Spatial ecology of an insect host-parasitoid-virus interaction: the winter moth (*Operophtera brumata)* and its natural enemies in Orkney

Simon Harold

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others

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Thesis abstract

Understanding the population dynamics of host-natural enemy interactions in spatially heterogeneous habitats may help mitigate the negative effects of environmental change. The aim of this thesis is to investigate the role of spatial processes in determining the distribution and abundance of two co-occurring Lepidoptera species of differing dispersal capabilities, and how this may affect interactions with natural enemies of differing transmission modes. *Operophtera brumata* is a resident species, attacked by a single parasitoid and three viral pathogens, all with differing transmission strategies; *Abraxas grossulariata* is a new coloniser, and may have escaped its parasitoid enemies. Both species feed on the same hostplant, *Calluna vulgaris*, in the Orkney isles, Northeast Scotland.

Natural enemy prevalence was rarely related to host density of either species at any scale. Significant host density dependence in infection was detected only among horizontally transmitted pathogens infecting *O. brumata,* and only during high density outbreaks. Parasitoid prevalence was significantly negatively related to *O. brumata* host density, although only during outbreak years, either due to satiation or competition from pathogens. Conversely, two years following an outbreak, parasitoid prevalence was found to be positively related to host density. There was no evidence that *O. brumata* escapes parasitoid attack at higher elevations, although newly colonising *A. grossulariata* populations may be susceptible to microclimatic variation, mediated by topography. Such susceptibility may be a result of unfavourable conditions at the northward margin of the species' range. A genetic signal of recent colonisation was detectable among *A. grossulariata* populations within Orkney when compared to mainland Britain. Genome scans also suggest that *A. grossulariata* may be under selective pressure from pathogenic infections, possibly due to their low genetic diversity. Local adaptation of *O. brumata* to individual patches was not apparent. The implications of these findings and potential areas of future research, are discussed.

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Chapter 1

General introduction

1.1 Population ecology of insect herbivores in space and time

A mechanistic understanding of the processes that cause fluctuations in populations of wild organisms in space and time is central to our ability to predict the dynamics of such populations, and the likely effects that ecosystem perturbations (e.g. climatic change, land use change, and habitat loss) may have (Benton *et al.*, 2006). The responses of organisms to such perturbations are typically complex, and variation in a range of environmental and demographic parameters may result in dramatically different population dynamics, which may have both immediate and/ or delayed effects; in turn, variation in the effects of these parameters may also give rise to similar dynamical responses, despite very different mechanisms of action (Turchin *et al.*, 1999; Lundberg *et al.*, 2000). As such, the major processes that act to regulate natural populations may be a complex of many dynamical interactions which may not be easily tractable. However, a holistic approach that integrates large-scale, long term field systems with theoretical models and controlled microcosm systems may represent the best method by which the complexity of natural systems may be studied and understood (McCallum *et al.*, 2001; Rushton *et al.*, 2004; Benton *et al.*, 2007).

As study organisms, insects are particularly suitable for this purpose, since their relatively short generation times, large-scale distribution and abundance of certain species, ease of culturing in the laboratory, and relatively simple physiology and behaviour, means that well-replicated long-term datasets are easily obtained, and theoretical models relatively simple to parameterise (Hassell, 2000; Srivastava *et al.*, 2004). Moreover, such studies may have practical implications, since many insect species are of considerable economic importance, both positively (e.g. pollinators of crops Kevan & Baker, 1983; biological control agents: Waage & Hassell, 1982) and negatively (e.g. crop pests Kogan, 1998; and vectors of human, livestock, and wildlife diseases: Hemingway & Ranson, 2000; Dobson & Foufopoulos, 2001). An understanding of their temporal and spatial dynamics, and the likely effects of human-induced environmental perturbations, may therefore be of great significance.

Most insect herbivore species may be broadly characterised by the large number of individuals produced in the juvenile stage, which may be subject to large losses, relative to the total number of individuals surviving to the reproductive, or adult, stage (Boyce, 1984). The dynamics of such populations over time may further be broadly classified into three groups, dependent on their characteristic patterns in abundance: i)

erratic fluctuations; ii) episodic outbreaks; and iii) abundance cycles (Hassell, 2000). The processes that act upon such populations—those that influence the number of births, deaths and recruitments—may again be subdivided into *endogenous* and *exogenous* processes: the former being those biotic processes that act within populations and which may be related to the abundance or density (e.g. competition for resources, space and reproduction: Hassell, 1975); the latter being those processes that are independent of density, and whose influence acts outside of the system as a whole, but which affect the survival and reproduction of individual organisms within it (e.g. abiotic influences, such as seasonality, temperature and rainfall: Andrewartha & Birch, 1954). However, one significant group of influences that may act both endogenously and exogenously, dependent upon the specific population interactions with host densities, are predators, pathogens and parasites (Ylioja *et al.*, 1999).

The biotic components that act on herbivorous populations may therefore be further sub-divided into top-down (those influences that occur from higher trophic levels i.e. natural enemies) and bottom-up (those from lower trophic levels i.e. resources) sources, whose relative strengths may vary within any population, and whose influences on regulatory processes may be wholly or partially partitioned into either group (Walker & Jones, 2001). These regulatory influences may come about through heterogeneity in a number of parameters that occur across all trophic levels (since herbivores may act as resources for consumer natural enemies), and may include the total availability of resources relative to population carrying capacity; the spatial availability of resources relative to the dispersal ability of the consumer; the spatial arrangement of resources patches and their relative quality, which again may be related to dispersal; and whether consumers are specialists or generalists (Latto & Hassell, 1988; Hanski, 1999; Hassell, 2000; Klemola *et al.*, 2002; Esch *et al.*, 2005; Hamback *et al.,* 2007).

The aim of this review then is to highlight the importance of the many different processes and influences that may act on natural populations, and the potential role of each in their regulation and stability in time and space. Particular reference is paid to the distribution and abundance of insect herbivores and their natural enemies in space, together with a consideration of the influences that resource heterogeneity, and abiotic effects may present. The focus will then be given to the specific study system investigated in this thesis: a two-host species complex of Lepidoptera feeding on a

shared hostplant, subject to attack by non-shared parasitoid and virus natural enemy species.

1.2 Predators, parasitoids, and pathogens

Top-down regulation by predators, parasitoids, and pathogens is thought to be an important component of the dynamics of insect herbivore populations, often displaying characteristic regular, coupled cycles in abundance as increases in prey herbivore abundance are followed by exploitation, and eventually self-limitation by higher trophic-level interactions. Such regular boom and bust oscillations are seen in many natural systems, including many forest Lepidoptera (Berryman, 1996; Liebhold & Kamata, 2000). However, a failure of top-down regulation may result in herbivores over-exploiting their resources, potentially leading to the occurrence of population outbreaks—large spatial and temporal variations in abundance—and subsequent damage to, and/or defoliation of, the hostplant (Wallner, 1987; Liebhold *et al.*, 1998; Gray *et al.*, 2000). The dynamics of these interaction patterns may typically be complex, and a theoretical understanding of the processes that may give rise to them—and the effect that altering various interaction parameters may have—is an important component in ultimately understanding what drives them (Liebhold *et al.*, 2000).

1.2.1 Predators and parasitoids

The earliest models to demonstrate that predator-prey oscillations were theoretically possible were by Lotka (1925) and Volterra (1926), which were based on a continuous-time framework that assumes birth and death processes are continually occurring, and that both predator and prey have overlapping generations; the rate of prey population increase therefore being a function of both the prey hosts (H) and predator (P) populations, following the law of mass action, or direct linear *density-dependence* (HP)—in this context, that predation varies in direct proportion to the product of the densities of both predator and prey. These models therefore demonstrated that the interactions between predators and prey could be neutrally stable, given that interactions were linearly density-dependent, and happened in continuous time. However, for many species, such assumptions may not be entirely valid; for example, temperate species may have seasonally synchronised discrete generations, and interactions among

predators may affect the ability of them to exploit prey in direct proportion to their densities. An alternative model to this was developed by Nicholson & Bailey (1935) that addresses the shortcomings of the initial Lotka-Volterra models by instead assuming that predators and prey were synchronised together, in discrete generations. These discrete-time models were motivated largely by observations from parasitoid interactions with their host prey, basing the mathematical assumptions on the attack rate of, rather than the number of encounters with, hosts.

Insect parasitoids themselves may be seen as functionally intermediate between predators and parasites, in that they exploit and deplete their hosts of resources, but unlike true parasites, kill them outright. Taxonomically, they are only found among the Diptera and Hymenoptera, although as a group, worldwide they are thought to comprise around 10% of the total number of metazoan species recorded (Hassell, 2000). Their apparent success as a functional group may be partly due to the broad range of life-history strategies exhibited among species: individual females may lay (oviposit) one or more eggs either on (ecto-parasitoids) or inside (endo-parasitoids) their insect hosts, which may also be passively ingested from eggs oviposited on hostplants; and females may attack either the egg, pupal, or larval/ nymphal host stages. Of the latter, attacked hosts may be either paralysed immediately, with all parasitoid larval development occurring within the host afterwards (idiobiont strategy); or attacked hosts may continue moving and feeding while the parasitoid larvae develops, feeding off the hosts' resources (koinobiont strategy). The developing parasitoid larvae may also be solitary (a single larva develops within each host) or gregarious (many developing larvae per host), and may even attack the larvae of other (primary) parasitoids developing within their herbivorous hosts (hyperparasitoids) (Godfray, 1994).

One of the important parameters introduced into the Nicholson-Bailey models compared to their continuous-time equivalents, was a more realistic estimation of the interactions among predators/parasitoids when prey proportions vary. This new parameter was therefore an estimation of the searching efficiency (α) of parasitoids, stating that, as host numbers increase, the proportion of hosts attacked increases, but at a decreasing rate. This formed the basis of a parasitoid 'competition curve' in which a Poisson distribution describes the proportion of hosts escaping parasitism. Such an effect therefore manifests itself in the generational time-lags in parasitoid attack rates, causing parasitoids to act in a *delayed*, but still density-dependent, manner (αHP). Such fundamental difference in the assumptions underlying parasitoid responses to host

density were also noted by Holling (1959), who termed them *functional responses*: direct density-dependent (i.e. mass action) responses he named Type I, which assumed a linear relationship with no upper limit; in contrast, Type II and Type III responses represented non-linear relationships (the former asymptotically decreasing; the latter sigmoidal), with an upper limit to prey consumption caused by constraints to the time spent searching for, catching, and consuming prey, as well as possible effects of satiation. Type III sigmoidal responses were therefore thought to be more common among higher vertebrates, as a result of predators learning to exploit prey more efficiently.

As a result of these alterations to the assumptions concerning how predators respond to prey densities, the original models appeared to show locally unstable population oscillations—in contrast to the neutrally stable Lotka-Volterra models. However, despite the inclusion of more realistic parameters, the observed instabilities in these models were often not seen in many laboratory and field-based populations (Hassell, 2000), suggesting that further realistic parameters were required. The introduction of the concept of *heterogeneity in risk* by May (1978), by modelling parasitoid attacks as being highly aggregated with a negative-binomial distribution, rather than a Poisson distribution as in the Nicholson-Bailey models, allowed populations to fluctuate more realistically around a locally stable equilibrium. Such heterogeneity in risk was assumed to arise as a result of either behavioural (e.g. parasitoids not foraging in the same areas in order to avoid competition) or numerical (e.g. pseudointerference among parasitoids arising from an increase in the number unsuccessful encounters with previously parasitized hosts, as the number of parasitoids increases) means.

1.2.2 Pathogens

Although also principle mortality agents in many insect populations (Dwyer *et al.*, 2004), pathogens differ from predators or parasitoids principally in their mechanism of action causing host mortality. Although the assumption of mass action among mobile, interacting parasitoids may be a simplification (since heterogeneity in attack rates may cause non-linear host-parasitoids associations due to functional responses), many types of pathogen may be passively acquired by hosts, as a direct product of contact with infected conspecifics, or infectious particles (Fuxa, 2004). However, modelling the

dynamics of such horizontally transmitted infections (i.e. infections transmitted *within* generations) may share many of the characteristics of the host-parasitoid systems described above: again, most models assume the mechanism of transmission is a mass action, or density-dependent, function of the number of susceptible to infected individuals, together with a transmission coefficient (β), which is a function of both the contact rate between susceptible (S) and infected (I) individuals, and the probability of successful transmission per contact. The transmission coefficient for most pathogens is usually assumed to be constant, and therefore the relationship is linear (βSI). However, unlike the situation among parasitoids and hosts, there may be multiple generations of infectives in the environment within only one generation of hosts, and therefore a parameter is included that also represents the population of potentially infective pathogen particles in the environment; the inclusion of a threshold density parameter is therefore also implicit in most host-pathogen models, in order to allow persistence of the pathogen. Such a parameter is fundamentally related to β, since pathogens with low transmission rates will only be able to persist in populations of high host density; pathogens with high values of β being able to persist more easily at lower host population densities (Anderson & May, 1981). The other parameters also included in these models are the rate of production of pathogen particles, the rate of decay of pathogen particles in the environment, the rate of disease-induced mortality, and the rate of host reproduction; as in host-parasitoid models, altering the magnitude of any of these parameters may change the dynamics between pathogen and host, potentially leading to: i) regulation to a stable equilibrium; ii) extinction of either the pathogen or the host; or iii) stable limit cycles, where pathogen and host may oscillate together (Dwyer, 1994). These models may also be extended to include a specific age-structure among the host population, which can be altered depending on the specific life-stage attacked by the pathogen; such models predict multigenerational host-pathogen cycles dependent upon the time-delays imposed, although a stable equilibrium may still be maintained (Briggs & Godfray, 1994). As such, pathogen cycles with host-related periods may share many of the characteristics of host-parasitoid dynamics, as described previously.

For any particular group of pathogens infecting hosts there is likely to be large variation in the above parameters, dependent upon the type of pathogen modelled and its mechanism of infection, making a range of dynamical outcomes possible. However, not all pathogens may be transmitted horizontally: sexually transmitted infections, as

well as pathogens vectored by other organisms, may both exhibit transmission functions that are independent of the density of host organisms in a population. Transmission among these pathogens is usually termed *frequency-dependent,* since the transmission rate is thought to rely on the frequency of infected hosts within any given population (I/N, where N is the total population size); usually modelled by the transmission term βSI/N (i.e. a function of the proportion of infected individuals, rather than density of infected individuals) (Ryder *et al.,* 2005). These types of infection differ from density-dependent ones in that they cannot influence host population sizes, and do not require host density threshold for persistence (Thrall *et al.*, 1993). However, for vectored pathogens in particular, there may be a range of potential transmission functions from direct density-dependence, to being a function of I/N^2 (Antonovics *et al.*, 1995); in fact for many types of horizontally-, sexually- or vector-transmitted infections, transmission may be unlikely to be either purely density-dependent or frequency dependent, but lie on a continuum between the two, varying according to host and pathogen type (Lloyd-Smith *et al.*, 2003; Ryder *et al.,* 2005). For vertically transmitted pathogens (those transmitted among host generations) too, transmission is not likely to be a function of host density, although the mechanism of persistence among this group through purely vertical means is thought to be unlikely, since any reduction in host fitness as a result of infection would result in selection for uninfected hosts, reducing the pathogen's frequency in the host population (Lipsitch *et al.*, 1995). Transmission and persistence is therefore thought to be mostly driven by either coinfection/interference with horizontally transmitted pathogens, or a partial component of horizontal transmission (Lipsitch *et al.*, 1995; Jones *et al.*, 2007).

1.2.2.1 Baculoviruses

Perhaps the most widely studied group of pathogens among insects are the Baculoviruses (Baculoviridae), a group of DNA viruses which, although also found among Hymenopteran and Diptera hosts, are most noted for their infections among Lepidoptera (Cory & Myers, 2003); partially due to their potential as biological control agents of crop pests (Moscardi, 1999), but also as model organisms to investigate the ecology of host-pathogen population interactions (Dwyer, 1992; Sait *et al.*, 1994). Each virus particle, or *virion* is composed of a lipoprotein membrane that surrounds the genome-containing *nucleocapsid;* each virion is surrounded by a crystalline

proteinaceous matrix (composed mainly of the protein *polyhedra*), known as an *occlusion body* (OB), which protect the virions from desiccation or degradation in the environment (Carruthers *et al.*, 1988). Baculoviruses may be differentiated into two taxonomic groups, according to the size of the infective occlusion bodies: the multi-virion *nucleopolyhedroviruses* (NPVs), and the smaller *granuloviruses* (GVs), which normally contain only a single virion, or virus particle (Funk *et al.*, 1997). However, the mechanism of transmission among both groups is largely by the same route: infectious OBs are ingested by the feeding stage of the host organism, where they pass into the midgut. The action of proteases and an alkaline gut pH breaks down the polyhedra that comprise the OB, releasing the infectious virions, which then pass through the peritrophic membrane and fuse with the columnar epithelial cells that line the midgut. Here, the DNA-containing nucleocapsid enters the cell cytoplasm, where viral DNA is injected into the cell nucleus, where viral replication begins (King & Possee, 1992). New nucleocapsids are formed, and exit the nuclear and cell membranes in the form of a lipid-enveloped non-occluded *budded* virus—a more highly infectious form of virus than OBs—which infected other tissues and organs as they disperse throughout the host haemocoel. Secondarily infected cells may produce both budded and occluded forms of the virus within nuclei, which eventually lyse, releasing further infections into the body. Once the majority of tissues and organs have undergone infection and lysis, and complete internal liquefaction of the host has occurred, the infected epidermis ruptures, releasing millions of infective OBs into the environment (often onto the hostplant resource), where they may be ingested by conspecifics, and a further round of transmission may begin (Cory & Myers, 2003; Fuxa 2004; Il'inykh, 2007). In addition to this, there is some evidence that baculoviruses can modify host behaviour by inducing infected hosts to climb to the highest tips of the host plant following infection, possibly in order to increase the spatial transmission OBs when the host body lyses, in a process known as *wipfelkrankheit* (derived from 'tree-top disease'; Goulson, 1997). Rainfall and/or predation by vertebrates and invertebrates may also increase the transmission of OBs in the environment, via localised run-off onto and vectoring, respectively (Vasconcelos *et al.*, 1996; D'amico & Elkinton, 1995; Cory & Myers, 2003). Early in the infection cycle, among some species, infectious OBs may also be shed into the environment through faeces (Cory & Myers, 2003), providing multiple alternative transmission routes.

Although the primary transmission of these viruses is therefore horizontal, many studies on Lepidoptera-Baculovirus interactions have suggested a secondary route of vertical transmission via *covert*, or *persistent* sublethal infections (Burden *et al.*, 2003). Such infections have been found in many systems, and molecular techniques have demonstrated that the virus may persist asymptomatically in all life-stages of the host (Hughes *et al.,* 1993), with the principal route of transmission thought to be either transovarial, or transovum: contamination within or on host eggs, respectively (Cory & Myers, 2003). Such chronic infections may arise as a result of successfully surviving a virus challenge, with the virus able to persist within the host even after pupation (Burden *et al.*, 2002). However, the effects of such sublethal doses may manifest themselves in reduced host fitness in the subsequent life-stages, with potential population-dynamic consequences (Sait *et al.,* 1994; Sait *et al.*, 1998). Additionally, some systems have demonstrated that covert infections may be triggered into fully overt infections under certain conditions, mainly driven by stress-induced responses e.g. to high host densities, resource limitation, or co-infection from other viral or parasitoid challenges (Hughes *et al.*, 1993; Fuxa *et al.*, 1999). However, it has also been suggested that persistent infections may confer some immunity benefits, with hosts potentially becoming resistant to subsequent conspecific infection (Burand *et al.*, 1986). Whether such triggering to an overt state is possible among truly latent infections, rather than infections that are maintained by a constant low level of replication, is not currently known (Hughes *et al.*, 1993). The effects on population dynamics of host and pathogen of infections with such mixed infection strategies is not well understood, and while vertically transmitted covert infections are known to be able to persist for multiple generations when not triggered into their overt state (Burden *et al.*, 2003), ecological theory would predict that the fitness consequences of such a strategy would preclude persistence (see above). Such a mixed infection strategy has been suggested to be an explanation of the observed epizootic patterns seen in many forest-Lepidopteran pests, as a mechanism of maintenance in years of apparently low infections between outbreaks (Myers, 1988); theoretical models of such infections have shown that the stability of such systems may be highly dependent upon the conversion rate of covert to overt infections, although extremes of either are likely to be destabilising (Boots *et al.*, 2003). However, when host stage-structure is accounted for, such covert infections may bring about stable, low-level persistence without large oscillations (Bonsall *et al.*, 2005).

1.2.2.2 Cypoviruses

Although the Baculoviruses are perhaps the most widely studied of the many types of viral pathogens that infect insects (Miller & Ball, 1998), other genera may have significant impacts on their host; one such genus, found only in insects, are the Cypoviruses (Cypoviridae).

Cypoviruses are double stranded RNA (dsRNA) viruses of the family Reoviridae, and as such may be identified by their characteristic genome profiles, or *electropherotypes* (Payne & Rivers, 1976). Most of the described species have been isolated from Lepidopteran hosts, although they may also be found among Dipteran hosts as well (Payne & Mertens, 1983). Morphologically, they are superficially similar to the nucleopolyhedroviruses (NPVs), containing a single-shelled capsid surrounded by a proteinaceous polyhedral occlusion body (Coulibaly *et al.*, 2007). Infection routes may be either horizontal or vertical: in the former, again infectious OBs are ingested by the host and capsids released after OBs polyhedra has been dissolved by the alkaline midgut. However, unlike NPVs the genome of cypoviruses is RNA-based and replication of capsids takes place principally in the host cytoplasm, rather than in the nuclei of cells (Payne & Mertens, 1983). As such, infections are rarely lethal and may persist chronically, resulting in reduced host fitness (Rothman & Myers, 1996), although early neonate larvae may be particularly susceptible and may suffer mortality from such infections (Payne & Mertens, 1983). Horizontal transmission occurs when infectious OBs are released into the environment in host faeces, although contamination of eggs, either transovarially or transovum, provides an alternative route of vertical transmission (Rothman & Myers, 1996).

1.3 Heterogeneity in natural populations

The inclusion of a component of heterogeneity in risk among host-parasitoid models may promote persistence and stability, by altering the effective attack rate (May, 1978; see above). The inclusion of such parameters is likely to better approximate the situation among naturally occurring populations, since organisms and their resources are rarely either attacked at a constant rate, or homogeneously distributed in time and/or space (Thomas & Kunin, 1999; Berggren *et al.*, 2009). Heterogeneity parameters then may take several, potentially non-mutually-exclusive, forms:

1.3.1 Phenotypic, genotypic and temporal heterogeneity

Variation in both host and natural enemy life-history traits is likely to have significant influences on the form of the population dynamics, according to the species' ability to evade or induce mortality, respectively (Hawkins *et al.*, 1997). Among natural populations there may be within-species variation in susceptibility to natural-enemy attack, which may be manifest in several, interactive, ways:

1.3.1.1 Age-related effects

Among parasitoids attacking hosts, there may be a specific life-stage for which there is a preference; because developing parasitoid larvae derive all of their resources from the host they parasitise, the size and quality of the resource each larvae represents may be directly related to the success of subsequent parasitoid generations—if the host resource is too small, the parasitoid larvae either may not derive enough resources to complete successful development (i.e. starvation), or they may suffer delayed fitness costs if they do survive and emerge (Godfray, 1994). Conversely, if the host is too big, the larvae may not be able to remove sufficient host body mass in order to successfully emerge; instead becoming trapped within the host integument where both host and parasitoid suffer mortality (Harvey, 1996). Such effects are most likely to occur among idiobiont strategists, since the size of the host represents the entire resource at the point of paralysis (Godfray, 1994). For hosts infected by pathogens, age may be of particular relevance according to the size of the pathogen dose that needs to be obtained in order to cause mortality. For most Lepidoptera infected by Baculoviruses, the minimum lethal dose is positively correlated with age (i.e. a smaller number of viral OBs are needed to be ingested in order to cause mortality) (Duan & Otvos, 2001). For some species, the difference between the lethal dose among early and late instar stages may be many orders of magnitude, or several million OBs (Sait *et al.*, 1994); however, among natural populations, later instars will consume far more (potentially infected) foliage, thereby increasing their exposure to the pathogen (Goulson *et al.,* 1995).

1.3.1.2 Resistance / virulence effects

In any exploitative consumer-resource interaction, antagonism among the two levels may occur; in the situation of insect hosts and their natural enemies, hosts may evolve different strategies by which to escape or minimise infection or attack. Such defensive mechanisms may be partitioned into three levels—*primary defences* include behavioural mechanisms, such as cryptic or warning colourations, shelter building or group living to avoid or prevent the initial attack; *secondary defences* include those active behavioural responses that may prevent attack once the host has been located, such as defensive spines, regurgitation and spitting responses, biting and/or falling from vegetation; and *tertiary defences,* which are those defensive mechanisms that act after the host has been successfully attacked (i.e. innate host immune responses) (Gross, 1993; Smilanich *et al.*, 2009). For many insect hosts being attacked by natural enemies, the innate immune responses may be very similar if attacked by either parasitoids or pathogens: a combination of both humoral (e.g. the production of antimicrobial molecules such a melanin) and cellular (e.g. haemocytic encapsulation) immune responses (Strand, 2008). The production of melanin is known to occur in some Lepidoptera species as a direct response to crowding, possibly because of the increased likelihood of density-dependent disease transmission, or the selective attraction of parasitoids—a process termed *density-dependent prophylaxis* (Wilson *et al.*, 2001). Melanin itself, as well as being toxic to many microorganisms, may also help strengthen the insect cuticle, possibly preventing parasitoid oviposition, or cuticular lysis following pathogenic mortality (Hajek & St. Leger, 1994).

In addition to the innate immune response, hosts may also resist pathogenic attack via an acquired immune response, following an unsuccessful, or sublethal, viral challenge. Among some Lepidoptera species such systemic, or developmental, resistance may have a strong age-related component, as older larvae (that require higher mortality-inducing viral dosages) are better able to overcome a secondary round of infection after suffering an initial challenge (Hoover *et al.*, 2002). Such age-resistant related interactions are not confined to the tertiary immune responses: both primary responses (e.g. nest building behaviour), and secondary responses (e.g. biting/ vomiting response), may be more frequent among younger and older Lepidoptera life stages, respectively (Gross, 1993).

These interactive age-resistance effects will, to some degree be mediated by a variable genetic component among individuals (e.g. the strength of the immune response) which may also manifest itself in phenotypic variation (e.g. the size of the host larvae relative to a parasitoid and its ability to fend of an attack). Such genotype-phenotype variation is also likely to be a component of the natural enemy attack response too: larger parasitoids may be better able to handle and subdue hosts than smaller ones (Gross, 1993); more virulent pathogens may be better able to overcome host immune responses and/or replicate faster (and therefore cause mortality) in the host than other, less virulent pathogens (Lipsitch & Moxon, 1997). Among Baculoviruses infecting Lepidoptera, genotypic variants of the same species are widespread, and are known to potentially have very different virulence among different populations (Hatfield & Entwhistle, 1988), as well as within the same population (Hodgson *et al.,* 2001), and even within the same host (Cory *et al.,* 2005). Such a pattern would suggest a high level of genome recombination when closely-related strains come into contact; a potentially important mechanism in maintaining high levels of genetic variation on which selection can act in the host-pathogen resistance-virulence interaction (Crozier & Ribiero, 1992; Myers & Rothman, 1995; *reviewed in* Fuxa 2004; Asser-Kaiser *et al.*, 2007).

1.3.1.3 Temporal effects

Temporal heterogeneity among host-natural enemy systems may also arise through a interaction with age-related susceptibility: especially for the situation among parasitoid attacks, *phenological asynchrony* (a mismatch in the time between host and parasitoid emergence) may alter the effective attack time in which parasitoids may oviposit within hosts, which in turn may have delayed consequences for parasitoid recruitment in the next generation (Godfray *et al.*, 1994). As such, there may also be interactive effects mediated by host age-structure, in that parasitoids emerging too early may find only hosts that are either too small to oviposit into, or else hidden or protected; conversely, parasitoids that emerge too late may find that the effective host population size has either diminished (due to transformation into the next stage e.g. larvae - pupae), or else the remaining hosts are too large or their defences too strong, making oviposition reduced, or not possible. The effects of this will be most pronounced among temperate

populations with discrete generations, since in continuous time, hosts will be available for oviposition at any stage of the life-cycle (Hassell, 2000).

For pathogens such as Baculoviruses, temporal asynchrony is likely to be far less important than among parasitoids, since the primary transmission routes are either a function of host movement and feeding behaviours (horizontal transmission), or not affected by time (vertical transmission). However, although a major source of infection among early instars may be from long-term persistent reservoirs of OBs in soil (Fuxa, 2004), outside of such reservoirs, OBs released onto foliage may degrade and/or become inactivated over relatively short periods of time if exposed to UV radiation, or certain plant phenolic compounds (Young & Yearian, 1974; Cory & Hoover, 2006).

Although the effects of each of these sources of heterogeneity may differ greatly in the magnitude and sign of their individual and interactive effects, it may still be possible to estimate the potential stabilising effects of heterogeneity parameters in host-natural enemy interactions: decomposition of discrete-time models reveals that the criterion for stability in most systems is explained by the *aggregation of risk* term, which is the equivalent of the square of the coefficient of variation (CV = variance/mean) of the number of searching parasitoids per patches (*see* Taylor, 1993; and Hassell, 2000 *for details*). The so-called CV^2 *rule* states that stability as a result of heterogeneity will be maintained if $CV^2 > 1$; a rule that approximates well for a wide range of host-parasitoid (Pacala *et al.*, 1990), as well as host-pathogen (Dwyer *et al.,* 1997), systems.

1.3.2 Spatial heterogeneity

Perhaps the most well studied example of heterogeneity among host-parasitoid and host-pathogen systems is the case of spatial heterogeneity: in a patchy environment, the relative risk of attack or infection among hosts may vary both within and among patches, with a quantifiable effect on the stability of the interaction (Hassell, 2000).

The earliest, and most influential, empirical demonstrations of the importance of spatial heterogeneity in the dynamics of predator-prey systems was by Huffaker (1958), who demonstrated that the introduction of barriers to movement could decrease the relative dispersal of predator to prey, in microcosm habitat mosaics of interacting species of predatory and prey mites. Such spatial heterogeneity created stable oscillations, facilitating coexistence of predator and prey, rather than the unstable

extinction events when the two species' interacted in a homogeneous microcosm habitat. Indeed, for most natural populations distributed among a landscape, it is likely that hosts and their natural enemies will be embedded in a complex, heterogeneous matrix of suitable and unsuitable habitat, and that local conditions may vary greatly both within and among discrete patches (Grosholz, 1993; Cronin, 2003). Both host and natural enemies may further be hierarchically structured into *metapopulations* (populations of populations), each with their own separate patch dynamics.

1.3.2.1 Local scale dynamics and spatial density dependence

The key component often used in assessing the stability of host-natural enemy interactions due to heterogeneity (the CV^2 *rule;* see above) is based upon the concept of the relative risk of attack or infection *per patch*, or the effect of heterogeneity on the aggregation of risk (Hassell, 2000). However, the definition of a patch is intrinsically linked to an assumption about size (i.e. unit area of space) (Girvetz & Greco, 2007), with the abundance of hosts and natural enemies within each patch therefore defining their respective densities (i.e. number per unit area). Hence, CV^2 may be partitioned into two components, based on whether the density of hosts per patch is i) influencing the regulatory behaviour of the heterogeneity parameter (*host density dependence*—which may be positive or negative) or ii) not influencing such behaviour (*host density independence*) (Jones *et al.*, 1993).

The patterns of within-patch (or *spatial*) host density dependence, and delayed-density dependence (caused by time lags in cycles or hosts and enemies), have been well studied in host-parasitoid interactions, and may be highly variable: both positive and negative density dependence, and density independence are all manifest in a broad range of host-parasitoid systems, habitats and scales (*reviewed in* Walde & Murdoch, 1988). It is likely that the majority of such interactions display patterns of negative spatial density dependence (Jones *et al.*, 1993), but that such findings may be more readily detected when the scale of the study small (Walde & Murdoch, 1988). Such variation in the spatial pattern of attack may be due to a number of potentially interacting reasons (see above), although it is likely that the patterns of aggregation of parasitoids relative to their hosts, may be most closely related to the form of the functional response for any two species host-parasitoid system (Hassell, 2000). However, a review of a number of studies that also investigated the potential stabilising

effects of varying levels of spatial heterogeneity in such systems (as measured by CV^2) found that heterogeneity was sufficient to regulate host populations in around ⅓ of the cases; 90% of which were density *independent* (Pacala *et al.*, 1990; Pacala & Hassell, 1991).

A major assumption in theoretical models that demonstrate stability in such systems is that parasitoids remain distributed in the same positions within host patches, within generations. However, empirical evidence has previously suggested that density dependence has no effect on stability, while density dependence may be destabilising, or have no effect (Murdoch & Stewart-Oaten, 1989). However, in theoretical models where the redistribution (i.e. free movement) of parasitoids among patches and within generations is accounted for, density dependence is found to be destabilising, whereas density independence is found to be a *stabilising* influence (Rohani *et al.*, 1994). This effect is thought to be largely due to variation in the spatial aggregation of risk, with parasitoids aggregating in areas of high host density at the beginning of each generation, but with aggregation being reduced over time as a result of the remaining healthy hosts becoming more evenly distributed later on in the season, due to within-generation movement.

Interactions between insect pathogens and host density have tended to focus more on the effects of density dependent transmission, or delayed-density dependent effect, rather than spatial density dependence *per se* (see above). The differing transmission modes of the two natural enemies (*active* parasitoid searching vs *passive* pathogen transmission) suggest that spatial interactions may be qualitatively different, with characteristic spatial patterns of infection. Of those studies that do report instances of spatial density dependence, or the effects of rearing densities on pathogen transmission, the results may be highly variable: among Lepidoptera-Baculovirus systems, some report positive, but delayed, effects (Fleming *et al.*, 1986), reduced susceptibility to infection at higher rearing densities (Goulson & Cory, 1995); higher rates of pathogen resistance when reared gregariously compared to solitarily (Reeson *et al.*, 1998); higher rates of immune response-related parameters in larvae reared solitarily (Wilson *et al.*, 2003); increase transmission efficiency with increased density (Vasconcelos, 1996; Knell *et al.*, 1998); and conversely, decreased efficiency with increased density (D'amico *et al.*, 1996).

Such variability in the responses of pathogens to host density suggests that some parameter of heterogeneity other than host density may be driving the patterns of infection observed in many systems. Indeed, it may be that the spatial distribution and density of pathogen particles in the environment could be of greater consequence than host density—Dwyer (1991) found that transmission of a nucleopolyhedrovirus infecting Douglas fir tussock larvae was greater when the pathogen (quantified as the density of infected cadavers) was clumped rather than uniformally distributed. However, crucially, this effect was mediated by heterogeneity in the age-distribution of larvae, since transmission to healthy early instars decreased with increasing patchiness of infected cadavers. Such an effect bears parallels with the within-generation host-parasitoid models described previously (Rohani *et al.*, 1994; see above), since the effect is thought to be driven largely by local dispersal of larvae within patches; later instars being more mobile than earlier stages, and therefore increasing their risk of encountering an infectious pathogen in the environment (Dwyer *et al.,* 1991). These suppositions are supported by better fitting theoretical models that incorporate non-linearity in transmission as a result of host heterogeneity in susceptibility, compared to models fitted with a linear transmission rate (Dwyer *et al.*, 1997). Equally, spatial heterogeneity as a result of pathogen aggregation, or *clumping*, may also explain the better fit of nonlinear models of pathogen transmission (D'amico *et al.*, 2005).

1.3.2.2 The problem with space

Spatial heterogeneity may therefore represent a complex and variable component in host-natural enemy dynamics, potentially with stabilising influences; however, as previously mentioned, the concept of patch definition and density may depend upon the scale at which it is viewed (Wiens, 1989; Levins, 1992), since density itself is a measure of abundance within space (Lewontin & Levins, 1989). Therefore, for any given individual organism, their perception of space—and therefore the density of, and the responses to, other interacting organisms—will depend on the extent at which it is viewed and quantified (Mayor & Schaeffer, 2005). As an example of such scale-dependency, the quantification of the measure of CV^2 for inferring the likely stability of host-natural enemy stability is based entirely on the investigators' definition of patch size, and their quantification of the total number of searching parasitoids within in it: different definitions of patch boundaries may lead to different values of CV^2 (*see*

Hassell, 2000). The same is therefore true for the quantification of spatial density-dependence among hosts and natural enemies, since the coefficient of variation will be derived from the same data. A large component of the variation in sign and magnitude of the response of parasitoids to host densities seen in the review of parasitoid spatial density-dependence studies by Walde & Murdoch (1988) could be attributed by the authors to the scale on which each study was conducted: negative density dependence more readily found at small scales; and positive density dependence at large scales. Many insect species are known to display differing responses to habitat availability at multiple scales (Krawchuck & Taylor, 2003; Dumbrell *et al.*, 2008), including parasitoids (Roland & Taylor, 1997), which may be related to species-specific body size. Predators and parasitoids are also known to respond to host densities at characteristic scales (Ives *et al.*, 1993; Norowi *et al.*, 2000), highlighting the importance of sampling at a range hierarchical levels (Crawley & Hails, 1992; Ray & Hastings, 1996). One of the few studies to investigate the role of spatial scale among Lepidoptera-Baculovirus systems, was by Cooper *et al.* (2003), investigating the hierarchical spatial structure of genetic variation of a nucleopolyhedrovirus infecting western tent caterpillars. Although the effects of scale on density dependence appear to be largely absent from the insect-pathogen literature, it seems likely that heterogeneity in the form of pathogen clumping, and a passive transmission strategy, may predispose such pathogens to high degree of small-scale scale-dependency.

A further problem of using a spatial approach to investigate ecological phenomena is the occurrence of *spatial autocorrelation* within data: nearby variables being more similar (positive autocorrelation) or less similar (negative autocorrelation) than expected by chance alone (Legendre, 1993). Such a phenomenon may arise simply as a product of the spatial arrangement of organisms within a habitat (e.g. herbivores clustering around areas of high resource quality); or through the existence of environmental gradients (e.g. organisms distributed along a slope may experience more or less favourable conditions, dependent on their spatial position on the slope, but that this favourability exists on a gradient from top to bottom). This may cause problems for the use of classical inferential statistics, which typically assume independence of variables, since any element—or sample point—will be more similar to adjacent elements than those farther away (Fortin & Dale, 2005). In some instances, testing for autocorrelation may reveal synchronous patterns in population dynamics, although this requires specific statistical procedures in order to disentangle pattern from artefact. Such

artefacts can arise through inappropriately designed field experiments, whereby sample points are insufficiently spaced apart from each other to avoid autocorrelation—known as *site redundancy* (Koenig 1999). The importance of accounting for such artefacts when analyzing spatially referenced ecological data was highlighted by McGeoch & Price (2004). In a review of analysis methods used to search for spatial density-dependence among a host-parasitoid system, the authors found that highly variable and contrasting conclusions could be drawn from the same set of data, dependent upon the analysis method used; they conclude by advocating a rigorously spatially explicit approach to avoid potentially misleading conclusions being drawn from such data.

1.3.2.3 Dispersal and metapopulation dynamics

In the previously described models, within-patch dispersal by hosts and their natural enemies may have important, stabilizing effects on population dynamics in spatially heterogeneous environments. However, in the same way that a spatially implicit perspective (i.e. one that does not account for within-patch dispersal) on population dynamics may be a simplification of the probable interactions that occur among natural populations, within-patch or single-patch models are a simplification of the probable interactions that may occur among freely dispersing organisms that are able to move among patches (Hanski, 1999).

In reality, many species occur in discrete patches of suitable habitat within any landscape or region, which may typically occupy areas much greater than the dispersal distances of the organisms that occur in them (Holt, 2003). However, if organisms are able to disperse between patches (i.e. locally), within-patch populations dynamics may be affected. This flux of emigrant and immigrant individuals within and among patches therefore form metapopulations (or populations of populations), all embedded within the same region (Levins, 1968; Hanski, 1999). These immigration and emigration rates, as well as colonisations and extinctions, will all be dependent upon the scale of these local interactions, which may be wholly or partially affected by local host density. For example, smaller, more isolated patches, may have lower colonization rates and/or be able to support lower densities of resources and consumers. Such processes are therefore likely to affect the potential within- and among- patch interactions between resources, hosts, and natural enemies in any region.

The importance of such scale-dependencies has been demonstrated theoretically in spatially implicit models of host-parasitoid metapopulations. When host dispersal is regional and parasitism is randomly distributed across all patches, the dynamics of each are characterized by unstable cycles (Comins *et al.*, 1992). Such complete mixing is, in reality, unlikely for most populations, and the overall effect is that all patches become perfectly synchronized over time, displaying perfect Nicholson-Bailey dynamics (Hassell, 2000). However, when demographic or environmental stochasticity (analogous to heterogeneity) is introduced into such models, the effect is to destabilize this synchronicity, and thereby promote persistence (Reeve, 1990; Adler, 1993; Taylor, 1998; Hassell, 2000). The effects of this stochasticity are resolved by modelling interactions on a local scale in spatially explicit terms using *lattice* models (i.e. models that utilise discrete time and space, but continuous populations; Kaneko, 1992; Hassell, 2000). In such models, asynchrony between patches is promoted by diffusive dispersal of hosts and parasitoids to neighbouring patches. For a wide range of parameters and degrees of asynchrony between local host-parasitoid populations, the dynamics of these metapopulations have been shown to be relative stable, although local population extinctions may become more likely if the habitat size decreases, due to disruption of the dispersal required to link the asynchronous local populations (Hassell, 2000).

The effects of such reduced dispersal may be manifest in the occurrence of host population outbreaks, as parasitoids are unable to exert sufficient regulation when their effective population size is reduced. However, if local populations exist within the spatial scale of host and natural enemy dispersal, these boom-and-bust outbreaks may be maintained, and may typically display travelling wave-like behaviour, especially in lattice models where dispersal is restricted to nearest-neighbour patches. These wave-like properties may give rise to characteristic spatial patterns among patches (e.g. spatial chaos, spiral waves, and crystal lattices), that depend largely on the relative dispersal rates of hosts and parasitoids (Comins & Hassell, 1996; Hassell, 2000). These types of spatial pattern formation, arising in such *reaction-diffusion* models, have been empirically verified in some field populations: for example, Maron & Harrison (1997) showed that when parasitoid dispersal is high relative to their hosts—in this case a mobile parasitoid attacking the larval stages of a Lepidoptera species with flightless female adults—the spatial spread of an outbreak could be suppressed via a higher ratio of parasitoids on the periphery of the outbreak zone, while other local populations further away were able to increase.

Similar reaction-diffusion models have been used to model the spatial spread of pathogens in the environment, which may also display travelling wave-like behaviour whereby the host density and the proportion of infected hosts follow a tightly coupled wave of expansion which suggests a form of coupled, delayed density-dependence (Dwyer, 1992). The rate of the spatial spread of such waves has previously been shown to be a function of the pathogen transmission rate; the rate of production of pathogen by hosts; the initial population density; the decay rate of the pathogen; and the death rate of the hosts, and may predict the true transmission rates seen among field populations well (Entwistle *et al.*, 1983; Dwyer, 1992). The obvious discrepancies between the dispersal rates of parasitoids and pathogens when attacking hosts means that we would expect within-patch processes to be characteristically different. Indeed, this appears to be the case: Dwyer (1994) was able to show, in a model parameterized from a Lepidoptera host-Baculovirus system, that the inclusion of density-dependent host reproduction *and* host movement behaviour in the larval stage increased the likelihood of regular host-pathogen cycles, which provided good fits to empirical data. Such models suggest, then, that larval dispersal may therefore play an important role in the stability of these cyclical interactions.

Local host dispersal may therefore play an important role in the dynamics of hosts and pathogens within patches. However, if the scale of these interactions is extended to include among-patch metapopulation influences, it becomes apparent that such interactions may be hierarchically structured at two further scales, due to variation in host resistance: hosts within patches among populations, and pathogens within hosts within patches among populations. In this sense, hosts may therefore act as effective patches for pathogen colonization, with extinction processes mediated by variation in susceptibility and resistance (Hess *et al.*, 2002). Within-patch occupancy and/or prevalence is therefore likely to be a product of pathogen transmission rates (which may itself be linked to host density), with prevalence among patches increasing with decreasing patch isolation (Hanski, 1999).

Such a scale-linked transmission function may therefore have important consequences for the particular host-pathogen interaction studied, dependent upon the relative dispersals of the interaction organisms. Disease persistence in spatially explicit models has previously been demonstrated to be highly dependent upon the spatial scale of pathogen dispersal (Thrall & Burdon, 1997). For example, in models of plant-

pathogen interactions, Thrall & Burdon (1999) found that disease persistence was highest at relatively local scales of dispersal, with much reduced levels of persistence at larger scales. However, such a large-scale pattern could also translate into disease-dependent reductions in the proportion of sites occupied by hosts. Such patterns therefore may translate into differences in *endemic* patterns at local scales, and *epidemic* patterns at larger scales, possibly mediated by resistance / virulence effects related to host-pathogen encounter rates. Indeed, investigation of transmission rates from a range of host-pathogen models suggests that small-scale, localized transmissions can drive large-scale epizootics at the metapopulation level (Fenton *et al.*, 2002).

Scale-dependent differences between hosts and pathogens may therefore influence evolutionary processes and interactions, such as host resistance and pathogen virulence: low virulence is predicted when both infectivity and host reproduction occur over local scales (Boots & Sasaki, 2000; Messinger & Ostling, 2009) due to a pathogen *self-shading* effect, whereby highly virulent pathogen strains surround themselves with a higher proportion of infected individuals, thereby reducing their effective transmission rate; the high virulence seen in many Lepidoptera-Baculovirus systems may therefore be a result of high host dispersal, relative to pathogen dispersal (Boots & Mealor, 2007). However, if host dispersal relative to pathogen dispersal is so great that pathogens are no longer able to colonise new hosts, such population interaction and regulation may fail. Such *enemy release* effects may potentially be facilitated by number of mechanisms, such as climate-induced host range expansions (Menendez *et al.*, 2008), or the human-mediated translocation of organisms outside of their native ranges (Wolfe, 2002), and may therefore be an explanation for the success of newly invasive organisms in novel habitats (Mitchell & Power, 2003).

The case for persistence may not be quite as straightforward for vertically transmitted parasites and pathogens, which ecological theory predicts should not be able to persist in populations due to their fitness-reducing effects (see above). However, theoretical models have shown that persistence may be facilitated if vertical transmission occurs together with a horizontal component of transmission (Anderson & May, 1981), or if there is some interference with co-infecting horizontally transmitted infections (Jones *et al.*, 2007). An alternative, spatially-related, mechanism may also exist whereby fitness-reducing, obligate vertically transmitted infections are able to persist in metapopulations, provided that infected individuals are able to colonise new patches (Saikkonen *et al.*, 2002).

1.4 Bottom-up and abiotic effects

The debate concerning whether 'the world is green' (natural enemy pressure reducing herbivore densities to levels below which they defoliate host-plants: Hairston *et al*., 1960), or whether 'the world is prickly and tastes bad' (physical and chemical defences of host-plants limiting herbivore infestations: Murdoch, 1966) has classically focused on the question of whether either of these processes are dominant in any particular community. Most contemporary ecologists agree that a combination of such bottom-up and top-down forces act together to regulate populations of herbivorous insects, although the extent to which the two interact in relative terms, and the factors that control whether one or either process is favoured, is still unclear (Hunter *et al*., 1997; Hassell *et al.*, 1998). In many previous studies, this has been the result of a failure to disentangle the respective roles of bottom-up and top-down effects that such a pluralistic approach requires, being in large parts down to a semantic failure to distinguish between density-dependent population *regulation* (allowing population persistence and the return to an equilibrium density), and other non-density dependent *influences* (the factors influencing the observed fluctuations in population density) that govern abundance.

1.4.1 Bottom-up factors influencing abundance

1.4.1.1 Phenology

Variation in larval host-plant development time can have a significant influence on herbivore populations, with most insects being adapted (via stabilising selection) to emerge when host-plant nutritional quality is maximal (Tikkanen & Lyytikäinen-Saarenmaa, 2002), thereby supporting high herbivore densities and causing high levels of defoliation (Hunter & Price, 1992). Individual variation in tree budburst phenology, however, can often mean that herbivore egg hatch dates are temporally asynchronous with their host plant budburst. This has negative ramifications for larvae that emerge both too early (when no food is available) or too late (when defoliation has occurred, unpalatable defences have been mobilised, or nutritional quality has diminished)

(Feeny, 1970; Watt & McFarlane, 1991). A polyphagous life-history may therefore be the result of a spreading-of-risk (or safety-net) strategy that includes feeding on alternative host species if emergence with the primary host is asynchronous (Wint, 1983).

1.4.1.2 Palatability

Although superficially it would appear that widespread defoliation by outbreaking insects would directly contribute to a reduction in host-plant quality, it may also be the case that indirect palatability compensation occurs. In this situation, a paradox arises, mediated by the host-plant, whereby insect herbivores increase their total consumption of the host-plant resource as a response to low quality or indigestible foliage (Kerslake & Hartley, 1997). In this way, constitutive defences may actually reduce host-plant fitness (Price *et al.*, 1980), which may be of particular significance among plants that are not adapted to a novel herbivore. Seasonal defoliation may also affect host-plant growth and reproduction in subsequent generations, creating time-lagged bottom-up consequences for the resident herbivore (Kaitaniemi *et al.*, 1999), influencing the timing of life-history traits, such as diapause induction (Hunter & MacNeill, 1997).

1.4.1.3 Plant architecture

The physical features of a particular host plant may also influence herbivore assemblages. As well as straightforward tactile defences, such as trichomes and pubescences (Levin, 1973; Rausher, 2001), the structural architecture of a plant may also play a significant role in influencing herbivore abundance (Lawton, 1983). For example, among Lepidoptera fauna associated with heather in Britain, Haysom & Coulson (1998) found that a significant progressive increase in larval diversity was correlated with plant height, more so than with either green shoot density, flower density, total plant cover, or age. In terms of overall diversity, this effect is thought to be due to a combination of size *per se* (larger plants are more likely to be colonised by insects) and resource diversity (a larger number of resource types can support a wider diversity of species), such as the provision of refugia and overwintering sites (Lawton, 1983).

1.4.2 Top-down factors influenced by bottom-up sources

1.4.2.1 Parasitoids

The relative strength of bottom-up and top-down influences may differ both temporally within habitats and spatially across them (Gratton & Denno, 2003), in a variety of permutations, including indirectly mediated effects. The availability of refugia via host-plant habitat complexity is one such example, whereby a bottom-up effect positively influences herbivore abundance via the indirect interruption of top-down processes. Similarly, the presence of physical plant defences (Levin, 1973); plant species composition within a habitat matrix (Cronin & Haynes, 2004); and heterogeneity of distribution in patchy environments (Walde & Murdoch, 1988) may also disrupt the temporal and spatial dynamics of an herbivore-parasitoid interaction, by hindering parasitoid search efficacy. Phenological asynchrony between parasitoid and host is also mediated by host-plant influences, although modelling suggests that this phenomenon has the ability to stabilise such interactions, thereby promoting persistence (Godfray *et al.*, 1994).

Other types of plant defence mechanisms may also alter top-down regulation: herbivore natural enemies can detect some volatile chemicals emitted by certain plants in response to tissue damage, with some species able to selectively attract host-specific parasitoids (De Moraes *et al.*, 1998). Indeed, chemical cues given off by oak when under attack from winter moths, are known to increase the level of parasitism and number of eggs laid by *Cyzenis albicans* (Roland *et al.*, 1989). Other allelochemicals, mobilised as anti-herbivore devices, may be sequestered by the attacking herbivore, making themselves either unpalatable to natural enemies, or presenting a chemical barrier to parasitoid larval development (Price *et al.*, 1980). In many cases this may create a trade-off in host-plant selection by ovipositing female herbivores (especially true of species that display a sedentary larval life-history) that balances the negative fitness consequences of plant defence mechanisms, against larval survival from natural enemy pressure, which may promote host-plant shifting to areas of enemy-free space (Björkman *et al.*, 1997). This effect comes under the umbrella of the *slow-growth, high mortality* hypothesis, which posits that slower growing larvae (mediated by plant quality and chemical defences) have a greater window of vulnerability (the temporal limits upon which natural enemies must attack) and are therefore subject to higher

mortality rates, than faster growing conspecifics (Osier *et al.*, 1996; Benrey & Denno, 1997).

1.4.2.2 Pathogens

As well as affecting parasitoids, top-down effects mediated by bottom-up factors can arise in host-pathogen systems too (*reviewed in* Cory & Hoover, 2006). As well as direct horizontal transmission via foliage ingestion, plant architecture has been shown to be an important factor promoting host-independent baculovirus persistence in the field, with foliar shading providing protection from UV irradiation, thereby allowing reservoirs of virus particles to persist from year to year (Raymond *et al.*, 2005). However, this effect is dependent upon the host plant, with some species conferring a negative effect on viral persistence through release of degradative foliar exudates (Young *et al*., 1977). Infectivity of a pathogen can also be influenced indirectly through diet-induced factors by altering the herbivores susceptibility to infection. Laboratory experiments have demonstrated that larvae fed on a high-quality diet, whilst more susceptible to infection, demonstrated greater survival rates than conspecifics fed on a poor-quality diet (McVean *et al*., 2002). The nutritional composition of the larval diet may be the key to this effect, with studies demonstrating that viral infection can induce behavioural self-medication responses that work to increase the protein content of the diet, conferring reduced mortality through an augmented immune response, and increased larval development rate (Hoover *et al.*, 1998; Lee, 2002 *sensu* Cory & Myers, 2003).

As well as the direct nutritional content of the host resource, phytochemicals can also play a role in host-pathogen interactions. Ingestion of secondary plant metabolites (e.g. phenolic compounds) can affect both rate of mortality and total mortality of larvae following viral infection (Farrar & Ridgeway, 2000): For example, experiments with the generalist lepidopteran *Operophtera brumata* (Lepidoptera: Geometridae) have demonstrated increased mortality when feeding on resources high in phenolic compounds such as Oak (Raymond *et al.*, 2002), which retain more pathogenic infectivity and persistence than habitats abundant in more palatable plants, such as heather moorland (Raymond *et al*., 2005). The precise mechanism involved in this interaction is unclear, with contradictory evidence demonstrating that phenolic compounds can also inhibit the infectivity of some viruses (Keating & Schultz, 1990).

This may be due to variability in phytochemical composition and mid-gut digestibility processes between species (Cory & Myers, 2003), and could play a role in the maintenance of viral genotypes within and between host populations (Hodgson *et al.*, 2002).

1.4.3 Exogenous factors

The role of abiotic factors in population ecology was first proposed by Andrewartha & Birch (1954; 1960), who stressed that inclusion of such exogenous processes was a *sine qua non* for understanding the regulation and abundance of herbivorous insect populations. Whilst this may not represent the modern consensus (Begon *et al.*, 1996), such density-independent factors (e.g. weather) remain an essential consideration, albeit a proximate one, when attempting to understand an organism's population ecology.

1.4.3.1 Temperature

Among the most studied of these is the effect of temperature. This is of particular significance given the threat of human-induced climate change, and the associated changes in development, survival, range and abundance that is predicted to be manifest in ecosystems across the globe (Bale *et al*., 2002).

Changes in phenology caused by climatic shifts are predicted to affect a huge variety of taxa (Visser & Both, 2005) although, regional differences in the timing of egg, larval and adult eclosion among Lepidoptera are thought to be controlled by both genetic *and* climatic influences (Kimberling & Miller, 1988; Peterson & Nilsson, 1998), being particularly influenced by severe winter temperatures (Holliday, 1985). Although some studies have postulated that elevated temperatures (in line with climate predications) would have little effect on the synchrony of larval emergence with host-plant budburst (Buse & Good, 1996), others have concluded that as little as a 2-5ºC rise in temperature is enough to cause complete asynchrony (Dewar & Watt, 1992), with changes in spring temperatures (rather than mean annual temperature) being of particular significance (Visser & Holleman, 2001). Although the differences between these results may be due to methodological problems (Watt & McFarlane, 2002), it is likely that such temperature changes will have greater ramifications for top-down influences, such as avian predators (Buse *et al*., 1999) and parasitoids (Nouhuys & Lei,

2004). Indeed, the autumnal moth (*Epiritta autumnata*) has previously been shown to have lower survival and egg-production indices, coupled with reduced larval parasitism, at higher temperatures (Virtanen & Neuvonen, 1999). It is also worth noting that the direct effects of temperature will only be manifest on non-larval parasitoid stages, as endogenous development always occurs in concert with that of the parasitized host (Gould & Elkinton, 1990).

1.4.3.2 Carbon Dioxide & UV-B radiation

As well as increased global temperatures, increases in levels of CO_2 are also predicted under climate change scenarios, although these are not thought to be of any great significance to the dynamics of insect herbivore populations (Buse & Good, 1996; Bale *et al*., 2002). Other human-induced effects include depletion of the ozone layer, leading to increased levels of ultraviolet-B (UV-B) radiation reaching the earth's surface. This can also be a result of reduced sunspot activity, which may have population dynamic consequences for hostplant feeding insects via the bottom-up: irradiated plants allocate more resources sequestering phenolic compounds that confer some UV-B protection, at the expense of phytochemicals that confer protection from herbivory (Selås *et al*., 2004). Such effects may be additive from the top-down too, as horizontally-transmitted viruses are particularly susceptible to UV degradation when in the occluded form (Cory & Myers, 2003; see above).

1.4.3.3 Altitude & latitude

It is worth noting that although Selås *et al.* (2004) found increased densities of outbreaking winter moths in relation to increased UV-B levels, these results were only apparent at higher altitudes. Such results are concurrent with other studies that posit changes in foliar chemistry along elevation gradients (Sparks & Ehleringer, 1997) as well as others showing similar correlations with temperature and relative humidity (Yarnes & Boecklen, 2005). This alone may mediate phenological mismatch between herbivore and host plant (Hodkinson, 2005), although studies along altitudinal gradients suggest that outbreaking Lepidoptera are able to match their phenology to that of their host plant, with the upper and lower range limits in some systems being regulated by snowfall and predation, respectively (Mjaaseth *et al*., 2005). This is in contrast to

latitudinal gradients, which tend to be more associated with factors affecting phenological synchrony, such as light availability and day length (Hodkinson, 2005).

1.4.3.4 Precipitation

Precipitation is another exogenous factor that may influence abundance, again due to indirect effects on host-plant phytochemistry. During periods of low rainfall or drought, concentrations of foliar nitrogen and defensive phenolic compounds have been shown to be reduced as a result of physiological stresses, with a concomitant direct correlation between low rainfall periods and insect herbivore damage (Shure *et al*., 1998). Conversely, Masters *et al.* (1998) demonstrated an increase in abundance of hoppers (Homoptera: Auchenorrhyncha) as a direct result of increased vegetation cover, via supplemented summer rainfall. However, the opposite was not true of drought conditions, which caused a decrease in vegetation cover, but no change in herbivore abundance. Although the indirect effects of precipitation seem to be largely confined to bottom-up sources, it may facilitate virus dispersal (D'Amico & Elkinton, 1995) and mortality rates— gypsy moth mortality from fungal pathogens has been shown to have a positive correlation with rainfall (Weseloh *et al*., 1993).

1.4.4 Effects on population processes

Although all of the above processes can be thought of classically as density-independent factors, Cooke & Roland (2003) argue that in populations of forest-tent caterpillars, winter temperature can act in a *partially density-dependent* manner, by directly affecting density-dependent behavioural and physiological processes. Although this concurs with Varley *et al.*'s (1973) life-table analyses--stating that winter mortality is the most significant factor regulating winter moth populations--there still appears to be no modern consensus regarding the most important factor regulating abundance. Opposing hypotheses regarding species the show large fluctuations in density include those originating from the observation that many forest defoliator species appear to be synchronised over disparate and wide geographic ranges. The so-called *climatic release* hypothesis suggests that outbreaks are triggered by successive periods of favourable weather that enhance larval survival and adult fecundity (Greenbank, 1956). An alternative to this is the *Moran effect*, which hypothesises that separate populations

undergoing different population oscillations, are able to be synchronised by a common exogenous perturbation (*i.e*. weather) (Moran, 1953). However, analysis of population data regarding the latter effect remains inconclusive, with some experiments giving a positive effect (*see* Hudson & Cattadori, 1999; Myers, 1998; Williams & Liebhold, 1995), whilst others suggest a greater role for biotic components as regulatory factors (Ims *et al.*, 2004).

1.5 The study system: two hosts, one foodplant, four pathogens, and a parasitoid

The particular system used in this study consists of two Lepidoptera species—the winter moth (*Operophtera brumata* L.), and the magpie moth (*Abraxas grossulariata* L.)—and their associated natural enemies. Although both species are polyphagous, and may or may not occur together on certain species of hostplant, in the Orkney Isles (northeast Scotland) they both feed primarily on the same shared hostplant: common heather (*Calluna vulgaris* L.(Hull)).

1.5.1 Orkney

The Orkney Isles (58°4′-59°2′N, 2°2′-3°3′W) consist of an archipelago of around 70 islands, separated from the most north-easterly point of the coast of mainland Scotland by around 10km. Its low topographic relief and proximity to the gulf-stream mean that the islands may experience a relatively mild annual climate, compared to both other areas at the same latitude, and in the nearby highlands of mainland Scotland. However, this also means that they may receive particularly strong, year-round, northerly winds. In general, the difference between summer and winter temperatures is relatively small (~10°C), although snowfall is common during the winter months. Some of the higher altitude exposed areas may support tundra vegetation species, suggesting that such equable conditions are not shared by all parts of the archipelago (Berry, 1985; Berry, 2000). Large areas of forest, and indeed trees in general, are almost entirely absent among all the islands, but for a few isolated areas of woodland and plantations; the majority of space being given over to improved grasslands or pasture, although around 29% is composed of heather moorland, which is often used by livestock farmers for

rough-grazing (Hanley *et al.*, 1996). Overgrazing of livestock may be contributing to the decline in heather moorland coverage in the islands (Simpson *et al.*, 1998), although a number of areas are designated as conservation reserves, as this habitat is able to support a number of species of moorland ground-nesting birds, including protected species such as hen harriers (*Circus cyaneus*), merlin (*Falco columbarius*), short-eared owl (*Asio flammeus*), and whimbrel (*Numenius phaeopus*). Large mammalian predators are completely absent from the islands, as are wild ungulates (Berry, 2000; Amar & Redpath, 2005). The Lepidopteran fauna of the Orkneys have been particularly well studied, and currently contain around 400-424 recorded species (Berry, 1985; Berry, 2000; Lorimer, 1983), representing approximately $^{1}/_{5}^{th}$ of the diversity of Britain as a whole.

1.5.2 Winter moth (Operophtera brumata)

The winter moth is a polyphagous, univoltine Geometrid that occurs throughout the UK on its primary larval food source, pedunculate Oak (*Quercus robur*). In Orkney, where the species is described as *resident,* populations have taken to feeding on heather (*Calluna vulgaris*), largely due to the absence of such deciduous woodland among the islands. Worldwide, it may also be found on Sitka spruce (*Picea sitchensis)* (Hunter *et al.*, 1991); Sycamore (*Acer pseudoplanatus)*; Beech (*Fagus sylvatica)* (Wint, 1983); Apple (*Malus sp.)* (Holliday, 1977); and Bilberry (*Vaccinium myrtilus*). Globally, they follow a holoartic distribution, limited by a lethal overwintering minimum temperature of -33°C (MacPhee, 1967) and have been the subject of numerous studies concerned with their populations in eastern North America (Embree, 1966), Central Europe (Visser & Holleman, 2001; Van Dongen *et al.*, 1994), Fennoscandia (Tikkanen *et al,*. 1998), and southern Britain (Varley *et al.*, 1973). Populations display congruous temporal development throughout this range, with differences in life-history stage development times being mediated by variation in both climatic and genetic variability (Kimberling & Miller, 1988)—egg stages are longer and pupal stages shorter, in more northerly latitudes (Holliday, 1985).

