeted “single species” habitat management on populations of other selected invertebrate and plant species on a degraded site (Site W)? (2) What are the patterns in re-colonisation rates of selected species? (3) What are the processes behind these patterns in re-colonisation rates of species? (4) Are *Viola* populations enhanced by increased populations of *M. sabuleti* or other *Myrmica* species, and is this facilitated by the dispersal of their seeds into microsites suitable for recruitment and / or reproductive success. (5) If *Viola* populations do benefit from an ant association, are these benefits manifested at the population level in other associated species such as *B. euphrosyne* and *B. selene* – two violet feeding fritillaries?

Two non-exclusive hypotheses are tested; (1), the population increases in *B. euphrosyne, B. selene, H. semele* and *Viola* species are attributable to habitat management which creates a warm, early successional niche in grassland which is shared by this guild of species. (2) The increases in the aforementioned species may be due to the direct or indirect effect of *Myrmica* ants manipulating the community. Population growth of *Viola* species on sites X and Y may partly be a result of increased ant densities; *V. lactea* and *V. riviniana* are myrmecochores.

My first hypothesis is addressed in Chapters 2 and 6. In Chapter 2, I describe the patterns of re-colonisation, changes in species assemblages and microclimate on sites prior to and during habitat restoration and management. In Chapter 6 the preferred egg laying niches of *B. euphrosyne* and *B. selene* in relation to *Viola* species growth forms, microclimate and co-occurring ant species are explored to find out if these share the early successional niche that is created by habitat management for *M. arion*.

My second hypothesis is addressed in Chapters 3, 4 and 5, which explore the direct and indirect effect of selected ant species on their micro community. In Chapter 3 the vegetation structure above ant nests is compared to non-ant nest spots. Firstly, this Chapter aims to establish if ants influence the vegetation near to their nests, and secondly, is there an association or co-occurrence between the *Viola* species and different ant species found on the study site? Behavioural bioassay experiments investigating the seed preference of *Myrmica* species and *F. fusca* ants in the laboratory are described in Chapter 4. This Chapter explores the mechanism of myrmecochory in vegetation manipulation. Chapter 5 addresses the effect of *M. sabuleti* on ant nest soil nutrients. Nutrient content of nest soil is compared to non ant nest soil in order to explain the higher densities of *Viola* species at nest spots. In addition to this, soil seedbank samples were taken from *M. sabuleti* nests and non-nest spots. *Viola* seed density was assessed and compared between nest and non-nest spots.

Chapter 2: Monitoring the re-colonisation of floral and faunal communities in degraded semi-natural acid grassland managed for the reintroduction of *Maculinea arion*

2.1 Introduction

In the UK and Europe, semi-natural grasslands and the specialist species they support are declining rapidly (van Swaay et al., 2006). Changes in agricultural practices are largely to blame, including chemical improvement using artificial fertilisers, herbicides and insecticides; physical destruction through cultivation or development; and abandonment (Walker et al., 2004). Many remaining grasslands are degraded, fragmented and isolated, making recolonisation by certain species all but impossible (WallisDeVries et al., 2002).

Fragmentation and isolation of habitat patches has led to the local extinction of species, and this is particularly the case for insects (Hanski, 1999). Insects respond rapidly to environmental change due to their short lifecycles (Bourn & Thomas, 2002), and many species are ill-adapted to modern landscapes due to their relative immobility and an inability to remain dormant though periods of unsuitable habitat conditions when their narrow niches are temporarily unavailable (Bourn & Thomas, 1993; Thomas et al., in press; Thomas, et al., 2001). Many invertebrate taxa have an intrinsic conservation value and play important roles in ecosystem processes (Watkinson & Ormerod, 2001). Previous studies have shown that insect community diversity has increased with habitat area (Steffan-Dewenter & Tscharntke, 2002), and extensive grassland management has been found to enhance both local and regional insect diversity (Di Giulio et al., 2001). Pöyry et al. (2005), report that intensive grazing is the most suitable method to maintain and restore grassland biodiversity. *Maculinea arion* and several other butterfly species that are at the northern edges of their ranges in the UK inhabit warm, ephemeral, narrow niches, whereas in central Europe the same species have broader niches and inhabit later seral stages in grassland (Thomas et al., 1998; Bourn & Thomas, 2002). This is due to the macro-climate being up to 3ºC warmer than that of the UK (Thomas, 1993). The narrower niches of many insects in the UK require intensive and frequent management to maintain them (Thomas, 1996). The habitat management on my study sites creates a mosaic of scrub and various sward structures, especially those containing warm, ephemeral, early successional niches.

Few studies have been undertaken to investigate the restoration of early successional niches in semi-natural acid grasslands (Walker et al., 2004). This Chapter describes the re-colonisation of site W by selected plant and invertebrate species which are of conservation interest. The site is undergoing restoration by scrub clearance, bracken control and grazing to re-create semi-natural acid grassland for the purpose of a reintroduction and or natural re-colonisation of *Maculinea arion*, its host ant *Myrmica sabuleti*, and other associated species.

Anecdotal evidence suggests that both alpha and beta biodiversity has increased on sites managed for *M. arion*. It is thought that the creation of early successional stages in acidic semi-natural grassland, through habitat management on these sites, may be responsible (Elmes & Thomas 1992)

2.2 Research Aim

The aim of this study is to measure the shift of the floral and invertebrate community in order to understand the species composition and rate of successional changes in the re-establishment of early seral, semi-natural, acid grassland. I test the hypothesis that population increases in *Boloria euphrosyne, Boloria selene, Hipparchia semele, Cicindela campestris* and *Viola riviniana* are attributable to habitat management, which creates a warm, early successional niche in grassland, which is shared by this guild of species.

2.3 Site Description

Site W is an abandoned semi-natural acid grassland habitat with a sloping south west aspect. The upper slope is rank semi-natural acid grassland, dominated by *Agrostis curtisii, Erica cinerea* and *Ulex europaeus. Viola riviniana, Viola lactea, Potentilla erecta, Thymus drucei* and *Polygala serpyllifolia* are also present in low densities. The lower part of the site is dominated by dense *Pteridium aquilinum* stands with a low density of *Viola riviniana* in the under-story.

Targeted habitat management began on this site in March 2003. The aim was to create a suitable site for the future reintroduction and/or natural recolonisation of *M. arion* and its associated species. Existing *M. arion* sites (sites X and Y) are 300m and 900m away from site W respectively. Prior to the commencement of habitat management, the site was split into 4 areas (figure 2.1);

W1. Area approximately 1775m^2. Sparse grassland, *Agrostis curtisii* and *Viola riviniana* present at low densities. *Rubus fruticosus* and *Pteridium aquilinum* dominant.

W2. Area approximately 1066m^2 .Tall rank semi natural acid grassland dominated by *Agrostis curtisii*, *Erica cinerea*, and *Ulex europaeus. Teucrium scorodonia*, *Viola riviniana, Viola lactea, Potentilla erecta, Thymus drucei* and *Polygala serpyllifolia* present at low densities.

W3. Area approximately 2877m^2 .Tall dense *Pteridium aquilinum* stand with occasional *Viola riviniana* present in under- storey.

W4. Area approximately 3004m^2 .Tall dense *Pteridium aquilinum* stand with occasional *Viola riviniana* present in under-storey.

Areas W1 and W3 underwent controlled burning in March 2003 to reduce the density of *P. aquilinum*. In June 2003, the regenerating *P. aquilinum* was cut and sprayed with the herbicide Asulam. The entire site was lightly grazed with cattle from May 2004 until July 2004; unfortunately grazing was not reinstated prior to the completion of my study. Controlled burning was undertaken on area W4 in March 2004; however, further follow up management was not pursued (Table 2.1).

Table 2.1: Timing of management treatments for each area of site W

	Burnt	**Cut & Sprayed**	**Grazed**
W1	March 2003	June 2003	May – June 2004
W2	n/a	n/a	May – June 2004
W3	March 2003	June 2003	May – June 2004
W4	March 2004	n/a	May – June 2004

Figure 2.1: *Maculinea arion* restoration site W. **W1.** Area approximately 1775m^2. Sparse grassland, *Agrostis curtisii* and *Viola riviniana* present at low densities. *Rubus fruticosus* and *Pteridium aquilinum* dominant. **W2. Area approximately** 1066m^2 .Tall rank semi natural acid grassland dominated by *Agrostis curtisii, Erica cinerea,* and *Ulex europaeus. Teucrium scorodonia, Viola riviniana, Viola lactea, Potentilla erecta, Thymus drucei* and *Polygala serpyllifolia* present at low densities. **W3. Area approximately** 2877m^2 .Tall dense *Pteridium aquilinum* stand with occasional *Viola riviniana* in the under- storey. **W4. Area approximately** 3004m^2 .Tall dense *Pteridium aquilinum* with occasional *Viola riviniana* in the under-storey.

Sampling in each study area of site W was stratified in proportion to the size of each area. Quadrats were positioned along transects, a method that has been widely used for many years (Kent & Cocker 1994). Thirty permanent 2x2m^2 quadrats were setup on site W (5 each in areas W1 and W2 and 10 each in areas W3 and W4), within which plants, ant species presence and soil temperature were recorded. Quadrats of 2x2m^2 are standard for NVC sampling. Recording of vegetation, ant species presence and abundance and soil temperature took place during the summers of 2003, 2004 and 2005 in most cases. Control quadrats (2x2m^2) were set up on the target site X; five in an existing *M. arion* grassland area and five in an unmanaged dense *P. aquilinum* area: these were also monitored to compare to the managed areas of site W.

2.4 Methods

2.4.1 Vegetation Sampling and Statistical Analysis

Percentage cover of the different plant species within each quadrat was recorded along with vegetation height in June 2003, 2004 and 2005. Individual *Viola lactea* and *V. riviniana* plants, the larval food plants for *Boloria euphrosyne* and *B. selene*, were counted and recorded within a central 1m x 1m quadrat (figure 2.2). Count data provided a quantitative measure of species abundance. Pre-management data were collected in February 2003 (2003a) on site W. These consisted of percentage cover of all plant species, absolute counts of *Viola* species, and vegetation height within each quadrat. However, it was not possible to use all of these data in the analysis due to the timing of data collection in subsequent years. Ideally, neighbouring quadrats would have received different management treatments to enable true rather than pseudo-replication. However this is not practically or politically feasible on this site. Moreover, foraging ants often stray a few metres into unsuitable habitats, and therefore edge and neighbour effects would be encountered by using small patches for treatments. May (1994) suggests that too many previous ecological studies have been carried out at a small scale, but recognises that larger scale ecological studies, in which changes in whole populations rather than subsets of individuals or behaviour are measured, are difficult to replicate due to scarcity of habitat and abiotic variation. Replication of sites was clearly not possible in this study since there were no other existing semi-natural acid grassland *M. arion* sites in the UK.

Vegetation data were analysed using Detrended Correspondence Analysis (DCA), available in CANOCO (Ter Braak & Smileau 1988) to investigate the impact of management treatments (burning, cutting, herbicide application and grazing) on the vegetation communities' within the different areas of site W. The control plots on site X were also included in the analysis.

Vegetation height measurements were analysed using the non-parametric Kruskal-Wallis statistical test followed by Mann-Whitney U tests, because the data were not normally distributed despite transformation. Kruskal-Wallis and post hoc Mann-Whitney U tests were used in the analysis of *Viola* species abundance due to the non normal distribution of the data.

2.4.2 Soil temperature

Soil temperature was monitored to assess the effect of habitat management on microclimate, in three randomly selected quadrats per compartment on site W using Tinytalk® data loggers. Data loggers were centrally positioned within the permanent quadrats (Figure 2.2); each was buried 1cm below the soil surface and recorded soil temperature every hour continually for 72 days. This depth was chosen since most *Myrmica* brood is kept at this level below ground (Elmes, 1982). At the end of the 72 days the data were downloaded in the field and the data loggers re-launched. Data logger locations were mapped and relocated using a metal detector. Comparative soil temperatures were also recorded on sites X and Y within optimal *M. arion* biotopes, and on site X in the unmanaged *P. aquilinum* area.

Soil temperature data from 15 April to 15 June for 2003, 2004 and 2005 were compared. Data were continuous between these dates for all sites and areas. Mean daily soil temperatures for each management treatment were calculated. Mean daily ambient temperature data from the Plymouth, Mountbatten weather station for 15 April to 15 June 2003, 2004 and 2005 were obtained from the Met Office to assist with interpretation of the results; soil temperature is intuitively related to air temperature.

2.4.3 Invertebrate sampling and Statistical Analysis

Changes in selected invertebrate species (Table 2.2) of high conservation interest were monitored on sites W, X and Y. The number of adult sightings was recorded per unit time across the site, a method that reliably measures differences in population size (Warren, Thomas & Thomas 1984). Counts were made of individuals of the following species observed within 5m of the recorder; *B. euphrosyne, B. selene, H. semele,* and *C. campestris.* Monitoring was conducted between the hours of 10am and 5pm, when ambient temperature was above 17°C and there was at least 60% sunshine, following standard Butterfly Monitoring Scheme (BMS) methodology (Pollard & Yates, 1993). Survey dates varied depending upon the flight period of the adults, (table 2.2).

Table 2.2: Invertebrate species surveyed on sites W, X and Y, and their adult flight periods. * *M. arion* flight period is detailed for information only.

Species	Adult flight period
Pearl-bordered Fritillary – *Boloria euphrosyne*	April – May
Small Pearl-bordered Fritillary – *Boloria selene*	May – June
Grayling – *Hipparchia semele*	May – August
Tiger Beetle – *Cicindela campestris*	March – September
Large Blue – *Maculinea arion**	June – July

Peak hourly count data of *B. euphrosyne*, *B. selene*, *H. semele* and *C. campestis* for each site were plotted and described. It was not possible to undertake statistical analysis on these data due to the low number of true replicates. I.e. at the site scale, individual time points within a site could not be true replicates (n = 3 or 4).

2.4.4 Ant baiting and Statistical Analysis

Standard ant baiting methodology (Wardlaw et al., 1998a) was followed in spring 2003, 2004 and 2005 and autumn 2003 on sites W and X to record changes in the dominant species present. Ant baits were placed at each corner of the permanent quadrat and checked after 30 minutes. Ant species were identified in the field with the aid of a hand lens. The changes in the presence of ant species at baits were compared at the quadrat level using Mc Nemar’s test of net change.

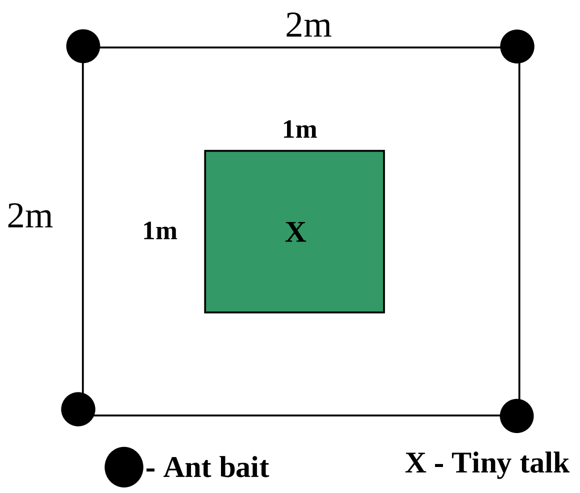


Figure 2.2: Diagrammatic representation of a typical quadrat used in the sampling. Percentage cover of the different plant species was assessed in the 2x2m^{2}quadrat. Counts of individual selected plant species were recorded in a central 1x1m^2 quadrat (green area). Four ant baits were set per quadrat and in 3 quadrats per compartment a data logger recorded soil temperature.

2.5 Results

2.5.1 Vegetation assemblage

Detrended Correspondence Analysis (DCA) is used to typify ecological community data. Data is detrended to eliminate the ‘arch effect’ which is a mathematical artefact that occurs when plotting data which lacks structure. Detrending involves dividing the first axis into segments (normally 26). The second axis is rescaled so that its mean is the same for each segment. Each segment has a measurement of 1 standard deviation unit. A difference of 4 standard deviation units indicates total community turnover (Hill & Gauch 1980). The axes are explained by ecological insight of the data or site.

Data for the plant species that occurred in quadrats at ≥0.5% cover were included in the DCA. The mean percentage cover for each species was calculated for each area of site W, and the grassland and *P. aquilinum* control areas on site X for each sampling event. Figure 2.3 shows the DCA ordination plot of the different plant species. Axis 1 (variance explanation, 24.3%, eigenvalue 0.66) is likely to represent the change in microclimatic conditions due to the habitat management. Axis 2 (explains 36.3% of the variance) and represents the shift in the community structure resulting from the different management treatments. Figure 2.4 shows the DCA ordination plot of each area within sites W and X in each year sampling took place. The coloured arrows indicate the shift in the vegetation community (as a result of management) for each area. Cross referencing figure 2.4 with the plant species from figure 2.3 indicates the plant species that have the greatest affect on the ordination; these are shown in bold on figure 2.4. For example, in 2004 *V. riviniana* and the ruderal ground flora that colonised area W3 due to the management (burning, cutting and spraying) 'pulled' the ordination for area W3 away from the *P. aquilinum*. However, in 2005 the ordination reverted back towards the *P. aquilinum* community due a lack of follow-up management. From figure 2.4 it is clear that the grassland and *P. aquilinum* controls do not change between years; therefore, the changes seen in the ordination of the other areas are not due to random fluctuations. In areas W1, W3 and W4 there is a large effect of *P. aquilinum* control, but in W3 and W4 the bracken returns rapidly. *P. aquilinum* control appears to be more successful in W1. The ordination plot shows that *V. lactea* is increasing in abundance in area W2. The habitat management is targeted towards the restoration and expansion of semi-natural acid grassland on site W. However, these results show that during the period of this study, this has not yet been achieved.

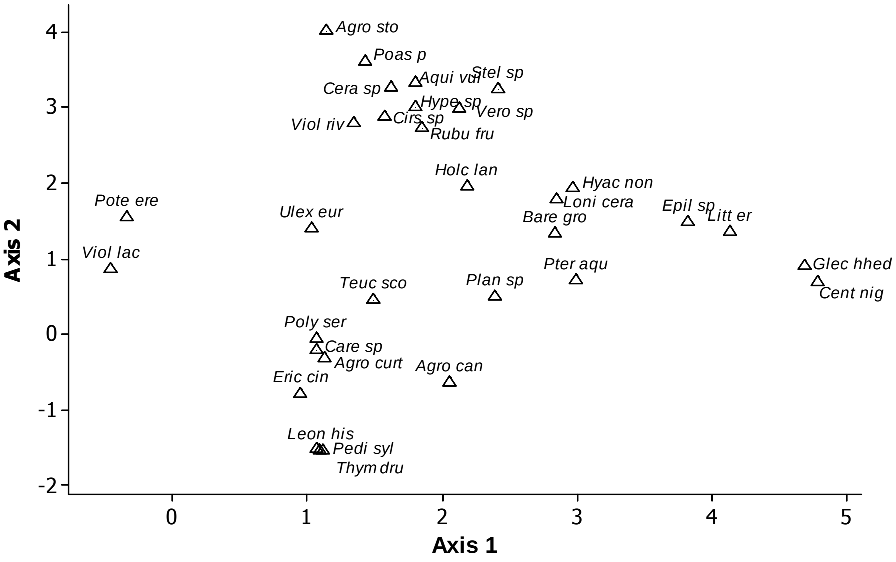


Figure 2.3: Detrended Correspondence Analysis ordination for mean percentage cover of species recorded within quadrats on sites W and X in 2003 -2005. Axis 1 (explains 24.3% of the variance) and represents the microclimatic conditions. Axis 2 (explains 36.3% of the variance) and represents the shift in the vegetation communities due to management treatments. Species names: Agrocan, *Agrostis canina*; Agrocurt, *Agrostis curtisii*; Agrosto, *Agrostis stolonifera*; Aquivul, *Aquilegia vulgaris*; Baregro, Bare ground; Caresp, *Carex spp.*; Centnig, *Centaurea nigra*; Cerasp, *Cerastium* spp.; Cirssp, *Cirsium* spp.; Epilsp, *Epilobium* spp.; Ericcin, *Erica cinerea*; Glechhed, *Glechoma hederacea*; Holclan, *Holcus lanatus*; Hyacnon, *Hyacinthoides non-scriptus*; Hypesp, *Hypericum* spp.; Leonhis, *Leontodon hispidus*; Litter, Litter; Lonicera, *Lonicera* spp.; Pedisyl, *Pedicularis sylvatica*; Plansp, *Plantago* spp.; Poasp, *Poa* spp.; Polyser, *Polygala serpyllifolia*; Poteere, *Potentilla erecta*; Pteraqu, *Pteridium aquilinum*; Rubufru, *Rubus fruticosus*; Stelsp, *Stellaria* spp.; Teucsco, *Teucrium scorodium*; Thymdru, *Thymus drucei*; Ulexeur, *Ulex europaeus*; Verosp, *Veronica* spp.; Viollac, *Viola lactea*; Violriv, *Viola riviniana.*

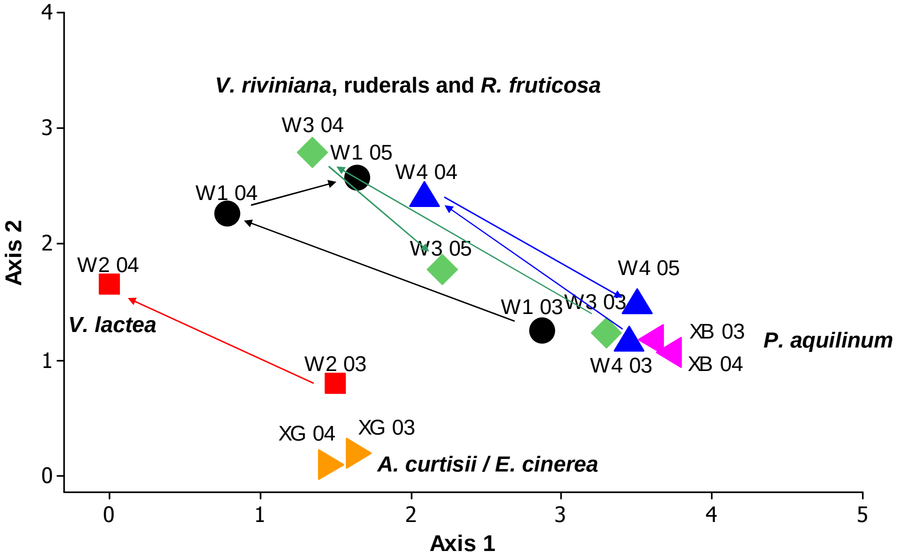


Figure 2.4: Annotated Detrended Correspondence Analysis ordination of the different treatment areas of site W; W1, W2, W3 and W4 and the site X grassland (XG) and *P. aquilinum* (XB) control areas. The arrows indicate the ordination of vegetation communities during the study period (03 – 2003, 04 – 2004, and 05 – 2005). The species that have the most effect on the ordination are in bold typeface (refer to figure 2.3). Axis 1 represents the microclimatic conditions. Axis 2 represents the shift in the vegetation communities resulting from the different management treatments.

2.5.2 Vegetation structure and soil temperature

<u>Managed versus non-managed *P. aquilinum* areas</u>

Both *P. aquilinum* areas W1 and W3 were burnt in the spring of 2003. The vegetation height data for these areas were compared to the unmanaged *P. aquilinum* area of site X. The burning of the *P. aquilinum* significantly reduced vegetation height within areas W1 (Mann-Whitney $W_1 = 15$, $P < 0.005$) and W3 (Mann-Whitney $W_1 = 55$, $P < 0.001$) in 2003 (table 2.2). The subsequent cutting and spraying of *P. aquilinum* re-growth in areas W1 and W3 in July 2003 also showed a significant reduction in vegetation height compared to the control area; W1 (Mann-Whitney $W_1 = 15$, $P < 0.01$) and W3 (Mann-Whitney $W_1 = 55$ $P < 0.01$).

Pre-management vegetation height data was available for area W4. This was compared to the control area of site X. Results show that vegetation height was significantly taller in area W4 before management (Mann-Whitney $W_1 = 105$, $P < 0.005$) and in 2004, after management (Mann-Whitney $W_1 = 96$, $P = 0.05$) (table 2.3). This indicates that the burning of *P. aquilinum* in area W4 was not as successful as that in areas W1 and W3. In addition to this, area W4 did not under go the follow-up management of cutting and spraying. This result highlights the importance of continued *P. aquilinum* management particularly when *P. aquilinum* is well established, as in area W4.

Table 2.3: Median vegetation height (cm) and Interquartile Ranges for areas W1 – W4 and site X target grassland (XG) and the *P. aquilinum* (XB) controls from 2003 to 2005. †Note that the area W4 measurements for 2003 are the pre-management measurements; this area was not managed until spring 2004.

	2003	2004	2005
W1			
Median	11.75	9.66	14.59
IQR	9.29 - 12.69	6.22 - 18.10	11.89 - 18.67
W2			
Median	15.63	19.13	Not recorded
IQR	13.31 - 35.47	9.04 - 28.56	Not recorded
W3			
Median	18.03	11.60	17.41
IQR	12.41 - 21.64	5.125 - 21.70	11.77 - 22.03
W4			
Median	155	135	11.531
IQR	144.75 - 164	117.5 - 150	6.61 - 17.73
XG			
Median	4.9	10	Not recorded
IQR	3.54 - 12.19	7.39 - 17.63	Not recorded
XB			
Median	110	110	Not recorded
IQR	110 - 117.5	110 - 117.5	Not recorded

It is well documented that there is a correlation between vegetation structure and soil temperature (Thomas et al., 1989). Table 2.4 shows that the soil temperature has increased (relative to the ambient temperature of each year) in areas where *P. aquilinum* has been managed compared to the un-managed *P. aquilinum* areas. The manipulation of the sward structure affects the micro-temperature at the soil surface and is crucial in determining the abundance and species of ants present.

Table 2.4: Mean soil temperature ° C ± S.E. from 15 April to 15 June for study sites and areas in 2003, 2004 and 2005. †Mean ambient daily temperature ºC ± S.E. from 15 April to 15 June for 2003, 2004 and 2005 as recorded at the Plymouth, Mountbatten weather station. Data supplied by the Met Office.

	2003	**2004**	**2005**
Site	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
W (whole site)	13.75 ± 0.15	15.03 ± 0.23	13.46 ± 0.15
W1	14.59 ± 0.32	15.64 ± 0.54	13.46 ± 0.30
W2	12.85 ± 0.25	14.37 ± 0.41	13.13 ± 0.26
W3	14.56 ± 0.29	15.46 ± 0.48	13.61± 0.30
W4	12.99 ± 0.23	14.66 ± 0.41	13.65 ± 0.31
X Grassland controls	14.35 ± 0.31	15.59 ± 0.45	14.44 ± 0.31
X *P. aquilinum* controls	12.36 ± 0.20	12.85 ± 0.33	11.84 ± 0.22
Y Grassland controls	14.71 ± 0.31	15.51 ± 0.45	14.17 ± 0.33
Air Temperature†	12.22 ± 0.29	12.73 ± 0.39	11.22 ± 0.24

Within site differences

Within areas W1 (Kruskal-Wallis; $H_2 = 3.75$, P = 0.154), W2 (Kruskal-Wallis; $H_1 = 0.27$, P = 0.6), and W3 (Kruskal-Wallis; $H_2 = 0.75$, P = 0.416), vegetation height did not differ significantly between years. However, the burning of *P. aquilinum* reduced the vegetation height in area W4 between 2003 (pre-treatment) and 2004 (Mann-Whitney $W_1 = 143$, $P < 0.005$), and between 2004 and 2005 (Mann-Whitney $W_1 = 155.0$, $P < 0.001$). Pre-treatment vegetation height data were not available for areas W1 and W3, but casual observations and photographs indicate that a similar reduction in vegetation height occurred within these areas. Comparing the vegetation height between areas W3 and W4, to assess the effect of burning, shows an unsurprising result. Area W3 was burnt in 2003, thus significantly reducing vegetation height compared to area W4, which was not burnt until the following year (Mann-Whitney $W_1 = 155$, $P < 0.001$). The same pattern was apparent in 2004 (Mann-Whitney $W_1 = 155$, $P < 0.001$). This suggests that burning *P. aquilinum* was effective at reducing vegetation height. However, no significant differences in vegetation height were found between areas W3 and W4 during 2005 (Mann-Whitney $W_1 = 87$, P = 0.19).

Degraded grassland area versus target grassland area

Vegetation height within the grassland area of site W (W2) was significantly taller than that of the managed target grassland area of site X (Kruskal-Wallis: $H_1 = 4.84$, $P < 0.05$) (table 2.3). However, in 2004 there was no significant difference between the areas (Kruskal-Wallis: $H_1 = 0.88$, $P = 0.347$). This is due to the median vegetation height doubling on the target grassland site. Additionally vegetation height did not differ significantly between years (Kruskal-Wallis: $H_1 = 0.27$, $P = 0.6$) within area W2 despite the introduction of grazing animals on the site (table 2.1 & 2.3).

2.5.3 Numbers of *Viola* species

Differences in *Viola* species within sites and between years

Total numbers of the UK Biodiversity Action Plan (BAP) priority species, the Pale Heath violet *V. lactea*, did not differ significantly between years across site W as a whole, (Kruskal-Wallis; $H_2 = 2.65$, $P = 0.266$). Conversely, the number of *V. riviniana* across site W increased significantly, (Kruskal-Wallis; $H_3 = 43.36$, $P <0.001$) between the pre *P. aquilinum* burn count in 2003 (2003a) and all subsequent years (table 2.5), representing a 22.5 fold increase during the whole study period. In contrast, *V. riviniana* densities were stable on the controls on site X during the same period (see table 2.6 below)

Table 2.5: Medians, Interquartile range (IQR) and significance of differences in numbers of *V. riviniana* recorded in 1x1^m quadrats on site W pre the *P. aquilinum* burn on areas W1 and W3 combined (2003a), 2003, 2004 and 2005. Mann – Whitney U tests had 1 degree of freedom.

	2003a	**2003**	**2004**	**2005**
Median	4	15	64	90
IQR	2 – 6.25	7.75 - 68	31 - 120	25 - 163
2003a				
2003	W = 492.0, P < 0.001			
2004	W = 409.5, P < 0.001	W = 724.0, P < 0.005		
2005	W = 420.0, P < 0.001	W = 652.0, P < 0.005	W = 769.0, P = 0.233	

Differences in numbers of *Viola* species within areas and between years

No significant differences in numbers of *V. lactea* were recorded within areas W1 (Kruskal-Wallis $H_2 = 1.09$. $P = 0.58$) or W2 (Kruskal-Wallis $H_2 = 0.01$, $P = 0.916$)

between years. Additionally, *V. lactea* did not colonise areas W3 or W4 during the study period. These results suggest that the microhabitat was not suitable for *V. lactea* in areas W3 or W4 (see Chapter 3 for further details), or that *V. lactea* was absent from the seed bank.

Conversely, the number of *V. riviniana* plants increased within all areas of site W between years; W1 (Kruskal-Wallis $H_3 = 9.73$, $P < 0.05$), W2 (Kruskal-Wallis $H_2 = 8.71$, $P < 0.05$), W3 (Kruskal-Wallis $H_3 = 20.10$, $P < 0.001$), and W4 (Kruskal-Wallis $H_3 = 10.56$, $P < 0.01$). Pair wise comparisons of between years and within areas were performed to display in detail where the differences occurred (table 2.5.).

Changes in *V. lactea* and *V. riviniana* numbers were also compared within the grassland and *P. aquilinum* control areas on site X. The data were not normally distributed after transformation, so the non-parametric Kruskal-Wallis test was used. The results show no significant differences in the number of *V. lactea* (Kruskal-Wallis; $H_1 = 3.72$, $P = 0.054$) or *V. riviniana* plants (Kruskal-Wallis; $H_1 = 0.01$, $P = 0.917$) between 2003 and 2004 within the grassland control area. The same pattern was observed within the *P. aquilinum* area; *V. lactea* (Kruskal-Wallis; $H_1 = 2.22$, $P = 0.136$), *V. riviniana* (Kruskal-Wallis; $H_1 = 1.00$, $P = 0.317$). Thus the increases in *V. riviniana* on site W can be attributed to habitat management.

Differences in the density of *Viola* species between sites

In 2003, the number of *V. riviniana* plants in area W2 was not significantly different from the grassland control area of site X (Mann-Whitney $W_1 = 32$, $P = 0.4$), nor did they differ in 2004 (Mann-Whitney $W_1 = 28$, $P = 1.0$) (table 2.7). However, in 2004 areas W1 (Mann-Whitney $W_1 = 15$, $P < 0.005$), W3 (Mann-Whitney $W_1 = 15$, $P < 0.05$), and W4 (Mann-Whitney $W_1 = 18$, $P < 0.01$) had significantly higher numbers of *V. riviniana* plants compared to the site X *P. aquilinum* control area (tables 2.6 and 2.7), suggesting that the *P. aquilinum* management on site W is having a positive effect on *V. riviniana* abundance.

Table 2.6: Medians, Interquartile range (IQR) and significance of differences in numbers of *V. riviniana* recorded in 1x1m^2 quadrats on site W within areas W1, W2, W3 and W4 pre *P. aquilinum* burn (2003a), 2003, 2004 and 2005. Mann – Whitney U tests had 1 degree of freedom.

	2003a	**2003**	**2004**	**2005**
W1				
Median	10	81	175	132
IQR	4.75 –15.25	25.5 –130	76.5 – 245.5	48 – 220
2003a				
2003	W = 12, ns			
2004	W = 10, P < 0.05	W = 20, ns		
2005	W = 10, P < 0.05	W = 23, ns	W = 30, ns	
W2				
Median	1.5	56	75	Not recorded
IQR	1 – 2.75	20.5 – 79	36.5 – 107.5	Not recorded
2003a				
2003	W = 10, P < 0.01			
2004	W = 10, P < 0.01	W = 22, ns		
2005	Not recorded	Not recorded	Not recorded	
W3				
Median	8.5	57.5	123.5	250
IQR	3.5 - 8.5	10 - 57.5	35.5 - 123.5	84.5 - 250
2003a				
2003	W = 64, P < 0.05			
2004	W = 47, P < 0.001	W = 72.5, P < 0.01		
2005	W = 54, P < 0.005	W = 69, P< 0.01	W = 79, P < 0.05	
W4				
Median	4.5	28.75	60.75	90
IQR	1 – 4.5	1.75 – 28.75	4.25 – 60.75	4.5 – 90
2003a				
2003	W= 67.5, ns			
2004	W = 60.5, P < 0.01	W = 95, ns		
2005	W = 55.5, P < 0.005	W = 85.5, ns	W = 91, ns	

Table 2.7: Medians, Interquartile range (IQR) for numbers of *V. riviniana* recorded in 1x1m^2 quadrats on the site X grassland and *P. aquilinum* control areas in 2003 and 2004.

	2003	**2004**
X Grassland		
Median	73	66
IQR	40.5 – 88	40.5 – 113.5
X *P. aquilinum*		
Median	0	0
IQR	0 - 0.5	0 - 0

2.5.4 Invertebrate recording: butterflies and Green Tiger beetle

All four study species colonised site W and two of them increased during these experiments (figures 2.5 – 2.8). None was present on site W in 2002; both the Pearl-bordered fritillary *B. euphrosyne* and the Small pearl-bordered fritillary *B. selene* colonised in 2003 and showed a steady increase in abundance from 2003 until my recording ceased in 2005. The Grayling *H. semele* and the Green Tiger beetle *C. campestris* colonised site W in 2004, but both species suffered a small decrease in abundance in 2005. In contrast none of the species colonised the un-managed *P. aquilinum* control area on site X even though the source populations were much closer. These results suggest that the targeted habitat management on site W has had a positive influence on these Biodiversity Action Plan priority insects.

Figure 2.5 shows the large increase in *B. euphrosyne* abundance from 2003 to 2005, this is similar to that observed on sites X and Y. *Boloria euphrosyne* was absent from site W in 2002. Counts of *B. euphrosyne* were greater on site Y in all years compared to sites W and X. It is unclear as to whether the increasing numbers of *B. euphrosyne* on site W are due to emigration from sites X and Y.

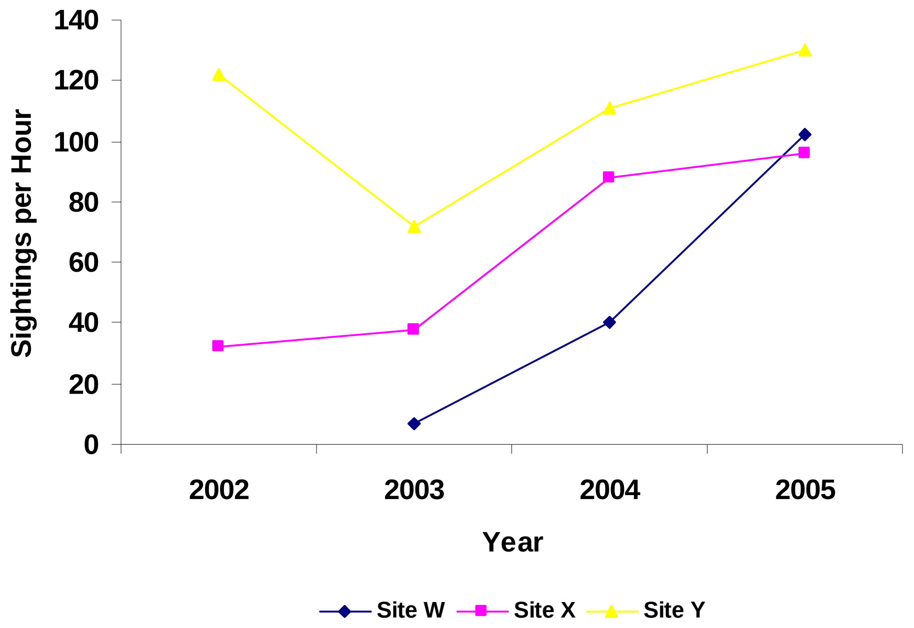


Figure 2.5 Sightings per hour during the peak flight period for *B. euphrosyne*, recorded on sites W, X and Y from 2002 to 2005.

B. selene was also absent from site W in 2002. This species colonised the site in 2003 and increased greatly from 2004 to 2005 (figure 2.6), a change that was mirrored on site Y, the most distant site from W. On site X, *B. selene* increased more gradually between years. From 2002 to 2004 site X had the highest peak counts per hour for *B. selene*, however, in 2005 counts were greater on both sites W and Y.

