Spatial Heterogeneity in Ecology

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Declaration

The work described here was carried out in the Department of Biological and Environmental Sciences at the University of Stirling. All the work is my own, unless stated otherwise, and it has not been submitted previously for a degree at this or any other institution.

Signed……. Date…….

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This PhD killed me.

General Abstract

This project predominantly investigated the implications of spatial heterogeneity in the ecological processes of competition and infection.

Empirical analysis of spatial heterogeneity was carried out using the lepidopteran species ***Plodia interpunctella***. Using differently viscous food media, it was possible to alter the movement rate of larvae. Soft Foods allow the movement rate of larvae to be high, so that individuals can disperse through the environment and avoid physical encounters with conspecifics. Harder foods lower the movement rate of larvae, restricting the ability of individuals to disperse away from birth sites and avoid conspecifics encounters. Increasing food viscosity and lowering movement rate therefore has the effect of making uniform distributed larval populations more aggregated and patchy.

Different spatial structures changed the nature of intraspecific competition, with patchy populations characterised by individuals experiencing lower growth rates and greater mortality because of the reduced food and space available within densely packed aggregations. At the population scale, the increased competition for food individuals experience in aggregations emerges as longer generational cycles and reduced population densities.

Aggregating individuals also altered the outcome of interspecific competition between ***Plodia*** and ***Ephestia cautella***. In food media that allowed high movement rates, ***Plodia*** had a greater survival rate than ***Ephestia*** because the larger movement rate of ***Plodia*** allowed it to more effectively avoid intraspecific competition. Also the

faster growth rate, and so larger size, of ***Plodia*** allowed it to dominate interspecific encounters by either predating or interfering with the feeding of ***Ephestia***. In food that restricts movement, the resulting aggregations cause ***Plodia*** to experience more intraspecific encounters relative to interspecific, reducing its competitive advantage and levelling the survival of the two species.

Spatial structure also affected the dynamics of a ***Plodia***-granulosis virus interaction and the evolution of virus infectivity. Larval aggregation forced transmission to become limited to within host patches, making the overall prevalence of the virus low. However potentially high rates of cannibalism and multiple infections within overcrowded host aggregations caused virus-induced mortality to be high, as indicated by the low host population density when virus is presented. Also aggregated host populations cause the evolution of lower virus infectivity, where less infective virus strains maintain more susceptible hosts within the aggregation and so possess a greater transmission rate.

The pattern of variation in resistance of ***Plodia interpunctella*** towards its granulosis virus was found using two forms of graphical analysis. There was a bimodal pattern of variation, with most individuals exhibiting either low or high levels of resistance. This pattern was related to a resistance mechanism that is decreasingly costly to host fitness.

Chapter 1

General Introduction

Aims

There will be two forms of biological heterogeneity discussed in this thesis. Most chapters will focus on the importance of within population spatial heterogeneity, while variation in resistance towards a pathogen is discussed in one chapter. Often the spatial aspects of ecology are only considered when mapping the distribution of species at broad scales. However the implications of the small-scale distribution of individuals within populations upon ecological processes are often ignored. Until recently, ecological systems were analysed using the simplifying assumptions of uniform physical environments and randomly mixing individuals. The 1990's and early 21st century have seen more realistic population structures being considered when we ask ecological and evolutionary questions. The work in my thesis attempts to contribute to this field. The approach is empirical, using an insect model system to examine the scaled effects of different spatial structures on pathogen transmission and intra- and inter-specific competition.

Plodia interpunctella

The lepidopteran moth species ***Plodia interpunctella*** (Hübner) is a member of the Pyralidae family. Its common name is the Indian meal moth, originating from the destruction of 'Indian' wheat in the southern states of USA. It has a global distribution resulting from human international trade and movement. The species is a tropical and warm-temperate species but is moderately cold hardy and can survive most winters in temperate climates (Arbogast ***et al***., 2002). It is predominately found in warehouses and barns where food is stored, and so is categorised as a 'stored product pest' along with over forty other moth species (Cox & Bell, 1985). Food

stores affected by ***Plodia*** infestation include dried fruit, cereals, oilseeds, groundnuts and botanicals (unrefined parts of medicinal plants).

There are five larval stages, each one identifiable by the size of the head capsule. Development can only occur at a temperature range of 18-35ºC (Bell, 1975). Larvae are negatively phototactic until they are about to pupate, and the pupal stage lasts 7-8 days (at 27ºC). Diapause, where development is delayed, can occur when temperatures are low, light periods are short and when population densities are high (Bell, 1976; Tsuji, 1959). This allows wild populations to survive cold winters, although laboratory populations display a reduced capacity for diapause. Larvae secrete a mandibular substance when they make head to head contact with other larvae. This substance acts to agitate larvae and so encourage larval wandering when found in large quantities (Corbet, 1971).

Adults live for approximately seven days and females lay, on average, 150-200 eggs (Lum & Flaherty, 1969) that hatch after 4-5 days (at 27ºC). Adult male fertility and female fecundity is reduced by exposure to continuous light or dark and temperatures above 33ºC (Lum & Flaherty, 1970). Adults are mainly nocturnal, with flight and reproductive activity peaking in the dark. Mating is initiated when females emit a sex pheromone chiefly composed of cis-9 trans-12-tera-decadienyl acetate (Kuwahara ***et al.***, 1971). The female lifts her abdomen between her wings and releases scent from the tip. Males locate females by following the pheromone trails and release their own in the final stages of courtship, eliciting timing behaviour in the female (McLaughlin, 1982).

Plodia interpunctella and its granulosis virus

Infection with a granulosis virus often results in larval mortality (Granados, 1980), however non-lethal, asymptomatic infection has been known to be theoretically important (Anderson & May, 1981; Boots & Norman, 2000; Onstad & Maddox, 1989). Sait ***et al***. (1994a) studied the effects of sublethal PiGV infection based on the dose and age dependent effects of larval exposure. They found strong, if indirect, evidence that sublethal infection causes a decrease in female fecundity and male fertility. Such consequences may be explained by the virus exploiting reproductive and other metabolically important tissues (O'Reilly & Miller, 1989; Silhacek & Oberlander, 1975).

As larvae develop through the five larval instars they become increasingly resistant to granulosis virus (PiGV) infection. This maturation resistance has two distinct phases and mechanisms. The first mechanism of maturation resistance acts from first to third instars and is a dilution effect (Sait ***et al***., 1994c). This may be a result of increasing body size reducing the surface-to-volume ratio of the midgut, and so increasing the probability of virus particles passing through the gut without attaching to target epithelial cells (Briese, 1986). Also the greater volume of food ingested as larvae develop from first to third instar further reduces the probability of infection (Engelhard ***et al***., 1991).

A different mechanism of maturation resistance seems to occur within fourth and fifth instars, a view developed because resistance here increases at a greater rate than body size (Sait ***et al***., 1994c). This additional maturation resistance renders fifth instars totally immune to infection (Boots, 1998). The possible explanations for this resistance in late instars include higher gut pH (Stiles & Paschke, 1980), greater gut wall sloughing (Kirkpatrick ***et al***., 1998), the short time available for viral

establishment before pupation occurs (Sait ***et al.***, 1994c) and changes in the physiology of the gut wall and tracheae induced by hormones (Whitlock, 1977).

Plodia has an additional mechanism of resistance beyond passive maturation, because changes in the prevalence of the resistance trait have been found in populations exposed to granulosis virus (Boots & Begon, 1993). Such resistance was associated with an increased development time, suggesting there is a cost to fitness associated with maintaining resistance. Although the precise mechanism of resistance is unknown, lepidopteran-virus studies have revealed a number of methods that may be potentially displayed by ***Plodia***. A well-studied insect phenomenon is gut wall sloughing, a process by which infected epithelial columnar cells are discharged into the gut lumen and replaced by maturing cells (Washburn ***et al***, 1998). This has been observed in many lepidopteran species (Briese, 1986) and has been shown to be a mechanism that can completely clear AcMNPV infection in ***Trichoplusia ni*** (Keddie ***et al.***, 1989). Sloughing is energetically equivalent to resistance through thicker gut walls, a mechanism discussed in Boots & Haraguchi (1999). Sait ***et al***. (1994a) suggested the rate at which lepidopteran larvae ingest baculoviruses might affect susceptibility, the idea being that slower feeding individuals may be able to survive exposure to virus. One example of this is found in larvae of the mosquito ***Culex quinquefasciatus***. Resistant individuals lower the rate of feeding when toxin produced by the bacteria ***Bacillus sphaericus*** is detected in the environment, so enabling the toxin to be tolerated (Rodcharoen & Mulla, 1995). However a study of the nucleopolyhedrosis virus of ***Trichoplusia ni*** found that the time taken for larvae to ingest the pathogen did not alter mortality (Milks 1997a).

One potential mechanism of resistance that has received attention is the cellular response to pathogens within the haemocoel. Foreign organisms such as fungi and

bacteria are surrounded and killed by haemocytes, which form a multicellular, melanised capsule (Ratcliffe ***et al***., 1984). This process has been seen to confer resistance to AcMNPV infection in ***Helicoverpa zea***, with infected tracheal cells being encapsulated and cleared in exactly the same way that bacterial and fungal infections are removed (Washburn ***et al***., 1996). To further support the role of haemocytes in lepidopteran resistance, they have also been thought to phagocytose granulosis viruses within ***Plodia*** and ***Cydia pomonella*** individuals (Begon ***et al***., 1993; Hess & Falcon, 1987). It is known that the enzyme phenoloxidase is important in this encapsulation process. Certain haemocytes release this enzyme when they come into contact with infected cells and it catalyses greater rates of non-self recognition and encapsulation (variations of this process are reviewed in Richards & Edwards, 2000). The costs associated with phenoloxidase activity are the production of toxic quinones and oxygen species that may damage the hosts own tissues (Nappi ***et al***., 1995; Slepneva ***et al***., 1999). However these costs may be minimised by the formation of the haemocyte capsule, which is a method of directly targeting these toxins at the infected cell (Russo ***et al***., 1996).

Plodia interpunctella and laboratory experiments

Plodia has been used in a large number of studies, many attempting to find suitable biological and artificial pest control methods. The species has also been widely used as an experimental model laboratory system. This is partly because of the ease at which ***Plodia*** populations can be maintained and manipulated within the laboratory environment. At temperatures above 20ºC and humidity levels as low as 25%,

reproduction and survivability rates are high. Also a wide range of food mediums can be used with no significant changes in ***Plodia*** behaviour and dynamics.

The species has been involved in studies examining the processes by which generation cycles are formed and the significance of stage-structured life histories upon population dynamics (e.g. Begon ***et al***., 1996; Bjørnstad ***et al***., 1998; Gurney ***et al***., 1983). The generation cycles associated with ***Plodia*** are driven by asymmetric competition between larvae at different developmental stages. Large, late instars can both cannibalise and outcompete small, early instars (Gurney & Nisbet, 1985). As cannibalism is a feature of ***Plodia*** intraspecific interactions, the species has been an important tool to analyse the general importance of cannibalism in forming the structure and dynamics of populations (Boots, 1998; Reed ***et al***., 1996).

The relationship between competition and species diversity has also been empirically explored using ***Plodia***. There are many potential competitors for ***Plodia***, as several other stored product pest species share a similar geographical range, climate tolerance and food preference (e.g. ***Ephestia cautella***, ***Ephestia elutella*** & ***Ephestia kuehniella***). There is good evidence that competition between species occurs in warehouses and the field (Soderstrom ***et al***., 1987; Vick ***et al***., 1987), often resulting in the dominance of only one species (Allotey & Goswami, 1992).

Although my thesis will only consider the ***Plodia*** specific granulosis virus, the species can form interactions with several other natural enemies. Hymenopteran parasitoids such as ichneumonids (e.g. ***Venturia canescens***) and braconids (e.g. ***Bracon hebetor***) lay their eggs in late larval instars (e.g. Sait ***et al***., 1995; Sait ***et al***., 1996). The egg hatches and the larval parasitoid kills its host. ***Plodia*** larvae are also susceptible to spore-forming bacteria such as ***Bacillus thuringiensis*** (Knell ***et al***., 1996, 1998a), due to the high pH in the gut environment. Sporulating Bt produce

crystallised insecticidal proteins within the bacterial cell wall (Aronson ***et al***., 1986). Larval midgut proteins activate these toxins, which bind to the brush border membrane of the midgut epithelium (Van Rie ***et al***., 1990). The toxin causes lysis of affected cells and, together with septicaemia from germinating bacterial spores, can result in the death of the host (Manthavan ***et al***., 1989). Other pathogens include fungi of the genera ***Entomophthora***, which infect individuals through the outer integument and spiracles, and protozoan microsporidians such as ***Nosema plodiae***. Microsporidian infection occurs through the ingestion of free-living infective spores that are excreted by previously infected larvae. Early instars display high susceptibility and infection often results in death, whilst infection of later instars results in sublethal reductions in reproduction (Onstad ***et al***., 1990).

Granulosis Virus

The pathogen used is the granulosis virus (reviewed in detail in Granados & Federici, 1986). It is a subgenus of the ***Baculovirus***, the only genera of the Baculoviridae family. This genus is the largest group of viruses pathogenic to arthropods, although granulosis viruses have only been known to infect lepidoptera. The two other ***Baculovirus*** subgenera are the Nucleopolyhedrosis and Nonoccluded viruses. Baculoviruses have received a great deal of attention because they have potential to be used as pest control agents (Moscardi, 1999). Although there is variation in the morphology and replication of granulosis viruses, there are a number of structures and behaviours distinct to the subgenus. The double stranded DNA is bound to a protein that enables the supercoiled genome to be condensed and packaged within a rod-shaped protein capsid (Kelly ***et al***., 1983). The nucleocapsid is 40-50nm in

diameter (Beaton & Filshie, 1976) and is formed of protein subunits forming rings spaced 5nm apart (Burley ***et al***., 1982). The nucleocapsid in enveloped by a trilaminar membrane 6-18nm thick, characterised by a lipid layer bound on both sides by layers of protein (Hughes, 1972). Between the enveloped nucleocapsid and the inclusion body is a matrix of protein molecules arranged into a cubic lattice (Harrap, 1972). The inclusion body is composed of the protein granulin that is transcribed by the viral genome (Summers & Smith, 1975). The occluded virion forms a distinct oval or elliptical shape (Tweenten ***et al***., 1981) and can persist in an ultraviolet free environment for considerable periods.

The most common method by which lepidoptera become infected with the granulosis virus is via the ingestion of occluded bodies in contaminated food, whilst less frequent methods of host entry include vertical transmission from parent to offspring (Kukan, 1999) and passage via the spiracles (Granados, 1980). ***Plodia interpunctella*** larvae primarily acquire infection through the cannibalism of infected larvae, which are moribund and easier to prey upon (Boots, 1998). Once the virions have been ingested, the protein inclusion bodies are rapidly dissolved by the action of the host's alkaline digestive juices (proteases) in the gut lumen (Nagata & Tanada, 1983). The foregut and hindgut are covered by ectodermal cuticle, restricting the sites of primary infection to epithelial cells in the midgut. The epithelium is lined by a peritrophic membrane, composed of chitin and protein, which prevents the epithelium from being damaged by the digestive process. The peritrophic membrane is not a major barrier to virions coming into contact with the epithelium because it possesses membranous pores and discontinuities, and is replaced at every moult (Begon ***et al***., 1993; Adang & Spence, 1983). The now nonoccluded nucleocapsids fuse with susceptible columnar cells and travel to the nucleus, possibly along cellular

microtubules (Granados, 1978). At the nucleus, viral replication occurs and capsids are enveloped in the cell cytoplasm (Summers, 1971). These nucleocapsids bud through the basal lamina of the midgut into the haemocoel, where secondary infections occur (Granados, 1980). Within the haemocoel, the hosts' fat body is a major site of secondary infections. Once the fat body is infected, the granulosis virus triggers the rapid mitotic proliferation of new fat body cells that can be infected after the next viral reproductive cycle (Walker ***et al***., 1982). It is possible that both haemocyte and tracheal cells act as important tissues by which the granulosis virus spreads through the host body in advanced stages of infection (Begon ***et al***., 1993; Benz, 1963). However the alternative explanation for virus particles found in haemocytes is that they have been phagocytosed in a defence response (Begon ***et al***., 1993).

The overt symptoms of granulosis virus infection appear after several days. Larvae cease to feed and developing a white colouration as the epidermis becomes infected (Hamm & Paschke, 1963). Growth stops and death occurs through a fatal liquefaction of host tissues. The fragile integument breaks and releases occluded virions into the environment. The period of time between initial infection and death depends on the health and age of the host, microhabitat temperature as well as the dose and virulence of the virus. Sublethal effects have been noted, where infection has a detrimental effect yet the host still completes its life-cycle (Rothman & Myers, 1996). It is possible that such sublethal effects can emerge as either a result of the host attempts to resist infection (e.g. Ratcliffe ***et al***., 1984) or a consequence of a non-lethal, limited infection of host tissues (e.g. Burand & Park, 1992). Latent infection can become overt when individuals are infected with an heterologous pathogen or become stressed through changes in diet, temperature and population density

(Longworth & Cunningham, 1968). A sublethal infection of male and female gonads might allow the virus to be transmitted vertically to the offspring of infected individuals (Burden ***et al***., 2002). This method of transmission may well be important for the persistence of the virus in small, sparse host populations where the opportunities for infection through ingesting occluded bodies are few (Burden ***et al***., 2002).

Baculoviruses have been shown to exhibit a great deal of genetic variation. Such variation has been found at a range of observations scales, from whole states down to individual hosts. At the scale of the individual, up to 24 different nucleopolyhedrosis virus genotypes have been found in one host (Hodgson ***et al***., 2001). This variation in the viral genome is generated by mutations, insertions, deletions and the ability for parts of the host genome to be incorporated (Crozier ***et al***., 1988; Crozier & Ribeiro, 1992; Martin & Weber, 1997). At broader scales, baculovirus strains have been shown to be more similar within USA states than between states (Shapiro ***et al***., 1991) because of geographical differences in founder strains and selection processes (Cooper ***et al***., 2003; Smith & Summers, 1978).

Spatial Heterogeneity

Most theoretical and empirical studies ignore the inherent spatial dimensions of real ecological systems and replace them with an assumption of ‘mean-field’ spatial homogeneity. The first mean-field assumption is that the environment in which the population exists is uniform. However in real populations, the distribution of preferential habitat and food tends to be irregular so forcing individuals into patches. The second mean-field assumption is that any one individual has an equal probability

of encountering any other individual within the population (e.g. Anderson & May, 1992). However in reality, individual dispersal is restricted meaning an individual will only experience a limited part of the whole environment. Therefore individuals are only capable of interacting with the limited number of conspecifics that are nearby. As a consequence of resource distribution and limited dispersal, real populations are heterogeneous with individuals experiencing different biotic and abiotic environments (Tilman ***et al***., 1997).

The spatial structure of a population or community varies depending upon the scale of observation (Husband & Barrett, 1996; Thomas & Kunin, 1999). There is a hierarchy of spatial structures, as individuals form patches, patches form subpopulations and subpopulations form complete populations. Each structure involves an ever larger part of the population and each potentially possesses different emergent properties. The finest scale of observation is that of the individual, the minimum unit of the population. Each individual organism is a discrete entity that can only interact directly with other organisms that are close by. The number of individuals that any one individual can interact with constitutes a contact neighbourhood, the size and stability of which depends crucially on the mobility of individuals. A non-mobile species will only be affected by a few, nearby individuals and the spatial position of the contact neighbourhood will persist over time. A highly mobile species will interact with many individuals and the large contact neighbourhood will be dynamic, with the position of neighbourhoods changing with each movement step.

At a broader scale of population observation, individuals are clumped into patches. Such patches may be caused through endogenous or exogenous forces, or a mixture of the two. Endogenous spatial heterogeneity is generated by the combination

of localised interactions and short dispersal distances. Variation in abundance at small scales is initially generated by localised interactions and then short dispersal fixes the altered abundances (Durrett & Levin, 1994b; Hanski, 1998; Kareiva, 1990; Keeling, 1999a). This can create a patchy population, where the density of individuals varies across space despite a uniform environment. Exogenous spatial heterogeneity is generated through environmental factors. Should preferential habitat and food be patchily distributed throughout the environment, the population will be correspondingly distributed. Endogenous and exogenous forces can interact, with a patch of individuals caused by short dispersal rapidly exploiting local resources and so forming a patchy distribution of resources (Williamson, 1981).

The broadest scale at which a population's distribution can be measured is where each patch belongs to a subpopulation and dispersal between subpopulations creates the whole (meta) population (Menéndez & Thomas, 2000). The concept of the metapopulation has its origin in landscape ecology and is a large-scale study of local populations based around 'islands' of suitable habitat. The metapopulation persists despite each subpopulation being vulnerable to extinction. It is possible to distinguish a patchy population from a metapopulation by examining the dispersal of individuals between population clumps. In a metapopulation, dispersal is rare, involves a long travel time (in proportion to life-span) and is associated with high mortality (Nachman, 2000). In a patchy population, dispersal between patches is common and involves little risk to the individual (Nachman, 2000; Roslin, 2000). Metapopulation structures have been found in habitats such as fragmented grassland (Harrison ***et al***., 1988; Thomas & Harrison, 1992) and interconnected ponds (Bengtsson, 1991; Sjögren, 1991).

A broad scale spatial structure functionally different to the metapopulation is the source-sink model (Holt, 1993; Pulliam, 1988). This is characterised by 'source' habitats where births are greater than deaths and 'sink' habitats where deaths are greater than births. Therefore the sink habitat population can only persist through immigration from the source habitat. This form of spatial structure, in the form of core-satellite models, has been used to understand the persistence of measles in human populations (Bolker & Grenfell, 1995a, b). The disease is endemic in the densely populated cities and movement maintains the presence of the pathogen in surrounding small towns that would be too small to allow persistence alone.

Life History Trade-Offs

Most chapters in this thesis study the effect of spatial structure on host-pathogen and competition systems. However Chapter 6 examines the variation of granulosis virus resistance within a population of ***Plodia interpunctella***. Variation in trait expression may indicate the presence of a trade-off between aspects of life histories. This is because should a trait carry no cost to fitness, then we would expect it to become rapidly fixed within the population. Stearns (1989) defined trade-offs as "the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another". Trade-offs can act through phenotypic mechanisms, with changes in resource allocation. However for a trade-off to have evolutionary consequences, the traits must be negatively correlated at the genetic level (Stearns 1992) by either pleiotropy or coincidental genetic linkage (Fellowes ***et al***., 1999; Reznick 1985).

When there is a fitness cost to pathogen resistance, the trait will only be selected for when there is a high frequency of contacts between susceptible hosts and pathogen. Host susceptibility is favoured in the absence of the pathogen because, with no pathogen, the costs of resistance outweigh the advantages. Therefore maintaining populations in the presence and absence of the pathogen enables any life history trade-offs to be measured. Insects have provided an effective method of testing trade-offs between resistance and other life-history traits, as the immune system is relatively simple. Insects lack lymphocytes and immunoglobulins (Gillespie ***et al.*** 1997) as well as antibody production associated with acquired immunity (Stevens ***et al***., 1997). Instead, cellular and humoral processes recognise and remove foreign invaders either by killing them or by isolating them from the rest of the body. Insects also exhibit rapid evolutionary responses to artificial selection in the laboratory, again making them a useful tool for studying costs to resistance. Rapid evolution can occur in many insect species because they have high growth rates and a low susceptibility to crowding, resulting in large carrying capacity reached at a slow rate from low densities (Boots & Bowers, 1999: Bowers ***et al***., 1994).

Boots & Begon (1993) maintained long-term ***Plodia interpunctella*** populations with and without PiGV. Populations exposed to the virus became 1.96 times more resistant than controls but suffered a calculated 15% reduction in fitness as development time increased and egg viability decreased. However the fitness costs outside the laboratory may be significantly lower because the increase in development time was correlated with an increase in pupal weight. The noctuid moths ***Spodoptera frugiperda*** (Fuxa & Richter 1989) and ***Anticarsia gemmitalis*** (Fuxa & Richter 1998) were selected for resistance towards their respective nuclear polyhedroviruses. In comparison to control populations, resistant ***Spodoptera***

frugiperda and ***Anticarsia gemmitalis*** displayed lower fecundity, lower egg viability and shorter adult lifespan. Also ***Trichoplusia ni*** females resistant to a nucleopolyhedrosis virus laid fewer eggs in comparison to susceptible females (Milks, 1997b).

Populations of ***Drosophila melanogaster*** resistant towards its parasitoids, ***Asobara tabida***, ***Leptopilina boulardii*** and ***Pachycrepoideus vindemia***, showed an 5-60% increase in the rate of parasitoid egg encapsulation (Fellowes ***et al.*** 1998a; Kraaijeveld & Godfray 1997). The costs associated with maintaining this higher rate of encapsulation were reduced adult fecundity, a lower feeding rate (Fellowes ***et al***., 1999) and a thinner puparial wall increasing the susceptibility to attack by the pupal parasite ***P. vindemiae*** (Fellowes ***et al***., 1998b). These trade-offs could only be observed when food levels were low and competition with susceptible strains was intense (Kraaijeveld & Godfray 1997). Also diamondback moth ***Plutella xylostella*** populations resistant to ***Bacillus thuringiensis*** displayed lower survival, fecundity and egg viability than susceptible populations (Groeters ***et al.*** 1994).

Examples of non-lepidopteran invertebrate trade-offs with resistance include the mosquito ***Aedes aegypti*** selected for resistance to carrying the avian malaria parasite ***Plasmodium gallinaceum***. The adults of resistant strains were smaller and laid fewer eggs (Yan ***et al.*** 1997). Resistant lines of the snail ***Biomphalaria glabrata*** to the schistosome ***Schistosoma*** mansoni displayed a lower fecundity than susceptible lines (Webster and Woolhouse, 1999).

There can also be trade-offs within the immune system, so that resistance to one pathogen confers susceptibility to another. Examples of this form of fitness cost include populations of ***D. melanogaster*** resistant to one parasitoid species, ***A. tabida***, becoming more susceptible to another species, ***L. boulardii*** (Benassi ***et al.*** 1998).

Mosquitoes incompatible with one species of ***Plasmodium*** were found to be susceptible to other species (Graves & Curtis 1982; Somboon & Takagi 1999). Also strains of mice resistant to ***Trichinella spiralis*** displayed an increased susceptibility to ***Trichinella musculi*** (Sorci ***et al***., 1997), a feature that may be common in vertebrate immune systems (Gill ***et al.*** 2000; Grencis, 1997).

One issue with experiments attempting to discover trade-offs between life history traits is that a range of important selection pressures present in the wild are often not considered within the confines of the laboratory. This means that apparently deleterious impacts on fitness may not actually reduce the survival and reproductive ability of individuals in the wild. For example, should a lower feeding rate be correlated with resistance, it is often seen as a cost capable of severely affecting the individual. However, there may be situations where large, well-fed, individuals are more vulnerable to predation, and so balancing out the perceived costs of resistance (Lanciani, 1975). Also the effects of environmental and demographic stochasticity may over-ride any costs of resistance. An example is the toxicity of ***B. thuringiensis*** towards the Gypsy Moth ***Lymantria dispar***, which depends on diet of the host. Pupae reared on Douglas-fir were more susceptible than those reared on Alder (Moldenke ***et al***., 1994). Another important consideration is that any costs of resistance can themselves be selected against, reducing any negative impacts on fitness. Studies into resistance of pesticides have shown that genetic mutations can occur to reduce associated deleterious effects of pleiotropic resistance (Lenski, 1988).

Thesis Outline

Chapter 2 focuses on the laboratory techniques used to change the movement rates of individual ***Plodia interpunctella*** and ***Ephestia cautella*** larvae. The food medium was altered to create different viscosity levels and the movement rates of larvae within the food types were measured. This was carried out with the aim of making food types that generate a series of relative larval movement rates, ranging from high to low. The development time and pupal weight of larvae fed on each food type were used to ensure different the food types had the same energetic value. The potential effects that movement rate change has upon the spatial structure of populations are then discussed. Chapter 2 includes an overview of the theoretical and empirical techniques used to study spatial heterogeneity.

Chapter 3 describes a laboratory competition experiment, using ***Plodia*** and ***Ephestia*** larvae. With knowledge of the individual movement rates gained from Chapter 2, we attempted to understand intra- and inter-specific competition at small cohort scales. The intensity of intraspecific competition is inferred from insect growth rate and mortality, with lower growth rates and higher mortality associated with higher resource and interference competition. Interspecific competition between ***Plodia*** and ***Ephestia*** is also tested by analysing the growth rates and mortality of larvae in different food types and at different densities. The results for the intra- and inter-specific competition studies are discussed in reference to the changes in spatial structure due to the different food viscosities. Chapter 3 also reviews the relevant theoretical and empirical literature concerning spatial heterogeneity and competition.

Chapter 4 uses the information gathered at the individual and cohort scale to study dynamics at the population scale. Long term ***Plodia*** time series data were gathered at three different food viscosity levels in the presence and absence of a

granulosis virus (PiGV). Population size, cycle length and number of infected cadavers are discussed in reference to competition intensity and infection rates caused by differences in population spatial structure. This chapter also includes a review of the empirical data concerning the relationship between spatial heterogeneity and host-parasite dynamics.

Chapter 5 describes experiments with selected granulosis virus extracted from populations maintained in Chapter 4. As different host population spatial structures can change the number of transmission opportunities for the virus, we might expect to see corresponding evolution in infectivity. Bioassays reveal the larval mortality rate associated with the concentration of virus particles within each strain. Larval mortality is used to indicate the infectivity because successful transmission results in overt infection and host death. This chapter also includes a review of the literature discussing the relationship between the evolution of parasite infectivity with patchy host spatial structure and localised transmission.

Chapter 6 focuses on a novel method of inferring the heterogeneity of parasite resistance within a population. ***Plodia*** larvae were exposed to a range of PiGV dose levels. Mortality across the dose range allowed the pattern of resistance variation to be assumed. Depending on whether the pattern of resistance in the ***Plodia*** population is gaussian or bimodal, we can potentially predict the shape of the trade-off curve between increasing resistance and the detrimental consequences to fitness. The implications of the pattern of variation and the corresponding shape of the trade-off curve are discussed, as are the limitations of the methodology.

Chapter 2

The Development of Spatial Techniques in the Laboratory

Introduction

The main aim of this thesis is to study the effect of population spatial structure on disease dynamics and competition. To this end, a series of laboratory experiments and simulations will be carried out. This chapter introduces the experimental procedures used throughout the thesis, as well as a discussion of the general, non-specific, methods by which spatial heterogeneity has been investigated. The annual number of published papers addressing the implications of spatial dimensions in ecology and evolution has increased dramatically since 1990 (Figure 2.1). Developments in computing power, the theoretical underpinning of biology and empirical data collections have all prompted an increase in the study of a wide range of biological heterogeneities. The spatial structure of many populations is irregular, with the density of individuals varying across the environment. This arises from the inability of individuals to disperse evenly through the entire environment and the patchy nature of favourable habitat. The central question in spatial ecology is whether the distribution of individuals is an important consideration when studying ecological processes such as competition, predation, and parasitism.

The simplifying assumption that individuals randomly mix in a uniform, homogeneous environment has been made in the classic studies of competition (Volterra, 1928; Macarthur & Levins, 1964), predation (Nicholson & Bailey, 1935) and infection (Kermack & McKendrick, 1927). Such deterministic mean-field models only consider the population densities at the global scale and are intrinsically unstable. This instability failed to match observations in natural systems, which tend to be characterised by stable, long-term population persistence.