Female adults display brachyptery (flightlessness) and show little discrimination between oviposition sites, attracting mates by releasing sex pheromones, which the winged males orientate toward. Males display protandry and may copulate up to seven times during their lifetime, whereas females typically copulate only once (semelparity),

leading to a male-biased operational sex ratio (the ratio of males to females ready to mate), with males primarily choosing mates based on female size-quality (Van Dongen *et al.*, 1998). Larvae, once established on a foodplant, remain monophagous until adulthood (Wint, 1983). Here, they feed for approximately 6 weeks, going through 5 larval instars (which may be identified by the width of the head-capsule), before overwintering as pupae in the soil, and finally emerging in winter (Hunter, 1998). Early instar larvae may build silken *nests* within the buds or shoots of the host plant (Edland, 1971); however, if emergence is under unfavourable conditions (e.g. poor foodplant quality), early instar larvae may disperse via *ballooning*—throwing out silken threads that get caught by prevailing winds (Bell *et al.*, 2005). Larvae are also known to display phenotypic variation (or *phase polyphenism*) in their degree of cuticular melanisation, from pale yellow or green to almost entirely black (Hagen *et al.*, 2003); such variation is known to be linked to the density of conspecifics, although it is not clear whether this is due to density *per se* (i.e. density-dependent prophylaxis), or directly to the effect of parasitioids and/or pathogens (Hagen *et al.*, 2006).

High density outbreaks of *O. brumata* larvae were first recorded on Orkney in 1981 (Picozzi, 1981), and have since become widespread in North-east Scotland where, as well as heather moorland, it has also been recorded on Bilberry and Sitka spruce (Kerslake *et al.*, 1996; Graham *et al,*. 2004; Hunter *et al.*, 1991). Population densities in moorlands can reach $1400m^{-2}$, whereas on oak peak densities are more often $250\text{-}300m^{-2}$ (Raymond *et al.*, 2002). Such outbreaks may sometimes cause localised death of the host plant, and are characterised by themselves being highly spatially restricted—densities of moths fall may to zero merely 50m away from severe outbreak sites (Hunter *et al.*, 1991). However, reports of outbreaks among moorlands in Orkney have also reported defoliation of heather across areas of up to eight hectares (Lorimer, 1983). Deciduous tree-feeding populations are also known to occur at outbreak densities, and may defoliate large areas of forest (Kaitaniemi *et al.*, 1999; Weslowski & Rowinski, 2006); such populations are also known to exhibit population cycles (Selas *et al.,* 2004; Nilssen *et al.*, 2007; Tenow *et al.,* 2007). However, whether heather-feeding populations exhibit similar dynamics is not currently known.

Among populations in mainland Britain, *O. brumata* may be attacked by a broad range of specialist and generalist predators and parasitoids (Frank, 1967; Hassell, 1969; Varley *et al.*, 1973). However, in Orkney, the only principle parasitoid is the solitary

endoparasitic Ichneumonid *Phobocampe tempestiva* (Holmgren) (Kerslake *et al.*, 1996; Graham *et al.*, 2006). Although predation other than from parasitoids is not explicitly investigated in the present thesis, the principle invertebrate predators of *O. brumata* in Orkney are Carabid and Staphylinid beetles, which feed on the pupal stage, and may exert some regulatory control amongst moorland populations (Raymond *et al*., 2002); and of the vertebrate predators that exist, starlings (*Sturnus vulgaris*), meadow pipits (*Anthus pratensis*) and common gulls (*Larus canus*) are known to feed directly on larvae (Picozzi, 1981), although the extent and frequency of such predation in this system is not currently known. It is also not clear whether any small mammal predation exists in the Orkney study system, although density-dependent pupal mortality has been recorded in similar outbreaking species (Tanhuanpää *et al.*, 1999).

Pathogenic infections among *O. brumata* populations other than Orkney have typically been underrepresented, or else entirely absent (with the exception of noted NPV infections in Embree, 1966; and microsporidian infections in Varley *et al.*, 1973), as parasitoid or predator interactions have been previously assumed to dominate. Among populations in Orkney, *O. brumata* may suffer infection from a number of different viral pathogens, including *O. brumata* Nucleopolyhedrovirus (OpbuNPV): a horizontally-transmitted species of Baculovirus. Different genotypes of OpbuNPV are known to occur in geographically separated populations within Orkney, doing so at prevalences ranging from 0.6-61%, which in some instances constitute the single major factor affecting host mortality (Graham *et al*., 2004). The effect of host-plant species on the infectivity of OpbuNPV has also previously been examined among *O. brumata* feeding on three different host plants, including heather (Raymond *et al.*, 2002). Here, the infectivity of virus was found not to differ between species, although oak-feeding populations died sooner and yielded more virus, than both heather- and Sitka spruce-feeding populations.

Also isolated from these same populations on Orkney as OpbuNPV were three novel species of Reovirus: two species of *Cypoviridae* (OpbuCPV18 and OpbuCPV19) and *Operophtera brumata* Reovirus (OpbuRV) (Graham *et al.*, 2006; Graham *et al.*, 2007). Both OpbuCPV18 and OpbuCPV19 are classic, occluded Cypoviruses that both exhibit vertical transmission modes, although OpbuCPV19 appears to be more prevalent among populations than OpbuCPV18 (Graham *et al.*, 2006). For this reason, only the former of the two is investigated in the present thesis (hereafter, CPV will correspond to

OpbuCPV19). In contrast, OpbuRV is a non-occluded Reovirus, although its exact taxonomic position is not presently clear. This species was found to be present in around 10% of sampled *O. brumata* larvae, although among the parasitoid *P. tempestiva*, the prevalence was 100% (Graham *et al.*, 2006; Graham *et al.*, 2008). Similar non-occluded viruses have been previously found to be vectored by parasitoids of other species, and are thought to occur mutualistically with the parasitoid, and play a role in suppressing the host larvae's cellular immune response upon oviposition (Renault *et al.*, 2005).

1.5.3 Magpie moth (Abraxas grossulariata)

A. grossulariata too, is a polyphagous univoltine Geometrid, distributed widely throughout the UK, as well as throughout mainland Europe and as far west as Japan (Hill, 1987). Its main foodplants are species of the genus *Ribes*, such as gooseberry (*Ribes grossularia*), although it may also feed on other shrubs and bushes, such as plum (*Prunus domestica*), hazel (*Corylus avellana*) and hawthorn (*Crataegus monogyna*). Egg batches are laid on the underside of leaves in the late summer, and take about two weeks to hatch; overwintering is therefore in the larval stage, and early instars may spend this time hibernating in sheltered areas, such as leaf litter. Following budburst in the spring, larvae emerge from hibernation and begin feeding. Final instar larvae may be relatively large (around 40-50mm), and as such may consume large quantities of vegetation, potentially causing localised defoliation; pupation usually takes around 4 weeks, with adults emerging in mid-summer.

A. grossulariata is one of the few species to display *aposematism* (warning colouration) among larval, pupal and adult stages of development, and is the only know British species to do so in the pupal stage. Such colouration may serve as a deterrent to predation, especially among vertebrate predators; to date, the only known avian predator is the cuckoo (*Cuculus canorus*) (Newman, 1851). This may be partly due to the sequestration of distasteful compounds, such as the cyanoglucoside *sarmentosin,* a bitter compound found in unusually large concentrations in all stages of development (Nishida *et al.*, 1994).

Recent studies of *A. grossulariata* are almost entirely confined to laboratory studies of their genetics (e.g. sex-linked inheritance; Doncaster, 1913; Doncaster, 1914) or the chemical ecology of their warning colourations (Nishida *et al.*, 1994; see above).

However, populations of the moth in Britain may have suffered widespread declines over the last century (Conrad *et al.*, 2006), and knowledge of their distribution, abundance, and population ecology is lacking severely. Despite these apparent declines, large-scale outbreaks of larvae have been reported over large areas of north Scotland over the last decade, particularly on heather moorland, with the distribution of larvae apparently confined to low-lying coastal areas of mature vegetation (Horsfield & MacDonald, 2004). In Orkney, the status of *A. grossulariata* has previously been one of *probable immigrant*, with only a single recorded sighting in the islands prior to 1981 (Lorimer, 1983). However, numbers of adults appear to have increased substantially since the early 1990s, and the first recorded larva among any island was in 2000, on the most southerly heather-dominated island, Hoy. In the following year, reports of increases in both larvae and adults of several orders of magnitude were reported within the same island, after which it has been recorded as widespread among the largest of the islands in the archipelago (Waring, 2006).

With the exception of the characterisation of some Cypovirus species isolated from individual *A. grossulariata* specimens by Payne & Mertens (1976), very little is currently known about the natural enemies that attack this species. It is likely that, like *O. brumata*, *A. grossulariata* is attacked by several species of parasitoid across its range; several species of Hymenopteran (Thorpe, 1930; Jackson, 1937) and Dipteran (Ford & Shaw, 1991) parasitoids have been reared from parasitized individuals. However, among larvae collected from Orkney since 2003, so far no parasitoids have emerged from any stage of *A. grossulariata* development (R. Graham, *pers. comm.*). However, Baculovirus infections have been observed among larvae during this time: AbgrNPV appears to be a highly genotypically variable species of pathogen (Graham, 2006), although its host-specificity among *A. grossulariata* is not presently known, and it is not clear whether the virus was acquired from existing reservoirs when the first colonisers arrived in Orkney, or whether it was brought over from previously infected populations during the colonisation process.

1.5.4 Heather moorland

Worldwide, heather (*Calluna vulgaris*) dominated moorland represents an increasingly rare and diminishing habitat type, under threat from increasing fragmentation, competition from encroaching grass species, and overgrazing (Hartley *et al.*, 2003;

Thompson *et al.*, 1995). Such habitat loss is of particular conservation importance, since it may harbour not only a wide variety of invertebrate and small mammal species (Gardner *et al.*, 1997) but also provide nesting and hunting grounds for game birds, raptors (Baines, 1996) and other protected bird species (Amar & Redpath, 2005; see above).

Heather moorlands are characterised by a blanket coverage of low-lying *C. vulgaris*, and are typically restricted to upland areas, or areas of poorly drained or nutrient deficient, acidic soils, where the presence of ericoid mychorrizae (that facilitate nitrogen fixation), give the plants a competitive advantage over other species (Harley *et al.*, 2003). However, the diversity and encroachment of other plant species onto moorlands may be mediated by the age of the moorland, in a process that moorland stewards and game keepers may try to manage by mowing, burning or grazing schemes (Meikle *et al.*, 1999). Growth of heather stands may be divided into four distinct stages: i) *Pioneer phase*, the earliest stage of growth (3-10yrs) for newly established seedlings. Here, heather forms a sparse coverage of low-growing (<15cm) pyramid-shaped individual plants in which other plants and bryophytes may be abundant; ii) *Building phase,* (7-13yrs) in which heather has maximal shoot production, forming a closed canopy (15-30cm) that blocks out many plant other species, outcompeting them for light and space; iii) *Mature phase,* (12-28yrs) in which maximal biomass goes into woody growth. Fewer green shoots means that overall canopy (30-40cm) cover is reduced compared to the building phase, and other species such as bryophytes may increase; iv) *Degenerate phase,* (>30yrs) in which minimal biomass goes into shoot production and plants may reach their maximal height (>40cm). However, the central braches of the plant may die off and the tall stems fall to the ground, creating large gaps in the canopy in which other plants (including new heather seedlings) may become established. Another separate stage may be categorises as *suppressed*, whereby normally healthy plants have the maximal sizes within each stage suppressed by extreme conditions, such as at high altitudes, or wind-exposed sites (Gimingham, 1972; Gimingham, 1985).

Compared to many other plant species, heather represents a relatively nutrient-poor resource for insect herbivores, since it contains relatively low levels of nitrogen and high levels of defensive compounds such as lignins and tannins (Kerslake *et al.,* 1996). Despite this, a wide diversity of Lepidoptera species may feed utilise it as a resource, although the exact number of species that do may be confounded by differences between species that are heather-habitat specialists, or generalist,

polyphagous feeders (Fielding & Coulson, 1995). Regardless of this, increases in both abundance and diversity of macrolepidoptera species feeding on heather has been positively associated with increases in the height, and therefore the stage of growth of, individual plants (Haysom & Coulson, 1998).

1.6 Thesis aims

The overall aim of this thesis is to investigate whether the spatial relationship between natural enemy prevalence and host density, distribution and demography, can be predictable by their mode of transmission. Specifically, consideration of how variation in the response of four different natural enemy species, all of differing transmission modes, to variation in host abundance at both local and regional scales in a natural field system, will be addressed. Whether these same relationships occur between different host species infected by the same family of pathogens, is also investigated. Further to this, an exploration of how genetic signals of host dispersal, and therefore spatial population structure, may influence these processes in two host species of differing life-histories and dispersal abilities, is also undertaken.

Large-scale, long-term studies of multiple host species attacked by multiple natural enemies are generally rare, with the focus of most studies being on addressing questions of how single types of natural enemy may impact on single species (Berryman, 1999). Although there are recent examples of studies where the scope of these interactions have been extended to include multiple natural enemies, or multiple hosts (Escribano *et al.*, 2000; Redman & Scriber, 2000; Dwyer *et al.*, 2004), some may do so over only small spatial extents, or in laboratory systems (Begon *et al.*, 1996; Sait *et al.*, 1996). Such systems typically ignore the variety of other trophic interactions that may be apparent in any complex system of interacting organism within a field-based study. Additionally, ignoring the spatial extent of the study may increase the chances of obtaining spurious results, by failing to capture the correct scale at which an organism may perceive its environment, and therefore respond to it. Spatial heterogeneity in any field-system may be an important component of the dynamics between host and natural-enemy, since it may introduce constraints to the rate at which hosts are exploited—an issue that may be overlooked in laboratory studies, or models systems that assume interactions are homogeneous. The ability to infer the scale of these specific responses

in any system, and the likely impacts of heterogeneity on host-enemy interactions may therefore be an important part of attempts to predict how host and enemy species may each respond to environmental changes.

The relatively closed system that Orkney represents means that inferences about regional-scale processes may be more tractable than among other, more open, field systems that may extend over many hundreds of miles (e.g. Elkinton, 1990; Liebhold *et al.*, 2000). The relatively simple Lepidoptera communities on the moorlands (compared with mainland Britain) may also be more easily manipulated, and the effects of natural enemies on the focal species in this study (*Operopthera brumata*) more easily understood, since it is attacked by a relatively simple community of natural enemies (i.e. only a single parasitoid species, and several viruses of differing transmission modes). How these natural enemies respond to changes in the distribution and abundance of hosts, may make this relatively simple compared with more complex multi-parasitoid communities. The continuous distribution of heather among the mainland may also facilitate a better approximation of the spatial scale of insect host-natural enemy interactions, since its low-lying nature means that spatial interactions are far more restricted than among other habitats, such as forest ecosystems, where the interactions may occur over a broader scale, in three-dimensions. This potentially allows an assessment of the specific scale of the interaction between host and enemy, which may be quantified and related to variation in transmission strategy as predicted through laboratory and theoretical models.

In particular, the spatial variation of vertically transmitted pathogens in space has received relatively little attention (*but see* Saikkonen *et al.*, 2002), probably because spatial interactions with hosts are not thought to be an important determinant of their transmission. Similarly, although pathogens vectored by parasitoids (and that act commensally or mutualistically with their parasitoid hosts) are well characterised among laboratory populations, very little attention has been paid to their ecological effects on the hosts they may facilitate parasitizing. Such an interaction may provide an important signal of the oviposition behaviour of parasitoids, when prevalence among hosts is solely determined after host mortality (i.e. unsuccessful oviposition attempts; probing but not ovipositing; or host immune responses overcoming parasitic attack). All of the above issues are addressed in the present study, providing an important link between theoretical predictions, laboratory observations, and ecological patterns in natural field populations.

The recent colonisation of the islands by *Abraxas grossulariata* also provides a unique opportunity to investigate the potential effects that species entering novel habitats may have on the endemic populations of interspecific competitors, and natural enemies alike. Range-shifting of species, especially Lepidoptera is widely predicted under models of climatic change, and have been observed in other systems (Parmesan, 2006; Jepsen *et al.*, 2008). The effects that such a potential shift may have on populations of *A. grossulariata,* and its natural enemies may therefore be highly applicable to those of other species, especially if those species cause detrimental effects to the newly-colonised habitat (Johnson *et al.*, 2006).

I address all of the above considerations by investigating host-natural enemy interactions in relation to enemy transmission mode, host dispersal ability, and spatial arrangement of habitats, at a range of spatial and temporal scales. In Chapter 3, I investigate whether demographic and spatial changes in the distribution and abundance of host species within-generations may influence the prevalence of a variety of natural enemy species, of differing transmission modes. How natural enemy species utilise hosts as resources for propagation will have population dynamic consequences for the recruitment of hosts and enemies to new cohorts. Therefore understanding these processes—and how they differ between different host and natural enemy species—is crucial for modelling and predicting the spatial and temporal dynamics of natural populations. Host age, density and spatial aggregation are all investigated in relation to risk of attack by a parasitoid, a horizontally transmitted virus, a vertically transmitted virus, and a virus vectored by the parasitoid. It is predicted that parasitoids and parasitoid-vectored viruses will have higher prevalence in late instar stages, whereas early instars may be more at risk from horizontally transmitted viruses. Vertically transmitted viruses are expected to show no pattern of prevalence at any particular age class. Risk of attack from parasitoids is expected to influence the spatial distribution of host larvae during their most susceptible stages, which may be detectable by increased aggregation.

In Chapter 4 the hypothesis is tested that the spatial pattern of natural enemy infection among hosts, and the prevalence of infection by each in relation to changes in host density, is related to its mode of transmission. Here, the spatial scale is increased to encompass a detailed local-scale examination of changes in the distribution of hosts, and infection by their natural enemies. Host interactions with natural enemies are known to be able to cause spatial patterning among hosts even in continuous habitats.

How natural enemies influence these spatial processes is not fully understood, and this study will aim to highlight how spatial scale of transmission may influence these processes. Parasitoids are predicted to display spatial patterning at larger spatial scales than their hosts, due to greater dispersal relative to their hosts, and to be aggregated in areas of high host density. This same pattern is anticipated to be true of its vectored virus. Horizontally transmitted viruses are expected to have spatial patterns of infection at smaller scales than their hosts, and be positively related to increases in host density, due to their passive transmission mode. Again, vertically transmitted viruses are not expected to have any relation to spatial scale, or host density. Additionally, a second study site is investigated that also harbours two populations of host Lepidoptera species, feeding on the same hostplant, but attacked by different horizontally transmitted viruses of the same family. Here, the hypothesis is tested that pathogens of the same transmission mode, but infecting different species, will display the same spatial pattern of infection, and relationship with changes in host density.

In Chapter 5, how host distribution is related to changes in the quantity and quality of available habitat within a region, is examined. Here, the hypothesis is tested that a recently colonising Lepidoptera species with greater dispersal ability will respond to changes in the availability of shared suitable habitat at larger spatial scales than a resident species that can disperse less far. The ability of a species to utilise available suitable habitat may have important population dynamic consequences, for example by escaping natural enemy attack, or dispersing away from areas where biotic or abiotic conditions are poor. Changes in abundance of the stronger disperser, a recent coloniser of the region at the edge of its natural range, are expected to be better correlated with changes in the availability of favourable microclimatic conditions, such as south-facing slopes. The resident species is not expected to correlate well with any topographic variable. Additionally, how prevalence of each of their natural enemies is related to changes in host density across the region is also investigated, in order to see whether this relationship is the same at both local and regional scales.

In Chapter 6, I utilise population genetic techniques in order to quantify the level of dispersal—inferred as levels of genetic isolation between spatially separate sites across a region—of two Lepidoptera species of differing dispersal abilities. Here, the hypothesis is tested that the less dispersive of the two species will display population genetic structure at smaller spatial scales than the more dispersive species. Dispersal ability of an organism will determine how it is able to respond to changes in the biotic

and abiotic conditions of a habitat, and will influence its interactions with natural enemies. For some species, this may also influence its ability to colonise novel habitats, which may have direct consequences for resident species if they share the same habitat requirements. Loss of natural enemy regulation through large-scale dispersal events, or encountering novel natural enemies in new habitats may also affect such populations. Knowledge of how species are likely to respond to such events may be important in predicting how organisms may respond to climatic change, and alterations in native ranges. The more dispersive species in this study is expected to show a genetic signal of recent colonisation of the region, as part of a northwards range-shift. Additionally, both species will be screened for any potential genetic markers that may be under selective pressure from environmental and/or pathogenic causes. Both a novel and well-established techniques for genome screening will be used, in which the newly invasive host species is expected to show greater signs of selective pressure in its newly colonised habitat.

Finally, in Chapter 7 I review the main findings from the present study and discuss their ecological relevance for other insect host-natural enemy interactions in spatially heterogeneous natural field systems. How the conclusions drawn from the present study may be extended into future areas of research, is also discussed.

Chapter 2

General methods

Only general methods, applicable to all data chapters, are given in the current chapter; for specific information on study sites, sampling methods, and statistical analysis, see individual chapters.

2.1 Larval sampling, collection, and storage

Larvae of both *Operophtera brumata* and *Abraxas grossulariata* were collected from a number of different heather moorland sites on three islands (Hoy, Mainland, and Rousay) in the Orkney archipelago, northeast Scotland, during May-July 2004-2009. The majority of sites comprise a blanket coverage of *Calluna vulgaris,* the primary larval foodplant. However, some sites contain varying quantities of bell heather (*Erica cinerea*) and bilberry (*Vaccinium myrtilis*), which larvae may also feed on. Larval abundance was quantified as the total number of larvae within a quadrat measuring 0.25 x 0.25m ($0.0625m^2$); a minimum of 10 quadrats were sampled per site, along a 10m linear transect, with the distance between each quadrat being 1m. Sampling designs for each chapter differ, although most are a modification of this basic quadrat-transect design (see individual chapters for details): Chapter 3 uses a single 10m quadrat-transect per sampling day; Chapter 4 uses nine 10m quadrat-transects end-to-end with varying distances between each, covering a total linear distance of 250m, per sampling year; Chapter 5 uses three parallel 10m quadrat-transects, each spaced 5m apart, creating a sampling square of $10m^2$ containing a total of 30 quadrats, per site; Chapter 6 uses the same sampling scheme as Chapter 4 for all sites except sites sampled from mainland Britain, which are not spatially quantified.

Within each quadrat in the field, all vegetation was removed and placed in sealed bags, with the site and spatial location of each quadrat labelled. All bags were then removed from the field, and the excised vegetation searched thoroughly for larvae of either of the two species, and the abundances per quadrat recorded. Any *O. brumata* larvae were then transferred to individual 12ml polypots containing sterile semi-synthetic diet (a modification of Hoffman's Tobacco Hornworm diet; Hunter *et al.*, 1984); *A. grossulariata* larvae were transferred to individual 12ml polypots containing non-sterile *C. vulgaris* shoots from the same site (since larvae of this species refuse to feed on semi-synthetic diet), to control for variation in the potential distribution of viral occlusion bodies (OBs) between sites. Frass was removed, and fresh shoots provided

every 3-4 days. All pots containing larvae were labelled with the site, quadrat and sampling date, and stored at 15-20°C until pupation or death. All pupae were then pooled by site and transferred to trays containing moist vermiculite, and stored at 16°C until adults emerged; any parasitoid pupae that emerged from larvae were also transferred to trays of vermiculite, pooled by site. Any emerging adult Lepidoptera or parasitoids, and all larvae that died prior to pupation, were then transferred to sterile, labelled 1.5ml Eppendorf tubes and stored at -20°C until further analysis.

2.2 Preparation of specimens

Preparation of larval specimens for DNA extraction was subject to minor modifications between years, in order to improve storage and archiving:

i) 2004-2005: Whole larvae were manually homogenised in 180µl of Buffer ATL (*see* Graham, 2006).

ii) 2006-2007: Individual larvae were cut in half using sterile wooden toothpicks; the abdomen was used for subsequent analysis (since this is the most likely site of viral replication), and the head and thorax kept for archiving at -80°C. The same procedure was used for adult specimens (see Chapter 6). Each sample was then homogenised in single-bead microtubes (Tepnel Life Sciences) with approximately 200µl lysis buffer, using a bead-beater (Qiagen TissueLyser) for 1 minute at 25 cycles per second.

ii) 2008: Whole larvae were transferred to labelled, sterile UV-treated 2ml screw-cap microtubes filled approximately ¼ full with GS2 glass beads, and 400µl media-prep H_20. Samples were then homogenised using a FastPrep®-24 tissue lyser (MP Biomedicals) at 4m sec^{-1} for 30 sec, and 200µl of the resulting homogenate transferred into sterile microtubes for DNA extraction. The remaining sample, plus glass beads, was retained at -80°C for archiving.

2.3 Nucleic acid extraction and purification

Again, differences in nucleic acid extraction and purification methods were used between sampling years, in order to improve high-throughput sampling:

i) 2004-2005: Total genomic DNA from each larva was extracted by the column-spin technique, using a DNeasy™ Tissue Kit (Qiagen) (*see* Graham, 2006).

ii) 2006-2008: Total nucleic acid content was extracted from each larva by the magnetic-bead technique, using a Nucleoplex™ plant DNA kit and a 96-well Nucleoplex Automated System DNA/RNA extraction robot (Tepnel Life Sciences). The standard manufacturer's lysis and purification protocol were used for all samples, with the omission of RNase solution to prevent degradation of double stranded RNA (dsRNA) viruses. Samples were then eluted into 120µl 10mM Tris in sterile 96-well plates, and stored at 4°C until further analysis.

2.4 Virus identification

Three different techniques were used to identify the presence or absence of different viruses within larvae, according to sampling year. The principal reasons for changes in technique between years were due to developments in primer design, partial genome sequencing, and high-throughput semi-quantitative screening methods.

i) 2004-2005: *Microscopy*

The presence or absence of OpbuNPV occlusion bodies (OBs) within each larva was identified by phase-contrast microscopy using x400 objective magnification (*see* Graham *et al.*, 2004; Graham, 2006).

ii) 2006: *RT-PCR*

To check whether nucleic acid extraction (above) was successful, 5µl of elutant was run on an ethidium bromide-stained 0.8% agarose/TBE gel at 50V for 1hr; the presence of nucleic acid being indicated by a bright smear at the leading edge of the gel, when visualised under a UV transilluminator or VersaDoc 3000 molecular imager (BioRad). In addition, the presence or absence of both CPV19 and RV were identified from the same gel electrophoretic image by their characteristic dsRNA genomic size-band profiles (*see* Graham *et al.,* 2006).

The presence or absence of OpbuNPV DNA in *O. brumata* and AbgrNPV DNA in *A. grossulariata* among purified host larval DNA was undertaken by reverse

transcriptase-polymerase chain reaction (RT-PCR) using specific primers, developed from the partial sequence of both OpbuNPV and AbgrNPV genomes (primers developed by R. Graham; *see* Graham, 2006). The primer sequences used were: OpbuNPV (5'-3') AATCGAGACGCGCTCATACG (Forward), GTCGCTACCAACCCAGCTGG (Reverse); AbgrNPV (5'-3') CAAGTACTACAAAAACCTGGG (Forward), GACACGGTTTACAAACGACTC (Reverse). All primers were received as freeze-dried synthetic oligonucleotides from MWG-Biotech, which were resuspended using Milli-Q H_20 to a stock concentration of 100pmol μl^{-1} and stored at -20°C until required. PCR reaction mixtures (50 µl per sample) were as follows: 31 µl Milli-Q H_20, 5µl 10x PCR Buffer (100mM Tris-HCl, 500mM KCl, 15mM $MgCl_2$, 0.1% gelatine), 1µl $MgCl_2$ (2.5M), 0.5µl dNTP (10mM), 0.5µl (1U) *Taq* polymerase (Sigma), 1µl (10 pmol μl^{-1}) forward primer, 1µl (10 pmol μl^{-1}) reverse primer, 10µl template DNA. All reaction mixtures were prepared under sterile laboratory conditions, and stored on ice prior to amplification. All RT-PCR reactions contained a positive (known concentration of virus) and negative (Milli-Q H_20) control, and were performed under the following reaction conditions, using a peQLab primus 96 advanced thermal cycler: initial denaturation at 95°C for 5 mins; followed by 25 cycles of: 95°C for 45 seconds; 94°C for 1 min; 55°C for 1 min; 72°C for 2 mins; and a final extension at 72°C for 10 mins. All PCR products were either used immediately, or stored at 4°C. 5µl of PCR product was then run on an ethidium bromide-stained 0.8% agarose/TBE gel at 50V for 1hr, and visualised using a VersaDoc 3000 molecular imager (BioRad).

iii) 2007-2008: *Real-time RT-PCR*

A multiplex real-time RT-PCR of Scorpian™ Probes (Sigma) were designed for each of the four virus species (OpbuNPV, AbgrNPV, CPV19, and RV), generated from partially sequenced polyhedra genes, details of which are given in Hussey *et al.* (2009). Each forward primer per virus was allocated a unique fluorophore reporter dye, which is detected separately during the PCR process; primer designs and fluorophores used for each species are given in Table 1. Changes in the fluorescence intensity of each forward primer-probe during the course of each cycle (as a result of loss of the fluorescent quencher during polymerase activation) is measured by fluorescence detectors, and should approximate an exponential distribution until all reaction products are used up. The use of a known positive reference standard can then be used to quantify the relative

amounts of viral DNA within each sample, and the presence of primer-dimers (which display different relative increases compared to true positives) detected and controlled for.

Positive dilution standards for each virus species were obtained from strong positive results obtained via viral screening from 2006 (see above). All positive viral standards were pooled together to create a mastermix of positive standards to use for each real-time RT-PCR run; a series of eight 1:5 serial dilutions of this mastermix standard was then used, using UV-treated DEPC H_20, to create the reference positive standard curve for each real time RT-PCR run. Before being added to the mastermix standard, each of the two dsRNA viruses were converted to cDNA using a Omniscript ® 200 reverse transcription Kit (Qiagen), which consisted of boiling for 5 min a mixture of 43.5µl DEPC H_20, 1.5µl (10pmol μl^{-1}) reverse primer, 30µl positive dsRNA virus sample, and 10µl 10x buffer RT. The mixture was then put on ice for 5 min, before adding 10µl dNTP (5mM) and 5µl reverse transcriptase, and finally incubating for 1hour at 37°C.

Nucleic acid extraction for each sample was quantified using a Nanodrop 8000 spectrophotometer, to ensure that the extraction process was successful. For each sample, the total 20µl reaction mixture consisted of: 10µl Platinum® *Taq* (Invitrogen), 5.2µl DEPC H_20, 0.35µl (10pmol μl^{-1}) each viral forward (Scorpian^{TM}) primer (x4), 0.35µl (10pmol μl^{-1}) each viral reverse primer (x4), 2µl sample host DNA. Four negative controls containing 2µl DEPC H_20 were also included in each run, in order to quantify the levels of background fluorescence in each reaction. Real time RT-PCR was then conducted using a RotorGene RG3000 (Corbett Research), under the following reaction conditions: 95°C for 30 seconds extension followed by 58°C for 30 seconds annealing (x45 cycles). Fluorescence levels during each PCR run were then visualised using RotorGene 6000 series software (v1.7). Logarithmic standard curves were generated automatically from the positive standards for each virus, and their best-fit regression lines checked by eye to ensure that all dilution standards had undergone successful PCR reactions. Any standards that had obviously not undergone satisfactory reactions were omitted. All Standard curves had R^2 values >0.9. Threshold values for true positives were calculated by firstly applying two automated techniques from the RotorGene software for removing samples that did not react in the expected exponential manner (e.g. primer dimmers): i) an automated threshold calculation in dynamic tube mode, with slope correction; and ii) removing outliers with <10% change in fluorescence relative to the largest change in any sample. Additionally, a conservative

secondary threshold was also applied to remove any values less than the mean + 2 standard deviations of all negative controls for each virus species, across all runs. This ensured that all negative, no-template controls in every run were below the final threshold used to detect true positives. Although these methods may therefore increase Type II error rates, it should ensure that Type I errors are almost entirely eliminated. Any sample with positive fluorescence values greater than this threshold are therefore deemed positive for the presence of viral DNA/cDNA; the relative quantities for each sample were then calculated, relative to the positive reference standards by extrapolation from the standard curves generated within each run, and the final relative quantity of viral DNA/CDNA within each sample calculated as the relative viral DNA concentration (ng μl^{-1} , assuming the first reference standard = 2 ng μl^{-1}) / concentration of host DNA (ng μl^{-1}, quantified using a Nanodrop spectrophotometer).

Virus	Primer	Primer sequence (5'-3')
OpbuNPV	*Forward*	[Joe] CGCCCGTATGAGCGCGTCTCGGGCG (BHQ-1) (HEG) GCAAGAAGCAGATGTGCGAA
	Reverse	CTCGGCGATCAGATACTTGTCAA
AbgrNPV	*Forward*	[6-Fam] CGGAGCCCAGGTTTTTGTAGGCTCCG (BHQ -1) (HEG) GTCGCACTTACGTGTACGACAA
	Reverse	CTTGCGCTTGGCGTTCTTAA
OpbuCPV19	*Forward*	[Cy5] CGAGGGCTCGGTCATGCCCTCG (BHQ-2) (HEG) GCACCCCAGCAAATTCGCAA
	Reverse	GCGTAGATGTATGAATCACGCGAA
OpbuRV	*Forward*	[Rox] CCCGCCTGCAGTGTTTTAGTATGTGGCGGG (BHQ-2) (HEG) GCTCCATTTCGCATACATCTTCCAA
	Reverse	GTCGTAACAATGAGTAAGGGTCGAA

Table 2.1 Primers used for each virus species when screening *Operophtera brumata* and *A. grossulariata* larvae for the presence or absence of infection. All forward primers are fluorescently labelled Scorpian™ probes; names in squared brackets indicate the different types of fluorophores used; (BHQ-1) and (BHQ-2) refer to the internal quenchers; (HEG) refers to the blocker.

Chapter 3

Within-generation insect host-natural enemy interactions

3.1 Abstract

Interactions between insect herbivores and their natural enemies may be dependent on variation in both demographic and spatial processes. For instance, parasitoids may preferentially attack later instar stages since they represent greater resources for their offspring. Conversely, among pathogens, earlier instars may incur higher rates of mortality per volume of infectious particles they come into contact with, since smaller lethal doses may be required. However, spatial heterogeneity in the distribution of hosts may disrupt these processes if hosts are harder to find, or if pathogens are spatially aggregated in the environment.

This study tested the hypothesis that older, larger instar stages are more susceptible to attack by parasitoids, and that earlier instar stages are more susceptible to infection from pathogenic viruses. Three differing types of viral pathogens are investigated, all with differing transmission modes. We test the hypothesis that prevalence of horizontally transmitted pathogens (OpbuNPV) will be positively related to changes in host density, parasitoids and pathogens vectored by parasitoids (RV) negatively related, and that vertically transmitted pathogens (CPV) have no relationship. The spatial distribution of each instar stage was also quantified, in order to assess whether heterogeneity in host distribution may be related to risk of mortality by parasitoids or pathogens.

Larvae of *Operophtera brumata* were sampled from a single site in Orkney every three days, spanning a maximum period of 25d (representing larval development stages from first to final instars), over a period of three consecutive years. Temporal- and instar-related changes in the aggregation of larvae were quantified using spatial autocorrelation statistics. OpbuNPV infections were quantified over all three years; parasitoids over the final two years; and all four species of natural enemy in the final year. Within-year larval abundance was highly variable, especially so when overall abundance was low. An apparent decline in total larval abundance was observed over the three years, with an increase in OpbuNPV prevalence. Parasitoid prevalence remained stable among the years and RV infections appeared to follow closely the pattern of attack by their vectoring parasitoid hosts. CPV infections displayed no obvious change in prevalence over time, and were not related to host density. No density-dependent infections or parasitism was detectable among any instar stage, although mid-late instar larvae were more susceptible to attack by all other natural

enemies, compared to earlier stages. Mid-instar larvae display a spatially aggregated spatial pattern compared to earlier and later instars, which may be a response to their increased risk of attack. Assumptions regarding the prevalence of natural enemies in relation to host density and age-structure in the field may therefore differ from predictions based on laboratory bioassays. Such interactions are likely to be system-specific. This may have important consequences for parameterisation of population dynamic models, although whether such patterns are true at larger spatial scales is not known. The potential effects of spatial and demographic heterogeneity on population dynamics between hosts and natural enemies, is also discussed.

3.2 Introduction

Parasitoids and pathogens are important components in the population dynamics of insect herbivores, both individually and interactively, with theoretical and empirical data positing a range of potential regulatory influences for each (Hassell, 1985; Godfray, 1994; Berryman, 1996; Dwyer *et al.*, 2004). Many of the original theoretical models proposed to explain the dynamics of these insect-natural enemy interactions deliberately made simplistic assumptions about the underlying processes involved, such as the relative overlap of generations (Lotka, 1925; Volterra, 1926), the random susceptibility of all individuals to infection or attack (Nicholson & Bailey, 1935), or the existence of a single susceptible stage that dies instantaneously upon infection (Anderson & May, 1981). However, the construction of species-specific life tables—often used to parameterise such models (Varley, Gradwell & Hassell, 1973)—suggested that there is a great deal of variability within and among the different life-stages of hosts in their susceptibility to attack by different natural enemies (Manly, 1977; Bellows, 1992; Hawkins, 1997), which may be fundamentally important in understanding the dynamics of the two.

Such heterogeneity in susceptibility among different host life stages may arise through a number of mechanisms related to life-history, including stage-specific attack (e.g. egg parasitism; Godfray, 1994) or variation in the susceptibility of different larval instar stages, both of which may vary according to the type of natural enemy. For example, 1st instar larvae of some Lepidoptera species may build nests in which to develop, or live gregariously to reduce the risk of parasitoid attack (Zaluki *et al.*, 2002). However, such behaviour may also lead to the selective attraction of parasitoids to an area of high host density (Wiskerke *et al.*, 1993; De Moraes *et al.*, 1998). Similarly, many bioassay studies of Lepidoptera larval-virus susceptibility demonstrate an age-related dose-response relationship whereby early instar larvae require a smaller lethal dose of virus than do later instars (Boucias & Nordin, 1980; Sait *et al.*, 1994).

Many species of parasitoids are also thought to make oviposition decisions based on the quality of their hosts (Godfray, 1994), which may be directly related to host size, and therefore age (Harvey *et al.*, 1994). However, larger, later instar larvae may be better able to defend themselves from parasitoid attack, either directly through defensive behaviours (Gross, 1993) or indirectly through a greater immune response compared to earlier stages (Bauer *et al.*, 1998).

As well as *stage-specific* heterogeneity in susceptibility, host-natural enemy populations may experience *spatial* heterogeneity in susceptibility, whereby hosts vary in their probability of encountering a natural enemy and therefore being subject to attack (Jones *et al.*, 1993). The importance of such variation for predator-prey interactions was first demonstrated by Huffaker (1958), who showed that predator encounter rates in microcosm systems were diminished after the introduction of spatial barriers—or prey refuges—to predator foraging, stabilising the host-enemy interaction. Both parasitoids (Walde & Murdoch, 1988) and pathogens (Dwyer, 1992; Dwyer, 1995) are known to be highly variable in space, although their responses to host variability may vary greatly due to their active and passive infection modes, respectively. Moreover, spatial heterogeneity in encounter rates may be influenced by stage-specific effects, such as variation in the susceptibility of instars stages with the availability of refuges from parasitoid attack (Sait *et al.*, 1997), or variation in the transmission rate within and among host instar stages (Hochberg, 1989; Dwyer, 1991).

Modification of host behaviours following infection may also influence the spatial distribution of infective pathogens in the environment (Goulson *et al.*, 1997), which may also be affected by host age, since older, larger larvae are likely to have greater virus yields upon death which may increase the density of infective pathogens per unit area. Such temporal and spatial variation in the responses of natural enemies to changes in host density—itself an explicit function of space (Lewontin & Levins, 1989)—have been a central component of host-parasite (Hassell, 2000) and host-pathogen models (May *et al.*, 1981), although the relative responses of natural enemies may be highly variable, and not necessarily positively related to host density, among both types of natural enemy (Walde & Murdoch, 1988; D'Amico *et al.*, 1996; Dwyer, 1994). The extent to which these processes are influenced by heterogeneity in age-related host susceptibility is not currently known, although it is likely to be an important component of the dynamics of many host species (Murdoch, 1977; Dwyer, 1991; Briggs & Godfray, 1994; Bonsall & Eber, 2001), since it may also alter other underlying population processes such as intraspecific competition for resources (Bernstein *et al.,* 2002*;* Cameron *et al.,* 2007) and alter the dynamics of populations over time (Benton *et al.*, 2006; White *et al.*, 2007).

The present study focuses on the within- and among-stage temporal and spatial dynamics of a single Lepidoptera species attacked by four different species of natural enemy (a parasitoid, a horizontally transmitted virus, a vertically transmitted virus, and

a virus vectored by the parasitoid) during the course of a single larval generation, at a single site over three consecutive years: *Operophtera brumata* is a univoltine Geometrid that feeds on heather (*Calluna vulgaris*) in Orkney, northeast Scotland. It passes through five distinct larval instars before pupating in the soil. Early instar larvae are known to build silken nests between the tips of heather shoots, possibly as a defence against parasitoid natural enemies (*pers. obs.*), however, whether such age-related behaviour manifests itself in variation in larval spatial distribution is currently not known. Its principal natural enemies are the generalist koinobiont endoparasitoid *Phobocampe tempestiva*, the horizontally transmitted nucleopolyhedrovirus OpbuNPV (Baculoviridae), and two species of Cypovirus (Cypoviridae) : CPV19, which is thought to be largely transmitted vertically; and OpbuRV, which is thought to have originated in *P. tempestiva*, but can be transmitted to *O. brumata* in a commensal manner, possibly to overcome host immune responses upon parasitoid oviposition (Graham *et al.*, 2004; Graham *et al.,* 2006).

Larval susceptibility to parasitism by *P. tempestiva* is expected to increase with larval instar, since large larvae may represent a greater potential resource for developing parasitoid larvae, which may have fitness benefits for the developing parasitoid; infections by OpbuNPV are expected to decrease with age, since the lethal dose of viral occlusion bodies (OBs) increases with age for most species of Lepidoptera larvae studied in laboratory bioassays. However, since early instars of *O. brumata* are largely confined to their nests, the probability of ingesting OBs may be reduced in this stage. The number of CPV19 infections is expected to stay relatively constant throughout the course of a generation, since transmission of vertically transmitted viruses is unlikely to change according to instar. The number of larvae infected with OpbuRV is expected to follow closely the relationship seen among larvae from which parasitoids emerge, since its main route of transmission is believed to be vectoring via *P. tempestiva*. The pattern of clustering of *O. brumata* is expected to follow closely the pattern of increased susceptibility with larval parasitism, with larvae showing a greater degree of small scale clustering, or aggregation, in order to find refuge from parasitoid foraging. Patterns of clustering are anticipated not to follow those of larval susceptibility to OpbuNPV infections, since transmission is unlikely to be affected by the ability of larvae to find refuges, since the virus is passively transmitted. However, infected late instar larvae may be more likely to climb to the tips of shoots, where OBs are likely to be more widely dispersed upon host death. Such a pattern of mortality may therefore be

detectable in a change in spatial pattern in the distribution of separate larval instars over time. Finally, natural enemy responses to host density are expected to show little or no response to variation in host density across the whole generation, since different larval densities are unlikely to be stable over time, and risk is anticipated to be stage-specific. However, when instar stages are investigated separately, OpbuNPV is expected to display a positive increase in the number of infections with increasing host density, within the most susceptible stage only. It is unclear at present what responses *P. tempestiva* may have to host density, although Graham (2006) found a negative relationship with increasing host density, which might be expected to hold true for the most susceptible stage in this study also. Such a pattern may also be expected among OpbuRV infections, although CPV19 infections are not expected to display any relationship with density, among any stage.

3.3 Methods

3.3.1 Sampling methods

O. brumata larvae were collected from a single site (Wideford) every three days during May and June of 2004 (13 sampling days), 2005 (10 sampling days) (data courtesy of R. Graham) and 2006 (11 sampling days), using the transect and quadrat sampling methods outlined in Chapter 2 (Methods). Wideford (58° 8'N, 3° 1'W) is a west-facing (slope 18 ±2°) area of dwarf heather moorland in the building phase of growth, 180 ±5 m above sea level, and subject to a low level of livestock grazing. For samples collected during 2004 and 2005, the total number of larvae within each instar stage was recorded for each transect only; for samples collected during 2006, the total number within each instar stage was recorded for each quadrat. Within years, consecutive sampling transects were positioned within ±1m of the previous transect to reduce spatial sampling errors; among years, transects were positioned away from areas of previously excised heather to reduce the effects of the previous year's sampling on the current year's larval density estimates.

All larvae were reared on sterile artificial diet until pupation or death (see Methods); all larvae not pupating or parasitized were screened for viruses using three different methods: a) the presence of OpbuNPV occlusion bodies in larvae dying of unknown causes during 2004 and 2005 was determined by examination of larval tissue

samples by phase-contrast microscopy, following the methods in Graham (2006); b) the presence of OpbuNPV in larvae collected from 2006 was determined by molecular RT-PCR screening using known primers; c) the presence of CPV19 and RV in larvae collected from 2006 was determined by gel electrophoresis of total genomic DNA of larvae following DNA extraction. Larvae from which the parasitoid *Phobocampe tempestiva* emerged were recorded during 2005 and 2006 only.

3.3.2 Statistical analysis

All statistical analyses were undertaken using R 2.9.0 (R core development team). All generalised linear mixed models (GLMM) were constructed using the package lme4 (Bates *et al.*, 2008); Getis-Ord G statistics were calculated using the package spdep (Bivand, 2009). All analyses were divided into two distinct stages, to investigate differences in larval behaviour and infection at the level of i) the total density of larvae; and ii) within each individual instar stage:

i) Total larval density

Clustering of the total number of larvae within each sampling transect among all years was quantified using Global G-statistics (Getis & Ord, 1992) at all neighbourhood sizes up to half the maximum distance of each transect (5m). Global G-statistics calculate a z-value (a measure of standard deviation away from a hypothesised normal distribution with mean zero and variance one e.g. z = +2 would represent two positive standard deviations from the mean) for the whole transect length based on the combined relative densities of larvae within each quadrat, compared to the surrounding quadrats, contingent on neighbourhood size. For example, at the 3m scale, a z-value is calculated for all quadrats within 3m of each focal quadrat, and a clustering value assigned based on their relative magnitudes; the global test sums all scores at this neighbourhood size and compares them to a null hypothesis of complete spatial randomness (i.e. no clustering). Therefore, the higher the z-score, the greater the degree of clustering of high values among all quadrats at that neighbourhood size. Significant negative scores indicate lower than expected clusters of values at that scale, or the juxtaposition of low values with a single high value at all distances within that scale.

Within each sampling year, the effects of larval density within each quadrat on the number of infections by each natural enemy over the whole sampling period was investigated using weighted binomial Generalised Linear Mixed Models (GLMM) with a logit link function of the ratio of infected to uninfected individuals per quadrat as response variable, and larval density per quadrat as explanatory variable. Sampling date and quadrat position were specified as random factors to account for temporal and spatial autocorrelation, respectively.

ii) Larval instar stage

To investigate whether there may be any spatial differences in the clustering of larvae within each instar stage (which may affect transmission dynamics through time), again global G-statistics were calculated for each instar stage, at all scales between 1m-5m (see above), and at all time points. Only data from 2006 was used, since between-quadrat variation among all instar stages was not available from the previous two sampling years. Significance values are given for each scale, based on the deviation from the null hypothesis of complete spatial randomness in clustering values.

Differences in susceptibility to attack by each natural enemy according to age was investigated using weighted binomial GLMMs, constructed as the ratio of infected to uninfected larvae as response variable, and instar stage as explanatory variable. For the sampling years 2004 and 2005, only the total number of larvae within each instar stage per transect is available, and so sampling date is the only specified random factor; for samples from 2006, larval instar stage within each quadrat is available, so random factors are specified as sampling date and quadrat position. For each GLMM model constructed, two alternative methods of comparing infection ratios over time was used: i) Treatment contrasts compare all values against infections among first instar larvae; since this stage is hypothesised to be least affected by natural enemy attack, this value therefore acts as a reference value against which all other values are based; and ii) Helmert contrasts represent a comparison of cumulative values of infection over time; since infection at each stage may be contingent on infection among previous stages, this method compares each stage with the mean of all combined previous stages. For example, infection among fourth instar larvae is compared against the mean of (1^{st} + 2^{nd} + 3^{rd} instars).

Similarly, the effects of larval host density on infection by each natural enemy within each instar stage is also compared (2006 data only), to investigate whether density-dependent natural enemy attack is stage-specific—an effect that may be masked when infection across all stages is combined. Again, weighted binomial GLMMs were fitted using ratio of infected : uninfected larvae as response variable, and host density as explanatory variable with a logit link function, for each separate instar stage. Again, random factors were specified as sampling time and quadrat position.

3.4 Results

i) Total larval density

Although the overall average quadrat density among *O. brumata* was larger during 2005 than 2004, variation around this value was slightly larger than in the previous year, suggesting that overall larval densities throughout the respective sampling periods were fairly similar (Table 1). During the following year, 2006, average larval densities during the same period more than halve, again increasing the variation around this mean value. The only natural enemy data available across all three sampling years is among OpbuNPV infections, which displayed a marked increase in the percentage of infected larvae over time across the three sampling periods, to a maximum infection of 18.3% among larvae during 2006. Parasitoid attacks during the two sampled years (2005 and 2006) remained fairly similar, although with a slight increase during 2006. Levels of CPV and RV infections among all larvae were low, being 1.2% and 3.1%, respectively.

				Natural enemies			
Year	**Start date**	**Total no. sampling days**	**Average quadrat density (± S.E.)**	**Parasitoid (%)**	**OpbuNPV (%)**	**CPV (%)**	**RV (%)**
2004	10/5	13	23.6 (1.2)	-	1.5	-	-
2005	16/5	10	26.2 (2.1)	1.1	8.7	-	-
2006	22/5	11	10.2 (2.3)	1.8	18.3	1.2	3.1

Table 3.1 Summary table of the average number of *O. brumata* larvae collected from Wideford over a single generation during the three sampling years. The start date for each sampling regime is given for each year, along with the total number of sampling days; each sampling point is separated by a three day interval. The total percentage of infection by each natural enemy across all larvae is also given. Dashed lines indicated where data were not collected.

Variation in *O. brumata* larval density over time during 2004 appeared to be fairly consistent throughout the sampling period, with peak quadrat densities reached after 5d (32.7 ±4.9 larvae per quadrat), followed by a steady decline in abundance, with the lowest densities found at 23d (15.9 ±3.2 larvae per quadrat) (Fig 3.1). Larval densities during 2005 appear to fluctuate more widely than during the previous year, with densities fairly equal at the beginning and end of the 19d sampling period, with a clear asymptote at the mid-point at 9d (38.5 ±7.3 larvae per quadrat). Abundance patterns during 2006 appear to follow the pattern seen during 2004 more closely than 2005, with low levels during the beginning of the sampling period, followed by a large peak density at the 5d mark (26.2 ±3.6 larvae per quadrat), followed by a steady decline throughout the rest of the period. Although the lowest density recorded throughout the sampling period was found at 13d (7 ±0.3 larvae per quadrat), it seems likely that this may be an anomalous result. Such a pattern is not reflected in previous sampling years, with the pattern of abundance among all other sampling points displaying a large asymptote followed by a long, steady decline in abundance with time. Such a result is interpreted as being due to a time lag in the egg hatch dates of neonate larvae, with the maximum quadrat density reached after all eggs have hatched; after this date, losses due to predation, competition, disease or exogenous mortality act so as to reduce the overall population size beyond these time points.

Although there were marked differences in the overall percentage of infection across all larvae between the three years, a pattern of increasing OpbuNPV infection with time is consistent among all years. Peak infection during 2004 was at 19d (6.3%), although this appears to be the only point at which infection increases beyond about 1% among all larvae. Infections by OpbuNPV among larvae from 2005 display a much clearer pattern of increase over the sampling period, despite fluctuating quite widely throughout the course of the 19d. Peak infection prevalence during this year was at 15 d (24.7%), after which infections decreased to levels similar to the beginning of the sampling period, below 10%. By far the clearest pattern of infection among all three years is evident in larvae from 2006, whose OpbuNPV infection prevalence appears to reflect the opposite of its variation in abundance: after an initially high prevalence within the first 3d, infections reach a minimum at 5d (3.4%), after which they increase steadily throughout the sampling period, culminating in the highest peak prevalence of infection among all time points, and all years (21d; 51.8%). Interestingly, despite some time points harbouring no evidence of OpbuNPV infections among any larvae, within

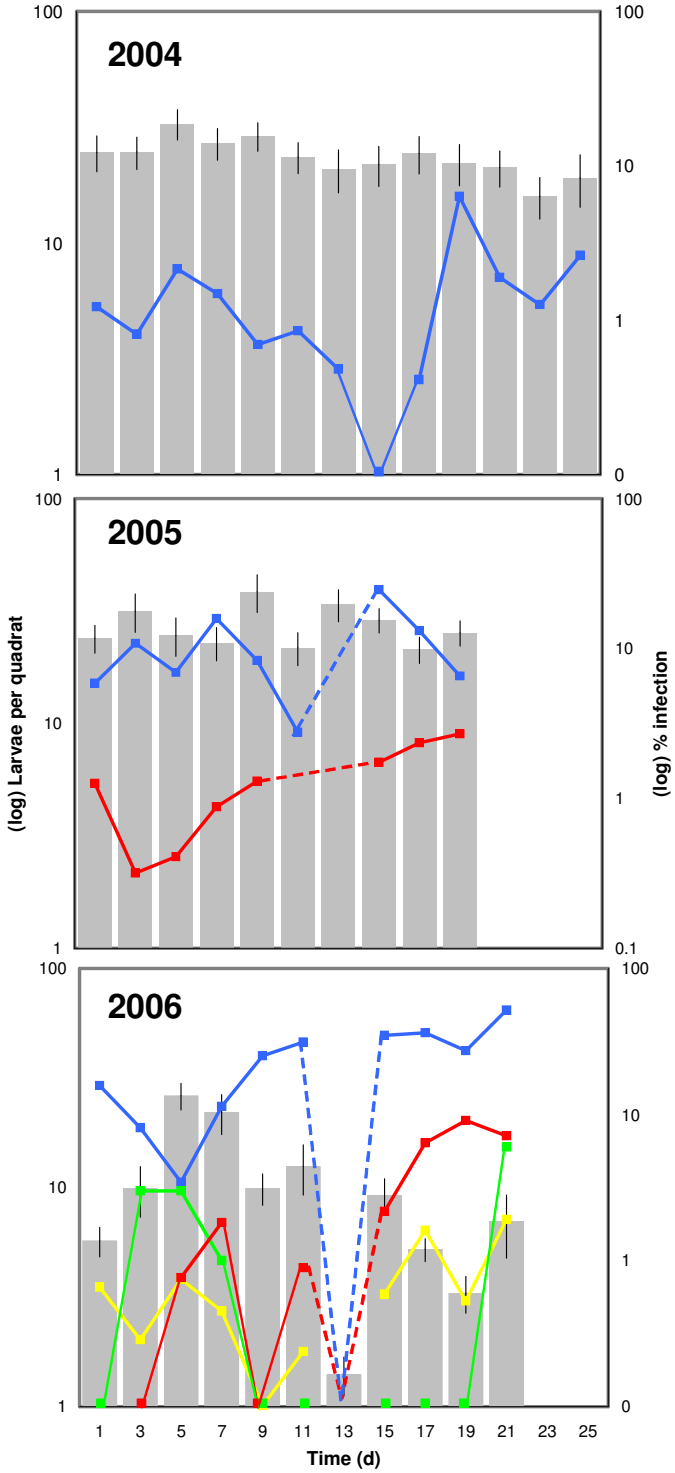


Fig 3.1 Change in (log) *O. brumata* larval density over time for the three consecutive sampling years 2004, 2005 and 2006. Grey bars indicate average number of larvae per quadrat ± 1 standard error (S.E.) at each time point (n= 10 quadrats per time point). ■ represents the average (log) % parasitism of all larvae by the parasitoid *P. tempestiva*; ■ represents the average (log) % infection of all larvae by OpbuNPV; ■ represents the average (log) % infection of all larvae by CPV; ■ represents the average (log) % infection of all larvae by RV. Dashed lines indicate missing or anomalous data points.

all three years there is evidence that *O. brumata* larvae are infected from the very first sampling point through to the very last, suggesting that infection is not wholly restricted within any time period over the course of a single larval generation.

Despite data on parasitoid infection prevalence only being available over two consecutive years, both 2005 and 2006 again display a high level of congruence in the patterns of attack throughout the host larval life cycle. Again, like OpbuNPV infections, attack by parasitoids appears to increase throughout the sampling time, in both years reaching a peak prevalence at 19d (2005 = 6.4%; 2006 = 9.09%). However, although this peak harbours the highest rates of parasitoid attack, it appears to be a secondary infection peak, with a primary peak, albeit low, occurring at around 7-9d among both sampling years. Whether this is a true peak, or sampling artefact due to some missing data is, however, not clear. Despite this, two primary infection peaks that are clear are among CPV infections during 2006. Infection prevalence among this species of natural enemy is fairly similar throughout the 3-7d sampling period, fluctuating at around the 3-5% mark. Infection then drops to zero for the middle period of sampling, only to increase again at the final sampling day, reaching a peak prevalence of 10.7%. Although RV infections similarly displayed an increase in prevalence during the final 3d of sampling, prior infections remained fairly consistent throughout the rest of the sampling period, also fluctuating around 3-5%. Again, peak prevalence was during 21d (7.4%).

Clustering of larvae within transects appeared to follow closely the patterns of variation seen in overall larval abundance within each year, with progressively larger variation in the magnitude and sign of clustering with each successive year (Fig 3.2). Larvae within transects during 2004 were positively clustered among all sampling days, with the exception of 15d, although even then z-values were low (maximum = -0.9; 5m scale). This suggests that larval densities were fairly uniformly distributed throughout each sampling quadrat, regardless of neighbourhood size, and that these densities were always high. With the exception of 1d (4 m scale; p= 0.04*), the majority of significant positive clustering during this year was concentrated at end of the sampling year, when larvae were oldest; for three of the last four sampling days, scales 1-4m showed significant positive clustering, indicating that densities of late-instar larvae were larger than expected than under a random distribution among all quadrats within a 4m radius. Similarly, during 2005, two of the last three sampling days showed highly significant positive clustering among all larvae within transects at all scales, with all of the last six sampling days (9d-19d) displaying positive clustering among all larvae at all scales

within 5m. However, unlike the patterns observed in 2004, larval clustering within the majority of the first four sampling days was negative, with two values at the 1m scale being significantly negative (1d, p = 0.033; 3d, p = 0.031). This suggests that larvae are significantly more spatially restricted within quadrats at all scales compared to a random distribution. Larvae within quadrats among all sampling points during 2006 show a much greater degree of heterogeneity in clustering than is apparent among other years, perhaps reflecting the larger variation in overall larval abundance during this year (Table 3.1; Fig.3.1). Early in the sampling year (1d-5d), larvae are positively clustered, although only significantly so on the first sampling day. By 7d and 9d, larvae are significantly spatially restricted within quadrats in at least one spatial scale, with a peak at 7d (5m scale; p = 0.009**). Again, although 19d displays a degree of negative clustering, larvae are also significantly positively clustered towards the end of the sampling year; significantly so at 17d (scales 2-5m, p<0.05*) and 21d (scales 1-2m, p<0.05*), suggesting a high localised abundance, neither spatially restricted, nor uniformly distributed.

OpbuNPV infections among *O. brumata* larvae during the 2004 sampling year displayed a weak positive response to increases in host density (Table 3.2; p =0.016*), although this trend was not apparent in any of the other two sampling years, despite there being a greater incidence of infection among all larvae during these latter years. This result seems unusual, given the low prevalence of infection observed during this year, and indeed examination of GLMM model residuals revealed an unusually high level of influence among a single quadrat at 19d which, when removed, negated the previous trend (p = 0.33). This result should therefore be treated with some caution; the lack of significant trend among other years suggesting that, across a whole larval generation, host density is not a significant factor governing the prevalence of OpbuNPV infection at this site. Similarly, no other trend was observed among any other species of virus or parasitoid infecting larvae in either 2005 or 2006, reiterating this same trend.

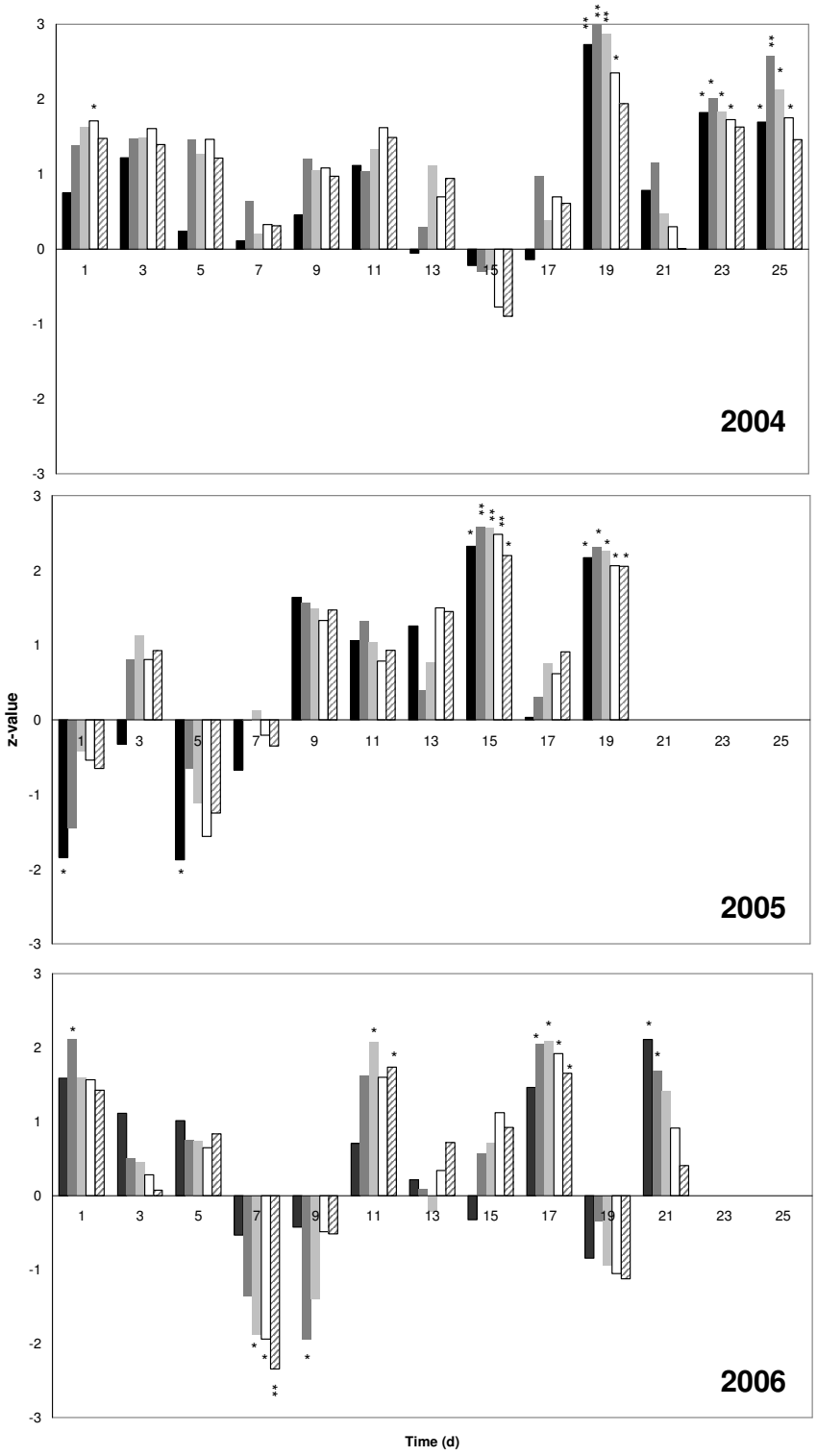


Fig 3.2 Global G statistics for *O. brumata* larval densities within each transect, for each year (2004, 2005 and 2006) over the whole sampling period, at five spatial scales. ■ represents 1m scale; ■ represents 2m scale; ■ represents 3m scale; □ represents 4m scale; ▨ represents 5m scale. Within each spatial scale, high z-values indicate that, across the whole 10m transect, larvae in adjacent quadrats have similarly high densities; low values indicate larval densities in adjacent quadrats are lower than expected. The significance of this assumption is tested against a null hypothesis of complete spatial randomness across the transect length. Significance codes are: *p<0.05; **p<0.01; ***p<0.001.