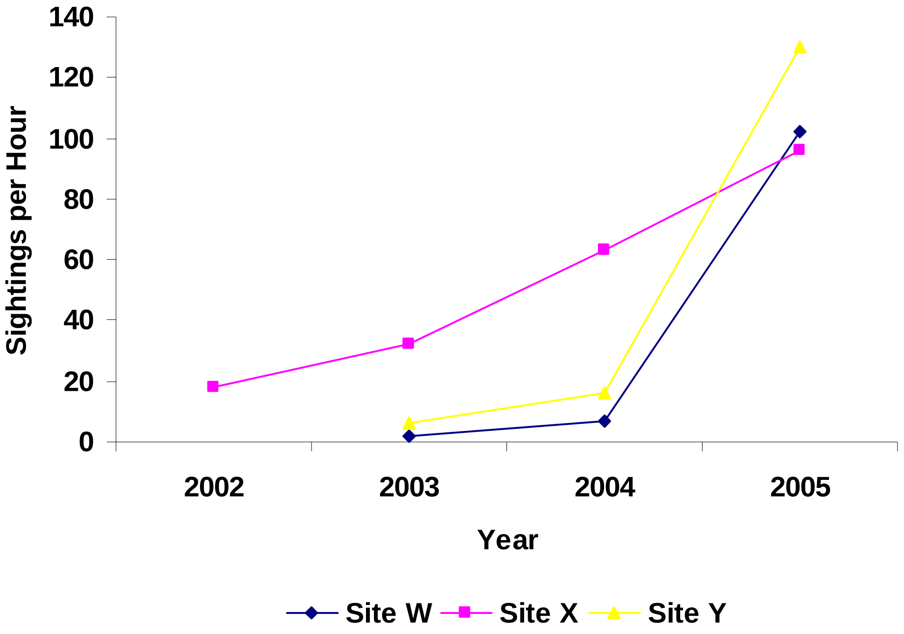


Figure 2.6 Sightings per hour during the peak flight period for *B. selene*, recorded on sites W, X and Y from 2002 to 2005.

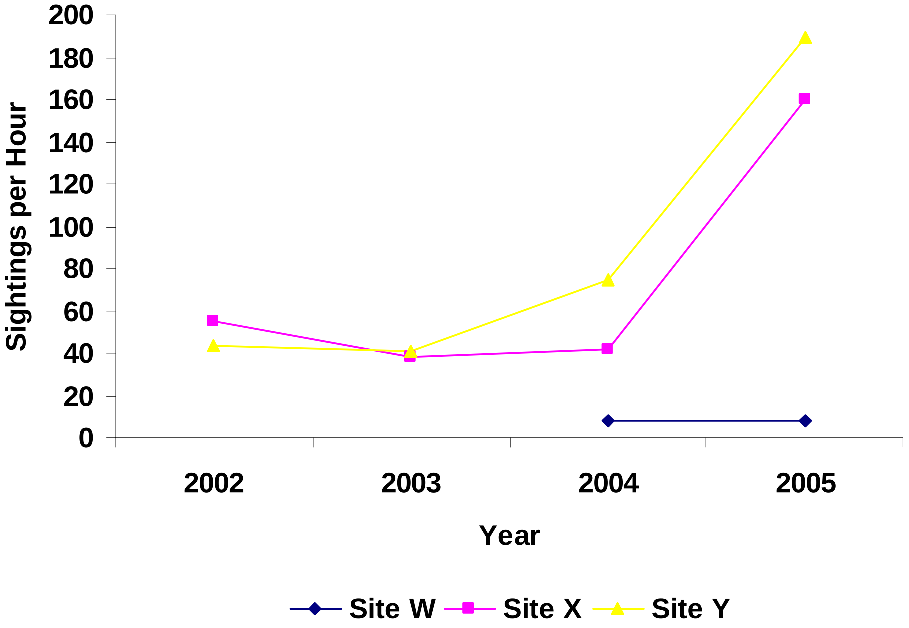


Figure 2.7: Sightings per hour during the peak flight period for *H. semele*, recorded on sites W, X and Y from 2002 to 2005.

H. semele was not present on site W in 2002 or 2003. It colonised site W in 2004, remaining in slightly lower numbers in 2005; in 2006, after my study ended, numbers more than doubled on site W (D J Simcox pers comm.). *H. semele* increased greatly on sites X and Y from 2004 to 2005 (figure 2.7).

C. campestris populations increased greatly on site X from 2002 – 2004, and on site Y from 2003 – 2004 (figure 2.8). In 2004, *C. campestris* colonised site W. However, in 2005 it experienced a small decline. This decline also occurred on sites X and Y, although due to the higher counts on these sites in 2004, the decline is more dramatic. This may be due to cooler weather in 2005; however, the steep decline indicates that this is more likely to be due to a population crash.

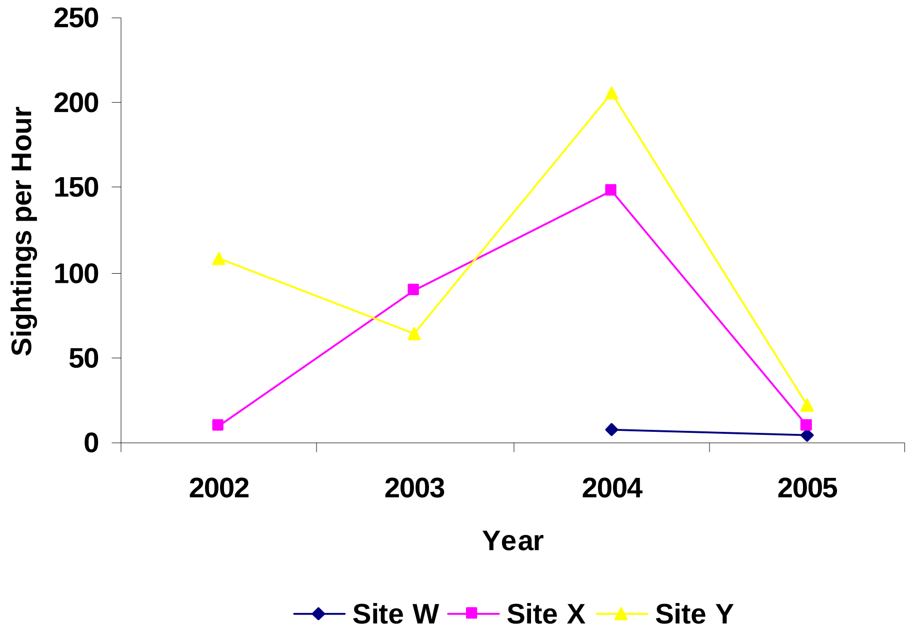


Figure 2.8: Sightings per hour during the peak flight period for *C. campestris*, recorded on sites W, X and Y from 2002 to 2005.

2.5.5 Ant baiting

Data were analysed using McNemars' test with the following equation:

$$\chi^2 = \frac{\left(\left|PA - AP\right| - 1\right)^2}{\left(PA + AP\right)}$$

Where PA is ant species present at time 1 (i.e. Spring 2003) but absent at time 2 (i.e. autumn 2003 or spring 2004 or spring 2005). Conversely AP denotes the number of baits where ant species were not present at time 1 (i.e. spring 2003) but were present at time 2 (autumn 2003, spring 2004 or spring 2005). The 5%, 1% and 0.1% critical values of χ^2 with 1 degree of freedom were 3.841, 6.635 and 10.828 respectively.

Area W1 (Sparse grassland and former *Pteridium aquilinum* area)

Formica rufa attendance at baits declined significantly from spring 2003 to spring 2004. However, abundance of this ant increased again in 2005 to autumn-2003 and almost spring-2003 levels (table 2.8 and figure 2.9). During this same time period *M. ruginodis* bait attendance increased to 85%, suggesting that *M. ruginodis* benefits in the absence of *F. rufa* and/or the change in vegetation structure. In spring of 2003, *M. scabrinodis* was absent from area W1, but was present at 10% of baits in all later samples. This result indicates that conditions are becoming suitable for *M. scabrinodis*, which requires a warmer micro-climate to that of *M. ruginodis*.

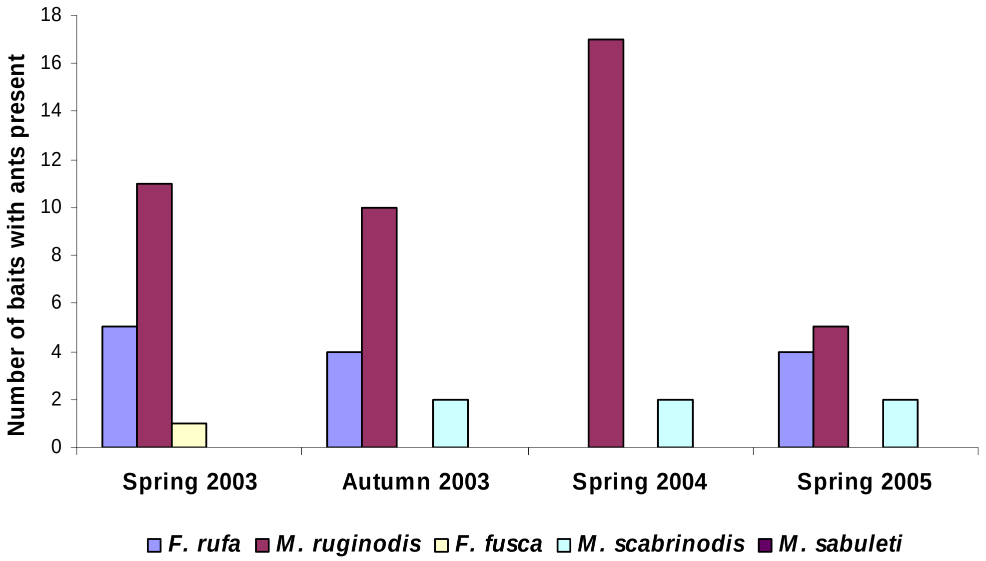


Figure 2.9: Number of baits attended by different ant species at the different sampling times in area W1. *F. rufa* was significantly less abundant at baits from spring 2003 to spring 2004 (χ_1^2 = 3.2, P <0.05). *M. ruginodis* increased significantly between spring 2003 and spring 2004 (χ_1^2 = 4.9, P < 0.05). *M. scabrinodis* increased between spring 2003 and autumn 2003 (χ_1^2 = 4.5, P <0.05).

Area W2 (Semi-natural acid grassland area)

In the spring of 2003, only three ant species, *F. rufa*, *F. fusca* and *M. ruginodis*, were detected by baiting. All three species declined significantly as W2 developed towards open grassland (table 2.8, figure 2.10). Over the same period, *M. sabuleti* (the host ant of *Maculinea arion*) and *M. scabrinodis* colonised the developing sward. Of these, *M. sabuleti* prefers shorter grassland niches where the micro-climate is significantly warmer to that occupied by *M. ruginodis* (Chapter 3).

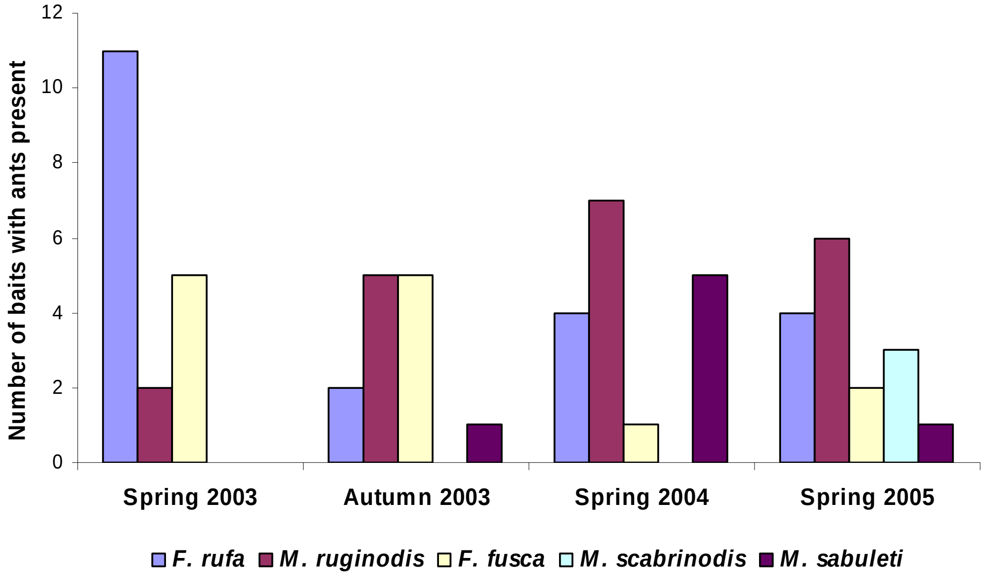


Figure 2.10: Number of baits attended by different ant species at the different sampling times in area W2. *F. rufa* decreased from spring 2003 to autumn 2003 (χ^2_1 = 5.818, P < 0.05) and from spring 2003 to spring 2004(χ^2_1 = 4, P <0.05). *M. ruginodis* increased between spring 2003 to spring 2004 (χ^2_1 = 4, P <0.05). Between spring 2003 and spring 2005 *M. scabrinodis* increased (χ^2_1 = 5.33, P <0.05). *M. sabuleti* increased from spring 2003 to autumn 2003 (χ^2_1 = 4, P <0.05) and from spring 2003 to spring 2004 (χ^2_1 = 7.2, P <0.01).

Area W3 (former *Pteridium aquilinum* area)

There was a significant decrease in *F. rufa* attendance at baits from spring 2003 to spring 2004 (table 2.8 & figure 2.11). *M. ruginodis* on the other hand decreased significantly from spring 2003 to autumn 2003. *F. fusca* was present at 5% of baits in autumn 2003, a significant increase from spring 2003 when this ant was absent. *M. ruginodis* is the most dominant ant in this area of site W.

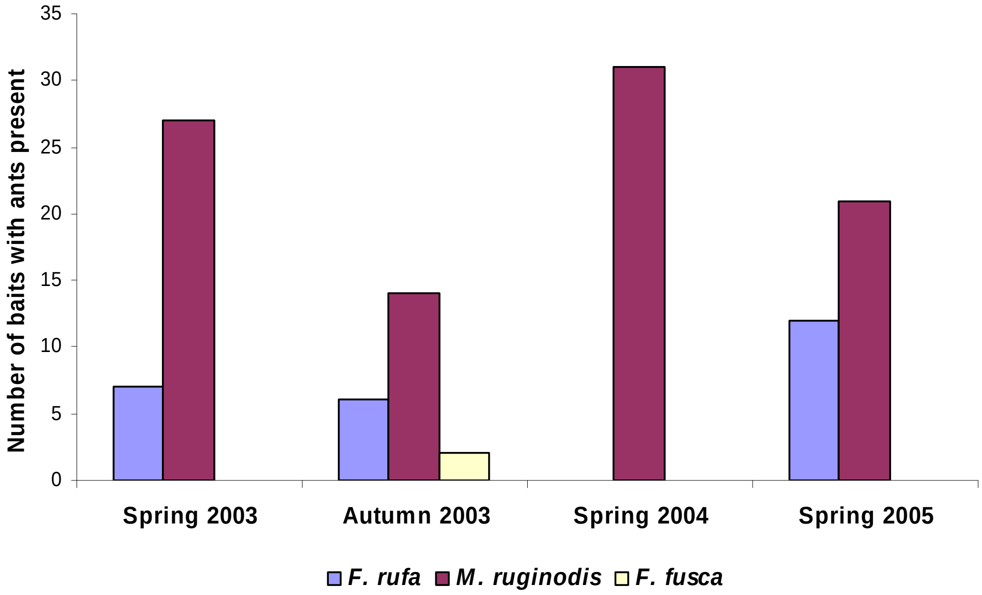


Figure 2.11: Number of baits attended by different ant species at the different sampling times in area W3. *F. rufa* decreased between spring 2003 and spring 2004 (χ_1^2 = 4.17, P< 0.05). *M. ruginodis* decreased between spring 2003 and autumn 2003 (χ_1^2 = 6.26, P < 0.05). *F. fusca* increased between spring 2003 and autumn 2003 (χ_1^2 = 4.5, P <0.05).

Area W4 (former *Pteridium aquilinum* area)

F. rufa shows a significant decline in attendance at ant baits between spring 2003 and autumn 2005 (table 2.8 and figure 2.12). *F. fusca* was detected in trivial numbers in one year. *M. ruginodis'* presence at baits was stable between sampling times up until spring 2005, when it showed a significant increase and was present at 55% of the baits (table 2.8 and figure 2.12). Area W4 was burnt in March 2004 and it is likely that the reduction in bracken density aided colonisation of this area by *M. ruginodis*.

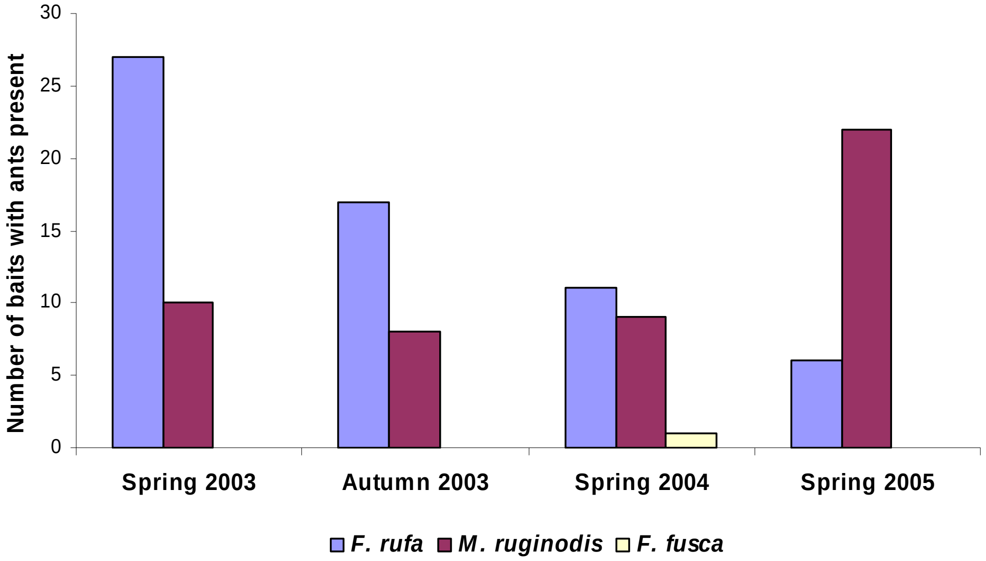


Figure 2.12: Number of baits attended by different ant species at the different sampling times in area W4. *F. rufa* declined between spring 2003 and autumn 2003 (χ_1^2 = 5.06, P <0.05), spring 2003 to spring 2004 (χ_1^2 = 12.5, P <0.001) and spring 2003 to spring 2005 (χ_1^2 = 19.05, P <0.001). *F. fusca* increased from spring 2003 to spring 2004 (χ_1^2 = 4, P <0.05). *M. ruginodis* increased between spring 2003 and spring 2005(χ_1^2 = 8.45, P <0.01).

Table 2.8: The number of times (and percentage of times) different ant species attended baits in each area of site W and the grassland and *P. aquilinum* control areas of site X at the 4 sampling times.

Area	Ant species	Spring 2003	Autumn 2003	Spring 2004	Spring 2005
		n (%)	n (%)	n (%)	n (%)
W1	*F. rufa*	5 (25)	4 (20)	0 (0)	4 (20)
	M. ruginodis	11 (55)	10 (50)	17 (85)	5 (25)
	F. fusca	1 (5)	0 (0)	0 (0)	0 (0)
	M. scabrinodis	0(0)	2(10)	2(10)	2(10)
	M. sabuleti	0 (0)	0 (0)	0 (0)	0 (0)
W2	*F. rufa*	11 (55)	2 (10)	4 (20)	4 (20)
	M. ruginodis	2(10)	5(25)	7(35)	6(30)
	F. fusca	5 (25)	5 (25)	1 (5)	2 (10)
	M. scabrinodis	0 (0)	0 (0)	0 (0)	3 (15)
	M. sabuleti	0 (0)	1 (5)	5 (25)	1 (5)
W3	*F. rufa*	7 (17.5)	6 (15)	0 (0)	12 (30)
	M. ruginodis	27 (67.5)	14 (35)	31 (77.5)	21 (52.5)
	F. fusca	0(0)	2(5)	0(0)	0(0)
	M. scabrinodis	0 (0)	0 (0)	0 (0)	0 (0)
	M. sabuleti	0 (0)	0 (0)	0 (0)	0 (0)
W4	*F. rufa*	27 (67.5)	17 (42.5)	11 (27.5)	6 (15)
	M. ruginodis	10 (25)	8 (20)	9 (22.5)	22 (55)
	F. fusca	0 (0)	0 (0)	1 (2.5)	0 (0)
	M. scabrinodis	0(0)	0(0)	0(0)	0(0)
	M. sabuleti	0 (0)	0 (0)	0 (0)	0 (0)
Control Areas					
X Grassland	*F. rufa*	0 (0)	0 (0)	0 (0)	0 (0)
	M. ruginodis	0 (0)	0 (0)	0 (0)	0 (0)
	F. fusca	2 (10)	1 (5)	0 (0)	0 (0)
	M. scabrinodis	3 (15)	4 (20)	1 (5)	1 (5)
	M. sabuleti	13 (65)	13 (65)	17 (85)	18 (90)
X *P. aquilinum*	*F. rufa*	14 (70)	5 (25)	0 (0)	Not recorded
	M. ruginodis	12 (60)	1 (5)	11 (55)	Not recorded
	F. fusca	0 (0)	0 (0)	0 (0)	Not recorded
	M. scabrinodis	0 (0)	0 (0)	0 (0)	Not recorded
	M. sabuleti	0 (0)	0 (0)	0 (0)	Not recorded

Site X Grassland Control Area

The attendance of *M. sabuleti* at baits increased significantly in 2003 to 2005 (table 2.8 and figure 2.13). This suggests that optimal habitat management for *M. sabuleti* was achieved. There were no significant changes in *M. scabrinodis* or *F. fusca* attendance at baits during the study period. The ant community within this target grassland area is very different compared to that of the site W restoration areas. *M. sabuleti* is the dominant ant within this grassland area. The maximum attendance for *M. sabuleti*, on site W was 25% in spring 2004. Unlike the restoration areas of site W, *F. rufa* and *M. ruginodis* were not detected during ant baiting on this target grassland site.

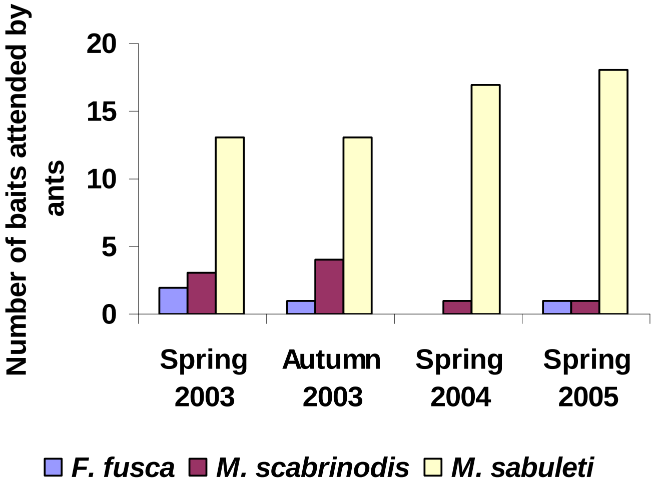


Figure 2.13: Number of baits attended by different ant species at the different sampling times in the site X grassland control area. *M. sabuleti* increased significantly from spring 2003 to spring 2004 (χ^2_1 = 4.2, $P < 0.05$) and from spring 2003 to spring 2005 (χ^2_1 = 7.2, $P < 0.001$).

Site X *P. aquilinum* Control Area

F. rufa decreased significantly in the *P. aquilinum* control plots in 2003 to 2004 (table 2.8 and figure 2.14). *M. ruginodis* attended significantly more ant baits in spring 2004 compared to spring 2003. The ant community in this control area is similar to that of the former *P. aquilinum* areas of site W, with both *F. rufa* and *M. ruginodis* being present.

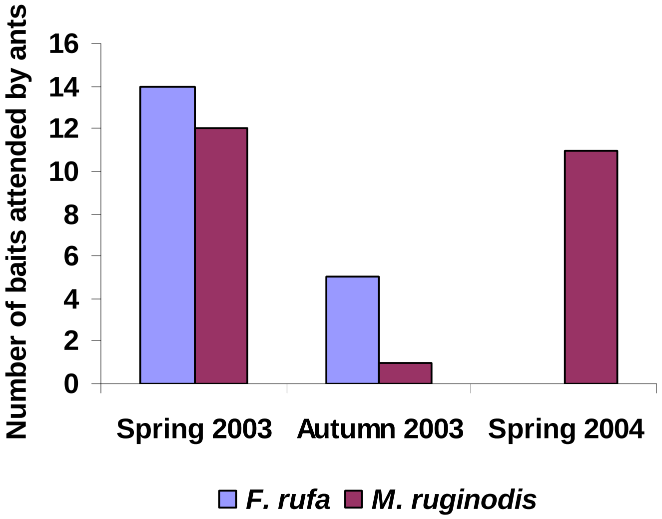


Figure 2.14: Number of baits attended by different ant species at the different sampling times in the site X *P. aquilinum* control area. *F. rufa* declined significantly between spring 2003 and autumn 2003 (χ^2_1 = 5.8, P <0.01) and between spring 2003 and spring 2004 (χ^2_1 = 5.1, P <0.01). *M. ruginodis* attendance at baits significantly increased between autumn 2003 and spring 2004 (χ^2_1 = 11.1, P <0.001).

2.6 Discussion

2.6.1 Habitat Management effects on Vegetation Assemblage

In the Detrended Correspondence Analysis ordination plot produced by CANOCO (figure 2.4), the *P. aquilinum* and grassland control plots on site X remained relatively static. This suggests that the changes that occurred within areas on site W are not due to random fluctuations and are a direct result of habitat management.

Vegetation assemblage in W1 and W3 changed significantly within the study period. Both areas showed re-establishment of semi-natural acid grassland as a result of earlier *P. aquilinum* burning, cutting and spraying. The removal of the dense litter layer, through burning, enabled recruitment of seedlings from the seedbank. In addition to this, areas of bare ground were created which allowed germination of buried seeds or seed rain (Fenner 1985). *P. aquilinum* management appears to have been more successful in area W1 compared to areas W3 and W4. However, *P. aquilinum* was less abundant in W1 prior to treatment. Mean percentage cover of *P. aquilinum* in each area was as follows; W1 (77%); W3 (100%) and W4 (98%). Despite this W1 did not revert back to the *P. aquilinum* community on the ordination plot. Area W4 reverted back to *P. aquilinum* community quicker than area W3, possibly due to the lack of follow up cutting and spraying of *P. aquilinum* after the initial burning treatment. The rhizomes of *P. aquilinum* store carbohydrates and if management ceases, the recovery of *P. aquilinum* is rapid (Lowday & Marrs et al,. 1992).

The grazing that was introduced on site W was not intense enough to see any major changes in vegetation assemblage in area W2. Until the grazing intensity of area W2 is increased, *M. arion* is unlikely to re-colonise the area in the near future due to the vegetation being too tall and hence soil temperature being too cool for high densities of the host ant *M. sabuleti*. The ordination plot shows that in 2003, area W2 and the site X grassland control plots were relatively similar; however, the grazing intensity must be increased to ensure the appropriate micro-climatic conditions are available for *M. sabuleti*.

2.6.2 Habitat Management effects on Vegetation Structure

The vegetation height within the *P. aquilinum* area on site X did not differ significantly during the study period. This indicates that the change in vegetation height that occurred within areas W1, W3 and W4 were directly due to habitat restoration management. The comparison of vegetation height between the site X grassland control area and area W2 showed some interesting differences. Vegetation height in area W2 in 2003 was significantly greater than that of the site X grassland control area; however there was no significant difference between sites in 2004. Although one might attribute this to the introduction of grazing on site W, in fact the vegetation height within the site X grassland control doubled. Additionally, despite the introduction of grazing animals on site W in May 2004, vegetation height increased within area W2 in 2004. However, the Interquartile range of the data was reduced (table 2.3). The convergence of vegetation height between these sites is largely due to site X growing taller. Had data been collected from area W2 and the site X grassland control area in 2005, I would expect it to show that vegetation height had increased significantly on site W due to the removal of the grazing animals in autumn 2004. The reduction of *M. sabuleti* and increase in *M. scabrinodis* on area W2 in 2005 reinforces this argument (see section 2.6.5 for further details).

The only significant difference in vegetation height across site W occurred within area W4; this result was due to the data including pre-management vegetation height. These data were included to act as a base line for area W4 and also as an additional control comparison between areas W1 and W3 which had received management in the previous season. What is clear from this comparison is that, unsurprisingly, vegetation height is initially reduced significantly after burning the *P. aquilinum.* The same results were seen when vegetation height between areas W1 and W3 were compared to site X *P. aquilinum* control vegetation height. The burning, cutting and spraying management reduced vegetation height in the managed areas in both 2003 and 2004. Additionally, the soil temperature in each of the managed *P. aquilinum* areas increased after management compared to the un-managed controls, an indication that vegetation structure was altered. However, in area W4, despite burning in spring 2004, there was no significant difference in vegetation height between this area and the *P. aquilinum* control area. This result highlights the importance of continued *P. aquilinum* management particularly in area W4 where *P. aquilinum* was well established. Had data collection been undertaken in the control *P. aquilinum* area in 2005, I would expect there to be a significant difference in vegetation height since the median vegetation height for W4 was reduced to 11.5 cm by 2005.

Despite there not being an overall decrease in vegetation height across site W in its entirety during the study period, it is clear from the CANOCO DCA ordination that vegetation assemblage altered. Fire temporarily destroys the dominant flora (Mallik & Gimingham, 1983). Ground flora re-establishment was possible in the absence of dense *P. aquilinum* stands. In other studies fire has been shown to increase species richness during the five years after the burn (Calvo & Luis, 2002; Borghesio, 2009). However, in order to control *P. aquilinum* domination intense management is required for at least seven consecutive years (P. O' Connor, pers comm.). Cutting *P. aquilinum* twice a year produces a favourable long term control (Cox et al 2007) as it reduces frond biomass (Snow & Marrs, 1997). The introduction of grazing animals at a higher density, and/or, for a longer period of time on site W would have had the effect of trampling the *P. aquilinum* and therefore broken up its regenerating stands, *P. aquilinum* is intolerant to trampling by cattle and ponies (Kirby 1992). In addition to this the gaps made by trampling the vegetation would have enabled greater natural recolonisation of plants from the soil seed bank (Rook & Tallowin, 2003; Pykälä, 2003).

These early results represent satisfactory progress to the restoration of semi-natural acid grasslands of high conservation value, and my observations of an overall reduction in *P. aquilinum* domination combined with a huge increase in ground flora that consisted primarily of *V. riviniana* are strengthened by the results of the CANOCO DCA ordination and the statistical analysis of *V. riviniana* data.

2.6.3 Changes in the numbers of *Viola* species

The numbers of *V. lactea* and *V. riviniana* did not differ between years within the site X grassland and site X *P. aquilinum* control areas. This is a clear indication that the dramatic changes in *V. riviniana* on site W are a direct result of the habitat management imposed upon the site. *Viola riviniana* density increased from 2003a to 2005 by 13.2%, 29.4% and 20% in areas W1, W3 and W4 respectively. A 50% increase in *V. riviniana* abundance occurred within area W2 from 2003 to 2004. However, burning as a management practice on the tallgrass prairies in the American mid-west did not have any significant effect on *Viola* species abundance (Swengel, 1997).

The burning of the *P. aquilinum* was followed by an increase in the abundance of *V. riviniana* in areas W1, W3 and W4. *V. riviniana* is a perennial plant (as is *P. aquilinum*) and perennials have been shown to respond rapidly to burning events (Calvo & Luis, 2002). Burning of *P. aquilinum* removes the dense litter layer as well as the above ground living biomass of the plant. Removal of litter has been shown to benefit *Viola palustris* in wet fenlands (Jensen & Meyer, 2001). The creation of bare ground enables seedlings to establish free from competition (Borghesio, 2009). It is well documented that *Viola* species thrive after *P. aquilinum* is burnt (Oates 1995). It may be that *Viola* seeds require heat to stimulate seed germination (Volg 1974). Further research to determine the effect of burning on *V. riviniana* seeds in the seedbank would be an interesting avenue of further research. Another factor may be increase in *Myrmica* ant species, which I show, in Chapters 3 and 4, to have a mutalistic association with *V. riviniana.* Thus, an increase in *V. riviniana* (and *Myrmica*) abundance also occurred after the *P. aquilinum* was cut and sprayed. It is likely that this further reduced the competition from the regenerated *P. aquilinum* and enabled *V. riviniana* establishment. I also noted that not only were *V. riviniana* more abundant within areas W1, W3 and W4 but the growth form or size class was noticeably different; prior to the management the *Viola* plants were much larger, most probably due to the competition for sunlight under the dense canopy of *P. aquilinum*. After the areas were burnt, *Viola*s were much smaller in size.

During the study period *V. lactea* did not colonise areas W3 or W4, perhaps reflecting its absence in the underlying seedbank. Another hypothesis is that the microclimatic conditions within these areas were unsuitable for their establishment (see Chapter 3 for further details). This species did colonise area W1 and was present in area W2 in small numbers. Although it is too soon to see any significant increase in *V. lactea* abundance, in due course, this species is likely to increase in abundance with increased grazing intensity, as grazing will create larger areas with suitable micro-climatic conditions.

2.6.4 Invertebrates and management

The huge increases in *V. riviniana* on site W coincided with the colonisation of *B. euphrosyne* and *B. selene* in 2003. *Viola* species are the larval food plants for both of these Red Data Book butterflies. In addition, the DCA ordination plot of plant species (figure 2.3) shows that areas W1, W3 and W4 (where the increases in *V. riviniana* occurred) also had an abundance of nectar sources for the adult butterflies. In another study the abundance of *Viola* was positively correlated with Fritillary butterfly presence (Swengel, 1997).

Both *B. euphrosyne* and *B. selene* were breeding on site W by the spring of 2004. *B. euphrosyne* showed a relatively large increase on site W during the study period this was mirrored on sites X and Y. Several other populations in the UK have also shown significant increases as a result of habitat management (Fox, et al. 2006). *B. selene* also underwent a large increase in numbers on site W from 2004 to 2005. Again this species was faring exceptionally well on sites X and Y.

In 2004, *H. semele* and *C. campestris* colonised site W. *C. campestris* is a ground-dwelling thermophilous insect which inhabits open sparsely vegetated areas with bare ground in which to oviposit (Pearson, 1988). During the study period areas W1, W3 and W4 all shifted towards early successional stage communities with an abundance of re-colonising acid-grassland plant species and bare ground. The decrease in *C. campestris* on site W in 2005 may be due to the regeneration of *P. aquilinum* caused by a lack of follow up management on area W4 or may be due to natural fluctuations in the species population. I suggest the latter; *C. campestris* also declined on sites X and Y in 2005.

H. semele larvae feed on certain grasses; exclusively *Agrostis curtisii* on these edge-of-Dartmoor sites. Eggs are laid in small tussocks of *A. curtisii* that are growing in small open patches of ground (Asher et al 2001; Thomas & Lewington 1991). The DCA ordination for Areas W1 and W3 show that *A. curtisii* was abundant in these areas in 2004 and 2005. In addition to this, I recorded the spread of *A. curtisii* from the edges of area W2 into areas W1, W3 and W4. However, these changes were not picked up in the quadrat data because quadrats were positioned along transects to minimise edge effects. On reflection, quadrats along the frontiers of the different areas may have been beneficial to my study to provide data about vegetative spread between areas. This would have given firm evidence to my more anecdotal observations.

Previous studies have provided evidence that *B. euphrosyne*, *B. selene*, *H. semele* and *C. campestris* have all experienced population increases on site X as a result of habitat management for *M. arion* (New et al., 1995; Thomas, 1999; Elmes & Thomas, 1992). The results of my study clearly show that these four species are benefitting from habitat restoration management for the future reintroduction or natural recolonisation of *M. arion* on site W. These sites, W, X and Y, are very important for *B. euphrosyne*, *B. selene* and *H. semele*, in the south-west of England (Fox, et al. 2006). Habitat management on these sites, and at the landscape scale must be maintained in the longer term ensure the future survival of these species (Bulman et al., 2007; Anthes et al., 2008). In the light of climate change, which will undoubtedly create unsuitable habitat and environmental conditions, species that occupy isolated habitat patches are vulnerable to local extinction (Hanski, 1999). Habitat management at the landscape scale will provide species with a network of additional refugia to colonise and move between, thus reducing the risk of local extinction and enabling long term persistence.

2.6.5 Ants and Habitat Management

The increase of *M. sabuleti* at ant baits within the site X grassland control area is encouraging; it indicates that the targeted habitat management is effective. In addition, the vegetation height within the site X grassland control area did not differ significantly during the study period; this again is a good indication that the grazing regime on site X is correct.

Within the site X *P. aquilinum* control area some changes in ant species did occur. Both *F. rufa* and *M. ruginodis* decreased from spring 2003 to autumn 2003; this may be due to the ants entering diapause in the autumn (Elmes pers comm.).

Changes in ant species assemblage within areas W1, W3 and W4 showed similar patterns. After the burning of *P. aquilinum, F. rufa* declined significantly whereas *M. ruginodis,* which had also been present in all areas, increased. The decline in *F. rufa* may be directly due to mortality as a result of the fire (Swengel, 2001), although one would also expect *M. ruginodis* to have declined significantly. Alternatively the niche created by the fire was more suitable for recolonisation by *M. ruginodis.* In areas W1 and W3 *F. rufa* was absent at baits in spring 2004, however, they were present at a small proportion of baits again in spring 2005. Although the vegetation structure data does not show any significant reduction in vegetation height between years for areas W1 and W3, largely as a result of the absence of pre-management data, the vegetation assemblage did change. Initially all the areas were tall dense *P. aquilinum* stands with very little vegetation at the ground level, with the exception of W1, where ground flora was sparse. The burning of the *P. aquilinum* removed the stands and dense ground litter layer (figure 2.4). This enabled the seeds from seed rain and from the seedbank to germinate and colonise the bare ground. This in turn created suitable conditions for the establishment of *M. ruginodis* ant colonies at the expense of *F.rufa*. *F. rufa* abundance in areas W1, W3 and W4 may have been reduced as a result of the *P. aquilinum* burn that created a warmer, drier and more open habitat, especially in 2004 when grazing occurred. Clear cutting of forests, which again creates open, warmer and drier habitats, have led to the local extinction of *F. aquilonia* (Vepsäläinen et al 2000). Additionally, the aphids and the extra floral nectaries that were abundant amongst the dense *P. aquilinum* were temporarily eradicated in areas W1, W3 and W4, thereby removing the main food sources of *F. rufa* (Brian 1977; Vepsäläinen et al 2000; Vepsäläinen and Wuorenrinne 1978). *F. rufa* is a territorial species that tends to exclude other species. The decline in *F. rufa* enabled *M. ruginodis* (a submissive species whose activity is suppressed by *F. rufa*) (Dauber et al 2006) to establish itself on the burnt *P. aquilinum* areas. However, the recovery of *F. rufa* in areas W1 and W3 in spring 2005 may be attributed to the removal of grazing animals and or the re-establishment of extra floral nectaries amongst the re-generating *P. aquilinum* stands.