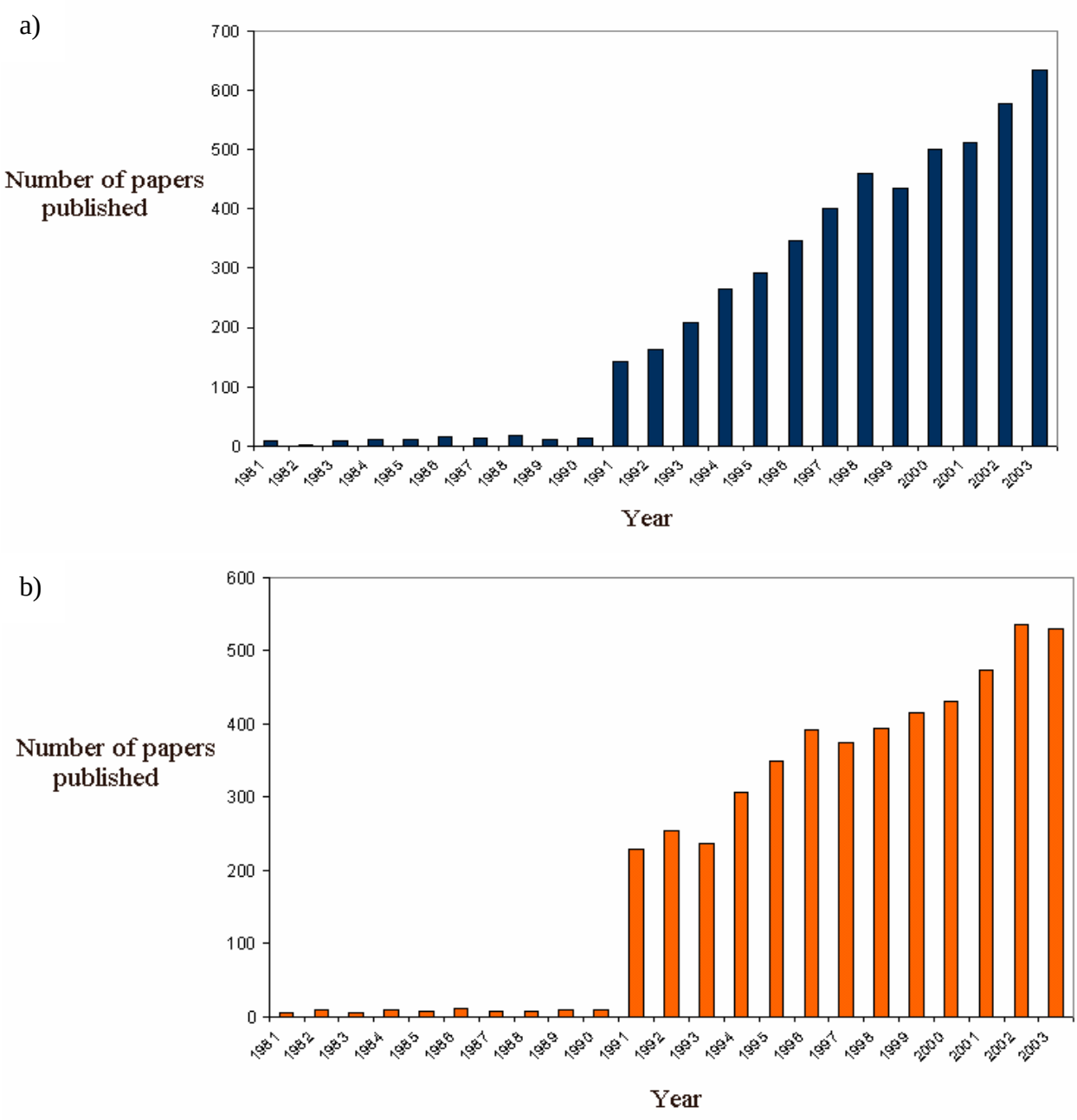

Figure 2.1 - The number of spatial biology papers published on Web of Science.

The search key words are a) ***Spatial*** AND ***Heterogeneity*** and b) ***Spatial/Space/Spatially*** AND ***Competition***

The number of spatially orientated biological papers was low until 1991. There was a rapid increase in 1991 and ever since there has been a gradual, yet significant, rise in the number of spatial studies. The development of theoretical tools and computational power has increasingly allowed ecologists to study the relationships between the spatial distribution of populations and dynamical ecological processes.

The potential that population spatial structure might be a factor generating the stability found in nature was highlighted in early laboratory experiments. Huffaker used a predator-prey mite interaction to replicate the instabilities found in theoretical models (Huffaker, 1958; Huffaker ***et al***., 1963). The prey species, ***Eotetranychus sexmaculatus***, could persist in the absence of its predator, ***Typhlodromus occidentalis***, but the system was rapidly driven to extinction when the predator was included. It was possible to render both prey and predator dynamics more stable by making food isolated and allowing ***E. sexmaculatus*** to disperse at a faster rate than its predator. This created local patches where prey populations increased exponentially in the absence of the predator and local predator populations went extinct in the absence of prey (Begon ***et al***., 1996). The spatial heterogeneities created by dispersal and habitat irregularity could generate a stable two-species interaction.

A series of spatially orientated theoretical models have been developed and used in a wide variety of ecological and evolutionary studies. There are three general types of spatial model: 1) reaction-diffusion; 2) metapopulation or patch and 3) individual-based cellular automata. Reaction-diffusion models are the most widely used method of simulating the effects of space in ecology. They are nonlinear, partial differential equations that are more tractable than cellular automata models. Space is treated as being implicit and continuous. The 'reaction' part of the model explains deterministic population dynamics within locations and 'diffusion' explains the movement processes linking dynamics across the environment. Such movement can range from simple passive diffusion to more complex forms of dispersal (Okubo, 1980; Okubo ***et al***., 1989; van den Bosch ***et al***., 1990). Population densities have to be large and therefore reaction-diffusion models tend to ignore interactions at small scales. Such models have been used to analyse both pattern formation (Murray, 1989)

and the effect patch size and shape has on the persistence of populations (Cantrell & Cosner, 1993).

A second form of spatial model is the metapopulation. The mathematics behind metapopulation theory, constructed by Levins (1969, 1970), provided an extension to the island biogeography concepts of colonisation and extinction (MacArthur & Wilson, 1967). Populations are defined not numerically but in terms of the occupancy or vacancy of local patches. The rate of population change is the difference between the rate empty subpopulations are colonised by dispersal and the rate other subpopulations go extinct. This simple model assumes that colonisation of one site can result from anywhere in the population, carrying capacity is reached instantly, subpopulations are equal in size and localised dynamics do not occur (Harrison ***et al***., 1995; Harrison & Taylor, 1997; Williamson, 1981). Incorporating these complexities to metapopulation models is at the centre of attempts to apply theory to practical conservation measures (Gilpin & Hanski, 1991; Hanski, 1989). Metapopulation structures have been found in several natural populations, including butterflies (e.g. Harrison ***et al***., 1988; Thomas & Harrison, 1992), frogs (Sjögren, 1991) and ***Daphnia*** species (Bengtsson, 1991). In these examples, suitable habitat is fragmented throughout the environment and intermediate rates of dispersal allow the population to persist despite localised extinction (Hess, 1996).

Probabilistic cellular automata are the third method of modelling spatial structure. Individuals occupy discrete sites within a homogeneous lattice, interacting predominately with locally positioned sites. As such, individuals only experience a small effective population, constituting a contact neighbourhood. Population dynamics are driven by the contact neighbourhood's effect upon the status of each of its constituting sites. This creates a complex array of heterogeneous patches across

the lattice, which can experience large, non-equilibrium fluctuations. The results of such models are explicitly stochastic as there is a finite number of individuals. A major problem with cellular automata is a tendency to be mathematically intractable, with analysis restricted to computer simulations. However pair approximation analysis (moment closure techniques) allows the mathematics of cellular automata to be simplified and rendered tractable. The method examines the correlations between interacting individuals by calculating the probability that any one site exhibits the same state as an adjacent site. The spatial processes within cellular automata have been shown to have a significant effect on ecological processes, such as parasite epidemics (e.g. Mollison, 1977) and host-parasitoid systems (e.g. Comins ***et al***., 1993), as well evolutionary processes (e.g. Boots & Sasaki, 1999; Rand ***et al***., 1995).

Associated with this spatial theory is a growing body of empirical data. It would be impractical to summarise the methods used in such experimental studies, as each natural system requires fundamentally different approaches to measure and manipulate the degree of spatial heterogeneity experienced by the participating species. Some methods associated with spatially orientated competition and host-parasite experiments will be reviewed in Chapters 3 and 4. It is possible to group the empirical approaches used in spatial ecology into three categories: 1) field work mapping of movement and distribution of populations (e.g. Harrison ***et al***., 1988; Thomas & Harrison, 1992), 2) analysis of migration and habitat coverage over time (e.g. Harrison, 1989; Kuussaari, ***et al***., 1996; Doncaster ***et al***. 1997; Turchin, 1998; Raynor & Cliff, 1999) and 3) the manufacture and imposition of a spatial structure onto a laboratory or field system (e.g. French & Travis, 2001; Huffaker ***et al***., 1963; Ims ***et al***., 2004). There are a number of unique problems related to the spatial study of ecology that any researcher must consider when formulating research plans. One is

that the scale of the study must correspond to the scale at which spatial heterogeneity produces a measurable effect. If metapopulation dynamics are important then the study must monitor subpopulations, uninhabited but suitable habitat and dispersal between sites. This has the potential to involve a large study area and the collection of a comprehensive dataset may prove impossible. Should fine scale spatial structure be important, collecting detailed information of habitat preference and individual movement at small scales might proof similarly awkward. Such difficulties are increased when considering broader community interactions, where species differ in their life-cycles, dispersal rates and responses to the environment. Also the importance of spatial heterogeneity may vary over time, with demographic or environmental fluctuations reducing or increasing the effect heterogeneity has on the variables of scientific interest.

The methods applied in this thesis primarily involve an attempt to generate endogenous spatial structure within a homogeneous food environment. I attempt to manipulate the larval movement rates of two phycitid moth species within a laboratory setting. By creating food media of differing viscosity, where movement rates are altered but feeding remains unchanged, it is possible to allow individual behaviour to generate different spatial structures at local and population scales. This is a rather rare experimental approach as it is the individuals themselves that create the spatial structure. A strictly defined spatial structure is not forced upon populations, as has been the case in previous laboratory based patchy- and meta-population experiments. The methodology described forms the basis for much of the thesis.

Methods

Plodia and Ephestia

Plodia interpunctella and ***Ephestia cautella*** are laboratory model systems that lend themselves to spatial studies. Adults lay batches of eggs directly in the food and the resulting larvae of both species spend much of their existence within the food medium provided. Only at the fifth and final instar do larvae display directed movement out of the food medium. By this time, larvae neither compete for food nor can their granulosis virus infect them. As larvae live within the food medium, it is possible to render this medium more viscous and therefore restrict larval movement. The methodology in this chapter describes the process by which food is made viscous and the effect this has on the movement and thereby the spatial structure of ***Plodia*** and ***Ephestia*** larvae. I also examine whether the manipulation of the media affects the life-history characteristics of the larvae.

The food medium

The standard food medium is a mixture of 400g HiPP organic harvest breakfast cereal, 80g brewers yeast, 100ml glycerol, 100ml organic clear honey, 0.5g sorbic acid (SA) and 0.5g methyl paraben (MP). The cereal, yeast and honey combine to create a nutritious medium. The glycerol adds moisture that prevents the negative effects of dryness on larval survival and development. SA acts as an antibacterial agent, important because ***Bacillus thuringiensis*** and other bacterial species frequently contaminate stock populations and long running experiments. Similarly, MP is a fungicide.

By adding water to this mixture, it is possible to change the texture of the food. When no water is added, the food possesses a soft, loose texture (Figure 2.2a). Adding distilled water makes the food harder and stickier, with the food clumping together into a dense mass (Figure 2.2c). One has to be careful about how and when the water is added to the mixture. Initially the cereal, yeast, SA and MP have to be mixed thoroughly in an industrial food mixer. To this, add the water and mix for no less than 10-15 minutes. Finally add the glycerol then honey, and mix until the texture of the food is constant. For food with high water amounts, this final mixing stage has to be observed carefully because if left too long, the food quickly hardens and becomes difficult to work with.

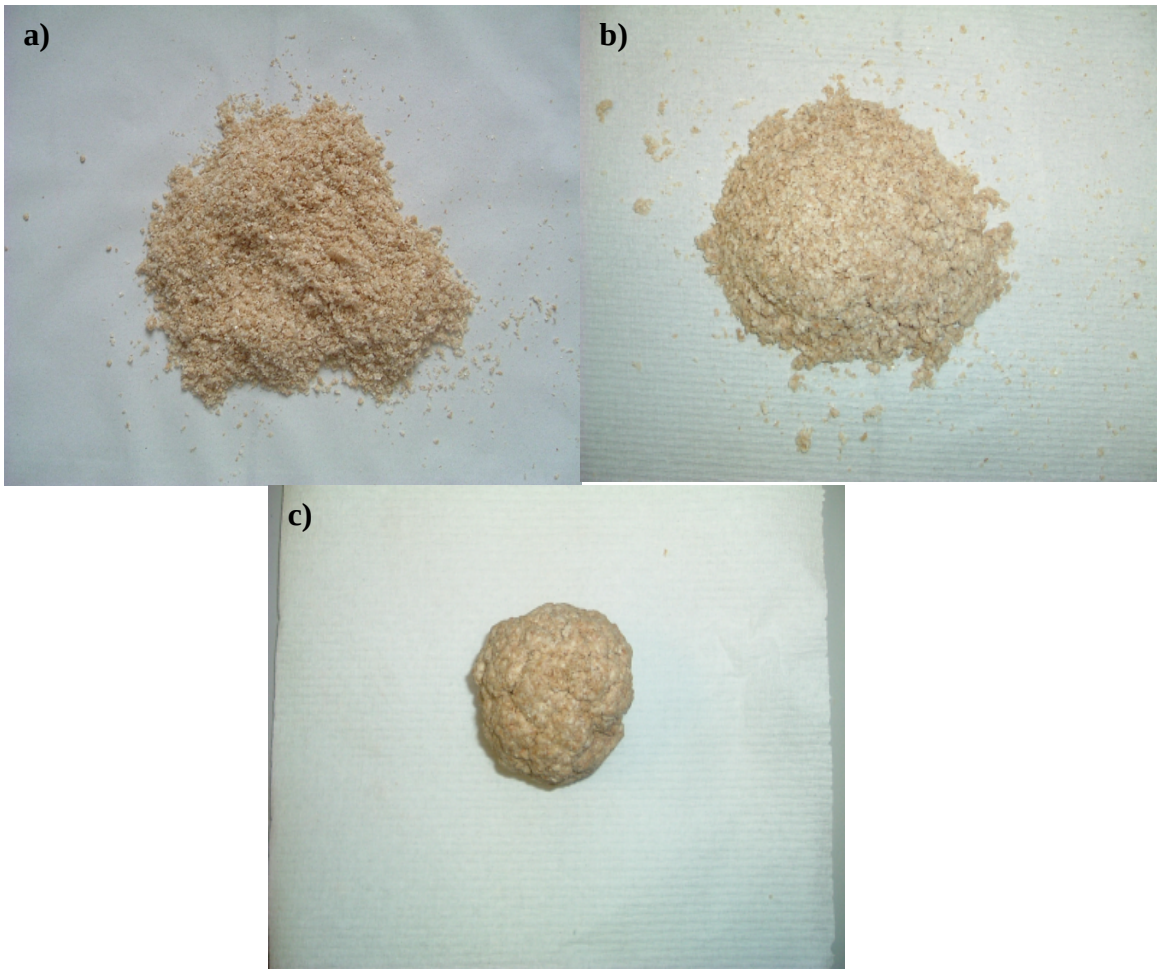

Figure 2.2 - Photographs of three food viscosity types
Food types are mixed with a) 0ml (Soft) water, b) 60ml (Intermediate) water and c) 140ml (Hard) water.
The food with no water is powdery and loose. As water is added, the food clumps together and hardens. Each food type produces significantly different ***Plodia*** larvae movement rates.

The effect of increasing food viscosity on larval movement rates

If the hardened food is to be useful, it must have a significant limiting effect on the movement of larvae. This was tested using equipment pictured in Figure 2.3. An 18 by 18cm cardboard structure was made, within which four confined lanes were delineated, each 18 cm long and 4cm wide. Food was added into each lane so the entire 18 by 4cm area was covered up to a depth of 0.5cm. The boxes were then placed in a plastic container, covered to prevent free adults from ovipositing eggs and left in an incubator for five days. This allows the sticky, dense food to dry out allowing the media to be consistently hard by the time larvae were added.

Movement rates were measured by adding first instar larvae at one end of each lane. Only one larva was placed in each lane and cardboard strips were weighted down on lanes to prevent larvae moving along the surface of the food or move between lanes. Each 4-lane box was placed in an aerated plastic container and left in an incubator at 27^0C and 35% humidity for either 12 days (***Plodia***) or 14 days (***Ephestia***). This ~2 week period covers the time taken by ***Plodia*** and ***Ephestia*** to develop from the first instar stage to the third instar. Then the food was carefully lifted from each lane and destructively sampled one centimetre at a time along its entire 18cm length in order to find the larva. The distance moved by surviving larva was measured. 40 ***Plodia*** larvae were tested in 6 food viscosity types, making a total of 240 individuals. For ***Ephestia***, 25 larvae were tested in 3 food types so that 75 individuals were tested in total.

Figure 2.3 – Method of measuring larval movement rates

Larval movement rates were measured using boxes as above. At one end of each lane, first instar larvae were placed within the food. ***Plodia*** larvae were left for 12 days and ***Ephestia*** larvae were left for 14 days, allowing larvae to develop into third instars. The distance travelled up the lane after this period was determined.

The effect food has on larval development time, pupal weight and survival

The next step was to discover whether making food viscous by adding water affected the growth rate and survival of ***Plodia*** and ***Ephestia*** larvae. If the viscous food is more difficult to eat or ingest, we would expect surviving individuals to experience the symptoms of malnutrition and stress; a longer development time, lower adult weight and death.

Development time for both species was tested by putting 80 grams of food with 0ml (Soft), 60ml (Intermediate) and 140ml (Hard) water in 3 perforated plastic containers, along with 80 recently hatched first instar larvae of either species. Development time was considered the time taken for first instars to develop into adults. A similar experiment was carried out to test pupal weight, with 50 newly hatched larvae placed in plastic containers with 80grams of each food type. To test the weight of individuals, pupae were chosen ahead of adults because adults can easily lose body parts, and therefore weight, during handling. Food was gently broken up by hand and pupae were removed with forceps.

The larval mortality associated with the three food types was analysed using a generalised linear model with binomial including the survival in both development time and pupal weight experiments. This model also included species (***Plodia*** & ***Ephestia***), and experiment type (development time & pupal weight) as covariates. The large amount of food given to larvae in both experiments negated the effect resource and interference competition had on development time and pupal weight. All containers were maintained in incubators set at 27ºC.

Results

Viscosity and movement rates

Initially six different levels of food viscosity were used to measure the distances travelled by ***Plodia*** larvae. The mean results are shown in Figures 4-6, with standard errors and the number of surviving larvae from which measurements were taken. The mean distance travelled was 10.63 cm (SE ±0.73) in food with no water, 5.08cm (Standard Error ±0.61) in food with 60ml water, 3.97cm (±0.61) with 80ml, 3.75cm (±0.46) in 100ml water, 3.48cm (±0.49) in 120ml water and 2.8cm (±0.26) in 140ml. A one way analysis of variance (with square root transformed data for homogenous variance) revealed there is a significant change in movement rates as the amount of water mixed into the food increases (ANOVA sqrt(movement): $F_{5,177}$ = 26.11, P <0.001), with increasing viscosity lowering movement rate. Analysis of the model coefficients showed there were significant differences between the movement in food with 0ml, 60ml and 140ml water (sqrt(movement): 0ml coefficient= 3.192, 60ml coefficient= 2.168, t= 5.857, p< 0.001; 60ml coefficient= 2.168, 140ml coefficient= 1.6, t= 3.07, p= 0.003). These three food types produce movement rates that are significantly different to each other, and so are used throughout the thesis. Food with 0ml water is called Soft Food, food with 60ml water is Intermediate Food and food with 140ml is Hard Food.

The movement of ***Ephestia*** larvae was measured in the three food viscosity types and is shown along with the ***Plodia*** results in Figures 4-6. The mean distances travelled were 3cm (SE ±0.65) in Soft Food, 2.62cm (±0.45) in Intermediate Food and 2.57cm (±0.33) in Hard Food. There is a small, statistically insignificant decline in ***Ephestia*** movement rates as the viscosity increases (ANOVA sqrt(movement): $F_{2,46}$ = 0.475, P =0.625).

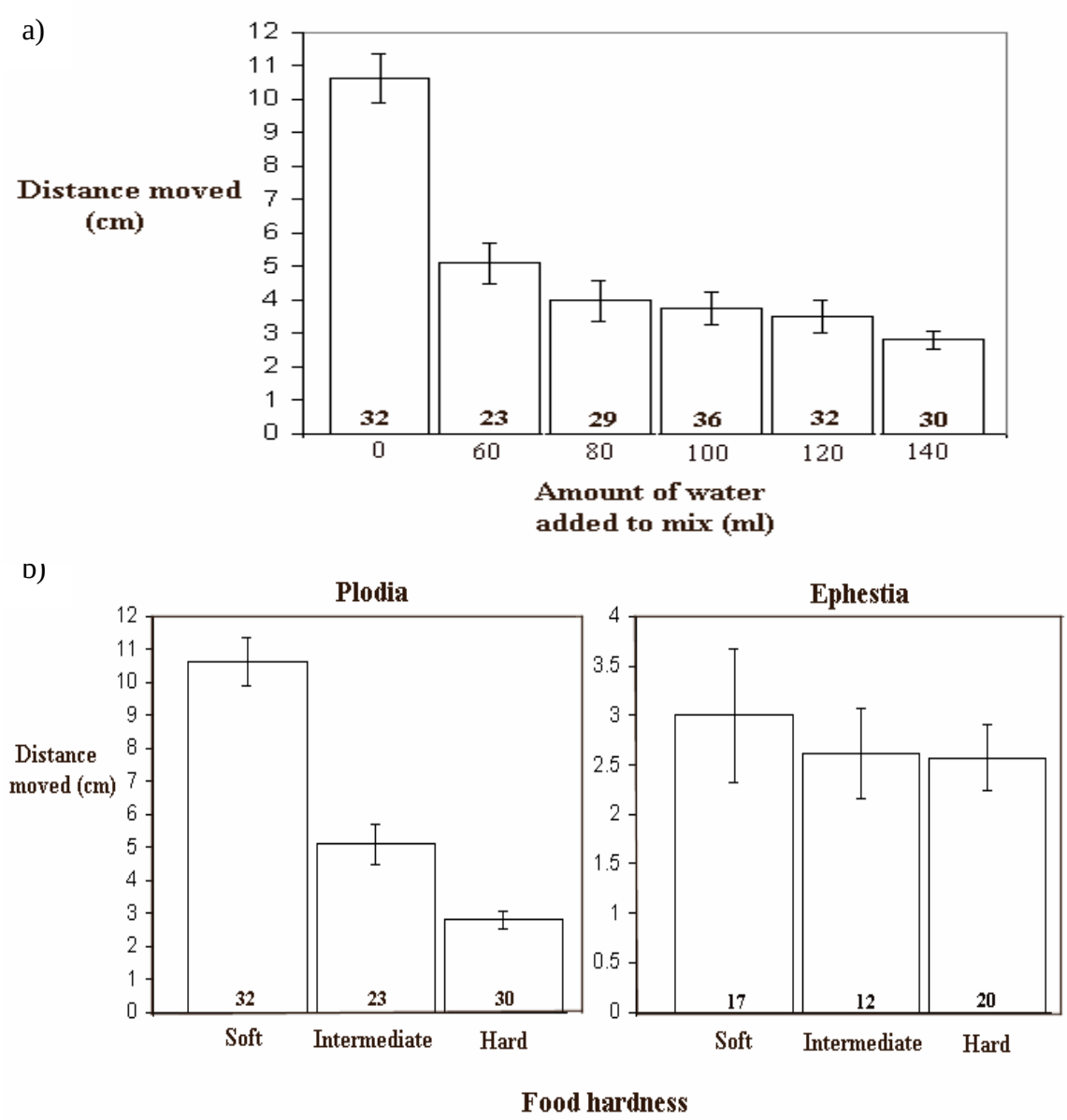

Figure 2.4 – Movement rates of larvae through different food types

a) The mean distance moved by ***Plodia interpunctella*** larvae with standard errors and number of larvae measured. Increasing the volume of water in food increases, so the distance moved declines. The three food levels that produce significantly different movement rates to each other were 0ml (Soft), 60ml (Intermediate) and 140ml (Hard).

b) The distance moved by ***Plodia*** and ***Ephestia cautella*** larvae in the three food types. The distance moved by Ephestia larvae does not show any significant relationship with food viscosity, despite a small decrease in distance travelled (ANOVA; $F_{2,48}$ = 0.235, P = 0.79).

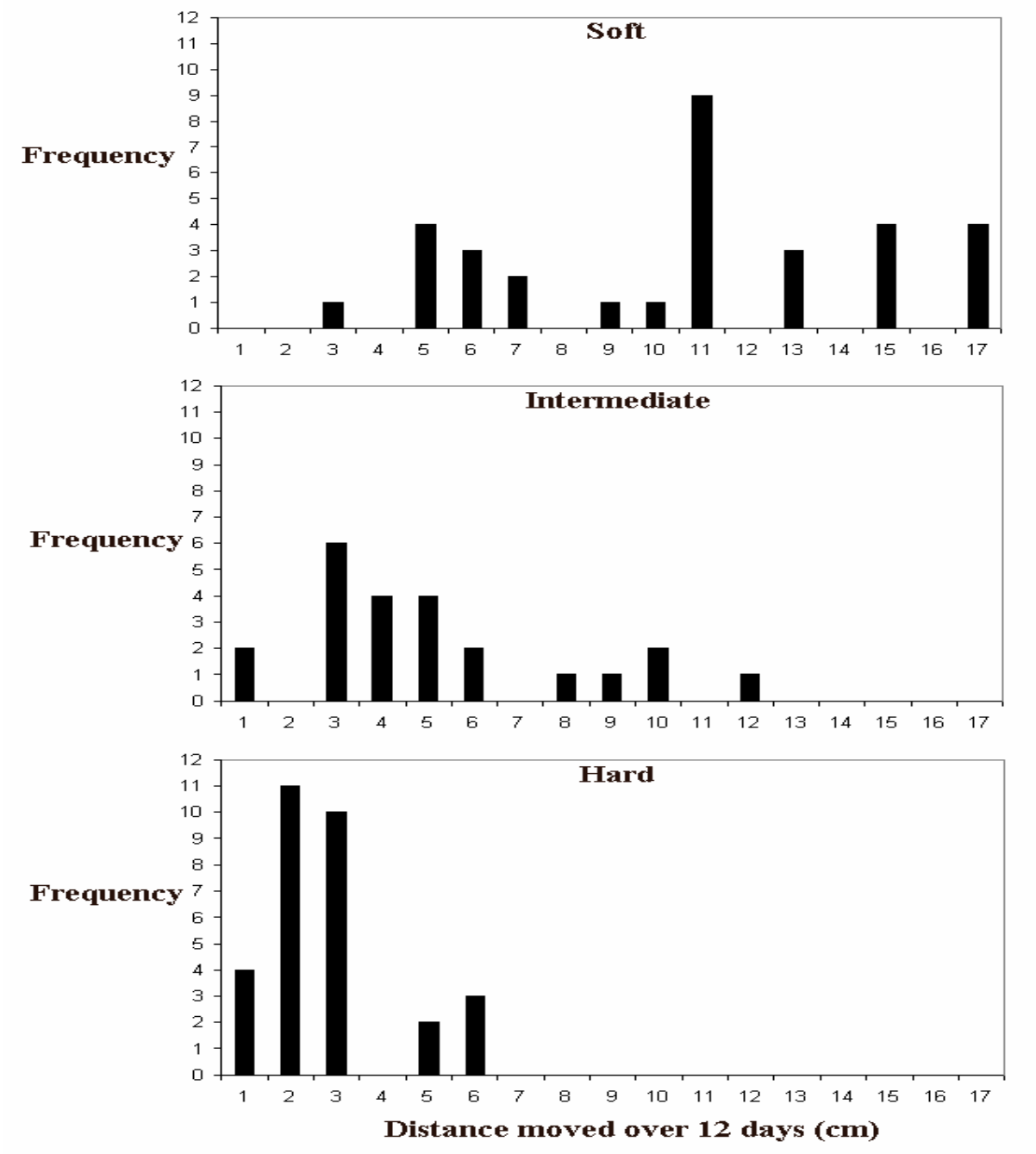

Figure 5a – Dispersal kernels of *Plodia* larvae in three food types

In Soft Food (0ml), the range of movement was 3-17cm and movement over short distances was as common as it was over long distances. In Intermediate Food (60ml), the range of movement was 1-12cm but the most frequent distances moved were 3-5cm. In Hard Food (140ml), the range of movement was restricted to between 1-6cm and most individuals only moved 1-3cm.

More viscous food acts to lower both the upper limit for larval movement and the most frequent distances travelled.

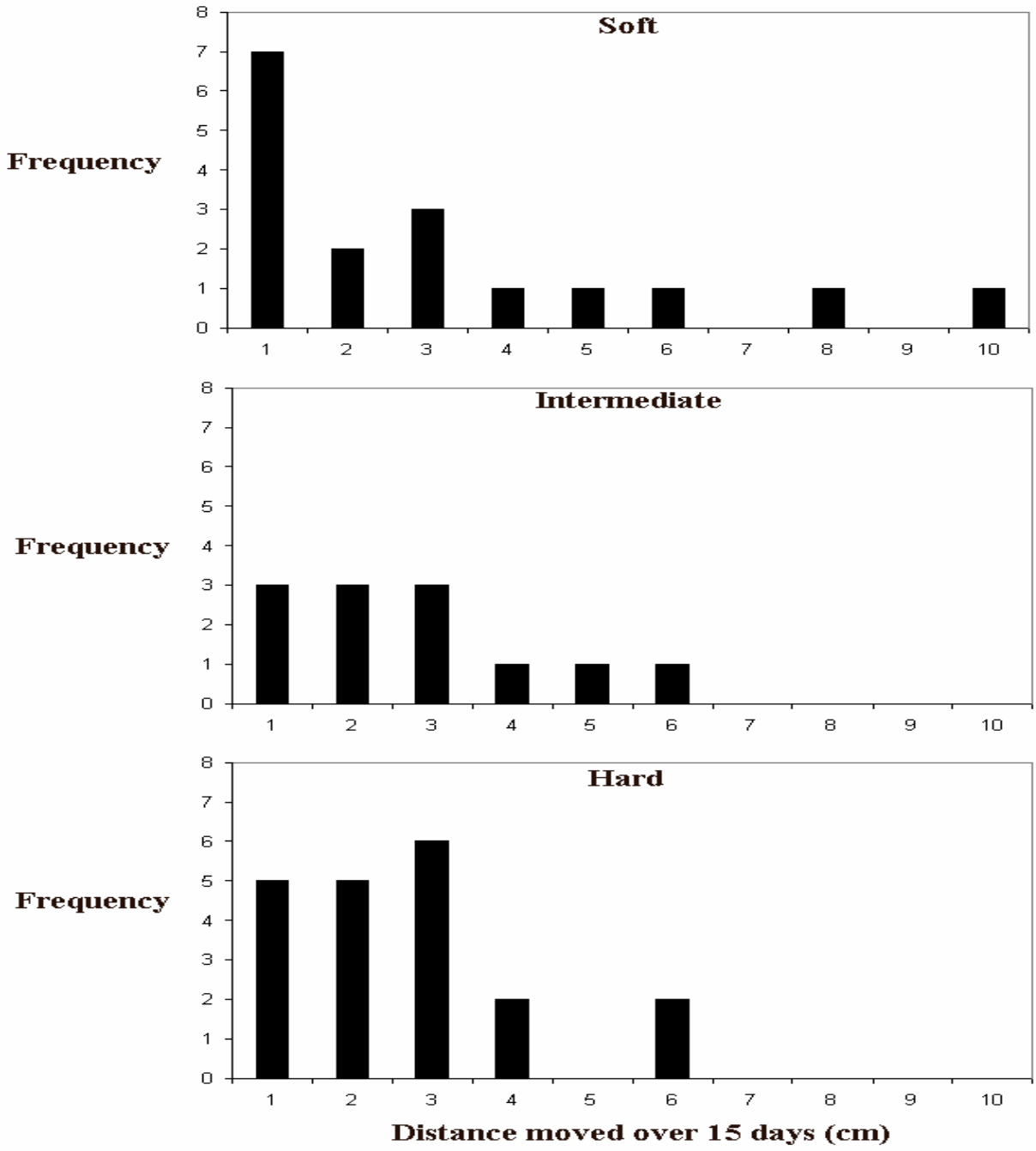

Figure 5b – Dispersal kernels of *Ephestia* larvae in three food types

In Soft Food (0ml), the range of movement was 1-10cm with most individuals travelling only 1-3cm. A minority of individuals go further and provide the kernel a long tail. The kernels for Intermediate (60ml) and Hard Food (140ml) were similar. Both show a range of movement of 1-6cm with most individuals moving only 1-3cm.