Year	Natural enemy	Estimate	LogLik	Δ logLik	p-value
2004	OpbuNPV	0.033	-67.3	3.02	0.016 *
2005	Parasitoid	-0.034	-37.67	0.59	0.07
	OpbuNPV	0.002	-78.46	0.08	0.67
2006	Parasitoid	-0.006	-33.68	0.03	0.78
	OpbuNPV	0.003	-88.41	0.04	0.79
	CPV	0.037	-25.45	0.68	0.27
	RV	0.008	-34.85	0.23	0.5

Table 3.2 Results of weighted binomial GLMMs of ratio infected : uninfected larvae vs total larval density per quadrat for *O. brumata* infected by each species of natural enemy over all sampling time points within each of the three consecutive sampling years (2004, 2005 and 2006). For each model, sampling time and quadrat position are specified as random factors. LogLik represents the (log) Likelihood of each model fit; Δ logLik refers to the change in (log) Likelihood of the final model compared to a null model with a constant explanatory variable. Significance codes are: *p<0.05; **p<0.01; ***p<0.001.

ii) Larval instar stage

The distribution of each larval instar stage over each of the three year's sampling periods was variable, although some general characteristics are shared between years (Fig 3.3); each instar stage appears to follow a normal distribution of abundance that does not appear to vary greatly, taking around 15-19d for all individuals to emerge and disappear from each age class. Although beginning of each sampling date varies between years (see Table 3.1), each regime was undertaken so as to approximate the same distributions within each year; there is good congruence between the distributions of each age class between 2004 and 2005, with sampling beginning about mid-way through the first instar age class. Fifth instars appear to be underrepresented in 2005 compared to the previous year, due to two days less sampling. In 2006, the beginning of the sampling period appears to have been before the peak in first instar abundance and, although this year appears to be more variable than previous years, the end point of sampling is around the mid-point of the fifth instar age class, as in 2004. It therefore seems that each year is adequately comparable as regards the distribution of age classes through time, with the possibly exception of the under-representation of fifth instars during 2005.

When all instar stage are combined within years (Fig 3.4), the abundance of larvae within each stage resembles that of the total larval abundance within quadrats over time (Fig 3.1), with the maximum larval abundance evident among second instar larvae, and decreasing thereafter to a minimum among fifth instars (with the exception of 2004, where densities did not differ substantially across all instars). Similarly, patterns of infection by natural enemies agree well with those when all larvae are pooled within quadrats; OpbuNPV prevalence in 2004 remained low throughout all age classes, peaking within 4th instar larvae (2.7%). Infection prevalence among other instars during this year were all around 1%, despite the slight increase among 4th instar larvae, binomial GLMMs revealed no significant difference between the ratio of infected to uninfected larvae compared either to 1st instar larvae, or to the cumulative mean of all previous instars ($p>0.05$; Table 3.3). In contrast, while infections during 2005 appeared to fluctuate widely over time pooled into quadrats, (with a steadily increasing relationship), when separated into age classes there appears to be a clear peak in prevalence among 3rd instar larvae infected with OpbuNPV (13.9%), after which prevalence decreases down to its lowest level, among 5th instars (4.2%). However, parameter estimates from binomial GLMM models suggest that, although not significantly different from prevalence among 1st instars, the peak infection age class is among 2nd instar larvae (Treatment contrasts; Estimate = 0.27). Compared to infections among 1st instar larvae, the only significant differences occur among 4th instars ($p = 0.03$*; Table 3.3), where infections were significantly lower among this age class. Similarly, when using Helmert contrasts, this age class had a highly significantly lower prevalence of infection than other age classes ($p<0.001$***). Despite the proportion of 5th instar larvae infected with OpbuNPV being even less than among 4th instars, no significant difference was detectable under the GLMM methods; this may be due to poor parameter estimates obtained from the low sample sizes: Fig 3.3 shows how few larvae were collected from this age class during 2005, and so estimates based on this age class should be treated with caution. Similarly, the large peak OpbuNPV prevalence among 4th instar larvae from 2006 suggests that this age class is more susceptible to attack from this natural enemy. However, prevalence among this age class were not significantly different from infections in either 1st instar, or 1st-3rd instar larvae; again GLMM model residuals revealed a single anomalous point of large influence among this age class which was skewing the total prevalence. In fact, parameter estimates from the model suggest that the largest prevalence occurs among 2nd instar larvae, which was

significantly greater than among 1st instars (p = 0.004**), as were infections among 3rd instars (p = 0.04*), although the latter was not different from the cumulative mean of 1st and 2nd instar infections (Helmert contrasts, p=0.27). When larvae reach their 5th instar age class, the overall prevalence of infection is significantly lower than both 1st instar larvae (Treatment contrasts; p = 0.02*) and the cumulative mean prevalence among all other age classes (Helmert contrasts; p<0.001***).

The prevalence of parasitoid attack among all larval age classes appeared to follow the same trend among both sampling years when parasitoids were recorded—one of increasing prevalence with increasing age. Prevalence of attack among larvae from 2005 appear to show little difference among instars 3-5, although GLMM parameter estimates clear place instar 4 as the most susceptible stage, harbouring the most emerging parasitoids (Table 3.3), being both significantly greater than among 1st instars (p = 0.02*), and when the previous three age classes are considered cumulatively (p = 0.002**). Interestingly, although not significantly different from parasitism among 1st instar larvae, 3rd instars have significantly higher prevalence of parasitoid attack when the cumulative mean of 1st and 2nd instars are considered together (Helmert contrasts; p = 0.016*). Parasitoid prevalence among larvae from 2006 appear to show a much more consistent pattern of parasitism over time, with the highest prevalence apparent among 5th instar larvae. Prevalence among this class was highly significantly greater than among 1st instar larvae (p<0.001***), as was prevalence among 4th instars (p = 0.013*), although this was not so for any other age class. However, when considering all age classes cumulatively, this relationship disappears, and no significant differences are found among any age classes (Table 3.3; 2006; Parasitoids; Helmert contrasts), suggesting that, although later instar stages appear to be more susceptible to parasitoid

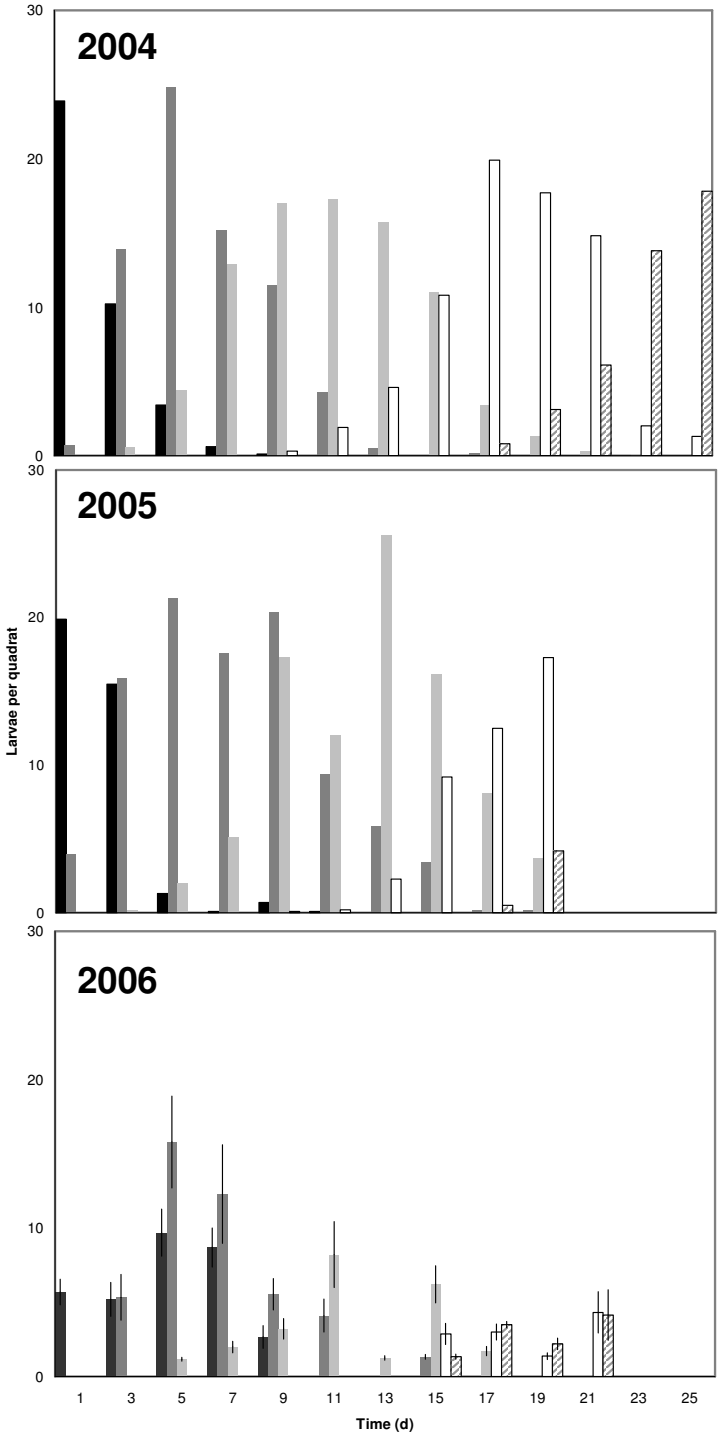


Fig 3.3 Changes in larval density within each instar stage over time, among the four consecutive sampling years (2004, 2005 and 2006). ■ represents average number of first instar larvae per quadrat; ■ represents average number of second instar larvae per quadrat; ■ represents average number of third instar larvae per quadrat; □ represents average number of fourth instar larvae per quadrat; ▨ represents average number of fifth instar larvae per quadrat. For 2006 only, error bars represent ± 1 S.E..

attack, this is not confined to any particular age class, and that this is manifest in a gradual increase in parasitoid prevalence with time.

Although CPV infections among *O. brumata* larvae remained fairly high among later instars, peaking among 4th instars (4.6%), no significant differences could be found between any of the age classes, either compared to 1st instars only, or the cumulative mean of all instars. In contrast, although patterns of infection by RV among all age classes appeared to follow a similar pattern to that among CPV infection (Fig 3.4), it is apparent that infections among 4th instar larvae were significantly greater than among both 1st instars (Table 3.3; Treatment contrasts; p = 0.04*) and all other age classes (Table 3.3; Helmert contrasts; p = 0.02*).

When the distribution of *O. brumata* larvae within quadrats (2006 only), is separated into their individual instar stages, clustering within each stage again seems to be highly heterogeneous (Fig 3.5), as it is when all age classes are combined (Fig 3.2; 2006). 1st instar larvae appear to be fairly uniformly distributed for the first three sampling days, with only a single positively significant clustering value at the 2m scale at 1d. However, by 7d larvae are highly significantly negatively clustered at scales 3-5m. Although such a pattern could be interpreted as being due to 1st instar larvae becoming more patchily distributed toward the end of their 1st growth phase, by 7d larvae are clearly still abundant within quadrats (Fig 3.3) and do not decline substantially until 9d, suggesting that this is an unlikely cause for the observed clustering of low values at this time. Similarly, 2nd instar larvae display a pattern of high, followed by low clustering within the first seven days of the growth phase, eventually becoming significantly positively clustered toward to end of this age class, when within quadrat densities are low. Clustering among 3rd instar larvae appears to display the direct opposite of that seen among the previous two stages; with negative clusters of low values around a peak of high value clusters during the maximal phase of growth, at 11d. Although the overall abundance of 4th and 5th instar larvae follow a very similar trend during 2006 (Fig 3.3), this was not evident in their respective clustering values: at 17d, 4th instar larvae display significant or highly significant clusters of high values at all scales, and while this trend does not extend into the preceding or proceeding sampling days, the final sampling day (21d) again displays positive clusters of high values, although only at small scales. In contrast, among 5th instar larvae, the only significant clustering values are negative ones, at larger scales on 19d, before and after which there appears to be little or no spatial pattern among larvae at this stage. In

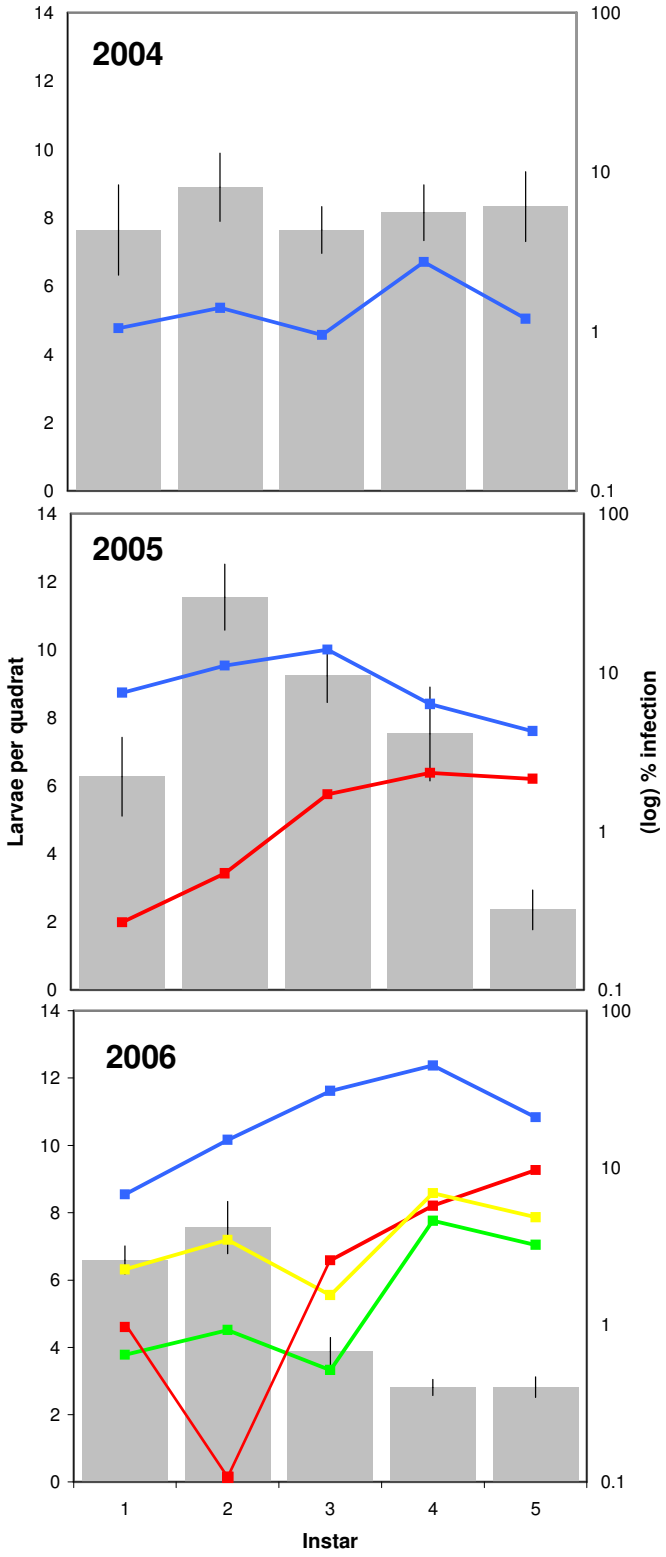


Fig 3.4 Changes in larval density per instar stage, and infection by four different species of natural enemy for *O. brumata* over the three consecutive sampling years (2004, 2005 and 2006). Grey bars indicate the average larval density per quadrat ± 1 S.E. for each instar stage (1-5). ■ represents the average (log) % parasitism of all larvae by the parasitoid *P. tempestiva*; ■ represents the average (log) % infection of all larvae by OpbuNPV; ■ represents the average (log) % infection of all larvae by CPV; ■ represents the average (log) % infection of all larvae by RV

			Treatment		Helmert	
Year	**Natural enemy**	**Instar**	**Estimate**	**P-value**	**Estimate**	**P-value**
2004	OpbuNPV	*1*	-4.74	-	-4.51	-
		2	0.28	0.70	0.14	0.70
		3	0.20	0.80	0.02	0.92
		4	0.92	0.23	0.19	0.12
		5	-0.27	0.77	-0.12	0.33
2005	Parasitoid	*1*	-5.93	-	-4.51	-
		2	0.71	0.52	0.36	0.52
		3	1.87	0.07	0.50	0.016 *
		4	2.38	0.02 *	0.38	0.002 **
		5	2.10	0.14	0.17	0.42
	OpbuNPV	*1*	-2.25	-	-2.61	-
		2	0.27	0.34	0.14	0.34
		3	0.03	0.93	-0.04	0.66
		4	-0.87	0.03 *	-0.24	<0.001 ***
		5	-1.23	0.14	-0.27	0.16
2006	Parasitoid	*1*	-4.63	-	-7.01	-
		2	-17.1	0.99	-8.56	0.99
		3	0.99	0.18	3.19	0.99
		4	1.83	0.013*	1.8	0.99
		5	2.39	<0.001***	1.19	0.99
	OpbuNPV	*1*	-1.85	-	-1.75	-
		2	0.96	0.004**	0.48	0.004**
		3	0.82	0.04*	0.11	0.27
		4	0.23	0.67	-0.09	0.38
		5	-1.52	0.02*	-0.4	<0.001***
	CPV	*1*	-5.64	-	-4.90	-
		2	0.32	0.72	0.16	0.72
		3	0.29	0.84	0.04	0.92
		4	1.93	0.12	0.43	0.10
		5	1.20	0.39	0.11	0.59
	RV	*1*	-3.77	-	-3.37	-
		2	0.45	0.34	0.22	0.34
		3	-0.39	0.58	-0.20	0.33
		4	1.17	0.04*	0.29	0.02*
		5	0.79	0.26	0.10	0.44

Table 3.3 Results of weighted binomial GLMMs of (log) ratio infected : uninfected *O. brumata* larvae by four species of natural enemy, among all instar stages over the three consecutive sampling years (2004, 2005 and 2006). For each model from 2004 and 2005, all instars are pooled from each transect, and random factors are specified as sampling time only; for models from 2006, infection among each instar stage within quadrats is used as the explanatory variable, with random factor specified as sampling time and quadrat position. "Treatment" represents the use of treatment contrasts within models, that compares each instar stage to infections among first instar larvae only; "Helmert" represents the use of Helmert contrasts within each model, that compares the mean of all previous infections to each consecutive instar stage starting at instar 1 and ending at instar 5 (e.g. Instar 3 vs mean[Instar 1+2+3]). Significance codes are: *p<0.05; **p<0.01; ***p<0.001.

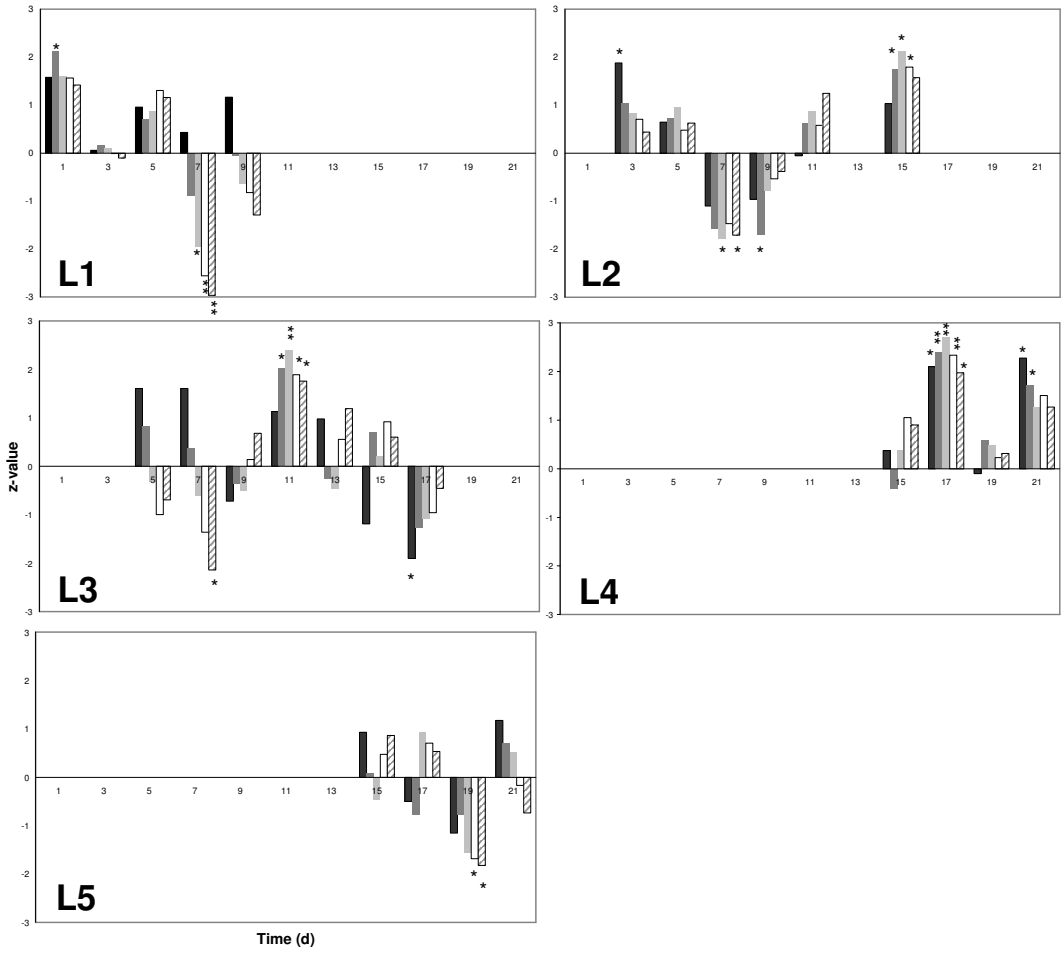


Fig 3.5 Global G statistics for *O. brumata* larval densities within each transect, for each of the five separate instar stages (where L represents each instar) over the 21 day sampling period during 2006, at five spatial scales. ■ represents 1m scale; ■ represents 2m scale; ■ represents 3m scale; □ represents 4m scale; ▨ represents 5m scale. Within each spatial scale, high z-values indicate that, across the whole 10m transect, larvae in adjacent quadrats have similarly high densities; low values indicate larval densities in adjacent quadrats are lower than expected. The significance of this assumption is tested against a null hypothesis of complete spatial randomness across the transect length. Significance codes are: *p<0.05; **p<0.01; ***p<0.001.

light of the large variation in clustering patterns among all individuals age classes during this sampling year, an overall pattern in spatial distribution is difficult to discern. However, it appears that among early instars at the beginning of their growth phase, larvae appear to change the spatial pattern from clusters of high values in the early stages, to clusters of low values in the later stages of growth; whereas, during their maximal growth phases, later instars appear to show the opposite effect, with clusters of high values when larvae are most abundant, and low values or random distributions at the early or late phases.

Although different larval instar stages appear to display age-specific patterns in their spatial distributions, no such effect was detectable when considering within-

quadrat density only (Table 3.4). Among parasitoids, the overall trend with increasing density was negative, apart from among 1st instars, which is consistent with the general trend when all larvae are pooled (Table3. 2), although no significant interactions were found among any age class. Similarly, OpbuNPV infections did not display any age-specific effects of density-dependence, although again the positive trend among most class is consistent with the total pooled effect. CPV infections displayed changing overall trends with different age classes, although not significantly so; and although RV infections among 2nd instar larvae showed a marginally non-significant positive effect of density, no other relationships were closely to significance, although all were positive.

Natural enemy	Instar	Estimate	logLik	ΔlogLik	p-value
Parasitoid	*1*	0.47	-5.03	2.1	0.19
	2	-	-	-	-
	3	-0.35	-13.4	1.64	0.15
	4	-0.14	-8.23	0.42	0.41
	5	-0.03	-7.92	0.06	0.74
OpbuNPV	*1*	0.02	-22.9	0.06	0.69
	2	-0.009	-44.6	0.1	0.64
	3	0.01	-37.2	0.03	0.79
	4	0.01	-25.6	0.02	0.85
	5	0.06	-8.5	0.69	0.23
CPV	*1*	0.02	-5.47	0.01	0.92
	2	0.07	-4.81	1.23	0.13
	3	-0.62	-2.90	0.81	0.38
	4	-0.14	-6.02	0.64	0.28
	5	0.07	-3.50	0.2	0.51
RV	*1*	0.09	-12.8	0.32	0.45
	2	0.04	-13.8	1.71	0.06
	3	0.05	-5.5	0.16	0.57
	4	0.18	-7.0	1.26	0.12
	5	0.03	-4.59	0.05	0.75

Table 3.4 Results of weighted binomial GLMMs of ratio infected : uninfected larvae vs density within each instar stage for *O. brumata* attacked by four different species of natural enemy during 2006. For each model, sampling time and quadrat position are specified as random factors. LogLik represents the (log) Likelihood of each model fit; Δ logLik refers to the change in (log) Likelihood of the final model compared to a null model with a constant explanatory variable. Dashes indicate the absence of infections among any larvae. Significance codes are: *p<0.05; **p<0.01; ***p<0.001.

3.5 Discussion

Larval abundance of *O. brumata* varied greatly both within and among years, with a general trend of decreasing abundance over time for both. Within years, OpbuNPV infections were highest among mid-instar larvae, and prevalence appeared to show an annual increase among all stages, as total abundance decreased. Data on parasitoid prevalence was only available for two of the three sampling years, although the patterns of attack were fairly consistent among the two, displaying an increasing prevalence with time, and being more prevalent among late instar larvae. There was no obvious change in CPV prevalence with time in the only year when it was sampled, with RV infections following a similar trend, apart from a significant increase among 4^{th} instars. The spatial pattern in distribution of larvae was highly variable within and among years, although early and late instar larvae appear to cluster into larger-scale areas of higher density, whereas mid-instar larvae appear to be more spatially restricted. Despite this, there was no significant relationship between total larval density and natural enemy prevalence, or among any individual instar stage.

Although such insect host- NPV interactions are not wholly consistent with laboratory studies of age-dependent responses to virus challenge, which show that earlier instars are far more vulnerable to virus challenge than later instars (Stairs, 1965; Boucias & Nordin, 1977; Ali & Young, 1991), it seems likely that this discrepancy may be due to the different sampling conditions among field-collected larvae. The susceptibility of larvae to viral mortality increases with increasing virus dose (Sait *et al.,* 1994), however, the size of the dose will depend on how many viral occlusion bodies (OBs) are ingested at that particular stage. Since OBs are deposited on foliage following the death of infected hosts, the probability of any larva encountering a lethal dose of OBs will be a function of the spatial distribution of OBs on host plants, and the quantity of host plant consumed by each stage (Payne *et al.*, 1981). Since *O. brumata* larvae are spatially restricted to nests in the early instar stages, and host plant consumption is less than in subsequent stages, it seems likely that among field populations, early instars have a reduced risk of infection than mid-late instars. Despite this, estimates of viral transmission among Lepidoptera in the field display contrasting results: Dwyer (1991) found that late instar larvae were more infective and more likely to become infected than earlier instars; however, Goulson *et al.* (1995) found no significant difference in

transmission rates among later instars even though earlier instars died more quickly. The observed reduction in infection prevalence among late instars in the present study may be due to a number of different, possibly interactive components: i) dilution effect, whereby the number of OBs required to cause larval mortality were too great among larger, late instar larvae. Sait *et al.* (1994) reported no observed deaths among 5th instar *Plodia interpunctella* despite a concentration of Granulosis Virus OBs 1x10^{6} times greater than that required to cause mortality among 1st instars, and Stairs (1965) found that 4th instar *Malacosoma disstria* larvae could survive NPV doses 2x10^{9} times greater than those needed to cause mortality in 1st instars; ii) systemic resistance, whereby larvae acquire increased immunity to viral infections as they age, both within (Hoover *et al.*, 2002) and among (Teakle *et al.*, 1986; Kirkpatrick *et al.*, 1998) instar stages, with older instars that have overcome sublethal virus challenges, potentially more likely to resist subsequent challenges, even though their encounter rate may be greater as they ingest more foodplant; iii) plant-mediated indirect effects, whereby phenolic compounds may act to inhibit or inactivate virus transmission within hosts (*reviewed in* Cory & Hoover, 2006). This may be particularly important for species feeding on host plants of relatively poor nutritional quality such as heather, which contain high levels of tannins that are known to inhibit NPV infections (Hunter & Schultz, 1993), since larger larvae will ingest larger quantities of the food plant. Indeed, compared to oak-feeding populations, heather-feeding *O. brumata* larvae are known to be less virulent and yield less virus, despite no apparent difference in infectivity (Raymond & Hails, 2007). However, even though variation in within-season plant nutritional quality may increase viral fitness by increasing the amount of foodplant needed to be ingested by larvae (and therefore increasing the potential OB encounter rate), this is unlikely to outweigh potential age-related factors *per se* in susceptibility to infection (Raymond & Hails, 2007). One potential caveat to the interpretation of these age-specific mortality factors is in the discrepancy between the point of infection and the time of death, since larvae may ingest a lethal dose in a prior stage to when collected in the field. The methodology used in the present study is therefore likely to overestimate age-specific susceptibility to virus mortality, although laboratory bioassay studies of NPV infections in *Malacosama neutria* have shown that this discrepancy is not likely to exceed a single moult (Magnoler, 1975).

The observed patterns in parasitoid attack suggest that 4th instar larvae are the most susceptible stage. Ecological theory would predict that older, larger hosts provide

more resources for developing parasitoid larvae, thereby increasing offspring fitness and survival (King, 1989; Godfray, 1994). Although the observed patterns found in the present study do not support this theory, the mechanisms underlying parasitoid host choice may be complex and highly variable among species (*see* Vinson, 1976). While such assumptions may be true for idiobiont parasitoids (that paralyse their hosts upon oviposition), this may not necessarily be the case for koinobiont species (larvae develop in free-moving hosts) such as *P. tempestiva* in the present study. For example, oviposition preferences in the koinobiont ichneumonid endoparasitoid *Venturia canescens* is not host-size or instar specific when parasitizing *P. interpunctella* (Harvey *et al.,* 1994), and although among both large and small host species, *Hyposoter didymator* (Hymenoptera: Ichneumonidae) did show increased development and survival, within host species there was little difference in prevalence among instars, despite decreased rates of parasitoid survival in final instar larvae (Reudler Talsma *et al.*, 2007). Indeed, some species may display little preference for host instar when they oviposit (Nofemela & Kfir, 2008), although it is likely that the earliest instars represent a poor quality resource for developing parasitoid larvae. Conversely, larger late instar host larvae may provide a high-risk resource for koinobionts, since parasitoid larvae may become trapped in the host integument unless they are able to consume the entire host before being able to emerge (Harvey, 1996); late instars may also be better able to mount stronger immune responses than earlier instars (e.g. encapsulation responses) following oviposition (Strand & Pech, 1995; Bauer *et al.*, 1998). Oviposition by adults may also be more difficult in later host instars, which may be able to mount stronger behavioural responses (e.g. defensive secretions) than earlier instar stages (Gross, 1993). Larvae of *O. brumata* are able to elicit both a physical and chemical response to handling by wriggling and emitting a defensive secretion (*pers. obs.*), although the extent to which such behaviours are able to deter potentially ovipositing parasitoids is not known for these two particular species. Larvae of *O. brumata* are also known to vary in their degree of cuticular melanism (Hagen *et al.*, 2003), which is known to develop as a response to parasitoid oviposition (Smilanich *et al.*, 2003). However, such responses have also been shown to be related to virus resistance (Wilson *et al.*, 2001) and density (Goulson & Cory, 1995), and the extent to which this variation is mediated by any of these factors in *O. brumata* is at present not clear (Hagen *et al.*, 2006).

The extent to which each instar stage is susceptible to parasitoid attack is again difficult to discern from the present study, due to the time lags between sampling and

emergence of parasitoids from each instar. Whilst there appears to be little evidence that *P. tempestiva* attacks, or at least is able to successfully emerge from 1st and 2nd instar *O. brumata* larvae, it is unclear whether 4th and 5th instars are the primary susceptible period in these species, since parasitoids emerging from 5th instars may have been initially attack during any of the previous instar stages. In the only other published study of these species, Graham *et al.* (2006) notes that all *P. tempestiva* adults emerged from 5th instar *O. brumata* larvae, consistent with the greater prevalence found in 2006. However, in the present study, the only significant interaction that is consistent between both sampling years is that among 4th instar larvae (although sample size is low among 5th instars during 2005). Parasitoid larval development times within different instars may vary greatly and extend across several instar stages (Harvey *et al.*, 1994; Godfray, 1994; Mironidis *et al.,* 2009); although it is not presently known what the species specific development times are among *P. tempestiva* parasitizing *O. brumata*, given the high prevalence among 4th instars, and the putative defensive mechanisms outlined above, it seems likely that a varying degree of risk exists between 3rd and 5th instars.

One possible indicator of oviposition behaviour that is independent of parasitoid emergence time is that of the prevalence of RV infections. RV is known to be particularly prevalent in *P. tempestiva* in Orkney, being present in 92% of individuals screened (Graham *et al.*, 2006), and is thought to confer mutualistic or commensal benefits for ovipositing parasitoids in suppressing the host's immune response (Renault *et al.*, 2005). The fact that the only instar stage to demonstrate significantly greater prevalence of RV infections was the 4th instar could therefore be indicative of the most susceptible stage for parasitoid attack among *O. brumata* larvae. Graham *et al.* (2006) found no evidence of vertical transmission of RV in *O. brumata*, so it seems likely from the presence of the virus in 1st and 2nd instars that these stages are susceptible to parasitoid attack, but that either successful development is less likely among these stages, or that parasitoids may be probing but not ovipositing, possibly because early instars represent a sub-optimal resource. CPV is also known to be present in *P. tempestiva*, although at far lower prevalence (< 5%), and always in co-occurrence with RV. It therefore seems likely that vectoring by parasitoids is not the primary transmission route for CPV, with vertical transmission being more likely (Graham *et al.*, 2006); as expected, there was no evidence of any stage-specific differences in CPV infections among *O. brumata* larvae in this study, consistent with this hypothesis.

Despite this, clearly further data are required from subsequent years to confirm whether these patterns are consistent across generations in this system.

Although there appeared to be no consistent pattern of clustering among either sampling year or instar stage, a general trend of clustering of high values was seen among both early and late instar stages, with intermediate stages displaying clusters of low values, possibly indicative of spatial restriction within quadrats. Although difficult to interpret, since larval abundance within each instar varies through time, a possible explanation for the observed patterns may be due to host utilisation of resources: it is likely that both early and late instar stages are found at the shoot tips of heather; 1st and 2nd instars build silken nests between shoot tips in which they spend all of these early developmental stages, whereas later instars are often found in the same areas, possibly utilising areas of newest growth which may be nutritionally superior (*pers. obs.*). If shoot tips therefore represent a limiting resource among these stages, it could be that larvae distribute themselves more evenly, although in clusters where resource quality is highest. Among mid-instar larvae, where risk from natural enemy attack may be greatest, small scale aggregations of larvae may represent a countermeasure against parasitoid attack, or decrease the encounter rate with viral OBs: aggregations of larvae, although sometimes acting as attractants for some parasitoids (Wertheim *et al.*, 2003), have previously been shown to not increase the risk of parasitism (Jumean *et al.,* 2008) possibly due to a dilution effect, or increase in the availability of structural refugia (Hassell, 2000). Indeed, if larvae in these intermediate stages are not aggregating on shoot tips, it may be because this increases the risk of parasitism, whereas aggregating in denser foliage may provide some degree of reduced risk from parasitoid foraging; Sait *et al.* (1997) found that first and second instar larvae of *Plodia interpunctella* had significantly reduced parasitism compared to later instars when structural refugia was available, with reduced parasitism across all instars compared to when refugia was absent. Similarly, small scale aggregations of larvae may have a reduced risk of encountering viral OBs that tend to be highly clumped as infected larvae die on hostplants (Cory & Myers, 2003): Dwyer (1991) found that transmission of an NPV infecting Douglas-fir tussock moths to early instars was reduced with increasing patchiness of infected hosts, while transmission to later instars was unaffected. However, without knowledge of the distribution of infective OBs in the environment, and any consistent spatial pattern among years, it is not clear that this is the exact mechanism driving these patterns.

Clumping of pathogens, specifically NPV infecting Lepidoptera, has been posited as a principal mechanism underlying the incidence of non-linear virus transmission (D'amico *et al.*, 2005). Although transmission rates in the present study have not been fully quantified, there were no significant incidences of spatial density dependence among any of the sampling years or instar stages, and any natural enemies. Previous studies have demonstrated density-dependent transmission among NPVs (Cory & Myers, 2003), with transmission being strongly affected by instar stage (Dwyer, 1991). However, it is doubtful that such a linear transmission process operates among all Lepidoptera-NPV interactions (D'amico *et al.*, 1996; Knell *et al.,* 1998), with evidence of spatial heterogeneity in OpbuNPV infection occurring in the present study, skewing parameter estimates in the GLMM. Among parasitoids too, spatial density dependence may be highly variable according to host species (Walde & Murdoch, 1998) and vary according to spatial scale (Hails & Crawley, 1992; Veldtmann & McGeoch, 2004). Attack rates among each natural enemy have already been demonstrated to have some stage-related specificity, and so pooled models of density across instars is unlikely to yield any clear relationship with host density. However, even though no relationship was found when densities were partitioned into individual instar stages, the fact that this was only possible to investigate using data from a single year (2006) makes this generalisation unsafe. Clearly prevalence among OpbuNPV and parasitoids was highly variable both within and among years, so it may be that some such density-dependent relationship exists; further data from subsequent years is needed.

Indeed, patterns in the abundance of *O. brumata* hosts and natural enemies over the three sampling years in the present study may be indicative of population cycles—host abundance decreases as natural enemy prevalence increases—which are known to occur in *O. brumata* populations in Fennoscandia (Tenow *et al.*, 2007). However, whether such cycles occur in heather-feeding populations is at present not known. Density dependence is a key parameter in the formulation of population dynamic models of insect natural enemy interactions, which may also include the effects of intraspecific competition for resources, and has been implicated in playing a role in the observed cycles of insect populations through interactions with both NPV-like viruses and parasitoid natural enemies (Anderson & May, 1981; Hassell, 2000; Liu & Li., 2006), which may also be affected by spatial processes such as host dispersal and the availability of refugia (Latto & Hassell, 1988; Dwyer, 1994). However, some models of insect-natural enemy dynamics have suggested that heterogeneity in susceptibility

among larval instar stages may be more important than spatial heterogeneity in predicting disease dynamics, especially when pathogen transmission is non-linear (Briggs & Godfray, 1995; Dwyer *et al.*, 1997). Such heterogeneity in susceptibility among different instar stages may therefore have important implications for the dynamics of host insects and their natural enemies, especially where multiple natural enemy species cause mortality among shared, or disparate, age-classes. Larger scale, longer term studies will inevitably be needed in the *O. brumata*–natural enemy complex in order to assess whether the spatial and temporal patterns in abundance observed in the present study are temporally stable, or exhibit the generational cycles predicted by mathematical models, and observed in other systems.

Chapter 4

Local scale insect host-natural enemy interactions

4.1 Abstract

The spatial scale at which natural enemies respond to changes in host abundance may be fundamentally linked to their transmission mode and dispersal relative to the host species. This in turn may have implications for the regulation of insect herbivores in natural populations, and may be manifest in quantifiable spatial patterns within patches. How such spatial responses relate to changes in host density may also be scale-specific, and mediated by variation in host immune responses.

Here, we test the hypothesis that different types of natural enemy will display characteristic spatial patterns in response to changes in host density due to their different transmission modes. Mobile parasitoids are expected to display a spatial pattern of parasitism at scales larger than that of their less mobile hosts, and that prevalence will be negatively related to increases in host density, due to saturation in attack rate at high densities. Conversely, horizontally transmitted pathogenic viruses are expected to exhibit patterns of infection at much smaller spatial scales than their hosts due to their passive transmission mode, and that prevalence is expected to be positively related to increases in host density. Viruses vectored by parasitoid wasps are expected to display similar responses to both the scale and density of that of their commensal hosts. Vertically transmitted viruses are not expected to display any spatial pattern of infection, or prevalence of infection to be related to host density. Whether these characteristic responses differ between host outbreak and non-outbreak years, between different species infected by the same family of pathogens, and whether host cuticular melanisation is a response to infection or parasitism by any of these agents, is also investigated.

Operophtera brumata larvae were sampled on a single day, from a single site in Orkney, over three years. Larval abundance within quadrats was recorded within a localised outbreak, with sampling extending out over a total linear distance of 250m. The spatial changes in larval abundance and natural enemy attack were inferred using spatial autocorrelation indices. A second, spatially separate sampling site was also investigated, in a single year, which supports both *O. brumata* and *Abraxas grossulariata*, in order to discern whether any host-pathogen scale responses could be generalisable among species.

O. brumata abundance displayed a wave-like pattern of localised host dispersal among years along the transect following an outbreak, eventually colonising local

patches where previously it was absent. Such a pattern may be driven to some extent by the high prevalence of natural enemies in the outbreak epicentre. Horizontally transmitted OpbuNPV prevalence was positively host-density dependent during the first two years of the outbreak, with spatial patterns of infection similar to the localised aggregation of hosts. Parasitoid prevalence was negatively density dependent in the outbreak year, but positive in the proceeding year, with a spatial signal greater than or equal to that of the host, but a prevalence signal that became remarkably similar to OpbuNPV in the latter year. Vertically transmitted CPV prevalence followed closely that of the localised aggregations of hosts, although its response to changes in host density was only manifest as a delayed effect. Parasitoid-vectored RV prevalence followed closely the spatial pattern of parasitoids among hosts, although it showed no sign of host-density dependence, at any scale. Spatial patterns of natural enemy attack among a spatially separate, low host density site showed no congruence with the outbreak site. Similar spatial patterns among *A. grossulariata* infected by AbgrNPV were also not evident, and it is likely that these effects may be localised, and site-specific. Patterns of parasitism among *O. brumata* varied greatly among the two sites, and this may be partially attributable to variation in the larval cuticular melanism response, which was explained better by variation in parasitoid prevalence than any other natural enemy, or host density.

Spatial patterns of natural enemy infection and prevalence in relation to host density appear to follow well predictions based on prior knowledge of transmission mode. However, this appears only to be the case during relatively rare host outbreak events. Host-natural enemy interactions in areas of low host density may not display such relationships, although different spatial patterns may occur at larger spatial scales than that examined in this study. The implications of these spatial interactions for the regulation of populations, and the interactions with host immune responses, is discussed.

4.2 Introduction

An explicit consideration of space is now known to be central to a mechanistic understanding of population ecological processes, since species' responses do not occur independently of their surrounding environment (Levin, 1992; Tilman & Kareiva, 1997), and may be sensitive to the scale at which they are viewed (Wiens, 1989). The occurrence of spatial patterns among species may be indicative of underlying ecological processes, which again may operate at different scales, influencing the distribution and abundance of organisms across landscapes (Turner, 1989; Dale, 1999).

For insect herbivores, the spatial arrangement of suitable habitat patches across a landscape may fundamentally influence their ecological dynamics (Fahrig & Paloheimo, 1988; Schöps, 2002; Chust *et al.*, 2004; Gripenberg & Roslin, 2005; McGeoch & Price, 2005), which in turn could scale up into higher trophic level processes (MacCauley *et al.*, 1993; Thies *et al.*, 2003; Denno *et al.*, 2005). For example, a growing body of evidence is emerging which demonstrates that mobile predators and parasitoids are able to respond to host habitat structure (Cappucino *et al.*, 1998; Cronin, 2003), and that these responses may be scale specific (De Roos *et al.*, 1991; Roland & Taylor, 1997). Such findings may have important implications for the biological control of certain pest insect species by their natural enemies (Waage & Hassell, 1982), which can also include horizontally transmitted viral pathogens, such as baculoviruses (Lacey *et al.*, 2001). Although as a group, baculovirus interactions with their (Lepidoptera) insect hosts have been well studied (Cory & Myers, 2003), their responses to spatial scale have been given less attention than among parasitoids (Dwyer, 1991; Cooper *et al.*, 2003), and even less attention has been paid to the spatial dynamics of vertically transmitted (Laitenen *et al.*, 1996) and vectored (Ostfeld *et al.*, 2005) viruses, with their insect hosts.

A key process underlying such host-natural enemy interactions, and therefore their capacity to regulate populations of pest species, is that of density dependence (Varley *et al.,* 1973; Anderson & May, 1981). Temporal and spatial density-dependent processes may act synergistically to bring about this regulation, and an explicit consideration of both is fundamental to an understanding of the dynamics of populations (Hassell, 1985; Hassell 1986; Hails & Crawley, 1992). However, detecting such responses among natural populations is often fraught with difficulties, not least in the definition of density itself (Lewontin & Levins, 1989). The scale at which different

organisms experience various ecological interactions may be highly specific, and definitions of density may vary greatly depending on the spatial extent over which these parameters are measured (Gunton & Kunin, 2007). Parasitoid responses to host density in space are known to be highly variable (Walde & Murdoch, 1988), although the conclusions drawn from many studies may be highly misleading if conducted at inappropriate spatial scales (Ray & Hastings, 1996; Veldtmann & McGeoch, 2004). Such spatial density-dependent processes, and the caveats that come with their study, may be highly important when attempting to understand the patterns and processes that underlie insect outbreaks, which are characterised by their high temporal and spatial variability (Wallner, 1987). Interactions with natural enemies play a key role in the dynamics of such outbreaks (Godfray & Briggs, 1995; Maron *et al.*, 2001), and regulation by a combination of agents with different transmission modes and responses to host density, may be crucial in stabilising and maintaining fluctuations in population abundance (Dwyer *et al.*, 2004).

Hosts, in turn, may respond to natural enemies by mobilising immune responses either as a direct result of, or countermeasure against, such attacks. In some species of Lepidoptera, such countermeasures are manifest in a change in phase polyphenism to a darkened colour morph via the production of higher levels of melanin in their cuticles (Wilson & Reeson, 1998). Melanin production is thought to be an effective part of the insect encapsulation response to infection by parasites and pathogens (Reeson *et al.*, 1998; Wilson *et al.*, 2001), although some species are known to induce such an investment in the immune response as a direct result of conspecific density, even when natural enemies are absent (Goulson & Cory, 1995), in a process termed density-dependent prophylaxis (Reeson *et al.*, 1998). Within hosts, there may also exist dynamic processes among natural enemies to overcome host immune responses (Vinson, 1990; Fuxa, 2004), as well as antagonistic and mutualistic interactions to ensure transmission of infective stages to subsequent generations (Hochberg, 1991; Bonsall & Benmayor, 2005).

In this study, the spatial pattern of a focal Lepidoptera species (*O. brumata*) is investigated both during, and in the years following, the occurrence of a localised outbreak on heather moorland in Orkney. The spatial pattern of interactions with four different types of natural enemy (one parasitoid and three viruses), all with different transmission modes is also investigated, in order to see if density-dependent processes are operating within any of these interactions, and if so, whether these responses are

contingent on the scale at which the interaction is studied. Evidence is also presented of *O. brumata* interactions with natural enemies and interspecific competitors from a similar site, but at much lower overall abundances, in order to investigate whether inferences made from the dynamics of hosts and natural enemies during outbreak years, are generalisable to patches with more stable spatial and temporal patterns. The heather moorland ecosystem provides an ideal opportunity in which to study local scale spatial processes, since in mature stands such as used in this study, vegetation coverage is largely continuous, and dominated by a single species (Gimingham, 1985). It therefore avoids many of the pitfalls inherent in having to account for host-plant heterogeneity in spatial pattern, when the focus of the study is on host-natural enemy interactions. *O. brumata* also is known to exhibit phase polyphenism in its degree of melanism, and this response has previously been found to correlate well with host density (Hagen *et al.*, 2003) and parasitoid abundance (Hagen *et al.,* 2006). In this study, melanism in this species is also investigated in response to viral infection, to see whether this provides an alternative, or supplementary, explanation for the occurrence of such phenomena among populations in Orkney.

The four different types of natural enemy investigated in this study are i) *Phobocampe tempestiva*: an generalist ichneumonid parasitoid, one of only two known to parasitise *O. brumata* in Orkney, and by far the most abundant; ii) OpbuNPV: a horizontally transmitted host-specific baculovirus; iii) CPV19: a vertically transmitted cypovirus; and iv) RV: a species of reovirus, thought to be largely vectored by *P. tempestiva* (Graham *et al.,* 2004; Graham *et al.*, 2006; Graham *et al.*, 2007; Graham *et al.*, 2008). Interspecific competition for the shared heather resource is also investigated through interactions of *O. brumata* with the recently invasive species *A. grossulariata,* a larger geometrid. Spatial patterns of infection and density-dependence are also explored among this species and its host specific, horizontally transmitted baculovirus, AbgrNPV.

Due to their high mobility relative to larval hosts, it is predicted that spatial patterns of infection by parasitoids will correspond to larger spatial scales than hosts, and show evidence of inverse density dependence as a result of predator satiation, especially in areas of high host density. In contrast, OpbuNPV infections are expected to operate at much finer spatial scales and show strong positive density dependence in infection, due to their horizontal transmission mode. CPVs are not expected to have any response to host density at any scale in the current year, although a positive delayed

effect of host density may be detectible due to vertical transmission from previous generations. RV infections are predicted to have spatial patterns and responses to host density very similar to that found among their parasitoid vectors, although the strength of the interaction, if any, is expected to be lower due to reduced transmission between vector and host. Parasitoids and OpbuNPV are expected to show signs of antagonistic intra-host interactions, manifest in negative correlations within spatial locations, as they compete for the same host as a resource. The degree of larval melanism is expected to be greatest where total infections from natural enemies are greatest, with infection by parasitoids or OpbuNPV (or a combination of the two) likely to elicit the greatest levels of immune response, manifest as increases in cuticular melanism. Total prevalence of natural enemies and the strength of density-dependent infections are anticipated to be greatest during outbreak years, and diminishing in subsequent years. Where host densities are spatially stable, little or no effect of density on natural enemy prevalence, especially among OpbuNPV, is expected, due to a decreasing effect of small scale pathogen transmission when host densities are more uniformly distributed in space. The presence of interspecific competition from *A. grossulariata* on the shared host plant is expected to disrupt the patterns of *O. brumata* host-natural enemy interactions, although patterns AbgrNPV infections are expected to show similar spatial patterns of infection to those seen among the *O. brumata*-OpbuNPV interaction.

This is the first time that spatial and density-dependent interactions have been investigated for a single Lepidopteran host attacked by four different natural enemies, of differing taxa and transmission modes. A novel method for investigating spatial density-dependence in host-natural enemy responses to changing spatial scale is also proposed, and the implications of the findings for insect host-natural enemy dynamics and regulation of natural populations, is discussed.

4.3 Methods

4.3.1 Study sites

Two sites were chosen due to their similar within-site habitat characteristics, and contrasting host-species abundances: Linnadale (58° 6'N, 3° 11'W) is a south-west facing (slope = 9 ±2°) area of mature heather moorland 156 ±10m above sea level (a.s.l.), with no obvious signs of grazing. *O. brumata* is locally abundant here,

displaying markedly higher densities than at any other site sampled in Orkney over the previous seven years. Small numbers of *A. grossulariata* occur at the site, although their densities are no greater than other Lepidoptera species, and are therefore not included in the present analysis; Wideford Cairn (58° 9'N, 3° 2'W) is a north-west facing (slope = 10 ±2°) area of mature heather moorland 93 ±10m a.s.l., also with no obvious signs of grazing. Both *O. brumata* and *A. grossulariata* are abundant here, and both species are included in this analysis to investigate any possible interspecific interactions that may occur due to their shared resource. Both sites are located on the south-west mainland of Orkney, and are >10km apart.

4.3.2 Sampling methods

O. brumata and *A. grossulariata* individuals were collected using the quadrat methodologies outlined in Chapter 2 (Methods), although with a modified linear transect design: a series of ninety 0.25m x 0.25m quadrats (each 1m apart) nested within nine 10m transects (each 10m apart), nested within three 50m regions (each 50m apart) were sampled (see Fig 4.1.), giving a total distance of 250m. For samples from Linnadale, one of these nested 250m transects was sampled each year during a single day in June of 2006, 2008 and 2009 (although no natural enemy data is currently available for 2009). For samples from Wideford Cairn, a 250m nested transect was sampled during a single day in June 2008. For both sites sampled, there were no obvious changes in topography over the 250m, and for samples among years, each transect was taken as close as possible to that of the previous year (error = <10m per quadrat among years). Heather height was measured as the distance from soil to shoot tip (to the nearest cm), at the centre of each quadrat. For *O. brumata* larvae (2008 only), the degree of cuticular melanism was subjectively recorded into three categories, following the methods of Hagen *et al.* (2003): (M1) non-melanic; (M2) melanic; (M3) intermediate.

All individuals were reared until pupation or death, as outlined in Chapter 2. All unparasitised dead individuals were screened for four viruses (*O. brumata:* OpbuNPV, CPV and RV; *A. grossulariata:* AbgrNPV), using molecular methods (*see* Chapter 2). Samples from 2006 were screened using standard RT-PCR methods; samples from 2008 were screened using multiplex real-time RT-PCR. A summary of the data collected from each site is given in Table 4.1.

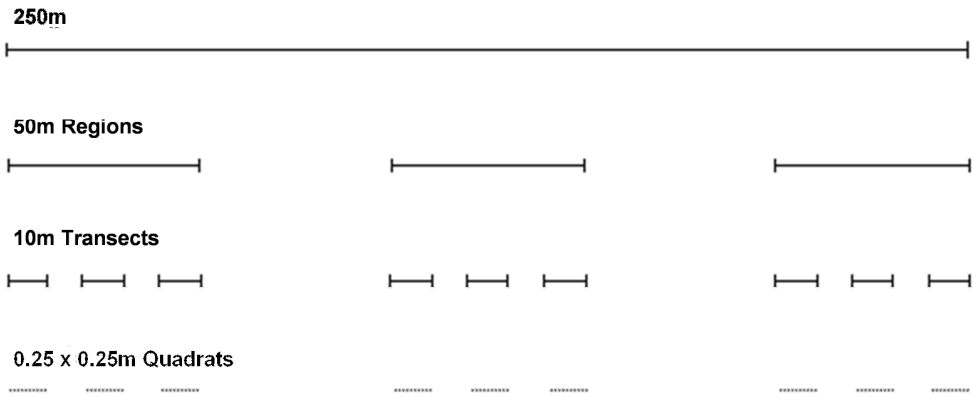


Fig 4.1 Sampling design for the nested linear transect. Data collected within quadrats only (n = 90); Quadrats, Transects and Regions correspond to the grouping levels used in subsequent statistical analysis.

						% *Instar*						%*Natural enemy*			
Site	**Year**	**Heather height (cm)** (±S.E.)	**Species**	**Total**	**Average quadrat density** (±S.E.)	**L1**	**L2**	**L3**	**L4**	**L5**	**Parasitoid**	**OpbuNPV**	**CPV**	**RV**	**AbgrNPV**
Linnadale	2006	-	*O. brumata*	1787	20 (3.7)	0	1	8	25	67	9	30.2	5.9	1.3	-
	2008	30.3 (0.6)		1262	11.9 (1.2)	0	2	21	56	21	8.9	35.5	2.1	2.9	-
	2009	31.3 (0.8)		1089	10.3 (0.78)	0	0	2	21	64	-	-	-	-	-
Wideford Cairn	2008	31.8 (0.8)		564	5.3 (0.44)	0	0.2	4	55	41	21.3	3.4	-	1.8	-
			A. grossulariata	139	2.7 (0.19)	-	-	-	-	-	-	-	-	-	20.1

Table 4.1 Summary of data collected from each of the two study sites over three years; % Instar refers to the percentage of the total number of individuals within each instar stage (1-5) at the time of sampling; % Natural enemy refers to the percentage of the total number of individuals infected with each natural enemy over the whole transect.

4.3.3 Statistical analysis

All statistical analyses were undertaken using R 2.9.0 (R core development team). All generalised linear mixed models (GLMM) were constructed using the package lme4 (Bates *et al.*, 2008); Moran's I autocorrelation tests and Getis-Ord G statistics were calculated using the package spdep (Bivand, 2009); Spline correlograms were constructed using the package ncf (Bjornstad, 2005); and Mantel tests were performed using the package vegan (Oksanen *et al.*, 2009). All analyses can be divided into the following stages:

i) Plant-insect interactions

To investigate whether patterns in insect abundance may be affected from the bottom-up, GLMMs were constructed using per-quadrat host density as the response variable and heather height as the explanatory variable, with a Poisson error structure and logit link function. Because heather is known to have a peak mature phase of growth before degeneracy (Gimingham, 1972), a quadratic covariate was also included in each model to look for possible non-linear associations. The scale used for the random effect (Quadrat, Transect, or Region), was chosen as the one that maximised the within-model variance, without residual spatial autocorrelation. This autocorrelation was assessed using Monte Carlo permutation tests (1000 iterations) of Moran's I autocorrelation statistic over all distances up to a maximum of half the total transect length (125m), to avoid small sample size biases at larger scales (fewer pairs of quadrats on which to calculate correlation values at greater distances). This statistic calculates a value for the spatial correlation (positive values being spatially similar; negative values being spatially dissimilar), and calculates a p-value based on the probability of finding the same autocorrelation value when compared to random permutations of the same data among all spatial locations. Model fits were assessed using log Likelihood values. Where models with a quadratic heather height covariate did not have a significantly better fit than models without the covariate, the latter was chosen as the final model due to parsimony.

Heather height data were not available for Linnadale from 2006, although models were constructed using data from both 2008 and 2009, assuming no appreciable difference between quadrats or years. This assumption was tested using a Mantel test with 10^4 permutations of the two distance matrices from each of these years.

ii) Spatial pattern analysis

Two approaches were used to assess the spatial pattern of host and natural enemy responses to space: in the first, relative hierarchical variance components were extracted from null models of each GLMM (models with a constant explanatory variable), according to different grouping levels of the random effect: either Regions; Transects within Regions; or Quadrats within Transects within Regions. Within each species or natural enemy, the scale at which most variation is evident should therefore be

indicative of the scale at which that species is responding either to its habitat, or its host. For host species, GLMMs were constructed using a Poisson error structure suitable for count data; for natural enemies, a sample-size weighted response of the ratio of infected to uninfected individuals, and a Binomial error structure, was used. Model fits were again assessed using log Likelihood values.

The second approach involved constructing spatial correlograms to assess the similarity of each pair of quadrats as a continuous function of distance, plotted as a smoothed spline curve. For different distances, the point at which the correlation function reaches zero indicates that (at that distance) pairs of values are no more similar than expected by chance, over the whole transect. It can therefore be inferred that this is the scale at which that species is operating. For host species, correlograms were constructed from the number of individuals per quadrat; for natural enemies, from the number of infected individuals per quadrat.

iii) Scale-dependent density dependence

Again, two different approaches were used to assess the incidence of density-dependence of infection for each natural enemy and, if such a relationship exists, whether there is evidence of spatial dependency:

Firstly, three separate GLMMs were constructed for each natural enemy, with the grouping level of the random effect reflecting grouping at each of the nested scales of the linear transect (Quadrat, Transect and Region; Fig. 4.1), using weighted binomial responses (as above) and host density per quadrat as the explanatory variable. By specifying the grouping level of the random effect at different scales among the transect length, any scale-specific differences in each natural enemy's response to host density should therefore be made apparent. The ability of the random effect to model the error variances at each scale sufficiently to remove any underlying spatial dependency in model residuals was quantified using Moran's I autocorrelation permutation tests using 10^3 iterations. Model fits at each scale were assessed using log Likelihood values, conditional on the absence of residual autocorrelation.

The second approach involved modelling the changes in host density between quadrats more explicitly than the previous approach since density and frequency are confounded, because the area of each quadrat is held constant. To do this, local Getis-Ord G-statistics (Getis & Ord, 1992) were calculated for each quadrat. This statistic

calculates a z-value (a measure of standard deviation) for each point, based on the relative values of its neighbours. Thus, quadrats with a low number of individuals relative to their neighbours will have lower (or negative) z-values; an equal number of individuals relative to their neighbours will give zero z-values; and high number relative to their neighbours will give higher z-values. Therefore, by changing the neighbourhood size upon which G is calculated, the relative densities within each quadrat can be calculated and standardised, according to spatial scale.

G-statistics were calculated for each quadrat for all values of the neighbourhood size between 1-125m, and GLMMs constructed for each natural enemy using weighted binomial responses, and local G statistics at each scale as the explanatory variable. The grouping level of the random effect used for each natural enemy was the linear transect scale that maximised the within-model variance. Residual spatial autocorrelation was assessed using Moran's I permutation tests, and Δ log Likelihood values (the change in log Likelihood relative to a null model) were used to assess which scale gave the best model fit for each natural enemy. Because of the large number of GLMM models constructed, a *post-hoc* Bonferroni correction for multiple comparisons was applied to assess the significance of p-values.

For both separate analyses, the delayed effect of density-dependence of infection at the Linnadale site was also investigated by constructing GLMM models of infection from 2008, with host density in 2006 as the explanatory variable, assuming spatial continuity of quadrats among years. Again, log Likelihood values were used to ascertain whether a delayed response to infection gave better model fits than the current year's density.

iv) Density dependence and melanism

To investigate whether host density *per se*, or natural enemy prevalence, was affecting the incidence of larval melanism in *O. brumata*, weighted binomial GLMMs were constructed based on the analysis of cumulative proportions of melanic to non-melanic individuals as response variable, following Hagen *et al.*. (2003). This involved constructing two separate models for each explanatory variable (host density, or number of infected individuals), to account for possible inconsistencies in the subjective cut-off point in classification of the intermediate (M3) colour morph: Model 1 = ratio (M1) : (M2+M3); Model 2 = ratio (M1+M3) : (M2+M3). The random effect used for each

model was the one that maximised the within-group variances; autocorrelation of residuals was assessed using Moran's I permutation tests; and model fits were assessed using log Likelihood values.

Due to the possibility that there may be interactive effects between different natural enemies, as well as colinearity in density-dependence (see above), further analyses were conducted to attempt to ascertain the causal mechanisms (i.e. whether melanism is a response to, or precaution against, natural enemy attack) driving the incidence of melanism. For samples from Wideford Cairn, the (arcsin square root transformed) proportion of viral DNA to host DNA within each individual was used as the response variable, with the melanism category of that individual as the explanatory variable, in a generalised linear model (GLM) with a quasipoisson error structure. In addition to this, binomial proportion tests with continuity correction were performed, comparing the pooled proportion of all infected individuals in each melanic category with the total number of uninfected individuals within the same category. Again, two models were used to correct for the possibility of subjective biases in the classification of intermediate colour morphs. However, because strict proportions are used in this approach (rather than success/failure binomial ratios), the two models differ slightly from those used under the GLMM approach: Model 1 = (M*X*) / (M*X* + M3); and Model 2 = (M*X* + M3) / (M*X* + M*Y* + M3), where M*X* = either non-melanic or melanic; M*Y* = the opposite category to M*X;* and M3 = intermediate colour morph. For example, to test whether the proportion of individuals of the melanic colour morph (M1) infected with OpbuNPV was significantly greater than the proportion of melanic individuals uninfected by OpbuNPV, the following two binomial proportion tests were conducted: Model 1 = [($M1_{infected}$) / ($M1_{infected}$ + $M3_{infected}$) vs ($M1_{uninfected}$) / ($M1_{uninfected}$ + $M3_{uninfected}$)]; and [($M1_{infected}$ + $M3_{infected}$) / ($M1_{infected}$ + $M2_{infected}$ + $M3_{infected}$) vs ($M1_{uninfected}$) / ($M1_{uninfected}$ + $M2_{uninfected}$ + $M3_{uninfected}$)]; The proportion of M1 vs M2 individuals infected by different natural enemies was also compared using the same methods.

v) Spatial association of natural enemies

Finally, spatial association between all pairs of natural enemies was investigated using partial Mantel tests to search for correlations between the number of individuals infected with each natural enemy over all spatial locations, whilst keeping host density

constant (to control for possible confounding effects of density dependence). The non-parametric approach was chosen due to there being no *a priori* reason for assuming directional causation. The significance of the spatial associations of the correlation matrices were assessed using 10^4 permutations of all spatial locations. The same approach was also used to test for spatial association between *O. brumata* and *A. grossulariata,* to investigate possible interspecific competition effects.