Myrmica scabrinodis inhabits a warmer niche than *M. ruginodis*, and was not detected in any area of site W during my initial sampling period (Table 2.8). By autumn 2003 it had colonised W1 but it was not detected in W3 until spring 2005. This suggests that the *P. aquilinum* management was more successful in this area compared to area W3. The cutting and spraying of the regenerated *P. aquilinum* in June 2003 created pockets of habitat that were suitable for the establishment of *M. scabrinodis* in area W1. My measurements of percentage *P. aquilinum* cover, taken in February 2003 (prior to management) show that the *P. aquilinum* was not as dense in area W1 (77% cover) compared to W3 (100% cover) and therefore there may already have been some ground flora established. This is also supported by the CANOCO DCA ordination which shows that both area W1 and W3 ordinate towards *R. fruticosus* and the re-colonising semi-natural acid grassland plants: however, W3 reverts back towards the *P. aquilinum* area within the ordination (figure 2.4). This trend also reflects the fact that *P. aquilinum* management, particularly in areas W3 and W4, was not continued.

The ant baiting results from W2 are disappointing. *M. sabuleti* increased significantly from autumn 2003 to spring 2004, but this was not due to the introduction of livestock grazing on site W in spring 2004 because vegetation height increased in W2 in 2004 (table 2.3). However, the animals' hooves may have opened up bare patches of ground within the rank vegetation which perhaps created spots for *M. sabuleti* nests. If the grazing intensity had been greater on site W during the study period, then the increase in vegetation height would have been arrested and *M. sabuleti* would probably have maintained a greater abundance. However, the removal of grazing animals in the autumn of 2004 meant the increase in *M. sabuleti* was short lived. This is supported by the establishment of *M. scabrinodis* on site W by spring 2005. *M. scabrinodis* is generally found in cooler niches than those of *M. sabuleti.* Vegetation height did indeed increase between spring 2004 and spring 2005, albeit not significantly. If the sward height increases by just two centimetres the soil surface temperature can be reduced by 5º C - 10ºC (Thomas et al., 1989). The relationship between vegetation height and soil temperature is explored further in Chapter 3. Unfortunately grazing was not reintroduced on the site until after I had completed this study. When this occurred, *M. sabuleti* increased rapidly from 0%-25% presence at ant baits in areas W1-3 in 2003-05 to 80% presence in summer 2006, and the first *M. arion* eggs were laid on the site in summer 2006 (D J Simcox, pers comm.).

2.7 Conclusion

The aim of this study was to measure the changing floral and invertebrate assemblages, and the rate of successional changes, in the re-establishment of early seral, semi-natural, acid grassland on previously abandoned sites dominated by *Pteridium* or *Ulex*. I tested the idea that population increases in *B. euphrosyne, B. selene, H. semele, C. campestris* and *V. riviniana* are associated with targeted habitat management for *M. arion* and *M. sabuleti*, which creates a warm, early successional niche in grassland that is shared by this guild of species. Subsequent Chapters explore the mechanisms involved in the increases of *Viola* and *Boloria* species. The close proximity of site W to sites X and Y is a key factor in the rapid re-colonisation rates of *B. euphrosyne, B. selene* and *H. semele.* Had the site been isolated, it is likely that these relatively immobile species would have taken decades to appear (Asher et al 2001).

The results from this Chapter confirm that habitat management for *M. arion* does benefit other rare species (Elmes & Thomas 1992). Even in the short period of time that monitoring was undertaken on site W, it is evident that the re-creation of early seral, semi-natural acid grassland creates niches that are exploited by this guild. The Red Data Book species *B. euphrosyne* and *B. selene* were the first specialist butterflies to colonise and breed on site W. *H. semele* (another Biodiversity Action Plan butterfly species) and *C. campestris* colonised a year later, when the habitat conditions were more suitable for them in terms of areas of compacted bare ground (essential for *C. campestris'* larval burrows) and sparse *Agrostis curtisii* in relatively open conditions for *H. semele* oviposition.

Clearer patterns were detected in the butterfly data compared to the ant baiting data. The butterfly species studied responded to restoration management quicker, confirming that they are early indicators of changing habitat quality (Thomas 2004).
In the semi-natural acid grasslands being studied, I also suggest that *M. arion* is an umbrella species as defined by Fleishman et al., 2000. The identification of the most sensitive species within the ecosystem, i.e. *M. arion*, adopts the focal species approach as suggested by Lambeck et al (1997). There has been much debate regarding the usefulness or validity of umbrella species for species conservation (McGeoch, 2007; Simberloff 1998; Roberge & Angelstam, 2004). The only practical example of successful conservation using the umbrella species approach has been the study of *M.*

arion in UK grasslands, namely sites X and Y (Thomas, 2007). The main reasons why this has been successful are because; the ecology of *M. arion* is clearly understood; the cause of its extinction has been rectified by creating suitable sites through management; and lastly these restored sites have been monitored for approximately 30 years (Simcox, et al., 2005; Thomas 2007).

Long term monitoring is vital for assessing changes in species abundance and habitat quality. An understanding of the ecology of threatened species and the interactions between species within the restored ecosystems is essential to ensure long term conservation (Thomas, 1994). The following Chapters explore the role of *Myrmica* ants as ecosystem engineers in the established *M. arion* grassland on sites X and Y, in order to further understand the complex interactions between the study species.

Chapter 3: Do *Myrmica* spp. and *Formica fusca* influence the vegetation assemblage above their nests in grasslands managed for *Maculinea arion*

3.1 Introduction

Several studies have shown that ants influence the vegetation above their nests (Elmes, 1991a). For example, King (1977) found that flora on the prominent mounds of *Lasius flavus* were significantly different from that of non mound vegetation. Conversely Elmes & Wardlaw (1982a) suggested that nest spot microhabitat features would determine the vegetation assemblage and the suitability for nesting *M. sabuleti*, a species that does not form obvious mounds. They did not find any evidence that plants above *Myrmica sabuleti* nests were responding to the physical changes to soils and vegetation that has been generated by the ants. Here I question their findings since *Myrmica* nests have been shown to increase local soil nutrients and organic matter (Petal, 1980; Beattie & Culver, 1983) and I suggest that in the longer term this is likely to influence above nest vegetation. Also, as described in Chapter 1, the gathering of myrmecochorous seeds by ants is likely to alter the above nest flora. Transportation of seeds by ants is expected to determine the spatial distribution of myrmecochorous plants, with these plants growing in greatest abundance close proximity of ant nests (Lack & Kay, 1987). For example, seed dispersal by ants has affected the distribution patterns of *Polygala vulgaris* and *Viola curtisii* in a primary dune valley in the Netherlands: adult plants were more abundant near to ant nests, and seedlings were confined to the nest mound (Oostermeijer, 1989). In addition to this, Kovar et al. (2001) found that in mountain grasslands there was a non-random distribution of myrmecochores; most were growing near to nests of *Lasius flavus, Tetramorium caespitum* and *Formica* spp. However conflicting results have been found in other studies. No spatial relationship was found between *Viola curtisii*, *Luzula campestris* and *Polygala vulgaris* and ant nests on the coastal grey dunes of Flanders (Belgium) (Lehouck et al., 2004).

In Chapter 2, I showed that densities of *Viola riviniana* have increased significantly on a study site where *Myrmica* ants had increased from low densities to become the dominant ant genus. *Viola* seeds possess an elaiosome and dispersal of these seeds may be facilitated by *Myrmica* or other ant species on the study site, thereby increasing *Viola* species densities near to ant nests. To test this hypothesis I will assess the vegetation above *Myrmica* species and *Formica fusca* ant nests and compare it with non-ant nest areas.

3.2 Research Aim

To determine whether the presence and abundance of selected plant species which possess elaiosomes (*Viola* species, *Potentilla erecta and Teucrium scorodonia*) and whether *Agrostis curtisii* which does not possess an elaiosome, are associated respectively with ant-nests or with spots away from *Myrmica* and *Formica fusca* nests in the field. I also investigated the niche association of different *Viola* species and *Myrmica* species.

3.3 Hypothesis

a) Although *Myrmica* spp. and *Formica fusca* do not noticeably alter the ground above and surrounding their nest sites, it is hypothesised that *Viola* species are more abundant near ant nests due (i) to the presence of an elaiosome on *Viola* seeds which causes ants to carry them to nests; (ii) to the probable exclusion of invertebrate herbivores from ground close to nests which creates enemy free space; and (iii) to higher nutrient levels near ant nests (Chapter 5).

b) *Viola lactea* inhabits a hotter niche than *Viola riviniana* and may therefore be associated with thermophilous species of *Myrmica* e.g. *Myrmica sabuleti*. *Viola riviniana* has a cooler niche and may be more associated with *Myrmica ruginodis,* an ant species that also prefers a cooler niche.

3.4 Methods

3.4.1 Introduction to Study Site

The study sites are semi-natural acid grassland on the edge of Dartmoor National Park. Some areas of the site have been undergoing habitat management and restoration from dense scrub and bracken since 1975 to recreate *Maculinea arion* grassland (Chapter 1).

The entire site is intensively grazed by cattle and ponies and undergoes rotational swaling (burning) of scrub. This creates a mosaic of heterogeneous habitat ranging from early successional stages to mature gorse stands. The flora is dominated by *Agrostis curtisii*, *Ulex europaeus* and *Erica cinerea*. The ant community is dominated by *Myrmica ruginodis*, *M. lonae*, *M. scabrinodis*, *M. sabuleti*, *M. scabrinodis* $_{\text{(HOT)}}$, *Formica fusca* and *F. rufa*. Only the first six ‘species’, all of which have a similar colony structure, were used to test hypotheses (a) and (b).

The niches of the main *Myrmica* spp. are well known with each occupying a distinct, partially overlapping, realised niche that can be defined by soil microclimate. (Elmes et al., 1998). However there is uncertainty about the taxonomic status of some *Myrmica* species, especially *M. scabrinodis* which was recently shown to consist of at least two (and perhaps several more) cryptic species (Ebsen, 2005). *M. scabrinodis* typically inhabits a relatively cool moist niche situated between, but overlapping with, those of *M. ruginodis* (cooler) and *M. sabuleti* (warmer). However on these study sites, an apparently identical ant which cannot (yet) be distinguished by chemical (S. Everett pers. comm.), morphological (G. Elmes pers. comm.) genetic (J. Ebsen pers. comm.) or physiological analyses (Randle & Wardlaw unpublished) also occupies a warmer and drier niche even than *M. sabuleti*. These two niches of *M. scabrinodis* are distinct, non-overlapping and predictable. For the purposes of this study, this latter form will be referred to as *M. scabrinodis* $_{\text{(HOT)}}$. Similarly, an ant resembling *M. sabuleti*, but with a much exaggerated projection on the antennal scape, was found in the cool, shady scrubland, beyond the optimal niches of *M. ruginodis*. The World’s two leading *Myrmica* taxonomists, G.W. Elmes and A. Radchencko (pers. comm.) cannot agree on the taxonomic status of this form, but both have provisionally identified it as *M. lonae*, which is it is called henceforth in this study. Further details regarding the division of *M. scabrinodis* types can be found in Elmes, et al. (1998).

3.4.2 Nest location and vegetation assessment

In 2004, a total of 93 ant nests were located using the standard methodology (Wardlaw et al., 1998b) of cake crumb baits covered with a plant pot saucer (10cm diameter). The baits were put out in optimum *Myrmica* foraging conditions (i.e. time of day and weather conditions) and checked 30 minutes later. Worker ants carrying crumbs were followed back to the nest. Ants were identified in the field using a x20 achromatic hand lens. Nest entrances were marked using garden canes and labelled according to ant species. Four *Myrmica* ant species were studied; *Myrmica sabuleti, M. scabrinodis, M. ruginodis* and *M. scabrinodis* $_{\text{(HOT)}}$. *Formica fusca* was included in the study as the only other ant present that had a similar abundance and colony structure to *Myrmica*. The high densities of ant nests on the site meant that location of paired non-nest spots in similar conditions was difficult. It was therefore necessary to take a stratified random sample across the site of non-ant nest spots in apparently identical habitat between the spots that contained marked nests. To identify these, cake crumb baits, put out during optimum *Myrmica* foraging conditions, were checked after 15 minutes for the presence of ants – if a nest was present at the baiting spot, the ants would have come to the bait in large numbers (>20 workers) within the given time. I am confident that if no ants attended the bait then the spot was definitely a non-nest spot. Eighty-seven non-nest spots were marked and numbered. Above nest vegetation was assessed within a $0.25m^2$ sampling hoop; *Myrmica* nests rarely extend further than $0.25m^2$ from the nest entrance (Elmes & Wardlaw, 1982a). Sampling hoops were centred on the nest entrance for nest spots and on the garden cane for non-nest spots. Vegetation assessment focussed on selected plant species, namely *Viola riviniana*, *V. lactea, Potentilla erecta*, *Teucrium scorodonia* and *Agrostis curtisii.* Visual estimates of percentage cover of *A. curtisii* and *Viola* spp. was recorded. Numbers of individual plants of *V. lactea, V. riviniana, P. erecta* and *T. scorodonia* were counted and recorded. The individual counting of plants is a more appropriate measure when comparing abundance of relatively low density species such as *Viola* spp. Turf height was recorded at four points within the sampling hoop using Stewart et al's (2001) direct method.

Williams (2001) recorded soil temperature and turf height at 70 ant nests of 5 different *Myrmica* species; *M. ruginodis, M. scabrinodis, M. sabuleti, M. scabrinodis* $_{\text{(HOT)}}$ and *M. lonae*. This data was analysed by me to ensure that any differences detected in my study were as result of differences between treatments (i.e. ant nest or non nest) rather than preferred niche differences of *Viola* spp.

3.4.3 Statistical Analysis

The data were analysed using General Linear Model ANOVA (GLM ANOVA) after count data were transformed using Log+1 and percentage cover data were arcsine transformed (Dytham, 2003). Pair-wise comparisons were performed using Tukey

95.0% Simultaneous Confidence Intervals tests to compare differences between ant species and non-nest spots.

There is a small yet significant difference between turf height and thus presumably temperature at ant nest and non-ant nest spots. This is possibly caused by sampling bias, which may have occurred when locating non-nest spots, despite the care taken to ensure that non-nests spots occurred within similar vegetation to nest spots. In order to eliminate this small potential bias, turf height was accounted for by using it as a covariate in all of the analyses.

3.5 Results

3.5.1 The relationship between turf height and ant nest temperature

Turf height is a significant predictor of ant nest temperature (figure 3.0; $F_{1, 70} = 64.79$, $P < 0.001$, $R^2 = 48.1\%$).

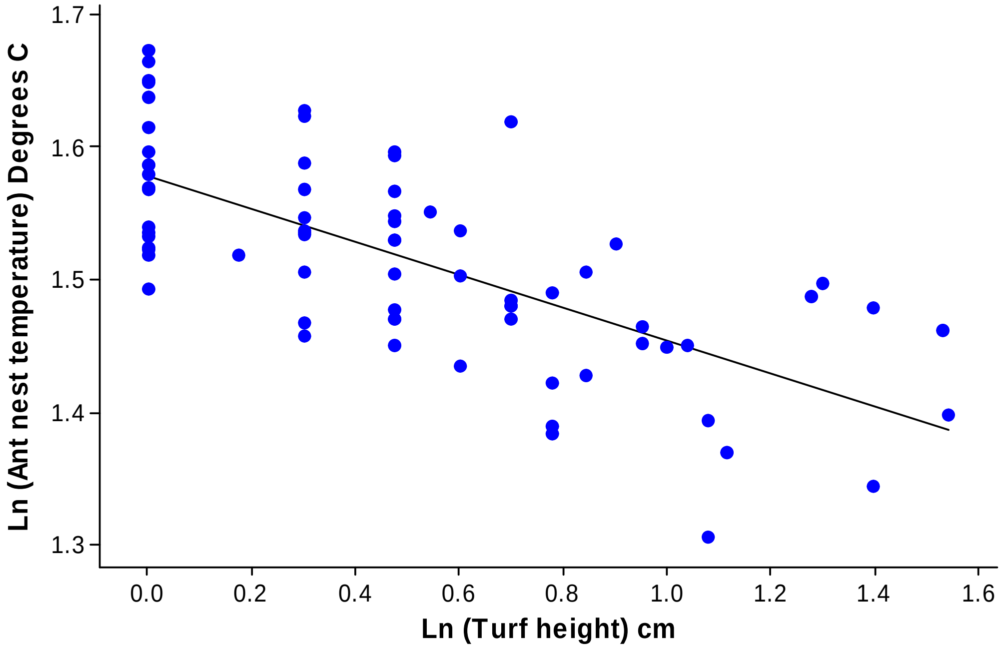


Figure 3.0: Correlation between Turf height (cm) and Ant nest temperature °C at 72 ant nest spots. ($F_{1, 70} = 64.79$, $P < 0.001$, $R^2 = 48.1\%$). Data is from Williams 2001.

2001 Data - Relationship between Turf height and Temperature

Figure 3.1 shows the relationship found between turf height and nest temperature and the distribution of the different ant species along the temperature gradient. There is a significant difference in turf height above different ant species nests (Kruskal-Wallis; H

$_4 = 54.48$, $P < 0.001$) and in temperature ($H_4 = 41.21$, $P < 0.001$) at different species' nests. *M. scabrinodis* $_{\text{(HOT)}}$ are found in the hotter shorter turfs, whereas *M. lonae* are found in the cooler longer turfs. All of the ant species studied were significantly different from each other with regard to turf height (table 3.0). Ant nest soil temperature differed between all ant species with the exception of *M. lonae* and *M. ruginodis*, and of *M. scabrinodis* $_{\text{(HOT)}}$ and *M. sabuleti* (table 3.1 and figure 3.1).

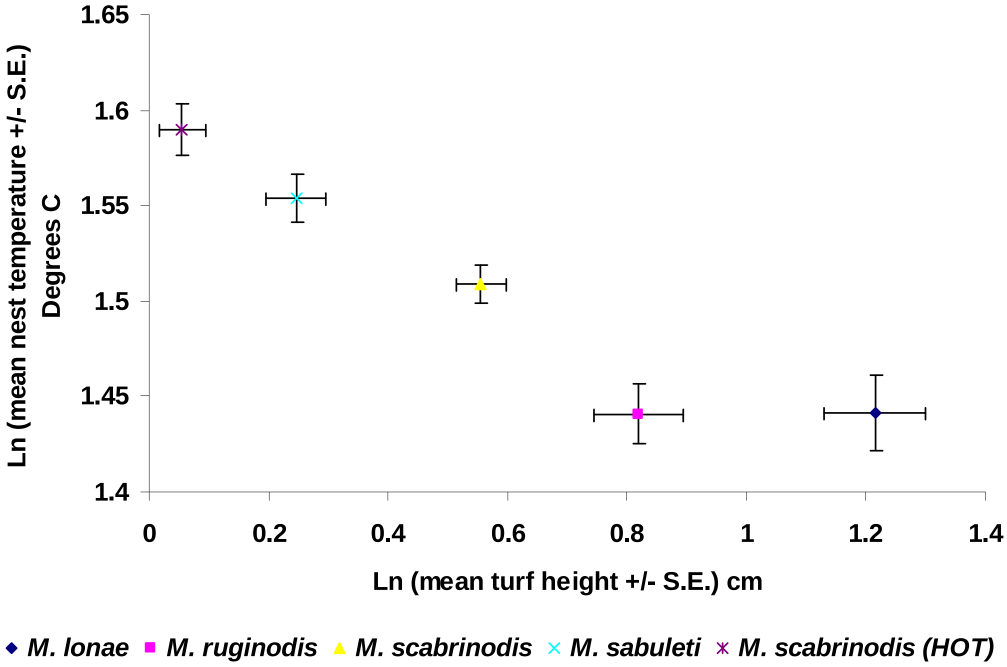


Figure 3.1: Correlation between Turf height (cm) and Ant nest temperature °C between 5 different ant species data. Data is from Williams 2001.

Table 3.0: Results of Mann-Whitney comparisons of turf height between 5 different ant species nest spots. Median, Interquartile range, test statistic (W value) and significance level (P value) are displayed. Mann-Whitney tests had 1 degree of freedom.

	Differences in Turf height between ant species nest spots				
Ant species	*M. lonae*	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)
Median	19	6	3	2	1
Interquartile range	9.5 - 29.5	3.75 – 11.25	3 - 5	1 – 2	1 – 1
M. ruginodis	W= 151.5 P = 0.0066				
M. scabrinodis	W= 179.5 P = 0.0001	W= 269.5 P = 0.0088			
M. sabuleti	W= 225.0 P < 0.0001	W= 372.0 P < 0.0001	W= 251 P = 0.0002		
M. scabrinodis (HOT)	W = 171.0 P < 0.0001	W= 299.5 P < 0.0001	W= 324.0 P < 0.001	W= 419.0 P = 0.0076	

Table 3.1: Results of Mann-Whitney comparisons of ant nest temperature between 5 different ant species nest spots. Median, Interquartile range, test statistic (W value) and significance level (P value) are displayed. Mann-Whitney tests had 1 degree of freedom

	Differences in Ant nest soil temperature between ant nest species				
Ant species	*M. lonae*	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* $_{(HOT)}$
Median	29	28.2	32.1	36.1	39.05
Interquartile range	25.9 – 30.4	24.25 – 30.23	30.2 – 34.5	32.33 – 39.1	34.6 – 43.7
M. ruginodis	W= 163, ns				
M. scabrinodis	W= 238, P = 0.0029	W= 296.5 P = 0.0019			
M. sabuleti	W= 379, P = 0.0002	W= 467, P < 0.001	W= 428.5 P =0.02		
M. scabrinodis $_{(HOT)}$	W= 231, P = 0.0001	W= 296, P < 0.0001	W= 143.5 P < 0.0004	W =299, ns	

2004 Data - Turf height as an indicator of different ant species niches

The 2004 result, confirmed that significant differences (GLM ANOVA; $F_{5,184} = 12.25$, $P < 0.001$) exist in mean turf height above different ant species' nest spots, and also compared non-ant nest spots.

Table 3.2 shows the differences in turf height above the nests of different ant species and also non-nest spots using Tukey's pair-wise comparisons. Nests of *M. ruginodis* and *F. fusca* were found in taller vegetation compared to *M. sabuleti*, *M. scabrinodis* and *M. scabrinodis* $_{(HOT)}$ nests. Turf height was again taller at *M. scabrinodis* compared to and *M. scabrinodis* $_{(HOT)}$ nests. These measurements confirm earlier measurements of sward / niche differences in these species (Thomas et al., 1998) and justify using turf height as a covariate in the GLM analysis.

Table 3.2: Results of Tukey's pairwise comparisons of differences in turf height between different ant species nests and non-ant nests. Mean ± S.E., test statistic (t) and significance level (P value) are displayed.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* $_{\text{(HOT)}}$	*F. fusca*	Non-Nest
Mean ± S.E.	22.61 ± 3.3	11.82 ± 1.17	8.79 ± 0.98	7.02 ± 1.39	8.26 ± 0.43	8.26 ± 0.43
M. scabrinodis	t = 3.422, P = 0.0098					
M. sabuleti	t = 5.700 P< 0.0001	t = 2.509, ns				
M. scabrinodis $_{\text{(HOT)}}$	t = 6.001, P < 0.0001	t = -3.331, P = 0.0132	t = -1.414, ns			
Formica fusca	t = -1.998, ns	t= 1.090, ns	t= 3.170, P = 0.0217	t = 3.854, P = 0.0022		
Non- nests	t = -6.326, P < 0.0001	t = -2.995, P= 0.0364	t = -0.133, ns	t = 1.461, ns	t = -3.538, P = 0.0067	

3.5.2 Spatial distribution of *Viola lactea* near to and away from ant nests

Percentage cover of *V. lactea above Myrmica* nests and non-nest spots.

For the combined *Myrmica* species, percentage cover of *V. lactea* was significantly greater (GLM ANOVA; $F_{1, 187}$ =9.82, P= 0.002) at ant nest spots (mean 1.44 ± 0.2) compared to non-nest spots (mean 0.7 ± 0.05).

Differences in numbers of *V. lactea* plants between ant species nests and non-nest spots.

When individual plant numbers rather than percentage cover are compared, *Viola lactea* is again significantly more abundant (GLM ANOVA; $F_{1, 187}$ = 25.73, P< 0.001) at ant nest spots (mean 16.22 ± 1.22) compared to non-ant nest spots (mean 12.4 ± 0.87).

Differences in percentage cover of *V. lactea* between different ant species' nest spots and non nest spots.

V. lactea was significantly more abundant above *M. scabrinodis* $_{\text{(HOT),}}$ *M. sabuleti, M. scabrinodis* and *F. fusca* nests compared to *M. ruginodis* nests (table 3.3). *V. lactea* was more abundant above *M. sabuleti* nests compared to non-nest spots.

Table 3.3: Differences in mean percentage cover (± S.E.) of *Viola lactea* plants between 5 different ant species nests and non-nest spots.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)	*F. fusca*	Non-Nest
Mean ± S.E.	0.32 ± 0.15	1.28 ± 0.28	1.93 ± 0.49	1.43 ± 0.28	1.58 ± 0.45	0.72 ± 0.06
M. scabrinodis	t= -4.057 P= 0.0010					
M. sabuleti	t= -4.800 P= 0.0001	t= -1.015 ns				
M. scabrinodis (HOT)	t= -3.916 P= 0.0018	t= 0.558 ns	t= - 0.265 ns			
Formica fusca	t= 4.205 P= 0.0006	t= 0.641 ns	t= -0.142 ns	t= 0.089 ns		
Non- Nest	t= 2.760 ns	t= -2.262 ns	t= -3.987 t= 0.0013	t= 2.531 ns	t= -2.429 ns	

Differences in numbers of *V. lactea* plants between ant species nests and non-nest spots

Again, a comparison between individual plants and ant species shows that *Viola lactea* was more abundant near to nests of *M. sabuleti*, *M. scabrinodis*, *M. scabrinodis* $_{\text{(HOT)}}$ and *F. fusca* compared to *M. ruginodis* nests (GLM ANOVA; $F_{5, 183} = 10.38$, $P < 0.001$) (table 3.4). Not only does this reflect the preference of *V. lactea* for warmer niches, but by using turf height as a covariate (see correlation between turf height and temperature in figure 3.0) in the analyses, this shows ‘true’ differences between ant species.

Table 3.4: Differences in mean (± S.E.) number of *Viola lactea* plants between five ant species nests and non-nest spots

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* $_{\text{(HOT)}}$	*F. fusca*	Non-Nest
Mean ± S.E.	4.00 ± 1.84	15.88 ± 1.99	18.00 ± 2.43	24.47 ± 2.33	15.62 ± 2.56	12.40 ± 0.87
M. scabrinodis	t= -5.724					
	P< 0.001					
M. sabuleti	t= -5.607	t= 0.165				
	P<0.001	ns				
M. scabrinodis $_{\text{(HOT)}}$	t= -6.198	t= 1.641	t= 1.910			
	P< 0.001	ns	ns			
Formica fusca	t= 5.245	t= 0.103	t= 0.238	t= -1.311		
	P< 0.001	ns	ns	ns		
Non- Nest	t= 4.752	t= -1.925	t= - 2.029	t= -3.538	t=-1.571	
	P= 0.0001	ns	ns	P= 0.0067	ns	

3.5.3 Spatial distribution of *Viola riviniana* near to and away from ant nests

Percentage cover of *V. riviniana* above ant nests and non-nest spots.

Again all ant nest data were pooled and compared with pooled non nest data. The percentage cover of *V. riviniana* was significantly (GLM ANOVA; $F_{1, 187}$ =10.76, P= 0.001) greater at ant nest spots (mean 1.65 ± 0.29) compared to non-nest spots (mean 0.51 ± 0.51).

Differences in numbers of *V. riviniana* plants between ant nests and non-nest spots

In contrast to their percentage cover, there was no significant difference ($F_{1, 187}$ =2.80, P = 0.096) between the number of *V. riviniana* plants above pooled ant nest spots (mean 15.28 ± 1.51 S.E.) compared to pooled non-ant nest spots (mean 8.10 ± 0.76 S.E.).

Differences in percentage cover of *V. riviniana* between different ant species' nest spots and non-nest spots.

When considering individual ant species, percentage cover of *V. riviniana* was significantly more abundant in 2004 at *M. ruginodis* and *M. scabrinodis* nests compared to *M. scabrinodis* $_{(HOT)}$ and non-ant nest spots (GLM ANOVA; $F_{5, 183}$ = 5.08, P <0.001) (table 3.5). This suggests a niche preference by *V. riviniana* for cooler niches on the study site and also that, when cooler niches coincide with *Myrmica* nest spots, *V. riviniana* is found in its greatest abundance within 25 cm of a nest.

Differences in numbers of *V. riviniana* plants between ant species' nests and non-nest spots.

There was a significant (GLM ANOVA; $F_{5, 183}$ = 5.02, P< 0.001) difference in the number of *V. riviniana* plants between ant species and non-ant nest spots. Again, a comparison of individual plants showed a similar pattern to their percentage cover, with *Viola riviniana* being more abundant on *M. ruginodis*, *M. scabrinodis*, *M. sabuleti* and *F. fusca* nests compared to those of *M. scabrinodis* $_{(HOT)}$ (table 3.6).

Table 3.5: Differences in percentage cover of *V. riviniana* between 5 different ant species nests and non nest spots.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)	*F. fusca*	Non-Nest
Mean ± S.E.	3.75 ± 1.49	1.70 ± 0.50	1.28 ± 0.37	0.37 ± 0.08	1.77 ± 0.56	0.51 ± 0.05
M. scabrinodis	t= 1.385 ns					
M. sabuleti	t= 2.264 ns	t= 1.204 ns				
M. scabrinodis (HOT)	t= 3.4970 P= 0.0077	t= -2.930 P= 0.0437	t= -2.131 ns			
Formica fusca	t= -1.219 ns	t= 0.034 ns	t= 0.995 ns	t= 2.5325 ns		
Non- Nest	t= -3.708 P= 0.0037	t= -3.305 P= 0.0143	t= -2.259 ns	t= 0.7451 ns	t= -2.538 ns	

Table 3.6: Differences in numbers of *V. riviniana* plants between five ant species nests and non nest spots.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)	*F. fusca*	Non-Nest
Mean ± S.E.	26.93 ± 5.85	19.80 ± 3.15	12.03 ± 1.93	3.47 ± 1.51	16.69 ± 3.20	8.13 ± 0.76
M. scabrinodis	t = 0.487 ns					
M. sabuleti	t = 1.868 ns	t = 1.893 ns				
M. scabrinodis (HOT)	t = 3.769 P = 0.0030	t = -4.251 P = 0.0005	t = -2.967 P= 0.0393			
Formica fusca	t = -0.427 ns	t = 0.013 ns	t = 1.523 ns	t = 3.642 P = 0.0047		
Non- Nest	t = -2.504 ns	t = -2.932 P = 0.0434	t = -0.909 ns	t = 2.62 ns	t = -2.232 ns	

3.5.4 *Viola* and *Myrmica* niche overlap

Combining the 2004 data for *V. lactea* and *V. riviniana* numbers, figure 3.2 illustrates the preferred niches of the two *Viola* spp. in this study along side turf height, the latter being an indicator of soil temperature. It is clear that *V. riviniana* prefers a cooler niche which coincides with the niche of the cooler loving ant species, *M. ruginodis* and *M. scabrinodis.* In contrast, *V. lactea* prefers a hotter niche which coincides with the niche of the hotter loving ant species such as *M. scabrinodis* $_{\text{(HOT)}}$. Both *Viola* spp. co-exist in abundance in a niche shared by *M. sabuleti*.

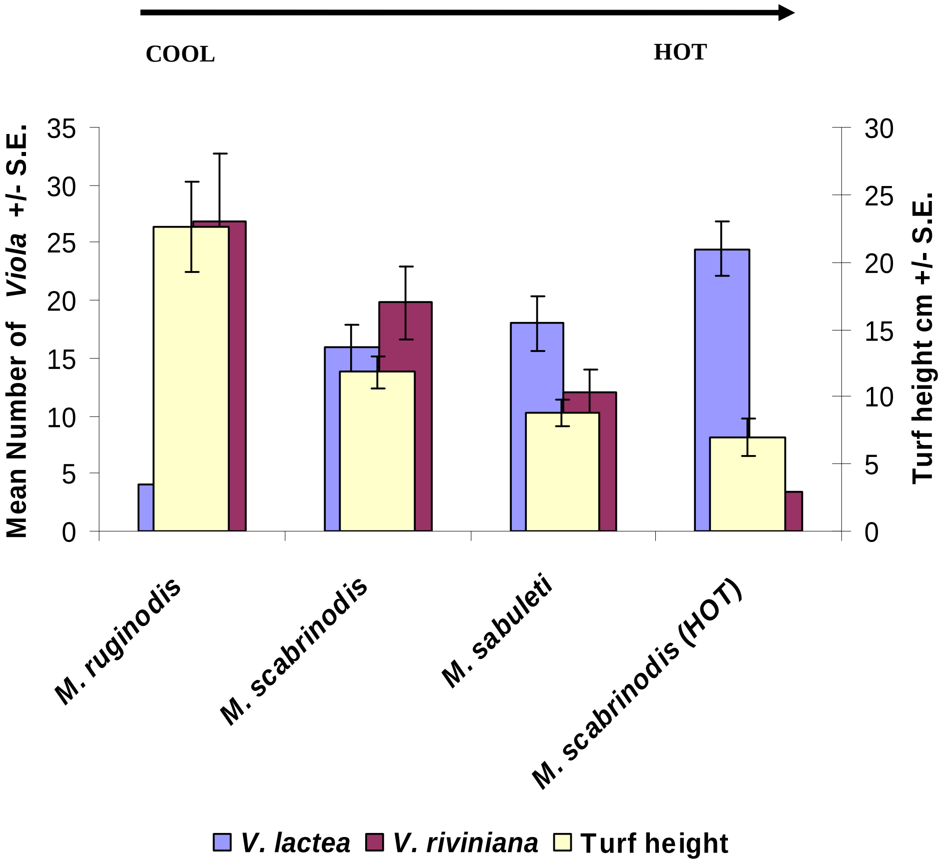


Figure 3.2: *Viola* abundance across a temperature gradient. Mean number +/- S.E. of *Viola* spp. and mean turf height +/- S.E. across known *Myrmica* niche temperature gradient. As turf height decreases, abundance of *V. lactea* (a hot loving species) increases. In contrast as turf height increases abundance of *V. riviniana* (a cool loving species) increases.

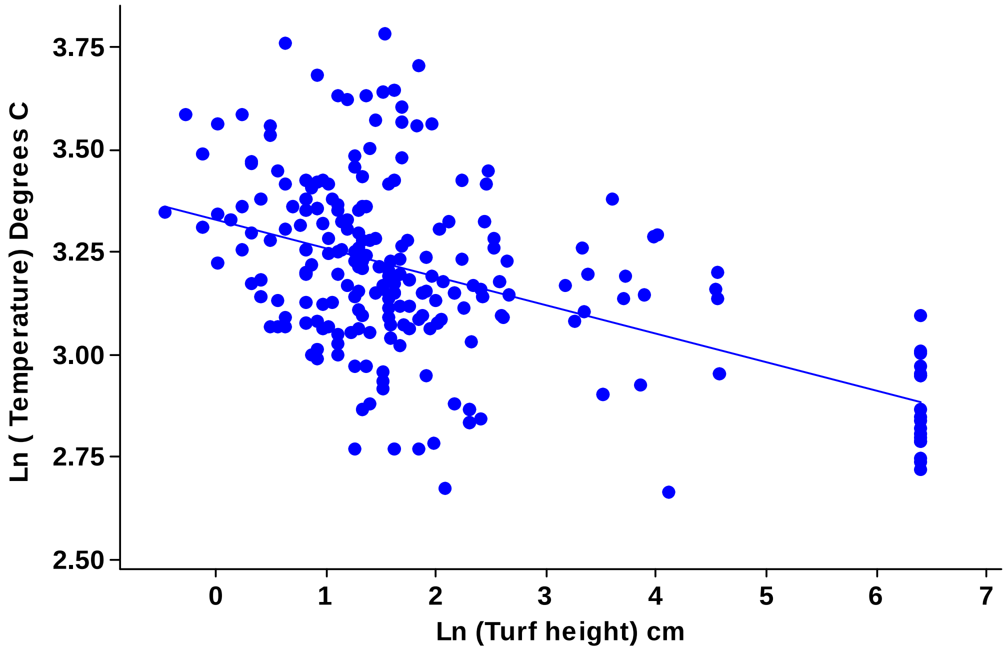


Figure 3.3: Correlation between Turf height (cm) and soil temperature °C at 221 *Viola* plants. ($F_{1, 220} = 76.09$, $P < 0.001$, $R^2 = 25.7\%$).

Figure 3.3 shows the relationship found between turf height and soil temperature at *Viola* plants. Turf height is significantly taller where *V. riviniana* occur compared to where *V. lactea* occur (Kruskal-Wallis; $H_1 = 16.87$, $P < 0.001$) (figure 3.4). Intuitively soil temperature is cooler where *V. riviniana* occurs compared to where *V. lactea* is found (Kruskal-Wallis; $H_1 = 34.55$, $P < 0.001$).