Making the food more viscous acts to restrict the upper limit of movement and so removes the tail found in Soft Food

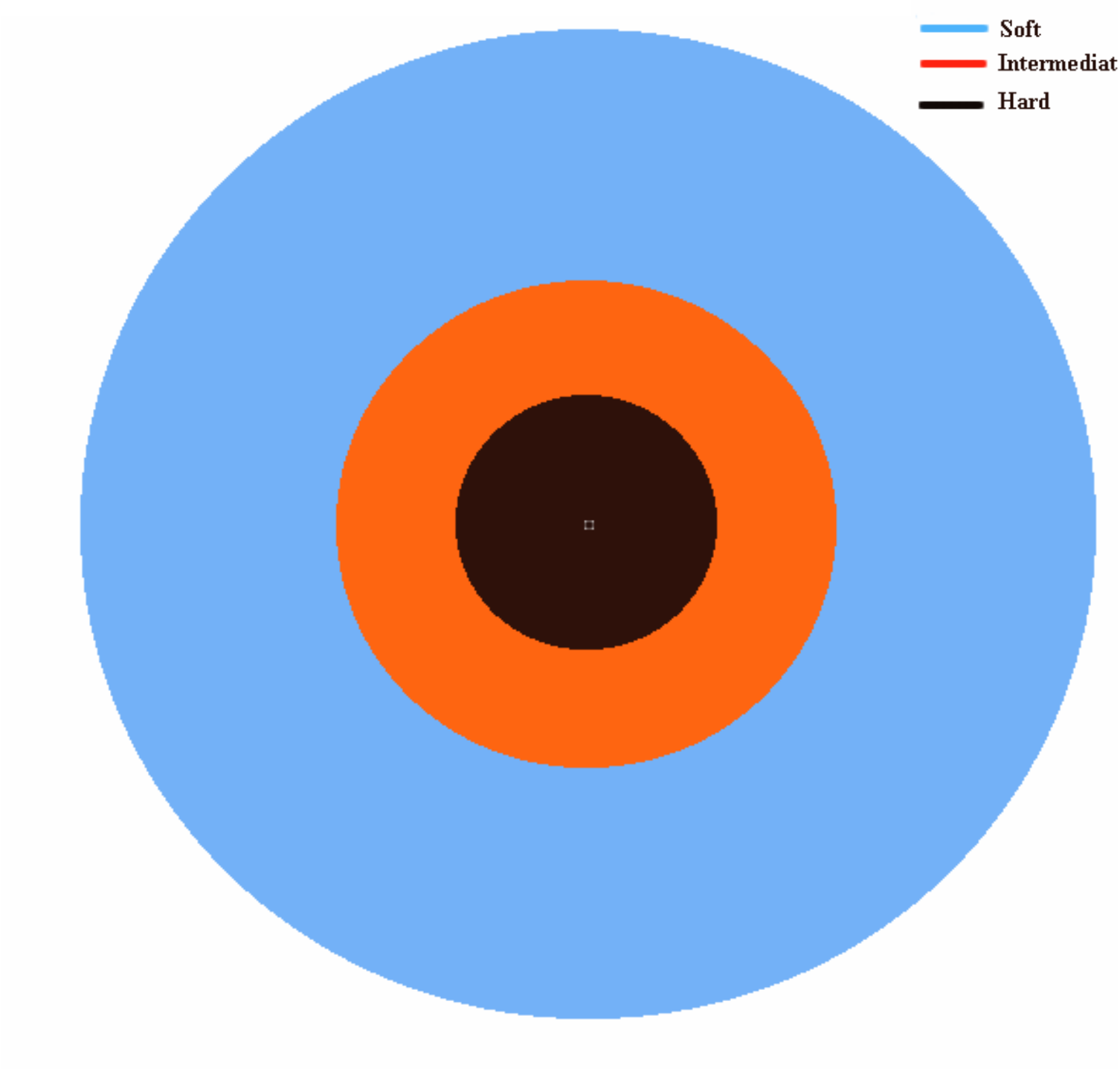

Figure 2.6a - The mean potential area *Plodia* larvae can experience in three food types

In Soft Food (0ml), the diameter of the area larvae could experience is 10.63cm, making the size of the total area 88.74cm (©r^2). In Intermediate Food (60ml), the diameter of the potential contact area is 5.08cm, therefore the total area is 20.26cm. In Hard Food (140ml), the diameter of the potential area is 2.8cm, creating an circle 6.15cm in size.

Viscous food forces individuals to experience a much smaller effective environment.

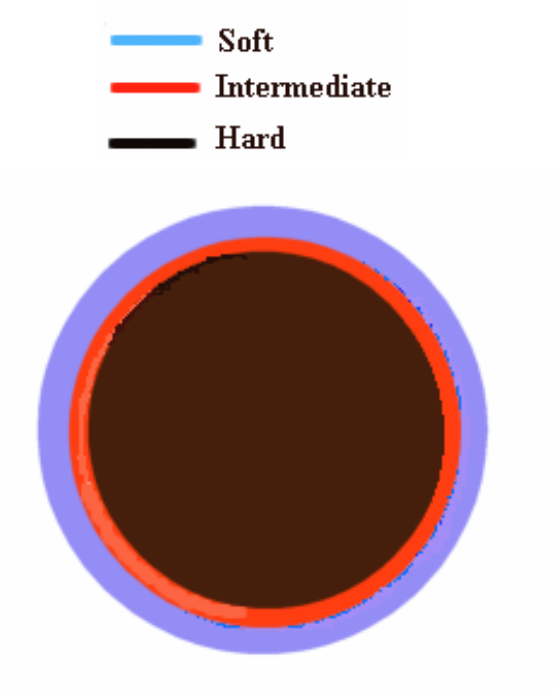

Figure 2.6b - The mean potential area *Ephestia* larvae can experience in three food types

In Soft Food (0ml), the diameter of the area larvae could experience is 3cm, making the size of the total area 7.07cm (πr^2). In Intermediate Food (60ml), the diameter of the potential contact area is 2.62cm, therefore the total area is 5.39cm. In Hard Food (140ml), the diameter of the potential area is 2.57cm, creating a circle 5.18cm in size.

Viscous food has a small effect in reducing the effective environment experienced by larvae.

The effect of food viscosity on development time, pupal weight and survival

The development time, pupal weight and mortality of ***Plodia*** and ***Ephestia*** were measured in the three food types Soft (0ml water), Intermediate (60ml water) and Hard (140ml). The mean times for development for ***Plodia*** larvae were 41.26 days (SE ±2.21) in Soft Food, 42.57 days (±2.13) in Intermediate Food and 40.56 days (±1.92) in Hard Food. This indicates that increasing the viscosity of food does not change the development time for ***Plodia*** larvae (ANOVA; $F_{2,121} = 0.22$, $P = 0.802$). Similarly, food viscosity had no effect on the development time of ***Ephestia*** larvae (ANOVA; $F_{2,188} = 0.44$, $P = 0.64$). The development times for ***Ephestia*** were 47.4 days (±1.56) in Soft Food, 45.43 (±1.53) days in Intermediate Food and 47.13 days (±1.66) in Hard Food. The mean development times for ***Plodia*** and ***Ephestia*** are shown in Figure 2.7a, with standard errors and the number of surviving larvae from which measurements were taken.

Mean ***Plodia*** pupal weights were 0.01261g ($\pm 5.81^{-4}$) in Soft Food, 0.01284g (SE $\pm 5.74^{-4}$) in Intermediate Food and 0.01314g ($\pm 5.62^{-4}$) in Hard Food. Therefore there was no significant difference in the pupal weight of ***Plodia*** fed on the three food types (ANOVA; $F_{2,177} = 0.23$, $P = 0.79$). For ***Ephestia***, mean pupal weights were 0.00955g ($\pm 3.47^{-4}$) in Soft Food, 0.00988g ($\pm 3.66^{-4}$) in Intermediate Food and 0.00971g ($\pm 3.63^{-4}$) in Hard Food. ***Ephestia*** pupal weight is not affected by food viscosity (ANOVA; $F_{2,93} = 0.21$, $P = 0.81$). The pupal weights for ***Plodia*** and ***Ephestia*** are shown in Figure 2.7b.

Regarding mortality, a generalised linear model with binomial errors found no significant effect of different food types on the survival of ***Plodia*** and ***Ephestia*** larvae for either the time ($F_{9,2} = 2.64$, $p = 0.274$) or weight experiments ($F = 0.58$, $p = 0.63$), as well as no interaction existing between food type and species ($F = 1.69$, $p = 0.371$).

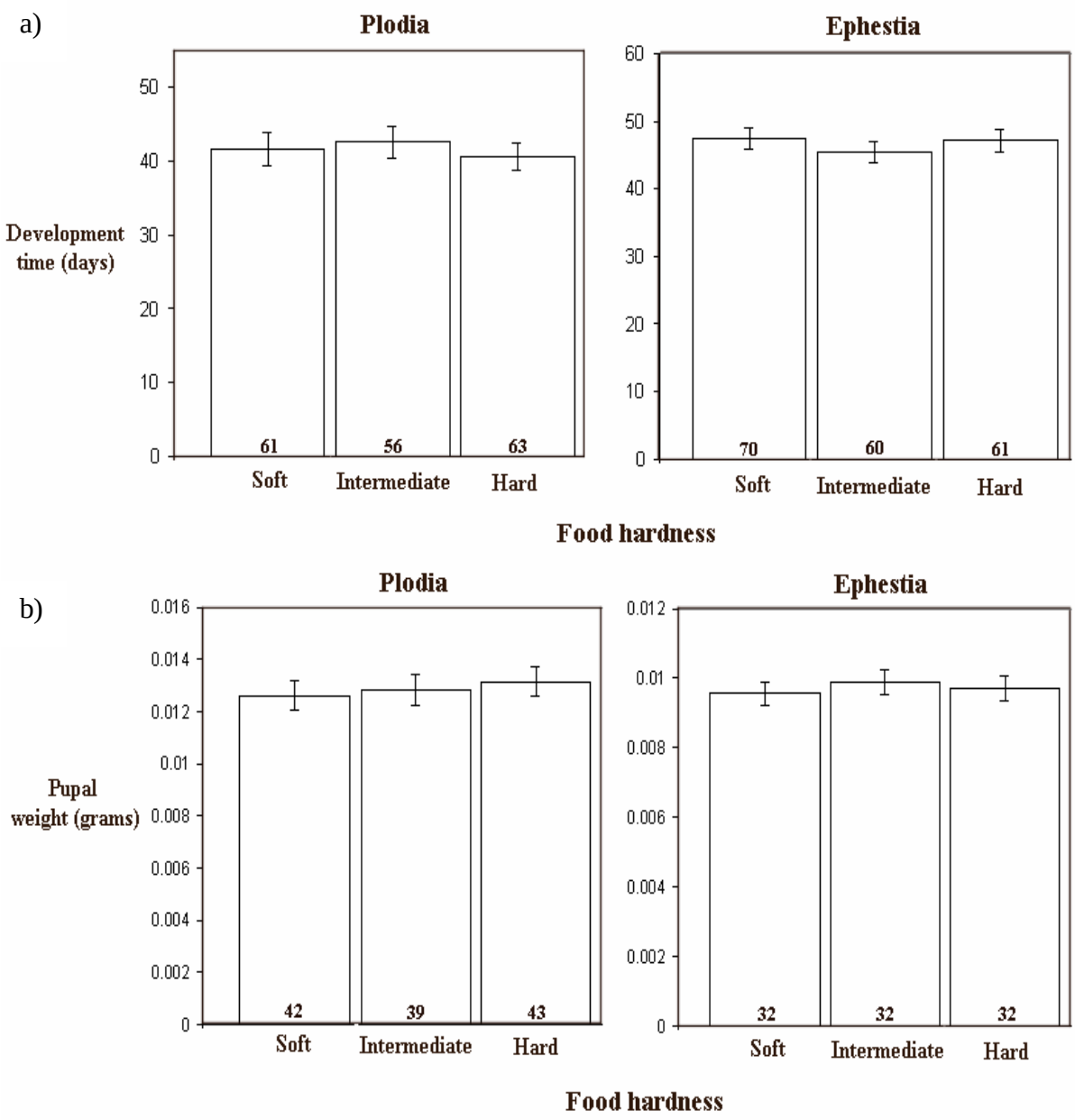

Figure 2.7 – Development time and Pupal weight on different food types.

a) The mean development time (+SE) of ***Plodia*** and ***Ephestia*** in Soft (0ml), Intermediate (60ml) and Hard (140ml) food, with the number of larvae measured. Food viscosity does not significantly change the development time of both ***Plodia*** (ANOVA; $F_{2,121} = 0.22$, P = 0.802) and ***Ephestia*** (DF = $F_{2,188} = 0.44$, P = 0.64).

b) The mean pupal weight (+SE) of ***Plodia*** and ***Ephestia*** in Soft (0ml), Intermediate (60ml) and Hard (140ml) food. Food viscosity does not significantly change the pupal weight of both ***Plodia*** (ANOVA; $F_{2,177} = 0.23$, P = 0.79) and ***Ephestia*** ($F_{2,93} = 0.21$, P = 0.81).

Discussion

An important question concerns how the addition of water produces such a significant effect on the texture and viscosity of the food. The point at which the water is added to the food is crucial, suggesting water added to the cereal-yeast mixture alters the way in which honey and glycerol combine to the mix. A possible explanation is that water forces the formation of small clumps of cereal-yeast, and honey attaches to the outside of these small clumps so sticking them together. As more water is added, the number of these clumps increases, so the effect of honey sticking them together becomes more apparent. This forces the food to become denser and hardens as the excess water evaporates. Water evaporation means the humidity of Hard Food is not significantly different to food with no water. One effect of such clumping of food is that resources may become unevenly distributed. This is countered by the long time (10-15 minutes in an industrial food mixer) the water is mixed into the cereal-yeast. Mixing makes small food clumps that are evenly distributed throughout the food, preventing any variation in food quality.

The results indicate that rendering standard medium viscous by adding water does have an effect on the movement rate of ***Plodia interpunctella*** larvae. Increasing the amount of water always resulted in some reduction of movement rate (Figure 2.4a), however a multiple comparison analysis showed there were only three water levels that produced significantly different movement rates. The movement rate was 10.6cm in food with no water, 5.08cm in food with 60ml water and 2.8cm in food with 140ml water (Figure 2.4b). The results from these three distinct levels of food viscosity (Soft = 0ml, Intermediate = 60ml and Hard = 140ml) were further examined to produce dispersal kernels (Figure 2.5a). The distances moved by individual ***Plodia*** larvae in Soft Food ranged from 3cm to 17cm. The frequency of distances moved was

relatively uniform, with as many individuals moving 5cm as moved 15cm. The dispersal kernel in Intermediate Food showed movement ranging from 1-12cm. Within this range, the majority of larval movement was restricted to 1cm to 5cm. Movement in the Hard Food ranged from 1-6cm, with most individuals only moving 1-3cm. Therefore, despite considerable movement variation within each food type, the upper limit and overall range of ***Plodia*** movement are both reduced with increasing viscosity.

The significant decline in movement rate was not matched for ***Ephestia cautella*** larvae (Figure 2.4b). For the three viscosity food levels there was a small but insignificant decline in mean movement rate. The mean distance moved by ***Ephestia*** larvae in Soft Food was 3cm, in Intermediate Food 2.62cm and in Hard Food 2.57cm. The dispersal kernels for ***Ephestia*** movement are shown in Figure 2.5b. In all three food types, movement is primarily restricted to 1-3cm. However the tail of dispersal in the Soft Food is long, with individuals moving up to 10cm., contrasting to the 6cm upward limit in Intermediate and Hard Foods. It appears the higher movement rate in the Soft Food is brought about by a minority of individuals capable of long distance dispersal, a capability removed by the increased viscosity of Intermediate and Hard Foods. The possible reasons why ***Plodia*** larvae move at a faster rate than ***Ephestia*** are discussed in Chapter 7.

For both ***Plodia*** and ***Ephestia***, there was insignificant difference in the development time, pupal weight and mortality of individuals fed on Soft, Intermediate and Hard Food (Figure 2.7). This indicates that adding water does not alter the nutritional value of the food nor has a detrimental impact on the ability of larvae to feed. Also the humidity of food has an important effect on ***Plodia*** and ***Ephestia***, with low humidity causing higher mortality and longer development times

(Johnson ***et al***., 1992). As no change was observed, this suggests the methods of inducing are not associated with a change in food humidity.

These results indicate the method of altering food viscosity only changes larval movement, so has potential to change the spatial structure of larval populations. Figure 2.6 demonstrates the size of area in which individuals, for both species, have the potential to experience in different food types. Regarding ***Plodia***, the mean potential area larvae can experience in Soft Food is 88.74cm, which is reduced to 20.26cm in Intermediate Food and 6.15cm in Hard Food. The effect is less dramatic for ***Ephestia*** with the mean potential area declining from 7.07cm in Soft Food to 5.39cm in Intermediate Food and 5.17cm in Hard Food. This shows how restricting larval movement may potentially cause aggregated clumping of individuals. Eggs are laid in batches and in Soft Food hatched larvae rapidly move away from the oviposition site and spread themselves across the environment. In the more viscous food types, hatched individuals may be unable to move away from the oviposition site and are therefore forced together into clumps.

The movement estimates discussed above are based on the assumption that larvae move in a straight line through the food media. In larval populations, this is unlikely to be a true reflection of larval dispersal, as larvae will primarily move to avoid the competitive interactions they experience with other larvae. As such, the direction of movement will be determined by the density and position of local conspecifics, meaning the true movement of larvae through the food environment is likely to be zig-zagged and may involve repeated movement through the same region of space. However the movement estimates discussed in this chapter do give an indication of the relative effects of food viscosity on movement and can indicate how

restriction determines spatial aggregation in combination with cohort and population scale experiments described in later chapters.

The assessment of movement rates was considered only for the period of time taken for individuals to develop from the first instar to third instar. However no measurements were taken of the effect food viscosity has on the movement of fourth and fifth instars, and therefore no direct inferences can be made about the spatial structure of later instars. This limitation in the methodology may be important. Early instars are crucial in host-granulosis virus dynamics because they lack maturation resistance and so are highly vulnerable to infection (Sait ***et al***., 1994c). However later instars also have an important role in infection because large, late instars that are infected with granulosis virus can be potentially cannibalised by a large number of healthy larvae. The high concentration of virus particles within large, infected larvae make it likely that many of the susceptible larvae that feed on the infected larva will ingest enough virions to become infected (Knell, ***et al***., 1998a). As a result, it is likely that late infected larvae will be the primary source of infection within a ***Plodia***-granulosis virus system and their spatial distribution may ultimately determine how differences in larval movement rate effect ***Plodia***-granulosis virus interactions. Also later instars are preferentially attacked by parasitoids such as ***Venturia canescens***, meaning further information concerning late instar movement would be essential for the use of the methodology presented here in the study of host-parasitoid interactions.

Later instars are also important in larval competition processes. Their size allows them to dominate competitive encounters with smaller instars, by either cannibalising or interfering with the feeding of early instars. Such age structured asymmetric encounters are crucial in driving the generation cycles characteristic of ***Plodia*** and ***Ephestia*** populations (e.g. Gurney ***et al***., 1983; Sait ***et al***., 1994b). Also

later instars have a tendency to move out of the food medium in search of suitable pupation sites, allowing them to disperse through the environment independent of food viscosity and so escape highly competitive regions in space.

Both ***Plodia*** and ***Ephestia*** have characteristics that make them highly suitable species for this type of experiment. Both species possess the ability to eat a wide variety of foods, ranging from soft, elastic foods (e.g. wheat) to hard, rigid foods (e.g. nuts). Therefore they already have the physical ability to gain nutrients from food types matching the texture of the mediums created in this study. Whether monophagic species could prosper equally well in soft and hard media is debatable. An important problem in studying spatial host-parasite interactions is the difference in how hosts and parasites disperse through the environment. In the ***Plodia***-granulosis virus interaction, the predominant infectious unit is the infected larva, which is cannibalised by non-infected larvae. Therefore another advantage of using ***Plodia*** in spatial studies is that the virus infectious unit possesses a fundamentally similar behaviour to potential hosts. If we change the spatial structure of the host population, the corresponding spatial structure of the virus population will be altered in a qualitatively similar way. On a more general note, both species are easily bred and maintained in laboratory incubators and controlled temperature rooms, with high survivability and reproductive rates found at 27ºC and a 24 hour light:dark regime of 16h:8h.

Chapter 3

Spatial Structure and Competition in Two Lepidopteran Species *Plodia interpunctella* and *Ephestia cautella*

Abstract

Larval competition experiments within and between two lepidopteran stored product pests ***Plodia interpunctella*** and ***Ephestia cautella*** were carried out in a range of food viscosities. Restricting the movement of individuals altered the intensity of intraspecific competition. In Soft Food, where movement rates were high, the growth rate and survival of larvae were both high, indicating less intense competition. In Hard Food, where movement rates were restricted, the intensity of competition increased, with fewer individuals surviving and those that did displaying lower growth rates. This difference is proposed to have occurred because high movement rates allow individuals to disperse through the food environment and avoid competitive encounters. When movement is low, individuals are forced into aggregations and are unable to move away from competitive encounters.

Interspecific competition in Soft Food always resulted in the dominance of ***Plodia*** over ***Ephestia***. This superiority was lost when movement was restricted, with no difference in the survival of ***Plodia*** and ***Ephestia*** larvae in the Hard Food. In Soft Food, ***Plodia*** move at a greater rate than ***Ephestia*** and so are therefore likely to be better able to escape competitive encounters and quickly exploit any competition-free space in the environment. In viscous foods, the reduced movement rate of ***Plodia*** prevents larvae from avoiding intraspecific encounters and the greater rate of competition induced ***Plodia*** mortality reduces the number of detrimental interspecific encounters experienced by ***Ephestia***.

Introduction

The ecological mechanisms that promote coexistence between competing species are of great interest to both theoretical and applied ecology. The processes by which species utilise resources and interact with competitors of different species determine the composition and evolution of community assemblages (Pacala & Levin, 1997; Tilman, 1996). Early modelling of the competitive exclusion principle suggested that superior competitors would always drive inferior competitors extinct and that therefore the number of species that did coexist is restricted by the number of resources (Buttel ***et al***., 2002; MacArthur & Levins, 1964; Volterra, 1928). This fails to describe much of the biodiversity in nature (Doncaster, 2001; Hutchinson, 1961) and a range of ecological mechanisms generating species coexistence have been proposed and discussed. The proposed mechanisms include population size fluctuations offering temporal refuges for inferior competitors (Chesson & Warner, 1981; Levins, 1979), intermediate sized disturbances preventing competitive exclusion (Grime, 1973; Mouquet ***et al***., 2002) and inter-species similarity in life-history traits (Chesson, 1991: Hubbell, 1979). A competition-colonisation life-history trade off, with inferior competitors possessing a higher dispersal rate than superior competitors (for empirical evidence for the trade-off see Armstrong, 1976; Hanski & Ranta, 1983**;** Tilman, 1994), can also theoretically promote coexistence, (Amarasekare & Nisbet, 2001; Bolker & Pacala 1999; Holmes & Wilson, 1998; Skellem, 1951). This allows the inferior competitor to exploit parts of the environment before the superior competitor and thereby avoid interspecific competition.

Spatial heterogeneity is another factor that may explain how a competitively inferior species can persist over time (Lehman & Tilman, 1997). Populations of

competing species tend to be aggregated, with individuals distributed in small, restricted areas. Such inhomogeneous distributions can form through both endogenous and exogenous processes. Exogenous heterogeneity is created by the patchy distribution of resources, leading to spatial variation in habitat quality (Doncaster, 2001; Molofsky ***et al***., 1999). A mosaic pattern of resources can generate aggregations and species segregation through species-specific preferences and responses to the spatially variable environment (Grubb, 1986; Ives, 1988). Endogenous heterogeneity is generated through the necessarily restricted dispersal of organisms through the environment. This can result in populations with irregular distributions that are entirely self-organised. The universal nature of both processes underlying spatial heterogeneity means that all community dynamics possess a spatial component.

The relationship between spatial heterogeneity and biodiversity has been the subject of a large number of theoretical studies, with a correspondingly diverse range of conclusions. In some models, it is the interaction between heterogeneity and life history trade-offs that produces coexistence (Skellem, 1951). This is due to species clusters being assumed to be dynamic, with interspecific interactions occurring at cluster edges. In the absence of life-history trade-offs, the superior competitor wins these interactions and so gradually spreads through the environment eliminating the inferior species (Neuhauser, 2002; Neuhauser & Pacala, 1999). Modelling aggregation caused through egg clutch laying also indicated that spatial heterogeneity alone was not enough to generate coexistence (Atkinson & Shorrocks, 1981). Since all the competing species experienced similar levels of intraspecific competition within egg clutches (Green, 1986), the inferior species failed to coexist without a competition-clutch size trade-off (Heard & Remer, 1997). Tilman (1994) showed that

competition-colonisation trade-offs within a heterogeneous environment could create a broad spectrum of coexistence that can persist indefinitely. The trade-off functions used were step-wise meaning the rate of inferior competitor displacement was constant, regardless of the superiority of the winning competitor. Adler & Mosquera (2000) smoothed the shape of these trade-off functions and found that the biodiversity was entirely dependent upon the steepness of the function. The conclusion of this particular work was that spatial heterogeneity was irrelevant, citing the sole importance of life-history trade-offs in generating coexistence.

However there are a number of models that suggest spatial aggregation can promote coexistence alone, without invoking life-history trade-offs. The requirement for trade-offs is removed by coupling localised dispersal with non-instantaneous displacement of inferior competitors (Higgins & Cain, 2002). This creates temporal and spatial refuges, so reducing the need for a colonisation-competition trade-off. In addition, high levels of intraspecific competition within aggregations of the superior species can reduce its population size to the extent that interspecific competition is reduced sufficiently to allow inferior competitors to coexist (Bolker & Pacala, 1999; Neuhauser & Pacala, 1999; Pacala, 1986; Weiner & Conte, 1981). When species have different dispersal rates, the sizes of their interaction neighbourhoods are also different. This can lead to a situation where an individual of the superior competitor is more likely to encounter an individual of a different species (Murrell & Law, 2003). The superior competitor outcompetes the inferior individuals, leading to increasingly less mixed populations. This in turn generates species aggregations that are both more stable and segregated, with clusters of the superior species unable to spread across the environment (Murrell & Law, 2003). Even if species aggregation cannot maintain an indefinite coexistence, it can delay competitive exclusion for long enough so non-

spatial mechanisms of coexistence can start to influence the community dynamics (Huston, 1979).

The contradictory nature of much competition theory is indicative of a significant lack of empirical research. This chapter attempts to study the importance of movement rates and aggregation in a competition system comprising of two phycitid moth species. ***Plodia interpunctella*** (***Plodia***) and ***Ephestia cautella*** (***Ephestia***) are cosmopolitan storage product pests with comparable life histories. As they share a similar range of climatic tolerance and food preference, it is likely the two species compete whenever their distributions overlap (Haines, 1981). A competition experiment by Allotey and Goswami (1992) found the outcome of competition depended entirely on which species colonised the food first and that coexistence never happened. The process of exclusion can be explained through the competitive effect of late instar larvae on early instar larvae. One species is introduced first and lays its eggs. The second species is introduced but its eggs and early instar larvae are both outcompeted and predated upon by the larger, older larvae of the first species. Founder exclusion explains why it is very rare to find both species infesting the same barn yet it is possible to find ***Plodia*** and ***Ephestia*** dominating barns within the same locality (Allotey & Goswami, 1992).

It is possible to alter the movement rates of ***Plodia*** and ***Ephestia*** larvae by changing the viscosity of the food medium. Figure 2.4 in Chapter 2 shows increasing food viscosity restricts the movement rate of larvae. Reducing the movement rate can potentially change the spatial structure of competing populations as individuals are forced to experience a smaller part of the environment and so become aggregated (Figure 2.6). Here I study the effect aggregation formation has on the intensity of intraspecific competition over different densities for both species. Interspecific

competition was studied over the course of one generation, with first instar larvae of both species placed in the same food at the same time to counter founder exclusion. In this coexistence experiment, changes in movement and spatial structure will be discussed in relation to changes in the relative levels of intra- and interspecific competition. It can be predicted that coexistence might be generated should restricted movement result in higher levels of intraspecific competition and a reduced number of interspecific encounters. Previous work with stored product pests has shown resource and interference competition can have significant effects on larval growth rate and survival (Mbata, 1990; Podoler 1974). Therefore we can use these characteristics as diagnostic signs indicating the intensity of intraspecific and interspecific competition (Anderson & Löfqvist, 1996).

Method

Plodia interpunctella and ***Ephestia cautella*** (Lepidoptera: Pyralidae) are cosmopolitan pests of stored food products. They are found globally and both species have a similar tolerance of food varieties and environmental conditions. Both species share a similar mean fecundity of ~190 eggs, of which ~90% successfully hatch (Allotey & Goswami, 1990). The design of the experiment was based on the protocol of Anderson and Löfqvist (1996). Competition intensity was measured at three density levels and three food viscosity levels. The numbers of first instar larvae used were 6, 12 and 24, so density ranged from low to medium to high. The three food types were Soft Food (mixed with no water), Intermediate Food (60ml water) and Hard Food (140ml water), produced according to the methods outlined in Chapter 2. The effect of food type has on the movement rates of ***Plodia*** and ***Ephestia*** larvae are

presented in Figure 2.4. For ***Plodia***, the mean distance travelled was 10.63cm (se ±0.73) in Soft Food, 5.08cm (se ±0.61) in Intermediate Food and 2.8cm (se ±0.26) in Hard Food. This significant reduction in ***Plodia*** movement rate was not matched for ***Ephestia***, with movement rates in Soft, Intermediate and Hard Foods 3cm (se ±0.65), 2.62cm (se ±0.45) and 2.57cm (se ±0.33) respectively. Food viscosity had no effect on the development time and adult weight of either species (Figure 2.7).

For the study of intraspecific competition, 6, 12 or 24 newly hatched first instar larvae of one or other of the species were placed upon 2-gram balls of food. For the study of interspecific competition, the density levels were the same but half of the larvae placed on each of the food balls were ***Plodia*** while the other half were ***Ephestia***. Each food ball was placed separately inside a sealed plastic pot to prevent emerging adults escaping. The time required for first instar larvae to develop into adults was used as a measure of the development time and each adult was removed, sexed and weighed at emergence. From these measurements, the growth rate of individuals was calculated by dividing adult weight by development time. The proportion of larvae that survived into adulthood was also noted. Larval growth rate and survival rate were used to indicate the degree of competition within different food types and density levels because the smaller amounts of food and space available under intense competition are associated with an increased probability of death together with surviving individuals exhibiting longer development times and smaller adult weights, and thus smaller growth rates (Mbata, 1990; Podoler 1974).

In all, there were nine density-food combinations: 6-Soft; 6-Intermediate, 6-Hard; 12-Soft; 12-Intermediate; 12-Hard; 24-Soft; 24-Intermediate; and 24-Hard. Each density-food combination was replicated twelve times for both the intra- and inter-specific competition experiments, making a total of 216 pots for the intraspecific

experiments (108 ***Plodia*** and 108 ***Ephestia*** pots) and 108 pots in the interspecific experiments. Blocking was carried out to prevent any differences between stock ***Plodia*** and ***Ephestia*** generations from confounding the results. Each block consisted of the nine possible density-food combinations and all the larvae used came from eggs laid by the same group of adults. All larvae were kept in incubators set at 27ºC and ~35% humidity, with a 16h:8h light:dark regime.

Statistical analysis was carried out in S-Plus. The effect of food type and density level on growth rates was analysed using a linear mixed effects model. Growth rate was included as a dependent variable and the covariates include food, density, sex, species and replicate, with replicate included as a random factor. Sex was not included in any interactions since we are interested in the effect food and density has on competition intensity. To avoid pseudoreplication, the factor replicate was nested within covariates. The data were square root transformed to generate a homoscedastic scatter pattern of residuals.

Survival in the intra- and inter-specific experiments was analysed using a generalised linear model with binomial errors. Survival was considered as the proportion of the individuals placed on food balls as first instars that survived competition and emerged as adult moths. The covariates in the generalised linear model were food, density, species and the square rooted mean growth rate of surviving individuals. Replicate was nested within covariates only in the analysis of the interspecific experiment in order to group the survival of the ***Plodia*** and ***Ephestia*** that were placed together in the same food balls. Nesting was not required for the analysis of survival in intraspecific competition because there was only one value of survival for each food ball.

For both mixed effects and binomial models, full models were fitted then insignificant interactions were removed using the S-Plus ‘update’ procedures. The tables of coefficients from the models were used to determine the effect of food type in the intraspecific experiment and the difference between species in the interspecific experiment. The treatment contrasts function of S-Plus was used to generate the coefficients. S-Plus initially presents the coefficient outputs for the generalised linear model in the form of logits (ln(p/1-p)). However in the results section, the logit coefficients are given in the direct form of the proportion of individuals surviving competition. Proportions were determined by transforming logits using the equation 1/(1+1(exp(coefficient))) (Crawley, 2002).

Results

The Effects of Food and Density on Growth Rate

Growth Rate in Intraspecific Competition

Table 3.1 shows the effect food type and density level have on the growth rate (adult weight/development time ratio) of ***Plodia interpunctella*** and ***Ephestia cautella*** larvae when experiencing solely intraspecific competition. Model coefficients are discussed.

	Df	Mean Sq	F	P
food	2	0.000045	11.713	<0.001
density	2	0.000135	35.056	<0.001
species	1	0.00824	2136.766	<0.001
sex	1	0.00512	1327.486	<0.001
replicate	11	0.0000078	2.011	0.024
food:density	4	0.0000234	6.058	<0.001
food:species	2	0.0000896	23.225	<0.001
food:density:species	6	0.0000207	5.374	<0.001
Residuals	1395	0.0000039		

Table 3.1. A mixed effects model examining the factors determining ***Plodia*** and ***Ephestia*** growth rate when experiencing intraspecific competition. Growth rate was square root transformed and replicate was fitted as a random factor nested within the other covariates.