4.4 Results

There was considerable variation in *O. brumata* densities along the entire 250m nested transect length at Linnadale during 2006 (Fig. 4.2), with the vast majority of larvae being found within the first 50m region, with peak densities occurring in the transect at 20-30m. This transect contained the largest quadrat density of *O. brumata* larvae (175) found across all years of sampling, and all sites. In subsequent years, this first region still continued to contain the highest densities of larvae, although at greatly reduced overall densities, with the other two regions increasing in larval densities. Among this first region, this translates into a 59% decrease in larval densities between 2006 and 2008; and a 74% decrease between 2006 and 2009. The overall spatial pattern in larval abundance therefore appears to one of a radial movement away from a peak centre (the first region), with small decreases in overall larval abundance (see also Table 4.1.). Most of those larva infected by any of the natural enemies also appear to be spatially restricted to this first region in 2006, being entirely absent from the region 200-250m. The highest densities of infection among all natural enemies is caused by OpbuNPV, especially where host densities are greatest. In 2008, larvae were infected by both the parasitoid, and all three species of virus screened, in the region 200-250m, indicating a spatial spread of infection following host larval abundance. Again, OpbuNPV appeared to be the greatest infective agent during this year, especially within the first 100m of the nested transect.

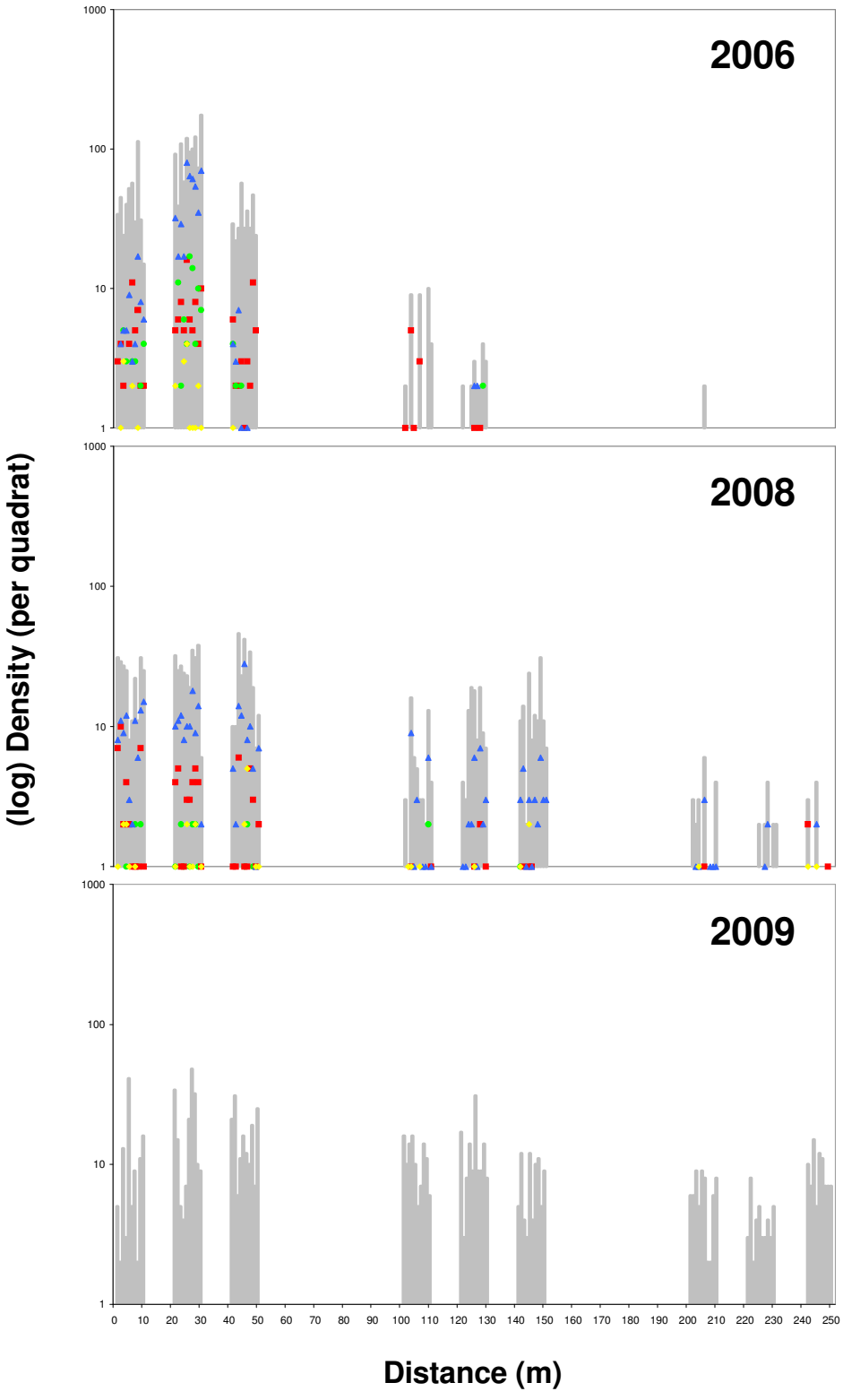


Fig. 4.2 Change in (log) density per quadrat for *O. brumata* (light grey bars) across all spatial locations from Linnadale during 2006, 2008 and 2009. Each bar represents a quadrat, corresponding to the spatial arrangement outlined in Fig. 4.1. ▲ represents number of individuals infected with OpbuNPV; ■ number of individuals from which parasitoids emerged; ● number of individuals infected with CPV; ♦ number of individuals infected with RV. Natural enemy data is not available from 2009.

For *O. brumata* larvae at Wideford Cairn (the non-outbreak site), although densities among quadrats appeared to be quite variable, there were no obvious differences in abundance among regions (Fig. 4.3). The most obvious difference between samples from this site and Linnadale was among the number of infected individuals—in contrast to Linnadale, the prevalence of OpbuNPV infections appear to be much lower, with the majority of infections coming from parasitoids. Again, infection by RV was relatively low, and CPV was entirely absent from all individuals from this site.

Although *A. grossulariata* abundance was much lower than *O. brumata* at Wideford Cairn (Fig. 4.3), it should be noted that the difference in size between the larval stages of these two species is fairly large, making direct comparison of their abundances difficult. What is noticeable however, is the apparent spatial restriction in abundance, being largely confined to the first region. This region also harbours the greatest number of individuals infected with AbgrNPV, with infection being absent beyond 103m from the first quadrat.

i) Plant-insect interactions

Although data on heather height is not available for Linnadale from 2006, models were fitted to *O. brumata* larval density from this year using 2008 and 2009 heather height data, under the assumption of no spatial difference between the pairs of years. Mantel tests found a significant positive correlation between the heather height values from these two years ($r = 0.13$; $p = 0.017$*), suggesting that this is a fairly robust assumption However, GLMMs found different effects when the two years were used as explanatory variables: both models had significantly better fits with a quadratic covariate, suggesting a non-linear relationship between abundance and height, although only height data from 2009 had a significant positive relationship with abundance ($p<0.0001$***) (Table 4.2). Similarly, data from 2008 suggested that *O. brumata* densities are significantly positively associated with increasing heather height when a quadratic covariate is used ($p=0.0004$***). There was no difference between models fitted with or without the quadratic covariate among the 2009 data, so the latter was chosen, again revealing a strong positive association with increasing heather height ($p = 0.0003$***).

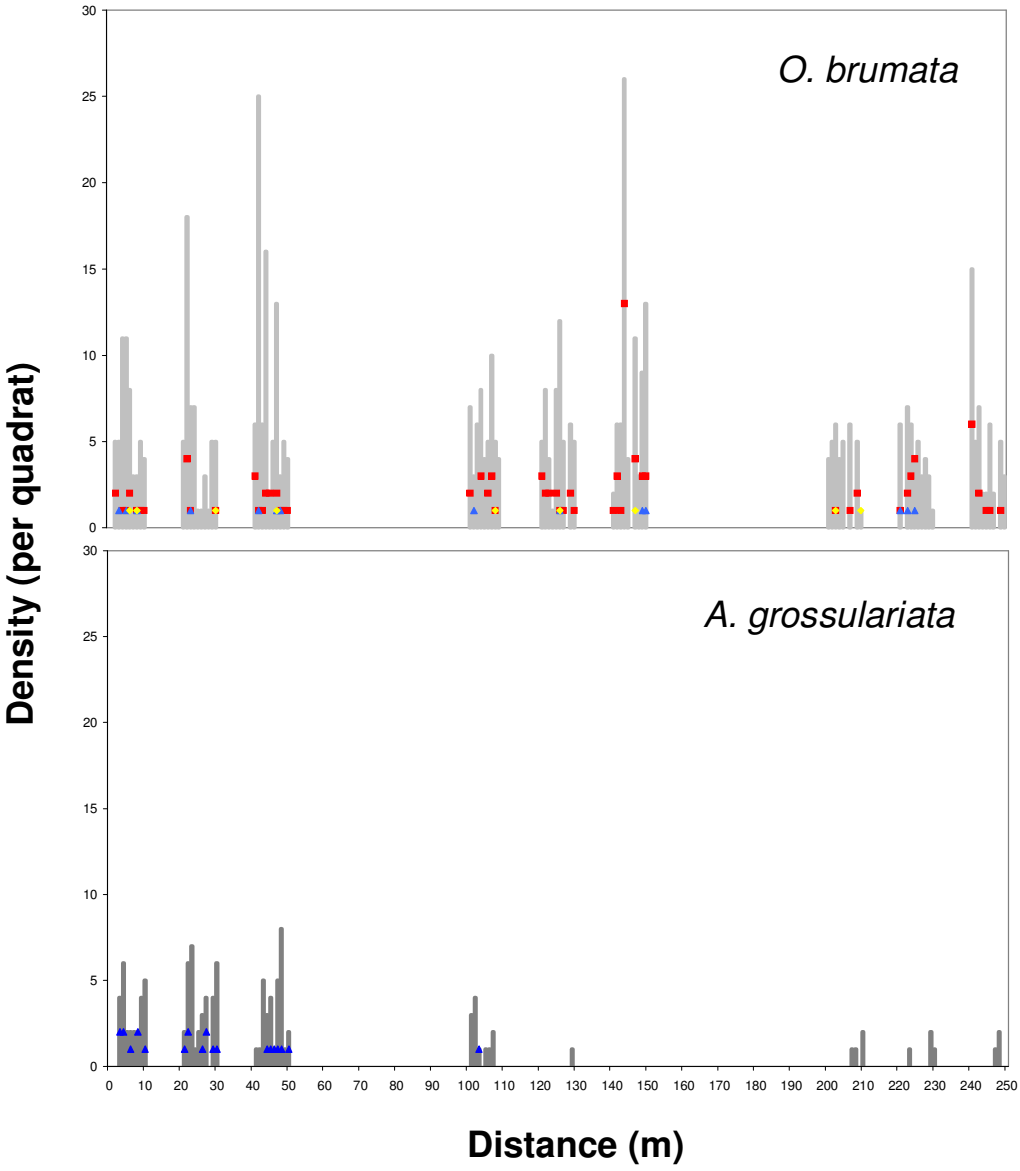


Fig 4.3 Change in density per quadrat for *O. brumata* (light grey bars) and *A. grossulariata* (dark grey bars) across all spatial locations from Wideford Cairn during 2008. Each bar represents a quadrat, corresponding to the spatial arrangement outlined in Fig. 4.1. ▲ represents number of individuals infected with OpbuNPV; ▲ represents number of individuals infected with AbgrNPV; ■ number of individuals from which parasitoids emerged; ♦ number of individuals infected with RV.

This significant relationship did not hold among *O. brumata* larvae from Wideford Cairn, although the overall trend was positive (Table 4.2). However, such a relationship was evident among *A. grossulariata* larvae (p=0.033*), whose relationship with heather height was best described by a linear relationship of increasing abundance with increasing height. All models had the largest error variances at the Transect scale except *O. brumata* from Wideford Cairn, and none of the models had significantly autocorrelated residuals (p>0.05; see Table 4.2).

Year	Site	Species	Random effect	Moran's I (p-value)	Effect size	logLik	Δ logLik	p-value
2006	Linnadale†	*O. brumata*	TransectΨ	-0.019 (0.82)	-0.004	-240.9	3.9	0.13
	Linnadale‡		TransectΨ	-0.019 (0.84)	0.054	-210.1	34.1	<0.0001***
2008	Linnadale		TransectΨ	-0.015 (0.6)	0.133	-168.4	14.1	0.0004***
2009			Transect	-0.019 (0.82)	0.054	-178.2	6.4	0.0003***
2008	Wideford Cairn		Quadrat	-0.015 (0.37)	0.019	-101.4	1.5	0.08
		A. grossulariata	Transect	-0.0195 (0.87)	0.031	-65.06	2.22	0.033*

Table 4.2 Results of GLMMs of host species abundance per quadrat vs heather height. † indicates heather height data taken from 2008; ‡ indicates heather height data taken from 2009; Ψ indicates models with heather height data including a quadratic covariate. Δ logLik is the change in log Likelihood relative to a null model.

ii) Spatial pattern analysis

In general, there was good agreement between the two methods used to describe spatial patterns of hosts and natural enemies at different spatial scales among all years at Linnadale: the majority of variance among total *O. brumata* larvae was partitioned almost equally between quadrat (1m) and region (50m) scale, with the exception of 2009, where the 65% was explained at the quadrat scale; for all three years, less than 1% of variance was partitioned at the transect (10m) scale (Table 4.3). This appears to follow the pattern of spatial restriction with time evident from the spatial correlograms of *O. brumata* density (Fig. 4.4), with zero correlation in 2006 reached at around 62m; 2008 at 54m. In 2009 zero correlation is reached at 97m, though correlation approaches close to zero at around 51m. This is further evidenced by the broadening confidence intervals at the zero correlation point over time, suggesting greater among-quadrat variation. The overall spatial pattern of host density therefore appears to be one of strong spatial restriction in 2006, up to a scale of around 50m; in 2008, more variance is evident at the regional scale, manifest in a secondary peak of positive correlation with an asymptote at around 130m; and in 2009, any spatial pattern is almost completely lost along the whole 250m transect length, with the majority of variation coming from among-transect heterogeneity in abundance.

The spatial pattern of parasitoid attack among the two years remained fairly consistent, with the majority of variance being attributable to the region scale, although in 2008 this was only marginally greater than variation at the quadrat scale. These findings are supported by the spatial correlograms, which show an almost identical

Year	Species	Natural enemy	Scale	% variance	logLik
2006	*O. brumata*		Region	44.9	-386.5
			Transect within Region	0.4	-1011
			Quadrat within Transect within Region	54.7	-334.6
		Parasitoid	Region	97.6	-41.86
			Transect within Region	2.4	-41.57
			Quadrat within Transect within Region	0	-42.13
		OpbuNPV	Region	38.9	-237.1
			Transect within Region	56.8	-94.42
			Quadrat within Transect within Region	4.3	-220.5
		CPV	Region	0	-77.87
			Transect within Region	11.1	-73.98
			Quadrat within Transect within Region	88.9	-67.05
		RV	Region	0	-19.17
			Transect within Region	0	-19.17
			Quadrat within Transect within Region	100	-19.17
2008	*O. brumata*		Region	50.9	-167.8
			Transect within Region	0.03	-426.8
			Quadrat within Transect within Region	48.9	-178.1
		Parasitoid	Region	54.6	-41.5
			Transect within Region	0.7	-49.2
			Quadrat within Transect within Region	44.6	-45.05
		OpbuNPV	Region	22	-52.77
			Transect within Region	0.002	-58.33
			Quadrat within Transect within Region	78	-52.48
		CPV	Region	27.5	-24.4
			Transect within Region	0	-24.84
			Quadrat within Transect within Region	72.5	-24.71
		RV	Region	0	-38.15
			Transect within Region	0	-38.15
			Quadrat within Transect within Region	100	-37.84
2009	*O. brumata*		Region	34.7	-198.9
			Transect within Region	0.05	-249.1
			Quadrat within Transect within Region	65.3	-191.6

Table 4.3 Hierarchical variance components analysis of *O. brumata* host density and density of infected individuals, at three different spatial scales from Linnadale in 2006, 2008 and host density only from 2009. Variance components were extracted from the random factors of null GLMMs specified at the different spatial scales. Log Likelihood values represent model fits, with values closer to zero indicating better fits, and are comparable only within hosts or natural enemies.

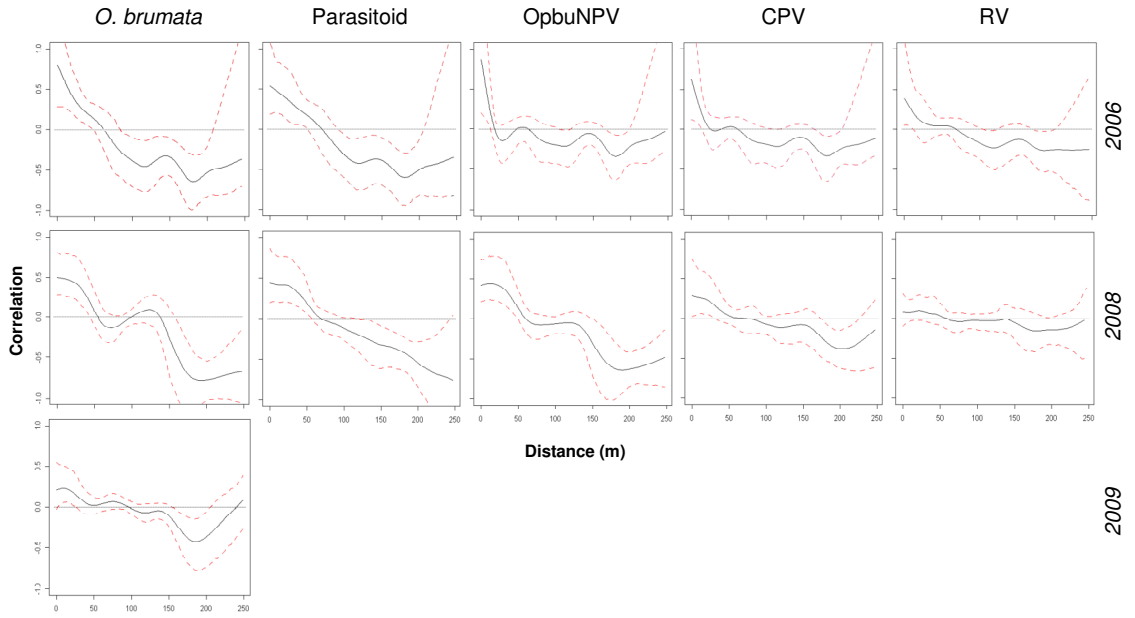


Fig. 4.4 Spatial correlograms of *O. brumata* densities and number of infected individuals across all distances of the 250m linear transect, and all three sampling years from Linnadale. Solid lines represent the spline smoothed mean correlation coefficients; dashed red lines represent 95% confidence intervals; the dotted line represents the point of zero correlation. The point at which the solid line crosses the zero correlation value can be inferred to be the scale (or distance) over which that host or natural enemy is operating.

spatial pattern among the two years, despite changes in the spatial pattern of host abundance, with the point of zero correlation being reached at 70m. The slight increase in the confidence limits during 2008, is possibly attributable to an increase in among-transect variation, in line with that found from the variance components. In contrast, infection of larvae by OpbuNPV displayed marked changes in spatial pattern among the two years, with the majority of variation in 2006 partitioned at the transect scale, whereas in 2008, less than 1% is attributable to this scale, having been transferred mostly to the among-quadrat scale. Although this could be attributable to a pattern of spatial restriction, the spatial correlograms suggest that it is in fact due to an increase in the scale of spatial response, from 18m in 2006 to over 60m in 2008; the increase in among-quadrat variation again attributable to heterogeneity of infection at such small scales.

The response of CPV infections to scale appears to follow a similar pattern to that of OpbuNPV infection, being spatially restricted to the transect scale in 2006, where zero correlation is reached at around 26m, and subsequently broadening out to around 63m in 2008, where around 28% of variation is explained at the region scale. Although these two responses appear similar, it is worth noting that the strength of

response (as measured by the strength of the correlation coefficient, and the confidence limits around it), appear to be much stronger among OpbuNPV than CPV infections. For RV, very little spatial pattern was evident at any scale, although this was consistent among the two years: all variation can be attributable to among-quadrat variation, and although the point of zero correlation was similar among years (2006 = 69m; 2008 = 58m), the large variation about the confidence limits and the flattened correlograms suggest very little, if any, response to space in infection by this virus.

These spatial patterns appeared to very different among *O. brumata* from Wideford Cairn, with no obvious pattern evident from the spatial correlograms (Fig. 4.4). The majority of variation (>95%) was explained among quadrats, indicating high levels of local variation, but low levels of region-scale variation (Table 4.4). Although there was no obvious spatial pattern evident in the correlogram among hosts infected by parasitoids across the 250m transect, in contrast to host spatial pattern, 24% of variation was partitioned at the region scale, suggesting a similar larger-scale spatial pattern of infection to that seen among parasitoids from Linnadale. Although the majority of variation was not partitioned exclusively at this scale, the region-scale model had a marginally better model fit compared to the quadrat-scale, although well within one log likelihood unit. Again, OpbuNPV infections showed no obvious pattern from the spatial correlograms, with the 95% confidence limits remaining not moving above or below the line of zero correlation along the entire length of the 250m study distance. Variance components for this hosts infected with this virus were similar to hosts themselves, being heavily partitioned at the quadrat scale, with very little difference (<1%) among transects or regions. Although all of the variance among hosts infected with RV was partitioned at the transect scale, there was no difference between model fits at any of these scales, indicating that overall levels of variation were small for this virus. Correlograms were also similar to the other natural enemies at this site, and RV from Linnadale, suggesting little or no response to space.

The spatial patterns of *A. grossulariata* hosts and those infected with AbgrNPV were very similar to *O. brumata*. Both had the majority of variation and best model fits partitioned at the region scale, although there appeared to be more among quadrat variation in host densities, than among infected individuals. Spatial correlograms of the distribution of both host and natural enemy were extremely similar, although the point of zero correlation was marginally greater among hosts than virus-infected host: 87m and 76m, respectively. Both patterns reached an asymptote of positive correlation at

Species	Natural enemy	Scale	% variance	logLik
O. brumata		Region	2.3	-109
		Transect within Region	2.3	-108.2
		Quadrat within Transect within Region	95.4	-89.4
	Parasitoid	Region	24.2	-56.64
		Transect within Region	0.2	-58.56
		Quadrat within Transect within Region	75.6	-56.87
	OpbuNPV	Region	0	-23.78
		Transect within Region	<0.001	-23.78
		Quadrat within Transect within Region	99.9	-23.78
	RV	Region	0	-22.14
		Transect within Region	100	-22.14
		Quadrat within Transect within Region	0	-22.14
A. grossulariata		Region	76.3	-22.07
		Transect within Region	0	-25.19
		Quadrat within Transect within Region	23.7	-24.51
	AbgrNPV	Region	99.9	-21.33
		Transect within Region	<0.001	-21.61
		Quadrat within Transect within Region	<0.001	-21.61

Table 4.4 Hierarchical variance components analysis of *O. brumata* and *A. grossulariata* host density, and density of infected individuals, at three different spatial scales from Wideford Cairn. Variance components were extracted from the random factors of null GLMMs specified at the different spatial scales. Log Likelihood values represent model fits, with values closer to zero indicating better fits, and are comparable only within hosts or natural enemies.

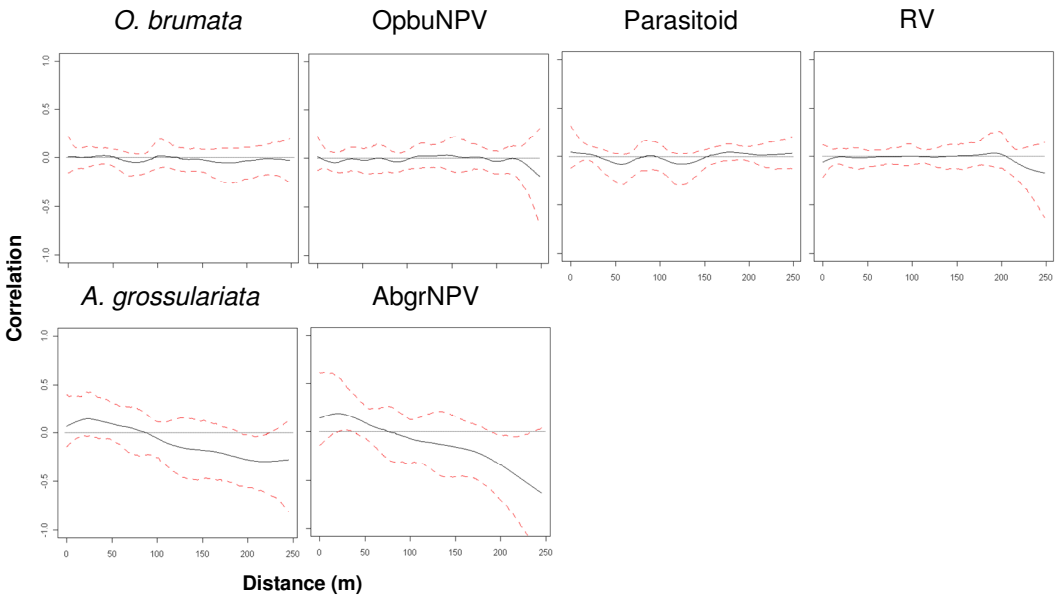


Fig 4.5 Spatial correlograms of *O. brumata* and *A. grossulariata* densities, and number of infected individuals across all distances of the 250m linear transect at Wideford Cairn. Solid lines represent the spline smoothed mean correlation coefficients; dashed red lines represent 95% confidence intervals; the dotted line represents the point of zero correlation.

iii) Scale-dependent density dependence

Again, there was broad agreement between the two different methods used to investigate natural enemy responses to host density: *O. brumata* larvae infected with parasitoids during 2006 displayed significant, although weak, negative responses to increasing host density across quadrats, regions, and transects, when GLMMs were fitted using host densities per quadrat (Table 4.5). Despite all models displaying equal model fits and effect sizes, models grouped at the region scale gave better fits relative to their null models, suggesting that parasitoid responses in this year tend toward larger scales. This inference is further supported by the models fitted with relative densities inferred from G statistics (Fig. 4.6), where model fits are virtually indistinguishable at all scales between 1m and 74m, after which the model fits become close to null. All of the models fitted within these scales showed a negative response to increasing host density, with the largest Δ log likelihood value occurring at the 4m scale ($p = 0.018$*). However, it should be noted that, there was no substantial difference between this model and any other model up to the 74m, and that despite all of these models being at $p<0.05$, none were significant after Bonferroni correction.

In contrast, *O. brumata* larvae infected with OpbuNPV displayed strong positive responses to increasing host density, although only at specific spatial scales. Models that specify both quadrat and region grouping levels were highly significant for positive density dependence in infection by this virus ($p<0.0001$***), whilst at the transect scale, no such relationship was found, with the overall effect being weakly negative. Of the two significant models, quadrat scale had the lowest log likelihood value, although the region scale model gave the largest change relative to the null model. This result is surprising, given the responses to scale evident from the G-statistic models, whereby all models between 1m and 19m were highly positively significant after Bonferroni correction, with the best fitting model being at the 13m scale (Fig. 4.6). There is evidently a strong response of OpbuNPV to host density at all scales below 20m during this year, and whilst the rest of the models at larger scales of significant at the $p<0.05$ level, the marked difference in log likelihood values between these two regions makes this response clear. Among the significant models within this peak region, there is a noticeable trough between scales 7m-9m whereby log likelihood values drop, before reaching an asymptote. Although these models are still highly positively significant, this

Year	Natural enemy	Scale	Moran's I (p-value)	Effect size	logLik	Δ logLik	p-value
2006	Parasitoid	Region	-0.021 (0.64)	-0.006	-37.26	4.6	0.002**
		Transect	-0.021 (0.66)	-0.006	-37.26	3.24	0.002**
		Quadrat	-0.021 (0.64)	-0.006	-37.26	0.47	0.002**
	OpbuNPV	Region	-0.02 (0.59)	0.013	-176.8	60.3	<0.0001***
		Transect	-0.02 (0.68)	-0.0008	-90.45	0.14	0.6
		Quadrat	-0.01 (0.26)	0.023	-60.81	7.54	<0.0001***
	CPV	Region	-0.02 (0.51)	-0.004	-76.43	1.44	0.09
		Transect	-0.02 (0.62)	-0.019	-58.36	16.17	<0.0001***
		Quadrat	-0.02 (0.55)	0.002	-44.77	0.04	0.78
	RV	Region	-0.02 (0.46)	-0.0008	-19.15	0.02	0.85
		Transect	-0.02 (0.45)	-0.0008	-19.15	0.02	0.85
		Quadrat	-0.02 (0.56)	0.0015	-18.41	0.02	0.85
2008	Parasitoid	Region	-0.016 (0.60)	0.0004	-41.5	0	0.97
		Transect	-0.014 (0.49)	0.012	-41.96	0	0.31
		Quadrat	0.008 (0.05)	0.027	-43.34	2.3	0.038*
	OpbuNPV	Region	-0.008 (0.25)	0.003	-52.77	0	0.62
		Transect	-0.008 (0.24)	0.008	-53.52	0	0.21
		Quadrat	0.006 (0.06)	0.016	-50.68	2.35	0.029*
	CPV	Region	-0.001 (0.13)	0.017	-24.4	0	0.35
		Transect	-0.003 (0.18)	0.017	-24.37	0	0.39
		Quadrat	-0.001 (0.12)	0.02	-24.33	0.5	0.33
	RV	Region	-0.02 (0.93)	-0.017	-38.15	0	0.28
		Transect	-0.02 (0.93)	-0.017	-38.15	0	0.28
		Quadrat	-0.02 (0.95)	-0.017	-37.72	0.43	0.37
2008 delayed	Parasitoid	Region	-0.016 (0.6)	0.0009	-41.47	0.03	0.78
		Transect	-0.015 (0.52)	0.005	-41.91	0.05	0.23
		Quadrat	0.0049 (0.056)	0.009	-42.25	3.39	0.009**
	OpbuNPV	Region	-0.009 (0.25)	0.0008	-52.8	0	0.68
		Transect	-0.0082 (0.25)	0.003	-53.5	0.02	0.154
		Quadrat	0.006 (0.051)	0.005	-50.38	2.65	0.02*
	CPV	Region	-0.012 (0.36)	0.013	-21.04	3.36	0.004**
		Transect	-0.012 (0.37)	0.013	-21.04	3.33	0.004**
		Quadrat	-0.012 (0.34)	0.013	-21.04	3.79	0.004**
	RV	Region	-0.023 (0.93)	0.0041	-37.72	0	0.344
		Transect	-0.023 (0.94)	0.0041	-37.72	0	0.344
		Quadrat	-0.023 (0.89)	0.0041	-36.18	0.4	0.383

Table 4.5 Results of binomial GLMMs of the ratio of infected to uninfected individuals vs host density among *O. brumata* larvae from Linnadale in 2006 and 2008; "delayed" indicates that density from 2006 was used as the explanatory variable to look for evidence of a delayed effect of density on infection. Scale represents the grouping level of the random effect; Moran's I refers to the test for autocorrelation of model residuals. Models with no autocorrelation have values of I close to zero, with the null hypothesis of complete spatial randomness ($p>0.05$) tested against random permutations. Δ logLik refers to the change in log likelihood values relative to the null model at that scale; significance levels of models are represented as $p<0.05$*; $p<0.01$**; $p<0.001$***.

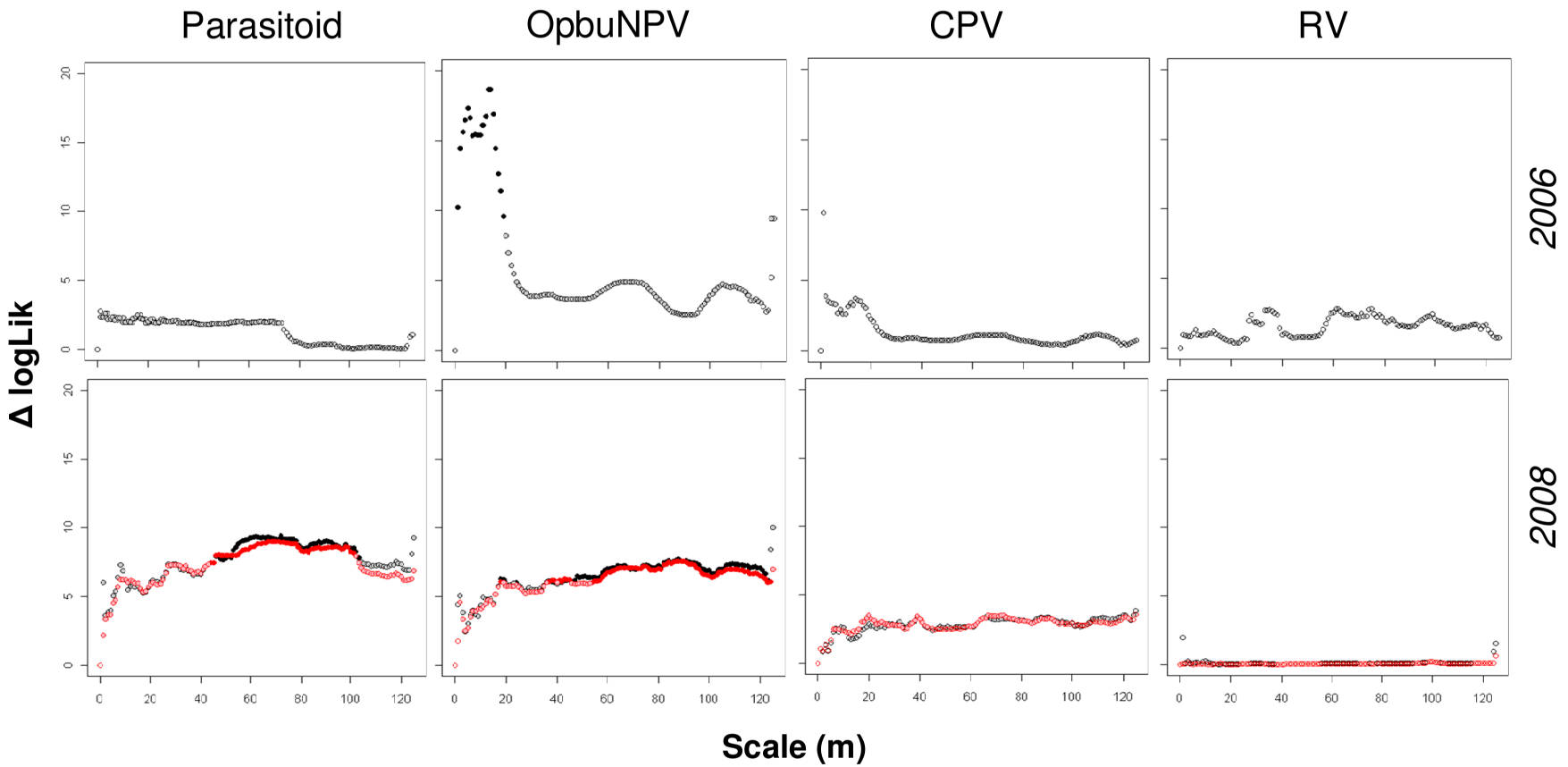


Fig 4.6. Change in log likelihood values with scale among binomial GLMMs of the ratio of infected to uninfected *O. brumata* individuals vs relative density (calculated as z-values of local G statistics at different neighbourhood sizes) from Linnadale in 2006 and 2008. Larger values of Δ logLik represent better model fits; Black points represent models fitted with host density explanatory variables from the same year as natural enemies; Red points on models from 2008 represent density from 2006 as the explanatory variable, to look for delayed effects. Solid circles represent significant models after Bonferroni correction ($p<0.0004$); light shaded circles represent models where $p<0.05$.

does suggest something happening at around the 10m scale that could explain the lack of significance when GLMMs are grouped at the transect scale.

This also appeared to be the case among *O. brumata* larvae infected with CPV, albeit with the opposite effect: a significant negative response to increasing host density was found at the transect scale (Table 4.5; $p<0.0001$***), with no effect at either quadrat or region grouping levels. Although at this scale, the overall model fit was lower than at the quadrat scale, compared to a null model at this scale, there was a substantially better fit. Despite the differences in scale-specific density dependence between CPV and OpbuNPV, and in the sign of the response, the GLMMs from G-statistics appeared to give a very similar pattern across all scales, albeit with a much reduced effect size. Similarly to the OpbuNPV response, the asymptotic response among CPV infection was found at the 14m scale ($p=0.014$*), with all models between 2m-18m having negative responses to density at the $p<0.05$ level. However, none of these models had p-values below the Bonferroni correction. Again, there was a trough in Δ log likelihood values between 7m-10m. All GLMM models of *O. brumata* larvae infected with RV displayed no response to host density, with all models have identical log likelihood values and identical Δ log likelihood values at all three grouping levels. For models constructed from G-statistics, there was no clear pattern of response to scale, and all models were non-significant ($p>0.05$). All models constructed across all scales and all natural enemies within this year had spatially uncorrelated residuals ($p>0.05$).

There did not appear to be much consistency in the sign or scale of response of any of the natural enemies to density between 2006 and 2008. Among larvae infected with parasitoids, although there was a significant response to host density ($p=0.038$*; Table 4.5), the response appeared to have changed between the two years, with 2008 showing a positive increase in parasitoid infections with increasing host density, but only at the quadrat scale. Parasitoid infections also showed a stronger positive response to delayed density dependence ($p=0.009$*), with a better model fit than the current years density. Although neither of these two significant models were significantly spatially autocorrelated, both had positive Moran's I values, which were only marginally non-significant at the $p>0.05$ level. Despite all parasitoid infection models constructed using G-statistic responses being significant at $p<0.05$, there was no support for the small-scale density dependence from the GLMM models described above. Although positively significant models were found after Bonferroni correction, these were all

grouped at much larger scales (between 44m-103m), with an asymptotic Δ log likelihood value at 72m ($p<0.0001$*). An almost identical pattern of positive density dependence was found at the same scales, although the asymptotic Δ log likelihood value was found at a slightly smaller scale of 67m. There was little evidence from these models that a delayed effect of density had greater explanatory power than the current year's density.

An almost identical pattern of scale-dependent infection to that of parasitoids was also seen among larvae infected with OpbuNPV during 2008. Significant positive quadrat-scale density dependence was apparent as both a contemporaneous ($p=0.029$*) and delayed ($p=0.02$*) effect, with the delayed-effect model giving a marginally better model fit. Again, no effect was seen at other grouping levels, and again, residuals showed marginally non-significant positive spatial autocorrelation. The pattern of scale-dependent positive density dependence was also similar to that among parasitoids, with all scales significant at $p<0.05$ level, and the majority of significant models after Bonferroni correction being at larger scales (36m -123m). However, the spatial spread of significant density-dependent infections appears to be greater than that among parasitoids, with significant models also occurring at 18m, among both current and delayed-effects models. There was no obvious difference between either of these two sets of models, with the exception of eight non-significant models between 46m-53m scales among the delayed-effects models.

No significant effect of current years density was found on the incidence of CPV infection at any of the grouped-level scales, although strong negative delayed density dependence ($p=0.004$**) was seen among models at all scales, with the quadrat scale giving a marginally better fit than other scales, compared to null models (Table 4.5). There was no evidence of spatial autocorrelation among any of these models. Despite this, no obvious pattern backing up these findings was evident from the G-statistic models (Fig. 4.6), although the highest Δ log likelihood value among all models at all scales was found among the delayed-effects model at 20m ($p=0.023$*), with most these models at other scales giving marginally better fits than those using current years density as the response. None of these models were significant after Bonferroni correction. None of the models of RV infection among both GLMM methods and all scales showed any spatial response to host density in either current year, or delayed-effect models. None of the models showed evidence of spatially autocorrelated residuals.

In contrast to parasitoid infections at Linnadale, there was no evidence of density dependent parasitism among *O. brumata* larvae from Wideford Cairn in 2008 (Table 4.6), under the standard GLMM method at either the quadrat, transect, or region scale. However, models using z-scores calculated from G-statistics revealed a highly fluctuating response to scale among infections from this natural enemy. The majority of models responding positively to increasing host density at the $p<0.05$ level occurred between 23m-101m, with a peak Δ log likelihood value at 51m ($p=0.0012$; Fig. 4.7). Despite this, none of these models satisfied the criteria for significance after Bonferroni correction.

Again, unlike results from Linnadale, none of the GLMM models of OpbuNPV infection under either the host density, or relative host density from G-statistics, displayed evidence of any response to *O. brumata* host density, at any scale. Models at the quadrat, transect, and region scale all had identical model fits (Table 4.6), and although there was a peak response to scale at 59m under the G-statistics models (Fig 4.7), this was not significant ($p=0.051$). Similarly, among larvae infected with RV, there was no response to spatial scale under either of the two GLMM methods, with Δ log likelihood values not increasing beyond a single unit under any of the models at any scale.

Species	Natural enemy	Scale	Moran's I (p-value)	Effect size	logLik	Δ logLik	p-value
O. brumata	Parasitoid	Region	-0.018 (0.73)	0.025	-55.47	1.17	0.12
		Transect	-0.019 (0.75)	0.019	-56.38	0.62	0.26
		Quadrat	-0.021 (0.87)	0.022	-55	0.47	0.33
	OpbuNPV	Region	-0.012 (0.37)	-0.022	-23.65	0.13	0.62
		Transect	-0.012 (0.34)	-0.022	-23.65	0.13	0.62
		Quadrat	-0.012 (0.35)	-0.022	-23.65	0.13	0.62
	RV	Region	-0.019 (0.76)	-0.08	-21.41	0.73	0.29
		Transect	-0.019 (0.77)	-0.08	-21.41	0.73	0.29
		Quadrat	-0.019 (0.74)	-0.08	-21.43	0.49	0.45
A. grossulariata	AbgrNPV	Region	0.002 (0.12)	0.012	-21.3	0.03	0.8
		Transect	0.05 (0.02*)	0.027	-21.45	0.16	0.57
		Quadrat	0.05 (0.02*)	0.027	-21.45	0.16	0.57

Table 4.6 Results of binomial GLMMs of the ratio of infected to uninfected individuals vs host density among *O. brumata* and *A. grossulariata* larvae from Wideford Cairn in 2008; Scale represents the grouping level of the random effect; Moran's I refers to the test for autocorrelation of model residuals. Models with no autocorrelation have values of I close to zero, with the null hypothesis of complete spatial randomness ($p>0.05$) tested against random permutations. Δ logLik refers to the change in log likelihood values relative to the null model at that scale; significance levels of models are represented as $p<0.05$*; $p<0.01$**; $p<0.001$***.

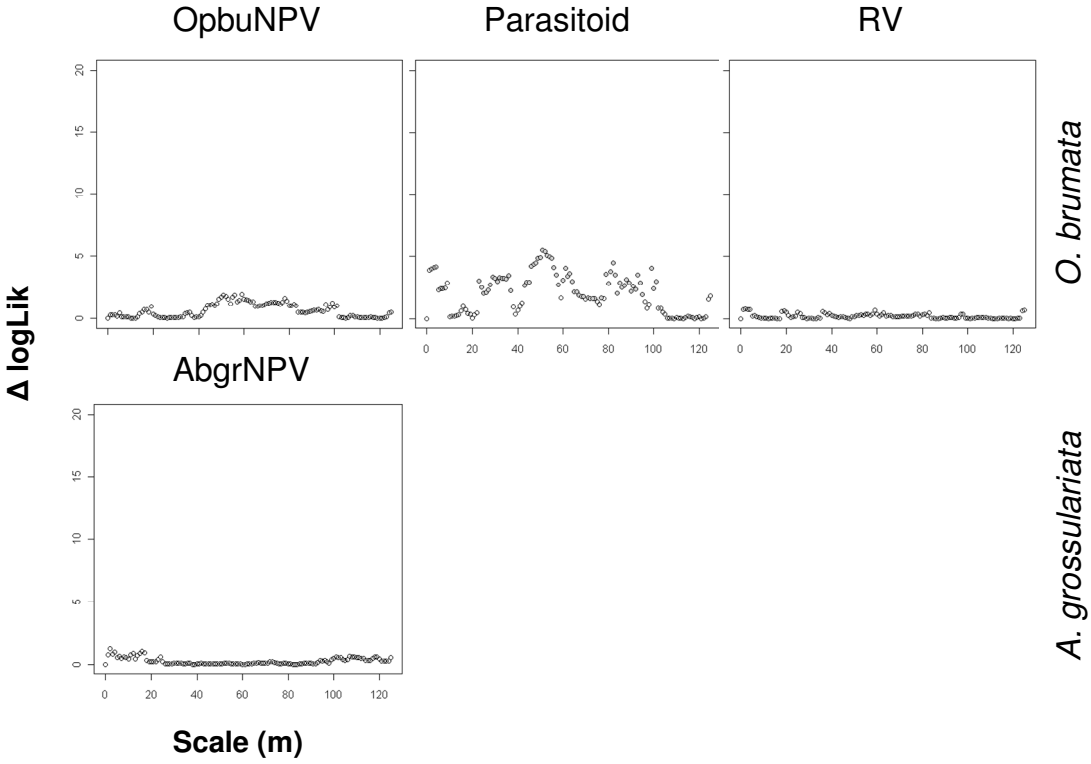


Fig 4.7 Change in log likelihood values with scale among binomial GLMMs of the ratio of infected to uninfected *O. brumata* and *A. grossulariata* individuals vs relative density (calculated as z-values of local G statistics at different neighbourhood sizes) from Wideford Cairn in 2008. Larger values of Δ logLik represent better model fits; Solid circles represent significant models after Bonferroni correction ($p<0.0004$); light shaded circles represent models where $p<0.05$.

This was also the case for *A. grossulariata* larvae infected with AbgrNPV—none of the models at the quadrat, transect, or region scale showed any response to host density, although among the two smallest scales, there was evidence of positive spatial autocorrelation among model residuals ($p<0.05$; Table 4.6), making inference at these scales unreliable. However, GLMM models of relative density from G-statistics appeared to confirm the lack of response to host density at any scale, with all models being non-significant at the $p<0.05$ level; and models at only two different scales having Δ log likelihood values greater than a single unit (2m and 16m; Fig. 4.7).

iv) Density-dependence and melanism

There was strong evidence of a density-dependent effect of an increasing degree of larval melanism with increasing density of *O. brumata* larvae within quadrats, under both models from Linnadale in 2008 (Table 4.7). The same models fitted using number

of larvae infected with parasitoids, also gave support for a density-dependent effect of increasing parasitoid density with increasing larval melanism. Both pairs of models

Site	Explanatory variable	Model	Moran's I (p-value)	Effect size	logLik	Δ logLik	p-value
Linnadale	**Host density**	1	-0.012 (0.39)	-0.036	-65.16	6.08	<0.0001***
		2	-0.004 (0.15)	-0.014	-21.13	2.57	0.0078**
	Parasitoid	1	-0.007 (0.2)	-0.22	-63.04	8.2	<0.0001***
		2	-0.005 (0.17)	-0.06	-20.99	2.71	0.013*
	OpbuNPV	1	-0.03	-0.03	-70.16	1.08	0.11
		2	-0.015 (0.57)	-0.01	-23.32	0.38	0.34
	CPV	1	-0.012 (0.34)	-0.11	-71	0.24	0.46
		2	-0.018 (0.7)	-0.06	-23.37	0.33	0.4
	RV	1	-0.013 (0.44)	0.18	-69.18	2.06	0.034*
		2	-0.018 (0.73)	0.045	-23.33	0.37	0.38
Wideford	**Host density**	1	-0.02 (0.88)	0.009	-50.98	0.09	0.67
Cairn		2	-0.02 (0.72)	0.012	-23.72	0.57	0.28
	Parasitoid	1	-0.02 (0.90)	0.009	-51.06	0.01	0.86
		2	-0.016 (0.60)	0.029	-23.59	0.7	0.24
	OpbuNPV	1	-0.02 (0.89)	0.06	-51.05	0.02	0.86
		2	-0.018 (0.70)	-0.072	-24.2	0.09	0.67
	RV	1	-0.02 (0.88)	0.39	-50.56	0.51	0.3
		2	-0.018 (0.67)	0.22	-24.29	0	0.92
	%NPV DNA	M1†	-	-2.53	-	-	0.16
		M2†	-	-3.54	-	-	0.57
		M3†	-	0.67	-	-	0.69

Table 4.7 Results of binomial GLMM models of degree of cuticular melanism vs density among *O. brumata* larvae at Linnadale and Wideford Cairn in 2008. Explanatory variables refer to either the total number of larvae, or the total number of larvae infected with each natural enemy, per quadrat. Model 1 refers to a response variable of the weighted ratio of M1 : M2 + M3 larvae per quadrat; Model 2 refers to a response variable of the weighted ratio of M1 + M3 : M1 + M2 + M3 larvae per quadrat (where M1 = non-melanic; M2 = melanic ; and M3 = intermediate degrees of melanism); Positive effect sizes therefore refer to an increase in non-melanic individuals with increasing density of hosts or natural enemies; Moran's I refers to the test for autocorrelation of model residuals. Δ logLik refers to the change in log likelihood values relative to the null model; significance levels of models are represented as $p<0.05$*; $p<0.01$**; $p<0.001$***.

displayed no sign of spatial autocorrelation of model residuals, although models fitted using parasitoid density as the explanatory variable had better model fits and larger effect sizes compared to those using total host density, especially under Model 1, with almost two log likelihood units difference between the two models. Neither OpbuNPV, nor CPV displayed evidence of any significant association of infection with degree of melanism, although the overall trend among both natural enemies was one of a positive increase in melanism with density of infected individuals. In contrast, larvae infected with RV had evidence of a decreasing degree of melanism with increasing density of

infected individuals, although this was only evident in one of the fitted models (Model 1; p = 0.034*).

O. brumata larvae from Wideford Cairn displayed no evidence of either host density, or natural enemy density-dependent effects on the proportion of individuals with melanised or non-melanised cuticles (Table 4.7). The trend among all models was one of increasing melanisation with increasing density, although this trend was not statistically significant. There was also no support for a relationship between the proportion of viral DNA found within individuals, and their degree of melanism, under any of the three subjective categories used. Despite this, there was evidence that a significantly greater proportion of individuals from which parasitoids emerged had melanised cuticles, compared to non-melanised cuticles, under both Model 1 (p=0.0002***) and Model 2 (p<0.0001***) when the total number of larvae infected by parasitoids was pooled (Table 4.8). No similar effect was found for either individuals infected with OpbuNPV or RV, despite similar proportions of melanic to non-melanic individuals being found within these two groups. Significant differences were found between both the proportion of melanic and non-melanic individuals when compared to the total proportion of uninfected individuals. Both melanic and non-melanic individuals showed the same significant trends, and changes in relative proportions, when compared to uninfected larvae. However, the differences in overall proportions between the two groups when comparing Model 1 and Model 2 fits suggests that differences in the number of larvae in the intermediate category may be confounding the results under these models, making firm conclusions difficult.

		M1 vs Uninfected	p-value	M2 vs Uninfected	p-value	M1 vs M2	p-value
Parasitoid	*Model 1*	0.10 0.32	<0.0001***	0.18 0.33	0..0002***	0.4 0.74	0.0002***
	Model 2	0.32 0.25	0.05*	0.33 0.25	0.024*	0.74 0.99	<0.0001***
NPV	*Model 1*	0.02 0.05	0.15	0.03 0.05	0.27	0.43 0.75	0.31
	Model 2	0.05 0.04	0.61	0.05 0.04	0.55	0.75 0.99	0.07
RV	*Model 1*	0.01 0.03	0.19	0.01 0.03	0.3	0.33 0.78	0.48
	Model 2	0.03 0.02	0.63	0.03 0.02	0.64	0.78 0.99	0.48

Table 4.8 Results of binomial proportion tests for differences in the total proportion of *O. brumata* larvae infected with different natural enemies vs total proportion of uninfected individuals, among all larvae under each category of cuticular melanism, and against each other. Model 1 = (M*X*) / (M*X* + M3); and Model 2 = (M*X* + M3) / (M*X* + M*Y* + M3), where M*X* = either total non-melanic or melanic larvae; and M*Y* = the opposite category to M*X*. Significance levels of models are represented as p<0.05*; p<0.01**; p<0.001***.

v) Spatial association between natural enemies

Among all natural enemies from Linnadale in 2006, all pairs of the three virus species (OpbuNPV, CPV, and RV) were significantly positively spatially associated (Table 4.9), with the association between OpbuNPV and RV being the strongest; and CPV and RV the weakest, being only marginally significant at the $p<0.05$ level. Only RV had any significant spatial association with parasitoid infection during this year, being strongly positively correlated (p=0.003**). Of these same associations, only those between OpbuNPV and CPV, and CPV and RV were still significantly positively associated at the same site in 2008. In contrast, during this year, there was a significant negative correlation between OpbuNPV and parasitoid infections (p=0.03*), but no significant association with RV between either of the two. This negative association between OpbuNPV and parasitoids was also evident from Wideford Cairn during the same year (p=0.003**), although there was no evidence of any other significant correlation between pairs of other natural enemies. There was also no evidence for a spatial correlation between densities of *O. brumata* and *A. grossulariata* at this site, although the overall trend was positive (Mantel r = 0.046; p=0.22).

			Mantel r (p-value)			
Site	**Year**		**Parasitoid**	**OpbuNPV**	**CPV**	**RV**
Linnadale	2006	Parasitoid	-			
		OpbuNPV	0.006 (0.39)	-		
		CPV	-0.05 (0.67)	0.54 (0.0002***)	-	
		RV	0.33 (0.003**)	0.31 (0.006**)	0.2 (0.05*)	-
	2008	Parasitoid	-			
		OpbuNPV	-0.1 (0.03*)	-		
		CPV	0.07 (0.16)	0.22 (0.01**)	-	
		RV	0.01 (0.33)	0.05 (0.19)	0.31 (0.001**)	-
Wideford Cairn		Parasitoid	-			
		OpbuNPV	-0.11 (0.003**)	-		
		RV	-0.05 (0.76)	0.07 (0.25)	-	-

Table 4.9 Results of partial Mantel tests for spatial association between pairs of natural enemies infecting *O. brumata* at all spatial locations along the nested linear transect at Linnadale and Wideford Cairn, whilst holding host density constant. *Mantel r* represents the correlation coefficient between the two natural enemies; p-values are calculated against a null hypothesis of complete spatial randomness after 10^4 permutations of the spatial matrices of each natural enemy across all spatial locations. Significance levels are represented as p<0.05*; p<0.01**; p<0.001***.

4.5 Discussion

Although a strong relationship was found between increasing *O. brumata* abundance and increasing heather height among all years at Linnadale, it is unclear whether resource-dependent traits are solely driving these patterns of abundance. Even with the caveat that proxy measures of height were used for the abundance-height models from 2006, it should be noted that the significant quadratic relationship between height and abundance only occurred during 2006 and 2008, when strong spatial patterning of host abundance was most evident. Given the fact that variance in host density was greater than variance in heather height during these years, it seems likely that it is the strong localised patterns of abundance, rather than heather height *per se*, driving these patterns. Strong positive relationships between Lepidoptera density and heather height have previously been found (Haysom & Coulson, 1998), although over a much greater range of heather heights and ages. Heather typically degenerates at heights beyond 40cm (Gimingham, 1985), and it seems likely that, given the fact that within site heather stands are likely to be the same age, the quadratic relationships found here are unlikely to be due to degeneracy. Despite this, even when spatial patterns of host were more evenly distributed (e.g. Linnadale 2009), a significant linear relationship was still evident. Although the same could not be said of *O. brumata* from Wideford Cairn, the overall positive relationship at this site suggests that localised changes in height could be influencing small-scale variability in host abundance. The fact that *A. grossulariata* also displayed a strong positive relationship also suggests that competition for the shared resource could be disrupting the *O. brumata* – heather relationship at this site. Clearly, further studies among a range of heather stands are needed to verify these conclusions.

Total natural enemy prevalence between the two years at Linnadale showed remarkable congruence, despite a marked change in total host density. Although the distribution of instar stages during these two years was slightly different, with the majority being L5 in 2006 and L4 in 2008, this congruence suggests that comparison between the two years is unlikely to yield biased conclusions. If anything, estimates of total host density are likely to be lower among *O. brumata* from 2006, due to increased losses from predation, and an unknown number pupating, at the point of sampling. Despite this, overall per-quadrat densities during this year were among the highest ever recorded for *O. brumata* feeding on heather, and are comparable to reports of outbreak

densities by Picozzi (1981) in Orkney, and Kerslake *et al.* (1996) from mainland Scotland. Although these reports suggest a large spatial extent to the feeding damage caused by these outbreaks (1-20+hectares), larval densities were found to be highly spatially restricted within sites, although comparison is problematic due to the smaller scales sampled within the 250m transect. At the time of sampling, no such large-scale extent of feeding damage was evident (pers. obs.).

What is evident from these temporal changes in *O. brumata* host density within Linnadale is the overall decline in abundance over time within the same sampling area, coupled with a decrease in the localised spatial pattern as larvae spread out into previously uncolonised areas. The result is perhaps surprising, given the flightless nature of female *O. brumata,* which we would expect to display high levels of spatial restriction. Wave-like patterns of spatial spread are known to have occurred in *O. brumata* (Tenow *et al.,* 2007), as well as other outbreaking (Harrison, 1997) and invasive Lepidoptera (Johnson *et al.*, 2006), although over much greater spatial and temporal scales. Local scale larval ballooning is thought to be the largest contributing factor explaining the spatial distribution of outbreaking tussock moths (*Orgyia vetusta*)—a species with similar life-history traits to *O. brumata*—with spatial variation not explained by either host plant quality, or predation (Harrison, 1997). Similarly, larval ballooning dispersal in gypsy moth (*Lymantria dispar*), and to a lesser extent larval crawling, has previously been demonstrated over similar distances to those over which *O. brumata* appears to be spreading at Linnadale (Dwyer & Elkinton, 1995). It therefore seems likely that either some response to conspecific density, host plant defoliation, or incidence of disease (Bell *et al.*, 2005) could be driving this localised expansion of larvae into potentially less suitable habitats, via ballooning or crawling.

Spatial escape from parasitoids has previously been demonstrated to be the best explanation for the origin of novel outbreaks of *O. vetusta* (Maron *et al.*, 2001), and although large outbreak densities do not appear to moving in a wave-like pattern at Linnadale, it seems likely that natural enemy pressure could be playing a part in driving the observed dispersal pattern. The fact that among both years studied at this site, parasitoids appeared to follow closely the spatial pattern of their hosts, even operating at larger spatial scales, corroborates other field studies demonstrating such spatial pattern formation among parasitoids in continuous habitats (Maron & Harrison, 1997), as well as theoretical models (Hastings *et al.*, 1997) that predict increasing rates of parasitism at the edge of outbreak zones, that could suppress the spatial spread of hosts from outbreak

epicentres. The explanation for such a spatial pattern could be that parasitoids are satiated within the outbreak epicentres (Harrison & Wilcox, 1995), which would certainly explain the pattern of significant inverse density dependence found among the parasitoids infecting *O. brumata* during 2006. An alternative explanation could be that parasitoids are being outcompeted by OpbuNPV within hosts, since multiple infections by either cannot be detected under the methods used in this study. Previous studies have shown that parasitoids may alter their oviposition behaviour in response to virus infected hosts (Sait *et al.*, 1996;Nguyen *et al.*, 2005), although whether parasitoids are able to survive to eclosion may depend on the relative timing of infection to parasitism (Escribano *et al.*, 2000; Matthews *et al.*, 2004). Other studies have concluded that intra-host competition between the two may be symmetrical, although the life-span of multiply infected hosts may be reduced compared to single infections (Hochberg, 1991). If this is also true in the present *O. brumata*-NPV-parasitoid system, we might expect greater detection of OpbuNPV at the expense of parasitoids, since any nonspecific mortalities from multiple infections are more likely to detect a molecular presence of OpbuNPV, providing replication of infectious particles is above the threshold detectible by PCR. Spatial associations between larvae infected with either of these natural enemies in this study were uncorrelated, so any inference concerning intra-host competition in this system is problematic. However, evidence from Linnadale and Wideford Cairn in 2008 suggests that, when host densities are not at outbreak proportions, there is a strong negative association between parasitoids and OpbuNPV, suggesting that under more stable conditions, such competition may be prominent. Further study in this area would be beneficial, although at present it may be prudent to propose a combination of intra-host competition, coupled with satiation, in explaining the spatial patterns of parasitoid infection in this system.

The response of parasitoids to host density among other studies appears to be highly variable (Walde & Murdoch, 1988), although some have found significant inverse density dependence at a range of spatial scales (Hails & Crawley, 1992). Others have found parasitoid responses to differ among spatial scales (Heads & Lawton, 1983; Norowi *et al.*, 2000), and whilst caution should be used in interpreting the GLMMs from this study due to possible spatial autocorrelation artefacts, models using G-statistics as a proxy for host density showed a marked change in the sign of response from negative in 2006 to positive density dependence at larger scales in 2008. Similar changes in the sign of parasitoid responses to both scale and host density over time have

previously been reported among a related species (*Phobocampe bicingulata*) parasitizing a similar host (*Eppirita autumnata*; Teder *et al.*, 2000), possibly as a result of differing dispersal abilities between the two. Theoretical models have predicted that such a discrepancy between highly mobile parasitoids and poorly dispersing hosts could contribute to the formation of stable spatial and temporal patterns in host abundance (Wilson *et al.*,1999), and has motivated calls for studies on host-parasitoid interactions to consider larger spatial scales (Cronin & Reeve, 2005). The vast majority of infections among larvae at Wideford Cairn could be attributable to parasitoids, demonstrating that such an interaction can be highly variable between sites. However, despite the stability in host spatial pattern across the entire length of the transect, it is unclear at this stage whether such a pattern is the result of regulation by parasitoids or not.

In contrast to the larger spatial scales in pattern and response of parasitoids to host abundance, infections by OpbuNPV appeared to have markedly different response to space across years, being highly spatially restricted during the outbreak year, and more spread out in the subsequent year. The positive density dependence found among infections by this natural enemy are perhaps not surprising, given its horizontal transmission mode (Cory & Myers, 2003), although the changes in spatial responses between different years are less clear. Despite this, such findings are in agreement with experimental (Entwistle *et al.*, 1983) and theoretical (Dwyer, 1992) evidence, which suggests that patterns of NPV infection among other outbreaking Lepidoptera species follow a "travelling wave" pattern among years, that extends out from a peak infection epicentre into a secondary diminished peak, before finally losing the wave-like pattern in an interference phase. The spatial dynamics of such a wave largely depend on the initial population size, transmission rates, susceptibility of uninfected individuals, and dispersal rates (Dwyer, 1992) and as such, may be highly variable. In a theoretical model parameterised from data for *O. brumata* feeding on Oak in mainland Britain, White *et al.* (2000) found four distinct wave-like patterns could be established from the host-NPV interactions, contingent on the relative dispersal abilities of hosts and virus at the leading edge of the wave, which could all lead to the formation of distinct spatial patterns in host abundance. The data presented in this study appear to support these other findings, and suggest an alteration in the spatial dynamics of host and virus, in response to density, mediated by local dispersal. Previous work has shown that the transmission of NPVs does not necessarily conform to a linear response to host density (D'Amico *et al.*, 1996; Knell *et al.*, 1998), especially when pathogens are highly

spatially restricted (D'amico *et al.*, 2005), and that the risk of infection can decline after an epidemic (Elderd *et al.*, 2008). Such changes in the temporal dynamics of disease in the regulation of hosts can influence the occurrence of population cycles (Liu *et al.*, 2007). Indeed, if it is the case that density- or disease-dependent mechanisms are driving hosts to disperse into poorer quality local habitats, it is possible that this too could influence the dynamics of populations of *O. brumata* within this site (McVean *et al.*, 2002).

In contrast, at Wideford Cairn it may be interspecific competition driving *O. brumata* into poorer quality habitats. Although temporal data is lacking for this site, the fact that both *O. brumata* and *A. grossulariata* utilise a shared resource suggests the possibility that some interspecific interactions occur between the two species. *A. grossulariata* larvae emerge prior to *O. brumata*, and are much larger at late instars, and therefore more likely to consumer larger quantities of the shared resource. Even though prevalence of AbgrNPV were fairly high at this site, there appeared to be no obvious spatial pattern to infection, largely appearing to follow its host distribution. There was also no detectable response to host density at any scale at this site. Although other studies have found indirect effects of pathogens and parasitoids among Lepidoptera on a shared resource (Redman & Scriber, 2000), no such effects were evident at this site and between these two species. At present, there are no known parasitoids of *A. grossulariata* on Orkney, and while AbgrNPV is known to have crossed over into *O. brumata* populations, it is unclear whether there is any pathogenic effect. No signal of interference or apparent competition is evident in this study, and at present it is not clear whether there is any spatial or temporal difference between AbgrNPV and OpbuNPV infections within their respective hosts. Clearly more extensive sampling is required in order to investigate this further.