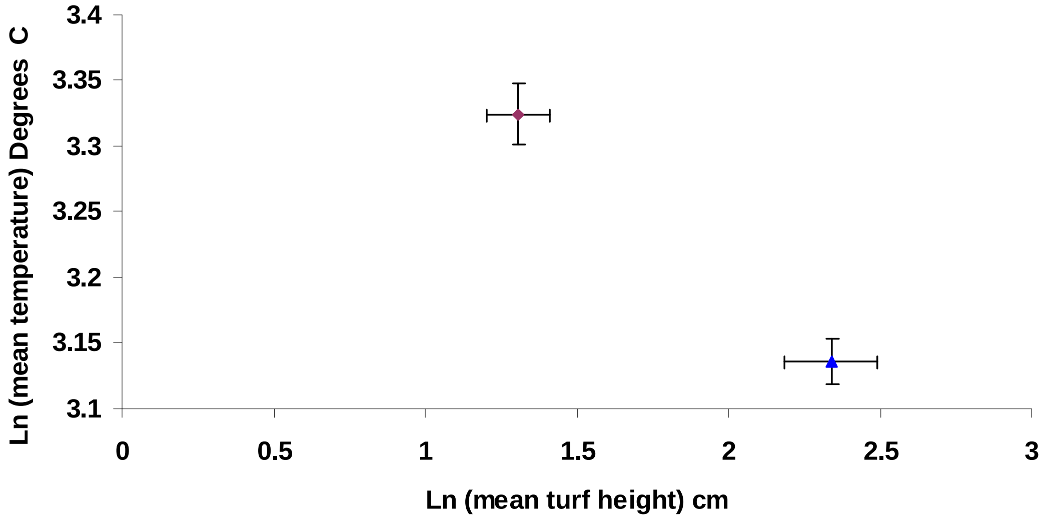


Figure 3.4: Correlation between mean turf height (cm) and mean soil temperature °C measured at *Viola lactea* ◆ and *V. riviniana* ▲ plants.

3.5.5. Abundance of *Agrostis curtisii* near and away from ant nests

Percentage cover of *Agrostis curtisii* near to and away from ant nests

The overall percentage cover of *A. curtisii* was found to be significantly greater (GLM ANOVA; $F_{1, 186} = 20.20$, $P < 0.001$) at non-nest spots (mean 59.31 ± 3.16 S.E.) compared to ant nest spots (mean 41.72 ± 2.21 S.E.). At a species level, *A. curtisii* cover was significantly greater at all non-nest spots compared to *M. ruginodis*, *M. sabuleti* and *M. scabrinodis* nest spots (table 3.7); it was also significantly more abundant at *F. fusca* nests compared to *M. ruginodis* nests.

Table 3.7: Differences between percentage cover of *A. curtisii* between 5 ant species nest spots and non-ant nest spots.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)	*F. fusca*	Non-Nest
Mean ± S.E.	21.36 ± 5.54	43.12 ± 4.77	42.86 ± 3.06	44.67 ± 4.74	55.42 ± 6.98	59.31 ± 3.16
M. scabrinodis	t = -2.866 P = 0.052					
M. sabuleti	t = -2.962 P = 0.0399	t = - 0.132 ns				
M. scabrinodis (HOT)	t = -2.806 ns	t = 0.466 ns	t = 0.368 ns			
Formica fusca	t = 3.865 P = 0.0019	t = 1.319 ns	t = 1.269 P = 0.8017	t = 0.798 ns		
Non-nest	t = 5.108 P< 0.001	t = -3.064 P = 0.0124	t = -3.402 P = 0.0041	t = 2.004 ns	t = 0.7794 ns	

3.5.6 Other plant species distributions in relation to ant nest and non-nest spots

Abundance of *Potentilla erecta* near to ant nests and non-ant nest spots.

Data were not normally distributed after transformation so were analysed using non-parametric Kruskal-Wallis test. There was no significant difference ($H_5 = 8.49$, $P = 0.131$) in the abundance of *Potentilla erecta* near to ant nests compared to non-ant nest spots.

Abundance of *Teucrium scorodonia*

The data were not normally distributed after transformation, so were analysed using a non-parametric Kruskal-Wallis test. There was a significantly greater abundance of *T. scorodonia* near ant nests compared to non-nest spots ($H_5 = 11.98$, $P = 0.035$); and a there appears to be a general trend for *T. scorodonia* to be more abundant near to *Myrmica* ant nests that inhabit cooler niches (table 3.8).

Table 3.8: Results of Mann-Whitney comparisons of percentage cover of *T. scorodonia* between 5 different ant species nest spots and non-nest spots in 2004. Median, Interquartile range, test statistic (W value) and significance level (P value) are displayed. Mann-Whitney tests had 1 degree of freedom.

Ant species	*M. ruginodis*	*M. scabrinodis*	*M. sabuleti*	*M. scabrinodis* (HOT)	*F. fusca*	Non-Nest
Median	1	1	0	0	2	0
Interquartile range	0 – 14	0 - 5	0 – 1.75	0 - 0	0 – 6.5	0 - 2
M. scabrinodis	W = 487.5 ns					
M. sabuleti	W = 863.0 ns	W = 1035.5 ns				
M. scabrinodis (HOT)	W = 181.5 P = 0.022	W = 592.5 P = 0.01	W = 1011.0 ns			
F. fusca	W = 198.5 ns	W = 476.0 ns	W = 834.0 ns	W = 166.5 P = 0.007		
Non-nest	W = 856.5 ns	W = 1616.5 ns	W = 2270.5 ns	W = 618.0 ns	W = 814.5 ns	

3.6 Discussion

Assessment of *V. lactea* and *V. riviniana* distribution showed that both species were more abundant or showed a higher percentage cover near to ant nests compared to equivalent non-nest spots.

V. lactea abundance was greater above the nests of ant species that inhabited the hotter niches in grassland compared to the ants living in the cooler niches. In contrast, *V. riviniana* abundance was greater above ant nests that coincided with the coolest available niches. These results were consistent when using either percentage cover or counts of individuals as the recording method.

The findings of my study are consistent with those of Lack & Kay (1987), Oostermeijer (1989) and Kovar (2001) who found that the spatial distribution of some myrmecochorous plants was influenced and determined by dispersal by ants, the nest areas having higher numbers of plants than surrounding soils.

<u>*Viola* niches</u>

Figure 3.2 shows the distribution of *Viola* spp. across a measured temperature gradient. It is clear that although both species co-exist across the temperature gradient, *V. riviniana* is more abundant at the cooler end of the spectrum and *V. lactea* is more abundant at the hotter region. Soil temperature is affected by turf height. The shorter the turf the more insolation reaches the soil and so soil temperature is increased, and vice-versa (Thomas, 1993). Therefore *V. lactea* is most likely to be more abundant in shorter swards and *V. riviniana* more abundant in taller swards. The results from this study show this clearly (figure 3.4).

<u>Ant and *Viola* niches</u>

Viola spp. exhibit clear niche preferences, as do the ants (figure 3.2). The study site has a natural gradient up the slope ranging from cool soils at the bottom to warm soils at the top, a gradient that is amplified by grazing and local variation in turf height. Turf height is well known to be a measure of microclimate (Thomas et al., 1998); and the results from turf height measurements taken during this study both confirm this and reflect the preferred *Myrmica* spp. niches based on a temperature gradient (figure 3.0). *V. riviniana* occurs at higher densities in the cooler available niches and is thus most abundant in the niche ranges of the cooler loving ant species *M. ruginodis* and *M. scabrinodis*. In contrast *Viola lactea* is more abundant in a warmer niche, which coincides with the *M. sabuleti* and *M. scabrinodis* $_{\text{(HOT)}}$ ant niche. Numbers of *V. lactea* plants were significantly more abundant above *F. fusca* nests compared to *M. ruginodis* nests; this suggests that *F. fusca* inhabits a warmer niche than *M ruginodis*. Additionally, *V. riviniana* plants were five times more abundant above nests of *F. fusca* compared to *M. scabrinodis* $_{\text{(HOT)}}$. This suggests that *F. fusca* inhabits a cooler niche than that of *M. scabrinodis* $_{\text{(HOT)}}$ and that *F. fusca* inhabits a niche where both *Viola* species co-exist. Obviously, other attributes of a niche than just temperature vary systematically along the slope, for example soil depth and moisture. However, temperature was found to be a useful correlate of niche space in this habitat (Thomas et al., 1998).

Ant effects on *Viola* distributions

At the extremes of the ant and *Viola* niches (based upon the temperature gradient) it is clear that sub optimal conditions for the different *Viola* spp. has a negative impact upon *Viola* densities despite the presence of an ant nest. However, when the optimum *Viola* niche coincides with ant nests, there are increased densities in *Viola* spp. compared to optimum niches without ant nests. For example, *V. lactea* is a hot loving species as is *M. scabrinodis* $_{\text{(HOT).}}$ *V. lactea* was 1.5 (log scale) times more abundant at *M. scabrinodis* $_{\text{(HOT)}}$ nests compared to pooled non-ant nest spots, but significantly less abundant at *M. ruginodis* (a cooler loving species) nests compared to pooled non-ant nest spots. In contrast *V. riviniana* (a cooler loving species) was 1.4 (log scale) times more abundant near to *M. scabrinodis* nests compared to non-ant nest spots. Using turf height as a covariate in the analyses has ensured that these are ‘true’ differences between ant species and *Viola* distributions, not just a reflection of niche differences. The non significant difference between *M. ruginodis* nests and non-nest spots is likely to be due to the small sample size which has high variance within the data.

Ant / *Viola* interaction

The results of these studies suggest that the presence of ant nests in optimum *Viola* niches is beneficial to *Viola* spp. distribution; there is an indication of some positive ant / plant interaction, which is examined in Chapter 4. *Viola* seeds have an elaiosome that is attractive to ants and a large body of evidence suggests that elaiosomes facilitate seed dispersal by ants (Giladi, 2006). Ants take the seeds to their nests, eat the elaiosomes, and discard the seed in or around the ant nest. Several possible reasons have been given for why plants that bear elaiosomes may benefit from their interactions with ants. (i) Transportation to a nutrient rich micro-site near to an ant nest. *Viola* spp. may be more abundant near to ant nests within their preferred niche than equivalent non-ant nest spots due to elevated soil nutrient levels. Previous studies of *Myrmica* have found that the accumulation of nutrients and of organic matter at ant nests is greater than at non-nest spots, (Petal, 1978), and that soil structure is altered resulting in improved aeration and drainage (Petal, 1978; Elmes, 1991a). The increase in organic matter and hence water retention capacity of the soil creates conditions that are ideal for plant root penetration, (Petal, 1978; Elmes, 1991a). This hypothesis is tested in Chapter 5. (ii) In addition to the relocation of the seed to a suitable micro-site, *Viola* seeds and seedlings may also gain some protection from the ants from above ground seed predators due to the removal of seeds to an underground refuge. (iii) The creation of enemy free space near to nest spots deterring invertebrate herbivores, and attacks by ants may protect *Viola* seedlings.

Myrmica spp. seed preference is investigated in Chapter 4, in order to explain the observed association between *V. riviniana* and cool-loving *Myrmica* spp., and that of *V. lactea* with thermophilous ant species. Are the ants exhibiting a seed preference based upon local adaptation and or experience, or do these species co-occur due to a niche overlap?

Agrostis curtisii

Agrostis curtisii does not posses an elaiosome and is less abundant near to *M. ruginodis*, *M. scabrinodis* and *M. sabuleti* nest spots compared to equivalent non-nest spots. In addition, *M. ruginodis* nests had significantly less *A. curtisii* compared to *F. fusca*, *M. sabuleti* and *M. scabrinodis* nests. This may be attributed to the soil conditions near to

M. ruginodis ant nests. *M. ruginodis* inhabits cooler areas that have a deeper topsoil, whereas *Agrostis curtisii* is more abundant on shallow podsols (Ivimey-Cook, 1959). Habitat management on the site is another factor. *Agrostis curtisii* rapidly colonises exposed ground after burning, (Ivimey-Cook, 1959). *M. ruginodis* does not occupy recently burnt patches due to biotic factors such as turf height and soil temperature. In addition the increased percentage cover of *V. riviniana* plants near to *M. ruginodis* nest spots may enable these species to out-compete *A. curtisii* in these areas, or ant nest spots may not be suitable for *A. curtisii* growth due to various biotic factors.

Other plant species

Despite there being no significant difference in *T. scorodonia* abundance near to and away from ant nests, there is a general trend for *T. scorodonia* to be more abundant near to *Myrmica* ants that inhabit cooler niches. *T. scorodonia* is intolerant to heavy grazing and does not persist in short turf (Hutchinson, 1968). This is reflected by its weak association with *Myrmica* spp. which occupy niches that have taller turf height, for example *M. ruginodis* and *M. scabrinodis*. *T. scorodonia* seeds possess an elaiosome which may or may not be attractive to ants. The seeds of *T. scorodonia* are shed in September which is at a time when ants are preparing for diapause and brood production has stopped and feeding is greatly reduced. This may explain why there is no significant difference in the abundance of *T. scorodonia* near to and away from ant nests – the seeds are not collected because they are available as food source at a time when the ants do not require them. It may also be that the species or genera of ants present on the study sites did not find *T. scorodonia* elaiosomes attractive, for in Chapter 4 I show that there are marked difference in the attractiveness of *Viola* seeds to ants of different genera.

Potentilla erecta was more abundant at *M. ruginodis* nests compared to all the other ant nests, and less abundant at *M. sabuleti* and *M. scabrinodis* nests compared to non-nest spots. This may be due to the seeds of *P. erecta* not being attractive to *M. sabuleti* or *M. scabrinodis* as a food source because of their chemical composition or size, or due to niche or other biotic differences.

Much work has been carried out investigating ant and seed preference. Generally, larger seeds with larger elaiosomes are preferred by ants (Oostermeijer, 1989; Mark & Olesen, 1996; Gunther & Lanza, 1989; Brew et al., 1989; Sheridan et al., 1996). However, Hughes & Westoby (1992a) suggest that carrying a large seed may expose foragers to increased risk of mortality as they are more conspicuous to predators. Therefore, the yield provided by the elaiosome has to outweigh the risk of foraging mortality. The larger the elaiosome, the more likely an ant is to retrieve the seed. I suggest that the size of the *Potentilla erecta*, *Pedicularis sylvatica* and *Polygala serpyllifolia* elaiosomes should be investigated as the size of these elaiosomes may not outweigh the risk of foraging mortality.

Another explanation to ant seed preference could be attributed to the central foraging theory. The overall assumption of central foraging theory is that the greater the energy expenditure to get to a foraging area, the more selective the ant is in choosing a food item to take back to the nest (Holldobler & Wilson, 1990). Seed size and elaiosome size are closely correlated (Edwards et al., 2006). From my own observations of *V. lactea* and *V. riviniana* seed and elaiosome size I suggest that elaiosomes on *Viola* seeds would be a more substantial resource when compared to *P. erecta*, *P. sylvatica* and *P. serpyllifolia.* Nevertheless it was surprising that more species with elaiosomes did not show the strong association with *Myrmica* or *F. fusca* that the *Viola* did. However, several previous studies investigating the spatial relationship between myrmecochorous plant distribution and ant nests have found that different ants exhibit specific seed preferences (Oostermeijer, 1989; Lack & Kay, 1987). Chapter 4 investigates the attractiveness of several seed types to *Myrmica* spp. and *Formica fusca* ants in the laboratory in order to explain the differences in plant assemblages near to and away from ant nests found in this study.

Chapter 4: Do *Myrmica* species and *Formica fusca* ants differentiate between myrmecochorous seeds found on grasslands managed for *Maculinea arion*?

4.1 Introduction

Myrmecochory is a mutualistic relationship between plants and ants: the former produce seeds which possess nutrient-rich appendages termed elaiosomes, while the ants provide a mechanism for seed dispersal (Gammans et al., 2005). It is considered that the lipid fraction of the elaiosome is the most attractive constituent, promoting seed collection by ants (Marshall et al., 1979; Skidmore and Heithaus, 1988; Brew et al.,1989; Gammans et al., 2006). Approximately 2000 - 3000 plant species are classified as myrmecochores (Brew et al., 1989; Hughes & Westoby, 1992b; Ohkawara et al., 1996). In general, seed and elaiosome size are strongly positively correlated (Edwards et al., 2006) and seeds with larger elaiosomes are more attractive to ants (Oostermeijer, 1989; Gunther & Lanza, 1989; Westoby et al., 1991) because of the nutrient content of a larger food reward (Brew et al., 1989). Additionally the removal rate of seeds increases with seed size and there is a weak correlation in removal rate and elaiosome size (Gorb & Gorb 1995). Similar results were found by Mark & Olesen (1996); seeds with larger elaiosomes were collected first. Even within a plant genus differences in seed attractiveness to ants is apparent; seed weight, elaiosome weight are seed removal rate was greater in *Viola selkirkii* compared to *Viola verecunda* (Ohkawara & Higashi, 1994). Other studies have found that the elaiosome/diaspore ratio is the factor which influences seed removal rate (Hughes & Westoby, 1992b; Gunther & Lanza 1989; Servigne & Detrain 2008).

The benefits of this mutualism to plants have been widely researched and are summarised in Chapter 1. In contrast, research into the benefits of this relationship to ants has been limited to date. Field studies by Morales & Heithaus (1998) showed that 65% of *Aphenogaster rudis* colonies that were fed seeds of *Sangunaria canadesis* produced more gynes (reproductive females), thus improving colony fitness. More recently, laboratory based studies by Gammans et al. (2005) found that feeding *Ulex europaeus* elaiosomes to artificial colonies of *Myrmica ruginodis* resulted in a 102% increase in brood production and an average 48% increase in larval weight when compared to control colonies. Berg (1975) found that myrmecochory is a generalised relationship between ants and seeds and that seeds are removed by a suite of ants that show no species specificity. Other studies have had similar findings: Beattie et al., (1979) found no differences between ant species and diaspore preference; Andersen (1988a) reports that seed dispersal distance and removal rate tend to be characteristic of the local site and therefore studies of single seed species can be used to characterise the general ant–seed relationship at any site. However, when investigating the fate of *Viola* seeds dispersed by ants, Culver & Beattie (1980) found that seedling emergence varied depending upon which ant genus removed the seeds (63% of seeds taken by *Formica* species emerged in contrast to only 28% taken by *Myrmica* species and 5% taken by *Lasius* species). Sernander's (1906 as cited by Lehouck et al. (2004)) laboratory experiments showing ants to be actively transporting seeds led to the statement that a spatial relationship between ant nests and myrmecochores is expected in the field. Such patterns have been observed in a number of field studies; Oostermeijer, (1998); Lack & Kay, (1987) and Kovar et al., (2001) found that myrmecochores were non-randomly distributed and growing near to ant nests. However, more recent field studies by Lehouck et al. (2004) showed no spatial relationships. While conflicting results have been found, what is clear is that myrmecochory has the potential to alter the assemblage of plant species near or around ant nests in some habitats.

Results from Chapter 3 show that the vegetation above ant nests differs from that of non-ant nest spots on two grasslands that were restored to support *Maculinea arion* in south-west England. Not all plant species whose seeds possess elaiosomes were more abundant at nest spots compared to non-nest spots, but both *Viola lactea* and *Viola riviniana* were significantly associated with ant nest, and each *Viola* species showed an increased association with the ant species that occupied its preferred niche. This Chapter investigates the attractiveness of different seed types to *Myrmica* species and *Formica fusca* in the laboratory to ascertain if myrmecochory is the mechanism responsible for the high densities of *Viola* species near to ant nests recorded in the field.

4.2 Aim

Williams (2001) found that *Viola lactea* and *Viola riviniana* were significantly more abundant near to *Myrmica* ant nests compared to paired non-nest spots. In 2003, laboratory experiments were carried out to investigate the relationship between the seeds of *Viola lactea*, *Viola riviniana*, *Agrostis curtisii*, *Potentilla erecta*, *Ajuga reptans* and the ants *Myrmica scabrinodis* $_{\text{(HOT),}}$ *Myrmica sabuleti*, *M. ruginodis* and *F. fusca*, in an attempt to explain the patterns found by Williams (2001) and described in Chapter 3. *F. fusca* was included in this study as it is the only other common ant species apart from the genus *Myrmica* that is found on the study site that also has a population structure of numerous small colonies. The only other abundant ant, *F. rufa*, occurs in a few extremely large colonies.

Having established in the first year that the seeds of both species of *Viola* were highly attractive to *Myrmica* but not to *Formica* ants in the lab, further experiments were designed in 2004 and 2005 to investigate whether the *Myrmica* species that co-exist most with each *Viola* species in the field found them more or less attractive than their congeners.

4.3 Methods

4.3.1 *M. sabuleti, M. ruginodis* and *F. fusca* seed preference - *Viola* spp. vs. *A. curtisii* (2003)

In July 2003 twelve ant colonies, comprising four each of *M. sabuleti*, *M. ruginodis* and *F. fusca,* were collected from site X. Four laboratory nests of each species were set up in clear polystyrene boxes (12cm x 8cm x 2cm) with 100 workers. The boxes had fluon (a non-stick chemical) wiped around the rims to prevent the ants from escaping. In addition to workers, *Myrmica* colonies were given brood (20 pupae, 30 larvae and 40 pre-pupae) to encourage foraging. *F. fusca* colonies had no brood since their nests do not contain it at this time of year (Elmes, pers comm). *Myrmica* colonies were fed 16mg *Drosophila* larvae and 7mg sugar per week. *F. fusca* colonies were fed 20mg *Drosophila* larvae and 10mg sugar per week to maintain the same ratio of food per biomass of these ants, whose workers are 40% larger than *Myrmica*. High mortality was recorded in *F. fusca* colonies in previous trials where they received the same quantity of food as *Myrmica* spp. (Randle unpublished). Food quantities are taken from Brian & Abbot (1977) and Wardlaw et al (2000).

Each artificial nest contained a damp sponge covered by a notched inverted plastic pot as a central nest area. This was connected to another polystyrene box (the foraging area) with a 1m length of plastic tubing (figure 4.1), representing the average foraging distance travelled by *Myrmica* in the field (Elmes, 1975). All colonies were maintained at a constant ambient temperature of 20°C to avoid confounding variables and given a 3 week acclimatisation period before observation began.

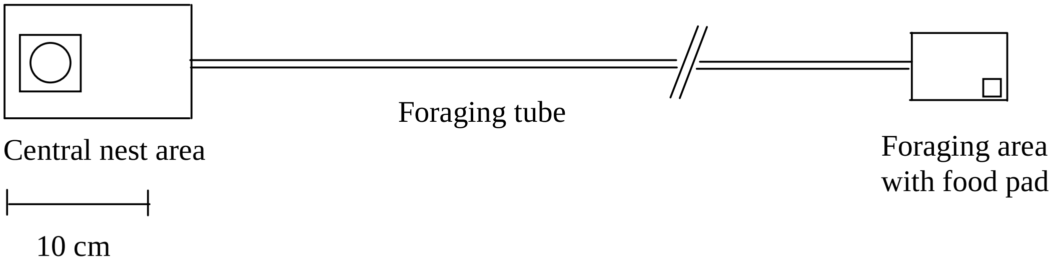


Figure 4.1: Experimental nest set up, central nest and foraging areas to scale Foraging tube was 1m long.

Twenty seeds, 10 *V. lactea* and 10 *A. curtisii* (trial 1) or 10 *V. riviniana* and 10 *A. curtisii* (trial 2), were placed in each foraging area of the four replicates of each ant species. Observational bioassays were undertaken for 1 hour to record ant behaviour and seed interactions. Each trial was repeated twice with an interval of 1 week between trials. Data were collected on the number of antennations, biting and carrying behaviours of ants towards the seeds of different plant species. Different behaviours are defined below:

Antennation - Worker ant examines and touches the seed with its antennae.
Biting **-** The ant uses its mandibles to investigate the seed and bites or chews at the elaiosome.
Carrying **-** The ant picks up the seed using its mandibles and moves it.
Retrieval of seeds from the foraging area and ultimate destination of seed after retrieval were also recorded during the one hour behavioural bioassay.

4.3.2 *Myrmica scabrinodis* $_{\text{(HOT)}}$ seed preference (2004)

Behavioural bioassays were used to determine if *M. scabrinodis* $_{\text{(HOT)}}$ has a preference for *V. lactea* seeds which may explain the high densities of *V. lactea* recorded above their nests (Chapter 3). Ten colonies of *M. scabrinodis* $_{\text{(HOT)}}$ were collected from site Y in July 2004 along with ripe seeds of *V. lactea, V. riviniana, P. erecta* and *A. reptans.* The number of workers, number of brood, food quantity provided per colony and experimental nest design were the same as in 2003. Twenty seeds, 5 *V. lactea*, 5 *V. riviniana*, 5 *P. erecta* and 5 *A. reptans,* were placed in each foraging area of the experimental nest. *P. erecta* seeds (which possess an elaiosome) were introduced into the experiment to determine if these seeds were less attractive to the ant species studied. If so, this may explain the lack of association between *P. erecta* and ant nest vegetation found in Chapter 3. *A. reptans* was used in the experiment since it is another plant species found on the study site which possesses an elaiosome. Observational bioassays followed the same protocol as those of 2003. Twenty seeds of each type were weighed to examine whether mass may explain seed preference, since Edwards et al (2006) found that seed size and elaiosome size are closely correlated. This would provide an indication into seed preference based upon seed weight.

4.3.3 *Myrmica ruginodis* and *Myrmica sabuleti* seed preference (2005)

In Chapter 3 I showed that *M. ruginodis* nests had high densities of *V. riviniana* above them. Here I test the hypothesis that *M. ruginodis* has a preference for *V. riviniana* seeds which could explain their observed association. *M. sabuleti* inhabits the niche where *V. lactea* and *V. riviniana* overlap. *M. sabuleti* was also used in this experiment because its realised niche is between *M. ruginodis,* which has a cooler niche, and *M. scabrinodis* $_{\text{(HOT)}}$, which has a warmer niche. In July 2005 ripe seeds of *V. lactea, V. riviniana, P. erecta* and *A. reptans* and ten colonies of *M. ruginodis* and ten colonies of *M. sabuleti* were collected from site Y. Again the number of workers, number of brood and food quantity provided to each colony and experimental nest design was repeated as in previous years. The seeds used and the observational bioassays in this experiment were consistent with the previous experiments. However, seed retrieval data was not recorded during this bioassay.

4.3.4 Statistical Analysis

All behavioural data were transformed (Log +1) to achieve normality. Percentage of seeds retrieved from the brood chamber was transformed using a standard arcsine square root transformation to achieve normality. Comparisons between different ant species behaviour towards the different seed species were made using GLM Analysis of Variance. Subsequent Tukey's Pair wise Comparisons were employed when significant results were found.

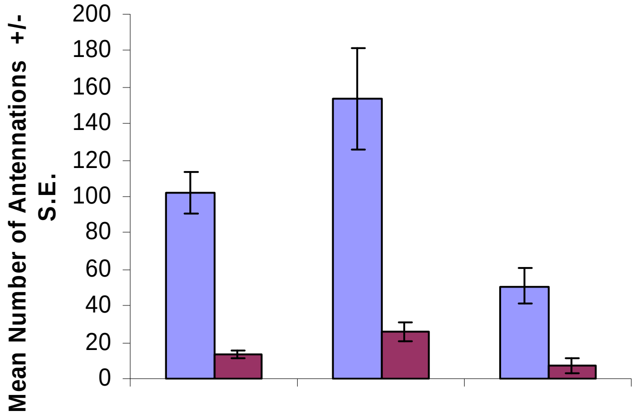
4.4 Results

4.4.1. *Myrmica sabuleti, Myrmica ruginodis* and *Formica fusca* seed preference (2003)

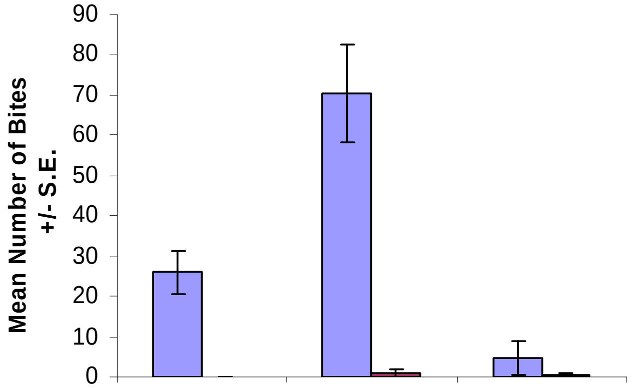
Seed preference: *Viola lactea* versus *Agrostis curtisii*

When presented with a choice between *V. lactea* and *A. curtisii* seeds, there are significant differences in ant behaviour towards the two plant species (figure 4.2). All 3 ant species antennated *V. lactea* seeds significantly more than *A. curtisii* seeds (GLM ANOVA; *M. sabuleti,* $F_{1,6} = 450$, $P<0.001$; *M. ruginodis* $F_{1,6} = 17$, $P=0.025$; *F. fusca,* $F_{1,6} = 72$, $P= 0.003$). In addition both *Myrmica* spp. bit and carried *V. lactea* seeds significantly more than *A. curtisii* seeds (*M. sabuleti,* $F_{1,6} = 170$, $P=0.001$; *M. ruginodis,* $F_{1,6} = 104$, $P= 0.002$). None of the ant species studied carried *A. curtisii* at all. *M. sabuleti* ($F_{1,6} = 371$, $P<0.001$; *M. ruginodis* $F_{1,6} = 2029$, $P<0.001$) carried *V. lactea* seeds significantly more than *A. curtisii* seeds. These differences are most likely attributed to the nutritional benefit of the elaiosomes on the *Viola* seeds.

Ant Antennate



Ant Bite



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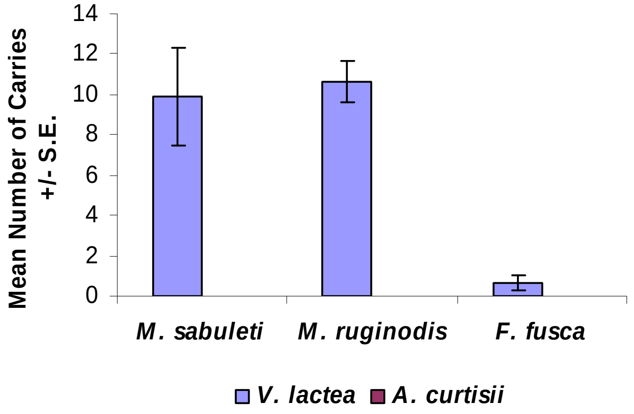


Figure 4.2: Comparison of mean number (± S.E.) of antennations, biting and carrying behaviours directed to *V. lactea* and *A. curtisii* seeds by the three ant species studied.

Differences between ant species interactions with *Viola lactea*

There were also significant differences observed between ant species in antennation (GLM ANOVA; $F_{2, 21}$= 5.23, P = 0.014), biting ($F_{2, 23}$ =23.45, P < 0.001) and carrying ($F_{2, 23}$ = 37.22, P < 0.001) behaviour towards *V. lactea* seeds (figure 4.2). Tukey's post hoc test showed that *M. sabuleti* (P =0.0483) and *M. ruginodis* (P = 0.0177) antennated *V. lactea* significantly more than *F. fusca*. *V. lactea* was also bitten more by *M. sabuleti* (P = 0.0008) and *M. ruginodis* (P < 0.0001) compared to *F. fusca*. Carrying of *V. lactea* seeds was significantly higher in *M. sabuleti* (P < 0.0001) and *M. ruginodis* (P < 0.0001) compared to *F. fusca*.

Differences between ant species interactions with *Agrostis curtisii*

Antennation behaviour towards *A. curtisii* seeds differed between ant species (GLM ANOVA; $F_{2, 23}$ = 8.63, P = 0.002) (figure 4.2). Post hoc tests using Tukey's test showed that *M. sabuleti* (P = 0.0285) and *M. ruginodis* (P = 0.0016) antennated *A. curtisii* significantly more than *F. fusca*. There were no significant differences in biting or carrying of *A. curtisii* seeds between ant species.

Retrieval of seed from foraging area

Retrieval of *V. lactea* seeds from the foraging area to the brood chamber differed significantly between ant species (GLM ANOVA; $F_{2, 20}$ = 18.82, P < 0.001; figure 4.3). Tukeys test showed that *M. sabuleti* retrieved almost 59% of seeds, (P = 0.0002) and *M. ruginodis* retrieved 65 % of seeds (P = 0.0001). In contrast *F. fusca* did not retrieve any *V. lactea* seeds. A 100% removal rate of the elaiosome from seeds retrieved by *Myrmica* spp. was observed whereas *F. fusca* did not remove the elaiosome from any of the retrieved seeds or from those left in its foraging arena. This suggests that *F. fusca* does not use the elaiosome on these seeds as a food source.

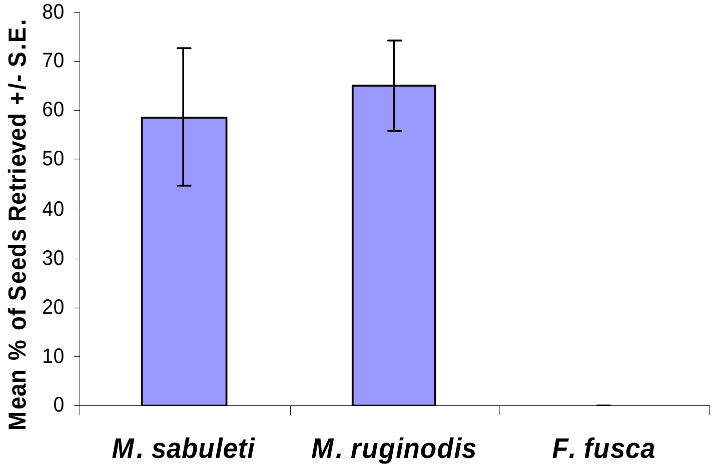


Figure 4.3: Mean percentage (± S.E.) of *V. lactea* seeds retrieved by the three ant species from the foraging area and taken to the brood chamber within the nest.

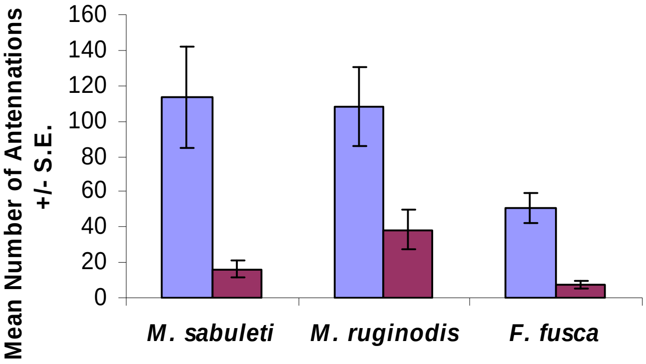
Seed preference: *Viola riviniana* versus *Agrostis curtisii* trial

As with *V. lactea*, *V. riviniana* seeds were more attractive to the ants compared to *A. curtisii* seeds (figure 4.4). All three ant species antennated (GLM ANOVA; *M. sabuleti*, $F_{1, 6} = 132$, P = 0.001 ; *M. ruginodis*, $F_{1, 6} = 23$, P=0.017 ; *F. fusca*, $F_{1, 6} = 75$, P= 0.003) and bit (*M. sabuleti*, $F_{1, 6} = 41$, P=0.008; *M. ruginodis*, F= $_{1, 6}$ = 397, P<0.001; *F. fusca* $F_{1, 6} = 19$, P= 0.022) *V. riviniana* seeds significantly more than *A. curtisii* seeds. However, only *M. ruginodis* carried *V. riviniana* seeds significantly more than *A. curtisii* seeds ($F_{1,6} = 58$, P=0.005). Again both *Myrmica* spp. interacted with *V. riviniana* seeds more that with *A. curtisii* seeds.

Differences between ant species interactions with *Viola riviniana*

Unlike *V. lactea* there were no significant differences in antennation of *V. riviniana* seeds between ant species. However, there were significant differences in biting (GLM ANOVA; $F_{2, 23} = 14.35$, P < 0.001) and carrying ($F_{2, 23} = 8.99$, P =0.002) behaviour between ant species (figure 4.4). Tukeys post hoc test once again showed that *M. sabuleti* (P = 0.0271) and *M. ruginodis* (P = 0.0001) bit *V. riviniana* seeds significantly more than *F. fusca*. *M. ruginodis* bit *V. riviniana* seeds significantly more than *M. sabuleti* (P = 0.0478). Carrying of *V. riviniana* seeds showed the same pattern as *V. lactea* with both *M. sabuleti* (P = 0.0278) and *M. ruginodis* (P = 0.0013) carrying significantly more *V. riviniana* seeds than *F. fusca*.

Ant Antennate



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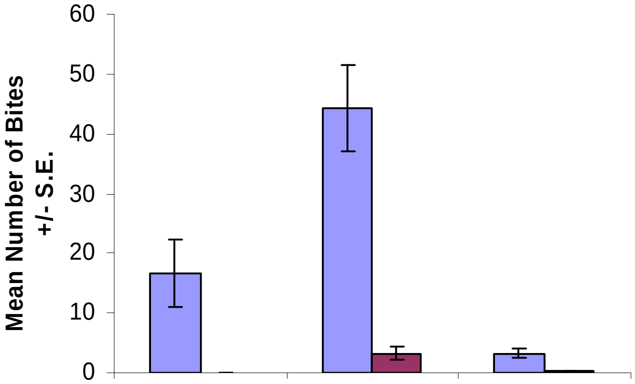

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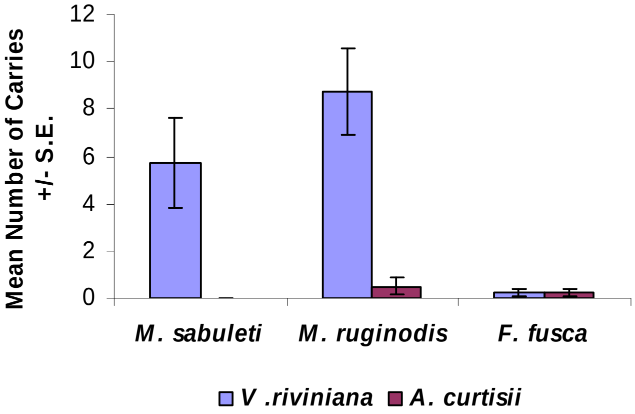


Figure 4.4: Comparison of mean number (± S.E.) of observed antennations, biting and carrying behaviour towards *V. riviniana* and *A. curtisii* seeds by the three ant species.