There is a significant interaction between food and density, suggesting the effect increasing density has on growth rate is different in Soft, Intermediate and Hard Foods. There is also a significant species interaction with food and density suggesting the way in which growth rate changes with density and food type is different for the two species. Such interactions are explored graphically in Figure 3.1 showing the mean growth rates (with standard errors). For ***Plodia*** in Soft Food, the growth rate at density 24 was significantly lower than at densities 6 and 12 (Soft Food-density: 6 coefficient = 0.0189, 24 coefficient = 0.0173, d.f. = 179, t = 2.263, p = 0.0235: 12 = 0.0189, 24 = 0.0173, t = 2.2951, p = 0.0229). The same relationship between density and growth rate was found in Intermediate Food, with the growth rate significantly lower at density 24 compared to other densities (Intermediate Food-density: 6 =

0.0186, 24 = 0.0172, d.f. = 179, t = 2.29, p = 0.0231: 12 = 0.0188, 24 = 0.0172, t = -3.792, p <0.001). In Hard Food, however, increasing density was not associated with any significant change in growth rate (Hard Food-density: 6 = 0.0179, 24 = 0.0172, d.f. = 179, t = 1.615, p = 0.106).

The relationship between food and density for ***Ephestia*** is broadly similar to that of ***Plodia***. In Soft Food, there was a significant decline in growth rate between density 12 and 24 (12 coefficient = 0.0151, 24 coefficient = 0.013, d.f. = 179, t = 2.05, p = 0.048). There was a different relationship in Intermediate Food, with the growth rates at density 6 and 12 both significantly larger than at 24 (Intermediate Food-density: 6 = 0.0152, 24 = 0.0111, d.f. = 179, t = 4.58, p <0.001; 12 = 0.0143, 24 = 0.0111, t = -2.561, p = 0.0127). In Hard Food, density had no significant effect on growth rate (Hard Food-density: 6 = 0.0102, 24 = 0.0107, d.f. = 179, t = 0.769, p = 0.445). Therefore in both species, increasing density forces growth rate to decline in Soft and Intermediate Food but no change is found in Hard Food.

Overall, the model coefficients show that ***Plodia*** exhibited a greater growth rate than ***Ephestia*** (sqrt(growth rate) x species: ***Plodia*** coefficient = 0.0177, ***Ephestia*** coefficient = 0.013, d.f. = 179, t = 14.17, p <0.001). Also the coefficients relating to the significant interaction between food and species reveals for ***Plodia***, increasing food viscosity failed to result in a significant decline in growth rate (Plodia-food: Soft = 0.0179, Hard = 0.0174, d.f. = 179, t = 0.479, p = 0.633). For ***Ephestia***, the growth rate in Hard Food was significantly lower compared to Soft and Intermediate Foods (***Ephestia***-food: Soft = 0.0136, Hard = 0.0108, d.f. = 179, t = 4.622, p <0.001; Intermediate = 0.0134, Hard = 0.0108, t = 3.922, p <0.001).

Growth rates in Interspecific Competition

The effect covariates had upon the growth rate of ***Plodia*** and ***Ephestia*** larvae when individuals competed in interspecific conditions is shown in Table 3.2. The coefficients from the model are discussed.

	Df	Mean Sq	F	P
food	2	0.000569	173.994	<0.001
density	2	0.000141	43.18	<0.001
species	1	0.0049	1498.04	<0.001
sex	1	0.00274	838.494	<0.001
replicate	11	0.00000515	1.573	0.102
food:density	4	0.0000188	5.734	<0.001
food:species	2	0.000106	32.415	<0.001
food:density:species	6	0.0000141	4.31	<0.001
Residuals	812	0.00000327		

Table 3.2. A mixed effects model examining the factors determining ***Plodia*** and ***Ephestia*** growth rate when experiencing interspecific competition. Growth rate was square root transformed and the random factor replicate was nested within covariates.

A significant interaction between food, density and species is apparent in the analysis and this three-way interaction is explored graphically in Figure 3.1. For ***Plodia***, the growth rate at density 24 was significantly lower than at other densities in every food type, with no difference occurring between densities 6 and 12 (***Plodia***-Soft-density: 6 coefficient = 0.01864, 24 coefficient = 0.0173, d.f. = 153, t = 2.059, p = 0.0412; Intermediate-density: 6 = 0.01852, 24 = 0.01711, t = 2.553, p = 0.012; Hard-density: 6 = 0.01797, 24 = 0.01663, t = 2.059, p = 0.0412). This ***Plodia*** food-density relationship was different to that found in intraspecific competition, where increasing density in Hard Food had no effect.

For ***Ephestia*** the growth rate at density 24 is significantly lower than other densities only in Soft and Intermediate Food (***Ephestia***-Soft-density: 6 = 0.01559, 24 = 0.01296, d.f. = 153, t = 3.05, p = 0.0033; Intermediate-density: 6 = 0.015106, 24 = 0.01108, t = 5.138, p <0.001). There was no change in growth with density in Hard

Food (Hard-density: 6 = 0.01034, 24 = 0.01054, t = 0.332, p = 0.74). This ***Ephestia*** food-density relationship was the same to that found in intraspecific competition, where increasing density reduced growth rates in the softer food types but had no effect in Hard Food.

Looking beyond the three way interaction to other aspects of the model output, the overall growth rate of ***Plodia*** remained significantly greater than that of ***Ephestia*** (sqrt(growth rate) x species: ***Plodia*** coefficient = 0.0177, ***Ephestia*** coefficient = 0.013, d.f. = 179, t = 14.17, p <0.001). In an additional piece of analysis, the growth rates of the two species were directly compared between intra- and inter-specific competition environments using a mixed model with growth rate square-rooted and replicate fitted as a random effect. For both species, there was no difference in the growth rate (***Plodia***-competition: Intra coefficient = 0.01768, Inter coefficient = 0.01769, t = -0.0852, p = 0.932; ***Ephestia***-competition: Intra = 0.013, Inter = 0.01264, t = 0.617, p = 0.5369).

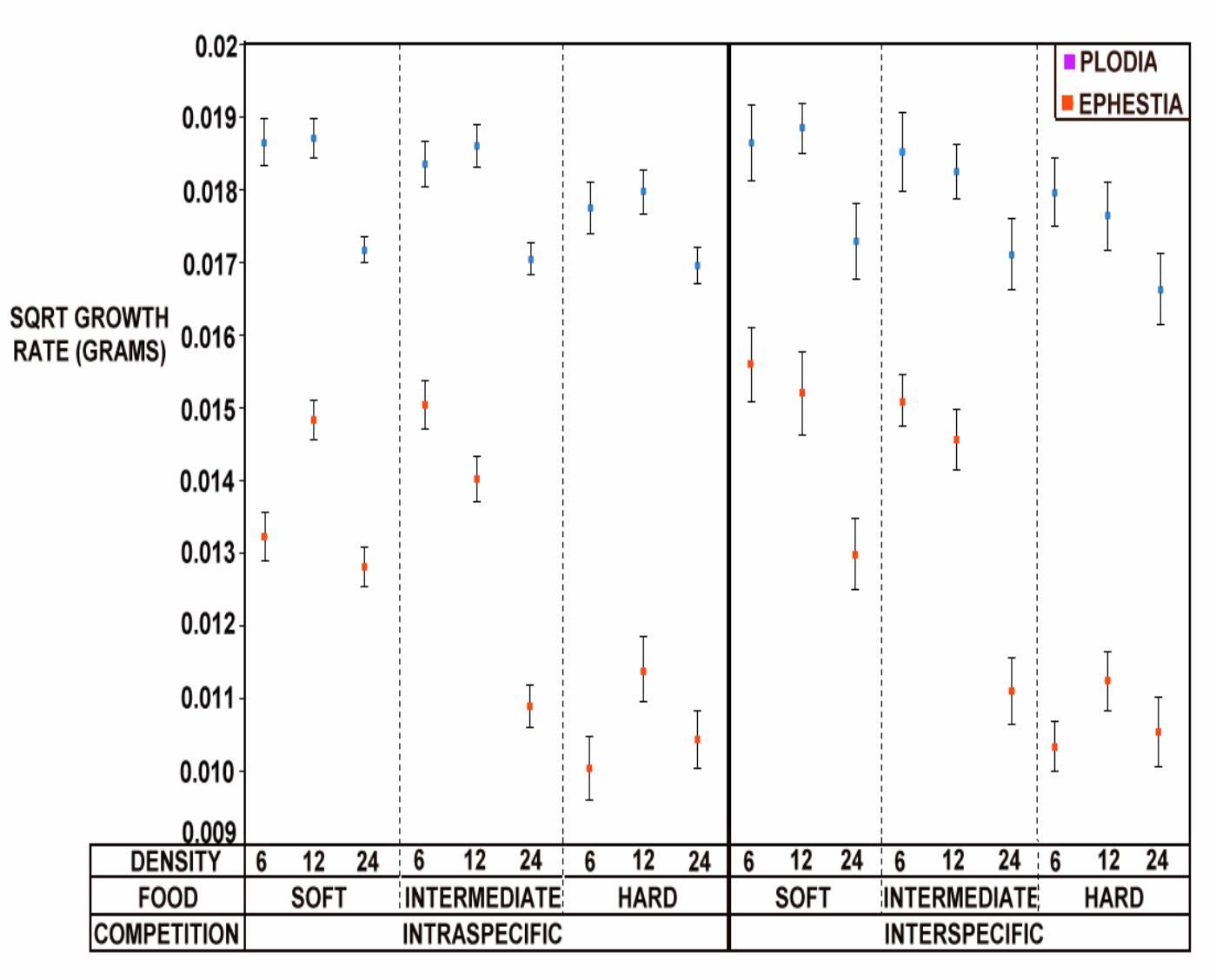

Figure 3.1 – The effect food and density has on the growth rates of *Plodia interpunctella* and *Ephestia cautella* in intraspecific and interspecific experiments.

Growth rate was considered the weight of adults divided by development time and were square root transformed to generate homogeneous variance. Mean growth rates are shown with standard errors. The blue points represent ***Plodia*** data points and the red represents ***Ephestia***.

In intraspecific competition experiments, increasing density caused the growth rate of both species to decline in Soft and Intermediate Food but there was no effect of density in Hard Food. In interspecific competition experiments, the growth rate of ***Plodia*** is larger than ***Ephestia*** in every food-density combination. For both species, increasing density caused significant growth rate declines in all food types except the insensitivity to density of ***Ephestia*** in Hard Food.

At high densities, increasing food viscosity reduces the proportion of ***Plodia*** surviving competition but increases the proportion of ***Ephestia*** surviving. This makes the difference between the two species insignificant in Intermediate and Hard Food.

The Effects of Food and Density on Survival

Survival in Intraspecific Competition

The influence covariates had upon the survival of ***Plodia*** and ***Ephestia*** larvae is shown in Table 3.3. The model logit coefficients transformed into proportions are discussed below.

	Df	Mean Sq	F	P
food	2	31.418	17.371	<0.001
density	2	47.004	25.989	<0.00
species	1	149.473	82.644	<0.00
growth rate	1	7.747	4.284	0.0398
replicate	11	1.36	0.738	0.71
density:species	2	23.264	12.863	<0.001
food:density:species	10	3.522	1.947	0.041
Residuals	197	1.809		

Table 3.3. A generalised linear model with binomial errors examining the factors determining ***Plodia*** and ***Ephestia*** mortality rate when experiencing intraspecific competition. Growth rate was square rooted.

The analysis reveals the effect of increasing density on species survival rate is dependent on food type and this three-way interaction is shown graphically in Figure 3.2. For ***Plodia***, increasing density was only associated with lower survival in Intermediate Food, with no difference occurring in Soft and Hard Food (***Plodia*** survival x Soft-density: 6 = 0.639, 24 = 0.67, t = 0.631, p = 0.529; Hard-density: 12 = 0.639, 24 = 0.441, t = 1.9, p = 0.065). In Intermediate Food, there was a significant decline in survival between density level 6 and 24 (Intermediate-density: 6 coefficient = 0.72, 24 coefficient = 0.51, t = 2.01, p = 0.0458).

For ***Ephestia***, increasing density resulted in reduced survival in Soft and Intermediate and Hard Food. In Soft Food, there was a significant decline in the proportion surviving between density levels 6 to 12 and 12 to 24 (***Ephestia*** survival x Soft-density: 6 = 0.903, 12 = 0.646, t = 2.75, p = 0.0063; 12 = 0.646, 24 = 0.326, t = 2.24, p = 0.025). In Intermediate Food, there was a significant decline in survival

between density 6 and 24 (Intermediate-density: 6 = 0.667, 24 = 0.194, t = 2.119, p = 0.0367). In Hard Food, there was a significant decline in survival between densities 6 and 24 (Hard-density: 6 = 0.333, 24 = 0.156, t = 2.717, p = 0.0072). Therefore for ***Plodia*** the only change in survival with density occurs in Intermediate Food, whereas in ***Ephestia*** density changes survival in all three food types.

Examining other features of the model reveals a significant difference in the overall survival of ***Plodia*** and ***Ephestia***, with ***Ephestia*** being characterised by a generally lower survival rate when experiencing intraspecific competition than ***Plodia*** (survival x species: ***Plodia*** coefficient = 0.599, ***Ephestia*** coefficient = 0.451, t = 3.091, p = 0.0023). Also food type had a significant effect on the survival of ***Plodia*** and ***Ephestia*** larvae, with survival greater in Soft Food than in Intermediate and Hard Foods (Soft = 0.643, Intermediate = 0.516, t = 2.85, p = 0.0048: Soft = 0.643, Hard = 0.397, t = 3.9591, p <0.001).

Survival in Interspecific Competition

The effect covariates had upon the survival of ***Plodia*** and ***Ephestia*** larvae when individuals competed in interspecific conditions is shown in Table. 3.4 and the coefficient outputs are discussed below, transformed from logits into proportions.

	Df	Mean Sq	F	P
food	2	1.339	1.118	0.329
density	2	47.849	39.935	<0.001
species	1	67.289	56.16	<0.001
growth rate	1	0.749	0.625	0.43
replicate	11	1.038	0.869	0.572
density:species	2	4.335	3.619	0.0286
food:species	2	29.523	24.64	<0.001
Residuals	205	1.198		

Table 3.4. A generalised linear model with binomial errors examining the factors determining ***Plodia*** and ***Ephestia*** survival when experiencing interspecific competition. Replicate was nested within the other covariates.

The significant interaction between food type and species is displayed in Figure 3.2. The proportions of ***Plodia*** and ***Ephestia*** surviving competition in Soft Food were significantly different compared to the survival in Intermediate and Hard Foods. For ***Plodia***, the proportion surviving competition in Soft Food was significantly larger than in the more viscous food types (***Plodia*** survival x food: Soft coefficient = 0.829, Intermediate = 0.715, t = 2.225, p = 0.0274; Soft = 0.829, Hard = 0.639, t = 2.9945, p = 0.0036).

For ***Ephestia***, the reverse was true, as the proportion surviving in Soft Food was significantly smaller than in more viscous foods (***Ephestia*** survival x food: Soft = 0.472, Intermediate = 0.625, t = -4.584, p<0.001; Soft = 0.472, Hard = 0.602, t = -3.917, p<0.001). Therefore the survival rate of ***Plodia*** is negatively affected by increasing food viscosity whereas ***Ephestia*** is positively affected by increasing food viscosity. This results in a convergence in the proportion of ***Plodia*** and ***Ephestia***

surviving interspecific competition, with the numerical difference between the species decreasing with food viscosity.

In a piece of additional analysis similar to that carried out for growth rates, the survival of the two species was compared between intra- and inter-specific competition environments using a generalised linear model with binomial errors. For ***Plodia***, there was no significant difference between the two environments (***Plodia***-competition: Intra = -0.599, Inter = 0.682, t = 1.438, p = 0.152). However, there was a significant difference for ***Ephestia***, as the survival in interspecific competition is greater than intraspecific (***Ephestia***-competition: Intra = 0.451, Inter = 0.566, t = -5.88, p <0.001). Further examination of the coefficients shows this difference for ***Ephestia*** is dependent on food type, with intraspecific survival greater than interspecific in Soft Food (***Ephestia*** in Soft Food: Intra = 0.625, Inter = 0.472, t = 4.42, p <0.001). The opposite is true in Intermediate and Hard Food, where survival is greater in interspecific competition (Intermediate Food: Intra = 0.456, Inter = 0.625, t = -4.02, p <0.001; Hard Food: Intra = 0.272, Inter = 0.602, t = -8.221, p <0.001).

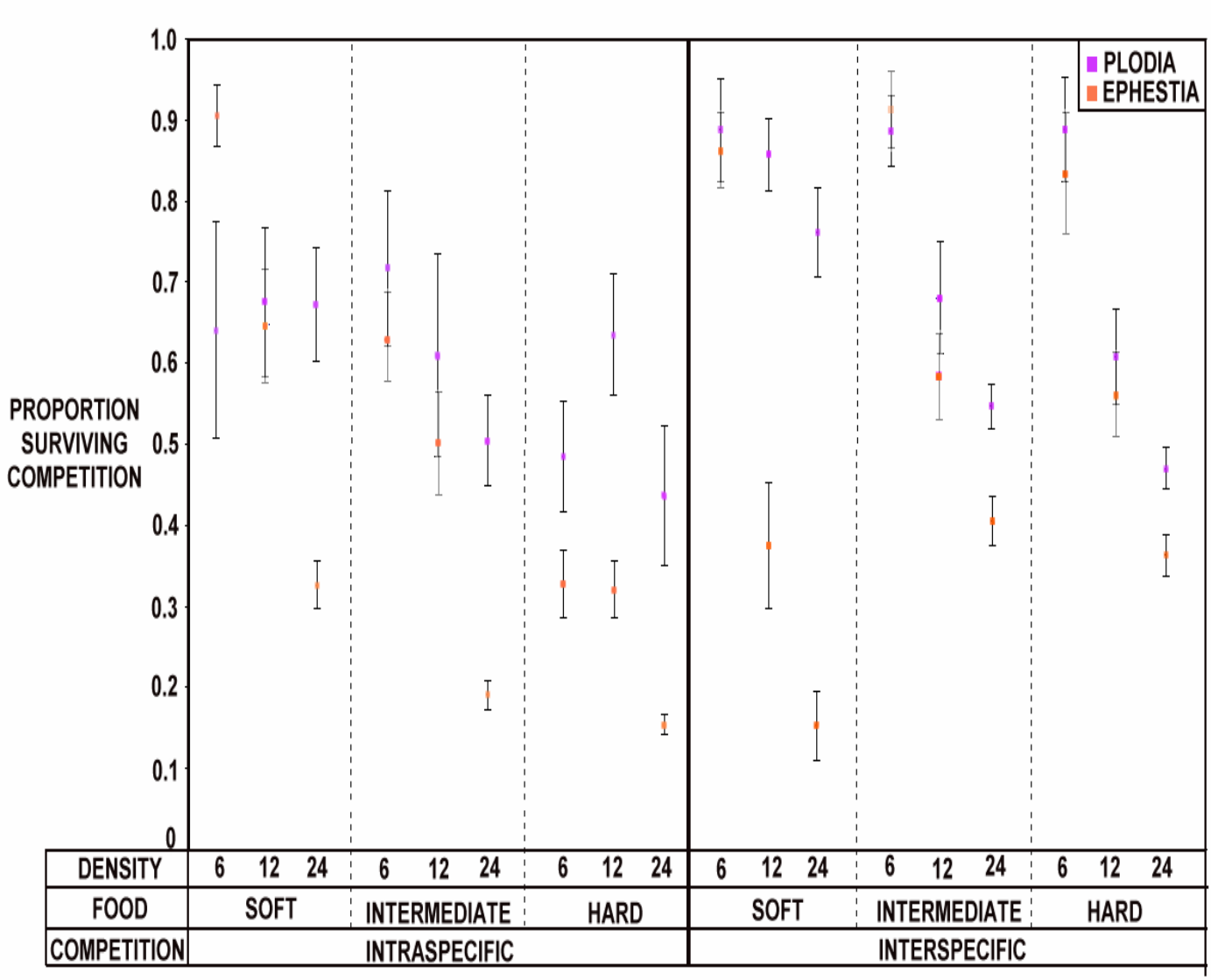

Figure 3.2 – The effect food and density has on the survival of *Plodia interpunctella* and *Ephestia cautella* in intraspecific and interspecific experiments.

Mean survival rates are shown with standard errors. The blue points represent ***Plodia*** data points and the red represents ***Ephestia***.

In intraspecific competition experiments involving ***Plodia***, increasing density only affected survival in Intermediate Food. For ***Ephestia***, density reduced survival in all food types.
In interspecific competition experiments, At high densities, increasing food viscosity from Soft to Hard reduces the proportion of ***Plodia*** surviving competition but increases the proportion of ***Ephestia*** surviving. This renders the difference between the two species found in Soft Food insignificant in Intermediate and Hard Food.

At high densities, increasing food viscosity reduces the proportion of ***Plodia*** surviving competition but increases the proportion of ***Ephestia*** surviving. This makes the difference between the two species insignificant in Intermediate and Hard Food.

Discussion

In this chapter, I studied the effects of restricted movement on intraspecific and interspecific competition in two lepidopteran species ***Plodia interpunctella*** and ***Ephestia cautella***. Growth rate and survival were used as measures for competition intensity, with larval resource and interference competition associated with decreased growth rates and increased mortality (Mbata, 1990; Podoler 1974). The intensity of competition of ***Plodia*** and ***Ephestia*** was studied at three different density levels and three food viscosity levels. The density levels were low (6 individuals), medium (12) and high (24) and the viscosity levels were Soft (0ml water), Intermediate (60ml) and Hard (140ml).

Intraspecific competition

For ***Plodia*** and ***Ephestia***, the survival and growth rate of larvae were greater in Soft Food than in more viscous food types. In Hard Food, the growth rate of larvae was lower than in Soft Food, although the decrease in ***Plodia*** was not significant (Figure 3.1). Also for both species the proportion surviving competition was significantly greater in Soft Food than in Intermediate and Hard Food (Figure 3.2). These changes in competition characteristics indicate the intensity of competition increased as the movement rate of individuals was restricted. A potential explanation for this is that high movement rates allow larvae to move away from competitive encounters with nearby conspecifics, and move into competition-free where they can feed without interference. Restricting movement, using more viscous foods, prevents larvae from moving away from competitive encounters and the reduced access to food and space causes lower growth rates and greater mortality.

The lack of sensitivity of ***Plodia*** growth rate to food viscosity, relative to ***Ephestia***, is perhaps counter intuitive considering the great effect food viscosity has on ***Plodia*** movement rate (Figure 2.4). However it might be explained by considering the fast growth rate of ***Plodia***, which results in individuals spending a shorter time in the larval state than ***Ephestia***. This reduces the degree of food and interference competition experienced.

The effect of increasing density in the three food types also indicates restricting larval movement causes the intensity of intraspecific competition to increase. ***Plodia*** and ***Ephestia*** growth rates significantly decline with density in Soft and Intermediate Food but there is no effect of increasing density in the Hard Food (Figure 3.1). Lets' first consider the reasons for declining growth rate within foods that permit relatively high movement. At low density, when there is ample food and space, such high rates of movement mean individuals have the ability to escape competitive encounters and disperse into competition-free environments. Through this 'race for space' process, individuals at low density can spread themselves evenly across the environment and minimise the detrimental consequences of competition. Therefore individuals have a rapid growth rate. At high density in Soft and Intermediate Food, the amount of food and space available for individuals diminishes. High movement rates no longer enable individuals to avoid encounters with conspecifics because there is little competition-free space available. The resulting intense resource and interference competition causes growth rates to decline.

In Hard Food, where movement rates are restricted, density does not result in any significant change in the growth rates of either species (Figure 3.1) indicating that increasing density does not change the intensity of intraspecific competition. This can be explained by considering competition at low densities. Despite the ample food and

space available low density, low movement means larvae are incapable of escaping competitive encounters and dispersing into the surrounding competition-free environment. Therefore increasing density has no further effect on the level of competition each individual faces and so growth rate remains the same.

The relationship between ***Ephestia*** survival and increasing density is the same as ***Ephestia*** growth rate, since density was only found to reduce survival in Soft and Intermediate Foods (Figure 3.2). This matches the explanation of the growth rate patterns, where restricted movement prevent individuals from avoiding competition and freely utilising the food resources available at low densities. The intense competition that results causes high levels of mortality that does not proportionately change at higher densities. However ***Plodia*** survival has a different pattern, with density only affecting survival in Intermediate Food. As previously discussed, increasing density in Soft Food did result in reduced ***Plodia*** growth rates and therefore more intense competition. However the very high movement rates of ***Plodia*** in Soft Food allow individuals to disperse rapidly through the environment so competitive encounters, though frequent, are short and so not associated with death. This means that mortality does not proportionately increase with density. In Intermediate Food, the moderately restricted movement still allows larvae to avoid competition at low densities but is not enough to permit individuals to successfully avoid long competitive encounters at high densities. This leads to a decline in the proportion that survive with density.

Coexistence between Plodia and Ephestia

The coexistence experiment examined larval survival and growth rates associated with interspecific competition between ***Plodia interpunctella*** and ***Ephestia cautella*** in different food types. Figure 3.2 shows the proportion of surviving ***Plodia*** and ***Ephestia*** in Soft, Intermediate and Hard Food at low (6), medium (12) and high (24) densities.

At low densities, there is no difference between the proportion of ***Plodia*** and ***Ephestia*** that survive interspecific competition. This is a result of the ample food and space available for individuals, causing the intensity of detrimental competition to be low. This pattern of coexistence breaks down at medium and high densities in Soft Food, with the number of surviving ***Plodia*** significantly greater than ***Ephestia***. The numerical difference is cancelled out by increasing food viscosity, with the proportion of ***Ephestia*** surviving increasing and the proportion of ***Plodia*** decreasing in Intermediate and Hard Food. In these viscous food types, there is no significant difference in the proportion of ***Plodia*** and ***Ephestia*** that survive interspecific competition.

In general this pattern can be explained by considering the growth and dispersal rates of the two species in different food types. In Soft Food, ***Plodia*** possess a greater dispersal rate (Figure 2.4) and growth rate (Figure 3.1) than ***Ephestia***. Being faster means ***Plodia*** are better able to escape intraspecific competitive encounters and find competition-free space. The higher movement rates allow a more uniform distribution of individuals to emerge, where interspecific encounters are likely to occur at a similar frequency to intraspecific encounters. Also the greater growth rate means ***Plodia*** larvae will be physically larger than ***Ephestia*** throughout development and so dominate any interspecific encounters. This asymmetric competition can result in

intense competition on ***Ephestia*** larvae through the disruption of their feeding and their predation by ***Plodia***. ***Ephestia***, with a slow movement rate, are unable to escape both intraspecific and deleterious interspecific encounters and so experience intense competition resulting in mortality.

In contrast to Soft Food, there is no significant difference between ***Plodia*** and ***Ephestia*** survival at higher densities in Intermediate and Hard Food (Figure 3.2). This is because increasing food viscosity reduces the movement rate of ***Plodia*** larvae, and so the species no longer has a significant competitive advantage over ***Ephestia***. As dispersal is limited, individuals are unable to escape competitive encounters and are forced into aggregations. This aggregated spatial structure results in ***Plodia*** suffering high rates of intraspecific encounters. The resulting decline in the intensity of interspecific competition allows ***Ephestia*** larvae increased access to food and reduces the degree of predation and interference from ***Plodia***. This work provides cautious support to theoretical models that suggest aggregation alone can promote coexistence (Higgins & Cain, 2002; Murrell & Law, 2003). However the experiment was only conducted over one larval generation, and therefore questions over long term coexistence remain to be asked.

Further analysis directly comparing the survival and growth rates of the species in intra- and inter-specific competition environments adds system specific detail to this general description of competition between ***Plodia*** and ***Ephestia***. Analysis of the proportion of ***Plodia*** surviving competition in different food types revealed there was not a significant difference between the competition environments. However for ***Ephestia***, significantly fewer individuals survived in interspecific competition in Soft Food compared to intraspecific competition, but this situation was reversed in more viscous food types. The proportion of ***Ephestia*** surviving interspecific competition

was larger in Intermediate and Hard Foods than in intraspecific competition. This shows the removal of the competitive superiority of ***Plodia*** in more viscous food is a result of more ***Ephestia*** surviving competition, rather than a decline in ***Plodia*** survival.

Regarding the growth rates of the two species competing together, the growth rate of ***Plodia*** was larger than ***Ephestia*** in every combination of food and density (Figure 3.1). Also the direct comparison between the two competition environments showed there was no significant change in the values of growth rate associated with each food type, and this is true for both species. Therefore there is no relationship between growth rate and survival in interspecific competition, as the greater survival of ***Ephestia*** in viscous foods is not matched by any increase in growth rate compared to either that of ***Plodia*** or the growth rate of ***Ephestia*** in intraspecific competition.

This difference between the survival and growth rate of ***Plodia*** and ***Ephestia*** indicates the possible mechanics of interspecific coexistence in more viscous foods over space and time. Chapter 2 Figure 2.4 shows increasing food viscosity restricts ***Plodia*** movement to a much greater degree than ***Ephestia***. The shorter dispersal of ***Plodia*** in Intermediate and Hard Foods results in individuals unable to avoid intraspecific encounters. These intraspecific encounters are intense because of the rapid development of body size asymmetries resulting from the large ***Plodia*** growth rate. These body size asymmetries cause those larvae with the most rapid growth rate to out compete and predate the smaller ***Plodia*** larvae they are locked into competition with. This explanation may account for the discrepancy in ***Plodia*** between the low survival and high growth rates found in Hard Food, with the few individuals that survive being those with the highest growth rates. At the same time, ***Ephestia*** also experience intraspecific competition but it is less intense due to the smaller

requirement for food associated with their lower growth rate. Also the intense intraspecific competition and mortality experienced by ***Plodia*** provides a window of growth for ***Ephestia***, where they can feed without experiencing the predation and competition associated with encounters with ***Plodia***. This window allows ***Ephestia*** to grow to a larger size than would be expected in Soft Food (where ***Plodia*** experience less intraspecific competition), and so are better equipped to survive competitive encounters with ***Plodia*** at later stages of competition. Therefore ***Ephestia*** survival is greater in more viscous foods because of a combination of the reduced number of ***Plodia*** encounters early in competition and the resulting larger ***Ephestia*** larvae being less vulnerable to predation at later stages of competition.

Previous empirical studies of spatial heterogeneity and competition

There are many mathematical models investigating competition within aggregated communities. However there is a significant lack of empirical data examining the implication of spatial structure on intra- and inter-specific competition (Rejmánek, 2002; Murrell ***et al.***, 2001; Stoll & Prati, 2001). Stoll & Prati (2001) tested the coexistence of four plant species when seeds were either sown randomly or in intraspecific clumps. Aggregation of species had the effect of reducing the biomass of the superior competitor and improving the performance of the other species. This result became clearer at higher densities, with increasing intraspecific competition benefiting inferior competitors. Another experimental study of plant aggregation found the competitive effect of barnyard grass on tomato yield was reduced and sometimes rendered negligible by clumped grass distributions (Norris ***et al.***, 2001). In contrast to regular and random distributions, aggregations of barnyard grass increased

the proportion of tomato plants evading interspecific competition, as well as increasing the effect of intraspecific competition upon barnyard grass. Such plant experiments have shown the more aggregated the population of competitors is, the more intraspecific interactions become dominant (Rejmánek ***et al***., 1992).

A rare study of animal competition was carried out to test the results of the Atkinson & Shorrocks (1981) model of aggregation through clutch laying. By using two species of ***Drosophila***, the inferior competitor could persist when egg laying was artificially restricted in space. The resulting aggregations may have promoted coexistence by increasing intraspecific competition and creating refuges that allowed the inferior competitor to persist despite local interspecific competition (Shorrocks, 1991). All these studies suggest that aggregated distributions result in increased intraspecific competition and reduced interspecific competition. However there are few studies that collect data for a long enough period of time to be sure that population aggregation is sufficient to produce long term species coexistence.

Chapter 4

Spatial Heterogeneity and the Dynamics of the Plodia interpunctella – Granulosis Virus system

Abstract

Populations of ***Plodia interpunctella*** were maintained in the presence or absence of the granulosis virus within three food viscosity types. In the no virus populations, competition between larvae determined the size and dynamics of populations. Adult females lay eggs in batches, causing populations to be initially aggregated. The high movement rates in Soft Food allow these aggregations to dissipate and so populations become evenly distributed. The resulting low larval mortality and high growth rates mean that Soft Food populations were characterised by large population sizes and short, regular 37.47-day cycles. The low movement rates in Hard Food prevent larvae moving away from their oviposition site and so populations remain aggregated. The intense competition within such aggregations causes high larval mortality and low growth rates. Therefore Hard Food populations were characterised by small population sizes and extended, 41.96-day cycles.

Restricting larval movement rates also had a significant impact upon the dynamics of the ***Plodia***-granulosis virus interaction. In Soft Food populations, the uniform distribution of larvae led to a high viral infection rate, resulting in lower larval growth rates and a correspondingly extended cycle periodicity, due to either sublethal infection or as a cost of evolved resistance. In contrast, the high contact rate within Hard Food population causes high rates of localised infection within larval aggregations, inducing great larval mortality and so preventing the asymmetric larval interactions necessary for the maintenance of regular population cycling.