Interestingly, it was not only OpbuNPV that appeared to display changes in the spatial pattern of infection between outbreak and non-outbreak years. Infections among CPVs showed remarkable similarities, although at much lower prevalence, to NPV infections between the two years. Surprisingly, the sign and scale at which these infections responded to host density was the opposite of that found among OpbuNPV. CPVs are thought to be largely transmitted vertically, and have only chronic or sublethal effects on hosts (Rothman & Myers, 1996), and previous studies on CPVs infecting *O. brumata* populations in Orkney have found a 54-94% vertical transmission rate from F_0 to F_1 generations (Graham *et al.*, 2006). The results from this study support

such an effect, with no effect of host density detected in the F_0 generation, and strong positive delayed density dependence being detected at all scales in the F_2 generation at Linnadale from 2008. However, the change in spatial pattern among scales is perhaps less easy to explain solely through vertical transmission. Tellingly, CPV and OpbuNPV infections were highly positively correlated in 2006, despite having opposing effects at different spatial scales. Multiple horizontal virus infections are known to alter the temporal patterns of density dependence in lepidopteran hosts (Bonsall & Benmayor, 2005), although the combined effects of horizontally- and vertically- transmitted viruses on the spatial patterns of density dependence are not clear. Although data on multiply infected hosts is not presented here, theoretical models have shown that persistence of a vertically transmitted parasite can be more likely when hosts are co-infected with horizontally transmitted parasites, due to interference (Jones *et al.*, 2007). It could therefore be possible that the apparent scale-sign discrepancy between CPV and OpbuNPV may reflect some form of ecological interference via CPVs conferring increased resistance to OpbuNPV-induced mortality; the scale discrepancy being indicative of the scale at which the interaction threshold between the two viruses is operating—very high densities and small spatial scales inducing greater horizontal transmission of OpbuNPV; other scales beyond these OpbuNPV epicentres facilitating increased CPV persistence. Detailed analysis of the extent of multiple infections at each of these scales will now be necessary in order to verify these suppositions, although they remain plausible mechanisms explaining the observed patterns of scale-dependency among these two viruses. Interestingly, this may also be a plausible explanation for the absence of CPV infections at Wideford Cairn, since if multiple infections are more likely to facilitate CPV persistence, the low levels of OpbuNPV observed here could be below the threshold needed to maintain this persistence.

Although RV prevalence among all sites were very low, there was little, if any, response to spatial pattern among larvae infected with this virus. RV has previously been isolated from parasitoids emerging from *O. brumata* in Orkney with 100% prevalence among all adults screened (Graham *et al.*, 2006), as well as other Ichneumonid and Braconid species, where it is thought to occur commensally via suppression of the hosts immune system upon oviposition (Renault *et al.*, 2005). Transmission of this virus to hosts is therefore thought to occur primarily through vectoring by parasitoids. Although a significant positive spatial association was found between parasitoids and RV from Linnadale in 2006, no evidence of such an association

was detectable among other years and other sites. Surprisingly, overall RV prevalence were no greater at Wideford Cairn than at Linnadale, where parasitoid prevalence were almost double. Prevalence of these two natural enemies have previously been shown to have a significant positive relationship among Orkney populations (Graham *et al.*, 2006), although this was not evident in the present study. It may be possible that reduced prevalence is a direct result of increased parasitoid success: if the number of unsuccessful parasitoid attacks is low, this may be reflected in low residual prevalence of its commensal virus. Simulations of pathogen-host interactions via vectoring have also shown that increasing spatial heterogeneity of hosts can cause pathogens and vectors to become decoupled (Caraco *et al.*, 2001), although it is not clear whether such an effect is occurring in this system—given the highly mobile vector, and the majority of host heterogeneity occurring at small scales, this seems an incomplete explanation for the observed data. Despite this, RV was also positively spatially associated with OpbuNPV and CPV at Linnadale in 2006, and CPV only in 2008. The causal mechanisms behind these associations could represent failed oviposition events by parasitoids—either probing of infected larvae and not ovipositing, or ovipositing but not emerging—via intra-host competition. Parasitoids have previously been shown to preferentially probe virus infected hosts due to their reduced defence reactions (Lopez *et al.*, 2002), although it is not known whether this is the case in this system.

Increasing density of parasitized individuals was also a good predictor of the degree of cuticular melanism among *O. brumata* individuals at Linnadale in 2008, being highly significant among both models constructed, to allow for changes in subjective melanism categories. Cuticular melanism among Lepidoptera larvae is a well documented response to increased risk of disease (Wilson *et al.*, 2001), and is known to confer resistance to viral infection (Reeson *et al.*, 1998). Host density-induced changes in the degree of melanism have been demonstrated in other species under laboratory conditions, independently of natural enemy attack (Goulson & Cory, 1995), in a process termed density-dependent prophylaxis (Wilson & Reeson, 1998). However, it is unclear whether the degree of melanism observed in *O. brumata* is a response to, or precaution against, natural enemy attack. Hagen *et al.* (2003) found evidence of strong density-dependence in the degree of melanism among *O. brumata* populations feeding on Birch in Norway, and a positive association between an increasing degree of melanism and parasitoid attack (Hagen *et al.*, 2006), but concluded that such an association was not indicative of a countermeasure against their attack.

The results of the present study therefore agree well with these other studies, although whether a positive association between increasing melanism and increasing parasitism is indicative of a lack of resistant to parasitoid attack, is less clear. Among the viruses screened here, only RV displayed any response to melanism. Surprisingly, increasing RV infections were weakly associated with less melanic forms of larvae. Although speculative, such an interaction could be mediated indirectly through parasitoid oviposition behaviour: given the lack of evidence that RV can be transmitted vertically through *O. brumata* generations (Graham *et al.*, 2006), it follows that occurrence of RV must be a proxy for parasitoid oviposition behaviour; and if cuticular melanisation is a response to parasitoid infection, any parasitoids probing, but not ovipositing, within hosts, are likely to leave a genetic signal of this through the deposition of RV particles. Any larvae not having to induce a melanisation response as a result of encapsulation of parasitoid eggs, is therefore more likely to be either intermediate, or non-melanic. A comprehensive review of parasitoid oviposition immune responses among a range of Lepidoptera species by Smilanich *et al.* (2009), found that species with strong immune responses—as measured by their degree of melanisation—had the lowest instances of parasitism. Clearly, correlation and causation are dynamic properties in such host-natural enemy immune responses, and it is difficult to separate one from another. However, despite the fact that no association was found between parasitoids and degree of melanism at Wideford Cairn, it is worth noting that the total number of melanic *O. brumata* larvae was greater at this site than either non-melanic or intermediate colour morphs, and far exceeded the ratio of the three colour morphs seen at Linnadale in 2008. The fact that parasitoid prevalence at this site were also more than double those of Linnadale during this year, and the fact that a significantly greater proportion of parasitised larvae were of the melanic form, suggests that some host-parasitoid mediated immune response is the most likely explanation for the observed patterns of melanism seen among *O. brumata* larvae at these two sites in Orkney. However, a potential caveat to these conclusions would be the observation that, although proportions of CPV and RV were not significantly different among melanic and non-melanic morphs under the binomial proportions test, their overall proportions were very similar to those among parasitoids. Small sample sizes could affect the Chi-squared estimate under this method (Crawley, 2007), and this remains a problem when studying natural enemies such as RV and CPV, that exist at only very small prevalence within populations. Clearly, more accurate methods of scoring the

degree of melanism among larvae would be an advantage in this context, although techniques such as digital image analysis (Wilson *et al.*, 2001; Smilanich *et al.*, 2009) are logistically difficult to implement in the field. Further experimental evidence through manipulative experiments would seem to be the best way of testing hypotheses such as these, generated from observational field data.

Further work is needed to verify many of the tentative conclusions proposed in this study. A major disadvantage is the small number of sites studied. By their nature, outbreaks are temporally and spatially patchy, making detailed study of them difficult. As well as continuing to monitor the present outbreak at Linnadale, such detailed within-site analysis is needed among other spatial locations to see if patterns of spatial host-natural enemy interactions are generalisable across sites. Implementing another spatial dimension to the analysis would also further enhance the present study, making speculations about the radial dynamics of travelling waves of host-natural enemy spatial expansion more explicit. The present study has also raised interesting questions about the possible implications of intra-host competition among natural enemies, and how this may be influenced by spatial location within outbreak zones, and after outbreak expansions. The molecular screening process used for virus detection does not, at present, allow for inferences to be made regarding pathogenicity, virulence, or mortality rates among the different natural enemies (with the exception of parasitoid mortality). For example, OpbuNPV is known to be highly genotypically variable in Orkney. Whether this variability translates into differences in infection rates is not currently known, and so at present, only inferences regarding spatial occurrence are possible. This makes assumptions regarding the potential impacts on population regulation of hosts problematic, although it may suggest fruitful avenues for further investigation. Finally, although one other interspecific competitor was included in this analysis, community-level responses of hosts and natural enemies also needs further investigation. At present it is not know whether the viruses and parasitoids that infect *O. brumata* have any effect on other lepidopteran hosts within the same moorland ecosystem. Apparent competition through shared natural enemies (Bonsall & Hassell, 1997), or indirect effects (Van Veen *et al.*, 2005) may both disrupt host-natural enemy dynamics, and could mask potential effects of density-dependent or spatial processes.

Chapter 5

Regional scale insect host-natural enemy interactions

5.1 Abstract

The distribution and abundance of organisms within a region may be related to the spatial variation in both the relative availability of suitable habitat, and its quality. Favourable population processes acting within these suitable areas, mediated by quality, will influence the births, deaths and recruitments, and therefore the total abundance of the species. However, the ability of an organism to exploit the most suitable patches will be a function of its ability to disperse away from unsuitable patches. Populations may therefore exist as heterogeneous metapopulations embedded in a matrix of suitable and unsuitable habitat, and the ability of natural enemies to exploit them within these patches will also be a function of their dispersal relative to their hosts. How enemies respond to the changes in host abundance within each patch may therefore also vary greatly within a region.

This study compares the responses of two Lepidoptera species of differing dispersal capabilities and life histories to changes in habitat quality and quantity of the same shared foodplant. *Operophtera brumata* is a resident species with flightless female adults, whereas *Abraxas grossulariata* is a newly invasive species with larger, fully-flighted adults of both sexes. Here we test the hypothesis that the less vagile of the two species will respond to changes in habitat availability at smaller spatial scales than the larger, more dispersive species. It is also predicted that more favourable microclimatic conditions at the local scale—mediated by changes in topography and vegetation cover—will be more correlated with increases in the abundance of the newly established *A. grossulariata* than *O. brumata* because of differences in overwintering strategy. Finally, whether natural enemy prevalence is related to host density across this regional scale is also examined.

Larvae of both *O. brumata* and *A. grossulariata* were sampled from 36 separate sites over two years that accounted for the total variation in topography within the region. The spatial scale at which each species' abundance best correlates with the total area of suitable habitat at each site was investigated by matching the model fits of variation in habitat area to variation in abundance. Changes in *O. brumata* and *A. grossulariata* abundance among years were highly variable, although the overall trend was increasing and decreasing, respectively. *O. brumata* showed signs that individual populations may be synchronised on a regional scale. Neither species responded significantly to changes in the scale of habitat availability. However, *O. brumata*

appeared to respond positively to increased elevation in the region, although whether this effect is mediated by co-linear patterns in grazing pressure, is not clear. Conversely, increases in *A. grossulariata* abundance were closely linked to south-facing aspects and taller heather, suggesting that microclimatic variables are mediating the successful establishment of this species among certain sites. *A. grossulariata* distributions also showed correlation in a southeast-northwest direction that may be a signal of its apparent northwards range expansion. How these two patterns may be linked is discussed. There was no evidence that local scale patterns in host-natural enemy interactions were translated into regional-scale patterns, although AbgrNPV infections among *A. grossulariata* larvae appeared to be clustered into a semi-regional hotspot of high prevalence. Despite this, there was no evidence that natural enemy prevalence was related to changes in host density, across any scale, and either species.

Exploitation of areas of suitable continuous habitat may not necessarily be related to a species dispersal ability. Variation in abundance may instead be mediated by variation in biotic and abiotic components of a site at local scales within a region, which will be a function of a species life-history and range. Predictions about how resident and immigrant species may respond under climate change should take into account local-scale habitat characteristics, which take into account changes in topography, microclimate, and land-use. The persistence of host-natural enemy interactions at regional scales in the absence of density-dependence is also discussed.

5.2 Introduction

The distribution and abundance of organisms may vary greatly in both space and time; an understanding of the processes that govern these changes is a central theme in ecology (Andrewartha & Birch, 1954; Gaston & Lawton, 1990; Brown *et al.*, 1995; Huston, 1999), and may be crucial in predicting and mitigating species responses to a changing environment (Parmesan, 2006; Berggren *et al.*, 2009). The spatial relationships between organisms in particular has received a lot of theoretical and empirical consideration (Hanski, 1999), providing a framework on which to understand population-level responses of species to the spatial arrangement of habitats in an ecosystem. Such responses may be influenced by a number of processes, such as the fragmentation or loss of existing habitat (Debinski & Holt, 2000), or spatial variation in factors that limit abundance (Gripenberg & Roslin, 2005; Gripenberg & Roslin, 2007). Therefore, it is often not clear whether the quantity, or quality of existing habitat—or an interaction between the two—is the principal factor governing abundance of an organism, and their likelihood of persistence at the population-level within any particular area of habitat (Krauss *et al.*, 2004; Summerville *et al.*, 2004; Dover & Settele, 2009): the quantity of available habitat may be important in governing how many individuals it is possible to support (Gaston *et al.*, 2000), although the spatial arrangement of distinct patches of habitat may affect the probability of any given patch being occupied (Krawchuck & Taylor, 2003). As a result, the responses of organisms to these habitat patches may be confounded by the scale at which they are viewed (Menendez & Thomas, 2000) due to variation in the dispersal capability of different species, which may also be related to body size (Roland & Taylor, 1997; Pinto & Keitt, 2008). On the other hand, quality of available habitat may affect both the abundance and persistence of species within patches by influencing the physiological and dynamical processes that act on, and regulate, populations (Thomas *et al.,* 2001a). Such influences may be broadly categorised into either biotic or abiotic effects, whose relative importance may change according to the species and habitat in question, and the trophic relationships of each of the interacting species within that habitat (Kamata, 2000; Abdala-Roberts & Marquis, 2007; Menke *et al.*, 2007; Dahlgren & Erhlen, 2009). For phytophagous insects, the direct effects of host plant quality as a resource for developing larvae may influence the fitness of individuals and the abundance of populations (Hunter & McNeil, 1997; Tikkanen *et al.*, 1999; Lill & Marquis, 2001;

Gripenberg & Roslin, 2005), whilst the indirect effects of variation in landscape and topography may influence the physiological and microclimatic conditions in which host plants are able to survive (Bennie *et al.,* 2008). This may, in turn, feedback into individual fitness effects for the developing herbivores, affecting survival (Virtanen & Neuvonen, 1999), larval and pupal development (Weiss *et al.*, 1988), and the thermoregulatory processes that limit species' ranges and multitrophic interactions (*reviewed in* Hodkinson, 2005).

Although heather moorland represents an increasingly threatened and diminishing habitat, often fragmented in lowland regions (Thompson *et al.*, 1995), among upland regions where it is most extensive it forms an almost continuous coverage, dominated by a single host plant species, *Calluna vulgaris*. The extent of coverage within any region may be influenced by many factors, including i) stand age: coverage is most extensive during the building phase (15-20 years) when shoot density is highest and there is little room for competing plant species (Gimingham, 1985); ii) Soil characteristics: plant diversity is lowest in wetter, more acidic soils, where ericoid mychorrizae of *C. vulgaris* can sequester nutrients unavailable to other species (Hartley *et al.,* 2003) iii) Grazing regime: increased browsing a new shoots and nitrogen deposition may facilitate the encroachment of competitive grass species (Hartley *et al.,* 2005). In turn, each of these characteristics may feedback into the invertebrate communities of the moor, according to habitat requirements. For example, leafhopper (Hemiptera) species may be affected by leaf nitrogen, and plant diversity; ground beetles (Carabidae) by soil conditions for pupation; Spiders and mites (Aranae) by abiotic conditions (Sanderson *et al.,* 1995); and moths and butterflies (Lepidoptera) by plant architecture (Haysom & Coulson, 1998). Although as a foodplant *C. vulgaris* is relatively nutrient poor (Kerslake *et al.,* 1996), the architectural complexity of different developmental stages may provide refuges from predators and parasitoids by increasing fine-scale spatial heterogeneity (Vanbergen *et al.*, 2007). The relative importance of each of these top-down or bottom-up influences for the distribution and abundance of a species is likely to depend largely on the particular system under investigation (Walker & Jones, 2001), and may also vary considerably in time and space (Gratton & Denno, 2003; Denno *et al.*, 2005).

For parasitoid and pathogen natural enemies attacking host species, the distribution and abundance of hosts within a habitat may similarly affect prevalence and persistence, operating as resource patches and undergoing metapopulation processes

(Hess *et al.*, 2002). Host patchiness and abiotic conditions may both affect natural enemy prevalence (Ram *et al.*, 2009), and therefore the ability of each natural enemy to persist within host species' in a landscape will be intrinsically linked to infection strategies, mediated through dispersal ability (Lopez *et al.*, 2005; Laine & Hanski, 2006; Greer & Collins, 2008). Such effects may be detectable through analysis of the spatial patterns of enemies in relation to their hosts (Maron & Harrison, 1997), cascading up through host plant effects (Benrey & Denno, 1997; Raymond *et al.*, 2002) and landscape-level influences (Cronin & Reeve, 2005).

This study investigates the spatial and temporal variation of two geometrid species of contrasting dispersal abilities, life-history traits, and range sizes. The principal factors governing their abundance and distribution among *C. vulgaris*-dominated heather moorland across the Orkney mainland is investigated, in order to discern whether scale-dependent habitat quantity, or local-scale variation in habitat quality (mediated through variations in topography and land use)—or a combination of the two—is more important in explaining their relative occurrences. The spatial patterns of infection by each of their putative natural enemies is also investigated, in order to establish whether patterns of infection are constant when scaling up from local (see Chapter 4) to regional scales.

The spatial and temporal distribution of *O. brumata* is expected to be relatively consistent among the two sampling years; although this species is known to display outbreaking behaviour, such occurrences are thought to be rare and, if manifest, highly locally restricted. The low dispersal ability of flightless females of this species is predicted to limit the temporal changes in abundance among sites, and distribution is anticipated to be relatively uniform due to the long establishment of this species in the region. However, some adaptation to local conditions is expected, manifest in areas of high host-plant quality, and low natural enemy prevalence (Harrison, 1994; Mopper, 1996). Soil characteristics are also anticipated to be important in determining their abundance, since this is where pupal development occurs.

For *A. grossulariata*, spatial and temporal variation is predicted to be much more pronounced; this species is occurring at the edge of its northwards range, having only recently become established on Orkney within the last decade. Its greater dispersal ability than *O. brumata* is expected to be manifest in a larger spatial scale of abundance and response to habitat cover, and its more recent adaptation to local conditions is expected to be associated with greater spatial variation in abundance (Holland *et al.*,

2005; Hamback *et al.*, 2007). Topographic conditions are predicted to play a larger role in determining where this species will occur, since the overwintering stage is larval, and at the edge of its northwards distribution (Thomas *et al.*, 2001b; Ward *et al.,* 2007; Kunin *et al.*, 2009). More sheltered, south-facing slopes where microclimatic conditions are more favourable are therefore expected to harbour larger abundances of *A. grossulariata* larvae. Soil conditions are not expected to be important determinants of distribution and/or abundance in this species, since pupal development is on the host plant, and during the warmer summer months.

Spatial patterns of natural enemy occurrence within both species are expected to be similar to that found at the local scale (see Chapter 4), being dependent on infection strategy: parasitoids are predicted to respond at greater spatial scales than their host (Brodmann *et al.*, 1997), and be more widely distributed than horizontally transmitted viruses (OpbuNPV infecting *O. brumata*; AbgrNPV infecting *A. grossulariata*). The latter is expected to be highly spatial restricted, due to its passive infection mechanism—infection occurring only when infected virions are ingested by conspecifics after host death on the same foodplant (Dwyer, 1991). For this reason, viral infection within sites is predicted to be positively host density-dependent for these two viruses, whereas for parasitoids negative density-dependence or density independence is anticipated to be the most likely outcome (as at local scales), due to saturation of host attack-rate at high densities (Walde & Murdoch, 1988). Finally, for the vectored virus (RV), infection is expected to mirror that of its parasitoid vector, although at lower infection prevalence.

5.3 Methods

5.3.1 Site identification

In order to examine the impact of topography and climate on species' distributions, 18 field sites were identified in heather moorland on mainland Orkney, stratified according to their topographical attributes: elevation, slope and aspect. Three separate classes for each attribute were assigned, based on the known occurrence of *O. brumata* on Orkney: the mean ± 1 standard deviation of each topographic attribute was extracted from a 10m resolution digital elevation model (DEM) of Orkney (EDINA Digimap), based on the

distribution of field sites outlined in Graham *et al.* (2004). Two further attribute classes were created based on all values less than or greater than this attribute class, to give a series of "low", "medium", and "high" attribute classes. A further 18 field sites were also identified as paired replicates, which also shared the same topographic attributes, giving a total of 36 field sites. Each pair of replicates were >10km away from each other, to avoid confounding effects of spatial non-independence from local sources (see Table 5.1.). Topographic maps of the intersection between each attribute class were extracted from the three separate coverage maps (elevation, slope, aspect), to create 18 separate coverages conforming to the attributes specified in Table 5.1, using ArcMap v9 (ESRI UK). The centroid point of the largest area of heather moorland (LandCover Scotland 1988) that fell within each class was then used as the primary replicate. Secondary replicates were chosen as the largest area of heather moorland in the same attribute class that was >10km away from the primary replicate. Each site is located in a >50m^2 area of heather moorland conforming to each attribute class, to avoid possible site identification errors in the field, and to reduce the possibility that each site lies at the extremes of any of the three attribute classes. All sites were located in the field using hand-held GPS units (EtRex UK) with an error ±5m. The distribution of all 36 field sites is given in Figure 5.1.

	Elevation (m)									
	0-70	*0-70*	*0-70*	*70-125*	*70-125*	*70-125*	*125+*	*125+*	*125+*	**Replicate**
NORTH (325-45°)	BoH	ET	AND	WL	SET	LY	MT	BR	BRA	*1*
	BS	HAS	QT	MM	SR	GH	PU	BU	AK	*2*
SOUTH (135-225°)	GW	SCW	WK	OV	RD	SRH	QF	MB	WH	*1*
	WT	QM	GOR	BB	BoW	FF	VN	BG	MLD	*2*
	0-6	*6-11*	*11+*	*0-6*	*6-11*	*11+*	*0-6*	*6-11*	*11+*	
	Slope (°)									

Table 5.1 Topographical attributes of each sample site within Orkney mainland. Each attribute is stratified into separate classes to ensure a uniform distribution of sample sites across all attributes. Replicates represent the same topographical attributes belonging to a spatially separate (>10km) site within the mainland. Site codes correspond to those given in Fig. 5.1. All sites were sampled during May-July 2007 and 2008.

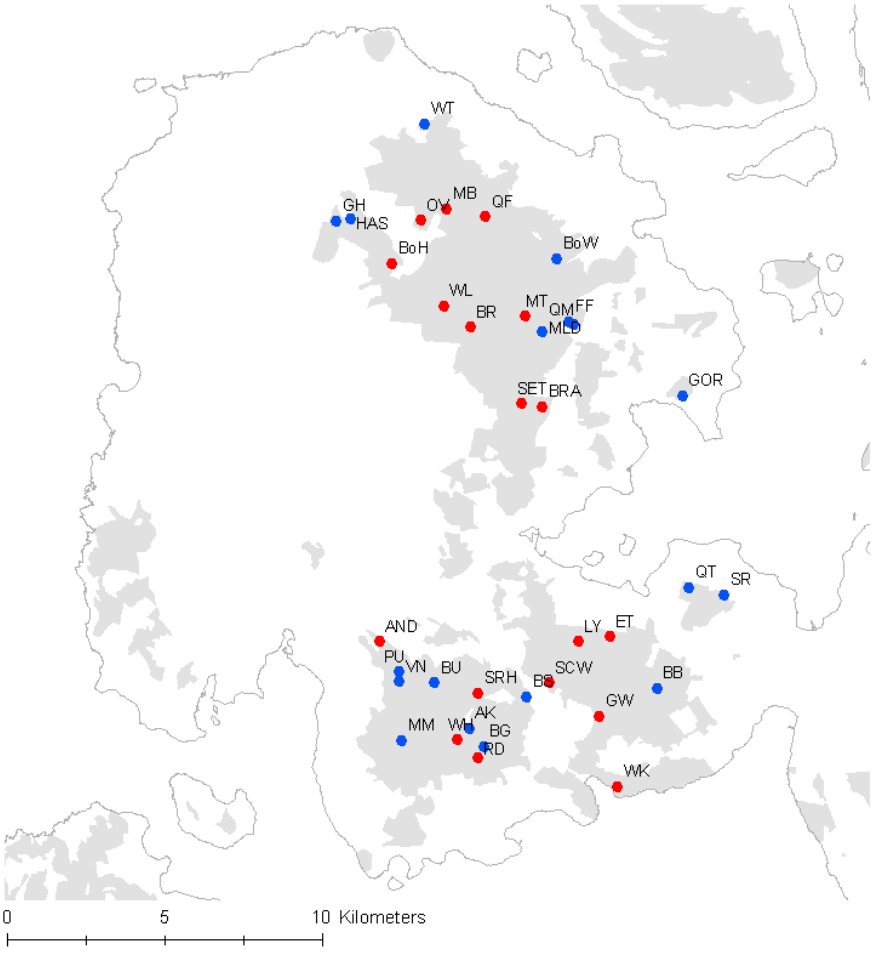


Fig 5.1 Geographical distribution of sample sites across mainland Orkney. Light grey shading represents areas of heather moorland. Site codes correspond to those given in Table 5.1. and represent different topographical attributes, stratified to cover variation in topography across the landscape. Red dots represent primary replicates, blue dots represent secondary paired replicates (see Table 5.1).

5.3.2 Sampling protocol

All sites were sampled between May-July during 2007 and 2008. Within each site, 30 replicate 0.25m x 0.25m quadrats were taken within an area of 10m^2 (three parallel 10m transects, placed 5m apart), following the sampling protocol outlined in Chapter 2 (Methods). Spatial sampling error between years was <1m; sites were sampled in a random order to avoid temporal errors in quantifying abundance that may occur due to changes in species' demography throughout the field season. *O. brumata* larvae were reared on artificial diet and *A. grossulariata* on heather collected from the same site. Virus prevalence among both years and both species was conducted using real-time RT-PCR as outlined in Chapter 2. Due to the large number of samples from three sites during 2008 (AK, BU, SET), a random subsample of 50 individuals was chosen for

viral screening, and prevalence calculated as the no. infected / (no. pupating + no. screening negative), following Jovani & Tella's (2006) recommendations for minimum sample-size thresholds (i.e. >30) when estimating parasite prevalence. Parasitoid data is only available from 2008.

Within each site, 10 different variables were measured that may influence the abundance and distribution of the two host species: i) heather height (m) was measured as the height from soil level to the tip of the highest shoot within the centre of each quadrat; ii) Shoot : wood ratio was measured as the ratio of the total wet mass of green shoots to woody stem tissue of heather excised from nine randomly selected quadrats within each site during 2007; iii) Soil moisture content was measured as the total % moisture loss after oven drying (40°C for 48 hrs) of the lower half of a soil core (0.05m diameter; 0.1m depth) taken from six randomly chosen quadrats within each site during 2007; iv) Soil Carbon content was measured as the loss on ignition of 1g oven dried soil (from the same soil cores used in iii)) (105°C for 3hrs) after ignition in a muffle furnace (550°C for 2hrs), following the methods of Hartley *et al.* (2003); v) Grazing index at each site was calculated as total number of sheep/ total area of rough grazing, after interpolating the centre point of each 2km grid square of Orkney mainland coverage, obtained from Scottish Agricultural Census data (EDINA; 2006) in ArcMap; vi) % heather moorland was calculated by extracting the number of 25x25m pixels of heather coverage (including wet, dry and undifferentiated heather, and blanket bog) in a circle of varying radii around each site, obtained from LandCover Scotland 1988 habitat map in ArcMap. Contemporary heather coverage was verified by comparing 1988 coverage to 2005 coverage from 25x25m resolution satellite images (LandSat TM, EDINA); vii) Solar radiation at each site was modelled using the Spatial Analyst extension in ArcMap v9 using 59° latitude and sky size 200, with ½ hourly intervals for each month over 2008, based on a 10x10m resolution DEM.

5.3.3 Statistical analysis

All statistical analyses were undertaken using R 2.9.0 (R core development team), except for windrose correlograms, which were constructed using Passage 1.1.2.3 (Rosenberg, 2001). All linear mixed effects models (LME) were constructed using the package nlme (Pinheiro *et al.*, 2008); generalised linear mixed models (GLMM) were constructed using the package lme4 (Bates *et al.*, 2008); Principal Components Analysis

(PCA) was conducted using the function prcomp (R core development team); Moran's I autocorrelation tests, Getis-Ord G statistics, and Spatial Autocovariates were calculated using the package spdep (Bivand, 2009); and spline correlograms were constructed using the package ncf (Bjornstad, 2005).

5.3.3.1 Spatial patterns in host distribution

Spatial patterns in the distribution of host species across the Orkney mainland were investigated using two different correlogram methods: i) spline correlograms calculate the correlation between each pair of points within sites over all distances in a straight line (see Chapter 4). The scale of spatial pattern of hosts is inferred as the point of zero correlation; ii) Windrose correlograms calculate the correlation between points in two dimensions by placing pairs of points into a series of concentric ring classes, based on distance and direction. This therefore represents a form of anisotropy analysis, to infer biases in the direction of spatial pattern of hosts. Correlation and significance values between classes are based on Moran's I spatial correlation coefficients, following the methods of Oden & Sokal (1986).

5.3.3.2 Factors influencing host abundance and distribution

To investigate whether either of the host species exhibit scale-dependent responses to habitat, the total number of pixels of heather moorland in a 25x25m resolution raster coverage were extracted from circles of varying radii around the centre point of each site. A total of 25 circles were extracted, in increments of 200m, between 200-5000m, and used as the explanatory variable in one of two sets of models: i) a binomial GLMM using presence/absence of the host species as response variable (to assess what determines whether each host species occurs in a particular area of habitat), with fixed effect parameters fitted using the Laplace approximation; and ii) a Gaussian LME using the log-transformed number of larvae per quadrat as response variable (to assess what affects changes in abundance when hosts are present), with fixed effects parameters fit by maximum likelihood. For both sets of models, space was incorporated by the addition of a spatial autocovariate, which incorporates neighbouring autocorrelation in the response variable as an additional distance-weighted function to the explanatory

variables, following the methods outlined in Dormann *et al.* (2007). Temporal autocorrelation was also incorporated into each model by including sampling day nested within year as random effects. Model fits at each scale were assessed using Δlog likelihood values (the change in log-likelihood of each model relative to a null model), and significance tests were determined after Bonferroni correction for multiple comparisons.

To investigate what additional factors may influence the abundance and distribution of the two host species, PCA was conducted on the 10 within-site habitat measures (elevation, slope, aspect, heather height, shoot:wood ratio, % heather cover in 1km radius, % soil moisture, % soil carbon, grazing index, solar radiation), in order to reduce the number of variables used in the model by combining those linear combinations that maximize the variance in the data. All variables that did not meet assumptions of normality were subject either to log-, logistic-, arcsine square root-, or squared- transformation in order to meet this assumption. The variable "Aspect" was subject to a transformation for linearising circular data ($0.5(\cos(\pi/180)(\text{Aspect}^\circ-30))+1$), whereby low values reflect more southerly aspects, and high values more northerly aspects, following the methods of Moisen & Edwards (1999). All those principal components then deemed sufficient to describe an adequate amount of variation (by maximising the explained variance, and minimising the number of components) were then subsequently used as additive and interactive explanatory variables in one of the same two models as described above for investigating scale-dependent habitat responses: i) a binomial GLMM of presence/ absence of host species within quadrats as response variable; or ii) a Gaussian LME with log number of larvae per quadrat as response variable. Both models contained additional spatial autocovariates and sample date nested within year as random effects. Minimum adequate models were fitted using a backwards stepwise procedure for removing non-significant terms, starting with highest-order interaction terms, until only significant variables are left, given no significant changes in deviance after removal. LME models were compared using likelihood ratio tests, and GLMM models using Chi-squared tests.

5.3.3.3 Insect host-natural enemy interactions

Natural enemy infection hotspots were identified using Getis-Ord G-statistics (Getis & Ord, 1992), calculated as z-scores (a measure of standard deviation of the data) given a

specified neighbourhood size—in this instance encompassing all sites within mainland Orkney—on the proportion of individuals infected within a site by each natural enemy. Significant infection hotspots are identified as those sites that lie outside the 95% confidence bounds of the global z-score.

Spatial patterns of infection by each natural enemy was also investigated by extracting the variance components from the random effects of null binomial GLMM models of the weighted ratio of infected to uninfected larvae per quadrat, either i) between sites; or ii) between quadrats nested within sites. These values are also compared with the variance components of host larval distribution extracted from Gaussian LME models. Spatial correlograms were also constructed of the number of infected individuals within each quadrat at different distances between 1 - 20 000m. The scale of infection is inferred as being the point of zero correlation between pairs of points.

The occurrence of density dependence in infection of each natural enemy was also analysed at two temporal and spatial scales, in order to investigate whether patterns in host-natural enemy attack vary according to spatial scale, and also to account for possible biases in model-fitting estimates when within-quadrat sample sizes are low: i) At the site scale, the weighted ratio of infected to uninfected larvae pooled from each quadrat within sites for each year is used as the response variable in a binomial GLMM with total number of larvae per site as explanatory variable, with the addition of a fitted spatial autocovariate. Sampling date is also specified as an additional random factor to account for unknown variation in infection across sampling dates (max. n=36); ii) At the quadrat scale, the same binomial GLMM models are repeated as in i), with the exception that the ratio of infected to uninfected larvae is measured within quadrats rather than pooled within sites, and the number of larvae per quadrat was used as the explanatory variable (max. n=1080). Models of delayed density-dependence in infection were also fitted to data at the site-scale, using number of larvae per site in the previous year as explanatory variable. Model fits between years were assessed using log likelihood values. Delayed density-dependence in infection was not investigated at the quadrat scale, due to large between-year variation in larval densities at this scale.

All GLMM and LME models were checked for residual autocorrelation by visual inspection of Moran's I correlograms of residuals vs distance, and global

autocorrelation in residuals was quantified using Moran's I permutation tests (10^3 iterations).

5.4 Results

5.4.1 Spatial patterns in host distribution

Among-year variation in the number of host larvae found at each site was far greater in *O. brumata* among sites than for *A. grossulariata*, with the former displaying around a four-fold region-wide population increase between 2007 and 2008; for *A. grossulariata* the opposite was true, with a region-wide decrease of around 60% between the same two years (Table 5.2.). During this period, among all natural enemy infections, the only noticeable differences in infection prevalence was among OpbuNPV infecting *O. brumata* larvae, with the total percentage of infections being reduced by around ⅓ during 2008 compared to the previous year. Despite this, the total number of infections among all larvae across the region remained about the same. Both RV prevalence in *O. brumata,* and AbgrNPV prevalence in *A. grossulariata* were not substantially different between the two years, and although data on parasitoid prevalence is only available for a single year, all these natural enemy prevalence tally well with more detailed within-site data from other sites on the Orkney mainland (see Chapter 4).

O. brumata larvae were present at 20 of the total 36 sites sampled during 2007, with the largest within-site density being found at AK, with 53 larvae (Fig 5.2, 2007a). Across the region, there was no obvious spatial patterning in abundance, with among-quadrat correlations fluctuating very little among all distance classes (Fig 5.2, 2007b).

					%Natural enemy		
Species	**Year**	**Total**	**Average Site density** (±S.E.)	**Parasitoid**	**OpbuNPV**	**RV**	**AbgrNPV**
O. brumata	2007	281	7.8 (2.4)	-	10.7	2.5	-
	2008	1247	34.6 (13.3)	8.3	3.73	2.5	-
A. grossulariata	2007	363	10.1 (3.2)	-	-	-	21.8
	2008	219	6.1 (1.9)	-	-	-	23.3

Table 5.2 Summary table of total number of *O. brumata* and *A. grossulariata* larvae sampled during 2007 and 2008 across all 36 field sites within mainland Orkney. % Natural enemy refers to the percentage of the total number of larvae among all sites infected by each natural enemy.

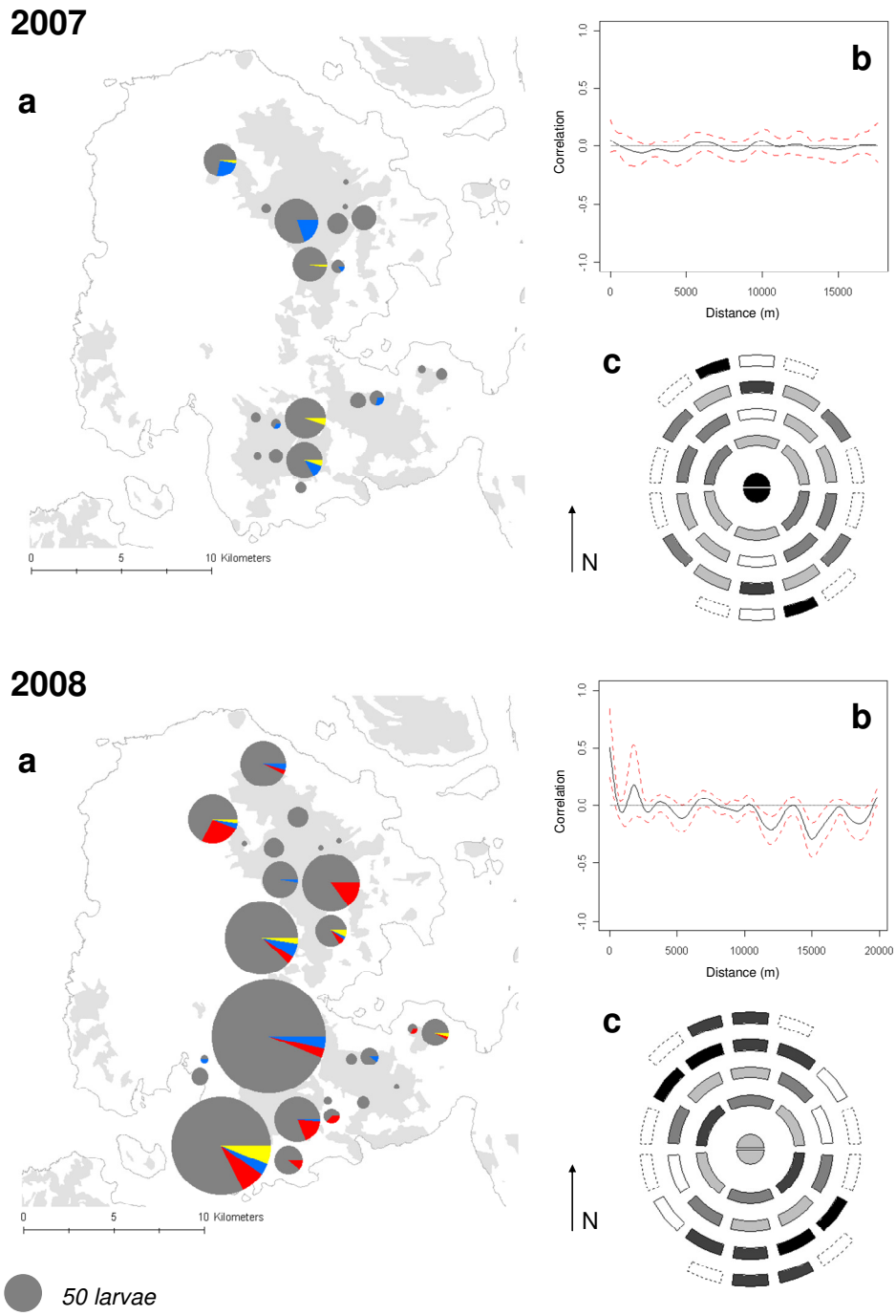


Fig 5.2 (a) Distribution and abundance of *O. brumata* larvae at 36 sites within the Orkney mainland during 2007 and 2008. Light grey areas represent areas of heather moorland. Individual circles represent separate sites; The size of each circle is proportional to the number of individuals within that site: ● = *O. brumata* larvae; ● = no. of larvae infected with OpbuNPV ; ● = no. of larvae infected with RV ; ● = no. of larvae from which parasitoids emerged (2008 only). (b) Within years, spatial correlograms represent the correlation between all pairs of quadrats within sites at all distance classes across mainland Orkney; dashed red lines represent ±95 % confidence intervals; the dotted line represents the point of zero correlation. (c) Windrose correlograms represent the correlation between all points in two dimensions across mainland Orkney, separated into discrete distance and direction classes; distance classes used (from inner to outer concentric rings) are 0-1000m, 1000-4000m, 4000-9000m, 9000-16000m, 16000-25000m; dark shading represents positive correlations, light shading represents negative correlations; full segments represent significant values of Moran's I autocorrelation coefficients; half segments represent non-significant autocorrelations.

Similarly, no significant spatial pattern was evident in two dimensions, although there was a slight positive trend in a north-northwest direction (Fig 5.2, 2007c). Among natural enemy infections, only six of the twenty sites where *O. brumata* were present harboured OpbuNPV infections, with prevalence ranging from 14.3% (BG; n=35) to 50% (BU; n=2). RV infections were present in four of the 20 sites, with prevalence ranging from 2.6% (SET; n=38) to 5.7% (BG; n=35). None of the infections by either natural enemy at any of the sites were identified as significant disease hotspots, outside the 95% confidence limits of all other infections in the region.

In contrast, the total number of *O. brumata* larvae found across the region was substantial increased in 2008 (Table 5.2), with the total number of occupied sites being 25 out of 36, and the largest within-site density being 391 larvae (BU; Fig 5.2, 2008a). This same site also displayed the greatest change in density between the two years, from only two larvae in 2007. Overall, 21 sites displayed increases in larval density between 2007 and 2008, with only six displaying decreases in larval density, the largest of which was a reduction from 20 larvae at FF in 2007 to zero in 2008. Nine sites showed no change in larval density between the two years, of which seven did not have any larvae present in either year. Spatial patterning at small scales was evident among quadrats sampled during 2008, with the point of zero correlation being reached at 627m. A secondary point of positive correlation is also visible at around 2500m, although the lower 95% confidence bound at this distance does not reach above zero (Fig 5.2, 2008b). Overall, there appears to be far more spatial variation in larval abundance within and between sites than during 2007. Again, two-dimensional positive correlations appear to be greatest in the north-northwest direction, especially at larger distances (>9000m), although not significantly so, suggesting a uniform or random distribution of larvae among all sites in the region (Fig 5.2, 2008c). Overall prevalence of OpbuNPV was less in 2008 than 2009 (Table 5.2), with infections found among ten of the 25 occupied sites, and prevalence ranging from 1.8% at BG (n=55) to 50% (AND; n=2). Interestingly, *O. brumata* larvae appear to have been entirely absent from the site with the second highest OpbuNPV prevalence, WT (13%; n=62), the mostly northerly of all the sample sites. RV was found among six of the 25 sites where *O. brumata* were present, which included three of the four sites where it was found in the previous year. The overall prevalence of RV among *O. brumata* larvae remained the same across all sites between the two years (Table 5.2), although the range of prevalence appeared to be greater in 2008, from <1% (BU; n=391) to 7.7% (BRA;

n=26). Parasitoids were present in almost half of sites where *O. brumata* larvae occurred in 2008 (n=12), with a broad range of prevalence from 2.8% (BU; n=391) to 60% (SRH; n=5), although other noticeable high prevalence also occurred at GH (39%; n=54), the most westerly sample site on mainland Orkney. Despite this, prevalence of infection by any of the three natural enemies surveyed did not lie beyond the 95% confident limits for the region, and are therefore not deemed significant disease hotspots (Fig 5.2, 2008a).

A. grossulariata larvae were found at 25 of the 36 sample sites during 2007, with a maximum within-site density of 82 larvae, found at BG (Fig 5.3, 2007a). Among-quadrat densities were autocorrelated up to a distance of 1011m, after which the spatial pattern fluctuates randomly around the point of zero correlation (Fig 5.3, 2007b). Among-site densities were also significantly autocorrelated at small scales in a north-south (0-1000m; I= 0.83, p<0.001***) and northeast-southwest (4000-9000m; I=0.88, p<0.001***) direction (Fig 5.3, 2007c). AbgrNPV was present in 10 of the 25 sites where *A. grossulariata* larvae were found, mostly concentrated in the southern moorland, with an average prevalence across all sites being 21.8% (Table 5.2). The highest prevalence were found at the sites BS (100%; n=2) and GH (79%; n=19), and the lowest at WH (5%; n=20). However, none of these sites were deemed disease hotspots, outside the 95% confidence limits for regional disease prevalence.

A. grossulariata larvae were both less abundant during the following year, and present at fewer sites (17 of the total 36; Fig. 5.3, 2008a). Of these sites, all but one (BoH) harboured larvae during the previous year. A total of 15 sites had densities lower than the previous year, the largest loss being at BG (2007 = 82; 2008 = 8) in the south, and the largest gain being at QF (2007 = 11; 2008 = 43) in the north, which was also the highest within-site density among all sites during 2008. A total of 14 sites showed no overall change in density between the two years, of which 10 did not contain *A. grossulariata* larvae in either year. Again, larval densities were spatially correlated up to a maximum distance of 1839m, fluctuating randomly at all distances beyond this, with the exception of distances greater than 19 500m. Beyond this point, densities are again correlated, possibly reflecting the fact that fewer larvae appear to be present in the central moorlands than in the northern and southern extremes (Fig 5.3, 2008b). Also consistent with the previous year, larval densities were significantly correlated in a northeast-southwest direction, however this time only within the 4000-9000m distance class (I= 0.28; p=0.02*). Beyond this, correlations between different distance and

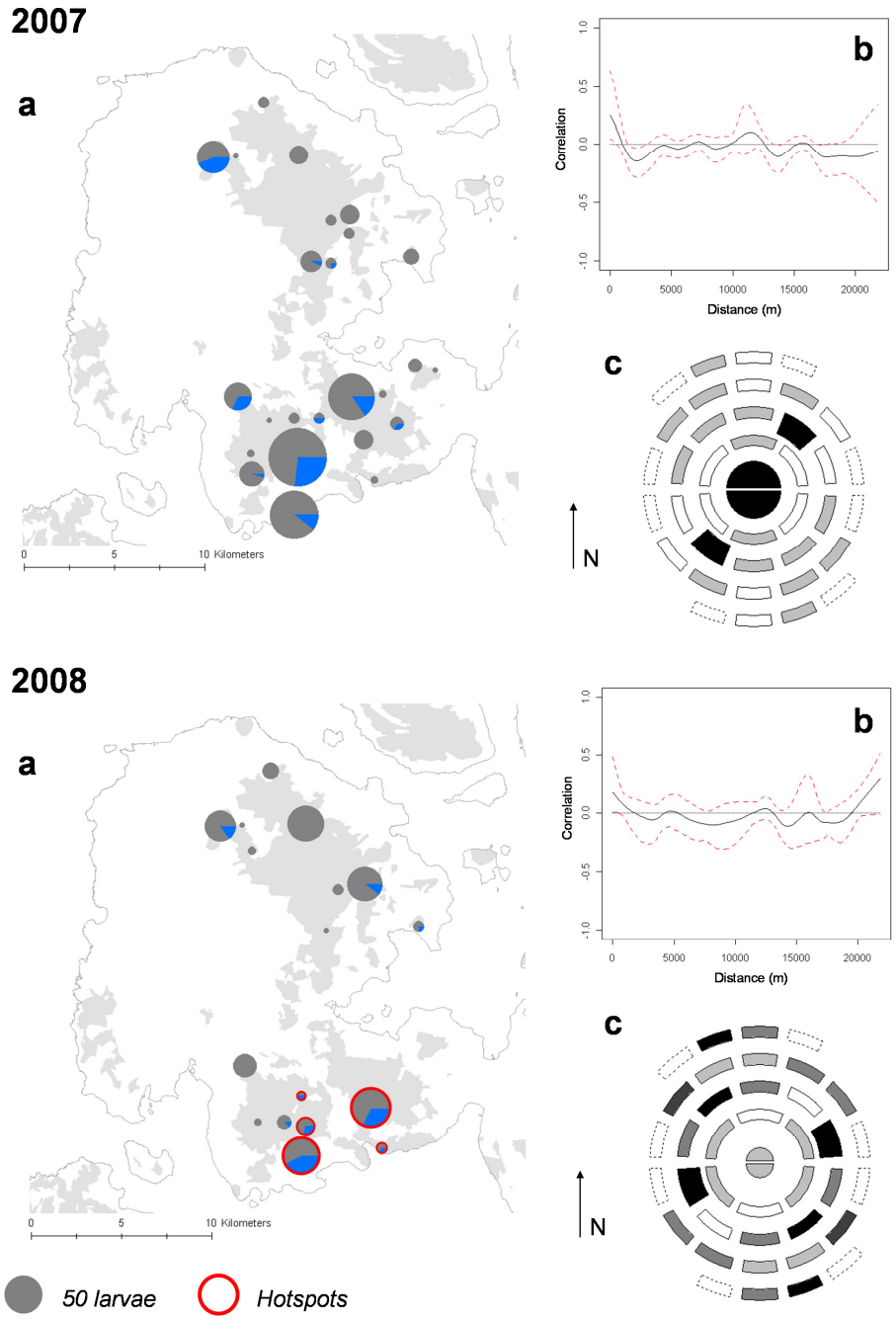


Fig 5.3 (a) Distribution and abundance of *A. grossulariata* larvae at 36 sites within the Orkney mainland during 2007 and 2008. Light grey areas represent areas of heather moorland. Individual circles represent separate sites; The size of each circle is proportional to the number of individuals within that site: ● = *A. grossulariata* larvae; ● = no. of larvae infected with AbgrNPV; Circles with red borders represent disease hotspots, where within site prevalence of infection is greater than expected compared to prevalence at other sites across the region, as calculated by Getis-Ord G statistics. (b) Within years, spatial correlograms represent the correlation between all pairs of quadrats within sites at all distance classes across mainland Orkney; dashed red lines represent ±95 % confidence intervals; the dotted line represents the point of zero correlation. (c) Windrose correlograms represent the correlation between all points in two dimensions across mainland Orkney, separated into discrete distance and direction classes; distance classes used (from inner to outer concentric rings) are 0-1000m, 1000-4000m, 4000-9000m, 9000-16000m, 16000-25000m; dark shading represents positive correlations, light shading represents negative correlations; full segments represent significant values of Moran's I autocorrelation coefficients; half segments represent non-significant autocorrelations.

direction classes appear to be more randomly distributed across the study sites (Fig 5.3, 2008c). The total prevalence of AbgrNPV infection among all sites remained fairly consistent between the two years, with a small overall increase in 2008 compared with the previous year (Table 5.2). Among all 17 sites where *A. grossulariata* larvae were found, AbgrNPV was present in 10 of these sites, only four of which harboured AbgrNPV infections in the previous year. Within-site prevalence ranged from 11% (FF; n= 36) to 74% (RD; n=23) and 100% (SRH; n=1), and appeared to be largely clustered in the southern moorlands, where six sites (BG, GW, RD, WH, WK) were identified as being disease hotspots, where AbgrNPV prevalence are greater than expected among all infections across the region (Fig 5.3, 2008a).

5.4.2 Factors influencing host abundance and distribution

Neither the presence/ absence of *O. brumata* larvae within sites, nor the density of larvae within sites, appear to show any strong responses to heather coverage, at any scale. All binomial models had log likelihood values greater than those of a null model with the same response variable, with the very smallest scale (200m) having the best model fit (Δ logLik = 2.2; p=0.037). The remainder of the models at higher scales did not substantially differ from the null model, and none of the models at any scale were significant below the Bonferroni threshold. Similarly, none of the Gaussian models of host density within quadrats had significant responses to heather coverage at any scale, although model fits were substantially better at smaller scales. None of the models were significant below p=0.05 threshold either, and although all Gaussian models had log likelihood values lower than the null model, it should be noted that this is most likely due to the addition of the spatial autocovariate, which, because it is derived from the response variable, accounts for a large proportion of the variation in model fits (Fig 5.4a).

Similarly, *A. grossulariata* displayed no clear response to heather cover among either the binomial presence/absence models or the Gaussian larval density models (Fig 5.4b). Although not substantially different among any scales, binomial responses did appear to peak at around the 1800m scale (Δ logLik = 1.5; p=0.08) and remain fairly constant at all scales beyond this point. Again, all Gaussian models had log likelihood values below that of the null model, with a similar pattern to that of *O. brumata,* with greatest model fits being at smaller scales, steadily decreasing as scale increases. The

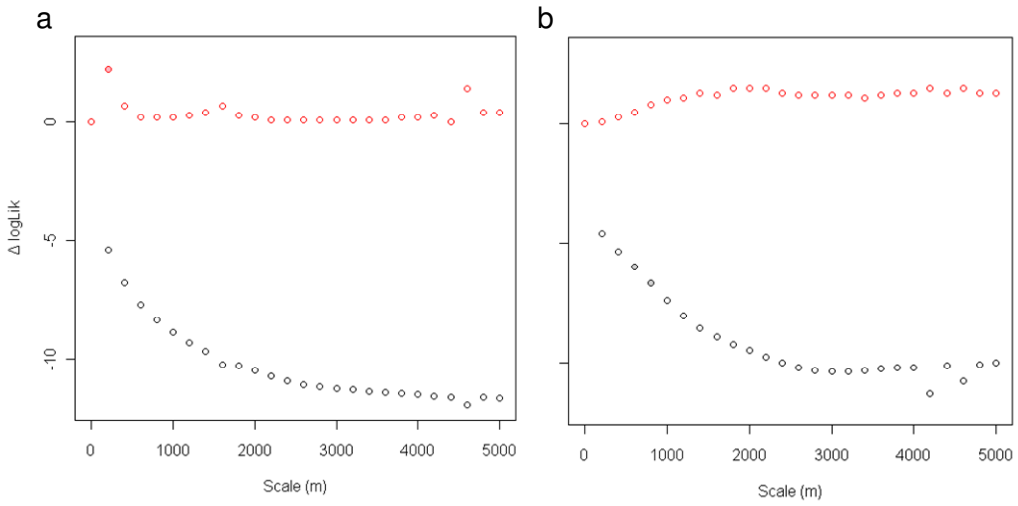


Fig 5.4 Scale-dependent responses to heather cover by a) *O. brumata* and b) *A. grossulariata.* Scale represents the circle radius of heather cover extracted from a habitat map of Orkney, with each circle centred over a sample site. Red circles indicate a binomial presence/absence GLMM model of larval occurrence within each quadrat, with sample year and sample day specified as random factors; Black circles indicate a Gaussian LME of (log) larval abundance within quadrats, with sample year and sample day as random factors. Within each scale-model, solid circles indicate significant models after Bonferroni correction ($p<0.002$) and light filled circles indicate models where $p<0.05$. Δ logLik refers to the change in log likelihood values of each scale-model relative to a null model.

best model fits were again at the 200m scale, although the only models that had responses below p=0.05 were at the 600m (p=0.043*) and 800m (p=0.044*) scales. None of the models were below the Bonferroni threshold.

Principal components analysis of the 10 within-site variables reduced the number of variables to be used in subsequent GLMM and LME models to just four, based on each principal component explaining greater than 10% of the variance of all variables. The cumulative explained variance of these first four components was 74% (Table 5.3), and this was deemed a sufficient number of components to maximise the explained variation whilst minimising the number of variables. PC1 contained three loadings (Heather area, Soil moisture, Soil Carbon) that were noticeably greater than most others, with all three having positive loadings of around the same magnitude (0.43-0.45). This can be interpreted as variation in different soil characteristics having similar, or collinear, properties which may be manifest in explaining where heather is most likely to be found—soils with high levels of carbon and moisture, as found in peatland moors, appear to be linked to larger areas of heather moorland than when carbon and moisture content is lower. The second principal component (PC2) had the greatest loadings among the three topographical variables elevation, slope and aspect.

	Transformation	PC1	PC2	PC3	PC4
Elevation		0.27	**0.52**	-0.17	**0.38**
Slope		-0.28	**0.50**	-0.11	0.28
Aspect	Linear	0.17	**-0.38**	**-0.50**	0.26
Solar radiation	Squared	0.11	0.25	0	**-0.70**
Heather height		-0.25	-0.22	**0.46**	0.22
Heather area	Arcsin √	**0.45**	0.34	0.13	0.14
Shoot : wood ratio	Arcsin √	0.33	-0.23	**-0.41**	-0.11
Soil moisture	Logistic	**0.44**	-0.11	0.08	0.07
Soil carbon	Logistic	**0.43**	0.02	**0.41**	-0.16
Grazing		-0.23	0.23	-0.37	**-0.33**
Standard deviation		1.78	1.25	1.19	1.10
% variance		32	16	14	12
Cumulative % variance		32	48	62	74
PC Name		Soil	Topography	Heather	Solar radiation

Table 5.3 Loadings from the first four principal components of a Principal Components Analysis (PCA) of 10 within-site variables among all 36 field sites on mainland Orkney, which together explain 74% of the variation among all variables. Transformation represents the data transformation performed on each variable prior to PCA; PC represents each principal component, with PC1 explaining the largest amount of variance. The three variables with the highest loadings within each PC are highlighted in bold, and form the basis of the generic name used for each PC when used as an explanatory variable in GLMM or LME models.

This is perhaps not surprising, given that both slope and aspect are derived from the DEM coverage of Orkney, although its is worth noting that the underlying trend among the three variables is for increasing elevation, increasing slope, and more southerly-facing aspects, as PC2 increases. PC3 is also associated with southerly-facing slopes as the largest loading, and increasing heather height as the second-highest. The next largest loadings are split equally between shoot:wood ratio (negatively), and soil carbon content (positively). Together, PC3 accounts for 14% of the total variation among all variables, and the colinearity in the data is interpreted as being largely driven by variation in heather: southern-facing slopes with greater soil carbon giving rise to taller, more woody heather. Finally, increased loadings on PC4 (12% explained variance) are most associated with decreases in the amount of incoming solar radiation within sites. This loading was substantially greater than all other loadings on PC4, and among all of the other principal components. For this reason, PC4 is interpreted as largely reflecting changes in this single variable. Other loadings on this axis that were collinear with decreasing solar radiation were: increasing elevation (0.38) and decreasing grazing pressure (-0.33). The fact that elevation appeared to correlate negatively with solar radiation is perhaps surprising, although it is worth noting that the changes in elevation

within Orkney are relatively small and therefore not likely to be influenced greatly by atmospheric influences. It seems most likely that this relationship is due to the colinearity on the PC4 axis of decreased solar radiation with more northerly facing aspects (Aspect = 0.26).

The presence of *O. brumata* among sites across the Orkney mainland was most strongly associated with the highest-order interaction term among all four principal components (p=0.010*). Although this makes interpretation of the meaning of such a result problematic, it does suggest that no single principal component is substantially driving the distribution of larvae among sites. Although such a result represents a minimum adequate model with the largest possible number of explanatory terms, the AIC value was substantially larger than that of a null model with no explanatory variables (ΔAIC=9). As AIC penalises less parsimonious models, this would suggest that such a result is unlikely to be derived from a type II error. Within this model, when single terms are considered, only PC3 and PC4 had significant associations with the presence of larvae. Although caution should be taken interpreting the significance of terms within a larger model, this does suggest that either of these two terms may be contributing greater explanatory power than either PC1 or PC2. Indeed, only when PC4 is removed from all three-way interaction terms, is there no longer a significant interaction (PC1:PC2:PC3; p=0.316) with larval presence, suggesting that changes in solar radiation, elevation, and grazing, may be driving the ability of *O. brumata* to persist in particular habitats, above any other of the measured variables combined in the PCA (Table 5.4; *O. brumata* binomial). Such an interpretation tallies well with that found among the Gaussian models of larval abundance within sites where *O. brumata* is present, with changes in abundance being most strongly associated positively with a two-way interaction between PC1 (Soil) and PC4 (Solar radiation) (Table 5.4; *O. brumata* Gaussian; PC1:PC4, p=<0.001***). This minimum adequate model has substantially more explanatory power than a null model (ΔAIC=71.2), and when included with single-order terms, only PC4 shows any significant association with changes in larval abundance (p=0.004**). Given such an association among two separate analyses, it seems likely that PC4 is exerting the most influence on *O. brumata* on mainland Orkney, and this is interpreted as a combination of reduced solar radiation at higher elevations and north-facing slopes facilitating the survival of increased numbers of *O. brumata* larvae, compared to other topographical characteristics. Reduced grazing pressure within sites may also contribute to the persistence of larger

	O. brumata				*A. grossulariata*			
	Binomial		**Gaussian**		**Binomial**		**Gaussian**	
	Estimate	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value
(Intercept)	-3.631	<0.001***	0.424	0.043*	-3.548	<0.001***	1.399	<0.001***
PC1 (Soil)	-0.198	0.133	-0.015	0.800	-0.382	<0.001***	0.123	0.003**
PC2 (Topography)	0.150	0.304	0.036	0.619	0.190	0.051	0.039	0.542
PC3 (Heather)	0.469	0.014*	0.099	0.075	1.088	<0.001***	0.386	<0.001***
PC4 (Solar radiation)	0.962	0.004**	0.150	0.004**	-0.131	0.288	0.010	0.856
PC1:PC2	0.271	0.026*					-0.121	0.006**
PC1:PC3	-0.118	0.210			0.242	0.001**	0.133	<0.001***
PC2:PC3	0.544	0.002**					0.155	0.024*
PC1:PC4	0.430	0.022*	0.168	<0.001***				
PC2:PC4	-0.638	0.015*						
PC3:PC4	-0.923	0.001**						
PC1:PC2:PC3	-0.112	0.316						
PC1:PC2:PC4	-0.437	0.045*						
PC1:PC3:PC4	-0.292	0.036*						
PC2:PC3:PC4	0.851	0.015*						
PC1:PC2:PC3:PC4	0.744	0.010*						
Spatial Autocovariate	5.404	<0.001***	<0.001	0.993	3.430	<0.001***	-0.641	<0.001***
Δ AIC	9		71.2		45		37.6	

Table 5.4 Results of GLMM (binomial) models of presence/absence of larvae within quadrats, and LME (Gaussian) models of number of larvae per quadrat, for *O. brumata* and *A. grossulariata* using the first four principal components from a PCA of 10 within-site variables (see Table 3) as interactive explanatory variables, with sampling date and sampling year as random factors. The highest order significant interaction term is given for each model, after backwards stepwise simplification. Spatial autocovariates within models account for residual autocorrelation, and are retained for each model; ΔAIC represents the change in AIC of the minimum adequate model, when compared to a null model with only a constant and the spatial autocovariate. Significance codes are p<0.05*; p<0.01**; p<0.001***.

local populations, possibly as a by-product of reduced stocking densities in more remote (i.e. high altitude) areas of moorland. Fig 5.5 shows the individual relationships between combined larval abundance within sites, and both of the loadings among the two significant principal components of the minimum adequate model. Also shown is the relationship between larval abundance and each of the three variables that contribute most to the definition of PC4 (solar radiation).