Differences between ant species interactions with *Agrostis curtisii*

As in the *V. lactea* and *A. curtisii* trial, antennation of *A. curtisii* seeds differed (GLM ANOVA; $F_{2, 23} = 4.29$, $P = 0.027$) between ant species (figure 4.4). Comparison between ant species using post hoc Tukey's test showed that only *M. ruginodis* antennated *A. curtisii* more ($P = 0.0212$) than *F. fusca*. Again, biting of seeds differed ($F_{2, 23} = 12.98$, $P < 0.001$) between ant species. *M. ruginodis* bit *A. curtisii* seeds significantly more than both *M. sabuleti* ($P = 0.0003$) and *F. fusca* ($P = 0.0017$). There were no significant differences in carrying of *A. curtisii* seeds between ant species ($F_{2, 23} = 1.14$, $P = 0.339$).

Retrieval of seed from foraging area

Once again significant differences (GLM ANOVA; $F_{2, 20} = 6.99$, $P = 0.005$) were detected between ant species in seed retrieval behaviour (figure 4.5). Follow up Tukey tests showed that both *M. sabuleti* ($P = 0.0164$) and *M. ruginodis* ($P = 0.0077$) retrieved more seeds than *F. fusca*. As observed in the *V. lactea* trial, *F. fusca* did not retrieve any *V. riviniana* seeds to the brood chamber. Fifty two percent of seeds were retrieved by *M. sabuleti* and 50 % by *M. ruginodis*. Again, *F. fusca* did not remove any elaiosomes from the seeds whereas both *Myrmica* spp. removed 100% of the elaiosomes. This suggests that *F. fusca* do not eat *V. riviniana* elaiosomes either.

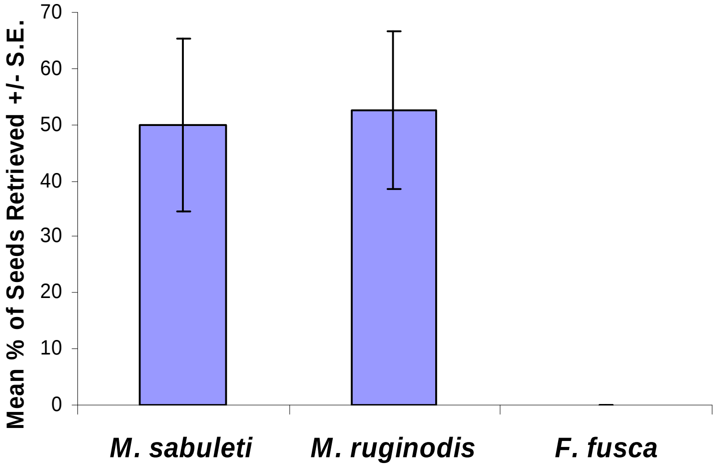
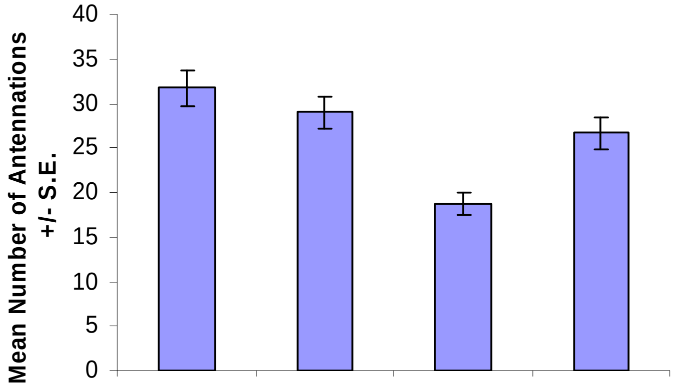


Figure 4.5: Mean percentage (± S.E.) of *V. riviniana* seeds retrieved by the three ant species from the foraging area and taken to the brood chamber within the nest.

4.4.2 *Myrmica scabrinodis* $_{\text{(HOT)}}$ seed preference (2004)

When laboratory colonies of *M. scabrinodis* $_{\text{(HOT)}}$ were offered a choice of *V. lactea*, *V. riviniana*, *P. erecta* or *A. reptans* seeds there were significant differences between the antennation responses of individual ant colonies (GLM ANOVA; $F_{9, 80} = 4.92$, $P = 0.001$) and in the behaviour of all colonies to different seed species ($F_{3, 80} = 15.78$, $P < 0.001$, figure 4.6). Post hoc testing using Tukey's test show that *V. lactea* ($P < 0.0001$), *V. riviniana* ($P = 0.0001$) and *A. reptans* ($P = 0.0022$) were antennated by *M. scabrinodis* $_{\text{(HOT)}}$ significantly more than *P. erecta.* Comparison of biting of seeds showed that there was no significant difference between ant colonies ($F_{9, 80} = 1.26$, $P = 0.300$) but there was a significant difference between seed species ($F_{3, 80} = 11.66$, $P < 0.001$). *V. lactea* ($P = 0.0001$) and *V. riviniana* ($P = 0.0053$) were bitten significantly more than *P. erecta* and *V. lactea* ($P = 0.0017$) was bitten more than *A. reptans*. When the frequency at which ants carried seeds was compared, there was no significant difference between ant colonies, but there was a significant difference ($F_{3, 80} = 8.52$, $P < 0.001$) between seed species. *V. lactea* was carried significantly more than *V. riviniana* ($P = 0.0013$), *P. erecta* ($P = 0.0072$) and *A. reptans* ($P = 0.0008$) (figure 4.7). These results indicate that the *Viola* spp., *V. lactea* in particular were more attractive to *M. scabrinodis* $_{\text{(HOT)}}$ ants than the other seeds.

Ant Antennate



Ant Bite

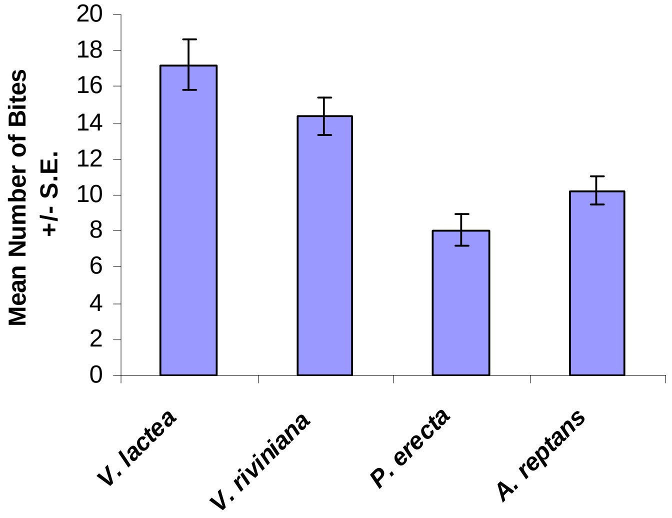


Figure 4.6: Comparison of Mean (± S.E.) number of observed behavioural events of *M. scabrinodis* $_{\text{(HOT)}}$ directed towards four seed species .

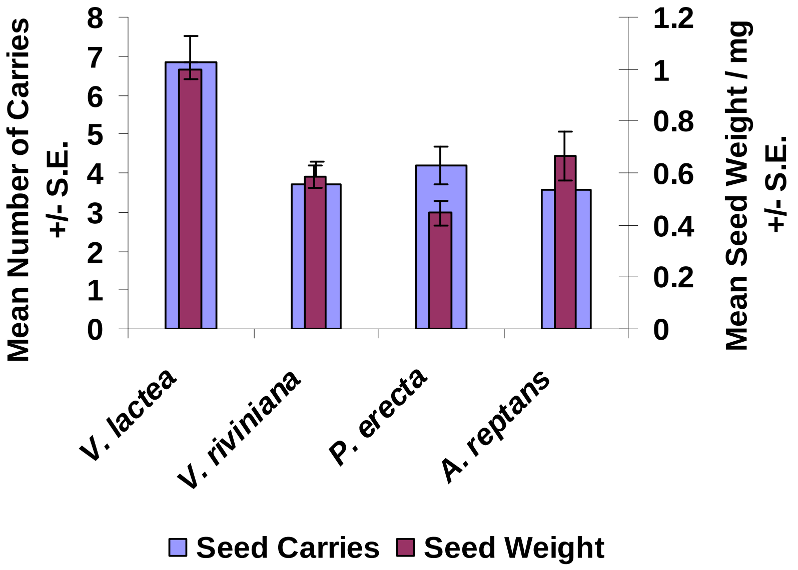


Figure 4.7: Comparison of mean number (± S.E.) of observed seed carrying events of *M. scabrinodis* $_{\text{(HOT)}}$ directed towards four seed species and their mean (± S.E.) seed weight (mg).

Seed Weight

Mean seed weights differed significantly between species (GLM ANOVA; F $_{3.76}$ = 15.56 P < 0.001). Post hoc Tukey's test showed that *V. lactea* seeds were significantly heavier than *V. riviniana* (P < 0.0001), *P. erecta* (P < 0.0001) and *A. reptans* (P = 0.0010). *A. reptans*' seeds were significantly heavier than *P. erecta* seeds (P = 0.0503), (figure 4.7). Edwards (2006) found that larger seeds had larger elaiosomes. Mark and Olesen (1996) found that seeds with bigger elaiosomes are generally more attractive to ants. However, my findings conflict with these previous studies; although *V. lactea*, the heaviest seed was carried significantly more than the other seeds; the lightest seed *P. erecta* was carried more (although not significantly so) than both *V. riviniana* and *A. reptans.*

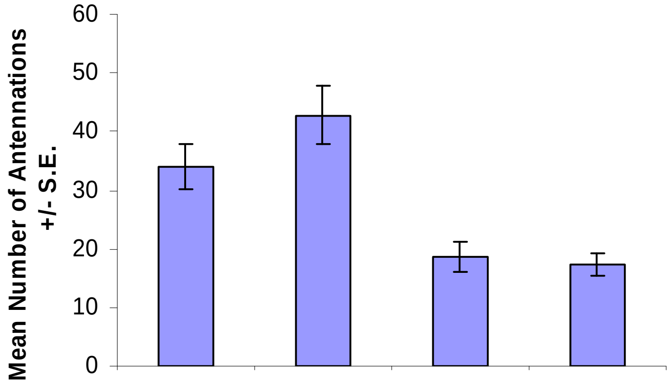
4.4.3 *Myrmica ruginodis* and *Myrmica sabuleti* seed preference (2005)

In field observations I found that *V. riviniana* is associated with *M. ruginodis* ant nests, *V. lactea* on the other hand were more associated with *M. scabrinodis* $_{\text{(HOT)}}$. The result from the *M. scabrinodis* $_{\text{(HOT)}}$ seed preference laboratory experiment suggests that this ant has a preference for *V. lactea* seeds, which could be a result of an evolutionary or learnt association, or simply a preference for the violet species possessing the larger elaiosome. In this experiment *M. ruginodis* and *M. sabuleti* were presented with seeds of *V. lactea*, *V. riviniana*. *P. erecta* and *A. reptans* to find out whether *M. ruginodis* and *M. sabuleti* have a particular preference for seeds of the plant they are most associated with in the field.

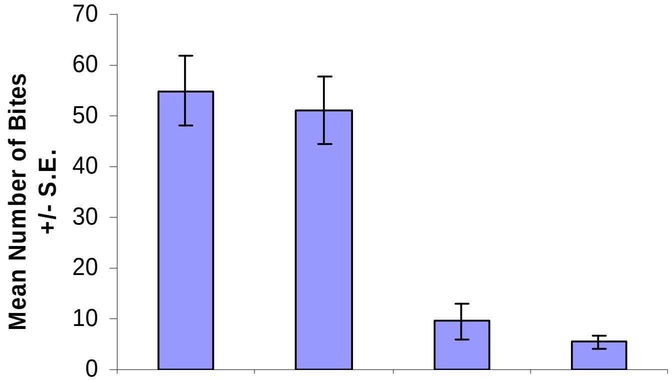
M. ruginodis seed preference

There were significant differences in the antennation (GLM ANOVA; $F_{3, 67} = 10.27$, $P < 0.001$), biting ($F_{3, 67} = 29.11$, $P < 0.001$) and carrying ($F_{3, 67} = 3.02$, $P = 0.036$) behaviour of *M. ruginodis* towards the different seed species (figure 4.8). Follow up Tukey test showed that antennation of *V. lactea* seeds was significantly greater than *P. erecta* ($P = 0.0109$) and *A. reptans* ($P = 0.0088$). *V. riviniana* was antennated significantly more than *P. erecta* ($P = 0.0002$) and *A. reptans* ($P = 0.0088$). *M. ruginodis* bit *V. lactea* significantly more than *P. erecta* ($P < 0.0001$) and *A. reptans* ($P < 0.0001$). Seeds of *V. lactea* were carried significantly more than *P. erecta* ($P = 0.0400$) seeds. These results are consistent with the findings of the 2004 experiments where *Viola* spp. seeds were antennated and bitten more than the other seeds offered as would be expected by another *Myrmica* spp. However, in contrast to the *M. scabrinodis* $_{\text{(HOT)}}$ experiments conducted in 2004, there was no significant difference between carrying of *V. lactea* and *V. riviniana* seeds ($P = 0.8760$). This therefore suggests that *M. ruginodis* does not have a preference for *V. riviniana* seeds, nor does it selectively choose the species (*V. lactea*) with the largest elaiosome.

Ant Antennate



Ant Bite



Ant Carry

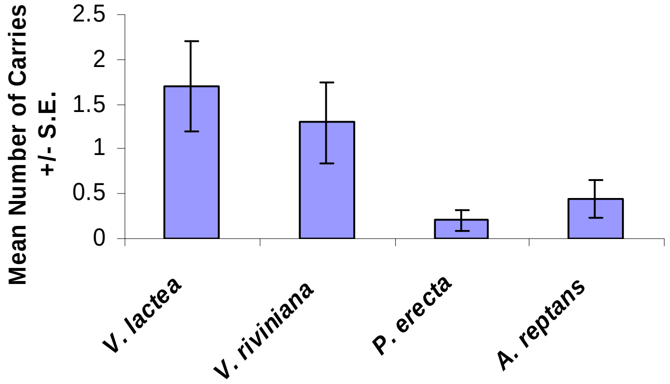
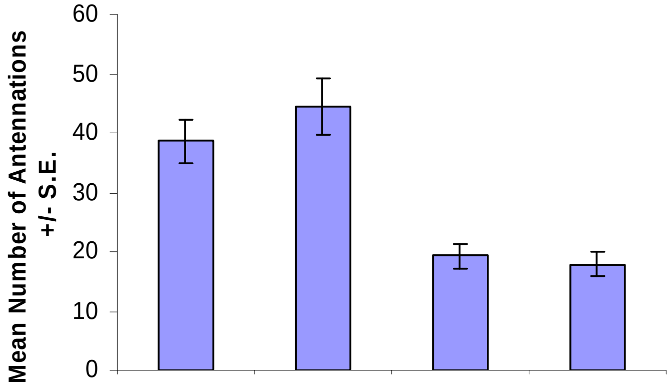


Fig 4.8: Comparison of Mean number (± S.E.) of observed behavioural events of *M. ruginodis* directed towards four seed species.

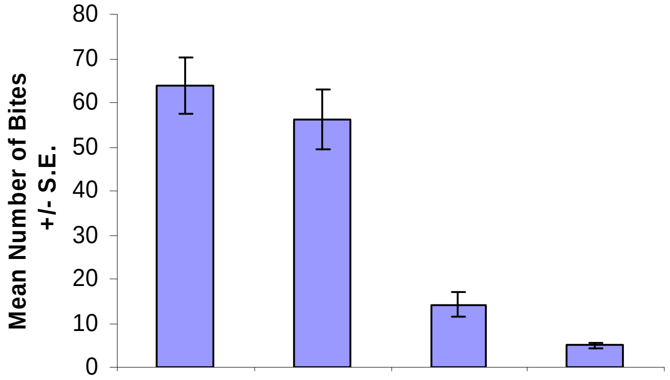
M. sabuleti seed preference

As with *M. ruginodis,* when presented with the different seed species, *M. sabuleti* demonstrated a significant difference in antennation (GLM ANOVA; $F_{3. 73} = 13.13$, $P < 0.001$), biting ($F_{3, 73} = 50.12$, $P < 0.001$) and carrying ($F_{3, 73} = 11.40$, $P < 0.001$) behaviours towards the different seed species (Figure 4.9). Post hoc Tukey's tests showed that *V. lactea* seeds were antennated significantly more than *P. erecta* ($P = 0.0012$) and *A. reptans* ($P = 0.0003$). Antennation of *V. riviniana* seeds was significantly greater than *P. erecta* ($P = 0.0001$) and *A. reptans* ($P < 0.0001$). *V. lactea* seeds were bitten more than *P. erecta* ($P < 0.001$) and *A. reptans* ($P < 0.0001$). *V. riviniana* seeds were bitten more than seeds of *P. erecta* ($P < 0.0001$) and *A. reptans* ($P < 0.0001$) and *P. erecta* seeds were bitten more often than *A. reptans* ($P = 0.0048$) seeds. *V. lactea* seeds were carried significantly more than any of the other seed species; (*V. riviniana*, $P= 0.0126$, *P. erecta*, $P = 0.0002$ and *A. reptans,* $P < 0.0001$). This indicates that *V. lactea* seeds are more attractive to *M. sabuleti* than the other seeds.

Ant Antennate



Ant Bite



Ant Carry

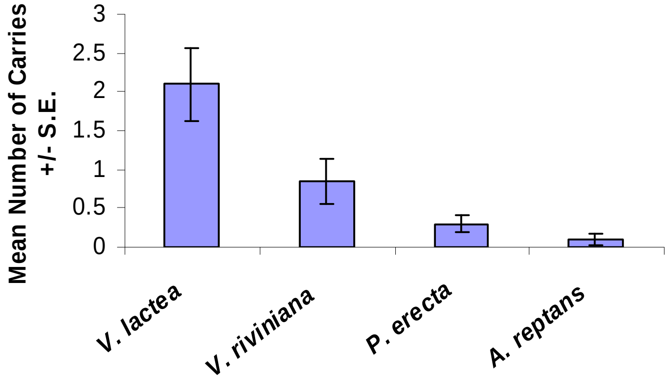


Figure 4.9: Comparison of Mean number (± S.E.) of observed behavioural events of *M. sabuleti* directed towards the four seed species.

4.5 Discussion

In the 2003 study, all three ant species clearly showed a preference towards the seeds of the *Viola* spp. over those of *A. curtisii.* This is probably due to the presence of an elaiosome on *Viola* seeds which attracts ants. However, a study carried out by Delatte & Chabrerie (2008) showed that the non-elaiosome bearing grass seeds of *Holcus mollis, Agrostis capillaris, Deschampsia flexuosa*, *Poa trivialis*, *Holcus lanatus* were readily collected by *M. ruginodis* in the French forest where their study was conducted. The fact that *Agrostis curtisii* is less abundant near to nest spots compared to non-nest spots in the field (Chapter 3) may be due to inter-specific competition; the increased abundance of *Viola* spp. near to nest spots may out-compete *A. curtisii* in these areas. On the other hand, ant nest spots may be less suitable for *A. curtisii* growth due to other abiotic or biotic factors.

Whereas all *Myrmica* species tested showed a strong liking for *Viola* seeds, observations of biting behaviour showed that *F. fusca* responded aggressively towards them; this may warrant further investigation into the chemistry of elaiosomes. Perhaps *Viola* seeds have evolved chemically to attract *Myrmica* and actually to repel other ant genera? If so, it is likely that ant specificity has evolved in other plant species; for example, *F. fusca* have been observed carrying *Ulex europaeus* seeds in the field (Gammans pers comm.) yet in the laboratory they show little interest in *Viola* species compared to the *Myrmica* species studied.

Another explanation may be that the small size of the elaiosome on *Viola* species compared to *Ulex europaeus* outweighs the effort in terms of energetics. Mark & Olesen (1996) found that seeds with larger elaiosomes were preferred by *M. ruginodis*. Hughes & Westoby (1992a) suggest that carrying a large seed may expose foragers to increased risk of mortality as they are more conspicuous to predators. Therefore, the yield provided by the elaiosome has to outweigh the risk of foraging mortality. The larger the elaiosome, the more likely an ant is to retrieve the seed (Oostermeijer, 1989; Gunther & Lanza, 1989; Westoby et al., 1991). I suggest that the relatively small size of the *Viola* elaiosome does not outweigh the risk of foraging mortality for *F. fusca*, which is a larger ant and is therefore more likely to carry seeds of other plant species that have larger elaiosomes, such as *Ulex* spp. However, if one applied central foraging theory to this experiment, *V. lactea* should have been the preferred seed type for *F. fusca* as it was the largest available seed (in weight, figure 4.7) and therefore would have the largest elaiosome (Edwards et al., 2006). In fact, *F. fusca* showed no such preference (figure 4.3). The findings of my study contradict those of Beattie et al., (1979) who found no differences in seed preference between *Myrmica punctiventris, Lasius alienus, Formica subsericea* or *Aphaenogaster rudis.* I therefore disagree with the statement that, "at any site, the study of a single seed species can be used to characterise the general ant – seed relationships that occur there" (Andersen, 1988a). Clearly, investigating only the role of *F. fusca* as a seed disperser on my study site would not have described the full ant and seed interactions that occur in this system. Further work in the laboratory and in the field should be undertaken to investigate the seed preferences of *F. fusca* to establish the role this ant has as a seed dispersal vector within its community. *F. fusca* acts as a seed disperser in beech-larch woodlands and has a significant effect on seed germination (Culver & Beattie 1980).

The second set of laboratory studies comparing ant preferences to *V. lactea, V. riviniana, P. erecta* and *A. reptans* found on the study site are consistent with the field data (Chapter 3). Both the 2004 and 2005 studies show that *Viola* seeds are more attractive to *Myrmica* spp. than the other myrmecochorous seeds in terms of antennation, biting and carrying behaviour. However, in the 2003 and 2004 experiments all of the elaiosome-bearing seeds were eventually collected by *Myrmica* spp. from the foraging areas and taken to the brood chambers equally, with the ants showing no specific preference. This phenomenon clearly aids seed dispersal, but in the field it is only *Viola* plants that are significantly more abundant near to ant nests compared to non-nest spots. This could be due to many reasons, including the close proximity (1m) of the brood chamber to the foraging area within the laboratory, which would mean that in terms of central foraging theory that all seeds are equally attractive in terms of energetics (Holder Bailey & Polis, 1987), whereas in the field seeds are dispersed more widely from the nest. There may also be a greater abundance of seeds in the field near to nests due to myrmecochory in previous years; my experiments had a limited number of seeds for the ants to collect. Finally, both *Viola* were abundant on the study sites, so if *Myrmica* colonies aggregated high densities of their seeds around their nests, the resulting violet plants may have outcompeted the seedlings of other myrmecochorous plants that were collected in smaller numbers (Culver & Beattie, 1980).

In the 2004 study, *V. lactea* was significantly more attractive to *M. scabrinodis* $_{\text{(HOT),}}$ the ant with whose distribution it overlaps most in the field (Chapter 3) compared to its attractiveness to other *Myrmica* spp. This may be due to a local adaptation between *V. lactea* and/or *M. scabrinodis* $_{\text{(HOT)}}$ that has evolved to enhance their mutualism, this is unlikely as species specific mutualistic interactions are rare (Stanton, 2003). Alternatively, it may represent previous learning by the colonies used in this experiment, since they were collected from the hot areas of site Y where *V. lactea* was abundant. However, *M. ruginodis*, which shows a preference for cooler microclimates, has higher densities of *V. riviniana* above nests compared to other ant species (Chapter 3). In laboratory conditions this species does not show a preference for *V. riviniana*, which may indicate a local adaptation being observed at site Y. However, seed preferences are influenced by foraging strategies not historical constraints (Peters et al., 2003), therefore local adaptation is unlikely to be a factor in the seed preference of *M. scabrinodis* $_{\text{(HOT).}}$ As *V. lactea* have heavier seeds than *V. riviniana* (figure 4.8), one might expect all *Myrmica* species to prefer seeds of this particular *Viola* species. However, only *M. scabrinodis* $_{\text{(HOT)}}$ the ant with which it associates in the field demonstrates a significant preference. *M. ruginodis*, which is associated with *V. riviniana* in the field, took more *V. riviniana* seeds compared to *V. lactea* but not significantly so.

Further studies to investigate the chemical composition of *Viola* elaiosomes compared to elaiosomes of *A. reptans* and *P. erecta* may indicate why *Viola* seeds are more attractive to the ant species studied. Chemical composition of elaiosomes differs between species (Fischer et al., 2008; Lanza et al., 1992). The size / mass of *Viola* elaiosomes may be a factor in *Viola* attractiveness to ants (figure 4.7). *Viola lactea* had the greatest weight of the seeds used in the 2004 experiment and may therefore have a larger elaiosome than the other species. Edwards (2006) found that seed size and elaiosome size are closely correlated, the bigger the seed the bigger the elaiosome. However, in the 2004 experiment I found that the lighter seeds of *P. erecta* were carried more than the heavier seeds of *V. riviniana* and *A. reptans*. In my study only 20 seeds of each type were weighed. I suggest that a larger sample size and a comparison of elaiosome to seed mass ratio be investigated in order to further explain the ant seed preferences found in these experiments. Ohkawara & Higashi (1994) investigated ant dispersal in two *Viola* species, *V. selkirkii* and *V. verecunda.* They found that mean seed weight, elaiosome weight, and seed removal rate was greater in *V. selkirkii* compared to

V. verecunda. They also showed that *Myrmica kotokui* took more *V. selkirkii* seeds than *V. verecunda* seeds. However, there was little difference in lipid and fatty acid composition of the elaiosomes. They concluded that *V. selkirkii* was more dependent upon dispersal by *M. kotokui* than *V. verecunda*. Perhaps *P. erecta* and *A. reptans* are less dependent upon ant dispersal than the *Viola* species studied in my experiment?

Conversely a study conducted by Servigne & Detrain (2008) found that the smaller seeds of *Chelidonium majus* were removed faster than the larger ones of *Viola odorata* by both *Lasius niger* and *Myrmica rubra*. This could be explained by the fact that the elaiosome of *C. majus* constitutes 50% of the overall mass of the diaspore (Imbert 2006). However, when the ant *Formica polyctena* was presented with these two seed types, this ant removed the *V. odorata* seeds more quickly (Gorb & Gorb 2002). Another study which contradicts my findings is that of Imbert (2006). Seeds of *Centaurea corymbosa* whose elaiosomes are 11 times smaller than those of *Euphorbia characias* were more attractive to ants. This suggests that relationship between elaiosome size and ant attraction is quite complex. In my study I can only elude that it maybe the seed size and thus the potentially larger elaiosome of *V. lactea* that accounts for the ant preference of this seed. Therefore an avenue for future work could be to investigate the elaiosome/diaspore ratio of the seeds in my study.

These laboratory studies have shown that *Viola* seeds are highly attractive to ants and the general trend for higher densities of *Viola* plants near to ant nests compared to non-nest spots (see Chapter 3) can in part be attributed to the presence of an elaiosome which acts as an ant attractant. Seeds are collected and carried back to the ant nest where the elaiosome is removed and the remaining seed may be discarded near to the nest (Culver & Beattie 1980; Higashi et al., 1989; Peters et al., 2003), suggesting a potential explanation for the higher densities of *Viola* plants near to nests compared to equivalent non-nest spots. These results are consistent with other studies that have found spatial relationships between ant nests and myrmecochores (Oostermeijer, 1989; Lack & Kay, 1987; Kovar et al., 2001).

One of the potential benefits to plants with myrmecochorous seeds is that seeds are transported to nutrient rich micro-sites such as ant nests (Beattie & Culver, 1983). In Chapter 5, I investigate whether or not *M. sabuleti* ant nests are more nutrient rich compared to equivalent non-nest spots and, if *Viola* seeds are more abundant in ant nest soil seed-banks compared to equivalent non-nest spots. This will determine if these are contributory factors to why *Viola* plants are more abundant near to ant nests compared to equivalent non-nest spots.

Whatever the mechanism behind the *Myrmica* / *Viola* interaction, there is one probable consequence – this interaction is likely to have a positive effect on fritillary butterflies whose larvae feed on *Viola* spp. In Chapter 6 the preferred oviposition niche of two species of fritillary butterfly are assessed to ascertain whether the observed increase in populations of these butterflies on the study sites is due to habitat management or as a result of the direct or indirect impact of ants.

Chapter 5: Do *Myrmica sabuleti* ant colonies influence soil chemistry and soil seedbank composition on a semi-natural acid grassland site

5.1 Introduction

5.1.1 Nest Chemistry

Ant activities such as nest building, the accumulation of food and detritus and the production of excreta can influence the physical and chemical properties of ant nest soil (Dostal, 2005; Frouz et al., 2003; Frouz et al., 2005). Many studies have found that ant nest soil differs from adjacent soil in terms of plant nutrients, temperature, soil porosity, organic content and pH (Beattie & Culver, 1983; Petal, 1978). Nests often provide conditions that increase germination and establishment success for seeds (Culver & Beattie, 1980; Beattie & Culver, 1983; Hanzawa et al., 1998). For example, experimental planting of seeds in ant nests has shown that *Corydalis aurea* plants develop faster, flower earlier and have improved over-wintering than those planted in adjacent soil (Handel & Beattie, 1990). These findings are consistent with Davidson & Morton (1984) who found that *Scleroleaena diacantha* and *Dissocarpus bilflorus* seedlings grew better on ant mounds compared to non-mounds. Root and shoot biomass was greater in seedlings of *Eucalyptus oblique* that were grown in soil from *Aphaenogaster longiceps* nests (Andersen, 1988b). *Formica canadensis* and *Myrmica discontinua* ant nests are richer in phosphorus compared to surrounding soils (Beattie & Culver, 1983). More recently, *Myrmica ruginodis* nests were found to be higher in phosphorus and potassium compared to non-nest soils (Gammans, 2005). *Polygala vulgaris* and *Viola curtisii*, whose seeds possess elaiosomes, have been found to be more abundant in ant nest soils compared to non-nest soils, and in addition to this, the nest soils in this study had enhanced levels of potassium, nitrate and potassium (Oostermeijer, 1989).

These studies support the nutrient enrichment hypothesis of myrmecochory. However, conflicting results have been found in other research. Rice & Westoby (1986) found that there were not elevated levels of available potassium or nitrate in soil close to the roots of emerging myrmecochorous seedlings. Bond et al., (1991) investigated the soil chemistry of *Anoplolepis* spp.; and concluded that seeds were transported to nutrient poor micro-sites.

5.1.2 Soil Seedbank

Seedling establishment is limited by the availability of suitable micro-sites (Dostal, 2005). Newly emerging seedlings are highly sensitive to competition from the surrounding vegetation. Grazing animals create gaps in the vegetation through trampling, thereby minimising competition (Bullock, 2000). Burrowing animals and insects also create gaps (Dostal, 2005). Sources of seed for plant re-colonisation are seedbanks, seed rain from adjacent vegetation and relocation by animals. Plant regeneration from the seedbank influences the colonisation and structure of plant communities. Seedbanks are vital for the restoration and conservation of plant communities (Thompson et al., 1997).

Despite the importance of seedbanks', few studies have researched the effect of ants on them (Dostal, 2005). Seed density and species composition within the seedbank are likely to be influenced by ants for several reasons. The relocation of seeds through myrmecochory is likely to alter seedbank composition; one would expect an increase in the density of the preferred species' seeds near to ant nests. Perturbation of the soil though nest building activities is likely to move seeds around within the soil profile. In addition, scarification of seeds and elaiosome removal has been shown to have a small yet positive effect on seed germination in some plants (Culver & Beattie, 1980).

5.2 Research Aim

In Chapter 3, I established that *Viola* species are more abundant on *Myrmica* ant nests compared to non ant nest spots. This can be attributed in part to the gathering of *Viola* seeds (Chapter 4), but may also be as a result of ant activity enhancing their nest soil chemistry or of increased survival in these spots due to their avoidance by insect herbivores for example. In this Chapter I investigate the soil chemistry of *Myrmica sabuleti* ant nests to find out if *M. sabuleti* nest spots are richer in plant nutrients than surrounding soils. In addition, the content of viable seeds near to *M. sabuleti* nests is compared to non-nest spots, to ascertain whether myrmecochory influences the species density and composition of the seedbank.

5.3 Hypotheses tested

Viola species are more abundant at *M. sabuleti* nest spots compared to non ant nest spots due to (i) an increase in soil nutrients, (ii) higher densities of *Viola* seeds in the seedbank as a result of myrmecochory.

5.4 Methods

5.4.1 Soil Nutrients

In July 2005, seven *M. sabuleti* nests were located using standard ant baiting methodology (Wardlaw et al., 1998). Paired non-nest spots were located 1 m away from the nests, where the vegetation structure, cover and micro-topography were indistinguishable from that around the nests. The numbers of *Viola* plants were counted at nest and non-nest spots to provide baseline data of the potential seed rain for the soil seedbank study. Nest and non-nest spots were excavated in a 30 cm x 30 cm square to a depth of 30cm. Ant nest samples were returned to the laboratory and the ants removed. The samples were then sieved to remove vegetation and stones. A sub-sample of 300mg was sent to Anglian Soil Sciences, Lincolnshire, for analysis of pH, potassium, phosphorus, ammonium nitrogen, nitrate nitrogen and moisture content.

5.4.2 Soil Seedbank

Seven *M. sabuleti* nests and paired non-nest samples were collected as above and sorted in the laboratory. The upper two centimetres of each sample were extracted using a sharp knife, representing the sector in which viable seeds were found to be concentrated in earlier studies (Bullock, 1996). The remaining sample, which provides a complete picture of the seedbank, was analysed separately. Samples were sieved through a 4mm mesh and air dried. Sub-sample volume was recorded and the soil then spread to a depth of 2cm in trays standing in a bed of washed sharp sand, and placed in a greenhouse. From August 2005 until August 2006, emerging seedlings were identified and counted to examine the association of soil seedbanks' with ants. All grasses, with the exception of *Agrostis curtisii*, were pooled and categorised as "other grasses", because of the difficulty of accurate identification of grass seedlings.

5.4.3 Statistical Analysis of Soil Nutrient Data

Data were analysed using the non parametric Mann-Whitney test. Although the samples were paired, analysis using a paired t-test was inappropriate due to the small sample size (Dytham, 2003).

5.4.4 Statistical Analysis of Soil Seedbank Data

Seedling index was calculated by dividing the number of emerged seedlings by the volume of soil sown multiplied by 1000 obtain the number of emergences per litre. These data were converted to proportions, arcsine transformed and analysed using One-way ANOVA.

5.5 Results

5.5.1 Soil Nutrients

There were no significant differences between *M. sabuleti* nest and non-nest soils with respect to pH ($W_1 = 67.5$, $P > 0.05$), phosphorus ($W_1 = 54.5$, $P > 0.05$), potassium ($W_1 = 53.5$, $P > 0.05$), magnesium ($W_1 = 52.0$, $P > 0.05$), ammonium nitrate ($W_1 = 53$, $P > 0.05$) or nitrate nitrogen ($W_1 = 49$, $P > 0.05$). However, soil moisture was significantly lower in *M. sabuleti* nest soil compared to non-nest spots ($W_1 = 32$, $P = 0.01$) table 5.1.

Table 5.1: Median, Interquartile Range (IQR) and statistical significance of chemical properties between *M. sabuleti* ant nest and non-nest soil.

	Nest	**Non-nest**	**Statistical Significance**
pH	4.4 (4.2 - 4.5)	4.2 (4.1 - 4.3)	$W_1 = 67.5$, $P = 0.06$
Phosphorus mg/l	10 (10 - 11)	10 (9 - 11)	$W_1 = 54.5$, $P = 0.84$
Potassium mg/l	151 (146 - 181)	162 (127 - 196)	$W_1 = 53.5$, $P = 0.94$
Magnesium mg/l	105 (96 - 126)	112 (99 - 119)	$W_1 = 52.0$, $P = 1.0$
Ammonium Nitrogen mg/kg	32.7 (15.7 - 57.8)	33.5 (18.1 - 41.6)	$W_1 = 53.0$, $P = 1.0$
Nitrate Nitrogen mg/kg	3.65 (3.63 - 15.9)	6.95 (3.19 - 21.7)	$W_1 = 49.0$, $P = 0.70$
% Moisture	24.1 (21.5 - 27)	29.7 (27.5 - 34.3)	**$W_1 = 32.0$, $P = 0.01$**

5.5.2 Soil Seedbank

A total of 1114 individual seedlings of 20 different species emerged from the soil seedbank; one species *A. curtisii* (the dominant grass of the study site) formed 56% of the emerging seedlings. There were 4 different myrmecochorous seeds in the seedbank (*Viola lactea, Viola riviniana*, *Ulex* spp. and *Potentilla erecta*). Nineteen percent of the seedlings were myrmecochorous, 25% of which emerged from *M. sabuleti* nest soil compared to 18% from non-nest soil.

M. sabuleti nest sub-samples combined versus Non-nest sub samples combined

Combining the emergence results from the upper and the lower samples for *M. sabuleti* nests and non-nests showed that there was no significant difference in the proportions of seedlings emerging from nest and non-nest soil (ANOVA $F_{1, 27} = 1.72$, $P = 0.201$) table 5.2. However, it is worth noting that *V. riviniana* was almost twice more abundant in ant soil compared to non-ant soil. The total number of myrmecochorous seedlings emerging from ant-soil and non-ant soil did not differ significantly (ANOVA $F_{1, 27} = 0.72$, $P = 0.405$), neither did the number of non-myrmecochorous seedlings (ANOVA $F_{1, 27} = 0.72$, $P = 0.405$) table 5.2.

Upper *M. sabuleti* nest soil compared to upper non-nest soil

A total of 16 species were identified from all the upper soil samples. Of these 11 species emerged from the *M. sabuleti* nest soil samples, in contrast 14 species were found in trays containing non-nest soil. Overall the proportion of seedlings did not differ between treatments (ANOVA $F_{1, 13} = 0.19$, $P = 0.67$). Emergence of *Viola riviniana* (ANOVA $F_{1, 13} = 7.03$, $P < 0.05$) seedlings was significantly higher from the upper *M. sabuleti* nest soil compared to the equivalent non-nest soil (table 5.3). However, adjusting the *P value* using Bonferroni's method, which eliminates type I errors resulting from multiple comparisons, the number of *V. riviniana* and seedlings are not significantly different ($P > 0.003125$) between treatments. Of the three species that were more abundant in ant soil, two had myrmecochorous seeds. Comparisons of the proportion of seedlings (ANOVA $F_{1, 13} = 0.19$, $P = 0.669$), myrmecochorous seedlings (ANOVA $F_{1, 13} = 1.00$, $P = 0.337$) and non-myrmecochorous seedlings (ANOVA $F_{1, 13} = 1.00$, $P = 0.337$) between nest and nest soils show no significant differences (table 5.3). This suggests that *M. sabuleti* is not concentrating myrmecochorous seeds in the seedbank near to their nests.