Introduction

The spatial relationships of natural enemy interactions have recently become the subject of a great deal of study. Much of the work has been theoretical and indicates the characteristics of many natural enemy systems cannot always be understood by considering populations to be spatially homogeneous (Tilman & Kareiva, 1997). Instead, it is the fine scale relationships within and between clumps of individuals that can be important. Spatially orientated approaches have been used to examine the persistence, dynamics and evolution of a variety of host-parasite interactions (e.g. Cliff ***et al***., 1981; Dwyer, 1992; Hassell ***et al***., 1991; Murray ***et al***., 1986). The empirical work in spatial heterogeneous systems has been restricted by a lack of general theory and the awkwardness of measuring the distribution and movement of individuals (Doncaster ***et al***., 1997; Harrison, 1989; Turchin, 1998). However the work that has been carried out supports many of the theoretical conclusions made and broadly falls into one of two categories: 1) parasite spatial heterogeneity creating spatial refuges for hosts and 2) host heterogeneity creating reservoirs for parasites.

Patchily distributed populations can change natural enemy interactions by generating new density dependent relationships (McCauley ***et al***., 2000). One arrangement where this change in density-dependence occurs is through the heterogeneous aggregation of a host population. Large and persistent host subpopulations act to maintain parasite presence despite extinction in smaller host subpopulations (Keeling, 2000a; Schrag & Mittler, 1996). Such spatial reservoirs have been observed in metapopulation structures of plant specific fungal parasites (Burdon & Jarosz, 1991; Burdon ***et al***., 1995; Ericson ***et al***., 1999; Thrall & Antonovics, 1995). The parasite is able to become endemic in large plant subpopulations and movement from these reservoirs sustain the parasite within

smaller subpopulations vulnerable to extinction (Antonovics ***et al***., 1994; Thrall & Antonovics, 1995). A parasite reservoir system has also been shown to be important for the dynamics of human measles (Bolker & Grenfell, 1995a, b). Towns and cities with high population densities support measles endemicity, and from these reservoirs the pathogen disperses to surrounding small villages and towns. The populations of small towns are too low to maintain measles themselves but the reservoir allows localised, short-lived epidemics to occur (Grenfell & Bolker, 1998).

Spatial heterogeneity in the distribution of the pathogen can also modify the existing density-dependence in natural enemy systems. An area of the environment can act as a refuge if a pathogen or predator population cannot invade or persist. (Abrams & Walters, 1996; Hassell & May, 1973; Hochberg & Hawkins, 1992). Refuges may have important consequences for a host-parasite interaction, with the refuge potentially acting to stabilise the dynamics (Hochberg, 1989; Hochberg ***et al***., 1990; McNair, 1986, 1987). In a refuge either a fixed number or proportion of the host cannot be infected, stabilising the interaction in two ways. First, the parasite is prevented from driving the host extinct because not all the host population is vulnerable. Second, movement out of the refuge provides new hosts for infection, so preventing parasite extinction (Lynch ***et al***., 1998).

Although the empirical support for the importance of spatial refuges is weak, the relationship between spatial refuges and host-parasite interactions has been examined across a range of species interactions. The parasitoid ***Venturia canescens*** attacks ***Plodia interpunctella*** hosts by sitting on the surface of the food medium, inserting its ovipositor and laying eggs in larvae (Harvey ***et al***., 1994; May & Hassell, 1988). If the food medium is over 0.5cm in depth, there is a region in which larvae cannot be parasitised. Without this refuge, the host is driven extinct by the parasitoid,

which then suffers the same fate (Begon ***et al***., 1995). With the refuge, the individual larvae that spend part of their life in the deeper food show a lower probability of becoming parasitised (Murdoch ***et al***., 1989) and the lowered parasitoid attack rate allows the system to persist indefinitely (Begon ***et al***., 1995). Bacteria-phage interactions have also been shown to be stabilised by host refuges (Schrag & Mittler, 1996). Bacteria that were allowed to grow near the side of the container were invulnerable to infection by two different phage types. This refuge allows both phage types to persist much longer compared to spatially homogeneous bacteria populations.

Spatial refuges do not need to be a fixed part of the environment in order to change victim-enemy interactions. Instead, refuges can be temporally dynamical, changing in position and size according to differential dispersal by enemies and victims. An example of this is found in laboratory herbivorous mite-predatory mite interactions, where the spatial structure of populations is dependent upon the arrangement of plants (Janssen & Sabelis, 1992; Janssen ***et al***., 1997; McCauley ***et al***., 2000; Zemek & Nachman, 1998). On individual plants, the prey species is rapidly driven extinct by the predator, which is followed by predator extinction (Pels & Sabelis, 1999). The same instability was found in multi-plant populations when dispersal for both species was unrestricted. Persistence is only observed when plants are subdivided into a group of interconnected patches. This arrangement alters the movement rates so prey species disperse locally and predators disperse globally, reducing the rate at which prey outbreaks are discovered by predators (McCauley ***et al***., 2000). The temporal lag between prey growth and predator discovery allows some prey subpopulations to increase and, before being rendered extinct by predators, repopulate nearby plants (Ellner ***et al***., 2001).

Within this context of limited empirical work, we examine the dynamics of a ***Plodia interpunctella***-granulosis virus (PiGV) host-pathogen interaction within different spatial environments. I attempt to generate endogenous spatial structure within ***Plodia*** populations by changing the dispersal ability of individual larvae. Populations were fed on food media of differing viscosity, which alters movement rates but not the ability of larvae to gain nutrients by feeding. Should different movement rates generate different spatial structures, we may be able to observe resulting changes in competition and infection processes in the population dynamics. This is a rather rare experimental approach as it is the individuals themselves that create the spatial structure. A strictly defined spatial structure is not forced upon populations, as is the case in laboratory based patchy- and meta-populations.

The dynamics of the insect species ***Plodia interpunctella*** have been the centre of much theoretical and experimental work (Begon ***et al***., 1996; Bjørnstad ***et al***., 1998; Bjørnstad ***et al***., 2001; Briggs ***et al***., 2000; Gurney & Nisbet, 1985; Gurney ***et al***., 1983; Jones ***et al***., 1990; Sait ***et al***, 1994b). They can be characterised by a cycle length that is a little longer than the generation time (Begon ***et al***., 1996; Sait ***et al***., 1994b). There are three significant density-dependent lags generating the population dynamics. A negative lag at week 2 dominates the dynamics and is caused by strong asymmetric competition between large and small instars, including the cannibalism of eggs (Gurney & Nisbet, 1985; Bjørnstad ***et al***., 1998). There is a less important negative lag at week 1 representing symmetric competition within early instars, and a positive lag at week 5-6 for the eggs laid by adults in the preceding generation (Briggs ***et al***., 2000). The addition of the granulosis virus of ***Plodia interpunctella*** (PiGV) causes a reduction in the ***Plodia*** population size due to pathogen-induced mortality and lower fecundity. Infection occurs primarily through the cannibalism of

infected larvae, so cadavers can be considered to be the primary infectious unit of infection (Boots, 1998). PiGV also extends the period of the host cycle, as development time increases due to either sublethal infection or as a trade-off with increased host resistance (Boots & Begon, 1993; Sait ***et al***., 1994a, b). However the strong competition between larvae prevents the host-pathogen interaction from becoming coupled. Therefore an increase in the density of infectious units in one generation does not have a correspondingly negative impact on the host population size in the next generation (Bjørnstad ***et al***., 2001). The virus only acts to change the existing density-dependence caused by competition.

Through rendering the food medium more viscous, the movement of ***Plodia*** larvae becomes increasingly restricted. Female adults lay their eggs in batches and if movement is restricted then individuals will be unable to move away from nearby kin. Therefore individuals may be forced into aggregations and the more movement is restricted, the more clumped the population distribution becomes. This aggregation process may have a number of significant effects on the dynamics of an insect-pathogen system, where the density of hosts (Carter ***et al***., 1983; Fleming ***et al***., 1986; Woods & Elkinton, 1987) and infectious units (Crawford & Kalmakoff, 1977; Entwistle ***et al***., 1983; Fuxa & Geaghan, 1983) determines the invasion and spread of disease. Within aggregations, the density of individuals will be high, so increasing both the contact rates between individuals and the competition between larvae for food and space. The aggregation of individuals has been shown to be associated with high levels of competition (Charnov ***et al***., 1976) and an increased vulnerability to infection (Dobson, 1988; Hochberg, 1991). Indeed, such aggregations may be crucial for the persistence of a pathogen (Hassell, 1978; Jones & Hassell, 1988; Tinsley & Jackson, 1986).

It is therefore likely that making ***Plodia*** populations more aggregated will change competition between larvae and the infection of susceptible larvae with PiGV. In terms of infection, the contact structure within aggregated populations may alter both the chances of susceptible hosts encountering infected cadavers (Dwyer, 1991) and that cannibalism will occur when this contact is made (Knell ***et al***., 1998b). Enforced aggregation may also increase the intensity of larval competition, with competition for food and space potentially resulting in a higher mortality rate and changes in aspects of ***Plodia*** life history, such as development time and reproductive rates (e.g. Mbata, 1990; Podoler, 1973). Increased rates of head-to-head larval contact within aggregations can also affect larval behaviour, due to the secretion of mandibular substance associated with agitation and dispersal (Anderson & Löfqvist, 1996; Corbet, 1971).

Therefore it should be possible to study the effect restricted movement has on the intensity of competition and the rate of infection by examining the population dynamics of ***Plodia*** populations in the presence and absence of PiGV. The cyclical behaviour of populations will be measured using autocorrelation function and spectral analysis. The density of populations will be determined using three measures; the mean number of adults counted each week, the mean density within population cycle and the mean amplitude of cycles.

Method

Populations of ***Plodia interpunctella*** were established using three different types of food media. The process by which food is made and the effect it has on movement rates is discussed in Chapter 2. Media with no water is called Soft Food, media with

60ml water is Intermediate Food and media with 140ml water is Hard Food. Increasing the amount of water added to the food medium increases the media's viscosity and reduces the movement rate of larvae (Chapter 2). Preliminary experiments show the average movement of ***Plodia*** larvae is 10.63cm (SE ±0.73) in Soft Food, 5.08cm (SE ±0.61) in Intermediate Food and is 2.8cm (SE ±0.26) in Hard Food (Figure 2.4). Changes in food viscosity had no effect on ***Plodia*** development time and pupal weight (Figure 2.7).

The ***Plodia*** used came from a strain donated by The University of Liverpool. Each population was started with 170g of food placed along the base of 20x20cm plastic containers. This food was divided into six sections. The ***Plodia*** populations were initiated using 15 fifth instar males and 15 fifth instar females, distinguishable through the visible male testes. For the with-virus populations, 12 late instar granulosis virus infected cadavers were placed on the food, 2 cadavers on each of the six sections. The lid of each container was perforated to aerate populations and net curtain sleeves were made to prevent adult moths from escaping whilst population counts were being made. Once started, populations were placed in incubators set at 27±2ºC and 35±5% humidity and maintained for 40 weeks.

9 ***Plodia*** populations were set without the granulosis virus (PiGV), three of each food type. 15 populations were set up with the granulosis virus, five of each food type. This unbalanced design was chosen in order to obtain more replicates with the virus, in the expectation a broader range of dynamics would be displayed. Every week, one section of food was removed and replaced so any particular section of food was replaced every six weeks. At the same time, all the dead adults were removed from each population and counted. As adults usually live no longer than a week, the dead adult count tells us the size of the population from the previous week. For the

with virus populations, the removed section of food was searched for PiGV infected larvae by breaking the food up by hand and carefully examining the food for larvae displaying a white colouration. This included recently infected larvae that exhibited a blotchy white epidermis. Those found were counted and placed back into the container on the fresh section of food, in order to maintain the host-pathogen interaction. First instar infected larvae were too small to be found or handled, so the larvae placed back into populations were aged second instar and above. The number of infected larvae within removed food sections gave an indication of the overall level of infection within populations.

The time series analysis was carried out in S-Plus. The autocorrelation function (ACF) was used to determine whether density dependent cyclical behaviour occurred and to give an indication of the length of cycles. ACF works by calculating the correlation coefficient between population values, describing how similar the population at one week is to the population at other weeks (Crawley, 2002). Spectral analysis was also used to provide a more subtle measure of cycle length. This method picks out the frequencies by which different cycle lengths appear in the dynamics, and the smoothed periodogram for each population is presented in the Results section (Turchin, 2003). The mean population size was considered the number of dead adult moths counted and removed from each population once a week. Amplitude was the difference between the number of moths counted at the lowest and highest points of each cycle. Cycle density was the total number of moths counted during each cycle. The effect of food type and infection on population dynamics was analysed by putting each population summary into a two factor general linear model and examining the coefficients output made using the treatment contrasts function of S-Plus. Regression

analysis of both the ***Plodia*** and PiGV populations revealed no significant trends that required detrending prior to time series analysis.

Results

No Virus Plodia Populations

In total, nine ***Plodia interpunctella*** populations were set up in the absence of the granulosis virus (PiGV) (see Table 4.1). Figure 4.1 shows the dynamics of ***Plodia*** in three Soft Food populations, a diet in which no water was added. Autocorrelation function (ACF) analysis shows there was evidence for cyclical behaviour of approximately 5-weeks and the spectral analysis indicated the mean length of these cycles was 37.47 days (standard deviation ±0.48). The mean adult population density was 148.81 (±10.46) per week, the mean number of adults within each cycle was 851.77 (±33.11) and the mean cycle amplitude was 380.52 (±26.6).

Figure 4.2 shows the dynamics of ***Plodia*** fed upon food with Intermediate viscosity, a mixture made with 60ml water. Again ACF analysis provides evidence for regular cyclical behaviour and the mean cycle length indicated by spectral analysis was 37.3 days (±0.09), which was not significantly different to Soft Food populations (Cycle Length coefficients; $t_{5,18}$ =-0.1586, p =0.876). In Intermediate Food, both the mean population density of 127.57 (±11.08) and the mean amplitude of 407.04 (±63.75) were not significantly different to Soft Food values (Population Density; $t_{5,18}$ =-2.082, p=0.052; Amplitude; t =-0.64, p =0.53). In contrast the mean cycle density of 721.33 (±62) was significantly lower than Soft Food populations (Cycle Density, t=-2.13, p=0.047).

Figure 4.3 shows the dynamics of ***Plodia*** populations fed upon Hard Food, a diet mixed with 140ml water. ACF indicated regular cycling of around 6 weeks and spectral analysis showed the mean cycle length to be 41.96 days (±0.79), significantly longer than both Soft and Intermediate populations (Cycle Length coefficients; Soft, $t_{5,18}$ = 4.03, p <0.001; Intermediate, t=4.19, p <0.001). The mean population density of 98.7 (±2.71) was significantly lower than both Soft and Intermediate populations (Population Density; Soft, $t_{5,18}$ =-4.91, p <0.001; Intermediate, t=-2.83, p=0.011). The mean cycle density of 621.37 (±42.26) was significantly lower than the value in Soft Food (Cycle Density; Soft $t_{5,18}$ =-3.77, p =0.0014) and the mean amplitude of 302.05 (±29.67) was significantly lower compared to Intermediate Food (Amplitude; Intermediate, $t_{5,18}$ =-2.53, p =0.021).

Food Type	Presence of Virus	Autocorrelation Function (weeks)	Spectral Analysis (days)	Population Density (mean ±sd)	Amplitude (mean ±sd)	Cycle Density (mean ±sd)
soft A	no virus	5	37.1	154.7 ±156.8	355 ±101.1	873.9 ±283.7
soft B	no virus	5	38	136.7 ±160.7	408 ±145	813.7 ±301.2
soft C	no virus	5	37.3	154.9 ±153.6	378.6 ±98.6	867.7 ±291.4
intermediate A	no virus	5	37.2	120.7 ±144.6	337.6 ±146.6	679.7 ±254.8
intermediate B	no virus	5	37.3	140.3 ±193	462.9 ±185	792.6 ±335
intermediate C	no virus	5	37.4	121.7 ±165.5	420.7 ±114.8	691.7 ±261.1
hard A	no virus	5	40.5	101.3 ±147.7	335.9 ±179.8	574.1 ±239.8
hard B	no virus	6	43.2	98.9 ±114.3	280.3 ±103.6	655.5 ±189.6
hard C	no virus	6	42.2	95.9 ±115.5	290 ±125.1	634.5 ±182.8

Table 4.1

A summary of the dynamics of ***Plodia*** populations fed on three food types in the absence of granulosis virus.

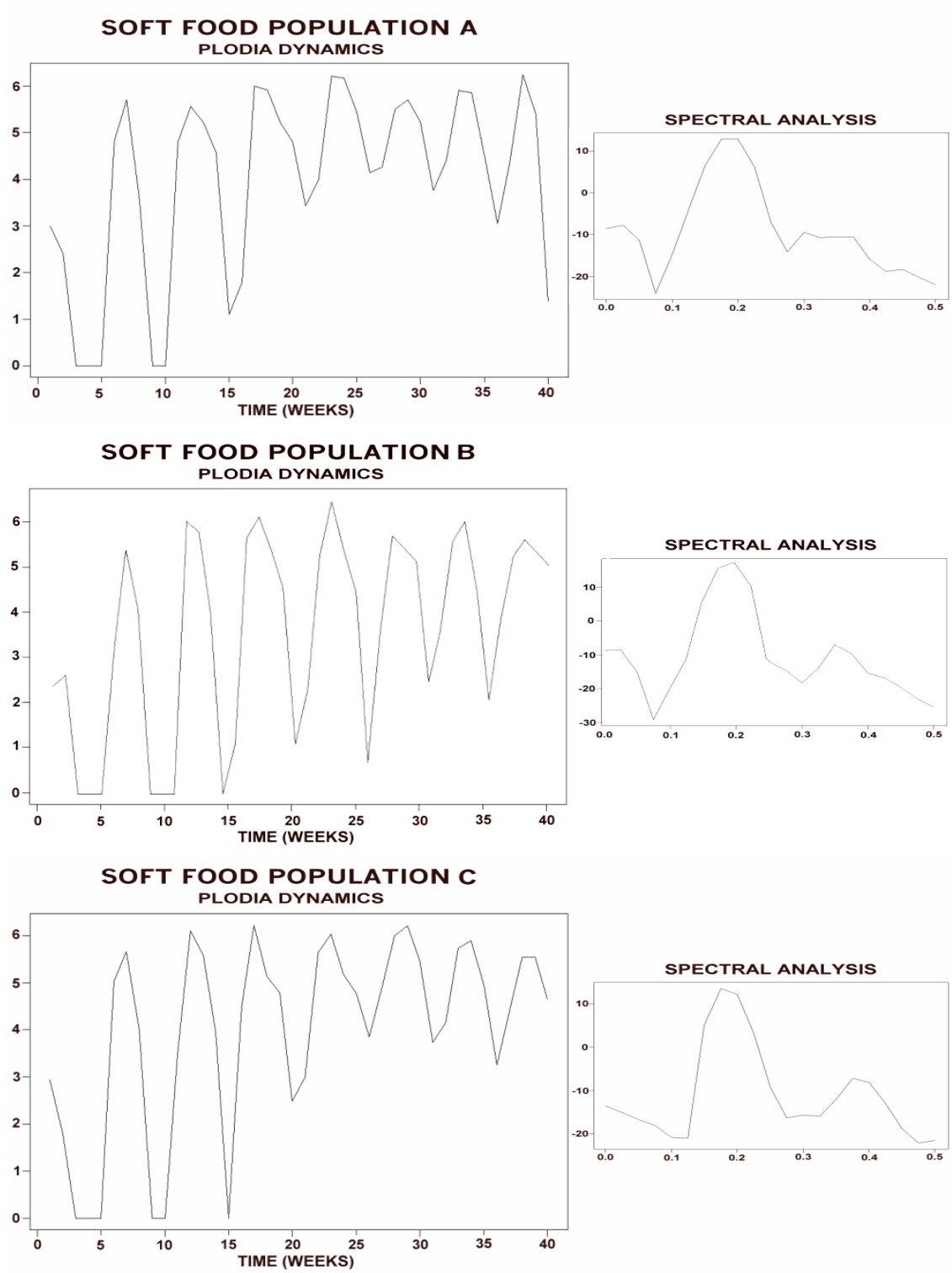

Figure 4.1 - Soft Food, no virus populations
The logged population dynamics of the three populations maintained in Soft Food in the absence of granulosis virus together with smoothed periodograms from spectral analysis.

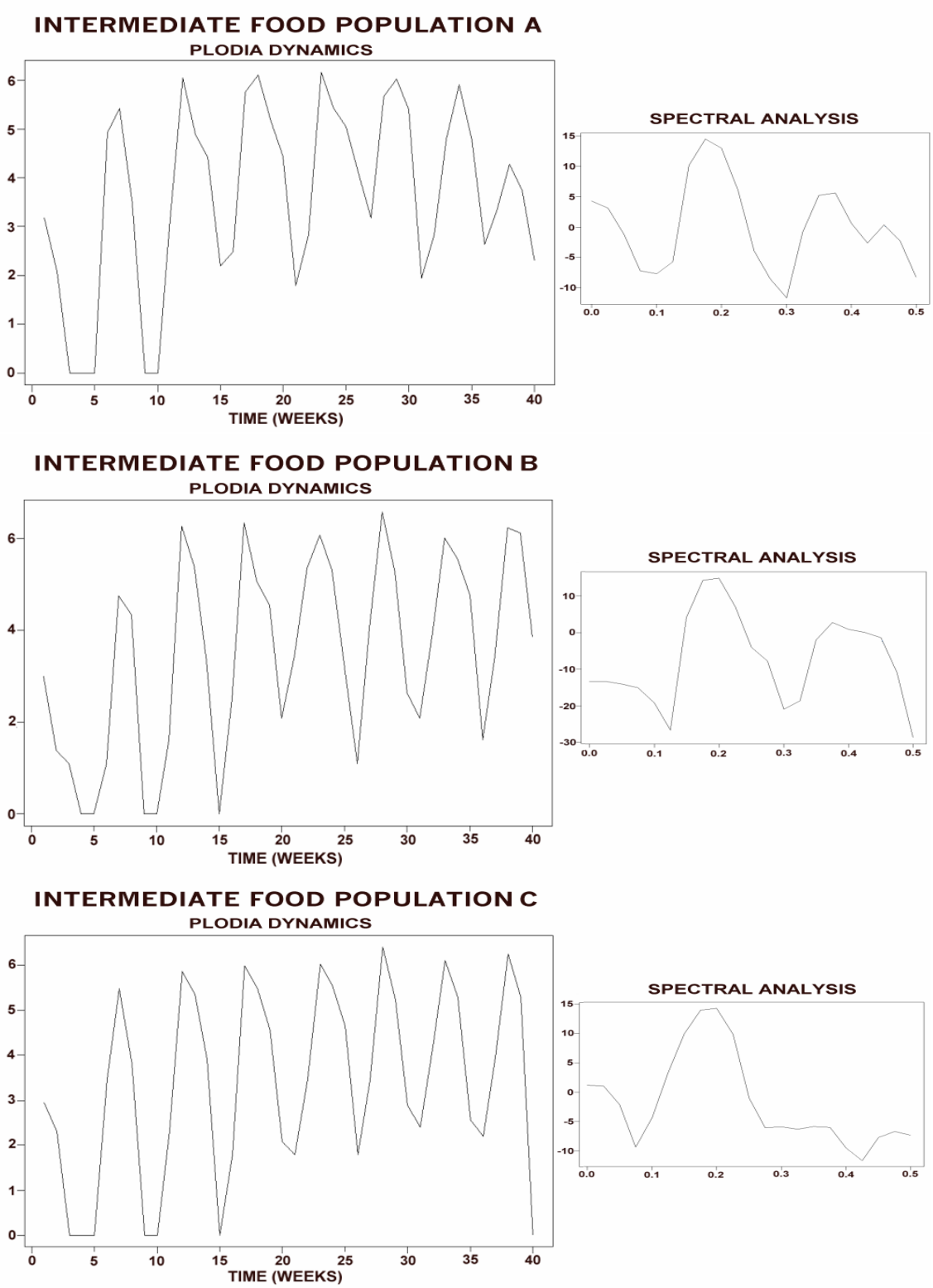

Figure 4.2 - Intermediate Food, no virus populations
The logged population dynamics of the three populations maintained in Intermediate Food in the absence of granulosis virus together with smoothed periodograms from spectral analysis.

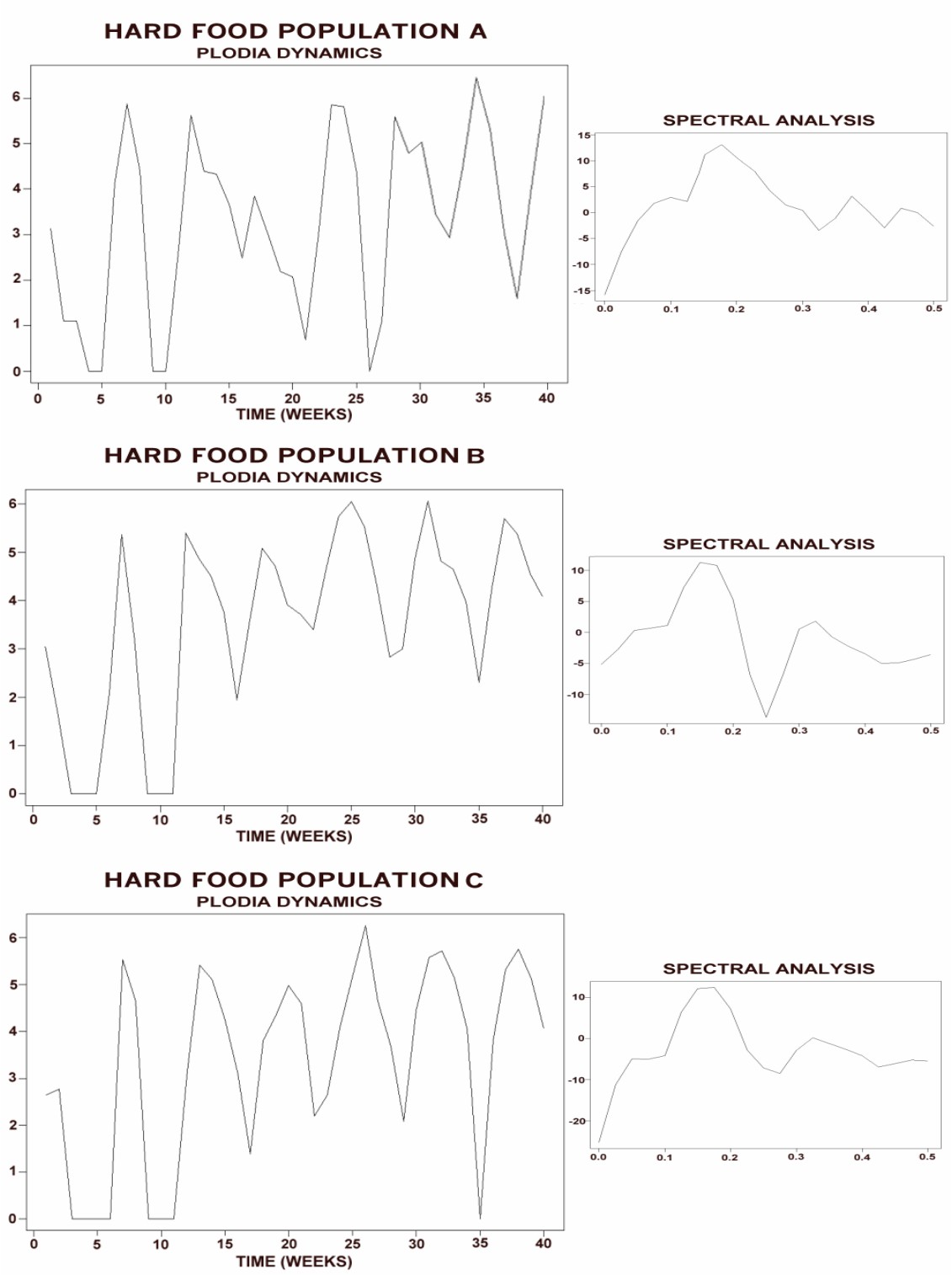

Figure 4.3 - Hard Food, no virus populations
The logged population dynamics of the three populations maintained in Hard Food in the absence of granulosis virus together with smoothed periodograms from spectral analysis.

With Virus Plodia Populations

There were fifteen populations of ***Plodia*** where granulosis virus (PiGV) was included, five populations for each of the three food types (see Table 4.2). Figure 4.4 shows the dynamics of ***Plodia*** and PiGV in five Soft Food populations. Autocorrelation function (ACF) analysis shows there was evidence for cyclical behaviour 5 weeks in length and the spectral analysis indicated the mean length of cycles was 39.88 days (standard deviation ±0.216), a significantly longer cycle than in no virus Soft Food populations (Cycle Length coefficients; $t_{5,18}$ =2.41, p =0.0266). The mean adult population density was 141.39 (±3.87) per week, the mean number of adults within each cycle was 826.3 (±33.55) and the mean cycle amplitude was 366.62 (±32.55). Compared to no virus Soft Food populations, there was no significant difference in any of these density measures (Population Density; $t_{5,18}$ =-0.813, p =0.427; Cycle Density; t =-0.647, p =0.647; Amplitude; t=-0.375, p =0.712).

Figure 4.5 shows dynamics of five ***Plodia*** populations on Intermediate Food in the presence of PiGV. Again ACF analysis provides evidence for regular cyclical behaviour in three of the five populations. The mean cycle length indicated by spectral analysis was 36.96 days (±2.08). The mean adult population density was 121.85 (±20.1) per week, the mean number of adults within each cycle was 714.17 (±115.7) and the mean cycle amplitude was 361.96 (±75.96). Compared to no virus Intermediate Food populations, there was no significant difference in any of these cycle and density measures (Cycle Length; $t_{5,18}$ =-0.265, p =0.794; Population Density; t=-0.813, p =0.427; Cycle Density; t =-0.237, p =0.816; Amplitude; t=-0.596, p =0.5584).

Figure 4.6 shows the dynamics of five ***Plodia*** populations on Hard Food in the presence of PiGV. ACF analysis shows there was no evidence for regular cyclical behaviour in such populations. The spectral analysis indicated the continued presence of generational cycles whose mean length was 41.25 days (±1.72). However the spectral analysis also provided evidence for the emergence of a new 20-week multigenerational cycle (as indicated with the peak in the power spectrum at 0.05 on the x axis), consisting of three generational cycles whose density appears to decrease as the multigenerational cycle progressed. In terms of density measures, the mean adult population density was 57.56 (±12.79) per week, the mean number of adults within each generational cycle was 379.43 (±85.7) and the mean generational cycle amplitude was 172.18 (±44.2). Compared to no virus Hard Food populations, all density measures saw a significant decline caused by the addition of PiGV (Population Density coefficient; $t_{5,18}$ =-4.507, p <0.001; Cycle Density; t =-4.425, p <0.001; Amplitude; t=-3.5, p =0.0025).

Food Type	Presence of Virus	Autocorrelation Function (weeks)	Spectral Analysis (days)	Population Density (mean ±sd)	Amplitude (mean ±sd)	Cycle Density (mean ±sd)
soft A	with virus	5	40	138.5 ±145.4	341.7 ±84.1	787.6 ±228.1
soft B	with virus	6	40	136.7 ±145.3	372.2 ±100.8	851 ±247.8
soft C	with virus	6	39.9	146.3 ±163.5	391.7 ±144.2	869.8 ±271
soft D	with virus	5	40	143.8 ±136.3	325.4 ±83.5	817.4 ±206.4
soft E	with virus	5	39.5	141.7 ±164	402.1 ±151.5	805.7 ±256.8
intermediate A	with virus	5	35	148.3 ±181.9	448 ±184.2	844.3 ±312.2
intermediate B	with virus	5	37.5	133.9 ±167.5	426.7 ±89.5	761.6 ±264.1
intermediate C	with virus	5	37.3	117.1 ±134.8	334.4 ±92.4	665 ±195.4
intermediate D	with virus	N/A	40	114.5 ±136.7	339.3 ±106.5	758.8 ±207.5
intermediate E	with virus	N/A	35	95.4 ±122.4	260.9 ±117.5	541.1 ±191.7
hard A	with virus	N/A	40	67.3 ±88.5	179.7 ±121.3	444.8 ±126.7
hard B	with virus	N/A	40	57.8 ±77.6	164.3 ±120.9	380.5 ±118.4
hard C	with virus	N/A	40	58.2 ±77.9	169.5 ±125.5	384 ±112.3
hard D	with virus	N/A	43.2	36.4 ±58.6	111.7 ±98.7	237.7 ±74.1
hard E	with virus	N/A	43.1	68 ±110	235.7 ±189.5	450.1 ±168.9

Table 4.2

A summary of the dynamics of ***Plodia*** populations fed on three food types in the presence of granulosis virus.

Virus Populations

The mean densities of PiGV populations are shown in Table 4.3. ACF and spectral analysis does not provide any evidence for regular cycling in viral populations. The mean density of infected larvae counted in food sections each week was significantly lower in Hard Food than in Soft and Intermediate Food populations. In Soft Food, the mean density of larvae was 5.18 (±0.982), 5.31 (±1.259) in Intermediate Food and 3.392 (±0.554) in Hard Food (Soft-Hard, $t_{2,12}$=2.91, p =0.013; Intermediate-Hard, t =3.12, p =0.0088).