In contrast to the binomial *O. brumata* model, the presence of *A. grossulariata* among sites was most strongly associated with a single two-way interaction between PC1 (Soil) and PC3 (Heather) (Table 5.4; p=0.001**). The additive single terms of each of these principal components were also the only two components to show any significant relationship under this minimum adequate model, PC1 being negatively associated (estimate = -0.382), and PC3 positively associated (estimate = 1.008). The explanatory power of this model relative to the null was also substantially greater (ΔAIC=45). Similarly, under a Gaussian model of (log) larval abundance per quadrat, the PC1:PC3 interaction was retained under the minimum adequate model as being

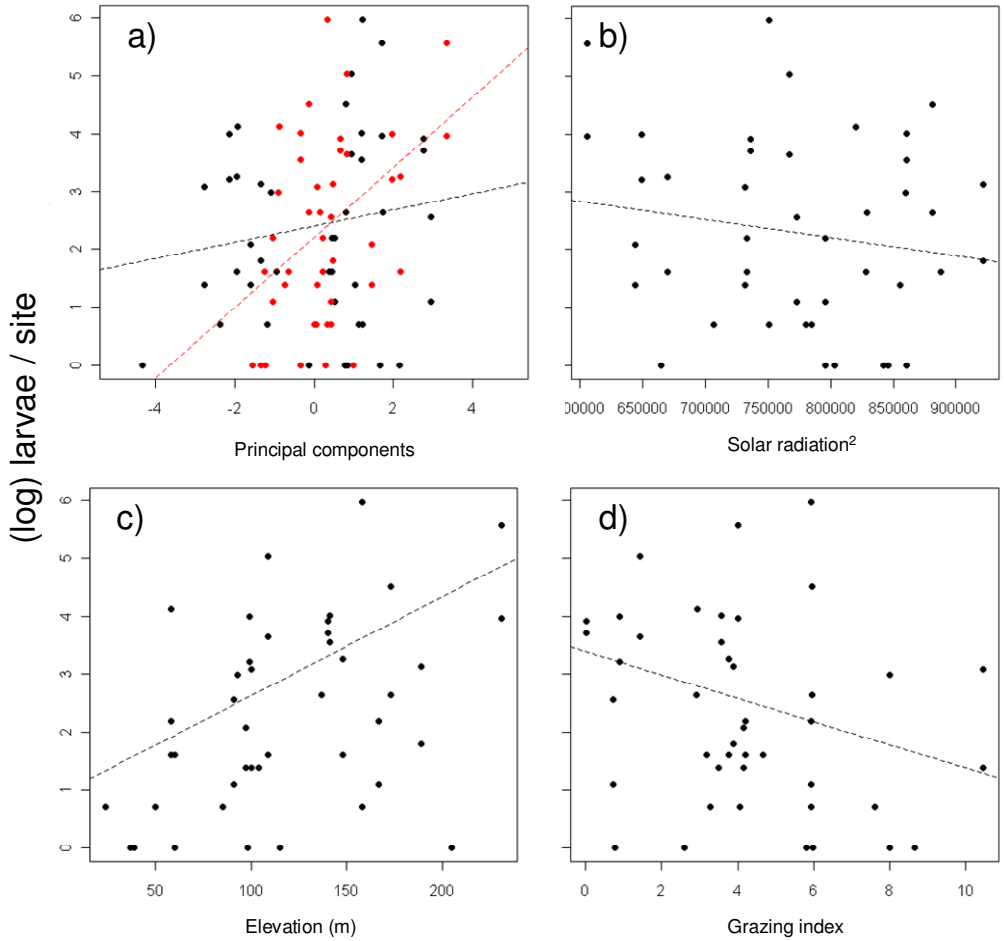


Fig 5.5 Relationship between pooled (log) larval abundance of *O. brumata* within sites and (clockwise, from top left) a) each of the significant principal components identified as having significant interactive associations with larval abundance per quadrat in a Gaussian LME (see Table 5.4). Black circles represent PC1 (Soil) loadings and red circles represent PC4 (Solar radiation) loadings; b) Solar radiation within sites after squared-transformation, as used in Gaussian LME; c) Elevation of each site above sea level (m); d) Grazing index of each site, as measured by number of sheep per hectare of rough grazing. Among all graphs, dashed lines are illustrative only, and represent the linear relationship of pooled larval abundance per site with each of the five different explanatory variables, derived from a linear model specifying no random effects.

highly significantly associated ($p<0.001$***). However, two other paired interaction terms (PC1:PC2 and PC2:PC3) were also retained, suggesting that changes in topography (PC2) may also be mediating this interaction. Again, the minimum adequate model after stepwise deletion has substantially more explanatory power than the null model ($\Delta AIC=37.6$). When viewed as single additive terms in the final model, PC2 does not show any significant association with changes in larval abundance, and whilst the positive association with PC3 (estimate = 0.386; $p<0.001$***) is congruent with the final binomial presence/absence model, under the Gaussian model PC1 appears to show a reversal in the relationship compared with the binomial, with an overall positive

association (estimate = 0.123; p=0.003**) under the Gaussian. This discrepancy between the two models is interpreted as possibly being due to a threshold being imposed by soil characteristics that limits where *A. grossulariata* are able to persist. For example, if soils are particularly water logged or contain high levels of carbon, the quality of the heather as a resource for *A. grossulariata* larvae may be insufficient to support even small numbers of larvae, which may then be entirely absent from such sites. Similarly, mobile larvae may disperse away from such areas before sampling. This could therefore create a discrepancy between models, whereby changes in abundance where larvae are already present is mediated by an interaction between soil characteristics (PC1) and heather characteristics (PC3), but only below a certain threshold where larvae are able to survive. It therefore seems likely that the most important principal component governing *A. grossulariata* abundance within sites is PC3 (Heather), which also contains a soil carbon component among the highest loadings. Figure 5.6 shows the relationship between pooled (log) larval abundance within sites for *A. grossulariata* against each of the two most significant interactive principal components (PC1:PC3), together with the breakdown of each of the four attributes with the highest loadings within PC3 (Heather). Clearly, southern-facing slopes appear to be supporting the highest densities of *A. grossulariata* larvae where they are present (Fig 5.6b), mediated by taller (Fig 5.6c), more woody (Fig 5.6d) heather, growing among more carboniferous soils (Fig 5.6e).

5.4.3 Insect host-natural enemy interactions

For *O. brumata* larvae in 2007, the variance in host distribution appears to be largely evenly split between both sites and quadrats (Table 5.5), reflecting a fairly uniform distribution of larvae at both small and large spatial scales. During this year, OpbuNPV infections show greatest variability between sites, possibly reflecting a localised spatial restriction of infections within sites. In contrast, RV infections during this year were most highly variable between quadrats. However, it should be noted that the log likelihood values among the two null models were identical, suggesting that this could be an unsafe supposition—the total number of RV infections among both sampling years was very low, making generalisations about infections difficult. During the following year, when *O. brumata* host densities had increased among almost all sites (see Fig 5.3a, 2008), the majority of variance among hosts was partitioned among sites,

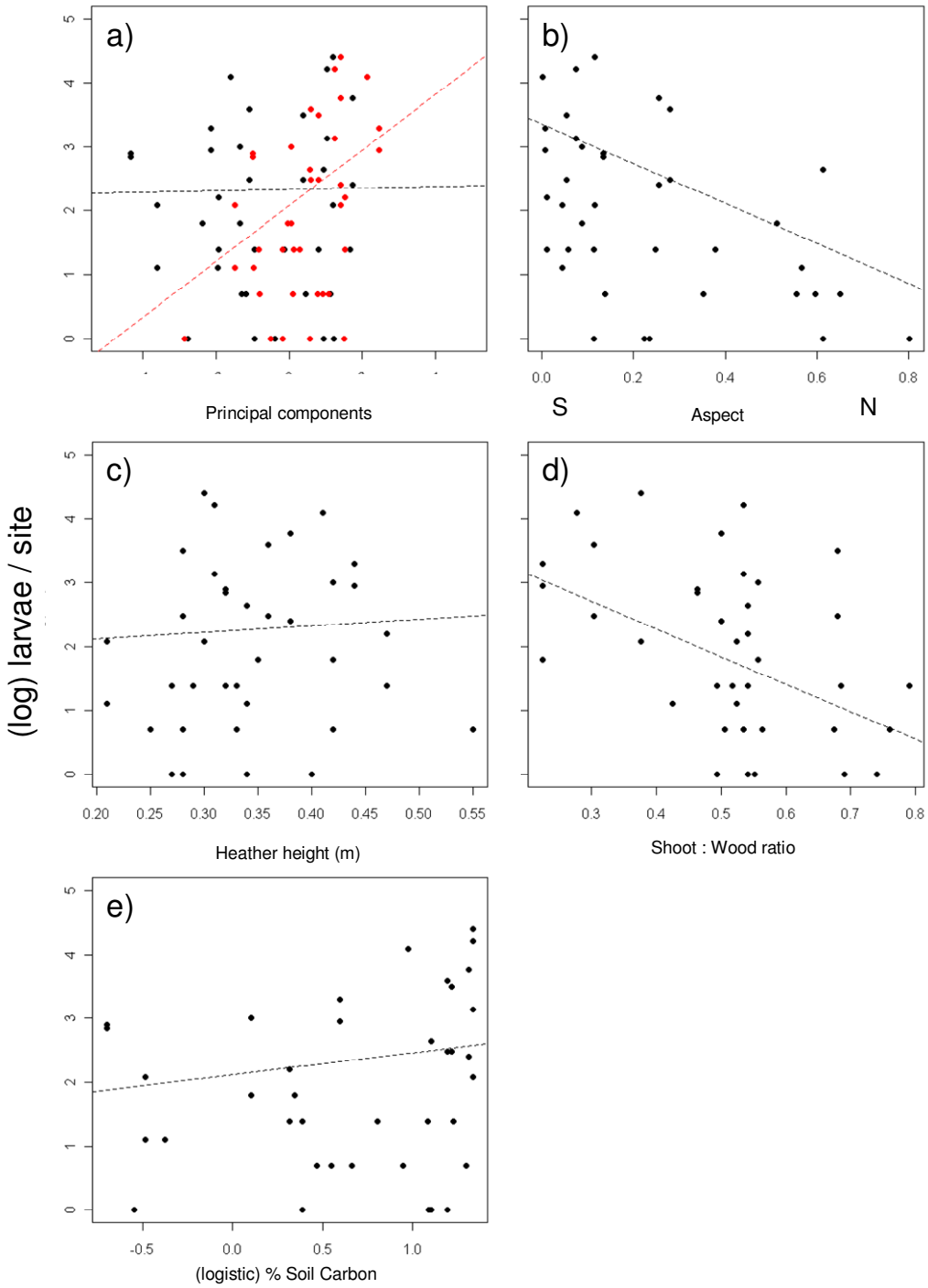


Fig 5.6 Relationship between pooled (log) larval abundance of *A. grossulariata* within sites and (clockwise, from top left) a) each of the significant principal components identified as having significant interactive associations with larval abundance per quadrat in a Gaussian LME (see Table 5.4). Black circles represent PC1 (Soil) loadings and red circles represent PC3 (Heather) loadings; b) Aspect within sites after linear transformation for circular data, as used in Gaussian LME; c) Average heather height (m) within each site; d) Average shoot:wood ratio of heather within each site, as measured by plant wet weight; e) % soil carbon after logistic transformation within each site, as measured by loss-on-ignition. Among all graphs, dashed lines are illustrative only, and represent the linear relationship of pooled larval abundance per site with each of the five different explanatory variables, derived from a linear model specifying no random effects.

Species	Year	Natural enemy	Scale	% variance	logLik
O. brumata	*2007*		Site	41	-65.73
			Quadrat within Site	59	-66.15
		OpbuNPV	Site	97	-47.94
			Quadrat within Site	3	-57.49
		RV	Site	<1	-21.07
			Quadrat within Site	99	-21.07
	2008		Site	97	-299.6
			Quadrat within Site	3	-610.4
		Parasitoid	Site	99	-112.9
			Quadrat within Site	<1	-143.1
		OpbuNPV	Site	<1	-56.18
			Quadrat within Site	99	-56.18
		RV	Site	89	-49.19
			Quadrat within Site	11	-46.7
A. grossulariata	*2007*		Site	91.5	-88.14
			Quadrat within Site	8.5	-103
		AbgrNPV	Site	99	-91.1
			Quadrat within Site	<1	-115.5
	2008		Site	99	-42.85
			Quadrat within Site	<1	-47.16
		AbgrNPV	Site	99	-61.65
			Quadrat within Site	<1	-87.49

Table 5.5 Hierarchical variance components analysis of *O. brumata* and *A. grossulariata* hosts, and infection by each natural enemy during 2007 and 2008, for all sites across the Orkney mainland. Variance components are extracted from the random effects of null models of each response variable, with Site or Quadrats within Sites specified as random factors. Host larval variance components are extracted from a Gaussian LME with (log) larval density per quadrat; Natural enemy variance components are extracted from a binomial GLMM of ratio infected to uninfected larvae per quadrat. logLik refers to the log likelihood of each model fits.

reflecting high localisation of changes in abundance at the site scale. Although data on parasitoid abundances is not available from the previous year, in 2008 the vast majority of variance among parasitoid infections was partitioned even more strongly than host larvae among sites, suggesting that infection prevalence by parasitoids is similarly non-uniformly distributed within the mainland Orkney, or else highly attuned to variance in host larval abundance. In contrast to the previous year (when total prevalence was higher, and host abundance lower), OpbuNPV variance in infection was almost entirely partitioned at the quadrat scale, possibly reflecting the general trend in decreased total infection prevalence, and the absence of localised infection hotspots within sites. RV

infections were largely partitioned among sites during this year, again possibly reflecting variance in host distribution.

Unlike the highly variable interactions between *O. brumata* hosts and their natural enemies, both *A. grossulariata* hosts and infections by AbgrNPV displayed remarkable congruence both within and among years—possibly reflecting the stability of host abundance and prevalence previously outlined in Table 5.2 and Fig 5.4. The majority of both host larvae and viral infection variances were partitioned among sites, reflecting high spatial variability within years across the region, but seemingly, low temporal variability in abundance and infection across the region.

Among *O. brumata* larvae, OpbuNPV infections during 2007 were spatially autocorrelated up to an initial distance of 775m, followed by a secondary correlation peak between 5000-8000m (Fig 5.7). Prevalence of OpbuNPV infections during this year across the region was fairly high during this year (Table 5.2; 10.7%), and when compared to the spatial correlogram of host abundance among sites (Fig 5.2b, 2007), there is little congruence, suggesting that OpbuNPV infections may be highly spatially variable both within and among sites. Indeed, the fact that less than ⅓ of sites where *O. brumata* larvae were found during 2007 sampling harboured OpbuNPV infections supports this, with the spatial patterns of infection reflecting the spatial aggregation of infections among the small number of sites where such infections occurred (see Fig 5.2a, 2007). The following year, when infection prevalence is reduced across the region (Table 5.2; 3.73%), there appears to be no spatial pattern in infection, despite the small-scale spatial patterning apparent among host larvae among quadrats (Fig 5.2a, 2008). This is perhaps surprising, given the overall increase in the number of occupied sites harbouring OpbuNPV infections (~40%), and may reflect a more uniform spatial spread of infection when prevalence are lower. The spatial pattern of RV infection among larvae during 2007 also appears to show no obvious spatial patterning, largely reflecting the uniform distribution of hosts during this year. In the following year, host spatial patterning was more variable at small scales than during 2007, and this appears to feed through into patterns of RV infection, with a small positive correlation among quadrats within 400m of each other. Infection prevalence among sites during 2008 was no different than the previous year (Table 5.2; 2.5%), suggesting that such a pattern is largely due to a small number of closely situated sites harbouring infection, despite the lack of any identified hotspots during this year (Fig 5.2a, 2008). Parasitoid infections during this year were spatially autocorrelated to mean distance of 1780m, representing

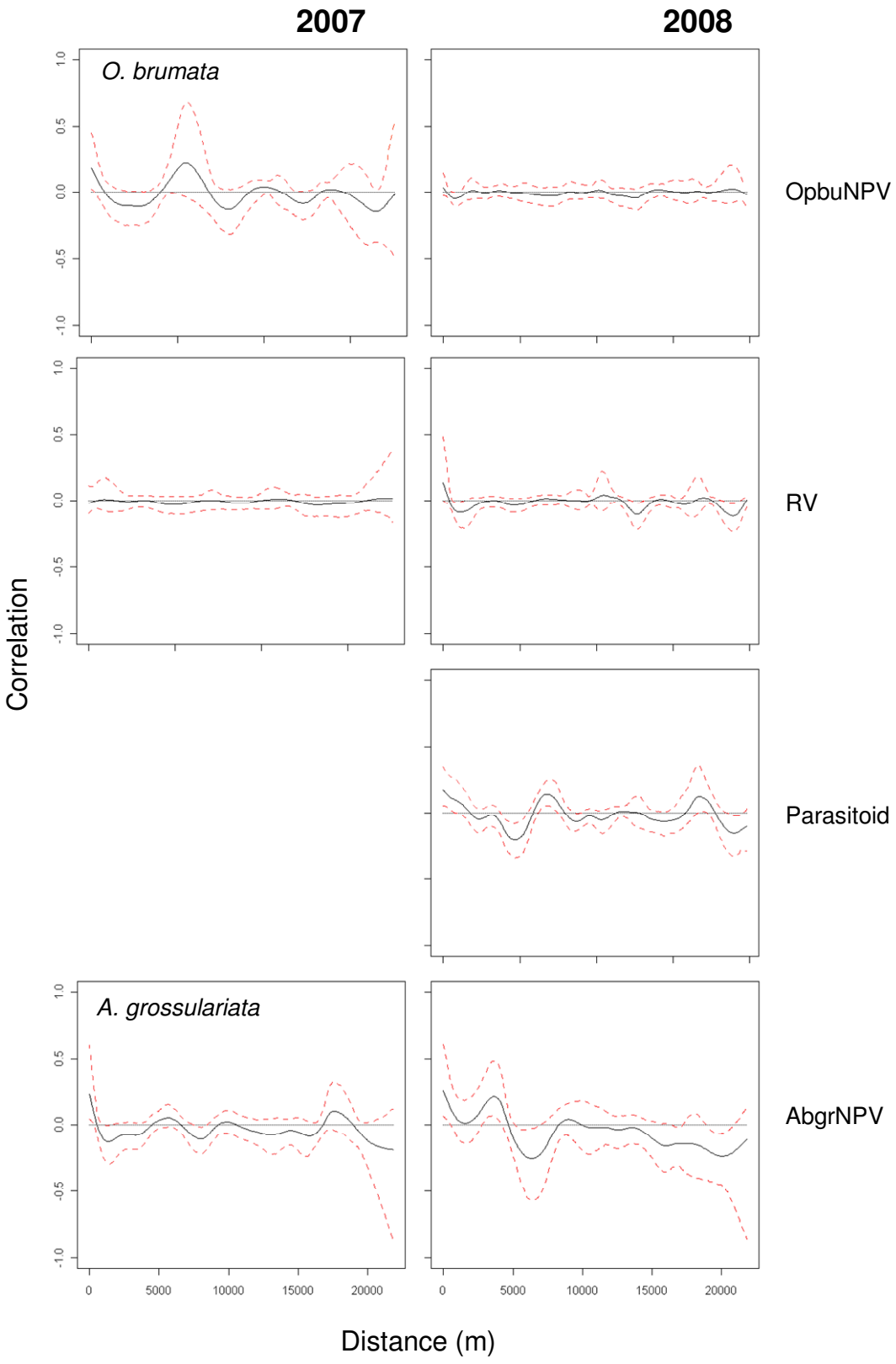


Fig 5.7 Spatial correlograms of number of *O. brumata* (above) and *A. grossulariata* (below) larvae infected with different natural enemies among all distances between pairs of quadrats within the 36 sampling sites across mainland Orkney. Solid lines indicate the mean correlation coefficient between pairs of quadrats at that distance apart; Dashed red lines indicate the 95% confidence limits of that coefficient; Dotted lines indicate the point of zero correlation;

spatial patterning at larger scales than either OpbuNPV and RV, and among *O. brumata* host larvae during both years of sampling. However, like OpbuNPV in 2007, there again appears to be a secondary point of positive correlations between 5000-10000m, although in the case of parasitoid infections, this is also accompanied by a tertiary peak at larger scales (15000-20000m). Although overall parasitoid prevalence in 2008 was less than OpbuNPV prevalence in 2007 (Table 5.2), the number of sites harbouring parasitoid infections was greater (Fig 5.2a; 50%), possibly conferring a more uniform distribution across the region, especially at larger spatial scales.

Despite relatively similar prevalence across the two sampling years, AbgrNPV infections among *A. grossulariata* larvae displayed little congruence in spatial patterning of infection between 2007 and 2008 (Fig 5.7). Infections during 2007 appear to follow closely the spatial distribution of hosts in that year (Fig 5.3a, 2007), with the exception of a small secondary correlation peak at around 17000m, being autocorrelated up to a mean distance 0f 527m—about half that among host larvae. Although initially, infections among larvae from 2008 follow that of hosts from the same year (Fig 5.3b, 2008), a secondary peak of infection is apparent, at distances just less than 5000m, after which the spatial pattern disappears. Such a pattern is most likely to be due to the apparent separation of sites where *A. grossulariata* are found into the relatively AbgrNPV-free north moorlands, and the hotspots identified in Fig 5.3b (2008) in the southern moorlands.

The fact that many of the spatial patterns of infection, among all types of natural enemy attacking both *O. brumata* and *A. grossulariata* larvae (Fig 5.7), appear to display little or no congruence with the spatial patterns in the distribution of hosts (Fig 5.3b, Fig 5.4b; 2007, 2008) across the Orkney mainland, is reflected in the lack of significant associations of infection with host density among almost all natural enemies, all scales, and all years (Table 5.6). The only significant association found among all the natural enemies was a positive density dependent association between *O. brumata* hosts and RV infections during 2007, at the quadrat scale (p=0.0063**). However, it should be noted that only eight RV infections were found among a total *A.* of 150 quadrats where *O. brumata* occurred during this year, making inferences of statistical significance particularly problematic regarding such small sample sizes. Although residuals were not autocorrelated within this model, the AIC value of this model was only within four AIC units of the null—below the seven AIC unit threshold whereby models are considered substantially different. Density dependent associations between

RV infections and *O. brumata* hosts also were not manifest at larger spatial scales, when host densities and infections are pooled by site (p=0.22).

Species	Year	Natural enemy	Scale	Moran's I (p-value)	Effect size	logLik	Δ logLik	p-value
O. brumata	**2007**	OpbuNPV	Site	-0.066 (0.5)	-0.025	-17.88	0.29	0.44
			Quadrat	-0.0068 (0.38)	0.071	-48.41	0.28	0.44
		RV	Site	-0.051 (0.36)	0.034	-3.836	0.89	0.22
			Quadrat	-0.01 1(0.68)	0.352	-16.8	3.81	0.0063 **
	2008	Parasitoid	Site	-0.0059 (0.17)	0.001	-23.05	0.04	0.76
			Quadrat	-0.0017 (0.22)	-0.012	-111.8	0.04	0.39
		OpbuNPV	Site	-0.043 (0.39)	0.001	-13.85	0.34	0.41
			Quadrat	-0.006 (0.72)	0.007	-55.93	0.07	0.76
		RV	Site	-0.059 (0.56)	0.034	-10.79	0.53	0.32
			Quadrat	-0.0059 (0.7)	-0.001	-42.16	0	0.98
	2008 delayed	Parasitoid	Site	-0.019 (0.23)	0.011	-23.66	0.18	0.55
			Quadrat	-	-	-	-	-
		OpbuNPV	Site	-0.039 (0.37)	0.007	-13.94	0.26	0.46
			Quadrat	-	-	-	-	-
		RV	Site	-0.05 (0.46)	0.028	-18.28	0.02	0.10
			Quadrat	-	-	-	-	-
A. grossulariata	**2007**	AbgrNPV	Site	-0.031 (0.28)	0.036	-24.38	1.49	0.11
			Quadrat	-0.0076 (0.51)	-0.045	-90.78	0.52	0.31
	2008	AbgrNPV	Site	0.02 (0.15)	-0.019	-16.38	0	0.12
			Quadrat	-0.015 (0.66)	0.023	-63.29	-63.31	0.86
	2008 delayed	AbgrNPV	Site	0.089 (0.07)	0.01	-18.28	0.02	0.82
			Quadrat	-	-	-	-	-

Table 5.6 Results of GLMM models of ratio of infected to uninfected individuals of *O. brumata* and *A. grossulariata* by different natural enemies vs host density at two different spatial scales and two different years. "Site" scale represents the pooled ratio of infected to uninfected larvae per site (max. n=36) as response variable and total number of larvae per site as explanatory variable, with additional spatial autocovariate, in a weighted binomial GLMM with sampling date specified as random factor; "Quadrat" scale represents the ratio of infected to uninfected larvae per quadrat (max. n=1080) as explanatory variable, with total number of larvae per quadrat as explanatory variable, with additional spatial autocovariate, in a weighted binomial GLMM with sampling date as random factor. "Delayed" represents the weighted binomial GLMMs of 2008 as specified above at the "Site", but with the previous years density as explanatory variable, to look for delayed effects of host density on current years infection. Moran's I represents the global residual spatial autocorrelation test, plus significance value, of each GLMM model (10^3 permutations); Estimate represents the overall trend in the model; logLik represents the log likelihood of each model fit; ΔlogLik represents the change in log likelihood value of each model relative to a null model with a constant and spatial autocovariate; Significance codes are p<0.05*; p<0.01**; p<0.001***.

No other significant associations were apparent among either *O. brumata* or *A. grossulariata* larvae infected with any other natural enemy, and host density either within the current year, or when considering a delayed density-dependent effect from the previous year. All models had ΔAIC values less than two, and can therefore be considered indistinguishable from null models. The overall trend in RV infections in 2008 was negative; among OpbuNPV, quadrat-scale infections in both 2007 and 2008, and among delayed-effects models, the overall trend with host density was positive (although this did change with scale); parasitoid infections at the quadrat scale had a negative trend, whilst delayed-effects and site-scale models were positive; and among AbgrNPV infections, the overall trend with *A. grossulariata* density changed both among years, and among scales within years.

5.5 Discussion

The high spatial and temporal variability among *O. brumata* larvae feeding on heather has been well documented among populations both within Orkney (Picozzi, 1981; Graham *et al.*, 2004) and in mainland Scotland (Kerslake *et al.*, 1996), as well as among populations feeding on deciduous trees in Britain and Scandinavia (Hunter *et al.*, 1991; Tikkanen & Roininen, 2001; Tenow *et al.*, 2007). What is perhaps most surprising is the magnitude of the differences in abundance between the two sampling years. None of the sampling sites during either year were identified as outliers across the region, so it appears unlikely that any could be classified as outbreak events (certainly no site sampled contained larvae at the densities illustrated in Chapter 4). Similarly, the lack of any obvious anisotropic spatial pattern, or large-scale spatial correlations among both years, suggests that whatever factor is most influencing such changes in abundance, it is most likely to be acting across the whole region, rather than localised. Forest pests such as *O. brumata* are known to fluctuate in regular population cycles (Klemola *et al.*, 2002; Tenow *et al.*, 2007), although this has so far not been demonstrated unequivocally among heather-feeding populations. The fact that the majority of sites sampled showed increases in abundance compared to the previous year, also suggests that some region-wide synchronising effect may be driving these changes in abundance. Such phenomena (e.g. Moran effects) are known to occur in other forest Lepidoptera (Klemola *et al.*, 2006), and *O. brumata* are known to display spatially synchronous dynamics among regions, although is it not clear whether such a climate-driven process occurs in this

species (Ims *et al.*, 2004; Nilssen *et al.*, 2007; Hagen *et al.,* 2008). The paucity of temporal data in the present study makes any suppositions about temporal dynamics problematic, although it seems clear that some process is occurring among all populations within the region, be it exogenous (e.g. favourable climatic conditions) or endogenous (e.g. escape from natural enemies, or natural population cycles), to explain the effect. Clearly further data over a number of years will be required to explore these processes in detail.

In contrast to the well studied *O. brumata* populations in Europe and North America, data on fluctuations in *A. grossulariata* populations are almost entirely absent. The reason for this may well be the fact that *A. grossulariata* larvae have only recently been cited in large numbers within the last decade, especially in northern Scotland, after their apparent host plant shift onto heather moorland. Horsfield & MacDonald (2005) report high densities of larvae within heather stands to be highly spatially variable (between $1m^2$ and 25 hectares), although without precise quantification of abundance. The present study suggests that such abundances are both less spatially variable, and less temporally variable than among *O. brumata* larvae, displaying reasonable consistency between the two sampling years across the region. However, like *O. brumata*, there appeared to be some degree of synchronicity among all sites, with the majority posting overall losses, rather than total abundance being driven largely by a single highly-variable site. Again, more data will need to be accrued before generalisations concerning population dynamics can be made in this system. Interestingly, *A. grossulariata* spatial patterns differed from *O. brumata* in their degree of isotropy, with the latter displaying consistent northwest-southeast correlations between sampling years. This species is known to have first been spotted in large numbers on the southern islands of Orkney before reaching the mainland, and expanding northwards, and it remains a possibility that such an anisotropic signal could be indicative of such a northward movement; the lack of signal among *O. brumata* possibly due to more stable dynamics within the region, as a result of longer historical establishment.

Despite such spatial and temporal variation, among neither species could this variation be attributed to the amount of available habitat, at any scale. Although previous work on *O. brumata* has found negative effects of habitat fragmentation on abundance in oak-feeding populations (van Dongen *et al.*, 1994; van Dongen *et al.*, 1998), reduced defoliation in mixed forests with high matrix heterogeneity (Weslowski

& Rowinski, 2006), and reduced outbreak occurrence in areas with increased suitable habitat (Tikkanen & Roinen, 2001), there was no evidence that increased areas of suitable habitat had any influence on the abundance of *O. brumata* in Orkney. Although the literature concerning the effects of habitat fragmentation on species abundance is extensive (*see* Fahrig, 2003), often evoking metapopulation processes (Hanski, 1999; Freckleton *et al.,* 2005), applicable to diversity as well as abundance (Chust *et al.*, 2004; Dumbrell *et al.,* 2008), and not necessarily negative (Grez *et al.*, 2004), such processes may not be of such relevance in this particular system—heather moorland exists largely as a continuous coverage across most of the Orkney mainland, with the north and south moors separated by only a few hundred metres. As such, dispersal driven metapopulation processes seem unlikely to be a major factor governing changes in abundance, certain among the sampling sites used in the present study. Even vegetational diversity is unlikely to have any influence, as in other invertebrate systems (Cappuccino *et al.*, 1998), since alternative food plants, such as deciduous trees, are almost entirely absent, and heather the dominant species among a mosaic of improved grassland. Similarly, there appeared to be little evidence of body-size related scale-dependency among the two species, as has been found in other invertebrates (Holland *et al.*, 2005)—although the presence of *A. grossulariata* among sites had slightly greater model fits at larger scales, this was not significant. Despite the lack of significance of these models, abundances of both species appeared to have stronger responses at small scales than at larger ones, in almost the same pattern, suggesting that, whilst habitat area *per se* may not be driving changes in abundance, some other localised within-site variable may be.

Although the presence/absence of *O. brumata* among sites could not be explained by a single combination of variables, it seems likely that variation in abundance is mediated by a combination of both solar radiation-correlated variables and, to a lesser extent, soil characteristics. Among the latter, such effects may be mediated either i) indirectly: increased fine-scale cover among wetter, more nutrient poor soils, where heather is able to outcompete grass species due to the presence of ericoid mychorrizae (Hartley *et al.,* 2003)—such variation in fine-scale cover may be important in driving among-site variation in abundance, over and above that of total cover at larger scales; or ii) directly: *O. brumata* larvae pupate in the soil, and therefore extremes of moisture—dry soils causing desiccation; wet soils causing saturation—may be important in governing the ability of individuals to survive the pupal phase within

any given site. However, soil characteristics, and competition from competing grass species, may be altered by land use changes such as livestock grazing. The decrease in *O. brumata* larvae within sites with higher grazing indices, as seen in the trend with PC4, suggests that this may be a pertinent factor affecting abundance within sites. Again, such an effect may be mediated either indirectly: increased mineralization of the soil through faeces increases competition from grasses; or directly: browsing of the green shoots not only creates less available resource for developing larvae, but livestock may also inadvertently consume eggs or larvae. In manipulative experiments, increased grazing and nitrogen deposition has been shown to reduce overall heather cover by 40-50% (Hartley & Mitchell, 2005), although crucially, variation in the magnitude of such effects may be mediated by soil type. Livestock grazing on heather moorlands within Orkney has increased greatly over the last two decades due to changes in land use policy, and it is likely that >15% of moorland may now be described as heavily overgrazed (Simpson *et al.,* 1998), with possible negative implications for the islands economy (Hanley *et al.,* 1996). Specifically, increased grazing has been implicated in the recent decline in hen harrier numbers, who hunt and nest in heather (Amar & Redpath, 2005). However, effects on invertebrate community diversity may vary according to species and life-history traits: increased grazing is known to improve hemipteran diversity through increasing plant diversity (Hartley *et al.,* 2003), whilst having positive and negative species-specific effects on carabid abundance (Gardner *et al.*, 1997). In the context of *O. brumata,* it seems most likely that increasing plant diversity via grazing would be detrimental to overall abundance, due to the likelihood that newly established species would be more likely to be inedible grasses, rather than palatable species such as *Erica cinerea* or *Vaccinium myrtillus* (Hartley *et al.*, 2003).

Although elevation and grazing loadings were collinear on PC4, it is difficult to partition the two factors into separate causal effects. It is likely that the grazing levels will be reduced in more remote moorlands with higher elevations, where the distance to low-lying farms is greatest; indeed, utilisation of heather moorlands for rough-grazing in Orkney is known to be more pronounced along the edges of moorlands than in the centre (Simpson *et al.*, 1998), although no significant interaction with proxy measures of isolation (% heather cover in 1km radius) were apparent on this axis. Studies from birch-feeding populations, where grazing pressure is not present, suggest that *O. brumata* outbreak zones are currently undergoing an increase in altitudinal limit, possibly as a response to climate warming (Hagen *et al.,* 2007). Such a phenomenon has

been noted in a number of other Lepidoptera species in Europe (Wilson *et al.,* 2005), and although the elevational distribution of these outbreak zones are similar to those surveyed in the present study (180-270m a.s.l.), they exist in slightly more northerly latitudes (69°40'N). If variation in elevational range is mediating variation in larval abundance in this species, it may be through a number of different mechanisms: temperature-mediated changes in egg survival in more exposed sites could influence the total number of eggs that hatch into the larval stage. Such an effect is known to limit populations of the closely related autumnal moth *Epirrita autumnata* in Fennoscandia (Tenow & Nilssen, 1990), although with a lethal egg temperature of -29°C (MacPhee, 1967), the relatively mild climates on Orkney make this unlikely. Mjaaseth *et al.* (2005) could also find no difference in larval development rates among *O. brumata* along an altitudinal transect of similar elevational range to the present study, despite greater larval abundance at mid to high elevations (~170m). Instead, the authors suggest that differential survival of late-eclosing adults in all but the most exposed sites could be driving the observed changes in abundance, through escape from avian and invertebrate predators. Some species of carabid predators are known to be more abundant at lower altitudes, where thermal conditions are more favourable (Straw *et al.*, 2009). Although carabid predators of *O. brumata* pupae are also abundant in high-altitude moorlands in mainland Scotland, causing inversely-density dependent mortality (Raymond *et al.*, 2002), it is not known what the present distribution of such predators is currently in Orkney. Hansen *et al.* (2006) also found no association with altitude and variation in pupal predation by shrews along an altitudinal transect in birch-feeding populations in Fennoscandia. Escape from parasitism among higher altitude sites, where parasitoids are less able to develop as larvae and/or forage as adults, has also been posited previously as a potential explanation of the occurrence of outbreaks among high altitude heather moorland (Kerslake *et al.*, 1996). However, the altitudinal range of moorland sites in Orkney is low compared to mainland Scotland, and the proportion of larvae parasitized at the highest altitude sites in this study (e.g. AK: elevation = 231m; % parasitised = 10%) was not significantly lower than among any other site sampled, making this unlikely.

A further possibility remains that altitudinal variation might mediate changes in the synchrony of larval emergence with host-plant phenology. Local adaptation to budburst is well known in both tree-feeding (Tikkannen *et al.,* 2006) and heather-feeding (Kerslake & Hartley, 1997) populations in mainland Scotland, although it is

thought that such temporal synchrony has little effect on larval survival and development in the latter habitat. Altitude has also been cited as being the major contributing factor in the timing of adult emergence among populations feeding of both types of host-plant, including heather-feeding populations within the Orkney mainland (Vanbergen *et al.*, 2003). Such responses in deciduous-tree feeding populations are thought to be driven largely by changes in the concentration of foliar nitrogen during the feeding period, being greatest just after the budburst, and growth of the new season's green shoots. However, *O. brumata* appear to be largely insensitive to variation in such traits (Kerslake & Hartley, 1997). Compared to broad-leaved plants, heather represents a poor nutritional resource for developing larvae, due to its low foliar nitrogen, and high concentration of tannins and lignins, causing heather-feeding populations to exhibit reduced body size and fecundity (Kerslake *et al.*, 1997; Vanbergen *et al*, 2003). Increases in the incidence of incoming UV-B radiation may affect this relationship, by inducing the host plant to allocate more resources to the accumulation of UV-B protective compounds (Lavola *et al.*, 2003) at the expense of herbivore protective compounds. Indeed, *O. brumata* have been previously shown to exhibit preferences for UV-B treated leaves (Lavola *et al.,* 1998), and such an effect has also been suggested as a causal mechanism in the pattern of population cycles seen in birch-feeding populations in Fennoscandia, also mediated by variation in altitude (Selas *et al.*, 2004). The strong negative association of changes in *O. brumata* abundance with changes in solar radiation on PC4 suggests that this may not be a plausible explanation for the patterns observed among Orkney populations. However, it should be noted that enhanced UV-B irradiation may also have detrimental effects to developing larvae, as well as host foodplants: some insect species may actively avoid areas of high UV-B radiation, as well as UV-irradiated foodplants (Mazza *et al.,* 1999), possibly as a result of DNA damage and immunosuppression (Paul & Gwynne-Jones, 2003). Crucially, the cuticle of *O. brumata* is known to transmit greater concentrations of harmful UV-B radiation than other Lepidoptera species feeding on the same host-plant (Buck & Callaghan, 1999), making it possible that larvae are either actively avoiding, or unable to survive in, sites in which greater concentrations of harmful UV-B radiation is incoming. Although such an effect may be mediated by an interaction with topography, with greater concentrations of solar radiation at higher elevations, the relatively small changes in elevation among the Orkney landscape make this unlikely to be a large contributing factor (Korner, 2007). Instead, such variation may be mediated through the

architecture of the host plant: broad leaved trees such as birch may provide greater levels of shading for developing larvae than low-lying, open moorland, meaning the benefits of irradiation in terms of host-plant palatability are negated. Clearly, the principal factors governing changes in *O. brumata* abundance in Orkney, and in other areas, is a complex process, probably driven by a number of competing and interactive components. Although the results of this study are able to suggest possible, likely sources of influence, more accurate, direct measures of these site-specific characteristics will need to be implemented, within an experimental framework, in order to shed more light on this issue.

Consistent with the interactions with *O. brumata* abundance, soil organic content appears to play a positive role in the abundance of *A. grossulariata* among sites—both an interaction with PC1, and a large loading on PC4, again suggest that this is an important component governing Lepidoptera abundance within Orkney moorlands. However, unlike *O. brumata,* there is no direct physiological link between larval survival and soil characteristics, since pupation occurs above ground. This suggests that such an interaction is mediated indirectly, again possibly due to changes in plant nutritional quality and/or architecture. Heather height and shoot:wood ratio were negatively correlated on the PC3 axis, which showed a significant association with both presence/absence, and abundance of *A. grossulariata* larvae. Such a result is consistent with the dynamics of heather, which allocate more resources to woody stem tissue as the plant ages, increasing in height and producing a blanket coverage (Gimingham, 1985). However, although the production of green shoots is maximal in earlier stages of growth (e.g. the building phase), the biomass of shoots in later stages is not reduced, meaning that the amount of available resource for herbivores is maximal; indeed, Haysom & Coulson (1998) report a significant positive relationship between height and green shoot density among moorlands in mainland Britain. This relationship also translated into increases in Lepidoptera abundance and diversity among several taxa, suggesting that this is an important determinant of larval abundance among moorlands. Lawton (1983) suggests that there may be generalisable patterns in plant architectural complexity in relation to invertebrate diversity and abundance, beyond simply the effects of biomass. As well as the effect of increased spatial cover on the ability of a species to exploit it host-plant, architectural complexity may exist independent of this as a resource—increased height may confer microclimatic benefits for developing larvae and pupae, by providing sheltered conditions in which to develop. Plant structural

complexity and height has previously been shown to be beneficial to some invertebrate species, through increasing the availability of favourable microclimatic conditions, such as increased temperature and humidity (Raghu *et al.*, 2004). Such an effect would seem to be particularly beneficial to species found in exposed moorlands, and perhaps more so for species such as *A. grossulariata* that overwinter in the larval stage.

Microclimatic conditions may also be mediated through topography as well as plant architecture, or through an interaction of both. The correlation on PC3 of both heather height and shoot:wood ratio with aspect suggests that such an effect may be pertinent in this system too: ambient temperatures are likely to be higher on more southerly-facing slope, and indeed increases in *A. grossulariata* presence and abundance appear to show a strong relationship with this particular topographic variable. Increasing heather height showed strong positive loadings on PC3 with south-facing slopes, so it may be that such an effect is mediated through variation in host-plant productivity in more favourable areas. However, the specific interaction with height and shoot:wood ratio (age-related effects) suggests that microclimatic conditions may be the main driver in this instance—ambient temperatures in heathlands have been shown to be >4-5°C higher on south-facing slopes than north-facing slopes, large enough to influence Lepidoptera population processes (Thomas *et al.*, 1999). Although such temperatures may be greater still where vegetation cover is less (e.g. shorter heather), the availability of shelter for developing pupae (to avoid desiccation) and overwintering larvae (to avoid exposure-related mortality), may be the crucial determinant of successful within-site establishment. Temperatures may also fluctuate less in the centre of taller, less open heather stands (Delany, 1953), creating a more stable environment in which larvae may develop.

Although a relationship with more woody heather is apparent in these *A. grossulariata* models, the possibility remains that such an effect may be an artefactual response to grazing pressures; although livestock browsing on green shoots would appear to be a likely factor in this, no such relationship was in evidence. However, *A. grossulariata* is known to displaying outbreaking behaviour in which heather stands may be almost entirely defoliated, with a large spatial variation in the damaged areas (Horsfield & MacDonald, 2003). Although not quantified, these same reports are consistent with a hypothesis that *A. grossulariata* is limited by local climatic conditions, being almost entirely absent from areas of moorland below 300m elevation, and on heather less than 15cm tall. These reports of heather damage by larvae of *A.*

grossulariata are among the first in these areas of north and north-west Scotland, with the species being described as an occasional migrant prior to this time (Lorimer, 1983). Such temporal and spatial changes in the pattern of abundance would appear to be consistent with an increase in the northward range of this species over the last two decades, and indeed this variation in traits that limit abundance in the newly invaded habitats remain consistent with traits associated with a high probability of invasion success (*see* Ward & Masters, 2007). The possibility also remains that the observed patterns in distribution may simply reflect the residual effects of such a northwards expansion, with dispersive adults more likely to encounter south-facing slopes as they advance—the northwest-southeast correlations observed in the windrose correlograms give weight to this supposition, given that they are independent of topography. However, again, these two processes may not necessarily be independent of one another,

The lack of a consistent pattern in attack among any species of natural enemy with host density, at any scale, is perhaps surprising given the strong responses observed in Chapter 4. Spatial density dependence among insect hosts and their parasitoids is known to be highly variable (Walde & Murdoch, 1988), and dependent on spatial scale (Hails & Crawley, 1992; Norowi *et al.*, 2000) and landscape structure (Cronin & Reeve, 2005). Indeed, a number of studies have demonstrated increases in parasitoid infections within patches of high host density, without finding significant density-dependence in prevalence (Sanchez *et al.,* 2009), with stronger responses to the occurrence of host habitat than host density (Esch *et al.,* 2005). The extent to which both *O. brumata* and *A. grossulariata* exist in distinct patches in heather moorland is unclear, although it seems likely that, given the almost blanket coverage across large areas of mainland Orkney, the effects of parasitoid aggregation within discrete host plant patches would seem to be less than among other systems where hosts exist among a matrix of unsuitable habitat. Cappuccino *et al.* (1998) found reduced parasitism rates among spruce budworm (*Choristoneura fumiferana*) hosts embedded in extensive forest habitat than in discrete host plant stands, suggesting that such predator aggregation may be reduced in continuous habitats such as heather moorland. The spatial pattern of infection among parasitoids in *O. brumata* across the Orkney mainland would seem to support such a hypothesis, highlighting the importance of considering landscape-specific contexts when investigating host-natural enemy traits.

Although the only positive relationship with density was found among RV infecting *O. brumata*, such a result should be treated with caution, due to the low sample size and relatively poor fit of the significant model compared to a null model. Positive density dependence would seem unlikely among this species of virus, unless the same effect was found among parasitoids too—Graham *et al.* (2006) found 100% prevalence of RV among all parasitoids screened that had emerged from *O. brumata* populations in Orkney, and it is thought that prevalence of this virus is almost entirely governed by changes in parasitoid, rather than *O. brumata*, abundance. Data presented in Chapter 4 would seem to support this theory, together with the fact that, in the present study, RV infections were only found among sampling sites where parasitoids were also present, regardless of host density. The lack of apparent spatial pattern among RV infections is also consistent when upscaling from the local to the regional scale. Surprisingly, given the strong spatial pattering found at the local scale among OpbuNPV infections (Chapter 4), no such patterns were evident at the regional scale among this host-natural enemy interaction. Spatial patterns were more evident when region-wide prevalence was highest, although this failed to translate into a significant association with host-density. However, region-wide AbgrNPV infections among *A. grossulariata* hosts displayed remarkable congruity with both local-scale prevalence (consistently ~20% infection at both scales), but similar spatial patterns of infection. Again, no effect of host density on infection prevalence—either in the current year, or through delayed effects—was detectable at either scale. Although infections among this species were not density dependent across the region, localised hotspots were identified that harboured higher prevalence of infection than expected across the region. The relationship between baculoviruses and their insect hosts is well studied, and known to be highly variable (see Cory & Myers, 2003; Fuxa, 2004) and, although the primary route of infection is horizontal—through ingestion of virions derived from dead conspecific hosts—such a transmission relationship is not necessarily thought to be directly proportional to host density (D'amico *et al.*, 1996). Indeed, fine-scale spatial restriction of virus particles is thought to be the main driver of non-linear patterns in the transmission of infections between hosts in some systems (D'amico *et al.*, 2005), and models parameterised from NPV-lepidoptera data have previously shown that small-scale variations in transmission rates can lead to large-scale epizootics in spatially structured populations (Fenton *et al.*, 2002). Such localised transmission events—either host outbreaks, or within-site disease hotspots—are consistent with the data presented in

this study, and Chapter 4. As a corollary, a potential explanation for the overall higher levels of infection observed in AbgrNPV could be fine scale differences in pathogen transmission strategies: AbgrNPV appears to liquefy host larvae (which are also larger) to a greater extent than OpbuNPV, whereby infected larvae remain largely intact when dead (pers. obs.). Such variation in transmission strategies might mean that OpbuNPV is much more spatially restricted than AbgrNPV within sites, with prevalence only increasing substantially during large population increases or outbreaks. Such density-driven infection thresholds would therefore be consistent with the scale-dependent patterns observed in Chapter 4, and could explain the large variation in prevalence across regions.

A potential confounding factor in the evaluation of spatial patterns of infection within and among populations, is that of the temporal scale of study. Populations that exhibit cyclical dynamics, such as *O. brumata*, often do so as a direct consequence of interactions with one or many natural enemies (Berryman, 1996; Klemola *et al.*, 2002; Dwyer *et al.*, 2004); in any stage in a population's cycle the relationship with each natural enemy may be different, and therefore not temporally consistent. As a pertinent example, Graham *et al.* (2004) found a significant positive association between OpbuNPV and *O. brumata* density within Orkney during a single years sampling, but found that this relationship disappeared when data was pooled from three years worth of sampling at the same sites (Graham, 2006). The high among-site variation in both host density and infection prevalence in both study species, locally and regionally in Orkney, would seem to be consistent with these findings, although whether this is due to cyclical dynamics will require further years of data. Heterogeneity in disease resistance within local populations, could also confound generalisations concerning infections among multiple sites: increased selection for disease resistance among populations undergoing epizootics could have implications for subsequent transmission dynamics across years, affecting both infection prevalence and host abundance (Cory &Myers, 2009). Indeed, OpbuNPV is known to be genotypically variable across the whole of the Orkney mainland (Graham *et al.*, 2004), so it seems likely that heterogeneity in resistance to different viral strains may have important dynamical consequences among spatially distinct populations. The magnitude and sign of natural enemy interactions with their invertebrate hosts is clearly a complex and dynamical one, and a number of studies have presented pertinent caveats to the conclusions that can be made from short term host-natural enemy studies (Teder *et al.*, 2000), including large scale studies involving *O.*

brumata (Bonsall & Hassell, 1995). Extending the present study to include long-term regional scale studies, potentially with the inclusion of habitat and landscape influences, and variation in disease resistance, would add greatly to the understanding of *O. brumata* and *A. grossulariata* population processes on Orkney.

Chapter 6

Spatial population genetics of winter moth (*Operophtera brumata*) and magpie moth (*Abraxas grossulariata*) in Orkney

6.1 Abstract

The dispersal capability of a species may be fundamentally linked to its ability to exploit novel, suitable habitat patches, or move away from areas of poor habitat. The potential metapopulation dynamics of any system are therefore a function of the relative dispersal capabilities of consumers to their resources. Quantifying a species' dispersal capability may therefore be crucial in understanding the dynamics of spatially structured populations of insect herbivores and their natural enemies. Population genetic tools may act as a useful guide to the potential dispersal ability of a species, since less dispersive species may display a pattern of restricted gene flow among spatially separate sites.

In this chapter, we test the hypothesis that a resident species (*Operophtera brumata*) with a flightless female stage will display a more spatially restricted population genetic structure than a larger, fully flighted, recently colonising competitor (*Abraxas grossulariata*). We test whether a genetic signal of recent colonisation of the Orkney isles from mainland Britain is detectable among *A. grossulariata* and not *O. brumata*, which has been resident in the isles for at least the last century. The hypothesis that *A. grossulariata* individuals are under greater selection pressure from novel pathogens and environmental effects than their resident competitors because they are at the northwards edge of their natural range, is also examined.

Individuals of both species were collected from a number of spatially and temporally separate sites both within the Orkney archipelago, and in mainland Britain. Amplified Fragment Length Polymorphism (AFLP) markers were used to screen individuals for population-specific genetic signals of relatedness, which were inferred from both spatially explicit, and non-spatially explicit Bayesian assignment tests. The hierarchical partitioning of populations, and distance-autocorrelated relatedness among populations was also investigated to explore how each species' populations are structured over local and regional scales. Further to this, a well established, and novel, method of screening for loci under selection was also implemented, in order to discern whether either species is under selective pressure from environmental or pathogenic effects.

Population structural analysis revealed that *O. brumata* populations were relatively well mixed within the Orkney mainland. Populations also differed far more markedly in time than in space. Among *A. grossulariata* populations, a potential genetic signal of colonisation from isolated north-coast populations was evident, as temporal

trends in population structuring suggest a south-north dispersal within Orkney. East coast populations were also more similar to Orkney populations than they were to west coast populations, which in turn were more similar to southern British populations, suggesting that eastern populations have become isolated over recent years. There was no evidence of a population bottleneck, although overall genetic diversity was extremely low among all populations, possibly as a result of widespread nationwide declines over the last century. Genome scans for loci under selection also revealed that *A. grossulariata* populations may be under selective pressure from viral pathogens, suggesting the possibility that populations acquired the AbgrNPV infections from Orkney, rather than carrying it from source mainland populations.

Population genetic analysis provides a useful tool for quantifying species dispersal across a region, which can be linked to their exploitation of suitable habitat and potential interactions with competing species and/or natural enemies. Genetic signals of gene flow may indicate how well mixed populations are across a region, and can provide details of levels of genetic diversity within a species. Populations of organisms undergoing climate-induced range shifts may both lose native natural enemies and gain novel ones. How they respond to this change may be governed by their levels of genetic diversity, if novel pathogen-mediated selection occurs in the new habitat. How this may affect future population processes, is discussed.

6.2 Introduction

Understanding a species' dispersal capabilities is key to understanding their current and future responses to environmental change, and their interactions with other organisms within an ecosystem (Roderick, 1996; Amarasekare, 2004). Habitat fragmentation, interspecific competition, sex-biases, and biotic and abiotic pressures, can all affect such dispersal mechanisms, and lead to genetic differentiation via the disruption of gene flow between populations (Bowler & Benton, 2005). Such differentiation can exacerbate population sub-structuring which can often leave a characteristic genetic signal (Orsini *et al.*, 2008), especially for invasive or range-expanding species (Rollins *et al.*, 2009). The spatial arrangement of habitats and organisms within these habitats is fundamental to a mechanistic understanding of the role of competition and coexistence within and among species (Hanski, 1999), and is crucial to understanding the spatial and temporal dynamics of disease spread in wild populations (Hess *et al.*, 2002).

Dominant genetic markers such as amplified fragment length poymorphisms (AFLP; Vos, 1995) are particularly useful for high-throughput screening of organisms when searching for possible signals of population structuring and genetic diversity when no *a priori* sequence information is available (Meudt & Clarke, 2007). Their high-level of reproducibility and the large number of markers produced also makes them well suited to hypothesis testing of possible selective pressures, where genome screening via other markers has not been implemented (Meudt & Clarke, 2007; Joost *et al.*, 2007). Robust statistical methods for their analysis are extensive and well documented (Bonin *et al.*, 2007), and whilst they have previously been used most widely for plants, bacteria and fungi (Bensch & Andersson, 2005), a number of studies have utilised the technique to look for genetic structuring among a variety of insect taxa (Salvato *et al.*, 2002; Grapputo *et al.*, 2005; Conord *et al.*, 2006; Schroeder & Degen, 2008; Timm *et al.,* 2008).

In this study, AFLP markers are used to investigate the population genetic structuring among *O. brumata* and *A. grossulariata* individuals from sites throughout Orkney and mainland Britain. A number of temporally and spatially separated samples are used to test current hypotheses about dispersal mechanisms, recent range-expansions and potential biotic and abiotic selection pressures acting on the two species. Spatial genetic structure is investigated at different spatial scales using a variety of different techniques. Bayesian assignments tests and metrics of population differentiation are

used to establish the most likely spatial and temporal arrangement of populations within the designated sampling sites; and spatial autocorrelation tests and analysis of molecular variance (AMOVA) is used to examine any potential isolation-by-distance effects, and partitioning of genetic variation at different spatial scales, respectively.

Finally, a genome scan of loci potentially under selection among both species is performed using two different techniques (population genomics and genome scans), to identify how each may be adapting to its current habitat. Although the population genomics approach to detecting outlier loci under selection is the most widely used method for AFLP markers, it lacks the ability to test predictions about the particular selective pressures operating on an organism. It also relies on a number of assumptions regarding the population genetics of each organism, and has previously been shown to have high rates of error if not used with caution (Caballero *et al.*, 2008). As a result, new analysis methods have been proposed that disregard any underlying evolutionary assumptions, and focus solely on statistical correlations (Joost *et al.*, 2008). Here, the latter method is extended so as to make the spatial dependency between sampled populations explicit, to rule out the possibility of spurious errors occurring through the similarity of more closely situated populations.

As *O. brumata* females are flightless, and the adults stages much smaller than *A. grossulariata* (see Chapter 1), it is predicted that that latter will be genetically differentiated at greater spatial scales. Within-population genetic diversity is expected to be higher among *A. grossulariata* populations, as their hypothesised greater dispersal abilities are predicted to be manifest in higher levels of gene flow among spatially separate populations. *O. brumata* are expected to show some levels of population substructuring, with separate areas of heather moorland acting as potential barriers to gene flow, and exacerbating levels of inbreeding. *O. brumata* populations are expected to remain temporally stable across genetic parameters due to their resident status. However, a genetic signal indicative of a northward range-shift is anticipated among populations of *A. grossulariata* relative to their source mainland populations, in line with contemporary accounts of their recent colonisation and establishment in Orkney. Both species are hypothesised to show some selective signal to pressures exerted by natural enemies such a nucleopolyhedroviruses (NPV), although *A. grossulariata* loci are expected to be under greater selective pressure, due to their colonisation of a new habitat with some potentially novel pathogens. They are also expected to be under greater selection by abiotic pressures, in line with the hypothesis that they are at the

northward limit of their current range, and therefore susceptible to colder climates in more exposed areas. Conversely, *O. brumata*, who overwinter as eggs, and are found throughout more northerly latitudes, are expected to be well adapted to the current climate, manifest in variation in topographic conditions.

6.3 Methods

6.3.1 Sample collection

For sites within Orkney, *O. brumata* and *A. grossulariata* larvae were collected by transect and quadrat methodologies, as in Chapter 5. Larvae were randomly collected within a $50m^2$ search radius from the two sites in north-east Scotland (RANGAG and CAITH; Table 1), and light traps were used to collect adult moths from the remaining three British mainland sites (BE, CT and WYT; Rothamsted light trap network). The spatial distribution of sites is illustrated in Fig 6.1.

All *O. brumata* larvae were reared on artificial diet (see Methods) and only those that died before pupation were used in the analysis (with the exception of LI and HU samples from 2004). Only those larvae free from known viruses (see Chapter 5) were used for subsequent analysis, to eliminate possible contamination from viral DNA. All *A. grossulariata* larvae collected were reared until adulthood on fresh heather. Only adult specimens were used, and are assumed to be free from substantial amounts of viral DNA. All samples were stored at -20°C prior to DNA extraction.

6.3.2 AFLP protocol

Tissue samples were obtained from *O. brumata* larvae following the methods described in Chapter 2. For *A. grossulariata* samples, adult abdomens were used for DNA extractions, and the head and thorax retained for archiving. Genomic DNA was extracted from both species using the magnetic-bead method as described in the General Methods chapter. Total concentration of DNA in each sample was then quantified using a NanoDrop 8000 spectrophotometer, and stored at 4°C.

						Life Stage	
Site	**Site code**	**OS Grid reference**	**Species**	**Year**	**N**	**When collected**	**Used for analysis**
Akla	AK	334023 1008117	*O. brumata*	2008	8	Larvae	Larvae
Beinn Eighe	BE	202400 862900	*A. grossulariata*	2006	8	Adult	Adult
Burgir	BG	334484 1007534	*O. brumata*	2008	10	Larvae	Larvae
Burn of Rusht	BR	334053 1020820	*O. brumata*	2008	8	Larvae	Larvae
Braes of Aglath	BRA	336344 1018293	*O. brumata*	2008	3	Larvae	Larvae
Buan	BU	332922 1009560	*O. brumata*	2008	9	Larvae	Larvae
Caithness	CAITH	308750 920750	*A. grossulariata*	2008	11	Larvae	Adult
Cothele	CT	242400 068100	*A. grossulariata*	2006	8	Adult	Adult
Fibla Fiold	FF	329819 1024182	*A. grossulariata*	2008	9	Larvae	Adult
Graemeshall	G	338480 1022194	*A. grossulariata*	2006	10	Larvae	Adult
Greeny Hill	GH	329819 1024182	*O. brumata*	2008	8	Larvae	Larvae
Groundwater	GW	338145 1008500	*A. grossulariata*	2008	10	Larvae	Adult
Heldale	HEL	327832 991846	*A. grossulariata*	2006	10	Larvae	Adult
			A. grossulariata	2007	6	Larvae	Adult
Hundland	HU	331033 1026442	*O. brumata*	2008	10	Larvae	Larvae
				2004	9	Larvae	Adult
			A. grossulariata	2006	9	Larvae	Adult
Linnnadale	LI	332306 1006331	*O. brumata*	2008	8	Larvae	Larvae
				2004	8	Larvae	Adult
Mildoe	MLD	336337 1020660	*O. brumata*	2008	9	Larvae	Larvae
Queefiglamo	QF	334541 1024310	*O. brumata*	2008	5	Larvae	Larvae
Scarra	SC	323878 1014139	*A. grossulariata*	2006	10	Larvae	Adult
Settiscarth	SET	335687 1018392	*O. brumata*	2008	9	Larvae	Larvae
			A. grossulariata	2006	10	Larvae	Adult
Springfield	SPRING	344050 1028850	*A. grossulariata*	2008	4	Larvae	Adult
Saverock	SR	342104 1012342	*O. brumata*	2008	7	Larvae	Larvae
Swartabeck	SW	337435 1007537	*A. grossulariata*	2004	8	Larvae	Adult
			A. grossulariata	2006	9	Larvae	Adult
			A. grossulariata	2008	8	Larvae	Adult
Rangag	RANGAG	318050 942150	*A. grossulariata*	2008	10	Larvae	Adult
Trumland	TR	342655 1028156	*A. grossulariata*	2007	10	Larvae	Adult
Wideford	W	340888 1011505	*O. brumata*	2008	9	Larvae	Larvae
Wideford Cairn	WC	340848 1012135	*O. brumata*	2008	7	Larvae	Larvae
Ward Hill	WH	333624 1007755	*O. brumata*	2008	5	Larvae	Larvae
Whitemire	WT	332609 1027245	*O. brumata*	2008	9	Larvae	Larvae
Wytham	WYT	445800 208800	*O. brumata*	2006	6	Adult	Adult

Table 6.1 Site codes and geographic locations of all samples, including year collected and life stage used

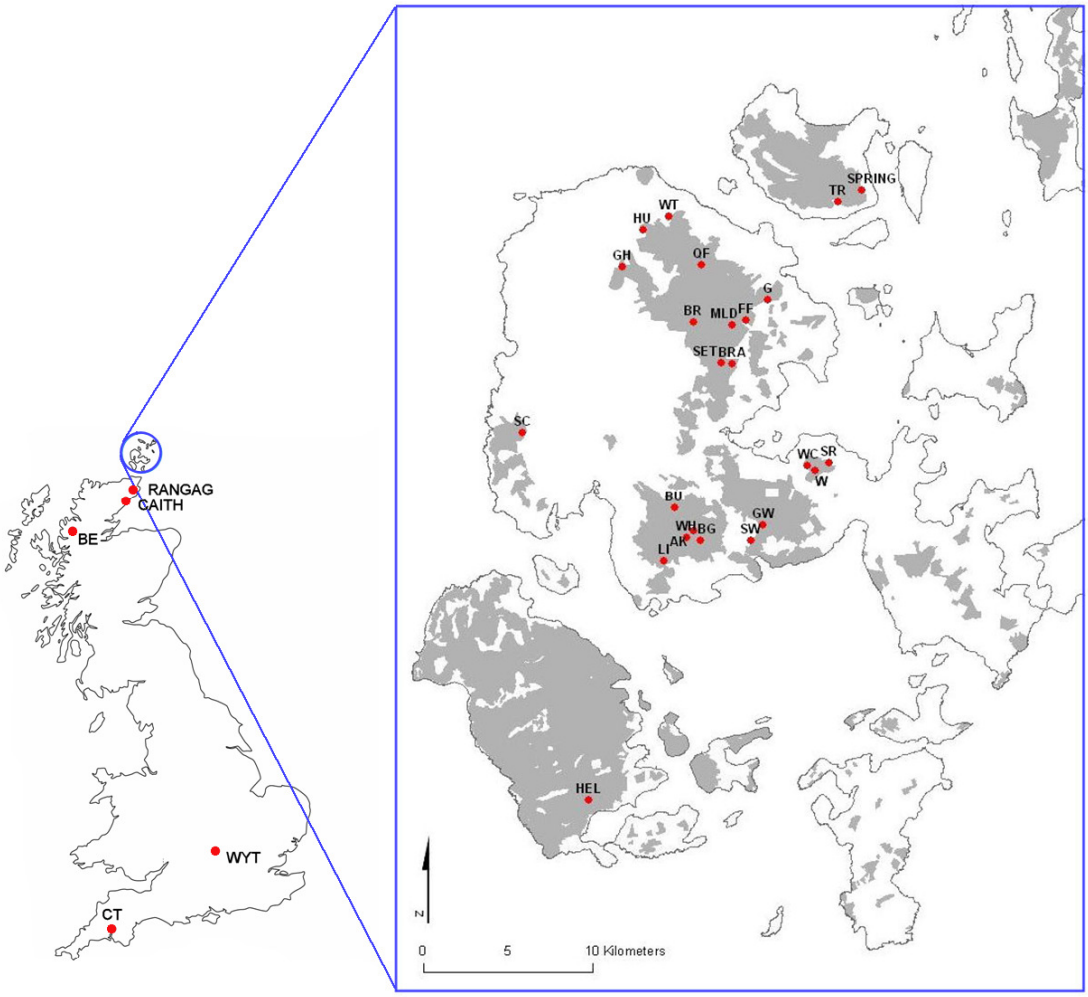


Fig 6.1 Geographical locations of all sampling sites in Britain and Orkney. Grey shading indicates areas of heather moorland (Orkney only).