Lower *M. sabuleti* nest soil compared to lower Non-nest soil

Fifteen species emerged from the lower sample of *M. sabuleti* nest soil compared to 18 species from the non-nest soil. The proportions of myrmecochorous seedlings and non-myrmecochorous seedlings did not differ significantly (table 5.3). These results again indicate that *M. sabuleti* is not affecting the soil seedbank composition deeper within the soil profile.

Table 5.2: Proportion ± S.E. of seedlings per litre of soil that emerged from *M. sabuleti* nests (upper and lower soil samples combined) compared to non-nests. * denotes myrmecochorous species.

	Median number of seedlings per litre	
	Mean proportion of seedlings per/ l ± S. E.	
Species	Nest	Non-nest
Agrostis curtisii	58.93 ± 5.38	57.34 ± 5.49
Betula spp.	1.778 ± 0.832	1.366 ± 0.765
Carex spp.	1.789 ± 0.741	2.284 ± 0.867
Cirscium spp.	0 ± 0	0.446 ± 0.446
Epilobium spp.	4.67 ± 2.05	1.24 ± 0.698
Erica cinerea	2.11 ± 2.11	2.04 ± 2.04
Filago spathulata	0.569 ± 0.329	1.432 ± 0.974
Galium spp.	0.162 ± 0.162	0.651 ± 0.476
Leontodon hispidus	0 ± 0	1.097 ± 0.9
Oxalis acetosella	0 ± 0	0.297 ± 0.205
Plantago major	0 ± 0	0.311 ± 0.217
*Potentilla erecta**	6.72 ± 2.03	8.96 ± 1.98
Rubus fruticosus	0.292 ± 0.292	0.813 ± 0.454
Sagina procumbens	3.03 ± 1.37	1.79 ± 1.32
Taraxacum agg	0 ± 0	0.408 ± 0.408
Ulex spp.*	7.87 ± 2.66	5.08 ± 1.24
Veronica spp.	0.146 ± 0.146	3.37 ± 2.04
*Viola lactea**	2.73 ± 1.51	2.43 ± 1.13
Viola riviniana *	6.96 ± 2.05	3.21 ± 1.32
Other grasses	2.249 ± 0.908	5.43 ± 2.31
Myrmecochorous seedlings	24.28 ± 4.34	19.68 ± 3
Non-myrmecochorous seedlings	75.72 ± 4.34	80.32 ± 3
Number of species	15	20

Table 5.3: Proportion ± S.E. of seedlings per litre of soil emerged from the upper and lower soil samples from *M. sabuleti* nests and non-nests. * denotes elaiosome bearing seed.

	Upper Soil Samples		**Lower Soil Samples**	
	Mean percentage of seedlings ± S.E.		Mean percentage of seedlings ± S.E.	
Species	Nest	Non-nest	Nest	Non-nest
Agrostis curtisii	60.9 ± 8.67	62.03 ± 6.82	56.97 ± 7.01	52.66 ± 8.76
Betula spp.	1.71 ± 1.41	1.85 ± 1.01	2.48 ± 1.43	0.246 ± 0.25
Carex spp.	1.096 ± 0.82	2.48 ± 1.24	2.52 ± 1.44	2.05 ± 1.08
Cirscium spp.	0 ± 0	0 ± 0	0 ± 0	0.893 ± 0.90
Epilobium spp.	2.86 ± 2.86	1.7 ± 1.32	6.48 ± 3	0.775 ± 0.55
Erica cinerea	0 ± 0	4.22 ± 4.22	4.08 ± 4.08	0 ± 0
Filago spathulata	0.28 ± 0.28	0.858 ± 0.60	2.86 ± 1.85	0 ± 0
Galium spp.	0 ± 0	0.325 ± 0.33	0 ± 0	1.301 ± 0.92
Leontodon hispidus	0 ± 0	0 ± 0	0 ± 0	2.19 ± 1.76
Oxalis acetosella	0 ± 0	0 ± 0	0.348 ± 0.35	0.246 ± 0.25
Plantago major	0 ± 0	0 ± 0	0.376 ± 0.38	0.246 ± 0.25
*Potentilla erecta**	8.8 ± 3.18	4.63 ± 2.51	11.18 ± 3.47	6.74 ± 1.84
Rubus fruticosus	0 ± 0	0.583 ± 0.58	0.357 ± 0.36	1.268 ± 0.83
Sagina procumbens	2.58 ± 1.49	3.48 ± 2.42	0 ± 0	3.57 ± 2.56
Taraxacum agg	0 ± 0	0 ± 0	0 ± 0	0.816 ± 0.82
Ulex spp.*	7.78 ± 2.77	7.96 ± 4.8	5.66 ± 1.78	4.5 ± 1.85
Veronica spp.	0 ± 0	0.292 ± 0.29	1.79 ± 1.41	4.96 ± 3.9
*Viola lactea**	2.04 ± 2.04	3.42 ± 2.37	2.83 ± 1.15	2.04 ± 2.04
*Viola riviniana**	10.13 ± 2.93	3.8 ± 2.51	1.78 ± 1.03	4.64 ± 2.41
Other grasses	1.84 ± 1.39	2.66 ± 1.26	0 ± 0	10.85 ± 3.65
Number of species	11	15	14	18
Myrmecochorous seedlings	28.75 ± 5.67	19.81 ± 6.53	21.45 ± 4.23	17.92 ± 4.47
Non-myrmecochorous seedlings	71.25 ± 5.67	80.19 ± 6.53	78.55 ± 4.23	82.08 ± 4.47
Total percentage of seedlings	**24.84**	**17.07**	**30.32**	**27.78**

Differences within *M. sabuleti* nest soil (upper v lower)

No significant differences were detected in the number of seedlings, species, myrmecochorous seedlings or non-myrmecochorous seedlings between the upper and lower *M. sabuleti* nest soil samples (table 5.4). However, numbers of seedlings in the upper soil sample were greater than in the lower samples. *V. riviniana* seedlings were more abundant in the upper portion of nest soil compared to the lower sample. These results suggest that the ants may be accumulating myrmecochorous seeds near the nests.

Table 5.4: Statistical significance of the proportions of seedlings, myrmecochorous seedlings and non myrmecochorous seedlings per litre of soil, compared within *M. sabuleti* nest samples and within non-nest samples.

	Statistical Significance	
Proportion of seedlings	Lower Nest Soil	Upper Non-nest Soil
Upper Nest Soil	$F_{1,13}$ = 1.73, P = 0.213	
Lower Non-nest		$F_{1,13}$ = 0.02, P = 0.877
Proportion of myrmecochorous seedlings		
Upper Nest Soil	$F_{1,13}$ = 1.33, P = 0.271	
Lower Non-nest		$F_{1,13}$ =0.59, P = 0.458
Proportion of non – myrmecochorous seedlings		
Upper Nest Soil	$F_{1,13}$ =1.33, P = 0.271	
Lower Non-nest		$F_{1,13}$ = 0.59, P= 0.458

Differences within non- nest soil (upper v lower)

Comparing the number of seedlings, species, myrmecochorous and non-myrmecochorous seedlings within the soil profile again shows no significant differences (table 5.4). Again, as with the *M. sabuleti* nest soils, greater proportions of seedlings emerged from the lower soil samples compared to the upper samples.

Viola abundance near to sampling locations prior to soil excavation

The number of *Viola* plants above soil sampling spots was recorded prior to soil sample excavation. There were no significant differences between the number of *V. lactea* (H_1 = 0.00, P = 0.949) or *V. riviniana* (H_1 = 0.15, P = 0.701) plants recorded above *M. sabuleti* nests compared to non-nest spots. The small sample size of ant nests versus non-nests may not have been large enough to show the pattern of significantly more *Viola* plants near ant nests described in Chapter 3. Table 5.5 shows the actual numbers of plants recorded in the field prior to sample excavation and the potential seed production of those plants. Seed production is based upon Moore (1958) who found that a single *V. lactea* plant produces an average of 500 seeds per year.

Table: 5.5: The number of *Viola* spp. plants recorded above *M. sabuleti* nests and non-nests in the field prior to soil excavation. One *Viola* plant produces on average 500.8 seeds per year. The potential seeds column is calculated from the number of plants recorded multiplied by 500.

	Number of *Viola* plants recorded in the field		
	V. lactea	***V. riviniana***	**Potential seeds**
***M. sabuleti* nest**	8	1	4500
	12	0	6000
	18	11	14500
	18	10	14000
	6	38	22000
	10	7	8500
	23	7	15000
Total	**95**	**74**	**84500**
Non-nest	2	1	1500
	16	8	12000
	12	4	8000
	23	6	14500
	3	19	11000
	13	26	19500
	26	17	21500
Total	**95**	**81**	**88000**

5.6 Discussion

There are five non-exclusive benefits of myrmecochory in plants: (1) fire avoidance (Holldobler and Wilson, 1990); (2) herbivore avoidance (Turnbull & Culver, 1983); (3) reduction of intra-specific competition (Holldobler & Wilson, 1990); (4) reduction of inter-specific competition (Holldobler & Wilson, 1990); and (5) relocation of seeds to nutrient rich micro-sites (Beattie & Culver, 1983; Petal, 1980; Oostermeijer, 1989; Folgarait, 1998). Ant nests are thought to be nutrient rich micro-sites due to the accumulation of detritus and organic matter (Folgarait, 1998). In this Chapter I studied benefit (5).

Soil Nutrients

The decreased soil moisture content at *M. sabuleti* nests detected in my study may be beneficial for *Viola* germination as these seeds are prone to mildew (Clive Groves, pers comm). It is unlikely that *Viola* species are more abundant at *M. sabuleti* nests compared to non-nests (Chapter 3) due to a change in soil nutrients as the soil chemistry results show no significant differences in nutrients between treatments. Studies by Rice & Westoby (1986) found that levels of available potassium or nitrate were not elevated in the soil near to the roots of emerging myrmecochorous seedlings. Bond et al., (1991) discovered that the soil chemistry of *Anoplolepis* spp did not differ from surrounding soils and that therefore seeds were transported to nutrient poor micro-sites.

These findings are contradictory to previous work which has shown that nests of *M. ruginodis* were higher in plant nutrients and soil moisture than surrounding soils (Gammans, 2005). I hypothesise that the differences in results may be related to the differing colony size of the two species. *Myrmica ruginodis* colonies are significantly larger, containing up to 6560 workers compared to those of *M. sabuleti* which contain a maximum of 2000 workers (Czechowski et al., 2002). However, on my study site *M. sabuleti* colony size rarely exceeds 200 workers (Thomas & Wardlaw, 1992). Activities of larger colonies will intuitively have a greater effect on surrounding soil compared to a smaller colony. However, previous research has shown that even small ant colonies differ from surrounding soils with respect to soil properties; Lydford (1963) as cited in Culver & Beattie (1980). One possible explanation is that the *Myrmica* colonies on these study sites are highly dynamic (Thomas, 1991), and non-nest samples may have been taken from spots that supported colonies in recent years. In addition, the study carried out by Gammans was conducted on Dorset heathland, an ecosystem that is exceedingly poor in nutrients (Webb, 1986), where increases in soil nutrients as a result of ant activities are more likely to be detectable than in acid-grassland habitats.

Soil Seedbank

The total number of species emerging from non-nest soil was greater than that of *M. sabuleti* nest soils. King (1976) also found that the viable seed content of non-nest soils was greater than in *L. flavus* ant mounds.

In my study *V. riviniana* and *Ulex europaeus* were two of the four myrmecochorous seedlings to emerge from the upper sample of soil *M. sabuleti* nests in greater proportions than from the equivalent non-nest soil. Although the results were not statistically significant, *Viola riviniana* abundance in the combined upper and lower nest samples was twice that of the combined non-nest sample. Additionally *V. riviniana* was almost three times more abundant in the upper *M. sabuleti* nest soil compared the control. This indicates that *M. sabuleti* may be gathering *V. riviniana* seeds particularly as there was no significant difference in the number of *V. riviniana* or *V. lactea* plants recorded above the nest spots compared to the non-nest spots immediately prior to soil excavation. *Viola lactea* was present in low numbers in both *M. sabuleti* and non-nest soils. This was surprising, particularly since in the field, *V. lactea* is associated with the warmer niches inhabited by *M. sabuleti* (Chapter 3) and *V. lactea* seeds are attractive to *M. sabuleti* in the laboratory (Chapter 4). Alternatively, the associations reported in Chapter 3 may not be due to ant activity at all, perhaps the vegetation assemblage and the suitability for nesting *M. sabuleti* is determined by the microhabitat features (Elmes & Wardlaw 1982a).

Both the proportions of seedlings and myrmecochorous seedlings were greater in nest-soil compared to non-nest soil on the study site. These findings are inconsistent with those of Dostál (2005) who compared the emergence of seedlings from the soils of *L. flavus*, *Formica* spp. and *Tetramorium caespitum* nests and control plots. Dostál found that control plots contained more seeds than ant mounds and that myrmecochores were most abundant in control plots. This may be due to seed predation, *Tetramorium caespitum* is a granivore (Gammans, 2006).

Seedlings were more numerous in the lower soil samples compared to upper samples for both nests and non-nests. This is inconsistent with the findings of Bullock (1996) who found greater numbers of viable seeds in the upper soil profile. However, I hypothesise that this result may be explained by the dynamic nature of the *Myrmica* species on the study site moving nest site. *Myrmica* species ant nest density on the study site is approximately 0.5 nests per m^2 (Thomas, pers comm.), therefore much of the site is influenced by ant activity. Additionally, Dostal (2005) found that the abundance and diversity of seeds declined with soil depth; however, this trend was not observed in ant mounds due to ant perturbation. He concluded that seed relocation by ants did not contribute significantly to the seedbank at his study site. Conversely my results suggest that *M. sabuleti* does contribute to the seedbank on my study site. The percentage of myrmecochorous seedlings in the upper *M. sabuleti* soil was 7% greater than that of the lower nest soil, whereas non-myrmecochorous seedlings were similarly numerous in both upper and lower portions of nest soil. It is also worth noting that in the upper soil, where ant influence is likely to be greatest, 9% more seedlings of myrmecochorous plants emerged from ant nest samples compared with the non-nest soil. This suggests that the presence of *M. sabuleti* nests may influence myrmecochorous seedling recruitment.

The results of this study are very interesting. In the field both *Viola* spp. are more abundant near to ant nests compared to non-nest spots (Chapter 3). However, the results of this study show that this is not due to relocation of *Viola* seeds to a nutrient rich micro-site. The reduction of intra-specific competition is another theory that can be discounted: in the field *Viola* spp. are more abundant near to ant nests compared to non-nests (Chapter 3). Although the total number of seedlings emerging from non-nest soil compared to *M. sabuleti* nest soil was not significantly greater, the number of species that emerged was. This suggests that inter-specific competition is reduced near to *M. sabuleti* ant nests.

The field data (Chapter 3) indicate that ant nests provide benefits to *Viola* spp. that non-nests do not. Previous work carried out by Hanzawa et al., (1988) and Davidson & Morton (1984) found that seedlings survive better on ant nests compared to non-nests, but so far a definitive explanation has not been reported. I hypothesise that the increased survivorship of seedlings near to ant nests compared to non-nests found by Hanzawa et al., (1988) and Davidson & Morton (1984) is due to worker ants that deter herbivorous insect predators during the seedling stage. The herbivore avoidance hypothesis could be tested in the field using barriers to exclude ground dwelling phytophagous insects, particularly when the plants are in their vulnerable seedling stage; these are likely to be the main herbivores excluded by *Myrmica* workers, which seldom climb more than 1cm above the soil surface (Thomas, 2002). Testing the herbivore avoidance hypothesis may also explain why higher densities of *Viola* spp. were also found near to *Formica fusca* ant nests (Chapter 3), although *F. fusca* did not carry *Viola* spp. seeds in the laboratory (Chapter 4), in the field the worker ants would deter invertebrate herbivores. In addition to this, the microbial properties of both *Myrmica* spp. and *F. fusca* ant nests may benefit the establishment of seedlings near to nests.

Increased levels of bacteria and fungi (Petal, 1978; Jakubczyk et al., 1972) have been found in ant nest soil compared to adjacent soils (Petal, 1978; Jakubczyk et al., 1972). However, processes such as humification are delayed (Petal, 1978). This suggests that the ants are suppressing the activity of Actinomycetales (humifying bacteria) (Petal, 1978). It is therefore reasonable to hypothesise that other bacteria and fungi occurring within the nest are manipulated by the ants. *Viola* spp. are prone to damping off (Clive Groves, pers comm.), soil borne bacteria and pathogens cause seedlings to rot at the soil level (Pedersen et al., 1999). These bacteria and pathogens may be less active within ant nests and therefore damping off is less of a problem for *Viola* spp. germinating near to ant nests thereby increasing *Viola* spp. density at nest sites. Again, this could explain why *Viola* spp. are more abundant near to both *Myrmica* spp. and *F. fusca* ant nests in the field. Investigating the microbial properties of different ant species nests' combined with testing the herbivore avoidance hypothesis is a very interesting avenue for future work.

An alternative hypothesis is that the removal of elaiosomes and scarification of seeds is the key to *Viola* spp. success on ant nests. It has been reported that the effect of scarification and elaiosome removal may increase the permeability of the seed testa to nutrients Mayer (1963) as cited by (Culver and Beattie, 1980). However, the effect of elaiosome removal on germination varies between seeds. Lobstein & Rockwood (1993) found a significant increase in germination rate when elaiosomes were removed from *Sanguinaria canadensis* seeds. Elaiosome removal also increased the germination rate of *Viola striata* by 80%. However, Blois & Rockwood (1986) found that elaiosome removal had no effect on germination of *Viola pensylvanica*. In preliminary trials I found that the removal of elaiosomes increased germination rates in *V. lactea* and *V. riviniana*, but the sample size was too small to detect any statistical significance. In contrast, Connor (2004) found the opposite. In both studies seeds were planted in ericaceous compost, perhaps a repeat experiment using ant nest soil would show more consistent results.

Chapter 6: Oviposition niche preference of *Viola*-feeding fritillary butterflies (*Boloria* spp.) in semi-natural grasslands managed for *Maculinea arion*

6.1 Introduction

The Pearl-bordered Fritillary, *Boloria euphrosyne,* lives in small colonies of up to a few hundred individuals. They occupy early successional habitats within dry open deciduous woodland with a history of continual coppicing, and in broadleaved woodlands with a mosaic of age structures created by clearing and replanting (Greatorex-Davies et al., 1992). In Northern and Western areas of the UK, breeding colonies are often found in well drained rough grasslands with bracken and gorse scrub (Warren, 1992), such as sites W, X and Y.

B. euphrosyne populations have suffered a 60% disappearance in their occupancy of 10km squares in the UK over the past 20 years, while surviving populations are, on average, about 10% the mean size of 1976. This makes it one of Britain's fastest declining butterflies; thus, it is a priority Biodiversity Action Plan species (Asher et al., 2001; Fox et al., 2006). This decline is attributed to habitat loss due to the reduction in traditional coppicing of woodlands and the abandonment of traditional farming on rough grazings. This species is increasingly restricted to rough grazing and scrubby grassland sites that have abundant bracken, gorse and violets, the larval food plant (Barnett & Warren, 1995a). But again these rough grassland sites are becoming unsuitable for *B. euphrosyne* due to changing management practices. On many sites grazing and burning have been abandoned, resulting in a tall rank sward with bracken and gorse domination, which shades out *Viola* plants and creates a build up of litter (Warren & Oates, 1994). Traditionally, bracken was collected and used as animal bedding, an activity that has long since ceased.

The adults of *B. euphrosyne* are univoltine and on the wing from late April until early June. During this time they feed primarily on *Ajuga reptans* (Porter, 1992; Asher et al., 2001). Female *B. euphrosyne* butterflies generally lay their eggs in areas with short vegetation where *Viola* species are abundant (Thomas & Lewington, 1991). Warren and Oates (1994) found *B. euphrosyne* breeding on the edge of Dartmoor where *Viola* cover was between 5-25%, grass species were sparse and the cover of dead bracken was high (40 -100%). In an open coppice habitat in Dorset, *B. euphrosyne* was found to be highly selective in choosing a suitable *Viola* on or near which to deposit an egg (Thomas et al., unpublished). However, Shreeve as cited in Porter (1992) states that *B. euphrosyne* females usually lay their eggs away from *Viola* and do not land on *Viola* plants before selecting an oviposition site. The egg stage of the lifecycle is between 11 and 20 days. *B. euphrosyne* over winters as larvae amongst leaf litter. The requirements of the larval stages of the lifecycle are far more specific than those of the adults. A warm microclimate is essential for *B. euphrosyne* larvae in order for successful development into the pupal stage of their life cycle (Barnett & Warren, 1995a; Thomas, 1989).

Despite being found in a wider variety of habitats compared to *B. euphrosyne*, the Small Pearl-bordered Fritillary, *B. selene*, has suffered a 52% decline in the UK over the past 20 years (Asher et al., 2001; Fox et al., 2006). *B. selene* can inhabit damper grasslands than *B. euphrosyne*, coppiced woodlands and woodland glades and clearings (Thomas et al., unpublished; Barnett & Warren, 1995b; Thomas & Lewington, 1991) Again habitat loss through the reduction of coppice management is responsible for this butterfly's national decline. The abandonment of grazing and burning activities on rough grassland sites has led to scrub, bracken and gorse encroachment and has created unsuitable conditions for the persistence of the butterflies (Warren & Oates, 1994).

B. selene adults are univoltine and are on the wing from mid May until the end of June. They coexist with *B. euphrosyne*, but in coppiced woodlands they select areas for oviposition that are at a slightly later successional stage (Thomas et al., un published; Barnett & Warren, 1995a; Thomas & Lewington, 1991). *B. selene* has been observed depositing eggs on host plants as well as at the base of *Festuca rubra* where *Viola palustris* was growing (Shreeve as cited by Porter 1992). Previous studies of oviposition sites have shown that females lay their eggs where there is 5-30% cover of *Viola*, grass cover of between 0-60% and dead bracken cover of 40-100% (Barnett & Warren, 1995b).

The habitat requirements and management regimes for *B. euphrosyne* and *B. selene* in woodland habitats are relatively well researched (Thomas et al., un published). However, less is known about their requirements in rough grasslands (Greatorex-Davies et al., 1992; Barnett & Warren, 1995b; Barnett & Warren, 1995a). The creation of an early successional niche through targeted management for *M. arion* on the study sites has resulted in an increase in the populations of these two rare fritillaries, *B. euphrosyne* and *B. selene* (Chapter 2). The management practices employed on these sites may be indicative of the niche requirements of these butterflies and could be used on other rough grazing sites.

6.2 Research Aim

On sites X and Y, both fritillary species have increased in contrast to severe national and regional declines, and this has been attributed to both the increase of *V. lactea* and *V. riviniana* at the sites and to the abundance of early successional growth-forms. Since I have already established that *Viola* seeds are ant dispersed (Chapter 4) and *Viola* plants are more abundant near to ant nests compared to non-nest spots (Chapter 3), this Chapter aims to identify the preferred oviposition niche of both *B. euphrosyne* and *B. selene* and to determine whether the increase in these two butterfly species is directly attributable to the increase in *Viola* species and / or to the character of the micro-habitat in which the new *Viola* plants are growing. Oviposition spot data will be compared between *Boloria* species to determine species differences. The hypothesis is that habitat management for *M. arion* creates a scarce niche shared by a guild of scarce of species, and that this is further enhanced by the presence of *Myrmica* ants in the ecosystem.

6.3 Methods

In May 2003, egg laying female *B. euphrosyne* butterflies were followed around site Y; 88 oviposition spots were marked with a cane and labelled. The attributes of the micro-habitats of a random sample of violets from across the violet population on sites were assessed in comparison to oviposition sites; 65 of the former were also marked with a cane and labelled (table 6.1). The characteristics of all selected plants were measured at the time of oviposition, including the random locations, using a circular quadrat of 0.04m² centred on canes. Turf height was recorded at four points within the sampling hoop (Stewart et al., 2001). Percentage cover of *Viola* spp., grasses, dead bracken and bare ground were assessed along with additional data on the number of individuals of *Viola* spp. and size class of the individuals. Size class was determined by the size and growth form of violets, (figure 6.1).

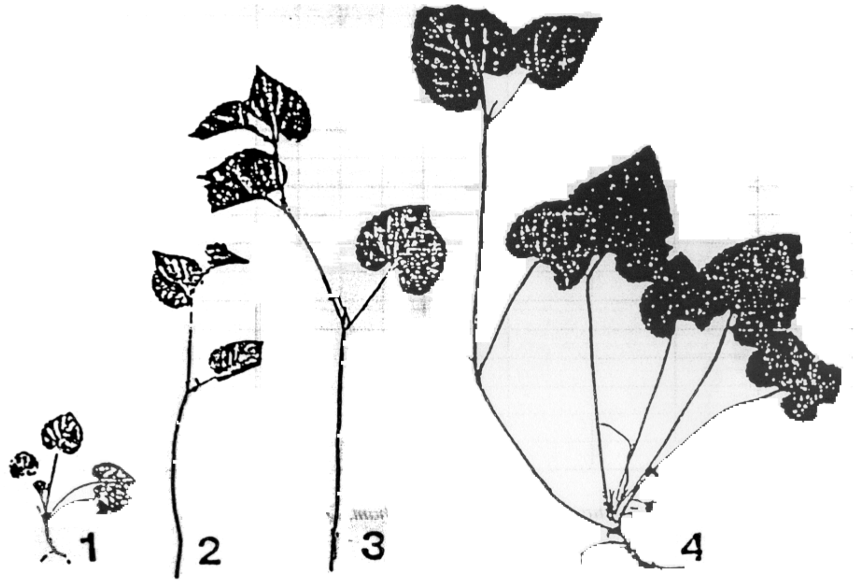


Figure 6.1: Violet size classes – 1. Small leafy compact violet, 2. Erect and less leafy violet, 3. Similar to stage 2 but has larger leaves and is more erect, 4. Larger leaves, sprawling and vigorous violet. The violets illustrated are actual size (Thomas et al., unpublished).

Soil temperature was measured using a temperature probe immediately adjacent to canes at a depth of 1cm. All temperature measurements were recorded on the same day. The location of each oviposition site with regards to current management practices was also recorded. Management on this site is cyclical, and was recorded as follows; 1 – Burnt in current year, 2 – Burnt last year, 3 – Burnt >1<2 years ago, 4 Burnt >2<3 years ago, 5 – Burnt >3 years ago.

In May 2004 *B. euphrosyne* oviposition spot preference was assessed on sites W and Y (table 6.1). On site Y, 52 oviposition spots were marked, while 59 oviposition spots were marked on site W. Fifty random violet locations were assessed on both sites. Sampling methodology followed the same protocol as in 2003.

Twenty two *B. selene* and 21 *B. euphrosyne* oviposition spots were assessed in May 2005 on sites X and Y respectively (table 6.2), to compare and contrast the preferred oviposition niche of each species. Standard ant baiting using sugar cubes was carried out at both *Boloria* species' oviposition spots to ascertain whether there was any niche association between *Myrmica* spp. and *Boloria* spp.

6.3.1 Statistical analysis

Data were transformed to meet the requirements of the principal component analysis. The following transformations were applied: arcsine-square root for percentage cover variables; Log_{10} for mean vegetation height and $\mathrm{Log}_{10}+1$ for violet size class. Principal component analysis (PCA) was used to reduce the variables collected into a smaller number of components that describe the underlying dimensions of the data (Pernetta et al., 2005). Principal Components with an eigen value of ≥ 1 were used in further analysis (Pernetta et al., 2005). Correlations of the original values to the PC values greater that 0.4 were considered important (Field, 2000). Analysis of Variance (ANOVA) was used to compare the resultant PC scores between *B. euphrosyne* oviposition spots with random violet sampling locations; *B. euphrosyne* oviposition spots between sites; and *B. euphrosyne* oviposition spots between years. *B. euphrosyne* and *B. selene* oviposition spots were also compared using ANOVA.

6.4 Results

6.4.1 *B. euphrosyne* oviposition spots versus random sampling locations, site Y 2003.

On site Y in 2003, microhabitat variables were recorded for a total of 88 oviposition spots and 65 random locations (table 6.1). Eleven variables were analyzed using principal component analysis; the four factors produced cumulatively explained 69.4% of the variance within the data (table 6.3). The PCA excluded missing values from the analysis and therefore data for 84 oviposition spots and 56 random locations were analysed. The first PC was negatively correlated with live vegetation cover (table 6.3). Oviposition spots occurred in areas where live vegetation cover was low compared to random locations. Positive principal component 2 scores indicated sites that had taller vegetation (figure 6.2). Negative scores were indicative of sites with high numbers of size class 2 violets. The third principal component was positively correlated with percentage cover of dead bracken and negatively correlated with percentage cover of grass (table 6.3 and figure 6.3). The fourth principal component was positively correlated with percentage cover of bare ground and numbers of size class 4 violets. There was a negative correlation with percentage cover of dead bracken (table 6.3).

Table 6.1: Mean (± S.E.) of each microhabitat variable recorded for *B. euphrosyne* oviposition spots and random locations.

			Percentage Cover					Violet Size Class				
	N	Vegetation height (cm)	Bare ground	Dead Bracken	Live Vegetation	Grass	Violets	1	2	3	4	Soil Temperature °C
Site Y 2003												
Random	65	175.87 ± 31.92	5.06 ± 1.36	4.23 ± 1.02	71.07 ± 4.38	33.26 ± 3.72	10.39 ± 1.66	0.42 ± 0.14	0.72 ± 0.15	0.89 ± 0.18	2.12 ± 0.24	20.74 ± 0.5
Oviposition	88	3.26 ± 0.22	13.06 ± 2.25	18.99 ± 2.39	53.15 ± 3.64	18.97 ± 2.40	4.47 ± 0.70	1.88 ± 0.24	1.72 ± 0.22	0.70 ± 0.12	0.81 ± 0.15	25.23 ± 0.5
Site Y 2004												
Random	50	126.52 ± 34.03	5.01 ± 1.51	6.29 ± 2.27	65.4 ± 4.66	29.6 ± 4.16	10.20 ± 1.32	1.92 ± 0.36	2.74 ± 0.6	0.94 ± 0.20	0.5 ± 0.12	27.75 ± 1.06
Oviposition	52	6.49 ± 1.22	4.51 ± 1.19	53.5 ± 5.11	27.01 ± 2.76	7.89 ± 2.03	4.94 ± 1.17	5.32 ± 1.57	1.14 ± 0.26	0.18 ± 0.07	0.06 ± 0.03	26.39 ± 0.67
Site W 2004												
Random	50	103.45 ± 31.13	23.8 ± 3.99	25.03 ± 4.27	39.1 ± 3.52	8.15 ±2 .48	18.21 ± 2.59	2.26 ± 0.43	1.37 ± 0.24	0.92 ± 0.25	0.74 ± 0.14	30.90 ± 1.19
Oviposition	59	12.07 ± 1.46	15.08 ± 2.9	37.42 ± 3.46	33.13 ± 3.15	0.90 ± 0.40	10.23 ± 1.23	1.25 ± 0.27	2.05 ± 0.26	1.91 ± 0.25	0.37 ± 0.08	25.94 ± 0.79

Table 6.2 Mean (± S.E.) of each microhabitat variable recorded for *B. euphrosyne* oviposition spots on site Y and *B. selene* oviposition spots on site X in 2005

Species			Percentage Cover					Violet Size Class				
	N	Vegetation height (cm)	Bare ground	Dead Bracken	Live Vegetation	Grass	Violets	1	2	3	4	Soil Temperature °C
B. euphrosyne	21	4.95 ± 2.73	35.95 ± 3.61	22.14 ± 4.47	42.14 ± 5.43	4.76 ± 1.12	19.52 ± 1.92	1.67 ± 0.20	2.14 ± 0.24	0.48 ± 0.16	0.00 ± 0.00	17.28 ± 0.14
B. selene	22	6.00 ± 0.64	5.91 ± 3.13	64.32 ± 6.32	29.32 ± 5.20	0.91 ± 0.53	13.41 ± 2.36	0.14 ± 0.14	0.55 ± 0.24	2.68 ± 0.51	0.09 ± 0.09	15.58 ± 0.16

Table 6.3: Principal component loadings, eigenvalues and percentage of variance explained by the first five PCA components on the microhabitat variables recorded on site Y in 2003. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000), and are presented in bold.

	Principal component loadings			
	1	2	3	4
Eigenvalue	3.0908	1.7331	1.6126	1.1914
Soil temperature° C	0.339	-0.227	-0.29	0.351
Vegetation height /cm	-0.272	0.471	0.184	-0.078
Dead bracken (% cover)	0.079	-0.27	**0.419**	**-0.501**
Live vegetation (% cover)	**-0.4**	-0.303	-0.333	-0.043
Grass (% cover)	-0.27	-0.191	**-0.58**	-0.161
Violets (% cover)	-0.364	-0.146	0.383	0.393
Bare ground (% cover)	0.398	0.186	0.018	**0.418**
Number of size class 1 violets	0.283	-0.368	0.065	0.068
Number of size class 2 violets	0.025	**-0.544**	0.239	0.083
Number of size class 3 violets	-0.246	-0.197	0.224	0.095
Number of size class 4 violets	-0.373	0.011	0.03	**0.491**
Percentage of variance explained	28.1	15.8	14.7	10.8

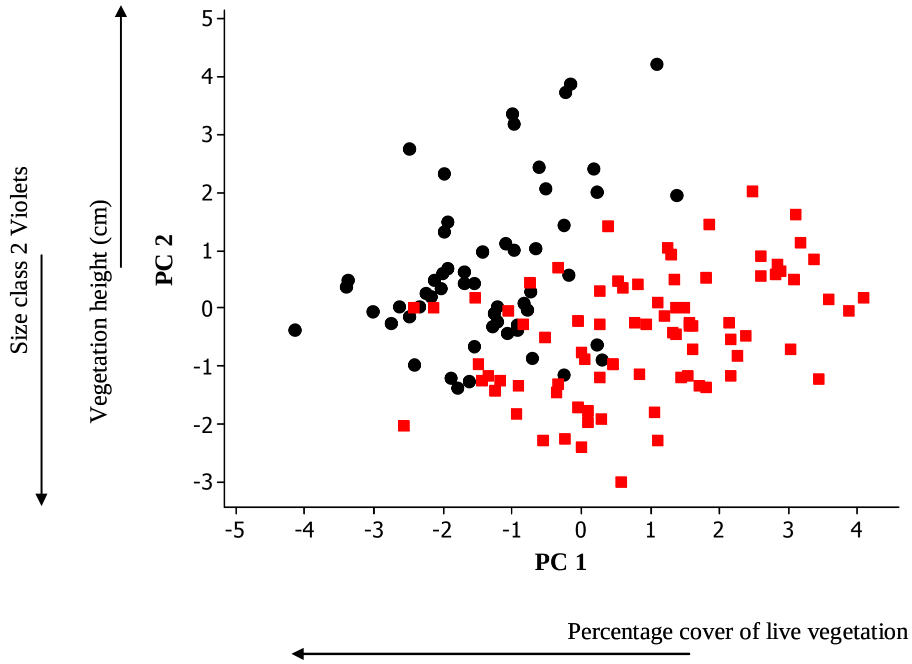


Figure 6.2: The relationship between PC1 and PC2 scores for 84 *B. euphrosyne* oviposition spots (🟥) and 56 random locations (●) on site Y in 2003

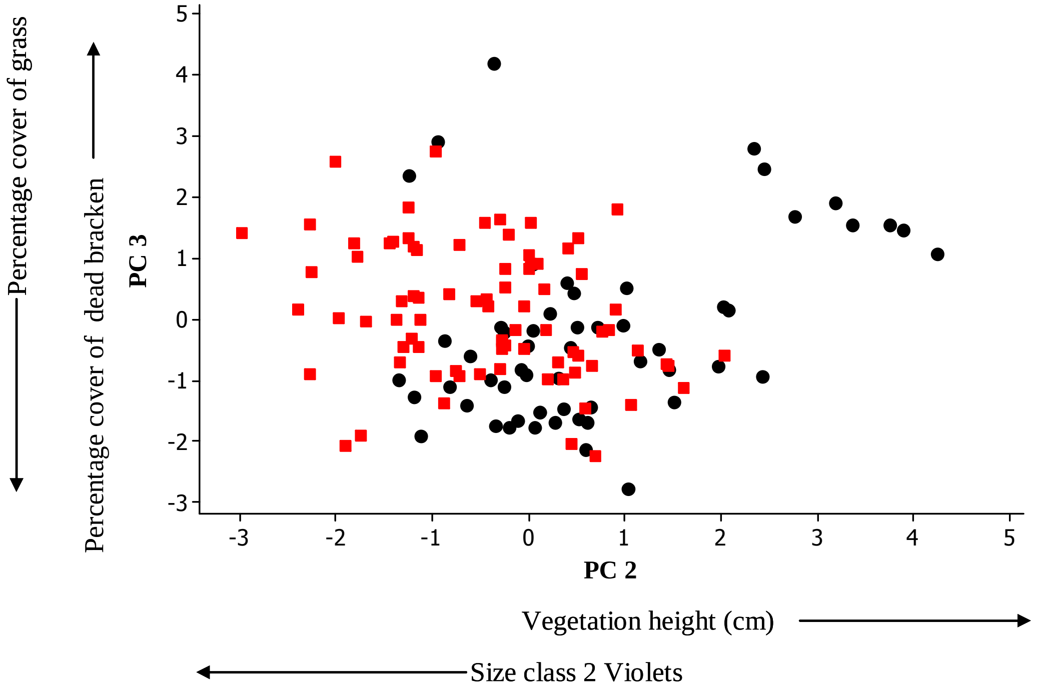


Figure 6.3: The relationship between PC2 and PC3 scores for 84 *B. euphrosyne* oviposition spots (🟥) and 56 random locations (●) on site Y in 2003

To assess whether *B. euphrosyne* oviposition spots were different to random violet locations the resultant PC scores were compared using Analysis of Variance (table 6.4). *B. euphrosyne*'s oviposition spots were significantly different to random locations for PC 1 ($F_{1, 138} = 90.40$, $P < 0.001$) and PC 2 ($F_{1, 138} = 30.20$, $P < 0.001$). However, there were no significant differences between oviposition spots and random locations for PC 3 ($F_{1, 138} = 3.26$, $P = 0.073$) and PC 4 scores ($F_{1, 138} = 1.87$, $P = 0.174$).