Food Type	Density of Infecteds per Food Section (mean ±sd)	Autocorrelation Function (weeks)
soft A	4.6 ±5.2	N/A
soft B	4.1 ±5.5	N/A
soft C	6.7 ±7.3	N/A
soft D	5.4 ±4.8	N/A
soft E	5.2 ±5.6	N/A
intermediate A	6.9 ±7.1	N/A
intermediate B	5.7 ±5.9	N/A
intermediate C	3.4 ±3.5	N/A
intermediate D	5.3 ±5.2	N/A
intermediate E	5.2 ±3.7	N/A
hard A	3.6 ±3.1	N/A
hard B	4 ± 3.2	N/A
hard C	2.5 ±3.1	N/A
hard D	3.3 ±3	N/A
hard E	3.5 ±3.1	N/A

Table 4.3

A summary of PiGV populations in three different food types.

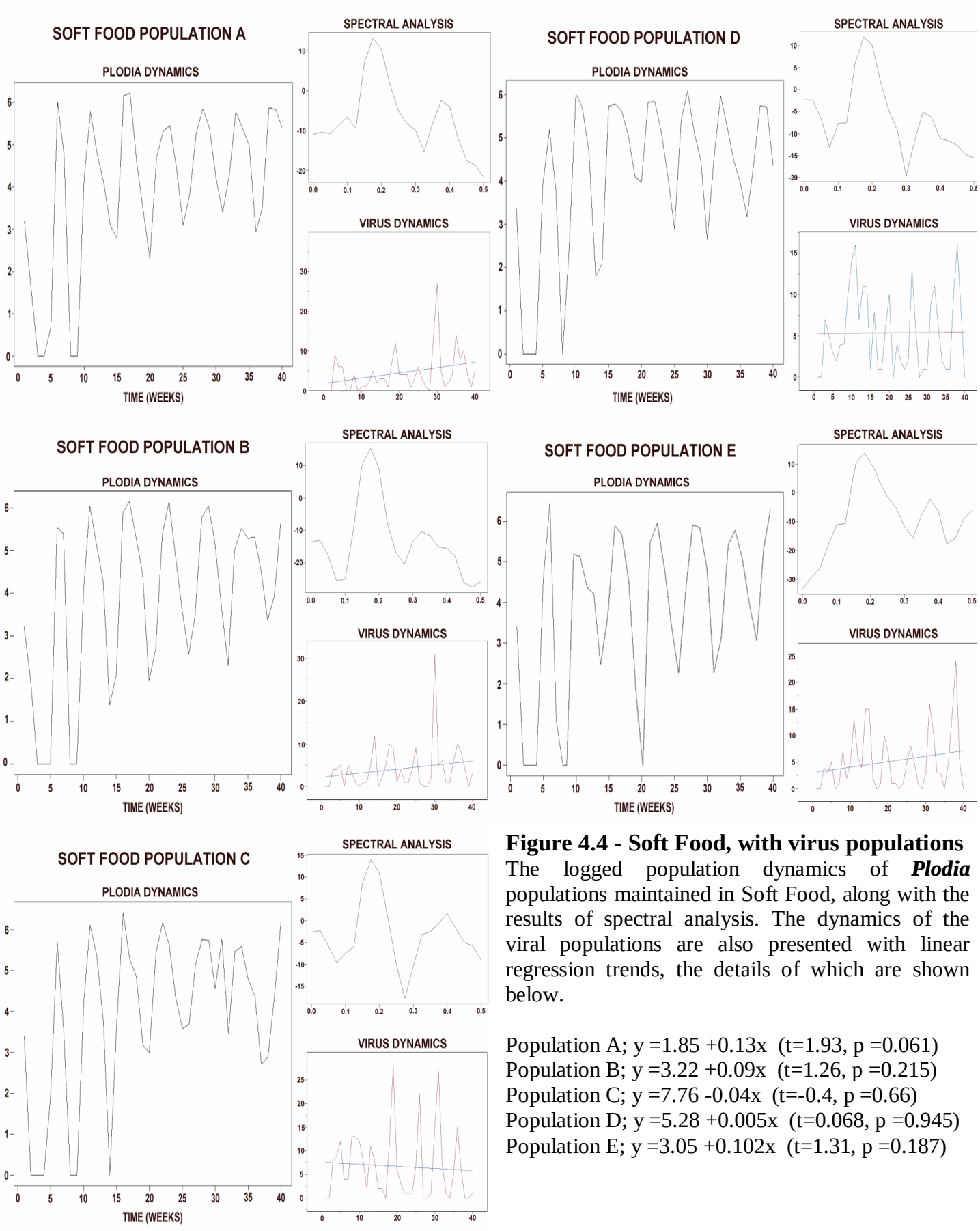

Figure 4.4 - Soft Food, with virus populations
The logged population dynamics of ***Plodia*** populations maintained in Soft Food, along with the results of spectral analysis. The dynamics of the viral populations are also presented with linear regression trends, the details of which are shown below.

Population A; y =1.85 +0.13x (t=1.93, p =0.061)
Population B; y =3.22 +0.09x (t=1.26, p =0.215)
Population C; y =7.76 -0.04x (t=-0.4, p =0.66)
Population D; y =5.28 +0.005x (t=0.068, p =0.945)
Population E; y =3.05 +0.102x (t=1.31, p =0.187)

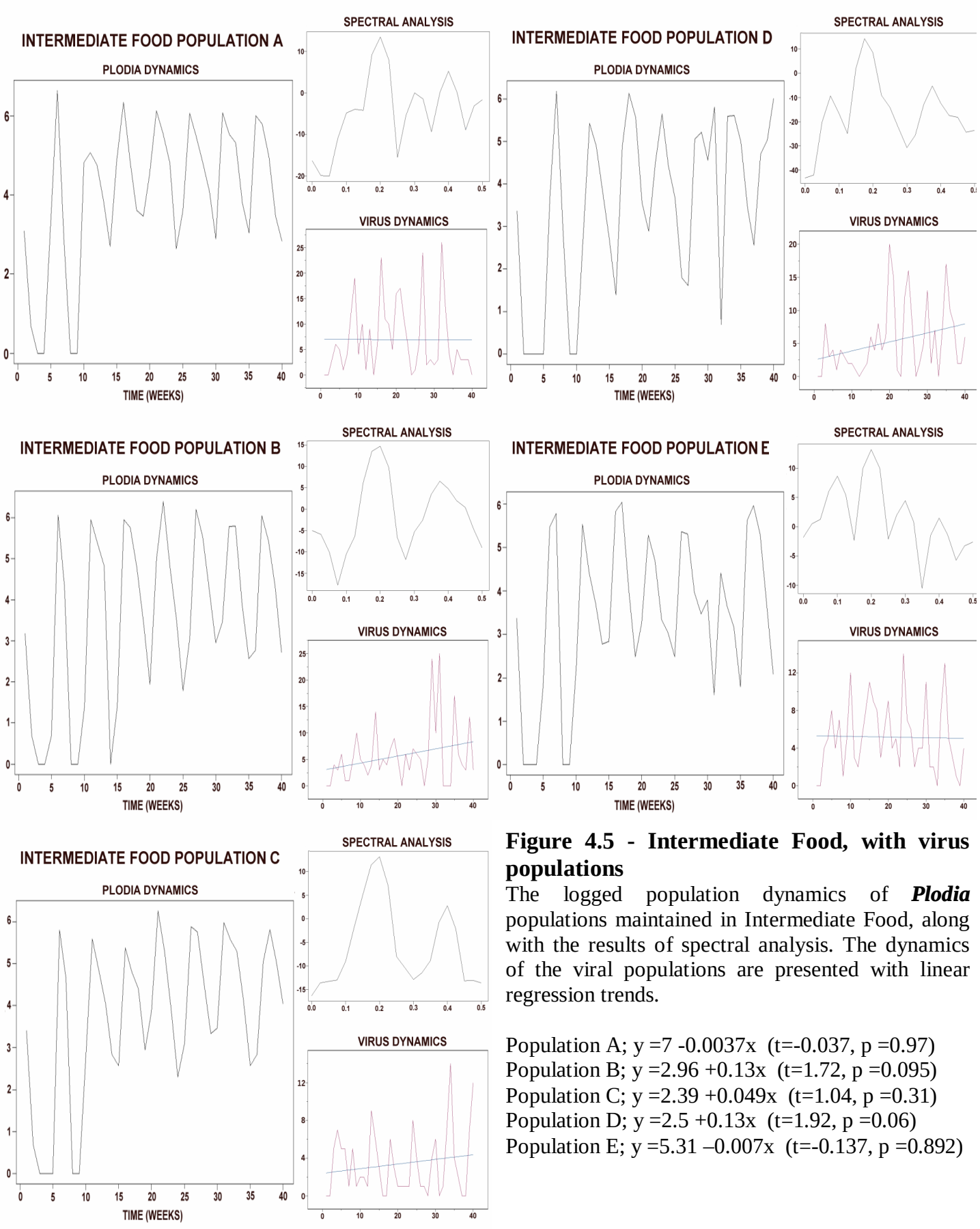

Figure 4.5 - Intermediate Food, with virus populations

The logged population dynamics of ***Plodia*** populations maintained in Intermediate Food, along with the results of spectral analysis. The dynamics of the viral populations are presented with linear regression trends.

Population A; y =7 -0.0037x (t=-0.037, p =0.97)
Population B; y =2.96 +0.13x (t=1.72, p =0.095)
Population C; y =2.39 +0.049x (t=1.04, p =0.31)
Population D; y =2.5 +0.13x (t=1.92, p =0.06)
Population E; y =5.31 –0.007x (t=-0.137, p =0.892)

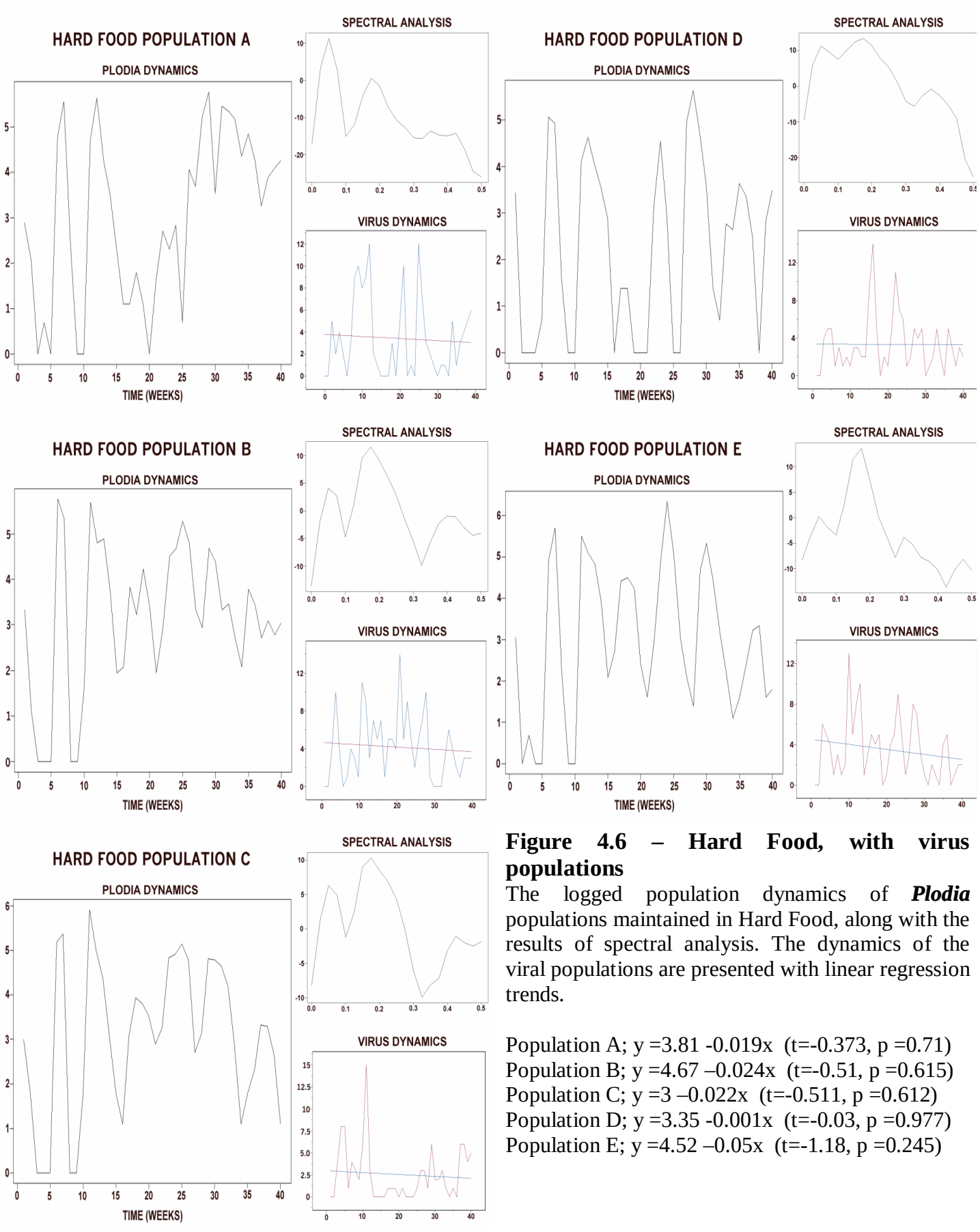

Figure 4.6 – Hard Food, with virus populations

The logged population dynamics of ***Plodia*** populations maintained in Hard Food, along with the results of spectral analysis. The dynamics of the viral populations are presented with linear regression trends.

Population A; y =3.81 -0.019x (t=-0.373, p =0.71)
Population B; y =4.67 –0.024x (t=-0.51, p =0.615)
Population C; y =3 –0.022x (t=-0.511, p =0.612)
Population D; y =3.35 -0.001x (t=-0.03, p =0.977)
Population E; y =4.52 –0.05x (t=-1.18, p =0.245)

Discussion

Spatial dynamics in the absence of PiGV

In comparison to Soft Food (Figure 4.1), Hard Food (Figure 4.3) produced distinct changes in the population dynamics of ***Plodia interpunctella***. The cycle length of populations in Hard Food was significantly longer than in Soft Food, with a mean increase of 4.49 days. Also the size of populations maintained in Hard Food was significantly smaller than Soft Food populations, as indicated by smaller weekly adult density, cycle density and cycle amplitude.

These changes can be explained by the effect that restricting the movement of larvae has on the intensity of larval competition. In Soft Food, individuals have a high rate of movement and so can rapidly move away from any competitive encounters. This generates a mixed population in which competition intensity is lowered by the ability of larvae to avoid it. In Hard Food, larval movement is restricted to such a degree that it is not possible to move away from competitive encounters. The resulting aggregation of individuals mean there is less food and space available, forcing competition to increase. Also, density-dependent cannibalism is likely to be higher in these aggregations than in the mixed Soft Food populations (Boots, 1998). The effects of an increased intensity of competition can be observed in the Hard Food dynamics. The smaller population size is a result of competition causing higher larval mortality and a lower reproductive rate associated with smaller, nutritionally deprived, adults. A longer cycle length is associated with the lower growth rates of larvae when resource and interference competition is high, as individuals require more time to develop from egg to adult. This is supported by the results of the competition experiments discussed in Chapter 3, where survival and growth rate of larvae in Hard Food were both lower than Soft Food (Figures 3.1& 3.2).

The difference in competition intensity, generated by restricted movement, is exacerbated by the oviposition behaviour of ***Plodia*** females. First, they select oviposition sites according to food quality as opposed to the larval density within the food (Anderson & Löfqvist, 1996). Second, adult females lay eggs in batches. This means the population of eggs is aggregated when oviposited, so guaranteeing high rates of competition between early instars. In Soft Food, larval movement dissipates such spatial aggregations rapidly. In Hard Food, these aggregations become fixed.

The dynamics of the Intermediate Food populations indicate competition processes falling between those found in Soft and Hard Food populations (Figure 4.2). The population density was significantly smaller than in Soft Food, by a mean value of 24.58 adults per week. This suggests the intensity of competition in Intermediate Food is greater, with mortality and reduced fecundity lowering population size. However this increase in competition is not reflected in a change in the periodicity, with the mean length of 37.3 days not significantly different to Soft Food values. An explanation for this difference is indicated in the competition experiments of Chapter 3, where different densities only produced different mortality rates in Intermediate Food (Figure 3.2). This suggests the intensity of competition experienced by larvae across Intermediate Food populations varies according to different local density levels. Within Intermediate Food populations, the moderate movement rates of larvae allow early instar larvae enough freedom to move away from competitive encounters with kin in the same egg batch. This is advantageous to larvae that experience a sparsely populated surrounding environment, as these individuals will be able to disperse away from the oviposition site, avoid competition and grow at a normal rate. This accounts for the similar cycle length in Intermediate populations compared to Soft Food. However because Intermediate Food restricts

larval movement, those individuals that experience a high-density surrounding environment will not be able to disperse far enough to escape this highly competitive region. These individuals account for the lower population density in Intermediate Food, as they will suffer high competition-induced mortality and those surviving to adulthood will be smaller and exhibit a lower reproductive rate.

Spatial dynamics in the presence of PiGV

Previous studies have shown the spatial structure of insect populations can have an important impact on the transmission of pathogens. Dwyer (1991) found that transmission of the nucleopolyhedrosis virus of the Tussock moth was dependent on the spatial distribution and density of infected individuals and the mobility of different larval instars. When the virus has a patchy distribution, slow moving early instars are less likely to become infected through low exposure to the pathogen. Later instars have a higher probability of becoming infected because they are highly mobile and experience a much larger portion of the environment, including pathogen aggregations (Dwyer, 1991).

In relation to the work carried out in this chapter, the addition of a granulosis virus creates a number of changes in the dynamics of Soft Food populations (Figure 4.4). The addition of the granulosis virus (PiGV) caused the length of ***Plodia*** population cycles to significantly increase by an average of 2.41 days. Measures of ***Plodia*** population size indicated that PiGV caused a small, but statistically insignificant, decline in population density (smaller by 4.98%), cycle density (smaller by 3%) and cycle amplitude (3.7%). These changes may be attributed to the effect of PiGV, which was highly prevalent in Soft Food populations (the mean number of

infected larvae was 5.18 per week). Larvae in Soft Food possess a high movement rate, generating a mixed population in which competition is relatively low and infection occurs evenly throughout the environment. Infectious units have the potential to encounter many susceptible larvae and so the virus has a high infection rate. This can explain the observed changes in cycle length and population size, as a high infection rate throughout the ***Plodia*** population may result in either selection for host resistance or the emergence of detrimental sublethal infection (Sait ***et al***., 1994b). One or both of these processes can result in a slower growth rate for larvae, significantly increasing the cycle length. Also both processes may result in a lower reproductive rate which, coupled with pathogen-induced mortality, might reduce the mean size of ***Plodia*** populations slightly.

Hard Food populations showed a number of dynamical differences when PiGV was included (Figure 4.6). Populations were significantly smaller, with the mean population density of 57.56 per week being 41.69% smaller than the ***Plodia*** density in no virus Hard Food populations. ACF analysis revealed all five with-virus populations lacked a regular periodicity, a feature unique to with-virus Hard Food populations. These changes might be attributed to the effect the aggregation of individuals has on competition, PiGV infection and the interaction between the two processes.

One apparent contradiction in the Hard Food dynamics is the very large reduction in population was caused by a small number of infectious cadavers. The mean weekly count of infected larvae was 3.39 in Hard Food, a significantly smaller number than counted in Soft and Intermediate Food. Yet the effect PiGV has in reducing population is much greater than the other food types. This amplified effect of infection may be due to the spatial distribution of hosts and infectious units, rather

than their overall number (Richter ***et al***., 1987). As individuals are forced into small patches by restricted movement, the localised density of these aggregations becomes high. Should infected cadavers exist within such a patch, the high density will increase the probability of susceptible larvae becoming infected. This higher infection rate within aggregations has two explanations. The first is there is a higher contact rate associated with high densities, which increases the chance of individuals coming into contact with the cadaver. Secondly, high local densities reduce the amount of food available to larvae, making it more likely they will cannibalise the primary cadaver or any secondary infected hosts (Polis, 1981; Knell ***et al***., 1998b) as well as the potential effect of reduced food making larvae more susceptible to infection (Steinhaus, 1958). As a result of higher infection rates, individuals within aggregations experience either pathogen induced mortality or deleterious sublethal effects. There may also be multiple infections, increasing the probability of infected individuals dying (Knell ***et al***., 1998b). However the epidemic is necessarily contained within the locality because there are few interactions between aggregations (Knell ***et al***., 1998a). Therefore, as the number of susceptible individuals declines through infection and maturation resistance, the force of infection falls and the localised epidemic fades out (Knell ***et al***., 1996). This may be the reason for the small number of infected larvae counted within the Hard Food populations.

Within infected aggregations of individuals, the reduced number of healthy individuals will lower the intensity of competition. This contrasts to the larval aggregations in which no virus was present, where the density of larvae is high and so competition is intense. The competition within these no virus aggregations is similar to that discussed in the no virus Hard Food populations. Therefore Hard Food with-virus populations can be characterised by two spatially segregated ecological

processes, competition and infection. Where there is infection, there is little competition. Where there is no infection, there is intense competition. Within this context, it is possible to explain the lack of regular cycling in Hard Food. The high infection rates in the with-virus aggregations causes the mortality of highly susceptible early instars (Sait ***et al***., 1994c). The intense competition in the no-virus aggregations causes the mortality of later, larger instars that require more food and more space and so are more susceptible to competition. The mortality of early instars through infection and late instars through competition remove the age structure of the larval population (Knell, 1998). This may result in a break down if the asymmetric larval interactions necessary for the discrete bursts of population characteristic of generational cycles. Also the lower population size in Hard Food might render the dynamics more sensitive to demographic stochasticity, such that regular cyclical behaviour becomes indistinct (Knell, ***et al***., 1998a).

There is also an appearance of weak multigenerational 20-week cycle in with virus Hard Food 20-week cycle, characterised by three generational cycles becoming smaller as the extended cycle progresses. One possible explanation for this cycle is the highly localised nature of transmission means PiGV prevalence is initially low, allowing the first larval generational to be largely unaffected by infection. The large number of eggs laid by this generation throughout the food environment acts to generate a correspondingly large, mixed larval population. A greater number of individuals will be positioned close to infectious larvae, so forcing infection rates and PiGV prevalence to increase. The mortality and reduced fecundity associated with infection causes the size of the second and third generations of ***Plodia*** adults to be progressively smaller. The low adult population at the third generation reduces the number of eggs laid and so the size of the resulting larval population is

correspondingly small. A density-dependent decline in PiGV infection at low larval densities allows more larvae to survive into adulthood and so generate the large adult population found at the fourth generational peak. This proposed pattern of infection might not found in the viral dynamics in Hard Food because the local nature of infection may make the weekly counts of infected larvae from one food section (1/6 of the total environment) unrepresentative of subtle changes in virus density across space and time.

As in the no virus populations, the dynamics of populations in Intermediate Food are complex (Figure 4.5). The mean density of ***Plodia*** populations on Intermediate Food with PiGV was 121.85, a 4.19% reduction in comparison to the no virus populations. The mean weekly number of infected larvae was 5.31, significantly larger than the Hard Food populations and similar to the Soft Food populations. These dynamical changes induced by PiGV in Intermediate Food are very similar to the changes in Soft Food. However the one difference between the two food types is the cycle length, which lengthens when PiGV is added in Soft Food but does not significantly change in the Intermediate Food. The Soft Food no virus populations had an extended periodicity most likely because the high rate of PiGV infection caused larval growth rate to decline through either sublethal infection or as a cost of resistance. Despite a similar number of infected larvae in the Intermediate Food, larval growth rate did not decline enough to alter cycle length. This difference might be explained by considering the movement rate of individuals in Intermediate Food.

In no virus Intermediate Food populations, the level of movement restriction gave individuals enough freedom to move from initial competitive encounters. At low densities this creates a lower contact rate between individuals and so a lower rate of competition. At high densities, an intermediary level of movement no longer confers

an advantage because individuals cannot move far enough to escape secondary competitive encounters within the dense, local environment. When PiGV is added to an Intermediate Food population, the relationship between density, movement and competition interacts with the infection process. In areas of low host densities the contact rate between individuals is minimal, meaning both the intensity of competition and the transmission of PiGV is low. Therefore host development time is not altered by either intense competition (like in Hard Food) or by pathogen-induced effects (like in Soft Food). In areas of high host density, individuals cannot avoid contact and so infection rates are high, lowering the density of hosts and so relaxing competition. The infection rate at high densities accounts for the high prevalence of infectious cadavers in the Intermediate food populations. However as the density in these areas is lowered overtime by infection, the rate of contact between individuals necessarily declines causing a density dependent reduction in PiGV infection. As a result, there is either limited selection for host resistance or few sublethal effects, corresponding to no increase in host development time.

Chapter 5

The Evolution of Baculovirus Infectivity in Spatially Structured Insect Populations

Abstract

Altering the movement of ***Plodia interpunctella*** larvae was found to change the infectivity of a species-specific granulosis virus. This was understood by considering the relationship between movement rate and the spatial range of transmission. When movement is restricted, the resulting population aggregation and localised transmission causes highly infectious strains to rapidly infect neighbouring a large proportion of available susceptible hosts, reducing infection opportunities and so lowering the transmission rate of the parasite. This process of 'self-shading' favours strains with lower infectivity, as such parasites are surrounded by a greater proportion of susceptible hosts. These strains possess a higher transmission rate as a result and so become prevalent. These results suggest that even subtle changes in the spatial structure of populations may constrain the transmission rate of infectious organisms.

Introduction

The emergence of new parasitic species and the evolution of virulent strains of endemic parasites can have severe effects upon both human populations and economically important species. General theory of parasite evolution shows that natural selection will act to maximise the basic reproductive ratio R_0: the number of secondary infections resulting from one infected host in a naïve host population (Anderson & May, 1991; May & Anderson, 1983). Parasite reproductive rate is a combination of transmission and virulence, with transmission acting to increase reproduction by increasing the number of infectious units and virulence acting to reduce it by lowering the number of infectious units through mortality. From the point of view of a directly transmitted parasite, transmission is the probability of an infectious unit successfully infecting a susceptible host upon contact (infectivity). We would therefore predict the parasite would evolve maximal infectivity and zero virulence (Levin & Pimental, 1981).

However, when there is a positive correlation between infectivity and virulence, and depending on the shape of the relationship, intermediate levels of transmission may be favoured (Ebert & Herre, 1996; May & Anderson, 1979). The mechanisms behind this correlation are assumed to be that a greater rate of replication within the host increases the potential for transmission, with more infective units released into the environment, picked up by vector species or passed onto neighbouring individuals by contact. However a high replication rate within the host also has the effect of inducing higher host mortality through increased tissue damage and the build up of metabolic toxins. Therefore the optimum reproductive rate of the parasite involves balancing the positive (transmission) and negative (host death) aspects of host exploitation. This ‘trade-off’ concept has become central to the study

of parasite evolution and has increasing empirical support. For example, a few host-parasite studies have directly shown positive relationships between the degree of replication within the host with increased parasite infectivity and detrimental effects on host health (Davies ***et al***., 2001; Ebert, 1994; Herre, 1993). Also several field studies provide indirect evidence of long-term changes in virulence that is consistent with the 'trade-off' concept (Ebert & Mangin, 1997; Ewald, 1993, 1994; Frank, 1996; Levin, 1996), including the human parasite HIV (Ewald, 1993) and the myxoma virus (see Bull, 1994 for review).

There are others factors not taken into account in the basic theory that may also select a level of infectivity different to that at the maximum reproductive ratio. For instance, when an individual host is co-infected by differently virulent strains, there may be within-host parasite competition (Bremermann & Pickering, 1983). This selects for strains with higher infectivity that quickly exploit and leave their host before experiencing the deleterious aspects of strain competition (Nowak & May, 1994; van Baalen & Sabelis, 1995). Another factor that may influence the evolution of infectivity is the spatial structure of host and parasite populations. Most work on parasite evolution makes the assumption the populations are homogeneously mixed and any susceptible host can become infected with any parasite in the population. However many natural host-parasite systems can be characterised by both localised transmission, where secondary infections are more frequent in neighbouring individuals of infected hosts, and patchy host distributions. This means only a small proportion of the host population is available to any one infectious unit. The relationships between the spatial aspects of real populations and the evolution of infectivity have been the subject of a limited series of papers (Boots & Sasaki, 1999, 2000; Claessen & de Roos, 1995; Haraguchi & Sasaki, 2000; Rand ***et al***., 1995).

Boots & Sasaki (1999 & 2000) modelled a host-parasite system using a lattice-based host structure and pair approximations to correlate the state of nearest neighbours. When host reproduction occurred only between neighbours, localised transmission favoured the persistence of strains with lower infectivity than when infection was global. This has two explanations. First, local transmission combined with high infectivity rapidly reduced the number of surrounding susceptible hosts by mortality (Boots & Sasaki, 1999). This process of 'self-shading' favours low infectivity, with the parasite maintaining a large number of surrounding susceptible hosts. Second, localised transmission creates distinct patches of susceptible and infected hosts. A highly infective parasite will rapidly infect and kill hosts within the patch before there is an opportunity to "jump" to a new patch of susceptibles (Boots & Sasaki, 1999). When host reproduction occurs globally, there is greater mixing of susceptible individuals within the lattice and so reducing the constraints 'self-shading' imposes on the spread of parasites. Therefore global reproduction allows a higher infectivity than local reproduction at the evolutionarily stable strategy (ESS) (Boots & Sasaki, 2000). However, even with completely global reproduction, local transmission constrains infectivity to some degree (Boots & Sasaki 2000).

In spatially structured models where the infectivity-virulence 'trade-off' is not invoked, similar changes in infectivity are observed (Haraguchi & Sasaki, 2000; Rand ***et al***., 1995). In a latticed structured probabilistic cellular automaton, Rand ***et al***. (1995) showed that localised transmission could result in a finite upper limit for infectivity, above which the parasite went extinct. This contrasts to mean-field models of homogeneous populations where there was no such limit on the evolution of infectivity. Haraguchi & Sasaki (2000) used pair-approximation techniques to show, in comparison to mixed populations, lower infectivity was favoured in lattice-

structured populations in which transmission was local (Haraguchi & Sasaki, 2000). Again it is the duel processes of 'self-shading' and reduced opportunities for "jumping" to new susceptible patches that select against high infectivity (Boots & Sasaki, 1999; Haraguchi & Sasaki, 2000).

The study of infectivity evolution in spatially structured populations has predominately been a theoretical exercise. However there are studies of host-parasite systems where changes in spatial structure, infectivity and virulence can be correlated. Serial passage experiments frequently result in increased virulence over time (Doroshenko ***et al***., 1996; John & John, 1994). One explanation for this is that each passage involves mixing the host population and disrupting any spatial restrictions on transmission (Haraguchi & Sasaki, 2000). Also, parasites with the capacity for long distance dispersal tend to possess a higher virulence than parasites transmitted over short distances (Ewald, 1991, 1993, 1994). A comparison between bacterial parasites reveals that water-borne species cause higher host mortality than those directly transmitted from host to host. Water-borne transmission allows the parasite to contact a larger proportion of the host population over a greater spatial range.

In this chapter, I present a selection experiment observing the evolution of an insect virus in differently structured host populations. Larvae of the moth species ***Plodia interpunctella*** can be infected with a species-specific granulosis virus (PiGV). Infection results from ingesting contaminated food, predominately involving the cannibalisation of infected larvae. Therefore the primary infectious unit for PiGV are infected host cadavers (Boots, 1998). There is a high mortality rate associated with infection, although sublethal effects can be observed when older larvae are infected (Sait ***et al***., 1994a). By using food mediums of different viscosities, the movement of

larvae is altered. This changes the spatial structure of host-parasite populations, with the high mobility in soft mediums allowing greater larval mixing and more distant dispersal of infectious units. Larvae in harder mediums are forced into overcrowded high-density patches, and it within such aggregations that localised PiGV transmission occurs.

Long-term host-parasite interactions were maintained in different food mediums, then destructively sampled to analyse the infectivity of selected viral particles compared to a stock strain. This was determined by finding the mortality rate associated with the concentration of virus particles. Mortality rate does not inform us about any change in virulence because overt infection always results in host death. Therefore the mortality rate solely reveals the probability of virus particles entering the host through the midgut and evading immune responses, and so indicates the strains infectivity.

Method

Populations of ***Plodia interpunctella*** and its granulosis virus were established using three different viscosities of food media. The process by which the food types are made and the effect they have on larval movement rates is described in Chapter 2. Media with 0ml water is Soft Food, media with 60ml water is Intermediate Food and media with 140ml water is Hard Food. Increasing the amount of water added to the food medium increases the media's viscosity and reduces the movement rate of larvae. Preliminary experiments show that the average movement of ***Plodia*** larvae from 1st to 3rd instar is 10.65cm (SE ±0.73) in Soft Food, 5.09cm (±0.61) in

Intermediate Food and is 2.8cm (±0.26) in Hard Food (Figure 2.4). Changes in food viscosity had no effect on ***Plodia*** development time and pupal weight (Figure 2.7).