The AFLP protocol was adapted from the methods of Wang & Porter (2004) and Meudt & Clarke (2005), and consisted of five distinct stages: i) Digestion of genomic DNA using restriction enzymes; ii) Ligation of adaptors to the digested DNA; iii) Pre-selective amplification of ligator-linked DNA; iv) Selective amplification of the pre-selective amplification product using standard primers and fluorescently-labelled primers; v) Visualisation of selective amplification products on an automated DNA analyser. All reactions were prepared under sterile laboratory conditions.

i) For each sample 250ng of genomic DNA was digested using 1µl MseI (10U/µl) (a frequent cutter) and 0.5µl of EcoRI (20U/µl) (a rare cutter) restriction enzymes, in a reaction mixture with 0.25µl BSA and 4µl NEB Buffer 4 (10x). This was then made up to a total volume of 40µl using Milli-Q H_20. The total reaction mixture was incubated for 3 hours at 37°C, followed by 20mins at 65°C to heat inactivate the enzymes.

ii) MseI and EcoRI adaptors were prepared by firstly annealing together complementary adaptors (MseI adaptors 1(5'-CTC GTA GAC TGC GTA CC-3') and 2 (5'-AAT TGG TAC GCA GTC TAC-3'); and EcoRI adaptors 1 (5'-GAC GAT GAG TCC TGA G-3') and 2(5'-TAC TCA GGA CTC AT-3')) at 95°C for 5mins and then leaving them to cool gradually to room temperature. To each of the 40µl restriction digests were then added 1µl MseI adaptor (1nmol/µl), 1µl EcoRI adaptor (0.1nmol/µl), 1µl NEB Buffer 4 (10x), 0.5µl T4 ligase (Promega), 1µl ATP (10mM) and 5.5µl Milli-Q H_2O, to make a final total reaction volume of 50µl. The reaction mixture was then vortexed briefly, and left to ligate at room temperature for at least 10 hours.

iii) 1µl of each ligation product was added to a mixture of 0.25µl dNTP (25mM each dNTP), 2µls 10x PCR buffer, 1µl EcoR I pre primer (5'-GAC TGC GTA CCA ATT CA-3') (10pmol/µl), 1µl Mse I pre primer (5'-GAT GAG TCC TGA GTA AC-3') (10pmol/µl), 0.2µl Taq (5U/µl) (Sigma) and the final reaction volume of 20µl made up with Milli-Q H_2O. Pre-selective amplification was then conducted under the following PCR conditions: 72^0C for 2 min followed by 20 cycles of {94^0C for 30 sec; 56^0C for 30 sec; 72^0C for 2 min}, and a final extension of 72^0C for 10 min. A 5µl aliquot of the resulting amplification product was then visualised on a 1% agarose gel to check that the amplification was successful.

iv) For the selective amplification step, four different combinations of two primer pairs (Eco ACA^{xxx}, Eco ACG^{xxx}, Mse CAT and Mse CAA; where ^{xxx} represents the fluorophore FAM) for both species were initially screened with a random subset of six *O. brumata* individuals and 10 *A. grossulariata* individuals. A further subset of two individuals of each species for each primer pair were run with undiluted (100pmol/µl) fluorescently labelled primer, to check for possible differences in fluorescence intensity among the AFLP profiles. The initial primer screening showed relatively minor differences among AFLP profiles for the different primer combinations. However, the combination Eco ACA^{xxx}/ Mse CAT showed more consistency in peak intensities and a more even distribution of peaks across the genome among both species than did other combinations, and was therefore retained for the subsequent analysis. The undiluted fluorescently labelled primer increased the level of background noise and spurious peaks, with marked differences in peak intensity at smaller fragment sizes. It was therefore rejected in favour of a 1:10 dilution, consistent with the unlabelled complementary primer.

The selective amplification reaction mixture thus consisted of the following: 1µl pre-selective amplification product (diluted 1:10 with Milli-Q H_2O), 2µl 10x PCR buffer (Sigma), 0.25µl dNTP (25mM for each dNTP), 2.5µl $MgCl_2$ (50mM), 1µl EcoRI 5'-FAM-GAC TGC GTA CCA ATT CAC A-3' (10pmol/µl), 1µl MseI 5'-GAT GAG TCC TGA GTA ACA T-3' (10pmol/µl), 0.2µl Taq (Sigma) (5U/µl). A final reaction volume of 20µl was made up with Milli-Q H_2O, vortexed, and run under the following touchdown PCR conditions: an initial denaturation of 94^0C for 2min, followed by 10 cycles of {94^0C for 30 sec; 66^0C (dropping by 1^0C per cycle) for 30 sec; 72^0C for 2 min}, followed by 25 cycles of {94^0C for 30 sec; 56^0C for 30 sec; 72^0C for 2 min}.

v) To prepare the selective amplification product for analysis, 0.5µl product was mixed with 0.5µl GeneScan 1200LIZ size standard (Applied Biosystems) and denatured in 9µl Hi-Dye formamide (Applied Biosystems) by heating to 95°C for 5mins and then immediately put on ice. Samples were then run on an ABI3730 Automated DNA Analyser (Applied Biosystems) under the following conditions: Pre-run voltage 15kV; Pre-run time 180 sec; Injection voltage 1.6kV; Injection time 15 sec; Run time 7000 seconds; Run voltage 8kV; Dye set G5.

6.3.3 Error rate analysis

All AFLP profiles were checked visually using the software GeneMapper (Applied Biosystems) to confirm that a suitable profile had been achieved. Any failed samples were repeated and, if failed again, were rejected from subsequent analysis. Of the retained samples, bin sets for scoring peaks were automatically assigned by GeneMapper from an analysis range of 100-999 base pairs (bp) and a cut off relative fluorescence unit (rfu) of 80. As such, all subsequent values of bp sizes and rfu values are quoted relative to this cut-off. All of these bin sets were inspected visually, and any that could not be scored unambiguously (e.g. due to possible fragment size homoplasy, or scoring of noise peaks) were removed.

To further quantify the error rate between different runs of the DNA analyser, a random subset of 15 *O. brumata* and 20 *A. grossulariata* samples were repeated from the DNA extraction stage, and their profiles subject to error rate analysis following the method of Whitlock *et al.* (2008), and the software AFLPscore. This method uses a semi-automated procedure to quantify the genotype scoring-error between AFLP profiles, and excludes all loci contributing high rates of error. By quantifying how many

loci may be retained according to different error thresholds, the user is able to make the inherent genotyping error transparent, and create AFLP profiles that are both objective and high-quality. All analyses were conducted using the absolute phenotype calling threshold method, using a series of models spanning 5-100% of all possible locus-calling and phenotype-calling thresholds, relative to the grand mean normalised peak heights (GMNPH) of all loci. The best model is considered to be the one with the lowest Bayesian and/or mismatch error rate, which still retains sufficient loci on which to conduct further analysis.

6.3.4 Non-spatial among-year analysis

Population genetic structure among populations and among years was analysed using the software STRUCTURE 2.2 (Pritchard *et al.* 2000), suitable for use with dominant markers (Falush *et al.* 2007). This method implements a Bayesian clustering approach for inferring population structure by assigning individuals probabilistically to their population of origin. The program assumes that loci are at Hardy-Weinberg equilibrium and that markers are unlinked within populations. No prior information regarding sampling origin is given to the program, with models being constructed according to the number of assumed populations (K) in the data set, from K_{min+1} to K_{max}. For *O. brumata* this was 2-19; for *A. grossulariata* this was 2-18. The true value of K was then inferred by calculating ΔK, the second order rate of change of the log probability of all K-models, following the methods of Evanno *et al.* (2005). This represents the uppermost hierarchical level of clustering within the data.

For both species studied the more complex admixture model was used (to allow for the possibility of mixed ancestry), assuming correlated allele frequencies within populations. Twenty repeat runs were conducted, each with a burn-in period of 10 000 iterations, and the number of Markov Chain Monte Carlo iterations (MCMC) set to 10 000. Cluster membership probabilities from the 20 repeated runs from the ΔK model were aligned using the cluster-matching and permutation program CLUMPP 1.1.1 (Jakobsson & Rosenberg, 2007), using the Greedy algorithm with 10^6 repeated random input orders. Results were visually represented using the program DISTRUCT 1.1 (Rosenberg, 2004).

Unbiased genetic diversity estimates within populations were calculated using the software AFLPsurv (Vekemans, 2002) and are given as the proportion of

polymorphic loci (PLP) and expected heterozygosity as Nei's gene diversity index (Hj), following Lynch & Milligan (1994). Spearman's rank correlation tests were performed to assess the independence of these two measures. The same software was also used to quantify genetic distances between populations, given as pairwise Fst values, computed from 500 permutations and 1000 bootstrap replicates. All of these measures are based on the estimation of allelic frequencies calculated via a Bayesian method with non-uniform priors (Zhivotovsky, 1999), assuming Hardy-Weinberg proportions. Consensus neighbour-joining trees (Saitou & Nei, 1997) of these pairwise F_{st} values were constructed from the 1000 bootstrap replicates using the program PHYLIP 3.68 (Felsenstein, 2005). Additionally, for comparison of heterozygosity levels between species, a random sample of 12 *Spodoptera frugiperda* and 12 *Spodoptera exigua* (Lepidoptera: Noctuidae) individuals from laboratory stocks (CEH Oxford) were subjected to the same processes as both *O. brumata* and *A. grossulariata*, as described above. Both of these stocks have been maintained from the same source individuals for >10 years and are therefore expected to be highly inbred.

6.3.5 Spatial within-year analysis

Very little is currently known regarding the dispersal rates and genetic admixture of both of the study species. For this reason, it was decided to conduct the spatial analysis among populations within sampling years, to eliminate the potentially confounding effects of temporal dynamics. Additionally, this reduces the possibility of any spurious results arising from possible template DNA degradation over time (Bensch & Andersson, 2005). For *O. brumata*, analysis was therefore restricted to samples collected in 2008 only; for *A. grossulariata*, two separate analyses were conducted on each of the samples collected in 2006 and 2008.

Spatial population genetic structure was analysed using the software TESS 1.2, a Bayesian clustering approach for inferring the highest level of clustering among a set of populations. This approach is analogous to that of STRUCTURE, although this algorithm includes a method whereby the spatial distribution of sample sites is included as a prior distribution of cluster labels, using a Hidden Markov Random Field (HMRF) on a spatial network to model the autocorrelation among populations (Francois et al., 2006; Chen et al., 2007). However, unlike STRUCTURE 2.2, TESS 1.2 has not been specifically developed to deal with dominant markers, and as such the AFLP input data

was modified by coding the presence/ absence of a band at any particular locus as a haploid allele, with the second allele coded as a missing value (Bonin *et al.,* 2007). For each sampling location within Orkney, random spatial locations were generated within a $10m^2$ (±1 standard deviation) search radius; locations outside of Orkney were generated within a 50 m^2 (±1 standard deviation) search radius, consistent with the sampling procedures and suitable for the TESS algorithm (Francois *et al*., 2006).

The inclusion of spatial information in the algorithm used by TESS (known as the Potts-Dirichlet model) also requires the inclusion of an interaction parameter (Ψ), which controls the importance given to spatial interactions, with values of Ψ greater than 1 considered as having a high level of spatial interaction. To establish the optimum value of Ψ for each spatial analysis, a number of pilot runs were conducted, using the Ψ values 0.5, 0.7 and 0.9, following the recommendations of Francois *et al*., (2006) and Chen *et al.* (2007). The best model is considered as the one with the consistently lowest Deviance Information Criteria (DIC), suitable for use in Bayesian analysis obtained by MCMC simulation. For each of these pilot runs, 10 repeats of each Ψ model were made using a burn-in period of 2000 iterations, followed by 10 000 MCMC iterations. For *O. brumata*, it was clear that the model using Ψ=0.5 gave a superior model fit, with lower DIC values for all values of K (2-16). For *A. grossulariata* from 2006, there was very little difference between models, although Ψ=0.5 gave the lowest mean DIC across all values of K (2-8). Similarly for *A. grossulariata* from 2008, there was very little difference between models across all values of K (2-8), and Ψ=0.7 was chosen as the final model due to its lowest mean DIC value. Once the Ψ value was determined for each model, 20 repeat simulations were run under the No Admixture model using 10 000 burn-in iterations and 20 000 MCMC sweeps, for all values of K. The optimum K value was then determined by visual inspection of the mean DIC value across all values of K, and is inferred as the point at which the mean DIC value for K reaches a plateau (Chen *et al.,* 2007). A further set of 80 simulations were run under the same parameters for the optimum K value, and the 20 highest likelihood runs (the Bayesian estimate) were retained. The cluster membership probabilities from these runs were then aligned using CLUMMP 1.1.1 using the Greedy algorithm with 10^6 repeated random input orders.

The presence of spatial genetic structure in the two species was investigated using the software SPAGeDI 1.2 to calculate the spatial autocorrelation between pairwise kinship coefficients with distance. This was based on the kinship estimator (F_{ij})

of Hardy (2003), specifically adapted for dominant markers. Although this method lets the user define the inbreeding coefficient (the departure from Hardy-Weinberg proportions), and since no prior knowledge of inbreeding is available for either species, this value was set to zero (Tero *et al.*, 2005). Although this may not be true strictly, the estimator is known to be robust to departures from the true level of inbreeding (Hardy, 2003). A total of 10 distance classes were created, of unequal width but with an equal number of individuals in each, using the software PASSAGE 1.1.2.3. Kinship coefficients per distance class ($F_{(d)}$) were calculated with 95% confidence intervals obtained from 10 000 random permutations of each distance class, to test for the overall presence of spatial structure. Because of the large discrepancies in distance between *A. grossulariata* sites from 2006, the two mainland Britain sites were used as separate distance classes, and the overall analysis conducted on a log scale. To assess whether this discrepancy had any major effect on the outcome of the analysis, it was repeated with these two sites omitted. Additionally, partial Mantel tests were performed to investigate evidence of a density-dependent or delayed density-dependent effect of within site density (measured as the total number of individuals found within a site, not the number used for AFLP analysis), and virus prevalence on heterozygosity Hj (see above). For *A. grossulariata,* both sampling years (2006 and 2008) were combined, and only sites within Orkney were used for the analysis, due to insufficient data at other sites. Permutation tests using 1000 replicates, keeping spatial location constant, were performed using PASSAGE 1.1.2.3.

To investigate how genetic structure is partitioned over different spatial scales, hierarchical Analysis of Molecular Variance (AMOVA) was performed, using the software Arlequin 2.0 (Excoffier *et al.*, 1992). This approach is based on the analysis of a phenotypic distance matrix (Bonin *et al.*, 2007), and separates the different covariance components into inter-regional differences, inter-site differences, and intra-site differences. For *O. brumata*, separate regions are defined as all the sites within an area of contiguous heather moorland (see Fig 6.1.); for *A. grossulariata*, two separate regions are defined: all sites either within, or outside Orkney. Significance values of the fixation indices (Φ) are given based on 1000 random permutations. Input data for use in the program was formatted using the software AFLPdat (Ehrich, 2006).

6.3.6 Genome scan for loci under selection

Two different approaches were used to investigate the existence of any possible candidate loci that may be under selection: i) Generalised Linear Mixed Model (GLMM) selection, and inference using Akaike's Information Criterion (AIC); and ii) a population genomics approach for the detection of outlier loci.

i) Generalised Linear Models (GLMs) provide a particularly useful tool for analysing binary datasets such as those generated from AFLP profiles (Bonin *et al.,* 2007), since they are able to relax the assumptions of simple regression analysis of normally distributed errors. Instead, GLMs can model these errors explicitly as binomial distributions, providing more robust hypothesis tests and avoiding the use of nonparametric statistics (Bolker *et al*., 2009). Such tests have recently been implemented to scan AFLP profiles for loci that may be under selection from environmental variables (Joost et al., 2007), and here their use is extended into a GLMM framework, which is able to model the error variances of defined groups and random factors. This has a distinct advantage compared with a GLM approach: although environmental variables may be recorded in distinct locations (spatial coincidence analysis), their inherent spatial dependencies cannot be accounted for explicitly in the analysis. Consequently, assumptions of independence of data points may be violated by spatial autocorrelation (Dormann *et al.*, 2007). Using GLMMs, point spatial locations may be coded as random factors in the statistical model, and their variances modelled independently of all other spatial locations (Crawley, 2007). Additionally, spatial statistics may be applied to the model residuals to quantify the extent of any spatial autocorrelation, should it not be dealt with sufficiently by the GLMM framework (Fortin & Dale, 2005).

Since many abiotic and biotic factors that may exert selection pressures do not act so independently of other factors, modelling each as independent explanatory variables could affect the incidence of Type I and Type II errors. Unlike previous genome scans using AFLP markers (Joost *et al*., 2007; Bonin *et al.*, 2006), here the interacting abiotic effects of landscape topography are investigated, defined as a combination of elevation, slope and aspect. Clearly all three of these factors are co-dependent. Similarly, biotic factors such as local host density and disease prevalence

may be co-dependent (Dwyer, 1994), and can be modelled as such using GLMMs (Bolker *et al.,* 2009).

The use of hypothesis testing in the context of genome scans from AFLP data is also particularly problematic. This is mainly due to the large number of loci being screened simultaneously with the same explanatory variables, which may also be large in number. Even with a large number of individuals sampled, binary data still only provides relatively limited amounts of variance on which to base statistical tests (Crawley, 2008). Additionally, comparison of a large number of models under the same conditions constrains the family-wise error rates of the null-hypothesis, and requires some form of significance-level adjustment for multiple comparisons (Storey & Tibshirani, 2003). Some of these adjustments, such as the most widely used Bonferroni-adjustment, may be overly conservative, and risk avoiding Type I errors at the expense of Type II (Moran, 2003).

An alternative approach to model selection, which lies outside the null hypothesis testing framework, is the use of AIC (Burnham & Anderson, 2002). Under this method, a combination of models are fitted, and their relative AIC values (ΔAIC) compared to a null model containing a constant only. AIC values are related to likelihood calculations, but penalise models with a greater number of explanatory variables, thus creating a trade-off between model fit and parsimony (Burnham & Anderson, 2002). Models with ΔAIC values within two units are considered the same; when $\Delta AIC > 4$, models can be considered to be different with 95% confidence (Richards, 2005); and models $\Delta AIC > 10$ have substantially more explanatory power. Use of the AIC method therefore allows for analysis of multiple comparisons without constraints, facilitates the use of interacting and additive explanatory variables, and avoids some of the biases inherent in the selection of single "best" models under stepwise model selection procedures (Whittingham *et al.*, 2006). Use of AIC for model selection and inference is therefore considered more suitable for large-scale genome scans, such as those produced from AFLP data. It has previously been used for scans using SNP markers (Gu *et al.*, 2007), although to my knowledge this is the first use of such an approach, as well as the first use of GLMMs, applied to AFLP data.

All statistical analyses were conducted using the package lme4 (Bates, 2009) in the software R 2.9.0 (R core development team). For *O. brumata,* only sample from 2008 were screened; for *A. grossulariata*, samples from 2006, 2007 and 2008 were pooled together. Topographic parameters were extracted from Digital Elevation Models

(DEMs) with a 10m resolution, using ArcMap 9 (see Chapter 5). Host density and natural enemy prevalence were measured as in Chapter 2, and included as explanatory variables from both the year collected and from the previous year, to check for current and/or delayed effects. All models were fitted using the Laplace approximation with a binomial error distribution, suitable for binary responses and likelihood-based inference (Bolker *et al.*, 1999). Site was included as a random factor to account for the spatial grouping of the data, and Moran's spatial autocorrelation tests performed on model residuals using the R package spdep (Bivand *et al.*, 2009) under 1000 random permutations. AIC values were extracted from each model and ΔAIC calculated as AIC_M-AIC_0, where AIC_M is the value for any given model and AIC_0, the value for the null model. Posterior probabilities are also given as Akaike weights, calculated as $\exp(-0.5\Delta AIC_M)/ \Sigma \{\exp(-0.5\Delta AIC_M)\}$. No other form of corrected AIC was deemed necessary, as binary data cannot be overdispersed (Crawley, 2008) and sample sizes for all models were adequate. The model with the largest ΔAIC value at each locus was retained as a good candidate model for selection, provided that it was >7. All other models within two AIC units of this model were also retained, and deemed not substantially different (Burnham & Anderson, 2002). All loci with less than 5% variability were omitted from the analysis. Similarly, all models that did not converge properly, or had binary fitted probabilities, were deemed unsafe and rejected from further analysis.

ii) The population genomics approach is the most widely used method for detecting outlier loci that may be under selection using data derived from AFLP markers (Bonin *et al.*, 2007). Under this method, genetic differentiation among populations is simulated under a coalescent model of neutral evolution, conditional on heterozygosity. Any loci with F_{st} values falling outside of the expected range of neutral drift is considered aberrant, and therefore a good candidate for being under divergent selection (Beaumont & Nichols, 1996). Unlike the locus screening using GLMMs, which require no prior assumptions about population structure or inbreeding, the population genomics approach relies entirely on good estimates of various population parameters on which to base the simulations. It is also not possible to test directly the selective pressure that may be acting on individual loci under this method, although it has been previously used to infer selection (Bonin *et al.*, 2006; Galindo *et al.*, 2009).

All loci were screened using the software DFDIST, the most widely used method, specifically adapted for use with dominant markers (Caballero *et al.*, 2008). Each simulation was run for 50 000 iterations to obtain 99% confidence intervals under the neutral model. Each site was considered a separate population with nine individuals, and the subroutine Ddatacal used to estimate the trimmed mean F_{st} following the recommendations of Caballero *et al.* (2008). The critical frequency for the most common allele was set to 0.99; the scale for the Zhivotovsky (1999) parameter was 0.25; the smoothing proportion was 0.04; and the value θ for the metapopulation was 0.04 (pilot simulations indicated that there was very little difference between models run with higher or lower values of θ). Input data for use in the program was formatted using the software AFLPconvert β0.3 (Rodríguez, 2009).

Finally, the incidence of linkage disequilibrium among loci was assessed using the software Arlequin 2.0. This method tests the non-random association of alleles at different loci, and is necessary to evaluate whether two or more loci that prove positive for selection under either screening method are inherited independently of each other. For each pair of loci, Fisher's exact probability tests on contingency tables were performed, and significance determined after a Bonferroni correction across all pairs of loci. A Markov Chain of 100 000 iterations, with initial dememorization of 1000 steps was used to explore the space of all possible tables (Excoffier *et al.*, 1992).

6.4 Results

6.4.1 Error rate analysis

Among all *O. brumata* profiles, GeneMapper created a total number of 352 bin sets, which was reduced to 124 after visual inspection; among *A. grossulariata* 336 bin sets were created, of which 145 were retained. Among eight no-template H_2O controls run concurrently with the positive samples, analysis with the *O. brumata* specific bin set scored an average 2.6% spurious peak error; with *A. grossulariata* scoring 1.3% spurious peak error. The majority of these spurious peaks occurred <150bp. From these initial bin sets, error rate analysis indicated that for *O. brumata* using a locus threshold of 1200 rfu (100% GMNPH) and a phenotype-calling threshold of 60 rfu (5% GMNPH) gave the lowest error rates across all combinations (Bayesian error = 6.1%; mismatch error = 3.03%). Under this model, a total of 44 loci were retained for further analysis

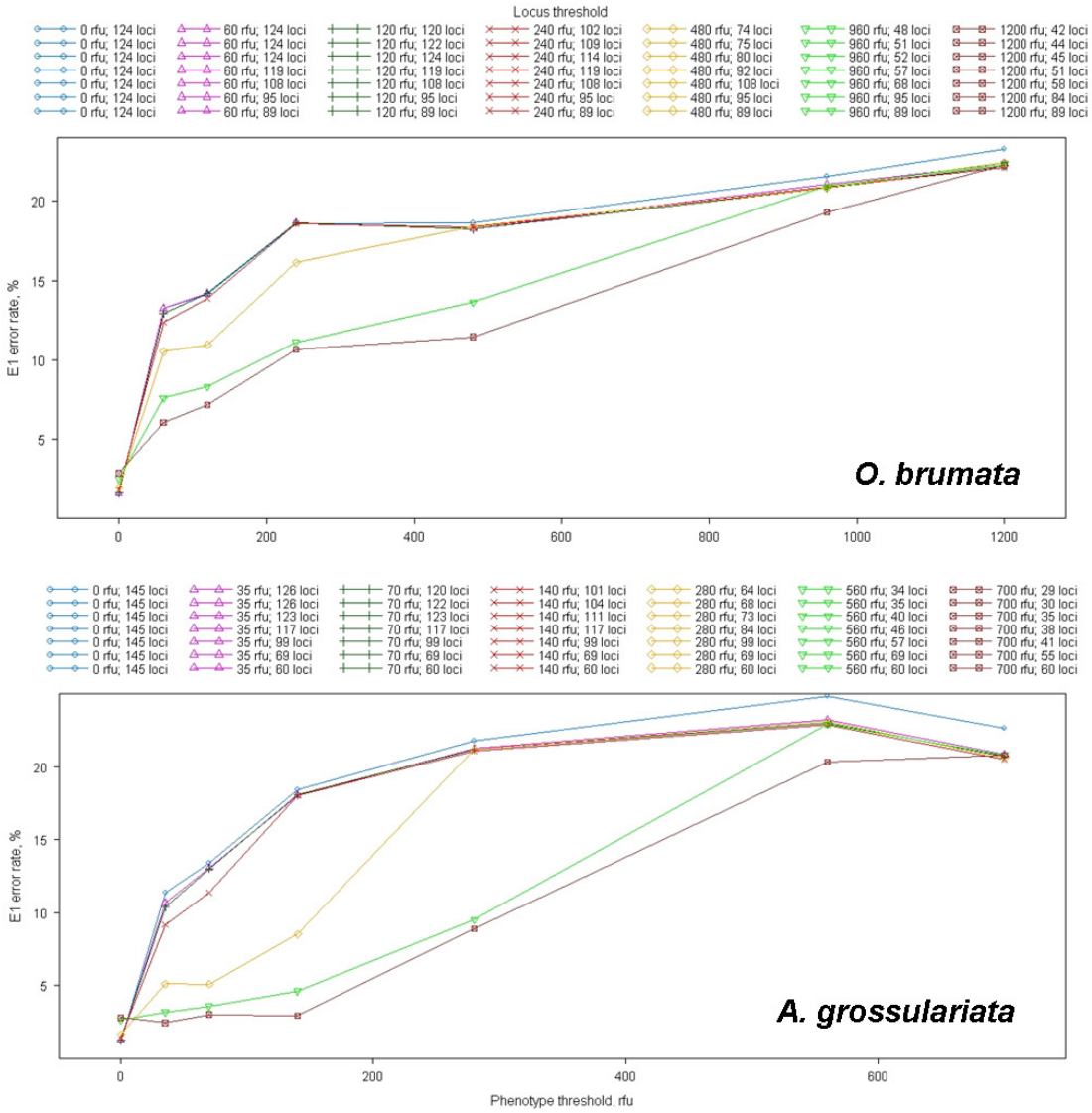


Fig 6.2 Bayesian ($\varepsilon_{1.0}$) error rates across all given locus- and phenotype-calling thresholds for both species. The largest threshold values represent the grand mean normalised peak height (GMNPH) across all loci for that species.

(Fig. 6.2), ranging from 55-851 bps in size. For *A grossulariata,* a locus threshold of 280 rfu (41% GMNPH), with a phenotype-calling threshold of 70 rfu (10% GMNPH) was chosen (Bayesian error = 5.1%; mismatch error = 1.37%), giving a final number of retained loci of 73, ranging in size from 55-978 bps. Although other scenarios gave lower error rates than the one chosen (Fig. 6.2), using these threshold values retained substantially more loci, whilst still remaining within acceptable error limits.

6.4.2 Non-spatial among-year analysis

For both species, the highest level of clustering was found at K=3 clusters, following the method of Evanno *et al.* (2005). This method showed substantial support for clustering at this level, with the modal value of all *O. brumata* models being >20 ΔK units greater than the next highest value. For *A. grossulariata,* this difference was >4 ΔK units. The differences in *O. brumata* cluster groups appeared to be partitioned most strongly by sampling year, with a strong tendency to cluster all individuals sampled from 2008 together into a single group, with a small number of individuals within some sites being associated more strongly with other clusters, most noticeably in the sites MLD and WH (Fig.6.3a). There is also a suggestion that the mainland Britain site WYT, collected in a different sampling year to all of the other samples, is relatively heterogeneous in comparison with other sites. It also does not appear to be as strongly differentiated from the other sites within Orkney, as the sites LI and HU collected in different sampling years. This could indicate either strong temporal differentiation within sampling sites, or some possible degradation of the DNA template used to score the AFLP data.

Similarly, among the *A. grossulariata* sites, around half of the samples collected in 2004 appear to be strongly differentiated from those collected from all other years. Samples collected from the same site on two subsequent years (SW06 and SW08), suggest a contrastingly high level of homogeneity among cluster membership probabilities in comparison. Unlike the mainland Britain sites used in the *O. brumata* analysis, these groups appear to be strongly differentiated from the Orkney sites, among all individuals sampled. However, there does appear to be a greater proportion of individuals sharing cluster membership with the Orkney sites at BE06, the site geographically closer to Orkney, than that at CT06 in southern Britain. Among the Orkney sites, there appears to be little cluster differentiation within sampling sites, with the exception of WC08, which contains about $^1/_3$ of individuals assigned to a different cluster. Other sites with at least one aberrant individual are HEL06, SC06, SE06, and FF08 (Fig 6.3b).

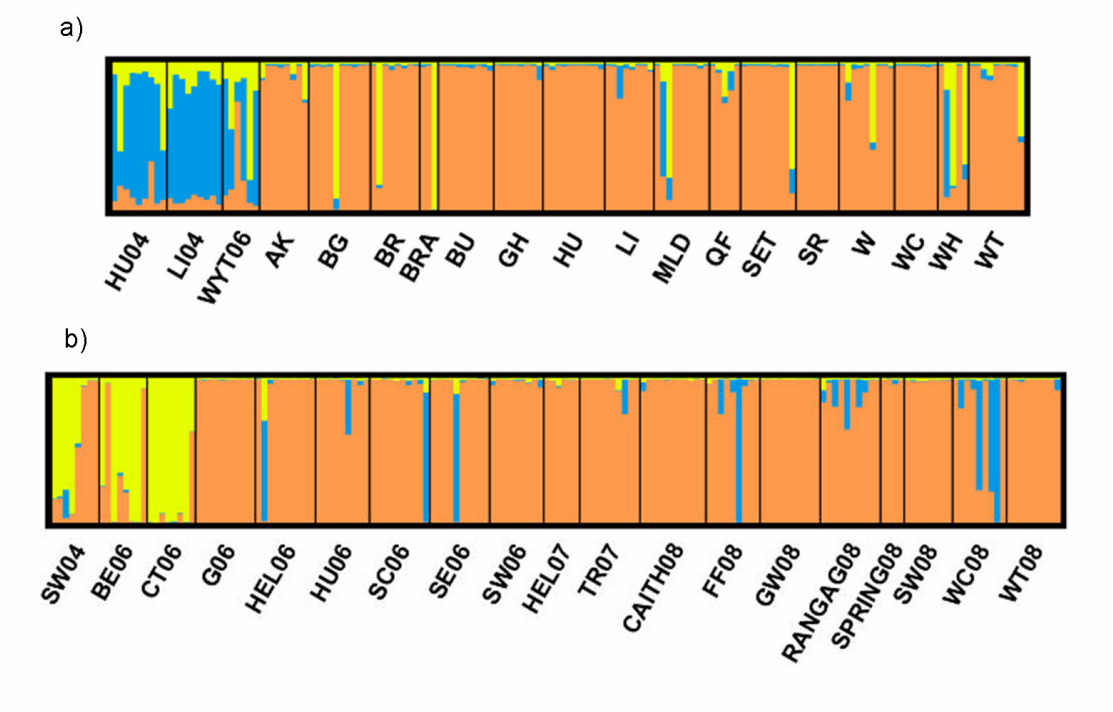


Fig. 6.3 Assignment probabilities of individuals of a) *O. brumata* and b) *A. grossulariata* to their inferred population of origin using the software STRUCTURE. Different colours represent assignment of individuals to separate populations. Each vertical bar represents a single individual. Site codes are given as in Table 1, suffixed by year of collection. For *O. brumata* individuals, site codes without numbers were all collected in 2008.

Population genetic diversity under Hardy-Weinberg proportions was markedly different for both species within sites. *O. brumata* had substantially higher levels of expected heterozygosity across all sites (average gene diversity within samples (H_w) ± S.E. = 0.333 ± 0.01) compared with *A. grossulariata* (H_w = 0.117 ± 0.05) (Fig. 6.4), although there was no obvious pattern of temporal and/ or spatial change in any of these values across any of the sampling sites. The proportion of polymorphic loci (PLP) was also substantially higher among *O. brumata* sites (0.73-1.0) compared to *A. grossulariata* (0.1-0.71), and restricted within a much narrower range. There was no evidence of correlation between Hj and sample size for either species (*O. brumata*: Spearman's rank correlation coefficient r_s=-0.18; p=0.46; *A. grossulariata* r_s=0.013; p=0.96), although PLP and sample size were positively correlated in *O. brumata* (r_s=-0.79; p<0.001) and uncorrelated in *A. grossulariata* (r_s=0.71; p=0.09). The two different measures of diversity (Hj vs PLP) were uncorrelated for *O. brumata* (r_s=-0.79; p=0.32), although for *A. grossulariata* this was not the case (r_s=0.91; p<0.001).

The highest levels of genetic diversity within *O. brumata* sampling sites were seen at BRA and WH, although these values still fell within three standard deviations of the mean across all sites. The mainland Britain outgroup WYT had marginally greater

genetic diversity (0.36 ± 0.02) than the mean across all sites, although again it did not differ substantially from those of other sites within Orkney. All three of these sites were polymorphic across all loci tested, as were the sites GH, LI, QF, SR and WC. There did not appear to be substantial differences in genetic diversity between either of the two sites sampled four years apart (HU and LI), when comparing both Hj and PLP, although Hj had decreased slightly in HU compared to HU04 (Fig 6.4a).

Similarly, levels of within-site genetic diversity appear to have declined in both *A. grossulariata* sites sampled across different years (Fig 6.4b). At the site SW, sampled at two year intervals, genetic diversity as measured by both Hj and PLP appears to have dropped substantially after the first sampling year in 2004, and although the rate of decrease is less, still decreased during the period 2006-2008. This pattern is also matched among the other site sampled across different years, HEL. This site was sampled over consecutive years 2006 and 2007, and again shows a decrease in overall diversity by both measures during this time. Despite these apparent trends, two sites from 2008 stand out as having relatively high levels of within-site diversity in comparison with all other sites across years—FF08 and WC08. Both Hj and PLP are clearly higher among these sites than all other samples from 2008, and from all other sampling years, indicating a much greater variation in among-site genetic diversity than that seen among *O. brumata* sites (see S.D. in H_w, above). These levels of diversity are also in excess of those seen from the mainland Britain sites BE06 and CT06, although Hj for BE06 is slightly greater than the average across all sites. The sites G06, GW08, and SPRING08 all had noticeably lower diversity estimates, at almost ½ the average of all other sites.

For the purposes of comparison with inbred populations, heterozygosity among the *S. exigua* samples (52 loci; $\varepsilon_{1.0} = 0.48\%$, mismatch error = 11.8%) was $H_j = 0.146 \pm 0.022$ (PLP = 21.2%); and among *S. frugiperda* samples (36 loci; $\varepsilon_{1.0} = 6.1\%$, mismatch error = 4.5%) Hj = 0.256 ± 0.027 (PLP=41.7%). All *O. brumata* populations had H_j levels greater than both of these species, and the overall H_w was substantially greater (see above). For *A. grossulariata,* none of the sites had H_j levels greater than *S. frugiperda,* and only five of the 19 sites had H_j levels greater than *S. exigua*. The overall H_w (± S.E.) across all sites was also lower than for both *Spodoptera* species.

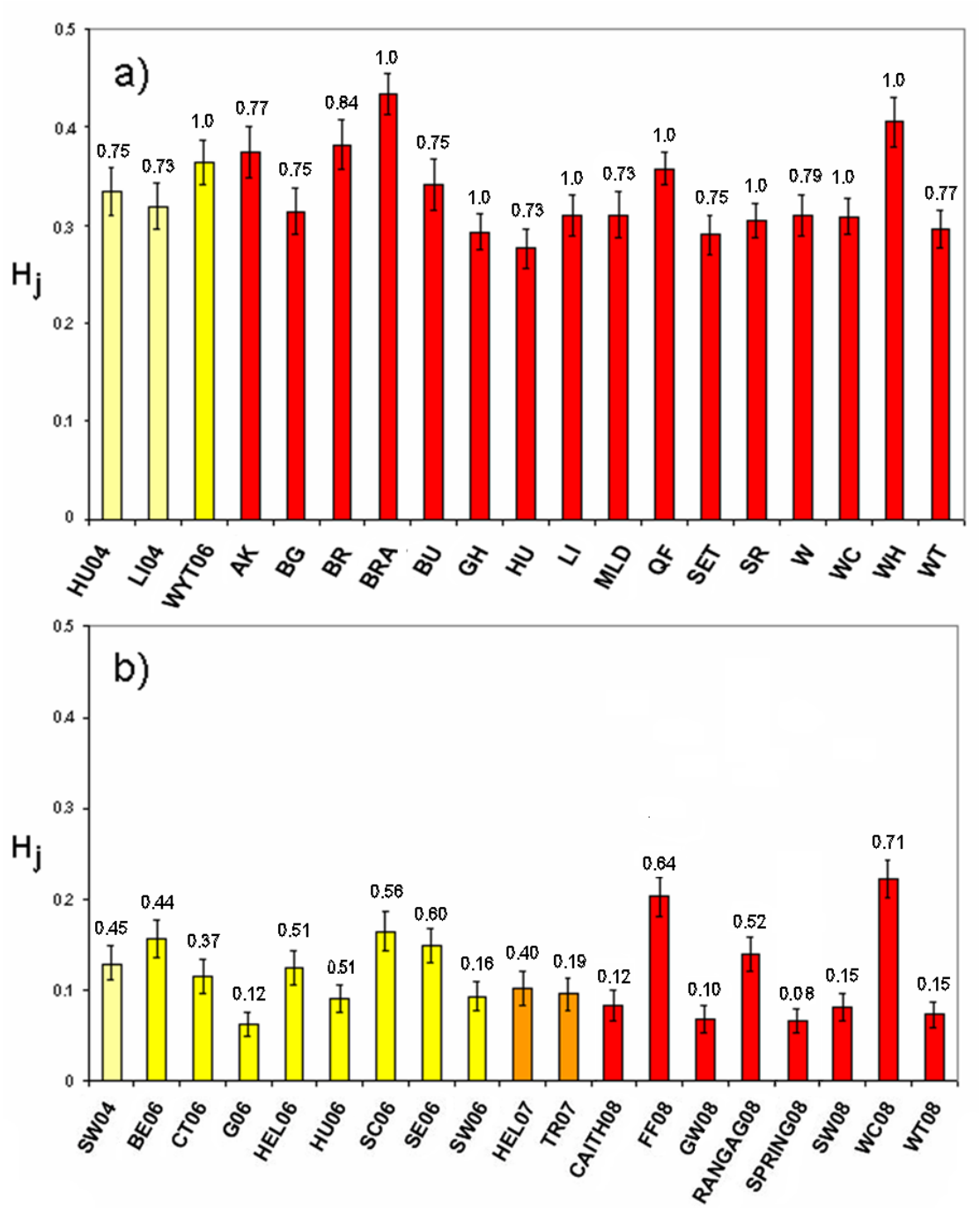


Fig 6.4 Expected heterozygosity under Hardy-Weinberg proportions (Hj) ± S.E. for a) *O. brumata* and b) *A. grossulariata*. Site codes are as in Table 6.1, suffixed by year of collection. This is also represented by the colour codes ■ 2004;■ 2006;■ 2007; ■ 2008. Numbers above each bar represent the proportion of polymorphic loci (PLP) within each site.

Genetic differentiation among populations was substantially lower in *O. brumata* (F_{st} = 0.0118) than *A. grossulariata* (F_{st} = 0.1272), although for both species, populations were significantly more differentiated than a random assemblage of individuals ($p < 0.001$). Among all *O. brumata* sites, only one branch was significantly differentiated with >95% bootstrap support, and contained the sites WH and BRA (Fig. 6.5a). Overall,

the two most genetically differentiated populations among all pairwise comparisons were the sites SET and BRA (F_{st} = 0.114 ± 0.025), which are also two of the closest geographically (Fig.6.1). The populations sampled during 2004 (LI04 and HU04) both cluster together along with the mainland Britain site from 2006 (WYT06) suggesting some temporal and/or spatial differentiation. However, this division is only given 59.6% bootstrap support. Differentiation between LI and HU within each sampling year was zero for both years among all bootstrap replicates. Similarly, all sites between SET and W on the main branch of the neighbour-joining tree show zero differentiation across 1000 bootstrap replicates, and together with the sites between LI and AK on the main branch (with the exception of QF), suggest a slight grouping of northern and southern moorland sites. The differentiation between SET and AK on the main branch is F_{st} = 0.018 ± 0.019.

Among all *A. grossulariata* sites, the most genetically differentiated sites were CT06 and BE06, the two mainland Britain sites furthest from Orkney geographically (Fig. 6.5b). This division from all other sites was supported across 100% of bootstrap replicates. The most highly differentiated of these two sites was CT06, which had the greatest genetic differentiation with WT08 across all pairwise comparisons (F_{st} = 0.516 ± 0.070). Both the sites SW04 and WT08 were differentiated from all other sites on the main branch of the neighbour-joining tree with 99.9% and 96.3% bootstrap support, respectively. The maximum pairwise differentiation among all other sites on the main branch was F_{st} = 0.073 ± 0.018, between WT08 and FF08. Among all of these sites, there appears to be a relatively heterogeneous mix of temporally and spatially distributed sampling sites. Surprisingly, the two mainland Britain sites closest to Orkney do not align themselves on the same branch, and in fact form sub-branches with other Orkney sites: RANGAG08 with HEL07 and SW08 (52% bootstrap support); and CAITH08 with SW06 (18.1% bootstrap support). The only other sub-branch formed in the tree is among the northern Orkney sites G06 and SPRING08 (26.1% bootstrap support), aligned closely to another northern site, WT08. None of the sites sampled over different years aligned themselves together on any of the branches, although there was not substantially greater differentiation between sites sampled a single year apart (HEL06 and HEL07 F_{st} = 0.015 ± 0.016) than sites sampled two years apart, with the majority of the differentiation coming solely from the samples from 2004 (SW04 and SW06 F_{st} = 0.219 ± 0.087; SW06 and SW08 F_{st} = 0.023 ± 0.028).

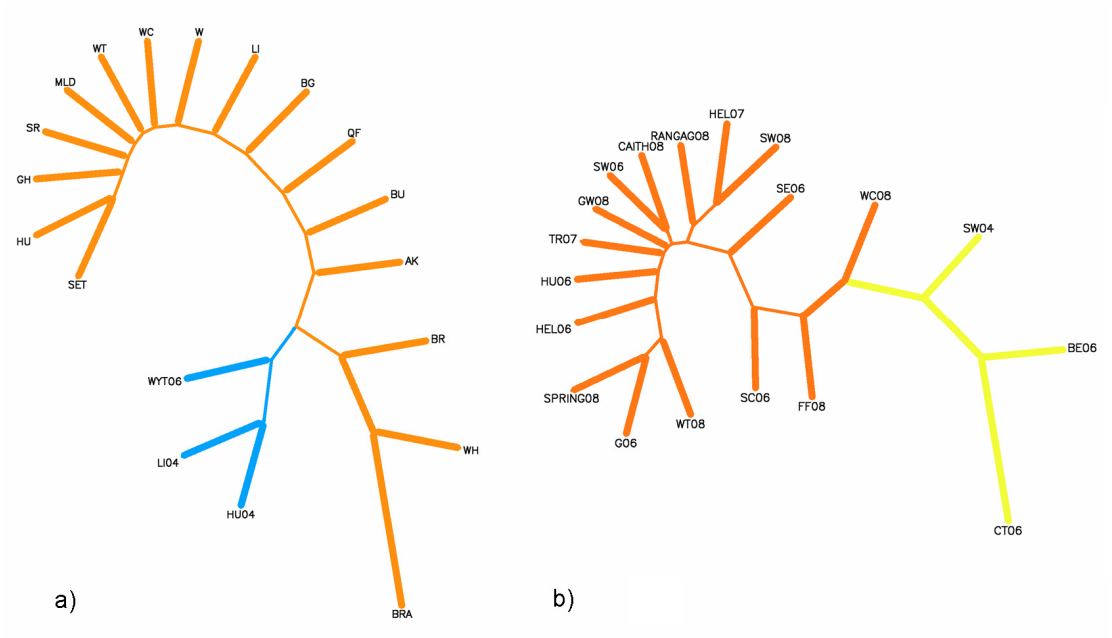


Fig 6.5 Consensus neighbour-joining trees of pairwise F_{st} values for a) *O. brumata* and b) *A. grossulariata*. Sites codes are as in Table 6.1, suffixed by year of collection. All *O. brumata* sites without suffixes were collected in 2008. Colour codes represent the largest cluster membership probabilities at each site, as in Fig. 6.3. Thick lines represent nodes with >95% bootstrap support. Branch lengths are relative to genetic distance.

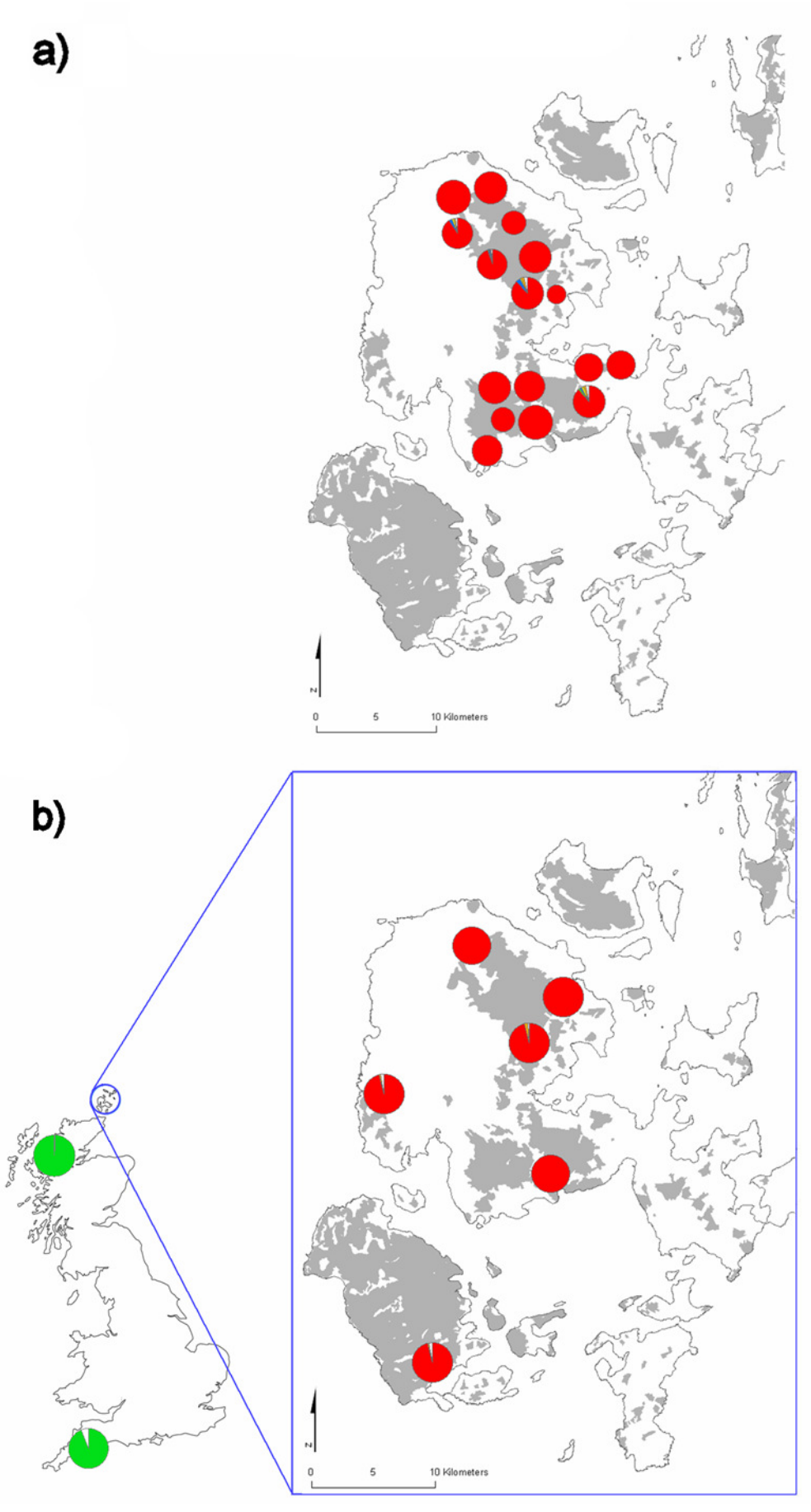
6.4.3 Spatial within-year analysis

Analysis of spatial genetic structure among *O. brumata* sampled from 2008 via spatial Bayesian clustering revealed strong homogeneity among all sites (Fig. 6a). The inferred K value for the maximum number of clusters was 6, although among all 16 sites, only four had cluster membership probabilities (Q) of less than 0.99 within the largest cluster (BR, GH, SET and W). Of these sites, W was the most heterogeneous (Q_{max} = 0.887, Q_{min} = 0.015) and also contained the highest probability among all non-maximal clusters (Q_{max-1} = 0.036), although SET had the lowest maximum cluster probability among all sites (Q_{max} = 0.875). There was little evidence of any spatial patterning among sites, although the largest heterogeneity of Q values appears to be partitioned along the south-western edge of the north moorland.

There was strong evidence for spatial genetic partitioning among all *A. grossulariata* sites sampled in 2006 (Fig. 6.6b), specifically among sites within Mainland Britain and within Orkney. The maximal K value for all permutations was K=6. Among these groups, all within-Orkney sites were assigned to a single cluster with probabilities between Q_{max} = 1 and Q_{max} = 0.950. The two mainland Britain sites each

had similar cluster membership probabilities, but assigned to different clusters (CT Q_{max} = 0.941; BE Q_{max} = 0.995). There was very little evidence of shared membership between these two groups, with the maximum probability of shared maximal cluster membership between an Orkney site (red) and a mainland Britain site (green) being Q = 0.006 (HEL). However, among all values of K clusters, the sites CT and SC shared membership of one cluster (white) with Q values of 0.059 and 0.034, respectively. Among all sites, HEL was the most heterogeneous across all six clusters, and G and SW the only sites completely homogeneous for a single cluster.

In contrast to 2006, among all *A. grossulariata* samples collected in 2008, there was little evidence of a spatial genetic partition at the scale sampled (Fig 6.6c), and no evidence that the mainland Britain sites were distinct from sites within Orkney. The maximal number of clusters within this year was K=4, and among all eight sites sampled, six sites contained cluster membership probabilities of Q_{max} = 1. Only the sites FF and WC were not assigned solely to a single cluster, although their cluster membership probability's within the same cluster as all other sites was still very high, being Q_{max} = 0.983 and Q_{max} = 0.965, respectively. The next highest cluster membership probability was within the site WC, but remained very low in comparison (Q_{max-1} = 0.027).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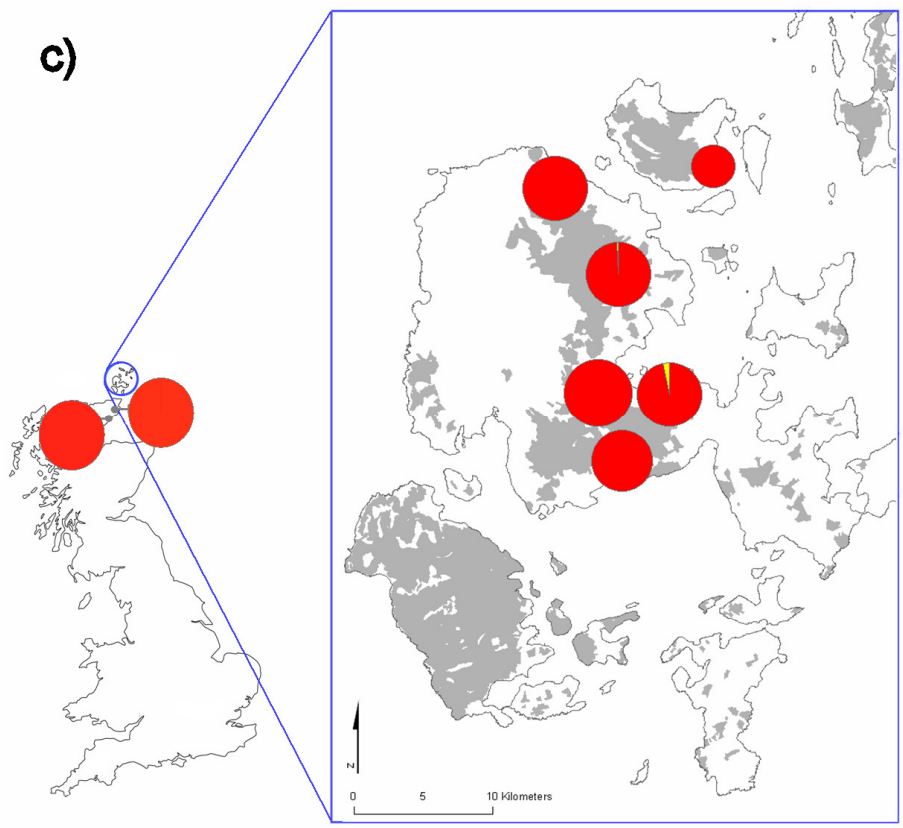

Fig 6.6 Assignment probabilities of a) *O. brumata* individuals collected in 2008 and *A. grossulariata* individuals collected from b) 2006 and c) 2008 to their inferred population of origin, using the software TESS. Different colours represent assignment to different clusters (K). The size of the circle at each site represents the relative sample size (within years) of individuals collected at that site. Grey shading indicates areas of heather moorland.

There was no evidence of any significant spatial structure among all *O. brumata* samples within Orkney in 2008 (Fig. 6.7a), compared to a random arrangement of individuals ($p > 0.05$). Individuals within sites were significantly more similar than random ($F_{(d)} = 0.038 \pm 0.015$; $p = 0.008$), although this relationship was not extended beyond the first distance class, at 3km. Mean pairwise kinship coefficients became negative beyond 5km distance, and were most dissimilar at a distance of 8km, after which they levelled off to zero again, suggesting a negative association between sites over this distance. However, none of these values were significantly different from random.

For all *A. grossulariata* sites sampled in 2006, the large discrepancies between pairwise site distances meant that all analysis was conducted on a log scale, with an unequal distance interval among the Orkney sites and the mainland Britain sites. The overall analysis among all sites and all distance classes revealed significant spatial genetic structure ($p = 0.016$) compared with a random permutation of individuals (Fig

6.7b). However, only individuals within sites were significantly differentiated ($F_{(d)}$ = 0.118 ± 0.035; $p < 0.001$), compared to random among all other distance classes. All sites within Orkney (distance classes 2-5; up to log(1.6)km) were positively associated, with a strong negative association with the mainland Britain sites (BE and CT). However, there appeared to be no obvious spatial pattern within these Orkney groups, with all mean pairwise kinship coefficients remaining stable at $F_{(d)} = 0.034 \pm 0.014$. A sharp increase in kinship estimates was evident between the distance classes log(2.3km) and log(2.9km), although not significantly so. These distance classes represent those between the sites BE and CT, and reflect a strong association between the two sites, relative to the sites within Orkney. Due to these large discrepancies between distance classes, the analysis was also repeated, removing both of these mainland Britain sites. When CT is removed only (the site further from Orkney), the overall spatial genetic structure is altered so that a significant association of decreasing kinship with distance no longer exists ($p = 0.092$), although kinship within sites remains significantly associated compared to random ($F_{(d)} = 0.053 \pm 0.025$; $p < 0.001$). Similarly, when BE is also removed, the significant association of kinship with distance disappears ($p = 0.753$), although under this scenario, kinship coefficients within sites are no longer significantly different from a random arrangement of individuals ($F_{(d)} = 0.002 \pm 0.010$; $p = 0.611$).

Autocorrelation tests among all *A. grossulariata* sites and all distance classes sampled in 2008 also revealed no significant spatial genetic structure ($p = 0.52$; Fig. 6.7c), although again kinship coefficients within sites were significantly more associated than random ($F_{(d)} = 0.024 \pm 0.012$; $p = 0.003$). Although there seems to be no overall pattern of genetic similarity with distance, kinship coefficients did reach levels very similar to that within sites at around the 100km distance class, after having been largely negatively associated in the intervening distances. Again, when the mainland Britain sites are removed and the analysis repeated, much the same pattern occurs, with the overall test of spatial association being non-significant ($p = 0.231$). However, unlike the samples from 2006, when samples are pooled to a within-Orkney only analysis, the within-site kinship coefficients are significantly more associated than random ($F_{(d)}$ = 0.028 ± 0.014; $p = 0.013$), indicating that the mainland Britain sites have little or no influence on the spatial genetic structure among sites.

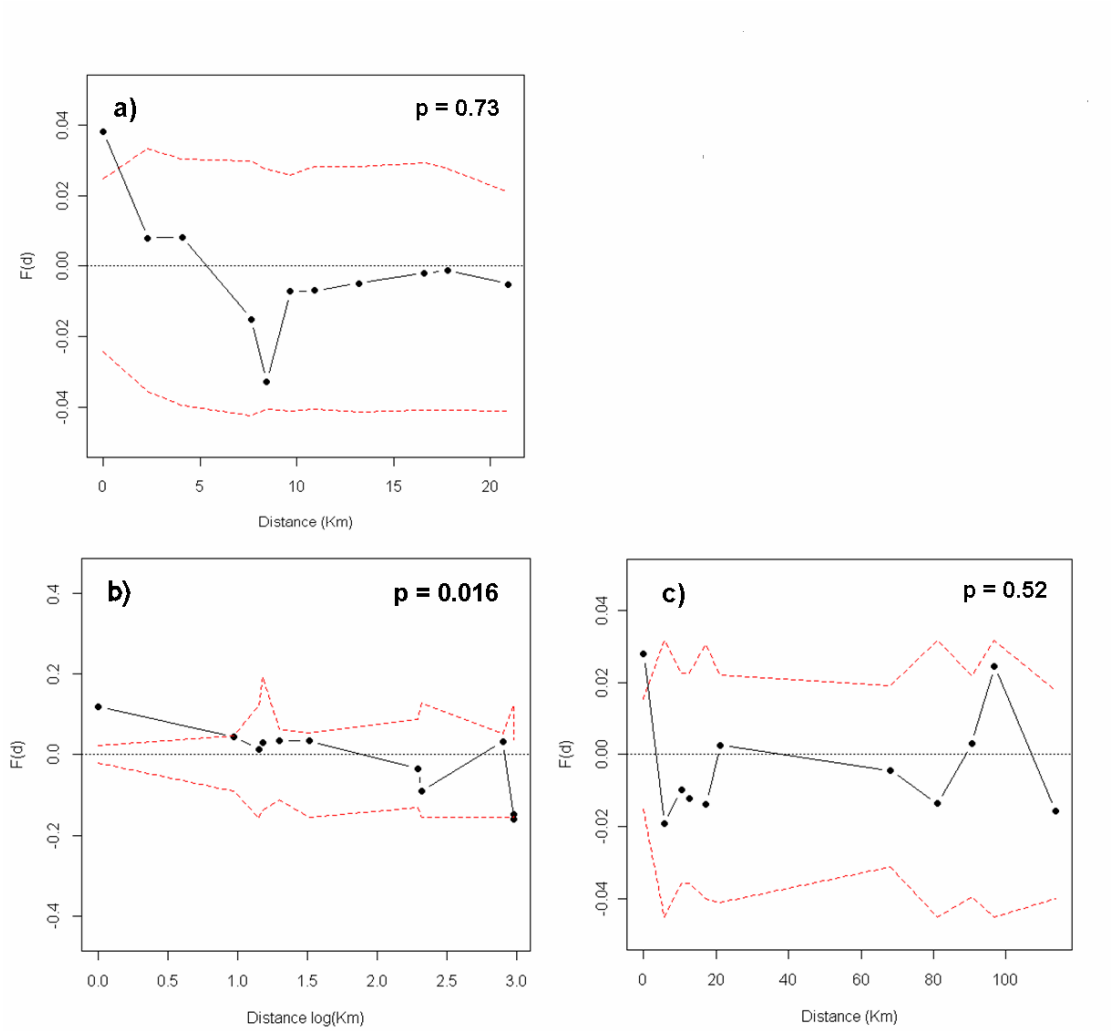

Fig 6.7 Correlograms of mean pairwise kinship coefficients $F_{(d)}$ at different distance classes for a) *O. brumata* samples from 2008; b) *A. grossulariata* samples from 2006; and c) *A. grossulariata* samples from 2008. Black points represent the maximum distance of each distance class, which have an equal number of samples. Dashed red lines represent 95% confidence intervals. P-values represent the probability that there is significant spatial structure among all spatial groups, in comparison to a random permutation of all spatial locations.

Partial mantel tests to investigate whether within-site density and virus prevalence had any effect on within-site heterozygosity (Hj) (while still accounting for any possible spatial genetic structuring) for all sites within Orkney, revealed that among *O. brumata* sites from 2008, there was no evidence of a within-year density effect (r = 0.059; p = 0.580), or a delayed effect from the previous year (r = -0.26; p = 0.766). Similarly, within-year virus prevalence (r = -0.147; p = 0.33) and previous-years virus prevalence (r = -0.128; p = 0.33) had no effect. For *A. grossulariata,* both years 2006 and 2008 were combined, since density and delayed density measures are independent of each other. Again, there was no evidence that either density within the current year (r = 0.0076; p = 0.967) or density from the previous year (r = -0.179; p = 0.229) had any effect on within-site Hj, compared to random permutations. Again, there was also no

evidence of a direct effect of virus prevalence (r = -0.058; p = 0.791) or a delayed effect of virus prevalence from the previous year (r = -0.100; p = 0.588) on the within site heterozygosity.

Hierarchical AMOVA revealed that for *O. brumata* from 2008, the vast majority of genetic variation is partitioned within sites (Table 6.2a), and suggests that the spatial arrangement of moorlands within the Orkney mainland has very little impact (<1%) on the genetic variation within this species. Total genetic differentiation among sites was low compared to when spatial groupings are considered separately, although the observed value was not significantly different from a random permutation of individuals among sites, suggesting that there may in fact be even lower differentiation among sites. In contrast, *A. grossulariata* samples from 2006 displayed far greater differentiation between spatial groups (Table 6.2b), with a fairly similar partition of genetic variation among regions and within sites. The fact that so little variation is attributable among sites within regions (1.39%) suggests that there is a large discrepancy between regional scales under this sampling regime. The total genetic differentiation among sites was marginally more significant than random permutations (p = 0.044), and suggests a fairly large differentiation, although again the fact that among region differentiation is so low suggests that this is almost entirely due to large regional differences among sites. When genetic variation is considered on a smaller spatial scale, as in *A. grossulariata* samples from 2008, the outcome appears very different. Similar to *O. brumata* samples from the same year, the vast majority of genetic variation is partitioned within sites (>95%; Table 6.2c). No variation can be attributable to regional differences in this instance, suggesting that samples from the two mainland Britain sites CAITH and RANGAG are not substantially different from those samples collected in Orkney. Again, the total genetic differentiation among sites is comparatively low (F_{st} = 0.014), although again, this value was not significantly different from a random permutation of individuals, suggesting high levels of admixture among all sites at these scales.