These results show that the microhabitat at *B. euphrosyne* oviposition spots consist of short vegetation that has a low coverage of live vegetation combined with high numbers of size class 2 violets compared to random violet locations.

Table 6.4: Mean Principal Component Scores (± S.E.) of *B. euphrosyne* and *B. selene* oviposition spots and random violet locations recorded in 2003, 2004 and 2005 on 3 adjacent sites in Devon.

		Mean ± S.E.			
Site Y 2003	***N***	**PC 1**	**PC 2**	**PC 3**	**PC 4**
B. euphrosyne oviposition spot	84	0.90 ± 0.17	0.68 ± 0.12	0.16 ± 0.12	-0.10 ± 0.12
Random spot	56	-1.35 ± 0.15	-0.45 ± 0.18	-0.24 ± 0.19	0.15 ± 0.14
Site Y 2004					
B. euphrosyne oviposition spot	46	0.97 ± 0.21	0.82 ± 0.09	0.06 ± 0.17	N/A
Random spot	48	-0.93 ± 0.22	-0.79 ± 0.26	-0.05± 0.17	N/A
Site W 2004					
B. euphrosyne oviposition spot	44	-0.50 ± 0.18	-0.42 ± 0.16	0.33 ± 0.13	0.12 ±0.12
Random spot	52	0.59 ± 0.27	0.49 ± 0.25	-0.39 ± 0.24	-0.14 ± 0.22
***B. euphrosyne* oviposition v oviposition**					
Site Y 2003	84	-0.45 ± 0.19	-0.37 ± 0.15	0.32 ± 0.12	-0.02 ± 0.13
Site Y 2004	46	0.81± 0.17	0.67 ± 0.23	-0.59 ± 0.17	0.04 ± 0.12
***B. euphrosyne* oviposition v oviposition**					
Site Y 2004	46	0.45 ± 0.25	0.45 ± 0.17	0.77 ± 0.14	0.18 ± 0.14
Site W 2004	41	-0.50 ± 0.22	-0.50 ± 0.26	-0.86 ± 0.21	-0.21 ± 0.17
***B. euphrosyne* v *B. selene* oviposition**					
B. euphrosyne	21	1.9 ± 0.28	0.41 ± 0.25	-0.10 ± 0.19	N/A
B. selene	22	-1.82 ± 0.29	-0.39 ± 0.32	0.10 ± 0.26	N/A

6.4.2 *B. euphrosyne* oviposition spots versus random sampling locations, site Y 2004.

On site Y in 2004 micro habitat variables were recorded for a total of 52 oviposition spots and 50 random locations (table 6.1) although owing to missing values data from 46 oviposition spots and 48 random locations were included in the analysis. The same variables as in 2003 were analyzed using principal component analysis; the three factors produced cumulatively explained 62.4% of the variance within the data (table 6.5). Principal Component 1 scores were positively correlated with the percentage of dead bracken and negatively correlated with live vegetation and grass cover (figure 6.5). Negatively correlated Principal Component 2 scores indicated sites with high numbers of size class 4 violets and increased vegetation height (figure 6.5). The third principal component was positively correlated with percentage cover of violets and increasing numbers of size class 1 violets and negatively correlated with percentage cover of grass.

Table 6.5: Principal component loadings, eigenvalues and percentage of variance explained by the first three PCA components on the microhabitat variables recorded on site Y in 2004. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000), and are presented in bold.

	Principal component loadings		
	1	2	3
Eigenvalue	3.0644	2.4376	1.358
Soil temperature ° C	-0.293	0.304	-0.135
Vegetation height /cm	0.157	**-0.509**	0.113
Dead bracken (% cover)	**0.409**	0.255	-0.106
Live vegetation (% cover)	**-0.45**	-0.191	-0.328
Grass (% cover)	**-0.44**	0.023	**-0.422**
Violets (% cover)	-0.301	-0.22	**0.545**
Bare ground (% cover)	-0.231	0.324	0.348
Number of size class 1 violets	-0.159	0.315	**0.481**
Number of size class 2 violets	-0.363	0.031	0.046
Number of size class 3 violets	-0.156	-0.316	0.036
Number of size class 4 violets	-0.021	**-0.439**	0.14
Percentage of variance explained	27.9	22.2	12.3

Analysis of Variance of the PC1 and PC 2 scores between *B. euphrosyne* oviposition spots and random locations reveals a significant difference ($F_{1,92} = 39.15$, $P < 0.001$) and ($F_{1,92} = 33.57$, $P < 0.001$) respectively. There was no significant difference in PC 3 scores between *B. euphrosyne* oviposition spots and random locations ($F_{1,92} = 0.20$, $P = 0.653$) (table 6.4).

These results again showed that *B. euphrosyne* oviposition spots coincide with areas where live vegetation is sparser, shorter, size class 4 violets are fewer and percentage cover of dead bracken is higher than random violet locations.

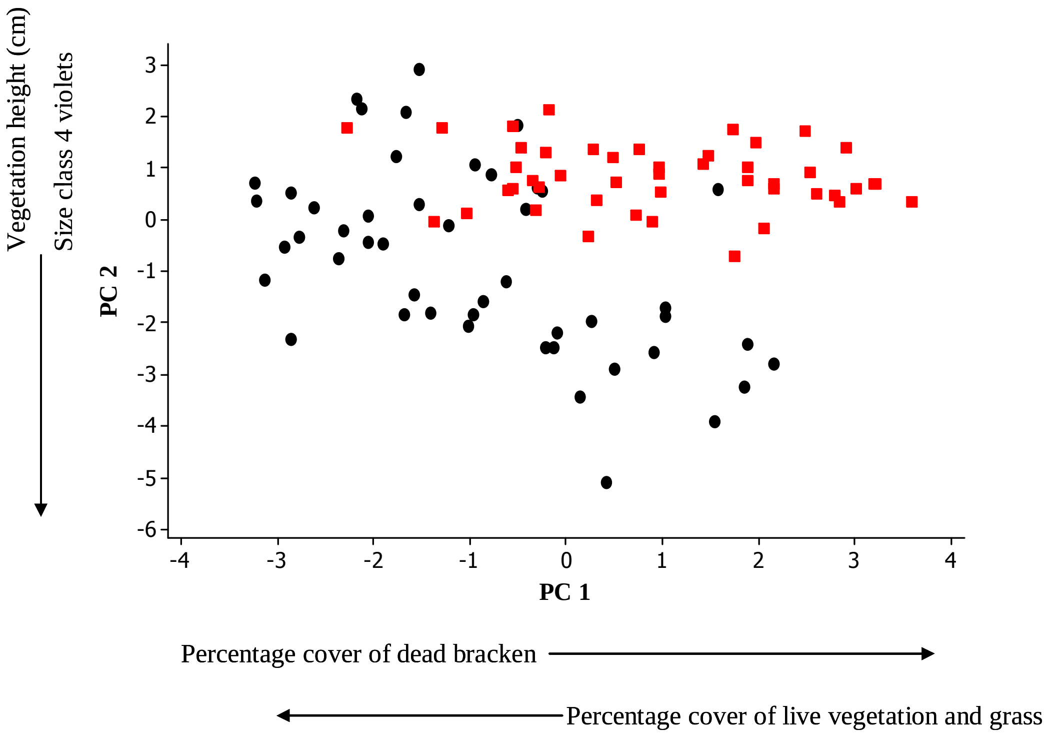


Figure 6.5: The relationship between PC1 and PC2 scores for 46 *B. euphrosyne* oviposition spots (🟥) and 48 random locations (●) on site Y in 2004.

6.4.3 *B. euphrosyne* oviposition spots on site Y years 2003 and 2004

The microhabitat variables for 88 oviposition spots recorded in 2003, and 52 oviposition spots recorded in 2004 on site Y were next analysed in combination using PCA. Again missing values were excluded and therefore 84 2003 oviposition spots and 46 2004 oviposition spots were analysed (table 6.1). The four principal components with eigen values greater than 1 explained 70.1% of the variation within the data (table 6.6).

Table 6.6: Principal component loadings, eigen values and percentage of variance explained by the first four PCA components on the microhabitat variables recorded on site Y in 2003 and 2004. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000), and are presented in bold.

	Principal component loadings			
	1	2	3	4
Eigenvalue	2.803	2.3487	1.3899	1.168
Soil temperature ° C	0.136	-0.328	0.17	-0.2
Vegetation height /cm	-0.083	**0.535**	-0.054	0.032
Dead bracken (% cover)	0.197	**0.483**	-0.235	-0.233
Live vegetation (% cover)	**-0.512**	0.011	0.071	0.27
Grass (% cover)	-0.378	-0.086	0.241	**0.539**
Violets (% cover)	**-0.419**	-0.086	**-0.45**	-0.267
Bare ground (% cover)	0.198	**-0.506**	0.093	-0.171
Number of size class 1 violets	-0.027	-0.258	**-0.66**	0.157
Number of size class 2 violets	-0.277	-0.155	-0.301	-0.14
Number of size class 3 violets	-0.35	0.095	0.22	**-0.402**
Number of size class 4 violets	-0.34	-0.043	0.252	**-0.484**
Percentage of variance explained	25.5	21.4	12.6	10.6

The percentage cover of live vegetation and percentage cover of violets were negatively correlated with PC 1. Vegetation height and percentage cover of dead bracken were positively correlated with PC 2; there was a negative correlation with bare ground (figure 6.6). Principal Component 3 scores were negatively correlated with size class 1 violets and the percentage cover of violets (figure 6.6 & 6.7). The percentage cover of grass was positively correlated with PC 4. Violets of size classes 3 and 4 were negatively correlated with PC 4.

Figure 6.7 indicates that oviposition spots in 2004 had low percentage cover of live vegetation which consisted primarily of size class 1 violets. In 2003 however, oviposition spots were higher in live vegetation cover, with fewer size class 1 violets.

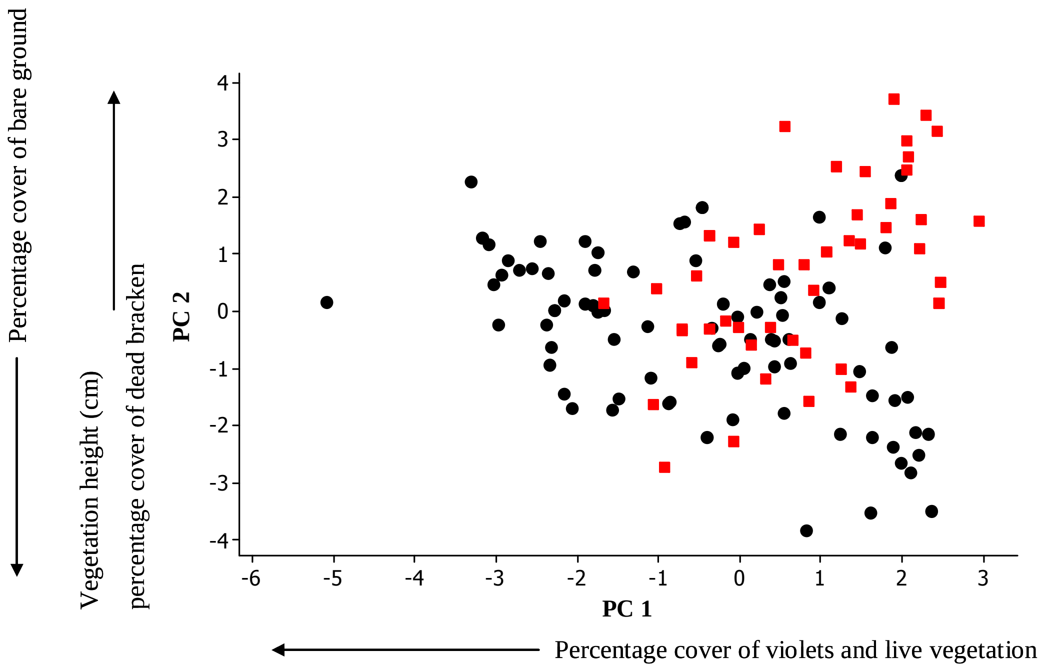


Figure 6.6: The relationship between PC 1 and PC 2 scores for 84 *B. euphrosyne* oviposition spots on site Y in 2003 (●) and 46 *B. euphrosyne* oviposition spots on site Y in 2004 (🟥).

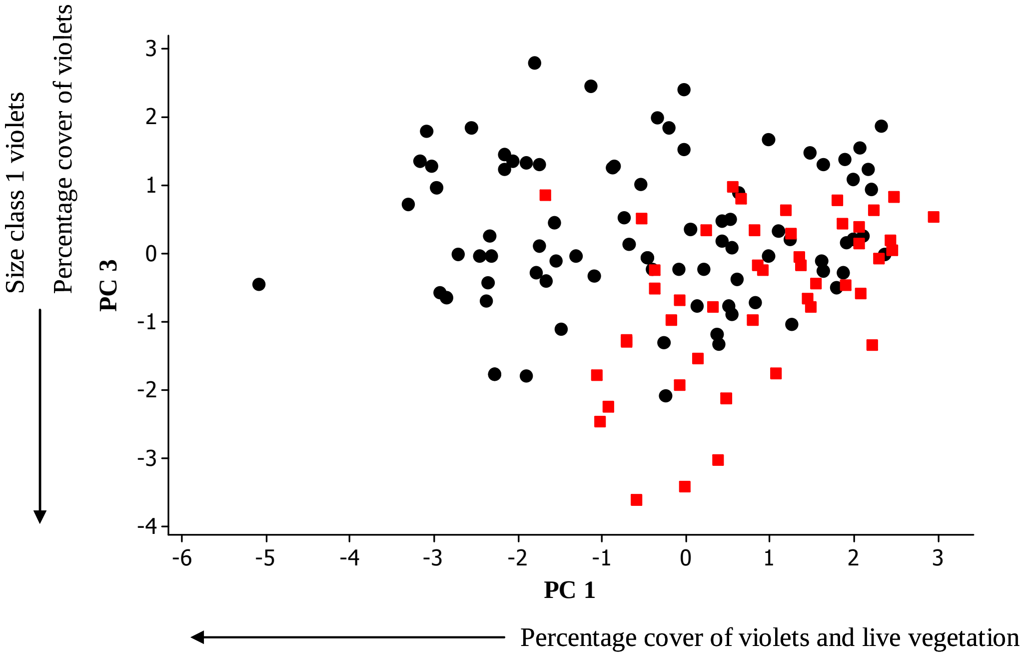


Figure 6.7: The relationship between PC 1 and PC 3 scores for 84 *B. euphrosyne* oviposition spots on site Y in 2003 (●) and 46 *B. euphrosyne* oviposition spots on site Y in 2004 (🟥)

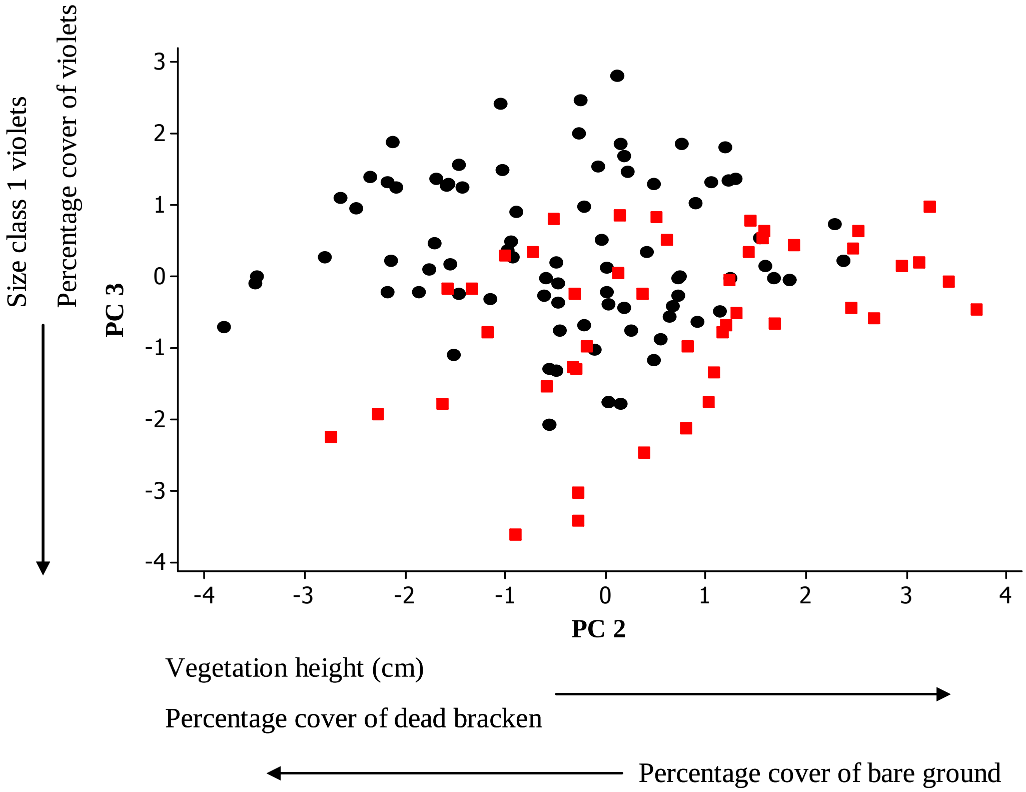


Figure 6.8: The relationship between PC 2 and PC 3 scores for 84 *B. euphrosyne* oviposition spots on site Y in 2003 (●) and 46 *B. euphrosyne* oviposition spots on site Y in 2004 (🟥)

Analysis of variance comparing PC 1 ($F_{1,128} = 19.16$, $P < 0.001$), PC 2 ($F_{1,128} = 15.14$, $P < 0.001$) and PC 3 scores for *B. euphrosyne* oviposition spots in 2003 and 2004 differ significantly ($F_{1,128} = 20.37$, $P < 0.001$). Between years there was no significant difference in PC 4 scores ($F_{1,128} = 0.08$, $P = 0.780$).

These results show that *B. euphrosyne* oviposition spots were significantly different between years. In 2003 oviposition spots had a high percentage cover of short live vegetation, high bare ground coverage and low numbers of size class 1 violets and low percentage cover of violets overall. Conversely in 2004 vegetation at oviposition spots was taller yet sparser and consisted of high numbers of size class 1 violets. Possible explanations for these differences can be found in section 6.5.

6.4.4 *B. euphrosyne* oviposition spots versus random sampling locations, site W 2004.

In 2004, 59 oviposition spots and 50 random spots were assessed on site W. Of these, 46 oviposition and 48 random spots were analysed by PCA owing to missing values. The variables recorded were the same as those recorded on site Y in 2004 and 2003, and were again compared using Principal Component Analysis. Four factors had eigen values greater than 1 and these explained 72.6% of the variance within the data (table 6.7).

Table 6.7: Principal component loadings, eigen values and percentage of variance explained by the first four PCA components on the microhabitat variables recorded on site W in 2004. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000) and are presented in bold.

	Principal component loadings			
	1	2	3	4
Eigen value	2.6606	2.1778	1.7603	1.3878
Soil temperature ° C	**0.51**	0.059	0.004	0.228
Vegetation height /cm	-0.368	0.059	-0.356	-0.23
Dead bracken (% cover)	-0.238	**-0.444**	0.064	0.33
Live vegetation (% cover)	-0.176	**0.601**	-0.065	-0.015
Grass (% cover)	0.084	**0.423**	0.195	**-0.421**
Violets (% cover)	0.116	0.371	-0.138	**0.6**
Bare ground (% cover)	**0.477**	-0.134	-0.104	0.033
Number of size class 1 violets	0.362	0.151	0.263	-0.016
Number of size class 2 violets	-0.203	0.151	**0.557**	0.14
Number of size class 3 violets	-0.312	0.144	0.222	**0.442**
Number of size class 4 violets	-0.012	0.185	**-0.607**	0.179
Percentage of variance explained	24.2	19.8	16	12.6

Principal Component 1 scores were positively correlated with soil temperature and percentage cover of bare ground (table 6.7 & figure 6.9). A positive correlation was apparent for live vegetation and grass cover for Principal Component 2 scores (figure 6.9). Percentage cover of dead bracken was negatively correlated with PC 2. Numbers of size class 2 violets were positively correlated with PC 3; whereas numbers of size class 4 violets were negatively correlated with PC 3 (figure 6.10 and 6.11). The fourth principal component scores were positively correlated with percentage cover of violets; increasing numbers of size class 3 violets and negatively correlated with percentage cover of grass.

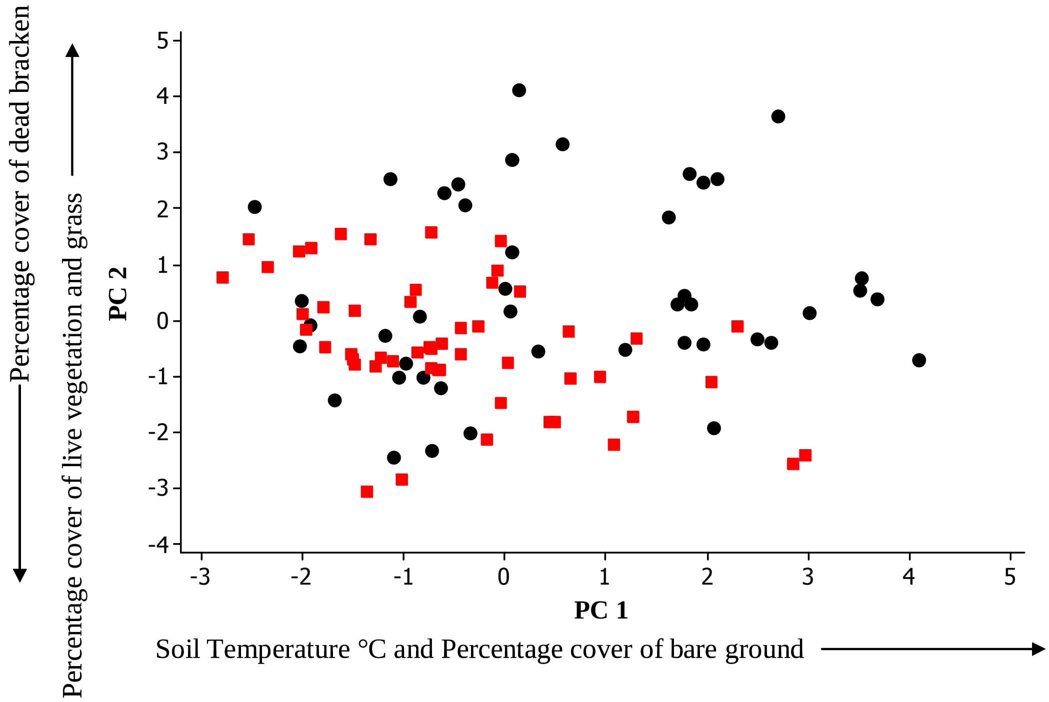


Figure 6.9: The relationship between PC 1 and PC 2 scores for 46 *B. euphrosyne* oviposition spots (🟥) and 48 random locations (●) on site W in 2004

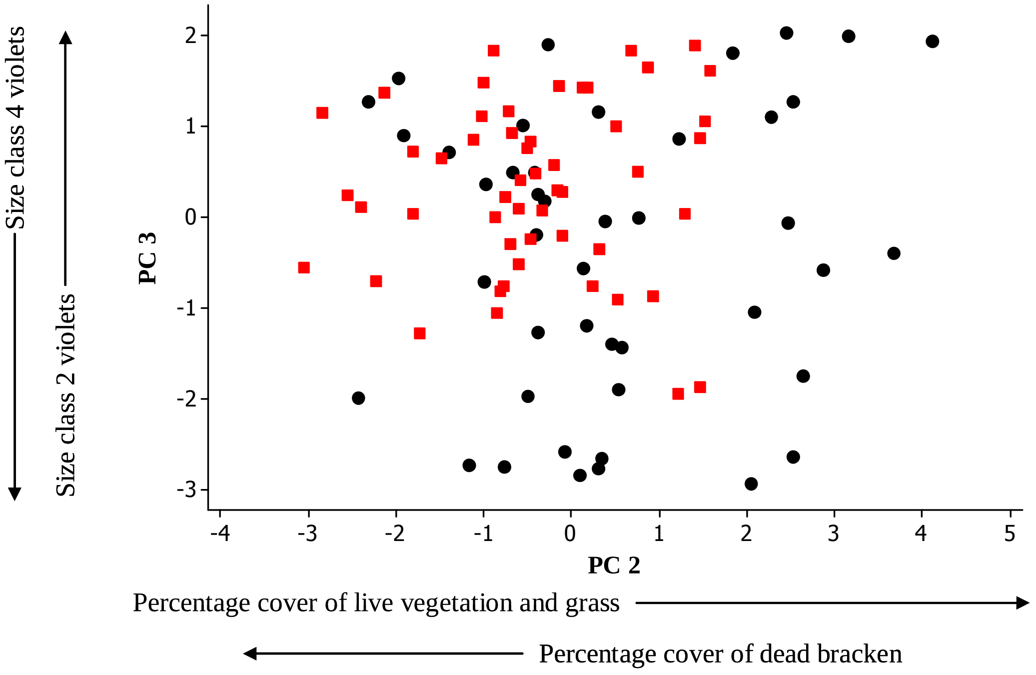


Figure 6.10: The relationship between PC 2 and PC 3 scores for 46 *B. euphrosyne* oviposition spots (🟥) and 48 random locations (●) on site W in 2004

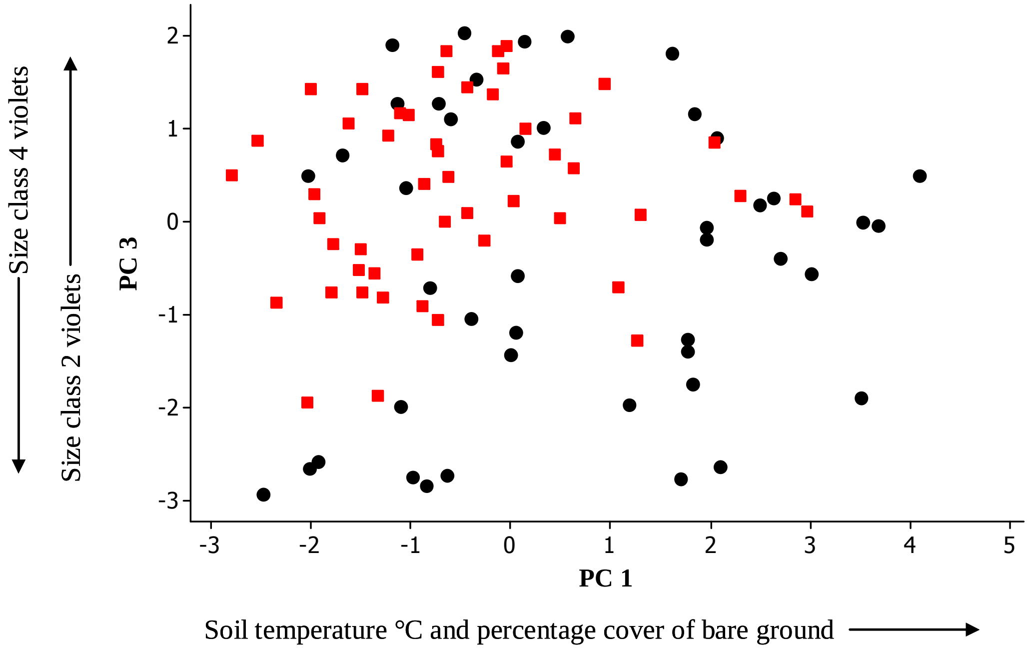


Figure 6.11: The relationship between PC 1 and PC 3 scores for 46 *B. euphrosyne* oviposition spots (🟥) and 48 random locations (●) on site W in 2004

The PC scores were analysed using one way ANOVA to compare *B. euphrosyne* oviposition spots and random violet scores. Oviposition spots and random location scores differed significantly for PC 1 ($F_{1,94} = 11.96$, $P = 0.001$), PC 2 ($F_{1,94} = 9.99$, $P = 0.002$) and PC 3 ($F_{1,94} = 7.37$, $P = 0.008$). There was no significant difference between oviposition spots and random violets for PC 4 ($F_{1,94} = 1.22$, $P = 0.273$).

B. euphrosyne oviposition spots occurred in areas that were significantly cooler, had higher numbers of size class 2 and higher percentage cover of dead bracken than random spots. These results appear to contradict those found for site Y in 2003 and 2004 where oviposition spots occurred in micro niches that had short sparse vegetation, where soil temperature is likely to be warmer compared to random violet locations (see Discussion).

6.4.5 *B. euphrosyne* oviposition spots on site Y 2004 and site W 2004.

Principal component analysis was performed on data collected in 2004 from 46 *B. euphrosyne* oviposition spots on site Y and 44 *B. euphrosyne* oviposition spots on site W (table 6.1). The four principal components generated explained 71.1% of variance within the data (table 6.7). Principal component 1 was positively correlated with percentage cover of dead bracken (table 6.6 & figure 6.12) whereas percentage cover of live vegetation and violets had a negative relationship with PC 1. Vegetation height was negatively correlated with PC 2. Percentage cover of grass was positively correlated with PC 3. Soil temperature and percentage cover of bare ground were both negatively correlated with PC 3. Increasing numbers of size class 2 violets were negatively correlated with PC 4; whereas high numbers of size class 4 violets had a positive relationship (figure 6.13).

Table 6.7: Principal component loadings, eigenvalues and percentage of variance explained by the first four PCA components on the microhabitat variables recorded on sites Y and W in 2004. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000) and are presented in bold.

	Principal component loadings			
	1	2	3	4
Eigenvalue	2.6602	2.2032	1.9543	1.0062
Soil temperature ° C	0.074	0.338	**-0.446**	-0.044
Vegetation height /cm	-0.047	**-0.473**	0.168	-0.392
Dead bracken (% cover)	**0.445**	-0.31	0.075	0.11
Live vegetation (% cover)	**-0.482**	-0.087	0.27	-0.042
Grass (% cover)	-0.199	0.317	**0.409**	0.064
Violets (% cover)	**-0.466**	-0.14	-0.232	0.207
Bare ground (% cover)	-0.107	0.307	**-0.508**	-0.254
Number of size class 1 violets	-0.249	0.325	0.261	0.387
Number of size class 2 violets	-0.36	-0.021	-0.013	**-0.546**
Number of size class 3 violets	-0.281	-0.38	-0.266	0.089
Number of size class 4 violets	-0.151	-0.305	-0.278	**0.513**
Percentage of variance explained	24.2	20	17.8	9.1

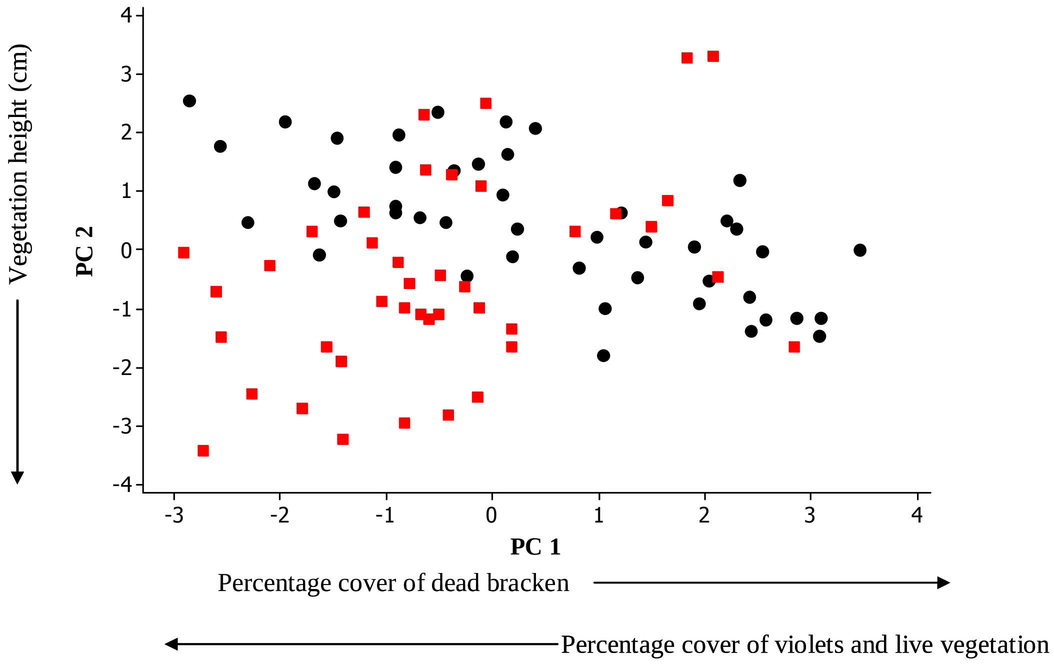


Figure 6.12: The relationship between PC 1 and PC 2 scores for 46 *B. euphrosyne* oviposition spots on site Y in 2004 (●) and 41 *B. euphrosyne* oviposition spots on site W in 2004 (🟥)

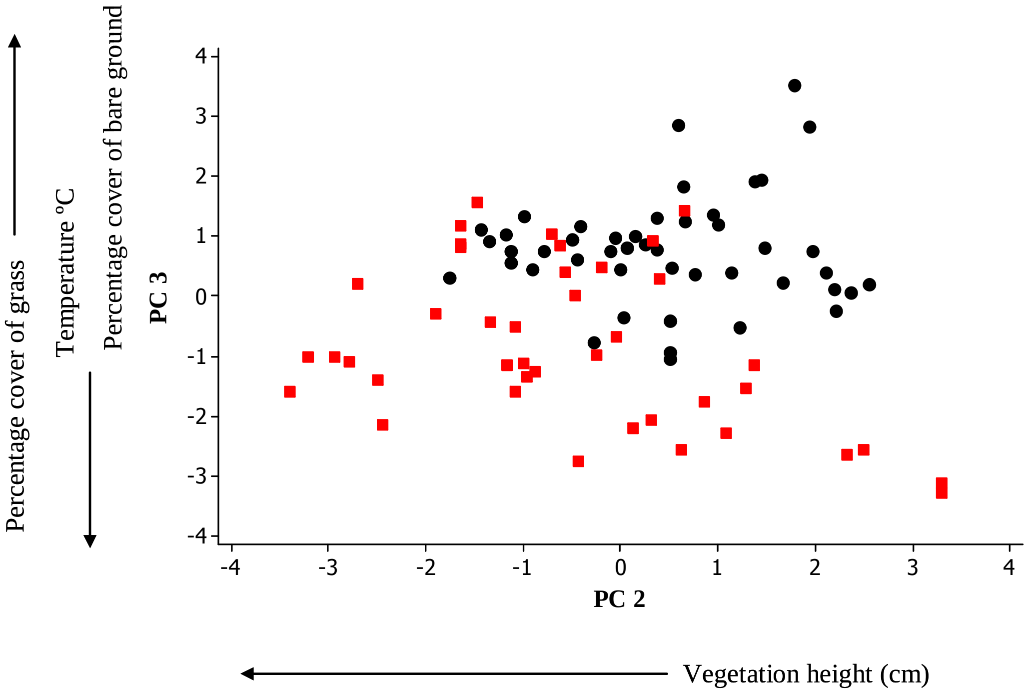


Figure 6.13: The relationship between PC 2 and PC 3 scores for 46 *B. euphrosyne* oviposition spots on site Y in 2004 (●) and 41 *B. euphrosyne* oviposition spots on site W in 2004 (🟥).

The resultant PC scores were compared between sites Y and W to determine if *B. euphrosyne* oviposition spots differed between sites. One way ANOVA shows that the PC 1 scores ($F_{1, 85} = 7.92$, $P = 0.006$); PC 2 scores ($F_{1, 85} = 9.82$, $P = 0.002$) and PC 3 scores ($F_{1, 85} = 44.71$, $P < 0.001$) differ significantly between sites. There was no significant difference in PC 4 scores ($F_{1, 85} = 3.41$, $P = 0.068$), this indicates that oviposition occurs near to size class 2 violets on both sites. These results suggest that *B. euphrosyne* oviposition on site Y occurs where there is a high percentage cover of dead bracken and a low percentage cover of live vegetation and violets. Conversely oviposition on site W occurs where there is less dead bracken and higher percentage cover of taller live vegetation.

6.4.6 *B. euphrosyne* oviposition spots on site Y in 2005 compared to *B. selene* oviposition spots on site X in 2005.

In 2005 the microhabitat was assessed at 21 *B. euphrosyne* and 22 *B. selene* oviposition spots (table 6.2). Three principal components with eigenvalues greater than 1 were generated by the PCA (table 6.8). Cumulatively they explain 75.4% of the variance within the data. Almost 50% of the variance is explained by PC 1 alone. However, none of the microhabitat variables have absolute component values greater than 0.4. Despite this the PC 1 scores do indicate a trend. The variables that have the greatest positive influence on PC 1 are size class 1 and 2 violets, percentage cover of bare ground and soil temperature º C. Dead bracken has the strongest negative correlation with PC 1 (table 6.8 & figure 6.14). Violets of size classes 3 and 4, percentage cover of violets and percentage cover of live vegetation were negatively correlated with PC 2. Violets of size class 4 were negatively correlated with PC 3 scores.

Table 6.8: Principal component loadings, eigenvalues and percentage of variance explained by the first three PCA components on the microhabitat variables recorded at *B. euphrosyne* and *B. selene* oviposition spots on sites X and Y in 2005. Component loadings with absolute values greater than 0.4 are considered important (Field, 2000) and are presented in bold.