Out of the fifteen long-term ***Plodia***-PiGV populations discussed in Chapter 4, twelve populations were destructively sampled at the end of the 40-week experiment. The populations sampled for cadavers were four Soft Food (a, b c, d), four Intermediate Food (b, c, d, e) and four Hard Food (b, c, d, e) populations. The methods by which these populations were started and maintained are detailed in Chapter 4 (p 81) and the dynamics of the twelve populations can be seen in Figures 4.4-4.6. Sampling involved crumbling the food by hand and removing and counting infected larvae. These were easily distinguishable from healthy larvae by their white colouration indicative of virus infection. Only heavily infected fourth and fifth instar larvae were selected for this experiment, meaning the number of virus particles extracted from each infected larvae was similar. Also larger instars were easily found and handled. The total number of larvae removed from each population at the end of the experiment is shown in Table 5.1, and is indicative of the virus population prevalence in differently viscous food environments.

Virus was extracting from infected larvae using sucrose gradients and centrifugation according to Smith & Crook (1988), with the methodology detailed in Appendix One. The relative concentration of virus particles in each extracted strain was determined using fluorescent activated cell sorting (FACS). This method involved making 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions of each of the twelve selected strains (making a total of 48 viral counts) and the stock strain, then dying the nucleic acid of the virus with Sybr Gold. Each dilution was run once through the FACS machine for 60 seconds, and the mean concentration of virus particles counted in each dilution is shown in Table 5.2. It would have been preferable to measure the total

number of virus particles in each sample, but time restrictions prevented the necessary preliminary work to be carried out. However, comparing the counts of the four dilutions made for each strain can act to test the veracity of the FACS output. Should the virus concentration of one strain at dilution factor 10^{-2} be greater than other strains, and the sampling error of the FACS method is low, then this should remain be the case in the other dilutions. Regarding the overall accuracy of FACS methods, work by Heather Rae at the University of Sheffield found calculations of total viral concentration gathered from FACS and more traditional Zeiss electron microscopy to be similar (Appendix Two).

Droplet feeding bioassays were then carried out with the selected strains and the original stock strain (Hughes & Wood, 1981). For the bioassay, each strain was diluted to 10% viral concentration using a solution consisting of distilled water, 0.1% Coomassie Brilliant Blue R dye and 2% sucrose. Larval cultures were started by allowing adults to lay eggs in abundant food for 24 hours, then waiting 12 days for the eggs to develop into early third instars. The third instar larvae were then placed individually into segmented petri dishes, covered with damp tissue paper to prevent dehydration and starved for one hour. Small droplets of the diluted virus were placed in each petri dish chamber using a 10μl Hamilton syringe, with the sucrose encouraging larvae to feed. Larvae were left to feed for a period and those that had fed enough to fill at least half the gut (as indicated by the blue dye) were removed and placed individually into a new segmented dish with fresh food. Dishes were maintained in incubators set at 27ºC and 35% humidity. Each treated larva was checked regularly and infected larvae were recorded and removed. Individuals that survived to adulthood were considered to have resisted infection.

For each viral strain, the larval numbers exposed and corresponding mortality rates are shown in Table 5.1. Thirteen bioassays were carried out, four testing the mortality of strains taken from Soft Food populations, four testing strains taken from Intermediate Food populations and four using strains from Hard Food populations. One bioassay tested the mortality associated with the stock strain that was used to initiate the presence of PiGV in ***Plodia*** populations. Three control bioassays were also carried out, one for each food type, to find a base level of mortality. Such bioassays found negligible mortality associated with the handling and exposure of larvae to dye/distilled water solutions (results not shown).

Results

Table 5.1 shows the number of larvae treated in each bioassay with a 10% solution of PiGV as well as the percentage larval mortality in each bioassay and the number of infected larvae removed from each ***Plodia***-PiGV population during destructive sampling.

Population Type	Infected Larvae Removed from Population	Number of Larvae Treated in Bioassay	Mortality in Bioassays (%)
Soft a	48	162	30.86
Soft b	56	189	53.4
Soft c	50	173	68.7
Soft d	54	169	49.7
Intermediate b	37	174	85.6
Intermediate c	23	182	52.2
Intermediate d	34	150	31.3
Intermediate e	33	147	19
Hard b	26	200	23.5
Hard c	16	193	35.8
Hard d	25	185	43.8
Hard e	21	146	2.7
Stock Strain	n/a	150	14.5

Table 5.1 – The number of larvae removed from each population from which granulosis virus was extracted and the mortality rate associated with bioassays testing the infectivity of each viral strain.

The concentrations of virus particles calculated through fluorescent activated cell sorting (FACS) and the associated infectivity are shown in Table 5.2. The infectivity of each selected strain and the stock virus was determined by dividing the respective bioassay mortality rate by the concentration of virus particles within the 10% viral solutions. This calculation shows the relative ability of virus particles from different populations to infect hosts and induce virus-induced mortality. A higher value shows that any one virus particle has a greater probability of successfully infecting a host, and therefore the infectivity is higher. Infectivity was calculated for the four dilutions

made of each strain (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}), to measure the potential effect of sampling error in the FACS method upon estimates of virus concentration.

Virus strain dilutions	Mean concentration of of virus particles (+se)	Mean Infectivity (+se): mortality/concentration
Soft -2	30571 (1764)	0.001636 (1.83e-4)
Soft -3	12699 (1934)	0.00435 (9.9e-4)
Soft -4	14013 (5500)	0.00601 (2.27e-3)
Soft -5	9610 (2147)	0.007173 (2.87e-3)
Intermediate -2	25816 (7023)	0.0018445 (2.96e-4)
Intermediate -3	14100 (1497)	0.003136 (6.95e-4)
Intermediate -4	11513 (574)	0.00402 (1.22e-3)
Intermediate -5	10522 (371)	0.00452 (1.49e-3)
Hard -2	40553 (11473)	0.000587 (1.42e-4)
Hard -3	23719 (8501)	0.001149 (3.66e-4)
Hard -4	12668 (1165)	0.002011 (6.12e-4)
Hard -5	11499 (302)	0.00227 (7.62e-4)
Stock Strain -2	12146 (NA)	0.00119 (NA)

Table 5.2 – The mean concentration of virus particles at each dilution level and the corresponding mean infectivity, as determined by dividing the mortality rate with the concentration. The strains tested include the original stock virus and the virus as selected in differently viscous food environments.

To measure the effect food viscosity had on infectivity across the dilution range, a two-way analysis of variance was carried out on the complete data set. Infectivity data was log transformed in order to generate homogeneous variance and normally distributed errors. Both food type and the dilution effect had a significant negative impact on infectivity (***Food type***, $F_{2,45}$= 6.602, P =0.0036; ***Dilution***, $F_{3,44}$ = 3.805, P = 0.0182) and there was an insignificant interaction between the two (***Interaction***, $F_{6,41}$ = 0.445, P = 0.844).

Analysis of the coefficients show the logged infectivity of strains maintained in Hard Food populations was significantly lower than Soft and Intermediate Food strains (Soft = 0.0062, Hard = 0.0029, t = 3.775, p <0.001: Intermediate = 0.0048,

Hard = 0.0029, t = 2.15, p =0.037), although there was no significant difference between Soft and Intermediate Food strains (Soft = 0.0062, Intermediate = 0.0048, t = 1.62, p =0.112). The dilution effect can be simply understood as an artefact of the decrease in viral concentrations as the strain becomes more diluted. The insignificant interaction term is important as it indicates that the effect food type has on infectivity occurs at all dilution levels, suggesting that any sampling error associated with FACS methods is not large enough to either obscure, or erroneously give the impression of, relationships between food type and infectivity. The negative relationship between food type and infectivity in the four dilutions is shown in Figure 5.1.

Comparing the infectivity of the stock strain to the selected strains (in Table 5.2), it is apparent that the stock's value is similar to the rate found in Soft and Intermediate Food and is larger than the value in Hard Food. This suggests the direction of selection has been to lower infectivity in Hard Food, rather than to increase infectivity in less viscous foods. Also, because there was no difference in the average size of infected larvae removed from the three food types (all were fourth and fifth instars), we can say that strains with a measured low infectivity do not boost their infectivity indirectly by increasing the size of infected hosts and so increasing the number of virus particles produced by infection.

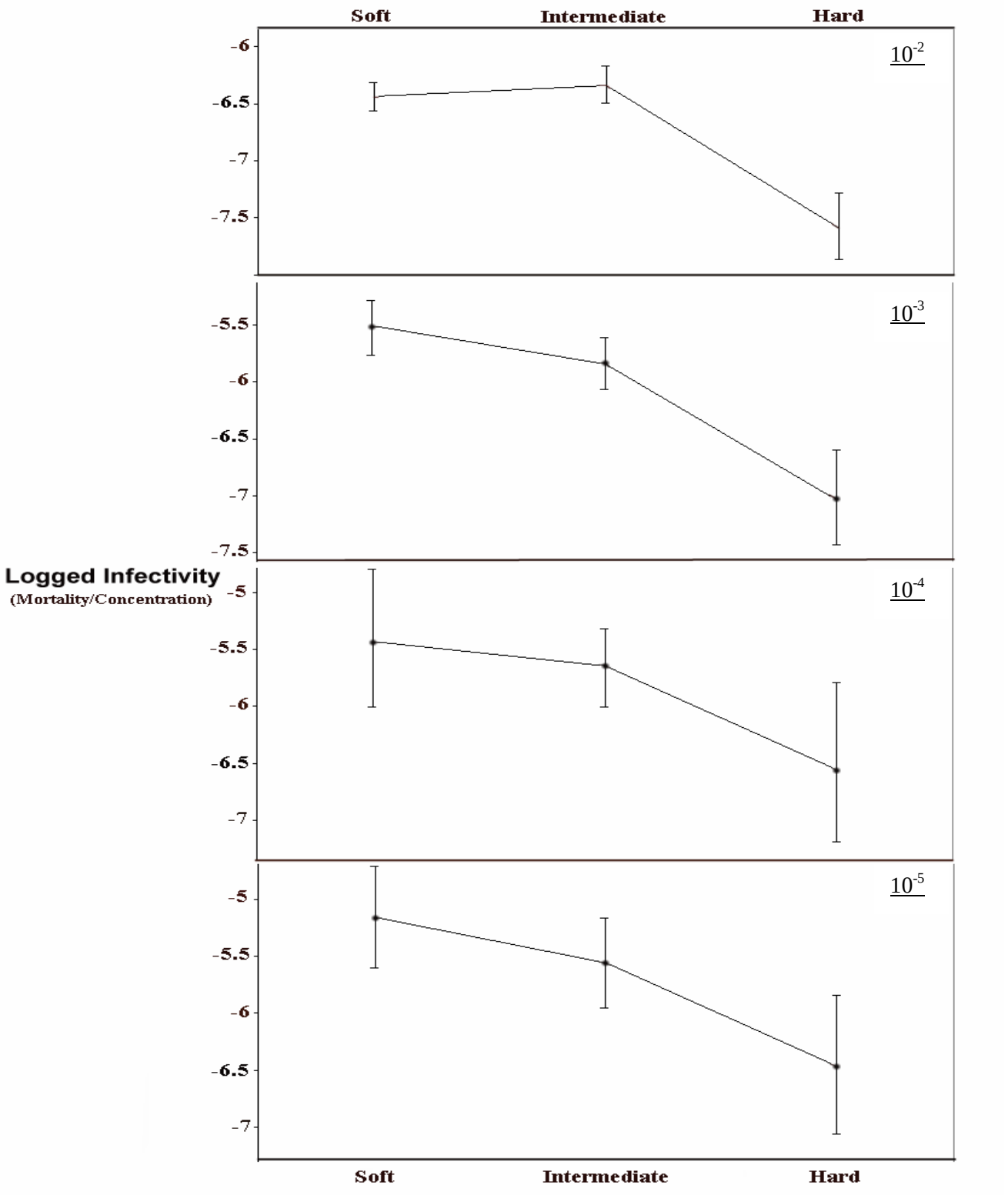

Figure 5.1 – Granulosis virus infectivity in Soft, Intermediate and Hard Food at four dilution levels.

Mean values of logged infectivity are presented with standard errors at four dilution levels. Regardless of the dilution, the relationship between infectivity and food type is similar. As food viscosity increases, infectivity declines (***Food type***, $F_{2,45}$= 6.602, P =0.0036).

The results shown in Tables 5.1 and 5.2 indicate the number of fourth and fifth instar larvae removed from populations and PiGV infectivity share the same relationship with food type, with both displaying significant declines with increasing food viscosity. Following from this, spearman's rank correlation analysis (in Figure 5.2) revealed significant positive relationships between infectivity and the number of larvae removed from populations in three of the four dilutions, supporting the link between the two factors.

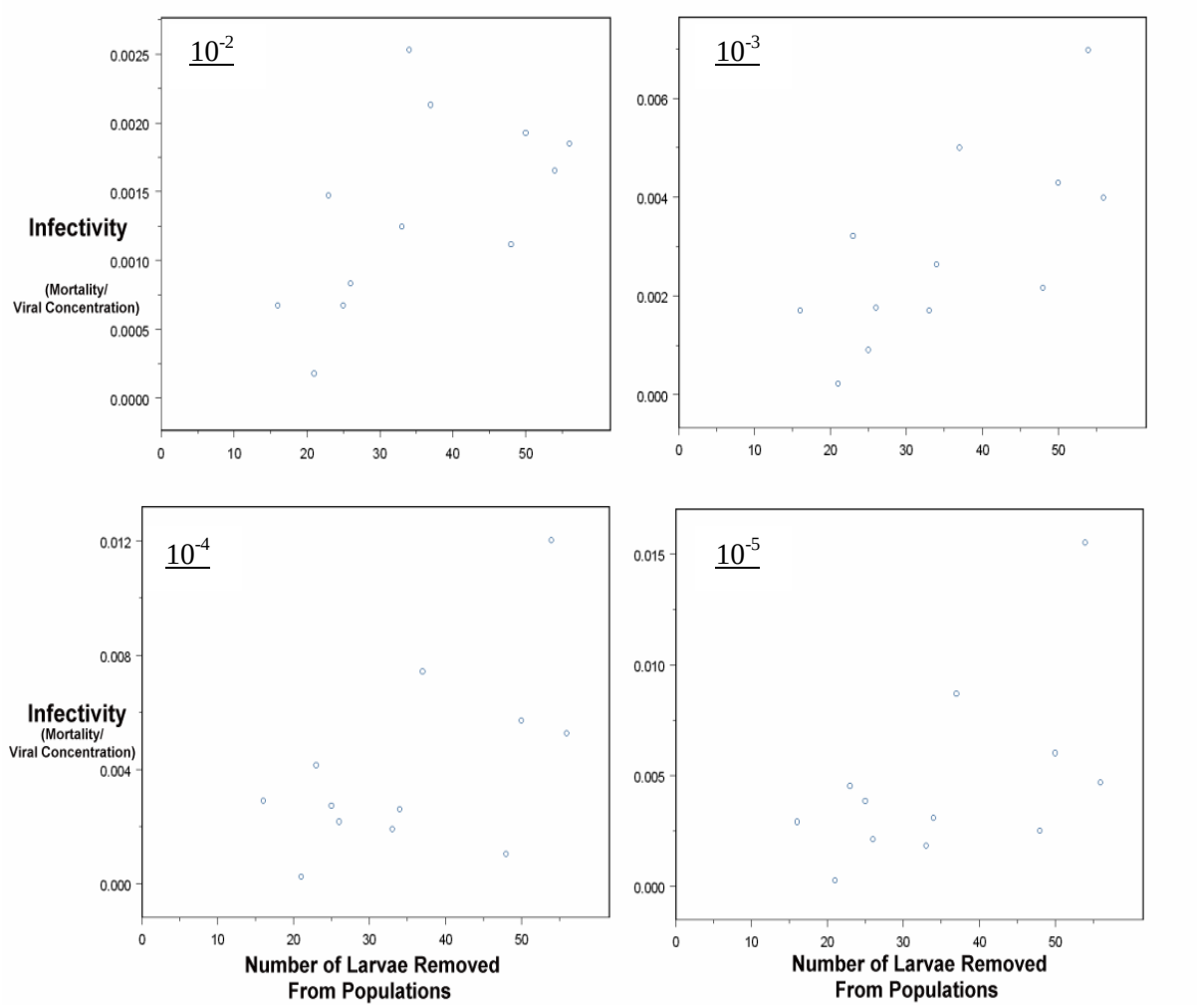

Figure 5.2 – The relationship between granulosis virus infectivity and infected larvae numbers

Spearman's rank correlation analysis reveals significant positive relationships between infectivity and the number of infected larvae in dilutions 10^{-2} ($r_S = 0.671$, $P = 0.0134$), 10^{-3} ($r_S = 0.734$, $P = 0.0077$) and 10^{-5} ($r_S = 0.594$, $P = 0.025$).

The results of the correlation analysis for dilution 10^{-4} were insignificant ($r_S = 0.483$, $P = 0.0561$).

Discussion

In this chapter, I examined the evolution of granulosis virus (PiGV) infectivity in host ***Plodia interpunctella*** populations. Previous work in Chapters 2, 3 and 4 has shown that Soft, Intermediate and Hard Foods produce significantly different larval movement rates and generate different spatial population structures. This chapter shows that lower movement rates and more aggregated populations are also associated with significant declines in the ability of virus particles to infect larvae (Figure 5.1). Figure 5.2 reveals the correlation between the number of infected larvae in populations and infectivity, with Hard Food populations characterised by low viral infected larvae numbers and low infectivity. This suggests that it is possible to understand changes in infectivity by considering how shorter dispersal distances of susceptible and infected ***Plodia*** larvae change both the spatial scale and rate of transmission.

In Hard Food, susceptible larvae can only disperse short distances and so can only encounter and cannibalise infected larvae within a very small region around their oviposition site. Also recently infected larvae are unable to move away from the location of initial infection, preventing viral strains from spreading through the environment. Such factors make host and virus populations highly aggregated, where the only hosts vulnerable to infection from infected larvae are those that occupy the same spatial aggregation. In this patchy form of population structure, virus strains with high infectivity will rapidly infect all the neighbouring susceptible larvae, a process accelerated by the high larval contact rate within overcrowded host aggregations. As all the nearby susceptible hosts become infected, the opportunities for new infections decline, so reducing the infection rate of highly infective strains. In contrast, strains with a low infectivity maintain a greater number of surrounding

susceptible hosts, meaning such strains have a higher transmission rate compared to more infective strains. Therefore, as populations become more aggregated, we see the evolution of lower virus infectivity.

The overall processes discussed above can be seen to be analogous to the relationship between localised infection and the evolution of infectivity described in theoretical work (Boots & Sasaki, 1999, 2000; Claessen & de Roos, 1995; Haraguchi & Sasaki, 2000; Rand ***et al***., 1995). In the ***Plodia***-PiGV system, parasite dispersal ability is dependent on its host, with infected larvae acting as the dispersal agent and primary infectious unit. Therefore by restricting larval movement, the dispersal ability of the virus is also restricted. As a result, viscous food types prevent the virus from "jumping" to new patches of susceptible hosts, forcing the virus to remain within its original host aggregation. The small number of hosts available for infection within such aggregations causes "self-shading" of highly infective strains, so favouring the evolution of lower infectivity (Boots & Sasaki, 1999, 2000; Haraguchi & Sasaki, 2000).

These results can be seen to be complimentary to the dynamical study of PiGV transmission carried out by Knell ***et al***. (1998b). They found the transmission coefficient, the probability of new infections occurring within a population, was dependent on the relative densities of susceptible hosts and infected larvae. When the density of infected larvae increased in relation to host density, the transmission coefficient of PiGV was found to decline. This was suggested to be a product of the rapid removal of susceptible hosts through infection, so preventing later interactions between host and PiGV resulting in new infections. Such changes in the dynamics of transmission due to host-PiGV density match the evolutionary changes in infectivity found in this Chapter. In aggregated Hard Food populations, strains with high

infectivity rapidly lower the density of surrounding hosts through infection, and thereby increase the local density of PiGV. The resulting reduction in transmission opportunities may well lower the transmission coefficient in the short term in a similar way to that found in Knell ***et al***. (1998b). However my work appears to suggest that selection acts in the long term to favour strains with low infectivity, a parasite trait that maintains a large local density of susceptible hosts and so confers a high coefficient upon the transmission dynamics of the strain.

Boots & Sasaki (1999) showed the ability of spatial structure to constrain parasite infectivity was not only manifested in highly structured populations. Populations that are nearly completely mixed still lead to lower infectivity than more mixed ones. The important question is whether even small degrees of spatial structure can have an effect on parasite evolution. Clearly, the spatial characteristics of the ***Plodia*** populations are a long way from the models, but it seems this different structure can still constrain parasite evolution. This is an important result because it shows that very subtle changes in mixing, within highly mixed systems, can lead to measurable changes in the evolution of parasites.

This chapter presents one of the first empirical studies of the effect host spatial structure and localised transmission can have on the evolution of parasite infectivity. Theoretical approaches have been the primary tool to analyse parasite evolution, but hopefully this work may encourage empiricists to develop further laboratory and field methods of testing the interaction between spatial structure, infection and the evolution of parasite infectivity and virulence. I show that even in homogeneous environments, changing the dispersal ability of hosts and parasites can significantly alter the selection pressure on parasites. A particular advantage of the laboratory ***Plodia***-PiGV system is that restricting the movement of larvae changes the population

structure of both hosts and parasites to a similar degree. This may not be the case in other host-parasite systems, where the small-scale spatial distribution of infectious units may be independent of the host distribution. Another strength of the ***Plodia***-PiGV system is that successful transmission results in overt infection and death, making the mortality rate an indicator of transmission. Systems where parasite infection is primarily associated with sublethal effects may require more complex experimental work to determine the infectivity and virulence traits of the parasite.

Chapter 6

Dimorphic Resistance Patterns Suggest a Decreasingly Costly Resistance Mechanism in a Model Insect System.

Abstract

Trade-offs between life history characters play a key role in shaping the evolution of individuals. Measuring the shape of these relationships directly is often impractical. Here, we use an indirect approach that examines the patterns seen within a population and then uses theory to infer the shape of the trade-off curve. Using a diet incorporation bioassay technique, we found a dimorphic pattern of resistance to a microparasite within a lepidopteran host population. A high proportion of individuals possessed a low level of resistance and a significant proportion had a very high level of resistance, but few individuals possessed an intermediate level of resistance. According to theory, this dimorphic pattern of resistance corresponds to a decreasingly costly impact on fitness of increasing resistance. The generality of the approach are discussed, along with the implications of the results to our understanding of the nature of innate resistance to parasites.

Introduction

A central idea to our understanding of life histories is that the evolution of particular fitness traits may be constrained by trade-offs with other life history traits (Roff, 2002). Furthermore, a key prediction of life-history theory is that the evolution of a particular trait is not just the result of the absolute strength of a trade-off but also of the functional form of trade-off relationships (Roff, 2002). The recent advent of adaptive dynamical evolutionary theory has further emphasised the importance of how costs and benefits change under different conditions (Geritz ***et al***. 1998; Bowers & White, 2002). This adaptive evolutionary theory recognises that trade-off relationships are unlikely to be exactly linear and that the shape of the relationship is important in determining the ultimate evolutionary outcome. In particular, the way that the costs and benefits vary determines both the convergence stability of the evolutionary system and whether evolutionary branching will occur (Boots & Haraguchi, 1999; Bowers & White, 2002). The shape of the trade-off curve may therefore predict the nature of variation in a trait that is maintained in a population, thereby determining whether polymorphism occurs. Despite the theoretical importance of the trade-off curve shape, there are few if any empirical studies that have accurately estimated its form (Roff, 2002). A detailed and rigorous measurement of these curves can therefore be seen as a key objective of evolutionary biology but direct measurement is usually impractical. Often a complex series of selection experiments featuring different resource levels is the only method of empirically determining the shape of these trade-off curves (Stearns, 1992).

Here we propose an indirect approach examining the patterns of variation at a population level and using this to infer the general shape of cost structures. Measuring phenotypic variation at the population level is usually straightforward relative to measuring the shape of trade-offs. If a theoretical model can be constructed that

predicts how different cost structures lead to particular patterns of population level variation, an empirical examination of the actual within population variation can be used to suggest particular costs structures. The functionality of this approach will depend on the quality of the theory, which will in-turn be determined by the knowledge of the genetic basis of the traits concerned. Here we give an example of this approach by examining the pattern of resistance to a parasite within a laboratory model insect system. This is then related to theory by Boots & Haraguchi (1999) that predicts the patterns of variation in resistance likely with particular shapes of trade-off curves. In this instance, our definition of the pattern of resistance is simply the proportions of a population exhibiting particular levels of quantitative resistance, ranging from susceptibility to low viral doses to resistance to high doses. Boots & Haraguchi (1999) found that if resistance was increasingly costly (Figure 6.1a), then the pattern of resistance would be expected to be gaussian around an optimal evolutionarily stable strategy resistance level (Figure 6.1b). Should the evolutionary trade-off curve be exponential, an increase in resistance from low to intermediate levels of resistance is associated with only a small additional cost to fitness. Therefore an ESS forms at intermediate resistance levels, beyond which the cost to any additional increase in resistance level becomes disproportionately high. Conversely, an asymptotic trade-off curve (Figure 6.1a) (costs reduce as resistance increases) is associated with a dimorphic pattern of resistance, with individuals in the population having either low or high levels of resistance (Figure 6.1b). An asymptotic curve selects against the evolution of intermediate resistance levels, with large fitness costs prohibiting the evolution of intermediate resistance from low levels but the low cost of additional resistance beyond intermediate levels favours the evolution of high resistance. Therefore there is evolutionary divergence from intermediate resistance.

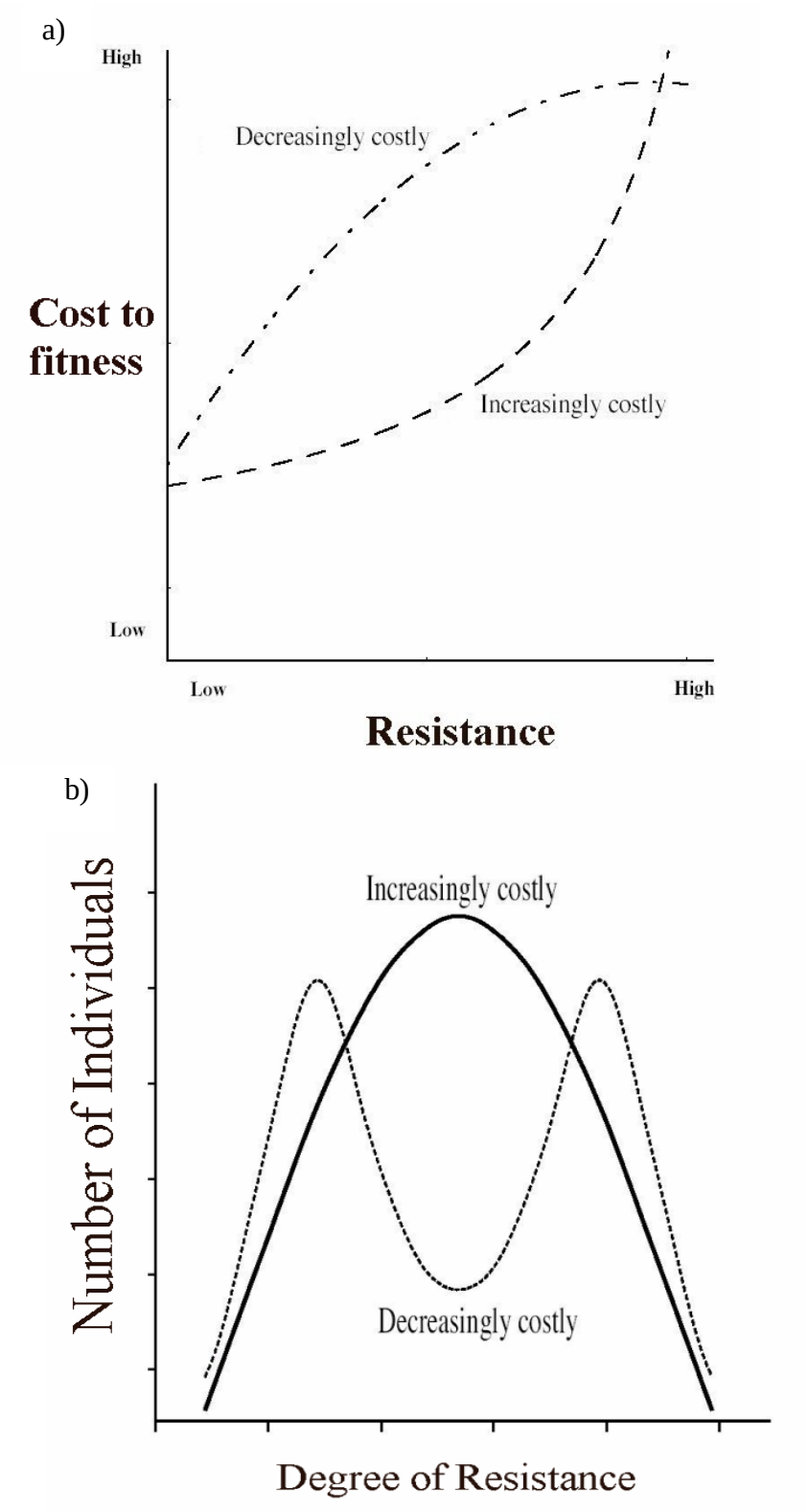

Figure 6.1 - Trade-off curves and the pattern of variation

a) Two potential trade-off curves: an exponential curve with costs increasing with the level of resistance; and an asymptotic curve with costs decreasing with the level of resistance.

b) The resulting pattern of variation with populations: an exponential trade-off produces a gaussian variation around and an asymptotic trade-off produces bimodal variation.

Method

The system: Plodia interpunctella and its granulosis virus

Plodia interpunctella is a well-studied phycitid species of lepidoptera. The larval stage is vulnerable to the granulosis virus (PiGV), a microparasite belonging to the family Baculoviridae. ***Plodia*** suffers a fitness trade-off associated with being resistant to PiGV, with resistant cohorts taking longer to develop and producing fewer viable offspring when compared to susceptible individuals (Boots and Begon, 1993). Bioassays were carried out using a similar technique to the one described in Vail & Tebbets (1990). The virus was prepared through diluting a stock supply with sterile distilled water (SDW). Twelve doses of the virus were administered through thoroughly mixing 4ml of diluted virus into 20g of food. The 12 doses were 0.01, 0.03, 0.05, 0.1, 0.3, 0.5, 1, 3, 5, 10, 30, and 50 μg/g, providing a range of viral challenges to the host. 50 first instar larvae were placed into each diet/dose mixture and resistant individuals were counted as they emerged as adults. All larvae were taken from one inbred, laboratory strain of ***Plodia*** to minimise genetic variation within samples. Altogether there were five replicates: each replicate consisted of the 12 dose levels and 1 control assay (with only SDW). The larvae were maintained at 27^0C in a 16h light: 8h dark regime.

Method of determining the within-population variation in resistance

We apply methods of statistical analysis more commonly used in pharmacological studies of drug metabolism in order to determine the pattern of resistance within the population. In these studies, the genetic variation in a metabolic trait is determined through sampling a population and creating a probit plot from the cumulated

frequency of the levels of trait expression (Eichelbaum & Woolhouse, 1985). The linearity of the probit plot is used to determine the pattern of variation, with linear plots corresponding to a normal (gaussian) pattern of variation while non-linearities are associated with other patterns (Penno & Vesell, 1983; Nakamura ***et al***., 1985). We use an updated method of these probit analyses and include a discussion of the how a pharmacological tool can be used in an ecological setting (Vessel & Gaylor, 1995). Jackson ***et al***. (1989) randomly generated data describing a range of patterns of variation, including normal, log-normal and bimodal distributions. From these data, they found the corresponding shape of the probit line. Therefore we can associate a particular probit plot to its underlying pattern of variation. Here we use logit analysis, which produces equivalent results to probit plots (Crawley, 2002). Using this technique in a laboratory based ecological experiment, such as this one, confers an advantages over its standard use in pharmacological studies as the degree of non-linearity in the logit regression can be accurately determined in our study using simple polynomial regression. This often cannot be carried out in pharmacology studies because of a lack of replication.

Results

The results of the five bioassay replicates are presented in Figure 6.2, showing the number resistant to infection at a range of virus doses. Blocking analysis shows no relationship between the shape of the data and the five replicated blocks (DF = 4, F value = 1.028, P = 0.403). The data was subjected to two forms of analysis: polynomial regression and generalised linear modelling with binomial errors (logit). The complete dataset, with all data points included, was found to contravene the data

requirements for both models. Regarding the polynomial regression, the variance was inhomogeneous and the residuals non-normally distributed. Regarding the generalised linear model, the residual deviance was much larger than the residual degrees of freedom meaning the variance was over-dispersed (quadratic model: residual deviance = 150.0546 on 57 degrees of freedom). Transformation was found not to improve the error and variance distribution and so outliers were carefully removed, with the output of models compared with and without certain outlying data points. Seven data points were eventually removed, solving the overdispersion (quadratic model: residual deviance = 53.7929 on 50 degrees of freedom) and making the error distribution normal and the variance more homogeneous. Therefore the data set used in the analyses contained 53 data points, some of which may be not be visible on Figure 6.2 due to overlapping.