6.4.4 Genome scan for loci under selection

A total of three loci were identified as being under selection from the biotic and abiotic variables screened under the AIC method used here for *O. brumata* (Table 6.3a). Of these three loci, three models each were considered as possible candidate models for

Source of variation	d.f.	Sum of Squares	% variance	Fixation indices Φ	p-value
a) *O. brumata* 2008					
Among moors	2	8.74	0.43	0.059	0.002
Among sites within moors	13	50.33	5.88	0.063	<0.001
Within sites	117	298.21	93.7		
Total	132	357.3		0.004	0.259
b) *A. grossulariata* 2006					
Among regions	1	58.50	40.15	0.0233	<0.001
Among sites within regions	6	28.31	1.39	0.415	<0.001
Within sites	66	209.56	58.46		
Total	73	291.37		0.401	0.044
c) *A. grossulariata* 2008					
Among regions	1	3.87	0	0.057	0.003
Among sites within regions	6	29.74	5.80	0.0444	0.006
Within sites	62	202.88	95.56		
Total	69	236.49		0.014	0.657

Table 6.2 Hierarchical AMOVA for each species at different spatial scales. For *O. brumata* (a), moors are defined as areas of contiguous heather moorland. For *A. grossulariata* (b and c), regions are defined as either Orkney or mainland Britain; d.f. represents degrees of freedom; Φ represents the fixation index at each scale (hence within site Φ is analogous to F_{st}); p-values represent the probability of a larger value of Φ being obtained by random permutations.

selection, out of a total of 44 loci and 34 different combinations of possible explanatory variables. None of these loci proved to be significantly associated with biotic variables (within site density, virus prevalence, and parasitoid prevalence) from the same sampling year, although two of the three loci proved positive for a delayed effect of density and virus prevalence (68bp and 91bp). The most likely candidate model for selection among locus 68bp was most strongly associated with virus prevalence from the previous year, an increase in which is concurrent with the loss of this locus. However, since the two alternative models (with an interactive and additive effect of density) are all within two AIC units, it is likely that density is an important factor governing this selection pressure equally with virus prevalence, despite a discrepancy in their effect sizes (Virus = -1.76; Density = 0.005). Similarly, for the locus at 91bp, the effect of virus prevalence and density is virtually indistinguishable, although in this case an increase in both variables (separately and additively) is associated with the loss of the locus. Again, the major effect size among these models appears to be driven by virus prevalence (-0.465) rather than density (-0.004). For the locus at 125bp, this was the only locus to screen positively for selection under any of the abiotic variables considered. The most likely candidate model is that containing just a slope explanatory

variable, although this is only supported by a posterior probability of 0.4. The other two candidate models, although less than seven AIC units different compared to the null model, cannot be considered substantially different from the most likely model. Both these models contain the same slope covariate, although additively with elevation, and slope covariates. Among these additive models, the slope variable has by far the largest effect size in comparison to the other variables, and it is likely that this is the most important factor affecting selection at this locus, with an increase associated with the loss of that locus. None of the nine candidate model's residuals were spatially autocorrelated (average Moran's I = -0.004 ± 0.007) compared to random permutations of residuals across all spatial locations ($p > 0.05$ for all models).

Among all 24 models screened across 73 loci for *A. grossulariata*, four models at 12 separate loci were deemed substantially better than the null (Table 6.3b). These four models consisted only of biotic variables measured in the previous year, in all possible interactive, additive and individual combinations. All candidate models had ΔAIC >7 in comparison to the null model, with four of the twelve loci having all candidate models with ΔAIC >10. The greatest level of support given to a single model was at locus 485bp, with the best candidate model having ΔAIC = -21.3 and a posterior probability of 0.78, indicating substantial support. This model consisted of a $Density_{(-1)}$*$Virus_{(-1)}$ interaction, with all other candidate model combinations being greater than two AIC units different, making it highly likely that this is the best candidate model. Overall this interaction is associated with the gain of the locus at this point. Among all other loci where the interaction between $Density_{(-1)}$ and $Virus_{(-1)}$ is among the candidate models, this combination has the least support and the lowest posterior probabilities. Other models with noticeably high posterior probabilities are the effect of $Density_{(-1)}$ at loci 297bp (0.71), and 177bp (0.70). Among all the best candidate models at each locus, the majority appeared to be associated with the loss of the locus at that point with an increase in the value of the explanatory variable. Of these, the biotic variable with the consistently highest level of support among all candidate models was that of $Density_{(-1)}$. However, again where $Virus_{(-1)}$ is also a viable candidate model, its effect size is consistently greater than that of $Density_{(-1)}$ across all loci, and among all additive models combining the two variables. There was no evidence of residual spatial autocorrelation among any of the 36 candidate models at the 12 loci (average Moran's I = -0.007 ± 0.006) compared to random permutations ($p > 0.05$ for all models).

Locus	$Density_{(-1)}$ * $Virus_{(-1)}$	$Density_{(-1)}$ + $Virus_{(-1)}$	$Density_{(-1)}$	$Virus_{(-1)}$	Elevation + Slope	Slope + Aspect	Slope
a) *O. brumata*							
68bp	-9.5 (0.19) 0.041	-10 (0.25) (0.005) + (-1.76)		**-11 (0.41)** **-1.47**			
91bp		-7.1 (0.19) (-0.004) + (-0.465)	**-8.4 (0.36)** **-0.006**	-8.3 (0.34) -0.68			
125bp					-6.7 (0.18) (-0.01) + (-0.26)	-6.9 (0.20) (-0.25) + (-11.39)	**-8.3 (0.40)** **-0.28**
b) *A. grossulariata*							
120bp		-9.8 (0.31) (0.07) + (-41.7)	**-10.5 (0.43)** **-3.69**	-9.4 (0.24) -0.002			
121bp	-11.1 (0.12) -0.102	-13 (0.33) (0.01) + (-5.65)		**-14 (0.54)** **-1.13**			
124bp		-9.4 (0.21) (-0.01) + (4.4)	**-10.9 (0.42)** **-0.006**	-10.07 (0.28) 0.09			
167bp		-10.1 (0.15) (-0.004) + (0.77)	**-12.1 (0.40)** **-0.003**	-11.9 (0.38) 0.15			
177bp			**-8.5 (0.70))** **-0.003**				
234bp	-7.5 (0.06) 0.15	-9.4 (0.16) (-0.0005) + (-1.07)	-10.9 (0.34) -0.002	**-11.4 (0.43)** **-1.15**			
259bp	-7.4 (0.07) 0.12	-9.4 (0.19) (0.007) + (-1.41)	**-10.7 (0.37)** **0.005**	-9.8 (0.24) -0.21			
265bp				**-8.7 (0.44)** **-3.06**			
275bp	-7.1 (0.08) -0.12	-9 (0.21) (0.005) + (-1.36)	**-10.2 (0.38)** **0.003**	-9.9 (0.32) -0.57			
297bp	-16.8 (0.29) -1.51		**-18.6 (0.71)** **-0.03**				
328bp	-7.3 (0.07) 0.004	-9.3 (0.18) (0.001) + (-1.73)	-10.1 (0.27) -0.002	**-11.2 (0.47)** **-1.58**			
485bp	**-21.3 (0.78)** **0.84**	-18.6 (0.20) (0.012) + (6.76)	-11.3 (0.005) 0.005	-12.3 (0.009) -2.18			

Table 6.3 Summary of all GLMM models where ΔAIC >7 compared to a null model. All models within two AIC units of any of these models were also included, and are deemed not substantially different. Individual loci are identified by their size (bp). For each locus, values are as follows: Top line = ΔAIC (Akaike weight); Bottom line = model coefficients. For models with multiple additive explanatory variables, separate coefficients are given in brackets. Positive coefficients indicate that an increase in the explanatory variable is associated with the gain of that locus. Biotic variables suffixed by (-1) indicate that the value has been obtained from the previous year (i.e. a delayed effect). Values in bold represent those models with the greatest support within each locus.

Among all 44 *O. brumata* loci screened under the coalescent approach for outlier detection, four loci fell outside the lower 99% confidence envelopes under the neutral evolution model (Fig. 6.8a). Of these, only locus 91bp also screened positive for selection under the AIC method. The other positive loci both fell within the 99% limit, although locus 68bp had the largest F_{st} value, conditional on heterozygosity, among all loci. Locus 125bp, that screened positive under abiotic selection pressures, had an F_{st} value very close to the trimmed mean across all loci.

Among all 73 *A. grossulariata* loci screened, eight loci fell outside of the 99% confidence envelope (Fig 6.8b). Of these, four were significantly greater than the upper

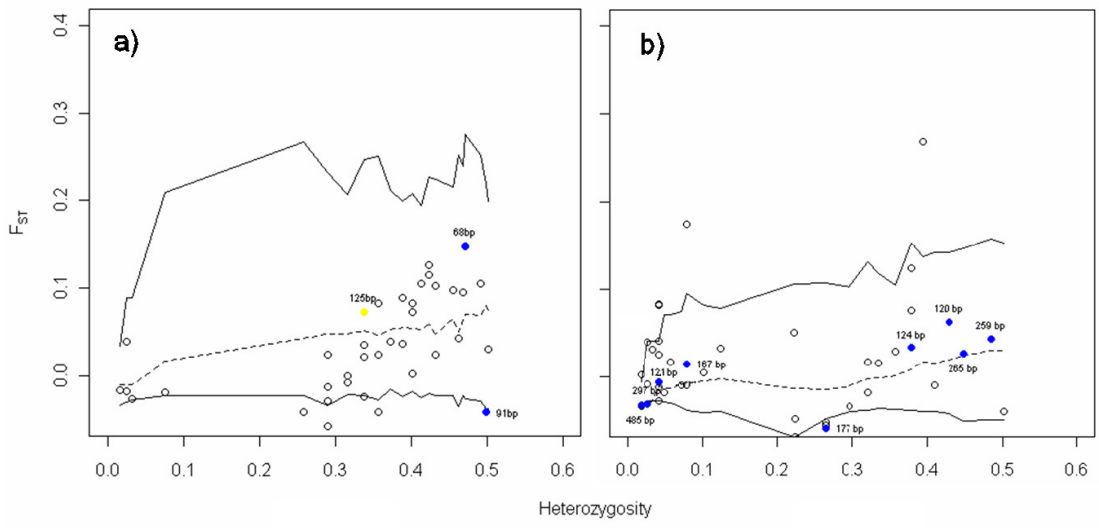

Fig 6.8 Coalescent models of F_{st} conditional on heterozygosity for all loci under the assumption of neutral evolution for a) *O. brumata* and b) *A. grossulariata*, using the software Dfdist. Dashed lines and solid lines represent the trimmed mean F_{st} and 99% confidence intervals, respectively. Circles represent individual loci. Blue circles represent loci that screened positive for selection via biotic pressures; yellow circles represent loci that screened positive for abiotic pressures (see Table 6.3). All positive loci are identified by their size (bp).

99% bound, although none screened positive for selection by any of the biotic or abiotic variables examined under the AIC method above. However, three of these twelve models were detected as outliers falling below the lower 99% bound: 177bp, 297bp and 485bp. All other models fell above the trimmed mean F_{st} across all loci, although not significantly so. Loci at 234bp and 328bp that proved positive for selection under AIC criteria were removed from the coalescent models, as they fell above the threshold value of 0.99 for the maximum allowable frequency of the commonest allele.

There was no evidence that any of the three *O. brumata* loci that screened positive for selection under the AIC method were linked (Table 6.4a). The average linkage value between pairwise comparisons of these loci (D) was 0.019 ± 0.009, and the overall percentage of positively linked loci across all 44 comparisons was 4.1%. The *A. grossulariata* samples had a slightly greater overall percentage of linked loci across all comparisons (13.3%), although the number of linked loci among those that screened positive under the AIC method was substantially greater (30.4%; Table 6.4b). Of these loci, only three were not linked with any others (167bp, 259bp, and 265bp). The locus with the greatest number of positive links to other loci was 120bp (D = 0.014 ± 0.009), and the average D-value across all pairs of AIC-positive loci was 0.008 ± 0.007.

a) *O. brumata*

	68bp	91bp	125bp
68bp			
91bp	NS		
125bp	NS	NS	

b) *A. grossulariata*

	120bp	121bp	124bp	167bp	177bp	234bp	259bp	265bp	297bp	328bp	485bp
120bp											
121bp	***										
124bp	***	NS									
167bp	NS	NS	NS								
177bp	***	***	***	NS							
234bp	***	NS	NS	NS	NS						
259bp	NS	NS	NS	NS	NS	NS					
265bp	NS	NS	NS	NS	NS	NS	NS				
297bp	***	***	***	NS	***	NS	NS	NS			
328bp	***	NS	***	NS	***	***	NS	NS	***		
485bp	***	NS	NS	NS	NS	***	NS	NS	NS	NS	

Table 6.4 Linkage disequilibrium among pairwise comparisons of loci that screened positive for selection (see Table 6.3). *** represents a significant positive association between pairs of loci (linkage equilibrium) after Fisher's exact tests and Bonferroni correction. NS represents a non-significant association (linkage disequilibrium).

6.5 Discussion

Genetic structure among populations of *O. brumata* were characterised by high levels of within-site genetic diversity, and low levels of among-site genetic differentiation. This is consistent with other studies of invertebrates that display outbreak behaviour (Baumann *et al.*, 2003; Conord *et al.*, 2006), although it should be noted that this study was conducted on a much smaller scale. Although only a single site was sampled outside of Orkney, the similar levels of genetic diversity together with relatively low levels of differentiation suggest a high level of dispersal among this species. Indeed, samples from some sites within Orkney were significantly more differentiated than sites sampled from different years and from different countries. Even considering such differences as host-plant species and phenological asynchrony (Kerslake & Hartley, 1997), it is surprising that such low levels of differentiation have been exhibited in this study, especially given that significant population differentiation in life-history traits has previously been found in this species (Vanbergen *et al.*, 2003). Research into the effects of habitat fragmentation on population genetic structure in *O. brumata* by van Dongen *et al.* (1998) are largely congruent with the findings of the present study, with very little differentiation found among populations, even at scales more than twice that of the present study (within Orkney). In their study, although differentiation was detected within fragmented areas of habitat, no effect was detected within continuous habitat. This is consistent with the finding here, and with other studies of fragmentation of

dispersal-limited invertebrates (Vandergast *et al.*, 2009) with only negligible levels of genetic variance between unconnected areas of moorland. The distances between moorland patches within Orkney are small relative to patch size, and despite a grouping of north-moor and south-moor sites by genetic distance, our findings here support the hypothesis that within-island heather moorland acts as a single homogenous habitat for *O. brumata*.

Although there was no significant effect of fine-scale spatial genetic structure among distance classes, such effects have been previously found in other Lepidoptera species (Schroeder & Degen, 2008), including at both small and large spatial scales in a species with a similar life-history (Wynne *et al.*, 2003). However, where differences in the dispersal capabilities of both males and females exist, as in *O. brumata*, results from other studies do seem to differ (Salvato *et al.*, 2002). The majority of genetic variance was partitioned within sites, and with relatively high levels of polymorphism among loci, it is perhaps surprising than no spatial restriction was detected. This could be due to the low sample sizes used; although the total samples size was within acceptable levels (Jump *et al.*, 2007), the mean number of samples per distance class, and the total number of loci screened was below that recommended from other studies (Cavers *et al.*, 2005). It is also worth noting that population genetic studies of plant species with wind-dispersed gametes typically display fine-scale spatial genetic structure, when analysed from AFLP markers (Takahashi *et al.*, 2000; Jump *et al.*, 2007). This is often coupled with high genetic diversity and low genetic differentiation, as found in this study. Since *O. brumata* larval stages are capable of wind-borne dispersal (Bell *et al.*, 2005) and females are flightless, this discrepancy may be due solely to large-scale male dispersal. However, despite this it seems unlikely that the low levels of differentiation found across all sites can be attributable to male dispersal alone. In a study on a similar scale with two related geometrid *Epirrita spp.* within the British isles, Wynne *et al.* (2003) found similar levels of large scale genetic differentiation among populations, attributing this to historical connectivity of post-glacial woodland constraining genetic drift, even in the absence of gene flow between populations. Similarly, *O. brumata* is a univoltine species that is likely to have expanded its range after this period, and is likely to have experienced similar genetic pressures.

In contrast, there was a clear differentiation between spatially separated populations of *A. grossulariata,* identified by both spatial and non-spatial Bayesian assignment tests, and bootstrapped F_{st} estimates. However, although this spatial

partitioning was only evident when geographic distances between Orkney populations and mainland Britain populations exceeded 200km, there was no detectable genetic differences between the sites BE and CT, which are separated by >800km. This suggests a significant separation between Orkney populations and the rest of mainland Britain. Large geographic features are known to cause significant barriers to gene flow (Hewitt, 1999), and there is evidence from other taxa that shows the mountainous regions that separate west and east Scotland are sufficient to cause genetic differentiation between populations (Zalewski *et al.*, 2009). When compared with other samples collected in the same year, this spatial separation of *A. grossulariata* sites explained around half the total genetic variance. However at smaller scales, again the majority of the variance was explained by within-site variation. Perhaps most surprisingly, genetic diversity within populations was also low; and on average, lower even than inbred laboratory stocks of two different species. Such low levels of diversity seem unusual, given that both males and females are capable of dispersal. Additionally, although dispersal capability in this species has not yet been investigated, it seems likely that, given their size (comparable to migratory species such as *Autographa gamma*), adults are most likely to be strong flyers. A mark-release-recapture experiment by Schneider (1999) in the noctuid *Heliothis virescens* found that males tended to disperse distances of around 10km, which translated into F_{st} estimates of around 0.002 among populations. These values are similar in range to those found among *A. grossulariata* populations in this study, and it is worth noting that distances of >10km would need to be covered in order to cross the Pentland Firth to arrive in Orkney. Since east Scotland and Orkney populations are indistinguishable (e.g. RANGAG08 and SW08: $F_{st} = 0.0064 \pm 0.0096$), it seems likely that these distances are well within the species' dispersal range. Studies of the Oak leaf-roller *Tortrix viridana* by Schroeder & Degen (2008) revealed similar levels of genetic diversity and differentiation among populations studied on a similar scale to those used in this study. However, crucially they uncovered spatial genetic structuring only within populations separated by up to 40m, consistent with site fidelity. This result was explained by the authors by observations of males mating with females from the same tree prior to longer-distance dispersal (Simchuk *et al.*, 1999). To my knowledge, such behaviour has not been documented in *A. grossulariata*, or in any other similar species (Reineke, 1999; Neve, *et al.*, 2000; Petenian & Neve, 2003), but could be a plausible mechanism to account for low levels of diversity among most populations, whilst still accounting

for high levels of admixture. The evidence in favour of this from the present study is the high degree of within population variation, and the significance of the intra-population kinship estimates. However, it should be noted with caution that this latter finding was not significant once the mainland Britain sites were removed (2006 only), and is also present in *O. brumata*, for which high levels of genetic diversity were found.

A further possible explanation for these findings relates to the possibility that *A. grossulariata* is currently undergoing a northward range expansion. There are no records of this species being anything other than a rare migrant to Orkney prior to 2000 (Berry, 2000), after which there appears to have been a large influx and establishment (Waring, 2006). The species has been recorded as widespread only in north-west Scotland during this time. However, after 2003, large outbreaks extending along the northern coast and towards Orkney have been reported (Horsfield & MacDonald, 2004). Crucially, it seems that during this period these populations appear to have been restricted to low-lying coastal areas only. Such a restriction could be consistent with a population bottleneck constraining gene flow between the mainland populations and the expanding range front, via a combination of corridor and/or jump dispersal (Wilson *et al.*, 2009). Indeed, non-spatial genetic structuring of Orkney populations contemporary with these reports (SW04) are more similar to the north-west populations than to current populations within Orkney. This raises the possibility that a small founding group of adults has become established within Orkney and north-east Scotland, and become genetically distinct from its mainland source population. However, population genetic theory would suggest that such a bottleneck would leave a signal of reduced genetic diversity relative to the source populations (Frankham, 1999), which is not in evidence from either expected heterozygosity, or the percentage of polymorphic loci, a potentially more sensitive indicator of historic bottlenecks (Amos & Harwood *et al.*, 1998; Luikart *et al.*, 1998).

Such losses of genetic diversity are well documented in newly invasive Lepidoptera (Johnson *et al.*, 2006) and other invertebrates (Grapputo *et al.*, 2005), and fragmented Lepidoptera populations (van Dongen *et al.*, 1998; Williams *et al.*, 2003)—both analogous situations to range expansions. Despite this, some studies of newly range-expanding and invasive species have detected little difference between source and range-front populations (Cheng *et al.*, 2008; Wilson *et al.*, 2009), although often these findings are characterised by initially high levels of genetic diversity among the source populations (Holland *et al.*, 2001). Highly vagile species that display low levels of

genetic variation could be explained by processes such as genetic drift causing fixation of alleles within a panmictic population (Holt, 2003). However, given the fact that source populations and Orkney populations are genetically distinct, this seems to provide an insufficient explanation. Such a phenomenon is also only expected among relatively small interbreeding populations (Frankham *et al.*, 2002). Previous work (see Chapter 5) as well as countrywide records (Hill, 1987) suggests that this is unlikely to be the case. Multiple introductions along an invasion front are known to maintain levels of diversity similar to source populations, even when source variation is already low (Marrs *et al.*, 2008), with even very low levels of gene flow operating between source and range margins (e.g. one immigrant per generation, Felsenstein 1983) sufficient to prevent population differentiation by genetic drift (Kunin *et al.*, 2009). An alternative possibility is that countrywide *A. grossulariata* populations have suffered a loss of genetic diversity due to population declines, but that such a decline is not manifest over the time scales used in this study. Molecular screening of the garden tiger moth (*Arctia caja*) from museum specimens revealed that this species has suffered dramatic declines in genetic diversity across all British populations over the previous century, and is currently undergoing a northwards range shift (Anderson *et al.*, 2008). Such losses of diversity are concurrent with widespread population declines, and are similar to the declines seen among *A. grossulariata* populations over the same period (Conrad *et al.*, 2006). Both species have similar life-histories (Skinner, 1998), and *A. caja* is also found in Orkney, albeit in much lower numbers (Berry, 2000; pers. obs).

Differences in host-plant feeding could be an explanation for the observed differences between the north-eastern populations and the rest of mainland Britain. The latter samples were all collected as adults from light traps, and whilst both traps were situated within moorlands, it is not possible to establish with certainty whether their larval stages had been feeding on heather alone. These mainland populations are probably far more likely to take advantage of a range of foodplants than those collected from Orkney, which contains very few trees and other shrubs that the larvae could feed on. This division may therefore reflect the selective effect of foodplant, restriction rather than an isolation by distance, or bottleneck effect. Indeed, if restricted gene flow is mediated by a bottleneck or range-margin effect, this could lead to increased selection for heather-feeding biotypes (Alleaume-Benharira *et al.*, 2006). Anecdotal evidence for such an effect exists from observations of F1 generation heather-fed larvae refusing to eat either novel foodplants or different heather ecotypes (Hesketh & Tyne, *pers*

observation). Local adaptation to foodplants has been previously reported in highly vagile invertebrate species with high levels of gene flow, on a regional scale (Peccoud *et al.*, 2009). However, whether this reflects a general phenomenon on a species level among *A. grossulariata*, or the beginning stages of adaptive deme formation (Mopper, 1996), will require further examination.

The low levels of genetic diversity seen among all *A. grossulariata* populations seem to present a paradox, given the high mobility and wide geographic range. As mentioned previously, local scale mating events could account for this via inbreeding depression (Charlesworth & Charlesworth, 1987), although evidence in favour of this is scarce. Another intriguing possibility is that of meiotic drive processes creating female-biased populations, thereby constraining the effective population size among populations. Research by Doncaster (1907; 1913; 1914) found evidence that F2 crosses of *A. grossulariata* with a pale colour morph (*A. grossulariata laticolor*) of the same species produced only female offspring. Subsequent research has ruled out the possibility that this phenomenon could be attributable to either parthenogenesis or male-biased mortality, and is thought to be due to cytoplasmic agents preventing male chromosomes from entering eggs (Majerus, 2003). Although reported distributions of the pale *laticolor* colour morph are almost entirely absent, these findings do suggest that some sort of sex-driven genetic effect could be operating in this species. All-female biases have previously been reported in other Lepidoptera species (Scali & Massetti, 1973; Ishihara, 1992; Majerus, 1981), and sex-linked differences have also been reported among populations of Lepidoptera analysed from AFLP markers (Reineke, 1999). However, no details of gender differences between individuals have been included in this present analysis, and so this theory remains supposition only.

An equally tenable explanation could simply be that the particular primer combinations used in the analysis were insufficient to detect the correct level of genetic diversity within populations, and were simply amplifying highly conserved regions of the genome. The recommended procedure for AFLP analyses states that ideally, a number of primer combinations should be used in conjunction (Bonin *et al.,* 2004; Bonin *et al.,* 2007). However time constraints limited the use of more than a single combination in this study. As a result, the total number of loci used for the analyses are typically lower than most published AFLP studies, and lower than the number recommended for robust analysis (Bonin *et al.*, 2007). Despite this, of the four primer combinations initially screened, the one chosen for the final analysis was clearly well

differentiated at most loci, with a good spread of identified loci throughout the genome, even at large sizes, which both fit in with recommended procedures. Additionally to this, error rate analysis revealed that the final AFLP profiles used for all species under analysis were all high-quality (Whitlock *et al.*, 2008), with error-rates typically lower than those even among the few studies that make their error rates explicit (Bonin *et al.*, 2004). It is also worth noting that all four species compared in the present analysis had profiles characterised by the same combination of primers, of which *A. grossulariata* had the greatest number of loci per profile. In this way, it seems unlikely that comparisons of genetic diversity between these species may be biased too greatly. The fact that two different inbred stock species were used for comparisons of genetic diversity also reduces the possibility that one or either species had spurious estimates. The Bayesian procedure for estimating heterozygosity from AFLP markers used in this study has also been shown previously to be the most reliable method of elucidating genetic diversity (Krauss, 2000; Bonin *et al.*, 2007), as it remains robust to departures from Hardy-Weinberg equilibrium (Kremer *et al.*, 2005).

For both *O. brumata* and *A. grossulariata,* some temporal discrepancies were evident within sampling sites. Among *O. brumata*, estimates of F_{st} suggested that individuals sampled from sites in 2004 were more closely related to individuals from mainland Britain than they were to individuals from the same sampling site, four years later. This analysis also indicated that some sites within Orkney (WH and BRA) were significantly more differentiated than either of these spatial and temporal outgroups. However, it is worth noting that these results are not consistent with those of the Bayesian assignment tests, which grouped all within-year Orkney sites together, and separated these three outgroups. Either of these methods could be sensitive to departures from the underlying assumptions of the algorithms used, although STRUCTURE is known to be robust to even low levels of population differentiation (Latch *et al.*, 2006), and as mentioned previously, the F_{st} estimates given under the Bayesian model are known to give the most reliable estimates (Bonin *et al.*, 2007). Further to this, these estimates are similar to those previously published from studies with *O. brumata* (Van San & Sula, 1993). In light of this, low sample sizes per site could be the most plausible explanation for these discrepancies, causing greater rates of error in the estimates. Degradation of template DNA quality could also contribute to increased error within AFLP profiles (Bensch & Andersson, 2005), although given the relatively short time periods used in this study and the storage method of specimens, this seems unlikely.

As mentioned previously, both F_{st} estimates and Bayesian assignment tests significantly differentiated the single *A. grossulariata* Orkney population from 2004 from all other Orkney populations, and more closely aligned it with populations from north-west Scotland and southern Britain, consistent with contemporary accounts of a recent range expansion. Temporal changes in population genetic structure within sites have previously been demonstrated in other invertebrate species from AFLP studies (Picard & Wells, 2009), and could be due to high mutation rates at certain alleles (Seabra *et al.*, 2009). The fact that both *O. brumata* and *A. grossulariata* both aligned the most temporally separate samples more closely with the most spatially separate samples could be indicative of the aforementioned template degradation. However, the fact that among *A. grossulariata* samples within the same year, two separate Bayesian assignment tests (STRUCTURE and TESS) independently identified the spatial outgroups as separate populations suggests that this is a true separation, and not just a methodological artefact (Rosenberg *et al.*, 2006). This was further supported by spatial correlogram analysis—which clearly indicated that the distance class containing BE and CT were more similar than among any other pairwise distance class—and hierarchical AMOVA, which partitioned almost half the molecular variance at this spatial scale. Other studies of Lepidoptera species utilising temporally separate samples among populations have similarly concluded that past demographic structure gives a strong genetic signal of current population structuring (Orsini *et al.*, 2008), highlighting the importance of investigating temporal as well as spatial variation among populations.

The success of many range-expanding and invasive species can often be attributed to release from the effects of native natural enemies in a novel environment (Colautti *et al.*, 2004). Although there was no detectable effect of current or previous-years virus prevalence on the genetic diversity of either species studied here (Ross-Gillespie *et al.*, 2007), some selective effects at certain loci were found for both species. Of these, the only selective effects were found from either previous-years density and virus prevalence, or an interaction of the two. This may reflect the fact that localised high densities of host species may be adversely affected by density-dependent transmission of pathogens (McCallum, 2001), which if mediated by high-mortality viruses could select for virus-resistant individuals (Reeson, *et al.*, 1998) among populations. The number of unlinked loci detectable as being under selection was greater for *A. grossulariata* than *O. brumata* across both locus screening methods, and may reflect either the increased selection against novel pathogens and natural enemies

in the new environment (Colautti *et al.,* 2004), or a reduction in the effective pool of disease-resistant genomes across the range-margin. Selection against mortality-inducing pathogens is likely to be high among most populations, although it difficult to deduce which is the case among *A. grossulariata* populations. Disease resistance among inbred populations, or those with reduced genetic diversity, is known to be far lower than in species with greater gene flow and diversity (Spielman *et al.,* 2004). Invasive and range-shifting species have also previously been shown to have greater numbers of loci under selection than native competitors (Mealor & Hild, 2006).

The fact that most of the positive selection models under the AIC model included previous-years virus prevalence in conjunction with density, suggests that the latter may be the primary agent of selection in this system. Mortality by nucleopolyhedroviruses (NPV) appears to be the primary agent of mortality among *A. grossulariata* populations in Orkney, as only a single parasitoid wasp is known to have emerged from any specimen collected (pers. obs.), and predation by birds is largely confined to a single bird species, the cuckoo (Newman, 1851; Nishida, 1994). Whether the NPVs present among these populations are novel or introduced is less easy to deduce. Viral mortality by NPVs primarily affects the larval stages of most moths (Cory & Myers, 2003), and since the most likely route across the Pentland Firth into Orkney would be by flying adult stages, it seems likely that the pathogens infecting nascent populations would be acquired from novel infections already present, possibly from other extant Lepidoptera species. NPVs known to cause mortality in *O. brumata* have previously been found in other Lepidoptera species in Orkney (Graham *et al.*, 2004), and the NPV causing mortality in *A. grossulariata* is known to be detectable in *O. brumata* (*unpublished data*). However, whether this is the case will require further investigation of the community of viruses present on Orkney heather moorland, and those in the mainland British range of *A. grossulariata*.

An alternative possibility is that covert infections of viruses that already infect mainland populations have been transferred from newly arrived adults into the F1 generation of larvae of the first arrivals to Orkney. Covert or persistent NPV infections are known to be transmitted transovarially (Cory & Myers, 2003) before being expressed as overt infections in the larval stages (Burden *et al.*, 2002). Any of a number of fitness-reducing processes, such as maternal fitness effects (Sait *et al.*, 1998), density-dependent fitness effects (Goulson & Cory, 1995), reduced fitness in novel environments (Sax & Brown, 2000), or slow-growth on foodplants of reduced

nutritional quality (Benrey & Denno, 1997), could all trigger covert into overt infections among these newly established populations. No data on virus prevalence currently exists for *A. grossulariata* populations outside of Orkney, and it is worth noting that all of the genome-scans were conducted only on populations within Orkney. Therefore, whether these effects of NPV-induced selection are operating outside of this range-margin, requires further investigation.

As well as NPV-induced selection pressures, one unlinked locus in the *O. brumata* genome screened positive for selection under abiotic pressures. Although overall there were three candidate models for this selective pressure, it appears as though the topographic variable slope was the main driver in this instance. All previous records suggest that *O. brumata* has been a fairly stable part of the Orkney heather moorland ecosystem for the previous century (Berry, 2005), often found in outbreak proportions (Lorimer, 1983; Picozzi, 1981; Graham *et al.*, 2004). Such a selective signal therefore seems at odds with a species that has been an established resident for so long. However, recent evidence from Fennoscandia suggests that *O. brumata* is also undergoing a northward range-expansion, largely mediated by climate warming (Jepsen *et al.*, 2008). As *O. brumata* in Orkney are at the northern end of their range limit (without crossing large expanses of water), it could be that such range shifts are manifest directly in elevational shifts to cooler climes (Hickling *et al.*, 2005; Hegen *et al.*, 2009), with a signal of this being shown by the elevation: slope interaction under the locus screening. Alternatively, the effect of slope could be mediated indirectly through the host plant. Steeper slopes would cause greater surface-run off and leaching of nutrients from the soil. *Calluna vulgaris* is known to have improved growth rates in soils of higher organic carbon content (Ward, 1971), and it could be that this provides sufficient pressure within *O. brumata* populations, raising the possibility of host-plant mediated deme formation in this species (Mopper, 1996). If this is the case, the effects of gene flow within marginal or isolated populations such as these could provide sufficient variance on which selection can act, without being overwhelmed by a high net influx of genes from core populations (Garant *et al.*, 2007), such as mainland Britain. Clearly, further research is required in this area to support these suppositions.

Despite these findings, very few of the loci identified as under selective pressures under both the GLMM and outlier-detection methods were in agreement. Of those that did agree, all of them were below the 99% confidence thresholds, meaning that differentiation at these loci was lower than expected, given the heterozygosity.

Crucially, for *A. grossulariata*, three loci were clearly identified as being outliers above the 99% threshold by the neutral evolution model. However, none were identified by the GLMM methods. It could simply be that there is some selective pressure on populations here that has not be measured for this species. A recent study by Joost *et al.* (2007) that studied the possible selective pressures acting on the pine weevil *Hylobius abietis* using a combination of genome-scan methods found broad agreement between the two different methods, when looking at the direct effects of abiotic factors, such as temperature, windspeed and precipitation. In this study, such measures can only be assumed indirectly, and it may be that an environmental variable not included in this analysis is driving the outlier behaviour at those particular loci. However, previous studies have shown topographic variable to be potent selective pressures in other taxa using AFLP markers (Bonin *et al.*, 2006), sufficiently able to affect genetic diversity among populations (Ohsawa & Ide, 2008). The fact that *A. grossulariata* overwinters in the larval stage make them particularly susceptible to winter temperature and other weather events that may not necessarily be correlated with topography. As far as possible, each site is chosen to give a broad range of topographic parameters. However, as Orkney is relatively low-lying (Berry, 2005), and *A. grossulariata* patchily distributed in space and time, it remains possible that the range of topographic measures was not sufficient to eke out any selective pressure that may be exerted on each of the species.

The population genomics approach to outlier loci detection, although widely used, is susceptible to errors in the underlying assumptions of the neutral model (Caballero *et al.*, 2008). Similarly, current locus screening methods that do not rely on such assumptions, do not make spatial dependence explicit, and are susceptible to Type II errors (Joost *et al.*, 2007). Although other genome-scanning procedures are in use for AFLP markers (Foll & Gaggiotti, 2008), here a model selection approach based on GLMMs and AIC was implemented. These methods are well established in ecology (Stephens *et al.*, 2007; Bolker *et al.*, 2009) but to my knowledge, have only ever been used in the context of genome scans when using SNP markers (Gu *et al.*, 2007; Skot *et al.*, 2007) and QTLs with binary traits (Coffman *et al.*, 2005). Although neither of the two genome scan methods implemented in this study were in broad agreement, the GLMM/AIC procedure remains a statistically robust method (Whittingham *et al.*, 2006), able to circumvent many of the problems inherent in using multiple univariate statistical models and stepwise procedures (Narum, 2006; Whittingham *et al.*, 2006).

Perhaps the largest drawback to this approach is the lack of available software to facilitate large scale genome scans across many loci. For a comprehensive study involving hundreds of loci screened for multiple interactive variables, the total number of possible models could be in the thousands, requiring very time-consuming post-processing of the statistical data. What is required now is a program that fulfils all of these crucial elements, whilst also eliminating the need to construct individual models for each locus vs variable test.

Clearly, much is still needed to be done to confirm many of the hypotheses raised in the present study. Explicit tests of each of the two species' topographical and climatological ranges, as well as natural-enemy mediated selective pressures are needed, both within Orkney, and outside of this possible range-margin. The inference made from population genetic studies can sometimes be susceptible to artefacts of the sampling scheme (Schwarz & McKelvey, 2009), and it is clear from the present study—especially in the case of *O. brumata*—that both increasing the spatial scale of sampling, including more among-site intermediates, and increasing the within-site sample sizes, could eliminate some of the inherent uncertainties. For *A. grossulariata*, determining if any sex-linked population artefacts are affecting genetic diversity estimates, or whether this is simply a primer-coding artefact, should be a priority. The use of multiple population genetic tools using bi-alleleic markers e.g. microsatellite or mtDNA (Bensch & Akesson, 2005; Meng *et al.*, 2008), could also reduce uncertainty in some of the calculated parameters. Similarly, for both species, understanding dispersal mechanisms—especially the mechanisms that could lead to such high levels of within-site variation—will also help to unravel some of the more complex population structuring mechanisms operating across spatial and temporal scales.

Chapter 7

General discussion

The overall aim of this thesis has been to determine if the spatial patterns of insect-natural enemy prevalence in relation to host density, distribution and demography, can be predicted by their mode of transmission. For this, I have utilised a model Lepidoptera species (*Operophtera* brumata) attacked by one parasitoid species, and three species of viral pathogen, all with differing transmission modes. Additionally, another Lepidoptera species (*Abraxas grossulariata*) attacked by a viral pathogen of the same family and transmission mode as the one that attacks *O. brumata* has also been studied, in order to test whether the observed patterns hold true for other species with differing life-histories.

How natural enemies respond to the spatial distribution of their hosts may be a key determinant in their role as potential regulatory influences—an interaction that may be mediated by the relative dispersal of hosts and enemies, determined largely by their mode of transmission. This, in turn will be affected by the spatial arrangement of habitat patches within the environment, and the variation in resource quality that each may represent for the herbivorous host. Exploitation of hosts within patches by enemies may be a function of host density, dependent upon the mode of transmission and the relative difference between natural enemy virulence and host resistance to attack. However, regulation may still come about if responses of natural enemies are independent of host density.

Understanding the factors that may give rise to these observed patterns and processes may therefore be fundamentally important in not only understanding species responses to present environmental conditions, but also to future perturbations such as land use change, habitat fragmentation and loss, and climatic change. The present study contributes to such knowledge by focussing on how different species of natural enemy may affect both native and newly-established populations of insect herbivore hosts, as a result of their interactions in space. The factors that govern the distribution of hosts in the environment may also be affected by factors wholly independent of their natural enemies, and these factors are also addressed.

7.1 Review of main findings

7.1.1 Spatial patterns of natural enemy attack and the role of host density, distribution and demography

Many insect herbivore outbreaks in space and time are thought to be driven by the loss of top-down regulation by predation, parasitism or the action of pathogens (Denno *et al.*, 2005), as they are likely to play an important role in driving host mortality in one generation, and therefore determining recruitment to the next. How natural enemy prevalence may be affected by changes in host density, spatial distribution of hosts, and host susceptibility to attack, may therefore all be important components of such regulation, and may vary greatly according to any particular natural enemy's mode of transmission or attack. This is the first study to directly compare natural enemies of four different transmission modes attacking a single host species, over several spatial scales, in the field. Whether such patterns are generalisable for natural enemies of the same transmission mode attacking different species was also investigated.

Early and late instar larvae of *O. brumata* appeared to be less susceptible to parasitism and infection from horizontally transmitted pathogens than mid-late stages (Chapter 3). Although ecological theory predicts that later instar stages may represent a higher quality resource for idiobiont parasitoids, the findings of this study indicate that a koinobiont strategy, such as that used by the parasitoid *Phobocampe tempestiva* investigated in this study, would make mid-late instars most susceptible, possibly due to a reduced risk of mortality during host pupation, or mortality from developing larvae becoming stuck in the host integument. Large variations in parasitoid prevalence appear to be rare in this system, occurring at around 10% among most populations and most years (with the exception of the site WC during 2008; Chapter 4), possibly due to large spatial and temporal variation in host abundance. However, this may not necessarily be the case for many host-parasitoid systems, which are known to display high variability in prevalence both within and among host and parasitoid species (Walde & Murdoch, 1988; Hassell, 2000). Similarly, the pathogen lethal dose that needs to be ingested by each larva will be relative to its body size and the amount of infective particles in the environment. It may therefore be that late instar stages do not encounter sufficient infective material to be detectable, or that their more developed immune systems are able to counteract any viral challenge that may occur. These discrepancies between

predictions based on laboratory bioassays and the results found among natural populations therefore highlights the importance of quantifying the risk that natural populations face, given the temporal and spatial heterogeneity apparent in the field.

In general, natural enemy responses in this study conformed well to predictions about host attack, based on knowledge of their differing transmission modes. However, there was no evidence that parasitoids were clustered in localised hotspots within the region (Chapter 5), although whether low density, endemic populations of *O. brumata* among sites are caused by, or a consequence of, low-level stable parasitism, is not clear. Evidence from other studies suggests that parasitoids of a number of species may aggregate in areas of high host density (Walde & Murdoch, 1988), and may restrict their spatial spread by operating at larger spatial scales than their hosts (Maron & Harrison, 1997). What does seem clear from this study is that interactions with hosts are rarely related to density, at any scale. Where they are (Chapter 4), it appears that this is solely due to the localised high densities apparent in an outbreak, suggesting that top-down failure from this source is unlikely to be wholly responsible for the occurrence of such high host densities. Such responses to host density appear to change according to the distribution of hosts during outbreak and proceeding years, and may be scale specific, responding to the spatial spread of hosts at larger scales. Such persistent low densities among most other sites, and host-specific responses during outbreaks, may be due to *P. tempestiva's* generalist life-history strategy: if this species is able to attack other Lepidoptera hosts within the same site, responses may instead be community-density-dependent, with *O. brumata* benefiting from apparent competition (Abrams *et al.*, 1998). Community diversity within each site may therefore represent a form of heterogeneity from which *O. brumata* escape overexploitation by parasitoids. However, this assumption will require further investigation.

There is evidence from both theoretical models and empirical studies that parasitoids may be able to restrict the spatial spread of outbreaking host species by responding at larger spatial scales than their hosts (Maron & Harrison, 1997; Hastings *et al.,* 1997). Spatial correlograms of parasitoid abundance, and scale-dependent responses to host density, both appeared to suggest that just such an effect may be manifest in the present study system (Chapter 4). However, there was little evidence that this extends much beyond the local scale. Generally, at regional scales, parasitoid responses were again at larger scales than hosts (Chapter 5), which has previously been found in forest Lepidoptera-parasitoids systems (Roland & Taylor, 1997). However, unlike these

studies, which sampled parasitoids independently of hosts, the exact scale of parasitoid dispersal among sites within the region in this study is difficult to discern, because prevalence measures were derived only from successful emergence from hosts.

Even more so than among host-parasitoid interactions, host-pathogen interactions—among horizontally transmitted infections—in this study appear to be governed at the local scale. Such a spatial pattern (smaller scales than the host) is predicted by theory, and reflects their passive transmission mode and therefore dispersal relative to the host (Boots & Sasaki, 2000). One interesting spatial pattern that emerged from these interactions, among both species of hosts, is the occurrence of local and regional differences in prevalence, according to within-site host density (Chapter 4; Chapter 5). Among *O. brumata*, the scale-specific density-dependence seen in the outbreak epicentre (strongly positive at transect scales) and two years later (strongly positive at "regional" scales; *see definition in* Chapter 4), suggests that horizontal density-dependent transmission may be non-linear and scale-dependent; that is, only when local host densities are sufficiently high, does the OpbuNPV act density-dependently.

Non-linearity in NPV transmission has been previously reported in other systems (D'amico *et al.*, 1996), and may be directly linked to the spatial distribution of pathogen particles in the local environment (D'amico *et al.*, 2005). The highly local spatial restriction may also be a result of pathogen self-shading (Boots & Mealor, 2007), whereby transmission to susceptible hosts may be retarded due to infectious particles surrounding themselves with cadavers of other infectious particles, thereby reducing passive dispersal by uninfected hosts. The consequences of this are that those pathogen strains with lower infectivity have a selective advantage, as they are able to surround themselves with a greater proportion of susceptible hosts than more infectious strains. Therefore the ability of a pathogen to persist among hosts will be a function of its spatial spread relative to its host, and therefore indicative of its transmission mode. More mobile hosts may therefore select for decreased pathogen infectivity, which may affect the ability to act as regulatory agents. Further work in this regard should focus on characterising the distribution and infectivity of pathogen strains within the same outbreak, to discern whether spatial variation is a direct result of variation in infectivity.

A similar effect may be seen for *A. grossulariata* populations infected by AbgrNPV, since localised disease hotspots were detected in the south mainland of Orkney during 2008, without apparent region-wide density-dependence in infection

(Chapter 5). AbgrNPV is known to liquefy hosts more readily than OpbuNPV, and the larger size of *A. grossulariata* larvae potentially means that successful viral mortality will yield a larger volume of infectious particles, spread over a wider area, than among OpbuNPV infections—suggesting a larger spatial scale of transmission. However, even as a result of greater virus yields per cadaver, the spatial scale of the distribution of hotspots during 2008 would appear to be too large to be attributable to pathogen liquefaction alone; it seems likely that some other process may be driving this semi-regional pattern. This may mean that specific pathogens may have specific spatially-related transmission strategies, even if they derive from the same family. Such a finding may have important consequences for the implementation of spatially-explicit theoretical models, which should take into account each species' dispersal capability and the consequences that this may have for transmission of its pathogens among hosts.

As expected, there was no evidence of any spatial structure or relationship to host density among vertically transmitted infections in this system; either within generations (Chapter 3) or at local scales (Chapter 4). Small sample sizes precluded the investigation of these viruses at the regional scales, which may itself be due to this transmission mode. However, at certain sites (e.g. Linnadale during 2006; Chapter 4), prevalence was relatively high. Some CPVs are known to have a horizontal component to transmission, through release of infective virions in host faeces, which may partially explain this effect. Interference from obligately horizontal pathogens have previously been shown theoretically to be able to promote persistence among vertically transmitted pathogens (Jones *et al.*, 2007), and CPV's lack of prevalence among regions where OpbuNPV is at low prevalence, or absent would certainly appear not to discount such a hypothesis. The apparently high rates of genetic intermixing among *O. brumata* populations (Chapter 6) may also suggest that persistence could be facilitated through metapopulation processes (Saikkonen *et al.*, 2002). However, if vertical transmission is via infected egg stages (transovarial or transovum transmission) then the flightless female stages of *O. brumata* may disrupt the potential for spatial spread to other interacting metapopulations. I am not aware of any theoretical models that have investigated such a situation, although the possible implications for long term CPV persistence in natural field populations merit further study. The question of how it is possible for obligately vertically transmitted parasites and pathogens to persist whilst reducing host fitness remains unresolved (Lipsitch *et al.*, 1995; Saikkonen *et al.*, 2002), although this study and theoretical models suggest that interacting natural enemies may

play an important role in this process, again highlighting the importance of multi-species, multi-enemy investigations.

As anticipated, OpbuRV infections were widespread, although not particularly prevalent within generations (Chapter 3), locally (Chapter 4) or regionally (Chapter 5). Especially on a local scale, prevalence appeared to follow closely the patterns of parasitoid prevalence, as expected from prior knowledge about its potential role as vector of the pathogen (Graham *et al.*, 2006). As predicted, there was little relation with host density, even when parasitoid responses did so. The potential transmission route of frequency dependence, as predicted from theory, remains valid, despite not being explicitly examined in this study (Antonovics *et al.*, 1995). Parasitoid-vectored insect pathogens have received very little attention in terms of field studies, and this is the first study that I am aware of to investigate their prevalence in a spatial context, in relation to both host density, and vector-parasitoid prevalence. It is likely that such pathogens play an important role in the successful oviposition of parasitoid species attacking Lepidoptera hosts, by overcoming host immune responses. This may therefore have population dynamic consequences for parasitoids as top-down regulatory influences. Further studies are therefore needed in order to verify the exact role they have to play in insect-population dynamics.

Overall, this thesis provides good evidence that spatial patterns of natural enemy attack, and their relationship with changes in host density, are predictable by transmission mode. Such an effect was most obvious during periods of high host-density, where it appeared that such interactions may be influencing the localised dispersal of hosts into previously uncolonised areas of habitat. Small-scale changes in host density and interactions with competing natural enemies may also alter these patterns, which was most evident among parasitoid wasps that appeared to change the sign of their relationship with increases in host density. However, when analysed over regional scales, such patterns did not appear to hold true, and it seems most likely that interactions with natural enemies in this system are governed at the local scale. This has important implications for future predictive models of insect population dynamics, which should take into account multiple, competing natural enemy species, and potentially flexible responses to local-scale changes in host abundance.

7.1.2 Host distribution and abundance in relation to dispersal and life-history

The ability of an organism to exploit areas of suitable habitat within a region will be a function of the spatial distribution of this habitat relative to the dispersal ability of the organism. This is also true for areas of unsuitable habitat, for example through the need to escape from natural enemies. How successful any species is at exploiting this suitable habitat will also be contingent on the resource quality at that site, and the suitability of it for survival and reproduction.

Another aim of this study was to investigate whether spatial and temporal variation in the abundance between two Lepidoptera species of differing life-history strategies, sharing the same host plant, could be attributable to either variation in bottom-up or top-down influences at both local and regional scales, and whether differences in dispersal ability would be observable in the scale at which they exploit areas of suitable habitat—specifically that the more dispersive of the two species (*Abraxas grossulariata*) would be predicted to respond at greater spatial scales than the less dispersive species (*Operophtera brumata*). Additionally, whether variation in abundance of either species was related to variation in habitat characteristics was also investigated. Here, we predicted that newly colonising species at the edge of their natural range would be more sensitive to changes in microclimate than a resident species, which would be detectable by greater correlations with topographic conditions such as south-facing slopes. The level of gene flow between spatially separate populations was also investigated, in order to see whether differences in the dispersal ability of the two species could be quantified by how genetically distinct different populations are across a region. Again, the less dispersive of the two species was expected to show isolation of populations at smaller scales than the more dispersive species, which was predicted to show a genetic signature of a recent colonisation of the islands from mainland source populations.

Populations of *O. brumata* larvae feeding on heather in Orkney appear to be characterised by their large spatial and temporal variation in abundance: within generations, among years (Chapter 3); locally, within and among years (Chapter 4); and across entire regions, within and among years (Chapter 5). Patterns of abundance within many species of insect herbivores are known to display high variability over time, principally as a result of the number of potential mortality factors that act upon them (Yamamura, 1999). For *O. brumata* and other epidemic or pest species that display

outbreaking behaviour, the dynamics of individual populations are thought to be characterised by relatively small fluctuations of individuals within consistently low-density populations, with some individual populations being prone to irregular high-density outbreaks (Wallner, 1984; Liebhold *et al.*, 2000). The patterns observed within the present study would certainly appear to fit well into such a category, and more specifically, with other studies of *O. brumata* feeding on heather (Picozzi, 1981; Kerslake *et al.,* 1996; Graham, 2006). Although the exact definition of what distinguishes an outbreak from a high-density fluctuation may be difficult to quantify, and may indeed be system specific (Myers, 1998; Radeloff *et al.*, 2000; Maron *et al.*, 2001), it is clear that the high densities seen in one site in particular (Linnadale during 2006; Chapter 4) are likely to fall under this category. Within-quadrat densities here are among the highest ever recorded for this species, and differ markedly—often by an order of magnitude—from most other sites in the region in later years (Chapter 4) and within the same year (*unpublished data*). In this instance, the issue of variation in larval abundance in space and time is perhaps best illustrated, since within this outbreak, larval abundance just a few hundred metres away fell to just a fraction of those recorded within the outbreak epicentre, becoming far more similar to the variations in abundance recorded among other sites in the region—including those where larvae were absent.

The difference in the spatial pattern of the scale of larval distribution among years fits well with theoretical models that predict a wave-like pattern of diffusive, local-scale larval dispersal by crawling (Dwyer, 1992), which may be partially driven by interactions with natural enemies. Spatial patterns in insect herbivore populations are known to occur in continuous habitats, such as those used in the present study, as a direct result of interactions with parasitoid natural enemies (Maron & Harrison, 1997). Although local scale changes in the total area of habitat available may be of some significance in determining the size of the local population (Chapter 5; Chust *et al.*, 2004; Holland *et al.*, 2005), a much stronger signal of variation in topography determining variation in abundance was detected among both Lepidoptera species. For *O. brumata*, Kerslake *et al.* (1996) surmised that the occurrence of high density populations at higher elevations in the Scottish highlands was the result of escape from parasitoid natural enemies, which could not survive in colder, more exposed habitats. However, this is unlikely to be the case in among populations in Orkney, since parasitoids are found among most sites in the region (Chapter 5). It seems most likely that either some climate-induced range-shift, or potentially co-linear grazing effects of

livestock grazing are driving these distributional patterns across the island. A large number of insect herbivore populations are likely to be affected by future changes in climate (Jepsen *et al.*, 2008) and land-use (Gardner *et al.*, 1997), and although this study is able to highlight the importance of investigating the impacts of these processes on the dynamics of insect herbivore populations, further work will need to be done to verify the hypotheses that have arisen from this work.

This need for further investigation is highlighted by the fact that *A. grossulariata* populations appear to be less affected by these processes than *O. brumata*, suggesting that even species sharing the same host plant may be subject to differing environmental pressures. Here, there appears to be some possible effect of resource-limitation acting on local populations. The strong response of this species to changes in the wood-shoot ratio of local moorlands may be partly due to such an effect (Chapter 5), although it also seems likely that age-related effects in the ability of each heather stand to provide a favourable microclimate for developing larvae may be more important. The prediction that the more dispersive species would respond to changes in habitat area did not hold true. In fact, this study found no difference in the scale at which both species responded to changes in the amount of habitat available, suggesting that such variation in the area of a continuous resource such as *Calluna vulgaris* may not be as important as local scale variation in habitat quality for either of these species. For *A. grossulariata*, variation in topography mediated by microclimatic conditions may be extremely important determinants of the recruitment of new larval cohorts of the species within each year, primarily because of their overwintering strategy in the larval phase. Such a finding is in line with predictions that the successful establishment of range margin species in novel habitats will be contingent on the availability of suitable conditions in which to survive and propagate, and adds to a growing list of insect species in which such establishment has occurred (Ward & Masters, 2007).

The high variability between regions seen in this species (both on the total number of occupied patches, and the number of colonisations and extinctions within patches and among years) compared to *O. brumata* supports the theory that dispersal is greater (i.e. over larger distances) in *A. grossulariata*. Such high vagility may buffer this species against the effects of livestock grazing, since populations may be more transient than *O. brumata*. It is this greater dispersal ability that has most likely facilitated the apparent recent colonisation of the Orkney archipelago by this species (Waring, 2006). The genetic signal of such a recent colonisation appears to have been verified by

population genetic analysis of *A. grossulariata* individuals sampled over broad spatial and temporal scales (Chapter 6): populations from Orkney are much more similar to east-coast populations than they are to west-coast populations, which in turn are more similar to populations from southern Britain..

The discovery of outlier loci that may be under selection in both species, suggests that there may some interesting evolutionary relationships occurring between pathogens and hosts (Chapter 6). In *A. grossulariata* in particular, whereby a number of (albeit in some, linked) loci appear to be positively associated with increases in viral prevalence, as well as other loci that appear outside the simulated bounds of neutral selection, there exists the possibility that true host-pathogen selection is occurring. It is not known whether *A. grossulariata* carried AbgrNPV infections among the first colonisers of Orkney from mainland Britain. However, such high levels of potential selection may be expected if this was the case, as novel, virulent infections select only those individuals that possess enough innate resistance to survive the viral challenge. Therefore, it seems that although the loss of parasitoid natural enemies among this species may have facilitated the process of successful establishment (Colautti *et al.*, 2004), the acquisition of novel, virulent pathogens to which populations have little or no resistance, may be conversely detrimental to population persistence, and may help explain the semi-regionwide patterns of disease hotspots seen in this species.

Overall, this study has shown that, while the distribution of insect herbivore species in continuous habitats may show high temporal and spatial variability, this distribution is likely to be more affected by local-scale interactions with natural enemies. Regionally, dispersal ability appears to play little part in how hosts exploit their host-plant habitat. However, the distribution and abundance of a species over this larger scale may be largely governed by within-site characteristics such as favourable climatic conditions, especially so for newly established species in novel environments. In addition to this, although loss of parasitoid natural enemies may have facilitated such successful establishment, encountering novel pathogens may add to increased selective pressure on these populations, illustrating the importance of investigating a range of natural enemies attacking invasive organisms. This study provides an important comparison of two related species of Lepidoptera on a shared host plant, under attack from very different biotic and abiotic pressures, and illustrates the importance of sampling at a range of spatial scales and natural enemies for future investigations of invasive and non-invasive insect herbivore populations under climatic change.

7.2 Future directions

Perhaps the largest barrier to inferences concerning the population dynamics of *Operophtera brumata* and its natural enemies is the lack of long-term data. Although the relatively short term frames used in the present study may only be able to provide a brief insight into the population processes that occur in this system, the significance of such findings need to be reviewed in the wider context of long-term population trends, and the likely effects that such processes may have. Such data is available for many insect-herbivore systems, especially forest-pest species (Watt *et al.,* 1997). However, whether the same processes that govern the fluctuations in distribution and abundance in these systems are the same as those that occur in the relatively nutrient poor, continuous, low-lying moorland habitats, is not clear. Data on outbreaks in this system have previously been gathered (Picozzi, 1981; Kerslake *et al.*, 1996), as well as interactions with natural enemies (Graham *et al.*, 2006), long term monitoring will need to continue in order to verify that such trends hold true over large time frames too, which will include the spatial effects of possible metapopulation processes among sites. The relatively closed island system, as well as the relatively low diversity of interacting natural enemies compared with mainland British populations makes this an ideal system from which to investigate such processes. The recent colonisation of the system by a novel species, *Abraxas grossulariata,* also adds an intriguing further level of potential dynamical effects on the population processes of the system. Although not explicitly treated in the present study, the likely interspecific effects of this colonisation for native populations such as *O. brumata* may have important wider consequences for many other resident and invasive species undergoing range-shifts..

As well as these competition-mediated effects, the potential effect of shared natural enemies between the two species is also an exciting area of research for the future. As mentioned previously, AbgrNPV has been found within populations of *O. brumata* on Orkney. Interestingly, in some instances this appears to be among areas of moorland not previously known to harbour *A. grossulariata* larvae in significant numbers—certainly not sufficient to explain the prevalence of such infections. Whether such infections are pathogenic to *O. brumata*, or simply acquired benignly or sublethally, is not known. A possibility it that the origin of AbgrNPV infections was within Orkney, from either an as yet unidentified source (i.e. another species of

Lepidoptera), or among *O. brumata* populations that have subsequently developed resistance. If either is the case, the evolutionary implications for the colonisation dynamics of *A. grossulariata* are intriguing. The fact that a number of loci were identified in these populations as potentially being under selection from pathogenic sources would suggest some evolutionary pressure being exerted on these populations, and the isolation and identification of the specific genes responsible should be a priority. Frequency-dependent selection of rare alleles among hosts is well known to occur within populations of co-evolving hosts and parasites/pathogens, and may be an important component of population cycles (Saccheri & Hanski, 2006).

Both AbgrNPV and OpbuNPV are known to be highly genotypically variable species (Graham, 2006; Graham *et al.*, 2006). However, such genotypic variability may only be of significance if it is translated into phenotypic effects, for instance in differences in pathogenicity and/or virulence to hosts. The lack of understanding of such variability—both widely, in the process of understanding what maintains such variation; and practically, in understanding what this variation means in terms of its likely dynamical effect on populations—is an obvious shortcoming of the present study, as both viral species are assumed to be genotypically homogenous, each with a constant rate of transmission and virulence. Single genotypes of Baculoviruses within hosts are probably rare, although there may be substantial difference between their pathogenicities in isolation (Hodgson *et al.*, 2001; Cory *et al.*, 2005) and when mixed (Hodgson *et al.*, 2004). Some species of NPV are known to also be hierarchically structured in terms of their genotypes (Cooper *et al.*, 2003). Whether such a pattern is evident among either *O. brumata* and *A. grossulariata* infections within Orkney will also require further work, as will a more detailed understanding of the spatial distribution and persistence of viral OBs—independent of putative host infections—in the environment may affect the likely occurrence of disease across the landscape. How changes to the resistance/ virulence associations between pathogens and hosts are mediated by the spatial context in which they interact (and the implications for persistence) has previously been studied in model systems (Boots & Sasaki, 2000) and empirically verified in Lepidoptera-Baculovirus systems. However, how the implications of such interactions translate into field populations has so far not been studied and, especially in the context of a outbreak/ epizootic event such as have been observed in the present study, could provide a suitable study system to test such hypotheses.

The existence of multiple, interacting, pathogens has also not been explicitly investigated within the present study. Clearly, each natural enemy does not act independently of the other species that also attack the same hosts. However, how each will impact on one another is not known. Studies of mixed infections of Baculovirus species within hosts are rare, and those that do show little effect on each respective prevalence (Fuxa & Geaghan, 1983). However, more recent theoretical and empirical investigations have suggested that such infections may alter the form of density-dependence, mediated through changes in the death rate of hosts (Bonsall & Benmayor, 2005). In some species latent infections are known to be triggered into their overt states following viral challenge from a different species (Cooper *et al.*, 2003), and as a result of oviposition by parasitoids. Conversely, parasitoids and pathogens may co-compete for ultimate control of the host as a resource (i.e. the outcome of competitive interactions is likely to binary), although the outcome of such interactions may be partly due to the relative timing of infection and parasitism: parasitism of infected larvae may have fitness consequences for developing parasitoids if infection reduces host fitness (Matthews *et al.*, 2004), and parasitoids may therefore actively avoid infected host larvae (Sait *et al.*, 1996). As previously mentioned, the likelihood of persistence of vertically transmitted pathogens may also be a function of co-infection with other pathogens (Jones *et al.*, 2007), although to what extent parasitoid-vectored viruses such as OpbuRV rely on infections within their secondary hosts (Lepidoptera) is not currently known (Renault *et al.*, 2005). The variety of different antagonistic, mutualistic and synergistic interactions within and among pathogens and parasitoids could therefore provide a fruitful area of future research, although the complexities inherent in such interactions may be better modelled theoretically, following parameterisation in the laboratory, or extrapolating from field studies such as the present one. Further to this, more detailed, possibly laboratory-based, manipulative experiments will be needed in order to elucidate the exact principles governing the cuticular melanisation response among *O. brumata* individuals. Smilanich *et al.* (2009) injected glass beads into developing larvae to simulate parasitoid oviposition; similar density-controlled experiments could easily be conducted, both using parasitoids and pathogens, in order to investigate these responses—and larval immune responses generally—more fully.

Very little is currently known about the host-natural enemy interactions of *A. grossulariata;* the ecological importance of its apparent colonisation of a novel habitat at the edge of its northward range may have broader significance under future climatic

changes (Parmesan, 2006) as an example of the likely trophic interactions and population dynamical effects of similar species experiencing the same conditions. Detailed studies of population life-history, including the temporal distribution of different larval instars, will now be needed for this species in order to verify the inferences about topographically-mediated local population persistent highlighted by this study. Populations of *A. grossulariata* are also known to have become established on the western isles of Scotland at around the same time that Orkney was colonised (Waring, 2006). However, whether their progress will increase northwards from Orkney toward Shetland—a distance of some 100km—will be interesting to monitor. Perhaps most interestingly, throughout the course of this study, the apparent lack of parasitism among *A. grossulariata* appears to have been altered: although only a single parasitoid emerging from a pupa was found from populations collected in Hoy during 2007, during the 2009 sampling year a number of parasitoid pupae have been found to emerge from parasitized larval hosts (*pers. obs*.). Continued monitoring of these same populations over the coming years will therefore allow an exciting opportunity to quantify the increase in abundance of a potentially novel host-parasitoid interaction in the field, and examine the effects on population processes that result. The sequence in which natural enemies invade established populations of hosts previously infected by other enemies is known to alter the outcome of their dynamics (Sait *et al.*, 2000); how such an invasion will affect the dynamics of this interaction will therefore be interesting to monitor, and compare to existing laboratory and theoretical studies. Field-based studies of insect-natural enemy interactions have typically been restricted to long-term monitoring of spatially pooled populations, or short-term investigations over large spatial extents, with the majority of such studies restricted to a single-host, single-natural enemy population interactions (Berryman, 1996).

The aims of this thesis were to investigate the variation in spatial patterns that exist among insect-natural enemy interactions among multiple species, and the potential population dynamical responses that occur as a result. Spatial processes may play important roles in the transmission and persistence of parasitoids and pathogens among hosts, mediated by dispersal. A vast range of synergistic dynamical processes are likely to be involved in the responses of populations to internal and external influences. Future studies should look to a more inclusive approach to analysis and prediction that includes all the complex dynamical interactions likely to be at work in natural populations.

Chapter 8

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