	Principal component loadings		
	1	2	3
Eigenvalue	5.2584	1.896	1.1495
Soil temperature ° C	0.308	0.078	-0.275
Vegetation height /cm	-0.278	-0.248	0.383
Dead bracken (% cover)	-0.392	0.112	-0.098
Live vegetation (% cover)	0.242	**-0.419**	0.323
Grass (% cover)	0.303	-0.182	0.374
Violets (% cover)	0.268	**-0.433**	-0.201
Bare ground (% cover)	0.365	0.263	-0.044
Number of size class 1 violets	0.369	0.044	0.175
Number of size class 2 violets	0.37	-0.041	-0.051
Number of size class 3 violets	-0.21	**-0.514**	**-0.642**
Number of size class 4 violets	-0.04	**-0.431**	-0.19
Percentage of variance explained	47.8	17.2	10.4

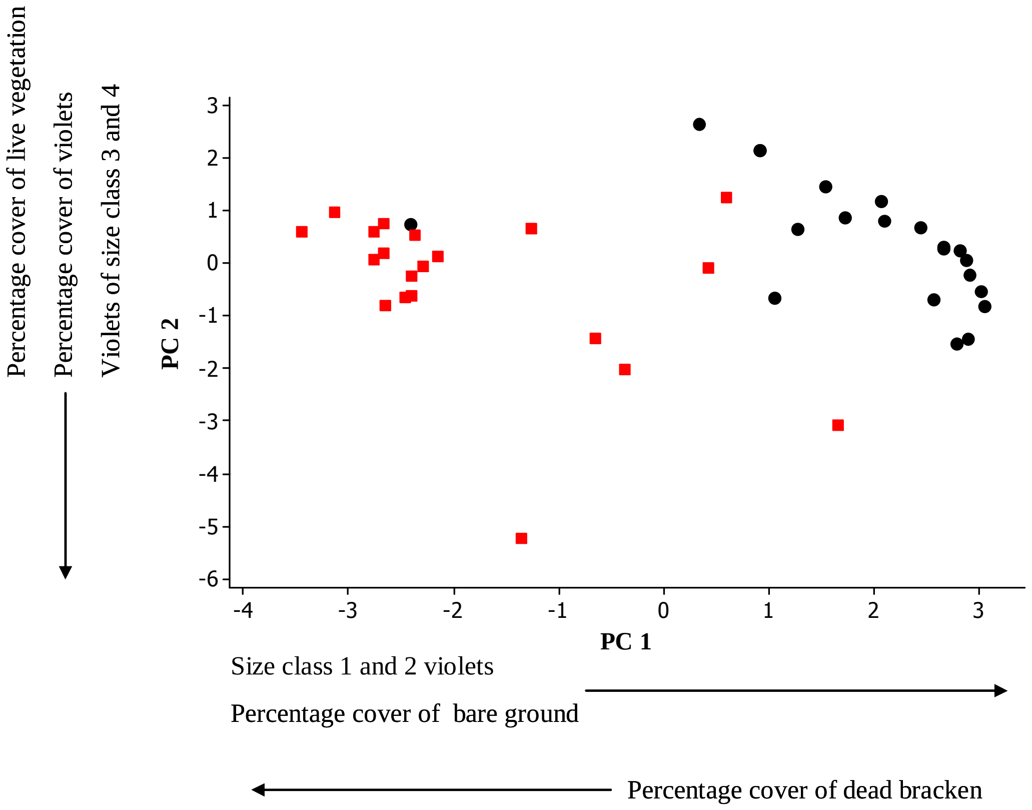


Figure 6.14: The relationship between PC 1 and PC 2 scores for 22 *B. selene* oviposition spots on site X in 2005 (🟥) and 21 *B. euphrosyne* oviposition spots on site Y in 2005 (●). N.B: None of the PC 1 variable scores have an absolute value >0.4, however the variables included on the PC 1 axis have the strongest influence and therefore illustrate a trend.

Comparing PC scores for *B. euphrosyne* and *B. selene* oviposition spots using one way ANOVA show that only PC 1 scores differ significantly ($F_{1, 41} = 86.28$, $P < 0.001$). There was no significant difference in PC 2 ($F_{1, 41} = 3.93$, $P = 0.054$) or PC 3 ($F_{1, 41} = 0.36$, $P = 0.555$) scores.

B. euphrosyne oviposition spots coincide with high numbers of size class 1 and 2 violets where there is a low percentage cover of dead bracken. Conversely, *B. selene* oviposition spots occur where the are fewer violets of size class 1 and 2; dead bracken is abundant and percentage cover of bare ground is low.

The ant baits at *B. euphrosyne* oviposition spots had 100% attendance by *M. sabuleti*, whereas *B. selene* oviposition spots had 100% attendance by *M. ruginodis*.

6.5 Discussion

The results of this study clearly show that *B. euphrosyne* does not oviposit in random locations. The same is true for many other species of butterfly including the Iolas Blue, *Iolana iolas* (Rabasa et al., 2005), the Marsh fritillary, *Euphydryas aurinia* (Liu et al., 2006; Bulman et al., 2007) and the Duke of Burgundy, *Hamaeris lucina* (Sparks et al., 1994; Anthes et al 2008).

In 2003 *B. euphrosyne* oviposition spots on site Y occurred where live vegetation was short, sparse and consisted of high numbers of size class 2 violets. In 2004 *B. euphrosyne* oviposition spots coincided with a higher percentage cover of dead bracken, a lower percentage cover of short live vegetation and grass and few size class 4 violets. However, size class 1 violets were more abundant at oviposition spots (mean 5.32 ± 1.57) compared to random locations (mean 1.92 ± 0.36).

The relationship between shorter vegetation and warmer soil temperatures was clearly highlighted in Chapter 3. Although in this study crude temperature measurements were taken, in most cases other factors such as short, sparse vegetation (which are indicative of a warm microhabitat) were variables which were more strongly correlated with oviposition spots than temperature. These conditions are indicative of an early successional niche. Dead bracken appears to be relatively important for *B. euphrosyne* oviposition because the larval stages use it for basking. However, the abundance of violets growing in the preferred size classes (1& 2) that occur in the warmer available niches are likely to be equally as important as dead bracken because violets are the larval food plant for *B. euphrosyne*.

The results from *B. euphrosyne* oviposition preference on site W are interesting. There was a preference for oviposition spots to be cooler than the random sample of violets assessed. I suspect that this is most likely owing to the very open conditions of the site. The bracken was burnt on this site in areas W1 and W3 in March 2003; W4 was burnt in March 2004. The well established dense stand of bracken and deep litter layer had prohibited the establishment of ground flora for a variety of reasons (see Jensen & Mayer 2001). The burning created large patches of bare ground upon which re-establishment of ground flora other than violets was slow. The removal of the litter layer in wet-fen meadows has been shown to enhance *Viola palustris* seedling establishment (Jensen & Mayer 2001). Although *B. euphrosyne* has shown a preference for warmer microclimates in which to oviposit on site Y (as is indicated by the short, sparse vegetation) it is likely that the openness of site W creates conditions that are too hot for oviposition, hence the preference for cooler oviposition niches to avoid the desiccation of eggs. Anthes et al., (2008) found that *H. lucina* exhibited oviposition electivity with regard to solar radiation and humidity on different sites in Germany. Despite the differences between sites in vegetation structure, on site W there was once again a positive correlation between oviposition spots and size class 2 violets, thus reiterating the importance of violets of the preferred size class for the larvae to feed upon. In other words on site W in 2004, the sward was so open and short that the small class 2 violets (figure 6.1) represented the tallest vegetation present during *B. euphrosyne* oviposition.

The conflicting results from the studies on site Y in 2003 and 2004 are interesting. Clear differences can be detected between *B. euphrosyne* oviposition spots in 2003 and 2004. In 2004, oviposition spots were found in areas with taller and sparser vegetation compared to 2003. In addition to this, the 2003 oviposition spots had fewer size class 1 violets and less overall cover of violets than in 2004. This difference is most likely due to the mean daily temperature being hotter in 2004 compared to 2003 (Chapter 2). The butterflies may have been laying their eggs where the risk of desiccation was lower (Anthes et al., 2008; Janz & Nylin, 1997). Although habitat management may be a contributing factor too. Most of the oviposition spots in 2003 occurred in areas that had been burnt the previous year, whereas in 2004 many eggs were laid in areas that had been burnt two months previously. Although vegetation height was taller and sparser in 2004 than in the previous year, it is likely that the microclimatic conditions at oviposition spots between years were very similar due to the relationship between vegetation structure and temperature (Chapter 3). Larval development is sensitive to micro-climate conditions (Anthes et al., 2008; Murphy, 2007). As for the higher numbers of size class 1 violets at oviposition spots in 2004, again this is likely to be due to the recent burning activities on the site, flushes of violets being common after burning (Oates, 1995).

The differences in oviposition spots between sites W and Y could be a result of the open and hot conditions on site W. Oviposition spots on site W occur in areas which are more vegetated, where there is less dead bracken compared to site Y. Violets of size class 1 are younger and are likely to occur in more open and hotter regions. On site W these regions are too hot for *B. euphrosyne* oviposition, hence an association with size class 2 violets which co-occur within the preferred *B. euphrosyne* oviposition niche on site W. This theory is supported by the fact that there was no significant difference in temperature or bare ground cover (PC 3) between sites W and Y. Therefore the microclimate at oviposition spots was very similar; this result reinforces the importance of warm microclimates for *B. euphrosyne* oviposition. Additionally, work carried out by Anthes et al., (2008) shows that *H. lucina* preferably oviposits on *Primula* species in shaded areas, with increased vegetation cover on south-facing slopes in central Europe.

One of the problems that was encountered when comparing *B. euphrosyne* and *B. selene* oviposition spots using PCA was the fact that despite PC 1 explaining almost 50% of the variance in the data, none of the microhabitat variables had absolute component value greater than 0.4. A larger sample size may have produced PC scores greater than 0.4. However, what is clearly evident is the strong influence of size class 1 and 2 (i.e. small, young) violets, a relatively high percentage cover of bare ground and high micro-temperatures in determining *Boloria* species oviposition. *B. euphrosyne* and *B. selene* differ in their preferred oviposition niches. Once again *B. euphrosyne* oviposition spots were positively correlated with violets of size class 1 and 2 whereas *B. selene* oviposition spots had a negative relationship with size class 1 and 2 violets.

B. selene oviposition spots occurred in areas that had a higher percentage cover of dead bracken where there was little bare ground. Although temperature did not have an absolute value greater than 0.4, it did show a trend towards being positively correlated with *B. euphrosyne* oviposition spots as opposed to *B. selene*. This suggests that *B. euphrosyne* oviposition spots are warmer than those of *B. selene.* This finding, combined with the fact that *B. euphrosyne* prefers a more open vegetation structure for oviposition, suggests that these two fritillaries inhabit different successional niches; *B. euphrosyne* an early niche, *B. selene* a slightly later one. Similar niche differentiation, albeit not at such a micro scale has been detected between *Boloria selene* and *B. bellona* butterflies that inhabit wet prairies in the Mid-west USA (Swengel, 1997). The results from the ant baiting at oviposition spots also supports this: *M. sabuleti*, an ant that prefers warm, early seral niches was present at all baits near to *B. euphrosyne* oviposition spots; *M. ruginodis*, an ant that prefers cooler later niches was present at all baits near to *B. selene* oviposition spots. However, it is unclear as to whether the high densities of the ants at oviposition spots is due to high nest density in the area or whether the oviposition spot coincides with the foraging niches of the particular ants. The baiting data suggest that it is the former, >30 ants attended baits in most cases.

In my study both *B. euphrosyne* and *B. selene* have been shown to be highly selective in choosing a suitable place in which to oviposit. *B. euphrosyne* preferred to lay eggs near to larval food plants that are relatively smaller than those chosen by *B. selene*. These findings are consistent with those of Thomas & Lewington (1991) and Warren & Oates (1994). The warm microclimate requirements of *B. euphrosyne* (Barnett & Warren, 1995a) were also confirmed by my study. However, my personal observations of *B. euphrosyne* oviposition conflict with those of Shreeve as cited by Porter (1992) who stated that *B. euphrosyne* usually lay their eggs away from violets. This behaviour has been observed in some fritillary species; the Regal fritillary *Speyeria idalia*, does not lay its eggs directly onto violets, nor are oviposition spots significantly different to random violet locations. However, they do choose micro-sites that are shady and near to the ground (Kopper et al., 2000). Laying eggs away from larval food plants immediately puts the newly eclosed larva in danger of predation and contradicts the theory that suitable oviposition spots are chosen to increase larval development and survival (Janz & Nylin 1997).

Previous studies have shown that violet density is important for *Boloria* oviposition; this is unsurprising as violets are the larval food plant for these species. However, as my study clearly shows, not all violet plants are suitable for oviposition. Both *B.*

euphrosyne and *B. selene* have a preference for violets of a particular size class. In addition to this, these preferred violets must coincide with the particular microclimatic conditions required by the different butterfly species for them to be selected for oviposition. Prior to this work only one previous study has shown that fritillary butterflies have a preference for certain size classes / growth forms within a specific niche (Thomas, 1989). These butterflies are not alone in their fastidiousness for oviposition sites. The Duke of Burgundy *Hamearis lucina* requires *Primula* spp. in the preferred microclimate upon which to oviposit (Sparks et al., 1994; Anthes et al 2008). The Lulworth Skipper, *Thymelicus acteon* and the Adonis Blue, *Lysandra bellargus* both exhibit an oviposition niche preference. *T. acteon* larval density was ten times greater in tussocks of *Brachypodium pinnatum* that was taller than 25cm compared to 5cm (Thomas, 1983b). *Hippocrepis comosa* is the larval food plant for *L. bellargus*, and oviposition only occurs on *H. comosa* which is growing in 1-4cm tall sward (Thomas, 1983a). The Brown Argus butterfly *Aricia agestis* also shows an oviposition niche preference; this butterfly lays its eggs on the ‘lush’ sheltered growths of *Helianthemum chamaecistus* (Bourn & Thomas, 1993).

The reintroduction of targeted habitat management for *M. arion* on sites X and Y is undoubtedly a contributory factor as to why *B. euphrosyne* and *B. selene* populations are increasing on these sites. The rotational scrub management that creates a habitat mosaic on these sites not only provides a large increase in *Viola* populations (Chapter 2) but also an abundance of the early successional niches required by *B. euphrosyne*, whilst the slightly later successional stages are available for *B. selene*. Within a month of burning the bracken on site W, both *B. euphrosyne* and *B. selene* were breeding there in large numbers.

Despite differences in the sites on which this work was carried out, it is clear that young violets that occur in suitable microclimatic conditions are vital for the persistence of *B. euphrosyne*. On many *B. euphrosyne* sites the bracken has become dense and the ground flora has been all but eradicated. The methods by which the bracken is managed do not seem to promote the ground flora. The violets remain sparse and the ground bare, thus the habitat is generally open. I suspect that suitable oviposition spots on these sites are rare, hence the small (or no) populations of *B. euphrosyne* that they can support. Prior to management activities, land managers and conservationists should assess the oviposition niche used by the butterfly on their particular sites (Anthes et al., 2008;

Bulman et al., 2007; Roy & Thomas, 2003). Once the preferred niche is identified, targeted management can be adopted to promote the required conditions. Promotion of the violets alone is not enough to maintain *B. euphrosyne* or *B. selene* populations. As with many other rare butterfly species, the promotion of the larval food plants at the preferred growth stage occurring in the precise narrow niche is intrinsic to their ultimate survival. Habitat management for rare Lepidoptera should therefore be aimed at creating optimum conditions for oviposition and subsequent larval survival.

Further work to establish the distance of oviposition spots from *Myrmica* spp. nests is required, as is investigation into the co-occurrence of *Myrmica* spp. nests and the abundance of suitable violets for oviposition near to nests. What is not clear from this study is whether high densities of *M. sabuleti* at oviposition sites have survival benefits for *Boloria euphrosyne* larvae. Higher densities of *Viola* plants near to nest sites may be attributed to the creation of enemy free space: this in turn may protect *B. euphrosyne* larvae from invertebrate predators. I hypothesise that protection from insect herbivores may be a benefit for *Viola* growing near to *Myrmica* nests. However, *B. euphrosyne* larvae are themselves insect herbivores, and further work to investigate the abundance and occurrence of insect herbivores near to and away from ant nests is required to clarify this hypothesis. Interactions between *M. sabuleti* and *B. euphrosyne* larvae should be researched. Some *Myrmica* colonies are more benign than others (Elmes et al., 2004), and *Myrmica* colonies that are infested with *Maculinea* caterpillars are generally more tolerant to other ant guests, (Thomas et al., in press). Therefore, *Myrmica* colonies may be benign to the presence of *B. euphrosyne* and other insect herbivores near to infested nest sites.

An alternative hypothesis to consider is that there may be a trade off; higher *Viola* densities around ant nests increase the larval food plant availability, thereby, reducing the overall effect of *B. euphrosyne* larval herbivory. The effect of herbivory near to *Myrmica* nests may be further reduced by the recruitment of *Viola* seed by ants (Chapter 4), which increases the already elevated numbers of *Viola* near to nest spots (Chapter 3). The myrmecochorous activity of the *Myrmica* ants on my study site has an indirect positive effect upon the *Boloria* spp. The ants are attracted to violet seeds due to the presence of an elaiosome. The ants take the seeds back to their nests, eat the elaiosomes and dispose of the seeds in or around the nest. Comparison of the vegetation near to *Myrmica* spp. ant nests with *Boloria* spp. oviposition spots would be an interesting avenue for future work. This may identify further interactions between *Myrmica* and *Boloria* species.

Chapter 7: Discussion

The following discussion includes a summary of the findings of this research. The impact of targeted management for *M. arion* and the wider application of *M. arion* as an umbrella species in conservation are discussed, together with the role of *Myrmica* spp. ants and their effect on *Viola* spp. and *Boloria* spp. within *M. arion* grasslands.

7.1 Impact of "single-species" habitat management on selected species

This thesis has shown that "single-species" habitat management for *M. arion* has had considerable benefits for a suite of rare declining species that occupy an early successional niche. Several of the selected invertebrates and plants are UK Biodiversity Action Plan or Red Data Book species. On site W, which had been abandoned farmland for more than 40 years, the management of *P. aquilinum* reduced vegetation height and *P. aquilinum* density. The creation of an open habitat allowed the ground flora to re-establish. The removal of the dense *P. aquilinum* litter layer enabled seedling recruitment from the seedbank; *V. riviniana* increased by 16% during the study period. Similar results have been shown for *V. palustris* (Jensen & Meyer, 2001). In conjunction with the rapid increase in *V. riviniana*, ruderal plant species which are valuable nectar sources re-colonised the site, thus providing the necessary components for *B. euphrosyne* and *B. selene* establishment.

The open habitat created by the *P. aquilinum* management and subsequent light grazing altered the micro-climate sufficiently to enable *B. euphrosyne* and *B. selene* to find suitable oviposition sites during the very early stages of site restoration. *H. semele* and *C. campestris* both colonised site W in 2004. However, the close proximity of sites X and Y can partly explain the rapid rates of colonisation. Had site W been more isolated from surrounding populations, the colonisation rates of the selected species would probably be greatly reduced, or non-existent without the artificial reintroduction of specimens of these relatively low mobility species (Hanski, 1999; Dover & Settele, 2009). The exact habitat requirements of *H. semele* and *C. campestris* are not fully understood, and would be a valuable area of future research. However, the co-existence of them with *B. euphrosyne* and *B. selene* within site W and on the neighbouring sites (X and Y) provides evidence that in semi-natural acid grassland sites, these four species form a guild that share the same early successional vegetation that is created by habitat management aimed specifically at *M. arion* and its host ant *Myrmica sabuleti*. Within this narrow sere, the species exhibited clear-cut niche preferences. *B. selene* was associated with the cooler range of the sere where *V. riviniana* and *M. ruginodis* are most abundant (Chapters 3 and 6). At the other end of the spectrum, *H. semele* is thought to oviposit on short tufts of *A. curtisii* in bare ground which are hotter (Thomas & Simcox, un-published data). In addition, the results from the ant nest vegetation research (Chapter 3) show that *A. curtisii* was more abundant above the nests of *M. scabrinodis* $_{\text{(HOT)}}$. As the name suggests, *M. scabrinodis* $_{\text{(HOT)}}$ is restricted to the hottest areas of site Y where the soil is thin and the vegetation short and sparse. *C. campestris* is also associated with dry open vegetation and therefore would also prefer the warmer range within the narrow niche. *B. euphrosyne* on the other hand is found within the mid and warm range within the sere: it coincides almost exactly with the niche of *M. sabuleti* and also where *V. lactea* and *V. riviniana* co-occur in the coolest and warmest parts of their respective niches (Chapters 3 and 6). Although some studies have shown a decline in species richness of grassland invertebrates under grazing management (Poyry, Luoto et al., 2006), the overall mosaic structure of *M. arion* habitat provides areas within the site that are suitable for less thermophilous grassland invertebrates. For the species on site W, their re-establishment is recent; habitat management needs to be maintained in order to sustain long term populations of the desired species. Indeed, continued monitoring of site W show substantial increases since 2005 in *B. euphrosyne*, *B. selene*, *C. campestris* and the rare thermophilous Lesser Cockroach, *Ectobius panzeri* and the Western Beefly, *Bombylius canescens,* as well as colonisation of the site by the High Brown Fritillary, *Argynnis addipe*, currently the UK's most threatened butterfly species.

7.2 The role of *Myrmica* spp. ants within *M. arion* grasslands

This study has provided valuable insights into the varied role of *M. sabuleti* in *M. arion* grasslands. Firstly, this ant is vital for the survival of *M. arion*. Secondly, *M. sabuleti* and other *Myrmica* species aid the dispersal of *Viola* species seeds. However, within the scope of this project it has been difficult to determine if *M. sabuleti* is a keystone species within *M. arion* grasslands. To answer that question unequivocally, eradication of ants within some quadrats on sites W and X would need to have been undertaken. This is impractical and the disruption of the habitat would be detrimental to *M. arion* and its associated species. The role of *M. sabuleti* as a keystone species could possibly be determined within a controlled environment facility such as the Natural Environment Research Councils ECOTRON.

The ants in my study are important ecosystem engineers (Elmes, 1991; Folgarait, 1998; Jones et al., 1994; Schuch, et al., 2008). The perturbation of soil, dispersal of seeds etc are vital for the maintenance of the habitat. The displacement of the *Myrmica* species by invasive ant species is likely to have detrimental effects, not only to *M. arion* populations but also on the myrmecochorous mutualism. Studies have repeatedly shown that invasive ant species such as the Argentine ant, *Linepithema humile* have a negative effect on seed dispersal mutualisms (Zettler et al., 2001). These ants not only displace the native species but also destroy the seeds that they collect or relocate them to micro-sites that are unsuitable for germination and subsequent seedling establishment (Zettler et al., 2001). Some invasive ant species actually aid seed dispersal of non-native invasive plant species (Ness, et al 2004). The presence of the invasive ant *Technomyrmex albipes* in Mauritius is preventing the pollination of a rare endemic plant, *Roussea simplex* (Hansen et al., 2009). The breakdown in this mutualism could potentially lead to the extinction of this plant. It is reported that in the near future the invasion of *Lasius neglectus* an ant which forms super colonies will become a global problem (Cremer et al., 2008).

Both *V. lactea* and *V. riviniana* were more abundant or showed a higher percentage cover on ant nests compared to otherwise identical spots. These findings are consistent with those of Lack & Kay (1987), Oostermeijer (1989) and Kovar (2001), who found that the spatial distribution of some myrmecochorous plants was influenced and determined by dispersal by ants, the nest areas having higher numbers of plants than surrounding areas. However, Schütz et al., (2008) found that the myrmecochorous plants were more numerous away from *F. execta* nests. Additionally, the *Viola* species have clear-cut, though overlapping niches across the temperature gradient on the study sites: *V. riviniana* was more abundant in the taller vegetation at the cooler end of the spectrum and *V. lactea* was more abundant in the shorter vegetation at the hotter region. My field studies revealed that the presence of ant nests in optimum *Viola* niches is beneficial to *Viola* species distribution; there is an indication of some positive ant / plant interaction. In some respects it is surprising that this micro-association with *Myrmica* nests could be detected at all, since *Myrmica* nests occur at densities of about 0.5 nests per square metre on sites X, Y and W, leaving little ground uninfluenced by their presence. I would therefore predict more clear-cut patterns on sites supporting lower densities of *Myrmica*.

Viola seeds have an elaiosome that is attractive to ants and a large body of evidence suggests that elaiosomes facilitate seed dispersal by ants (Giladi, 2006). My laboratory experiments clearly showed preferences and specifically in the ant-plant interactions, at least at the genus level. Thus all *Myrmica* species tested were attracted to both *Viola* species more than they were to the other elaiosome-bearing seeds they were given. Furthermore, *M. scabrinodis* $_{\text{(HOT)}}$ showed a species-level preference in the laboratory for *V. lactea*, the *Viola* which its niche overlaps the most. *F. fusca* ants on the other hand did not retrieve any *Viola* seeds in the laboratory trials. Further work in the laboratory and in the field should be undertaken to investigate the seed preferences of *F. fusca* to establish the role this ant has as a seed dispersal vector of other plant species within this ecosystem. In addition, investigating the chemical composition of *Viola* species, *A. reptans* and *P. erecta* elaiosomes may give an indication as to why *Viola* species seeds are more attractive to the *Myrmica* species than to *F. fusca*. Some studies have revealed that bigger seeds have larger elaiosomes (Edwards, 2006; Gorb & Gorb, 1996; Mark & Olsen 1995). Comparing the mass of the elaiosome on different seed species studied would therefore be another interesting avenue of future research.

The benefit of *Viola* elaiosome consumption for *Myrmica* species would be interesting to study. Gammans, (2005) and Morales & Heithaus (1998) found that ant colony fitness was improved when ants were fed elaiosomes. If the same were true for *M. sabuleti* this could be beneficial to *M. arion's* future survival.

Five non-exclusive benefits to myrmecochorous plants are regularly cited: (1) fire avoidance (Holldobler & Wilson, 1990); (2) predator avoidance (Turnbull & Culver, 1983); (3) reduction of intra-specific competition (Holldobler & Wilson, 1990); (4) reduction of inter-specific competition (Holldobler & Wilson, 1990); and (5) relocation of seeds to nutrient rich micro-sites (Beattie & Culver, 1983; Petal, 1980; Oostermeijer, 1989; Folgarait, 1998). In my study I tested the fifth hypothesis. However, significant differences in soil nutrients between *M. sabuleti* ant nest soil and non-nest soil were not revealed. Therefore it is unlikely that *Viola* species are more abundant at *M. sabuleti* nests compared to non-nest spots due to an increase in soil nutrients. I also investigated the affect of myrmecochory by *M. sabuleti* on seed bank composition; *V. riviniana* was one of three myrmecochorous seedlings to emerge from the upper sample of soil from *M. sabuleti* nests in greater numbers than from the equivalent non-nest soil. Additionally, the number of myrmecochorous seedlings in the upper *M. sabuleti* soil was 8% higher than that of the lower nest soil. This suggests that the seed dispersal activity of *M. sabuleti* may influence myrmecochorous seedling recruitment near to nests. The reduction of intra-specific competition is another theory that can be discounted: in the field *Viola* spp. are more abundant near to ant nests compared to non-nests.

Predator avoidance was not addressed in this thesis; but this is a potential reason for high densities of *Viola* species near to ant nests compared to non-nest spots especially since *Viola* species were also more abundant near to *F. fusca* colonies. It is likely that there is a different mechanism which accounts for this; the laboratory experiments indicate that *F. fusca* does not have a mutualistic relationship with *Viola* spp. Previous studies have shown that seedling survivorship is greater above ant nests compared to non-nests (Hanzawa et al., 1988; Davidson & Morton, 1984). However, to date a definitive explanation has not been reported. I hypothesise that the increased survivorship of seedlings near to ant nests compared to non-nests is due to worker ants that deter insect herbivores from young seedlings. The herbivore avoidance hypothesis could be tested in the field using barriers to exclude ground dwelling phytophagous insects, particularly when the plants are in their vulnerable seedling stage; these are likely to be the main herbivores excluded by *Myrmica* workers, which seldom climb more than 1cm above the soil surface (Thomas, 2002). In addition to this the relocation of seeds to ant nests makes them inaccessible to seed eating invertebrates (Ohkawara, et al., 1997) and mammals (Turnbull & Culver, 1983).

The reduction of inter-specific competition was partially addressed in this study. However, the total number of seedlings emerging from *M. sabuleti* nests compared to non-nests was not significantly different. Therefore it is reasonable to conclude that inter-specific competition is not reduced near to ant nests. Fire avoidance was not examined within this thesis but again it would be an interesting area of future research and may explain why *Viola* species are more abundant near ant nests compared to non nest spots. Many myrmecochorous plants are found in fire prone habitats, seeds that are taken below ground into an ant nest are protected from destruction by fire (Holldobler & Wilson, 1990; Hughes & Westoby, 1992b). Perhaps *Viola* species are adapted to fire, hence their population increase after burning. Chapter 3 strongly suggests that ant nests provide benefits to *Viola* species that non-nests do not, although the possibility remains that it is the ants themselves that build nests in spots where *Viola* species (for whose seeds they have a penchant) grow in the greatest abundance (Elmes & Wardlaw, 1992a).

Ants manipulate bacteria and pathogens that occur within their nests (Petal, 1978; Jakubczyk et al., 1972). *Viola* species are prone to damping off (Clive Groves, pers comm.). Therefore bacteria and pathogens may be less active within ant nests and therefore the occurrence of damping off is reduced. A study into the bacteria and pathogens within ant nests and non nests may help to explain the high densities of *Viola* spp near to ant nests.

Scarification of seeds could also be the key to *Viola* species success on ant nests. Many studies have been undertaken with mixed results Mayer (1963) as cited by Culver and Beattie, (1980); Lobstein & Rockwood (1993); Blois & Rockwood (1986). A more recent study showed that elaiosome removal enhanced germination by 30% (Leal et al., 2007). Connor (2004) and I undertook preliminary trails to test this hypothesis. We had conflicting results but we used ericaceous compost as the germination stratum; perhaps a repeat experiment using ant nest soil would show more consistent results.

7.3 Interactions between *Myrmica, Viola* and *Boloria* species

Despite not fully elucidating the mechanism behind the *Myrmica* / *Viola* interaction, the consequence of this interaction had a positive effect on *Boloria* spp. butterflies whose larvae feed on *Viola* leaves. The observed increase in populations of these butterflies on the study sites was attributable to habitat management, which created an early successional niche with a great abundance of *Viola* spp. Coupled with that was the direct effect of *Myrmica* species ants which aided *Viola* species seed dispersal and *Viola* species abundance through myrmecochory. However, despite the 16% increase in *Viola* species density caused by the habitat management and *Myrmica* species ants, not all of the *Viola* plants were suitable for oviposition by the two fritillary butterflies.

Both *B. euphrosyne* and *B. selene* showed clear oviposition preferences in terms of foodplant structure. *B. euphrosyne* preferred *Viola* spp. plants of size class 1 and 2, which are seedlings and young plants recruited from the soil seed bank. *B. selene* showed a preference for *Viola* spp. plants of size class 3 and 4, which are mid-aged violets that inhabit a later successional stage than those of size class 1 and 2. Differences in soil temperature between the different species' chosen oviposition sites also highlighted the preference for different successional stages. Oviposition sites were also positively correlated with percentage cover of dead *P. aquilinum*. Ant baiting at oviposition spots reinforced the oviposition niche preference of the two *Boloria* spp; *M. sabuleti* (an ant that prefers earlier, warmer niches) was present at all baits near to *B. euphrosyne* oviposition spots. *M. ruginodis* (an ant that prefers cooler, later niches) was present at all baits near to *B. selene* oviposition spots. Prior to this work, only one previous study has shown that *Boloria* spp. butterflies have a preference for certain size classes / growth forms within a specific niche (Thomas, 1989).

7.4 *M. arion* as an indicator and umbrella species

Although single species conservation for the threatened Scops Owl, *Otus scops* has had wider conservation benefits in the Alps (Sergio et al., 2009), this study is the first to shown how single species conservation for a butterfly benefits several other rare taxa. *M. arion* fits the criteria for classification as an umbrella species, defined as "species whose protection serves to protect co-occurring species" (Fleishman et al., 2000). *M. arion* is a successful umbrella species because it inhabits a seral stage or niche that is rare and has greatly declined in unfertilised semi-natural grasslands across the northern half of Europe (Morris et al., 1994; Thomas, 1993). Restoring ant abundance and continuity of this niche has enabled a diversity of other species adapted to this type of habitat to increase, whereas by definition they too have been declining through loss of habitat. Additionally, the ecology of *M. arion* is clearly understood, habitat management mistakes which did not help prevent its extinction have been rectified and the restoration sites have been monitored for 33 years (Simcox et al., 2005).

However, the usefulness of *M. arion* in wider conservation practice has limitations. The species is not widely distributed due to its highly specialised life cycle. Not all grasslands in the UK are suitable for reintroduction of the species; however, what is clear is that by understanding the intricacies of its ecological requirements, successful remedial habitat management and reintroduction of the species is possible. It is these factors that make *M. arion* a model species for conservation. However, the use of different *Maculinea* species as indicators across Europe has potential. Wet heathland sites in Belgium that are occupied by *M. alcon* are reported to be significantly richer in wet heathland species and in red list species (Maes & Van Dyck, 2005). In addition to this Spitzer et al., (in press) has recently described *M. arion* as an umbrella species in the Czech Carpathians. They also found that occupied sites had richer butterfly and plant diversity, many of which were specialist species. As with the findings of Maes & Van Dyck (2005), a greater number of red list species were also present on *M. arion* sites.

If one selected a specialist species in any given habitat whose ecology was similarly widely understood and applied the same principles, it is likely that this species too could be an umbrella for its specific habitat. This strategy may well help the UK or any other country for that matter, reach their targets to reduce / halt biodiversity loss (Dennis et al., 1997; McGeoch, 1997). For example, in the UK 151 species of moth are listed as Biodiversity Action Plan species. Obviously, it is impractical to adopt measures to conserve every individual species (Hilty & Merenlender, 2000). However, what is feasible is to select what are thought to be the specialist species within given habitats or guilds, research their exact habitat requirements and adopt the appropriate habitat management strategy (Maes & Bonte, 2006). This may of course be a lengthy process, but at least the exact conservation and habitat management practices will be discovered, rather than the misguided, hit and miss actions that often occur (Warren, 1992b). By adopting such measures many more umbrella species are likely to be revealed, and at the same time a wider proportion of our diminishing biodiversity saved. The use of umbrella, indicator and/or surrogate species has been widely adopted in recent years due to the 2010 target to reduce biodiversity loss (McGeogh, 1997). One successful example of an indicator species is that of the Beaver, *Castor canadensis* which has been found to be a good indicator for breeding frogs and other amphibians in Alberta, Canada (Stevens et al.,2007).

One of the biggest threats facing biodiversity is that of climate change, it is predicted that by 2050 15-37% of global insect species will be extinct (Dennis et al., 2007; Thomas et al., 2004). The implication of climate change on the future habitat management for *M. arion* and its associated species is not fully understood. However, these species are at the northern edges of their ranges in the UK, hence their requirements for warm ephemeral narrow niches (Thomas, 1996). There is the possibility that the area of suitable habitat in the UK will increase. In continental Europe these species have broader niches due to the macro-climate being 3ºC warmer on average compared to the UK. Under these conditions the intensity of management is much reduced, requiring only perturbations every 5 - 10 years on flat rather than only on south-facing terrain (Thomas, 1993; Thomas et al., 1998). This in turn will maintain the micro-climatic conditions required by the species. Once again this highlights the importance of long term monitoring on sites to assess for changes in habitat quality and abundance.

7.5 Implications for conservation of *Boloria* spp. and other butterflies

Violets that occur in suitable micro-climatic conditions are essential for the persistence of *B. euphrosyne* and *B. selene*. Many butterfly species are fastidious when it comes to selecting suitable oviposition sites (Thomas, 1983b; Thomas 1983a; Bourn & Thomas 1993; Sparks et al., 1994). Prior to habitat management activities, land managers and conservationists should assess the oviposition niche used by butterflies on their particular sites (Anthes, et al 2008). Once the preferred niche is identified, targeted management can be adopted to promote the required conditions. In the case of *Boloria* spp. promotion of the violets alone is not enough to maintain *B. euphrosyne* or *B. selene* populations. As with many other rare butterfly species the promotion of the larval food plants at the preferred growth stage occurring in the precise narrow niche is intrinsic to their ultimate survival (Thomas, 1991; Rabasa et al., 2005; Liu, et al 2006).

Investigations into the distance of oviposition spots from *Myrmica* spp. nests are required. Co-occurrence of *Myrmica* spp. and *Boloria* spp. in relation to the abundance of suitable violets for oviposition near to nests should also be researched. High densities of *M. sabuleti* and *M. ruginodis* at oviposition sites may have survival benefits for *B. euphrosyne* and *B. selene* larvae respectively. Higher densities of *Viola* plants near to nest sites may be attributed to the creation of enemy free space: this in turn may protect *B. euphrosyne* and *B. selene* larvae from invertebrate predators, so long as they themselves can co-exist with ants. A benefit for *Viola* spp. growing near to *Myrmica* spp. nests may be protection from insect herbivores. However, *Boloria* spp. larvae are themselves insect herbivores and further work to investigate the abundance and occurrence of insect herbivores near to and away from ant nests is required to clarify this hypothesis. The possibility of interactions between *M. sabuleti* and *B. euphrosyne*, and *M. ruginodis* with *B. selene* larvae should be researched. Colonies of *M. sabuleti* that are infested with *M. arion* caterpillars are generally more tolerant to other ant guests (Thomas et al., in press). This may have implications for other insect herbivores that occur near to infested *M. sabuleti* nests. The increase of larval food plant availability near to *Myrmica* spp. ant nests, a result of seed recruitment by the ants, may reduce the overall effect of *B. euphrosyne* larval herbivory. Comparison of the vegetation near to *Myrmica* spp. ant nests with *Boloria* spp. oviposition spots would be an interesting avenue for future work. This may identify further interactions between *Myrmica* and *Boloria* spp.

Two other rare UK butterflies, the Duke of Burgundy, *Hamearis lucina*, whose preferred larval food plant is the Cowslip, *Primula veris* (Anthes et al., 2008) and the Heath Fritillary *Melitaea athalia*, whose larvae eat Cow wheat, *Melampyrum pratense* (Warren et al., 1984), have declined by 58% and 46% over a 10 year period respectively (Fox et al., 2006). It is extremely interesting that the larval food plants of both butterflies produce elaiosome bearing seeds. The role of ants in these species' habitats should be investigated, the ants may be vital in aiding seed dispersal of the larval food plants. *M. pratense* is a Biodiversity Action Plan species (Dalrymple, 2007). The seeds of this plant are dispersed by *M. ruginodis*, *F. fusca* and *F. rufa* (Heinken & Winkler, 2008). However, Dalrymple, (2007) reports that ant aided seed dispersal was not observed during her study in the Central Scottish Highlands. The future survival of *M. pretense* is dependent upon its dispersal ability (Winker & Heinken, 2007). I propose that habitat management which took into account the ant species' habitat requirements is likely to benefit *M. pratense* and ultimately help to conserve *Melitaea athalia*. Further studies to investigate the relationships between guilds of species could provide valuable knowledge which will aid the conservation of species and reduce the rate of biodiversity loss.

8. References

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