The results of the logit regression are shown in Figure 6.2a. A cubic polynomial logit regression gives the best fit ($Y= 0.203 – 3.6X – 1.42X^2 – -0.76X^3$, DF = 49, T value = -2.53, P = 0.01). The slope of this regression line is steepest at low and very high virus concentrations, and is relatively flat at intermediate dose ranges. According to the previously discussed work of Jackson ***et al***. (1989), this line corresponds to a bimodal pattern of resistance (Figure 6.1b). The accuracy of this cubic fit was tested by jacknifing the logit regression, which examines the influence of outlying data points on parameters. The mean jacknife estimates for the cubic fit were $Y= 0.2065 – 3.54X – 1.367X^2 – 0.728X^3$, and these slope estimates were all smaller than their respective regression predictions by 1.58%, 4.09% and 4.55%. The slightly smaller jacknife estimates were still large enough to be considered as explaining a significant degree of variance (T value = -2.32, P = 0.02 on 49 d.f.) and the jacknifed cubic regression remained the simplest model. Bootstrapping also

indicated that the cubic regression is robust to outlying data points, with the 95% confidence limit for the cubic term having the end points –1.24 and –0.104 and so excluded zero.

The second form of statistical analysis also reveals a bimodal shape of variation. Figure 6.2b shows the number of individuals surviving a viral challenge over a range of doses. The cubic regression line fitted to this data explains 75.7% of error and maximum likelihood analysis shows that the cubic is the simplest necessary model as it is not significantly different to the quartic (L. Ratio = 1.04, p = 0.31) but is a better fit than the quadratic (L. Ratio = 6.83, P = 0.009). The slope of the cubic regression line can be used to determine the proportion resistant over the dose range of the experiment, assuming that individuals resistant to one dose are also resistant to all doses below it. The line slopes steeply through the low dose region of the graph, which means there are a high number of individuals with their maximum level of resistance at low doses. In other words, there is a proportion of the population that is killed at low viral doses, and therefore has a high susceptibility to infection. Over intermediate doses, the slope levels off, signifying that the individuals resistant to these doses are also resistant to higher doses. This means there are few individuals with a maximum level of resistance in this intermediate range. At high dose levels the slope once again declines, indicating there is another distinct proportion of the population susceptible only to these higher doses. The interpretation of both logit and polynomial graphs therefore reveal a bimodal pattern of resistance within the ***Plodia*** population.

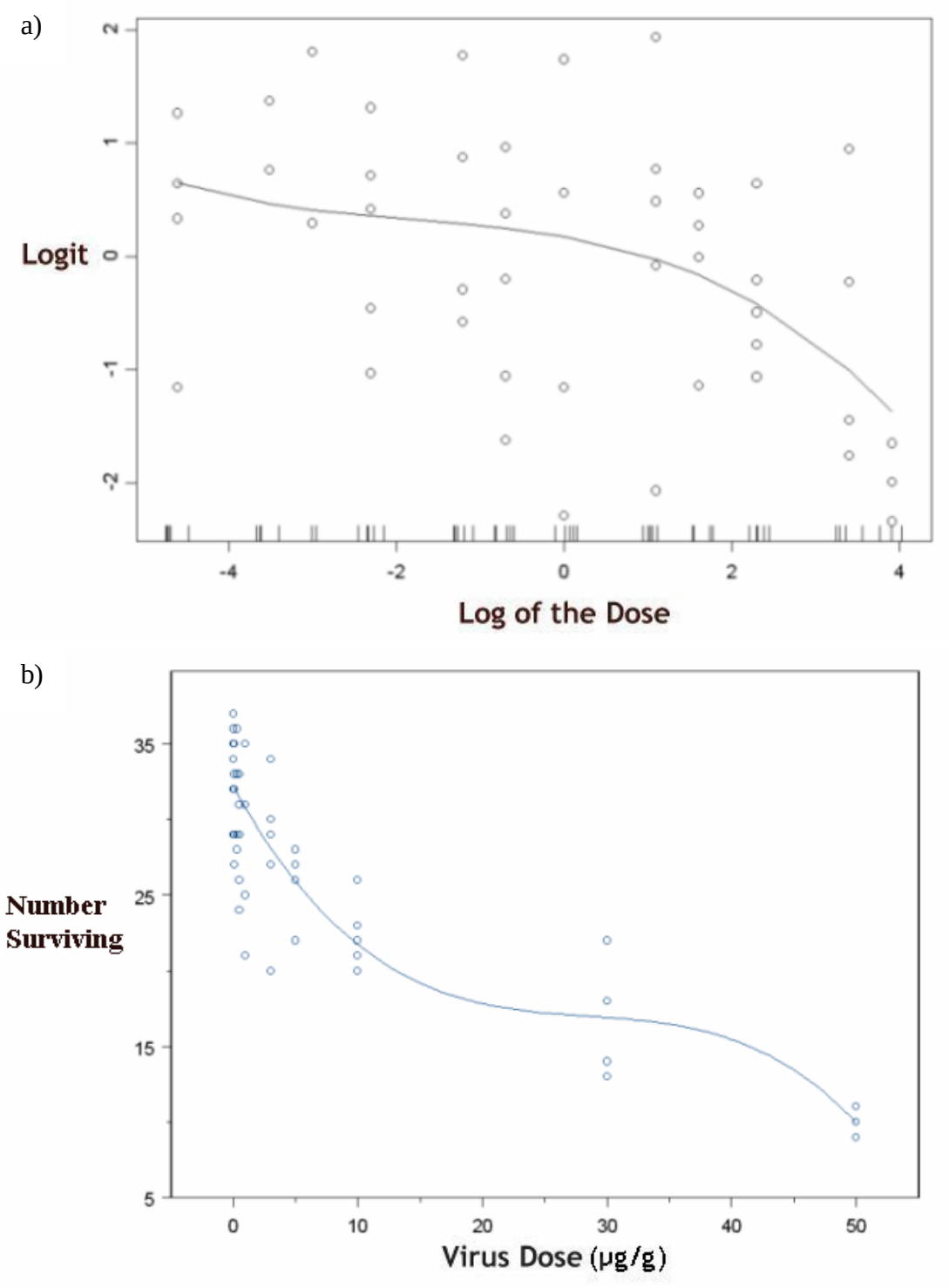

Figure 6.2 – The results of the bioassay

a) Logit analysis. The cubic polynomial regression equation is $Y= 0.202 – 3.6X – 1.42X^2 – -0.76X^3$ (DF = 49, T value = -2.53, P = 0.01). The regression lines' steepness over low and high dose ranges indicates that the pattern of variation is bimodal (Jackson ***et al***., 1989).

b) Polynomial regression. The cubic regression through this data is the line of best fit (DF = 49, T value = -2.49, P = 0.016). Its equation is $Y= 27.5 – 43.73X + 10.3X^2 – 9.26X^3$. The shape of the slope also indicates a bimodal pattern of variation within the population (see Results).

Discussion

Resistance towards a granulosis virus in ***Plodia interpunctella*** has been shown to carry a cost such that there is a fitness trade-off with respect to other aspects of life history (Boots & Begon, 1993). The aim of this study was to infer the shape of the trade-off curve associated with resistance. Using mathematical theory constructed by Boots & Haraguchi (1999) it was possible to infer the trade-off curve by finding the pattern of resistance in a population. A bioassay indicated a dimorphic pattern of resistance, with most individuals possessing either a low or high level of resistance and very few with an intermediate level (Figure 6.1b). Since other possible causes of variation in resistance, such as stochasticity and spatial heterogeneity, have been minimized in this experiment, it is possible that this pattern is caused by the associated cost structure. According to theory, a dimorphic pattern of resistance relates to an asymptotic trade-off curve shape where the rate of costs to fitness decrease as resistance increases (Figure 6.1a).

This paper presents a potential method for studying both the shape of the trade-off curve associated with resistance and the variation within populations resulting from this cost structure. The concept of trade-offs existing between different components of life history is a fundamental aspect of evolutionary ecology, yet the exact nature of such trade-off relationships is very difficult to find empirically (Stearns, 1992). This paper, in conjunction with Boots & Haraguchi (1999), shows that it might be possible to infer the shape of the trade-off curve simply from standard bioassay techniques, using logit and polynomial regression analysis to discover the variation of resistance within a population. Particular patterns of resistance within a species are generated by specific trade-off curve shapes, which are determined by the mechanism of resistance. Therefore estimating the cost structure of resistance using

these methods means that we gain insights into the mechanisms of resistance within the host. This follows because a particular mechanism implies particular cost structures (Boots & Haraguchi, 1999).

The indirect nature of these concepts means the methodology requires careful application and interpretation. Without studies examining the genetic basis to resistance and the variation of such genes in populations, it is difficult to confidently state that the pattern of ***Plodia*** resistance is caused solely by the trade-off curve. Despite the quantitative nature of ***Plodia*** resistance, it is possible that bimodal variation emerged through broad gene-for-gene interactions between ***Plodia*** and PiGV. If this were the case, any within-population variation would be a product of the ratios of corresponding host and parasite genes rather than phenotypic variation resulting from the cost structure. Another potential explanation of the variation is that the relationship between parasite dose and infection is non-linear, generated by transmission and virulence thresholds. However in this experiment, infection was observed at each dose level suggesting the absence of any transmission thresholds within the experimental dose range. Also virulence thresholds, where high parasite loads produce non-linear effects on host mortality, are perhaps more likely to be observed in macroparasitic infections where sublethal effects of infection are commonly used as the measure of host susceptibility. The methods presented in this chapter may be useful in identifying non-linearities in parasite transmission and virulence in well-understood gene-for-gene systems, where the genetic susceptibility within each host strain is uniform.

A final consideration is the potential application of this methodology for sampling the variation of resistance within field populations. Populations can be subdivided into patches, with the population density at each patch dictated by habitat

and interspecific interactions. In this instance, the most suitable method of sampling the variation would involve initially concentrating sampling effort on the largest subpopulation with no recent history of extinction and recolonisation. This approach would allow most of the variation to be captured whilst minimising the effects of spatial stochasticity. Smaller subpopulations might then be sampled to ensure the upper and lower limits of the initial variation discovered were representative of the population as a whole.

For clarity, this work has focussed on the particular trade-off with resistance to parasites. However the general approach of using theory to predict patterns that arise from particular cost structures and then examining the patterns directly at the level of the population has good applicability. Indeed recent general theory (Bowers & White, 2002) suggests that the curvature of trade-off functions, interpreted in terms of accelerating or decelerating costs, have a good general predictive ability beyond host-parasite interactions. Measuring trade-off curves directly will always be difficult, but tightly controlled examinations of population level variation may help us understand fundamental selective pressures.

Chapter 7

General Discussion

This thesis examined two forms of ecological heterogeneity. In terms of spatial heterogeneity, the implications of different spatial structures upon competition and parasite infection were investigated using both insect laboratory systems and probabilistic cellular automata. In terms of life history heterogeneity, novel applications of statistical methods were used to determine the variation of resistance within an insect population.

Methods of Investigating Spatial Structure

This thesis focussed on methods of creating different forms of spatial heterogeneity in laboratory populations of ***Plodia interpunctella*** and ***Ephestia cautella***. The approach involved changing the ability of individual larvae to disperse through a homogeneous food environment. Changing the viscosity of the food medium generated different movement rates and the method was successful because it incorporated two important aspects of the insect's life history: 1) larval movement predominately occurs within and through the food medium; 2) both species are capable of feeding and surviving on a wide range of food types. These factors meant that changing food viscosity would alter larval movement rates and yet not affect their ability to feed and develop. In ***Plodia***, the larval stages critical for determining infection and competition are early instars, which have yet to show full maturation resistance towards the granulosis virus and are highly sensitive to the amount of food available. Crucially, it is these early instars that are most affected by the viscosity of food, with later instars naturally moving out the food to search for pupation sites. Therefore, in this system, increasing the viscosity of food has a clear, significant impact on competition and infection processes. Future work with other systems would require an understanding of the

stages in a species' life history that are critical in determining the dynamics of ecological processes. Then techniques would have to be implemented to change the movement rate of these particular stages. The importance of this is shown in the ***Plodia*** populations maintained on Soft Food (Chapter 4). At the egg stage, populations are intrinsically aggregated because eggs are laid in batches throughout the environment. The rapid movement of hatched larvae soon breaks down this aggregated distribution and a more homogeneous structure emerges. It is this relatively homogeneous larval structure that is important in determining the overall dynamics, not the aggregated egg structure, because competition and infection only occur at the larval stage.

Many previous attempts to generate different population spatial structures in the laboratory have involved plants, either to test directly (e.g. Norris ***et al***., 2001) or to create different spatial distributions for plant parasites or herbivorous species (e.g. Burdon & Jarosz, 1991; Janssen & Sabelis, 1992). Also, the distributions of animal populations in the field are often measured by the position of the plant species known to provide food or habitat to the animal (e.g. Harrison ***et al***., 1988). The advantage of using plants in spatial studies is that they are stationary, so the experimenter can measure their position and easily monitor the state of individuals or patches over time. As a result of this work, much of the emphasis in spatially orientated experimental studies has been on exogenous forms of spatial heterogeneity, where populations are divided into distinct, discontinuous clumps. In contrast, this thesis shows the importance of endogenous forms of heterogeneity, where individual dispersal and local interactions generate different, continuous spatial structures despite a uniform, homogeneous environment. Endogenous heterogeneity is normally difficult to create, as it requires each individual in the population to be monitored and

manipulated. My work solves this problem by using preliminary experiments (as in Chapter 2) to gain an understanding of the relative behaviour of individuals, then inferring the effect of such behaviour at broader cohort and population scales. Exciting future work may involve methods of creating endogenous heterogeneity but in variable, resource patchy environments, such that both endogenous and exogenous factors can be combined.

The methodology developed in this thesis was laboratory based, using insects in microcosms to study population structure. An advantage of this approach is that different spatial structures can be easily replicated. Also the Soft Food populations provide an excellent spatial 'control', because the population structure of larvae is relatively homogeneous. This means both the degree and the effect of patchiness in other food types can be easily estimated in comparison to the homogeneous control. It is the small environment size associated with the insect microcosms that allows homogeneous structures to emerge in Soft Food, because larger scale experiments would be too big for individual larvae to travel through a significant proportion of the food.

The disadvantages associated with this laboratory microcosm approach are that the spatial structure is not directly observed, instead it is inferred from larval movement rates and competition and infection processes. This means that dynamical, temporal changes in spatial structure are missed. Such spatial patterns may have a significant impact upon host and host-parasite dynamics and could explain some of the variation found between replicates in the competition and infection experiments discussed throughout the thesis. To overcome this problem, the direct monitoring of at least some individuals is required, together with destructive sampling of populations and the mapping of individuals. Also the preliminary experiments in

Chapter 2 show there is considerable variation in movement among individuals, with some ***Plodia*** larvae moving 18cm and some 3cm (Figure 2.5a). The implications for such variation upon the fate of individuals are unknown and again require monitoring selected larvae. It is difficult to see how this may be possible in microcosm experiments with highly mobile, short-lived individuals moving through a homogeneous environment. Also the small size of the environment, which offers some advantages, can also be seen as a limiting factor in interpreting the laboratory results. This is because potentially important long-tailed dispersal of both insects and parasites cannot be replicated in 20x20cm food environments. Such dispersal may be important for baculovirus spread, with wind and bird vectors acting to spread virus particles long distances (Entwistle ***et al***., 1983). Adult moths can also move relatively large distances, and this may play a very important role in defining the spatial distribution and dynamics of natural host-parasite populations.

Differences between *Plodia* and *Ephestia* Movement

The preliminary experiments in Chapter 2 show a large difference between the movement rates of ***Plodia interpunctella*** and ***Ephestia cautella***. Over a period of development from first to third instar, ***Plodia*** larvae travelled an average distance of 10.63 ± 0.73cm in Soft Food, with ***Ephestia*** only travelling an average 3cm ± 0.65 (Figure 2.4). The different larval movement rates of the two species may be explained as an evolutionary response to the different oviposition behaviours of ***Plodia*** and ***Ephestia*** female adults. ***Plodia*** females preferentially lay eggs in food that is high in quality, with high larval densities not deterring oviposition (Anderson & Löfqvist, 1996). Therefore it is probable that ***Plodia*** larvae exist in local environments

surrounded by many conspecifics, making competition for food and space intense. A high larval movement rate might then enable individuals to escape local competition and disperse into less stressful competition environments.

It is possible that ***Ephestia*** oviposition behaviour is different to ***Plodia***, as a study of the sister species ***Ephestia kuehniella*** found adult females actively avoid laying eggs in densely populated larval regions (Anderson & Löfqvist, 1996). Females select oviposition sites by detecting the presence of mandibular pheromones in the food, a substance that is released when the heads of larvae make contact (Corbet, 1971). The more pheromone there is in the food, the more larvae are present. Should ***Ephestia cautella*** behave the same way, the lower movement rates of larvae could be considered to be the optimum to avoid competition. A hatched larva would experience competition mainly from kin, as a result of the low number of nearby individuals from other egg batches. A low to moderate movement rate is all that is required to avoid this, with larvae moving into the surrounding, competition-free, environment. Indeed, a high movement rate for ***Ephestia*** might be detrimental, as the more distance individuals travel the greater the likelihood of encountering competition from unrelated individuals.

Competition and Space Structure

Chapter 3 shows that changing the spatial structure of larvae can significantly change the nature of interspecific competition. ***Plodia interpunctella*** was shown to be a superior competitor to ***Ephestia cautella*** in Soft Food (Figures 3.1& 3.2). In this food type, ***Plodia*** larvae outcompete ***Ephestia*** for two main reasons: 1) a faster growth rate allows ***Plodia*** to develop faster and so dominate interspecific encounters; 2) a faster

movement rate allows ***Plodia*** individuals to avoid intraspecific encounters and more rapidly exploit available resources. The importance of movement rate in conferring a competitive advantage is shown in Hard Food, where the movement rates of ***Plodia*** and ***Ephestia*** are similar. Here the competitive advantage of ***Plodia*** larvae disappears, with no significant difference found in the numbers from either species surviving competition (Figure 3.2). Although movement rate determines the outcome of competitive encounters when first instar larvae of both are placed together in the laboratory, in the wild the outcome is determined predominately by founder competition (Allotey and Goswami, 1992). In founder competition, the species that first colonises a region will have the advantage because its hatched larvae will be able to exert asymmetric competition on the eggs and young larvae of a secondary invading species. Therefore the dispersal rate of the two species, although critical in determining the outcome of interspecific competition in my work, has likely not evolved in response to community selection pressure. Rather, it has more likely evolved through the need to avoid intraspecific competition associated with the egg laying behaviour in the adult stage.

Broadly, these results show the importance of dispersal in determining the outcomes of competition between species. The dispersal ability of species has often been incorporated into competition studies by using a competition-colonisation, life history trade-off. This trade-off involves the distinct separation of dispersal and competitive ability, with individuals with high movement rates being weak competitors. However in ***Plodia-Ephestia*** laboratory interactions, it is the movement rate that defines competition, with the species capable of moving the furthest distance being the best competitor. Here, the dominant species is the one best able to avoid

competitive encounters, and this is perhaps true of many competitive interactions between mobile species that share similar life histories and habitat preference.

There has been a large body of work attempting to appreciate the relationship between the coexistence of competitors and population spatial heterogeneity (Bolker & Pacala, 1999; Neuhauser & Pacala, 1999; Pacala, 1986; Weiner & Conte, 1981). The experiments in Chapter 3 show that aggregating the distribution of species can reduce interspecific encounters and increase intraspecific encounters, resulting in the greater survival of the inferior competitor (***Ephestia***). However this experiment only examined small-scale competition over one generation. Therefore larger scale studies are required over longer time periods to understand whether aggregated distributions have the potential to induce long-term coexistence of competing species. Such experiments will reveal whether population aggregation can lead to species segregation and the threshold "arena size" below which coexistence cannot occur (Steinberg & Kareiva, 1997). Measuring the threshold "arena size" and relating it to specific aspects of the insect system will be crucial for broadening the use of the experimental approach, because it might indicate the scale of study required to observe segregation and coexistence in other competition systems. Populations of ***Plodia*** and ***Ephestia*** could be maintained in different food types then destructively sampled to analyse both the overall number of both species and the small-scale spatial structure of larval populations. The small-scale larval structure may also reveal important spatial patterns associated with the dispersal ability of larvae, different adult behaviours and the coexistence or otherwise of competing species.

Infection and Host Spatial Structure

The effect of different spatial structures upon a host-parasite interaction was studied in Chapters 4 and 5. In Soft Food populations, ***Plodia*** larvae movement rates were high, making the population structure relatively homogeneous. This allowed a large proportion of the population to encounter larvae infected with granulosis virus, causing a high viral prevalence in populations (Figure 4.4). The effect of homogeneous host-parasite interactions on the ***Plodia*** host was a significant reduction in growth rate, either due to the evolution of resistance or to sublethal infection. The effect on the virus was to maintain the relatively high infectivity of the initial stock strain (Table 5.2).

In Hard Food populations, ***Plodia*** larvae movement rates were low forcing individuals within populations into overcrowded aggregations. The resulting highly localised nature of infection caused the population prevalence of the virus to be low. However, high contact rates within aggregations caused local infection and multiple infection rates to be very high, explaining the large parasite-induced decline in Hard Food population sizes (Figure 4.6). Aggregated host and parasite spatial structures were also associated with the evolution of lower viral infectivity (Figure 5.1), because strains with less ability to infect hosts maintain a larger number of surrounding susceptible larvae. This causes strains with low infectivity to possess a greater transmission rate compared to highly infective strains, which rapidly infect neighbouring susceptibles and reduce the number of infection opportunities.

These results allow broader conclusions to be made about the methods required to understand the effect parasites have upon host populations. Field populations are often fragmented due to the patchy distribution of suitable habitat, with fragments linked by migration. In such aggregated populations, the overall prevalence of a

parasite may not give a true indication of its effect on the host dynamics. This is because a parasite with low overall prevalence may have a very high local prevalence within individual host patches, due to localised overcrowding and high host contact rates. As a result, the localised mortality and sublethal effects associated with infection may significantly reduce the host population size despite only a small number of patches containing the parasite and the evolution of less infective strains. This idea has been previously expressed in relation to highly virulent parasites, which have a significant effect on host mortality but remain at a low prevalence in the population (McCallum & Dobson, 1995). Such evolutionary mechanisms may be difficult to distinguish from the ecological mechanisms of localised infection within host patches.

Another potential difficulty with studying aggregated host-parasite interactions is the relationship between host evolution and parasite-induced mortality. Should the highest parasite-induced mortality be associated aggregated hosts, we might expect to see significant evolutionary changes in host resistance. However, in this environment, the highest mortality rate is also associated with low overall parasite prevalence. Low population prevalence means the selection pressure for increased resistance is weak for a large proportion of hosts. Also should resistance be associated with a cost to fitness, susceptible hosts will be superior competitors in the absence of the parasite and so further restrict the evolution of host resistance. Therefore spatial heterogeneity may separate the parasite's effect on population size and host evolution, with evolution only occurring alongside low parasite-induced mortality.

The work in this thesis suggests that spatial heterogeneity needs to be incorporated into host-parasite studies if the interaction is to be completely understood. This is especially true when the dispersal of the parasite is associated

with host movement. In the laboratory ***Plodia interpunctella***-granulosis virus interaction, the primary infectious unit is infected larvae. Therefore the spread of the virus through the environment depends directly upon the movement of such infected larvae. This results in a distribution of the parasite that matches the host distribution. Parasite's, whose transmission and spread is associated with free-living stages or vectors, may have a spatial distribution that is independent of the host. In such cases, the spatial heterogeneity in the parasite cannot be inferred from host heterogeneity and so requires separate spatial analysis.

There have been a number of theoretical studies linking the creation of habitat corridors in fragmented habitats to an increase in the spread of disease (Hess, 1996; Keeling, 1999). The work in this thesis suggests that conservation measures restricted to connecting small, overcrowded host patches may render such patches more vulnerable to parasite-induced extinction. Instead, increasing the size of patches, in conjunction with corridor formation, may be the best conservation method. This is because high densities of hosts within a restricted area will lead to high host contact rates and so high rates of direct parasite infection. Increasing the size of habitat patches has the effect of increasing the area available to individuals, reducing both the host contact rate and density, so lowering the parasite infection rate. Habitat corridors will then enable density-dependent emigration from patches in order to prevent host density and infection rate rising, so reducing the probability of parasite-induced, local population extinction.

Variation in Life History Traits: Resistance

Chapter 6 focussed on heterogeneity in the expression of resistance within a population of ***Plodia interpunctella*** towards its granulosis virus (PiGV). A series of replicated bioassays found the mortality rate associated with twelve viral doses and the shape of polynomial regression and logit plots (Figure 6.2) were analysed to determine the shape of variation within the population. A dimorphic pattern was found with most individuals having either a low or high level of resistance (Figure 6.1).

A dimorphic pattern of resistance within the population may have a variety of implications for the dynamics and evolution of the host-parasite interaction. In the absence of a virus, the highly resistant proportion will be outcompeted by susceptible individuals with a faster development time and larger reproductive capacity (Boots & Begon, 1993). Indeed, the variation in resistance found within the stock population is only maintained because the large quantities of high quality food supplied reduce competition between the two classes. An introduction of a virus with intermediate or high virulence will result in the removal of the susceptible proportion with only the resistant individuals persisting (Boots & Haraguchi, 1999). This may cause the mean level of resistance to change quickly, with resistance initially increasing as the susceptible population is removed by the virus, followed by a potential decline in the level of resistance should the virus then be lost from the population.

A number of mathematical and simulation based models have examined the interaction between a susceptible and a resistant class of hosts, together with a non-evolving parasite. Several gene-for-gene coevolutionary models have generally concluded that the presence of a parasite can create a stable polymorphism within the host population (reviewed in Sasaki, 2000). This stability occurs because the

susceptible class maintains the presence of the parasite, which the resistant hosts could not do alone. The consequence of parasite maintenance is that a selective advantage remains for the resistant phenotype, so preserving the host polymorphism (Antonovics & Thrall, 1994; Bowers ***et al***., 1994).

Concluding Remarks

This study highlights the importance of the spatial structure of populations in determining the outcome of competition and infection processes. Laboratory techniques to change the movement of individual ***Plodia interpunctella*** larvae created different levels of population aggregation. Increasing aggregation had the effect of making intraspecific competition more intense, which changed the survival and development of larvae at cohort and population scales. An increase in intraspecific competition when movement was associated with a decline in interspecific encounters, thereby encouraging greater coexistence with the inferior competitor ***Ephestia cautella***. Aggregated population structures were associated with lower granulosis virus prevalence and the evolution of lower infectivity. However, in such populations, the virus had a greater effect on the host because of high, localised infection rates within overcrowded host clumps. Finally, graphical analysis was used to analyse the results of a bioassay with ***Plodia interpunctella*** and its granulosis virus. A dimorphic pattern of resistance was measured, characterised by most individuals possessing either a low or high level of resistance.

Appendices

Appendix One - GV Extraction from *Plodia interpunctella*

Day before extraction…

Make up sucrose gradients – Series of solutions - 40%-65% in 5% gradations. Concentrations made up on weight basis – e.g. 40% solution = 40g sucrose with 60g solvent (60ml). Solvent is either water or TE with 100^{th} volume of 10% SDS (added to reduce aggregation of occlusion bodies). Note- highest concentration may need to be heated to dissolve sucrose (50 degrees C for few hours). Solutions then transferred in series to centrifuge tubes. Volume of tubes around 14ml so 2ml of each concentrations added to each tube, beginning with the highest conc. – results in layers of different solutions with interface lines visible between them. These are left overnight at room temperature for gradients to form.

Step 1…

Before beginning the extraction or handling of the infected cadavers a solution of sodium carbonate is made up. This is used to clean equipment following contamination with occlusion bodies – all glassware is transferred here after use. Concentration should be around 0.05-0.1M. Infected larvae are then ground up in solution of TE or water. Volumes aren't particularly important, as long as the larvae are submerged. A white solution is produced of insect tissues and virus occlusion bodies. The solution is then transferred to eppendorfs - around 2/3 full roughly, using a dropper pipette.

Eppendorfs roughly balanced in Micro Centaur centrifuge so that equally filled pairs placed opposite in the rotor. Virus/insect tissue solution may be redistributed between eppendorfs if needed. Tubes then spun at 3000rpm for 1 minute to pellet most of the insect tissue, leaving the virus occlusion bodies suspended in the

solution. The resulting supernatant is removed using a pipette and transferred into a clean eppendorf. As much supernatant should be removed as possible without taking up the pellet of insect tissue (however, if it is, it doesn't matter, it'll be separated out later). Note – don't block tip of pipette with insect tissue! Could result in rest of pipette becoming contaminated! Note - If more virus is required, more TE can be added to the eppendorfs of insect tissue and these can be whirlimixed and the above centrifuge process repeated again. The resulting virus supernatant can then be added to the supernatant tubes. The removed supernatant then consists of virus solution with the bulk of the insect tissue removed. To pellet the virus occlusion bodies the eppendorfs are spun in the micro centrifuge again (balancing them as before), this time at 13 000rpm for 3 minutes.

Step 2…

The pellets of virus OBs are suspended in 0.5ml of TE or water by pumping the pipette, then removed by pipette from the tubes and transferred carefully to the surface of the sucrose gradient ultra centrifuge tubes. These tubes should be as full as possible – about 5mm from the top – otherwise they may collapse! Before the ultra centrifuge, the tubes should be pair balanced accurately by weighing and if tubes need to be matched the required volume of TE or water may be added. Technically, opposite pairs must be matched to within 0.5g.

Once tubes are prepared they can be transferred to the rotor buckets and the lids screwed on. The buckets are then hooked onto the rotor pins, free hanging. Note – the tubes are Beckman ultra clear centrifuge tubes and the centrifuge is the Beckman XL 100. With buckets attached, the rotor is transferred to the centrifuge – the notches

on the rotor base fit in at right angles to the centrifuge pins. The centrifuge is then set at 20 000rpm for one hour at 20 degrees C.

Step 3…

After centrifuge a white band of virus OBs is visible in the tube. The solution above this band is carefully removed using a dropper pipette, beginning with the pipette tip on the surface and working down, rotating the tube to pick up all surface layers. Care must be taken not to pump the pipette and thus mix up the layers. This solution is disposed of into a bottle to be autoclaved. When there are a few mm of solution remaining above the virus band, small amounts of virus can be seen being sucked towards the pipette – the virus band should now be removed. A new dropper pipette should be used. The tip of the pipette is immersed inside the virus band and the band is drawn off with one motion if possible, rotating the tube to remove OBs from the sides and avoiding squirting back into the tube (Note – to increase purification of the virus, the last step of centrifugation should be repeated on the virus band removed). The separated virus band is then transferred to a new ultra centrifuge tube, not more than 1/3 filling it, taking care not to transfer too much sucrose solution. This is then diluted with TE or water to fill the tube. Mixing of the solution is ensured by pumping the pipette used. As before, the tubes are filled to 0.5mm from the top, pair balanced and transferred to the ultra centrifuge – 15 000rpm for 20 minutes at 20 degrees C.

Step 4…

Following centrifugation, the virus OBs will be visible as a white pellet at the bottom of the centrifuge tube. The supernatant is removed, again by pipette, avoiding

removing as much of the virus pellet as possible. 250µl of water are added to the pellet by pipette and the solution mixed. This can then be added to a container for storage.

Appendix Two – Comparison of Virus Counting Methods

Traditional techniques for calculating the concentration of granulosis virus particles within a solution involved transmission electron microscopy, using 0.01mm depth counting chambers. Such counting chambers are no longer made with the necessary slide part for dark field illumination, so a 0.02mm depth chamber was used to compare the results of electron microscopy to fluorescent activated cell sorting (FACS) methods. Chambers with a greater depth make virus counting more difficult, therefore to reduce the variance the focus was adjusted and several people counted the same sample (personal communication from Dr. Doreen Winstanley at Warwick Horticulture Research International). The methods used are listed below:

1. Vortex and sonicate the virus sample briefly before use. Transfer 2ul of the viral suspension onto the grid of the counting chamber. Press a toughened coverslip firmly onto the sample until Newton rings occur.

2. Count the viral PiGV using a Zeiss microscope fitted with an oil condenser, a 20x Objective and a 10x eyepiece using dark field illumination. Only count the shining particles moving by Brownian motion.

3. Dilute the virus sample to give approximately 100-200 PiGV per large square (64 small squares). Prepare duplicate samples at this dilution. Carry out two counts at each dilution. For each of the 4 individual counts, 4 large squares (i.e. 64 small squares) are counted diagonally across the grid.

4. Use the following equation to determine the total number of PiGV: x 105 x dilution factor

5. Calculate the standard error of the mean for the counted samples.

Heather Rae and Rebecca Finley at the University of Sheffield compared the total concentration of two PiGV strains as calculated by the microscopy techniques above with FACS methods.

Strain	Microscope	FACS
1	9.58E+08	1.36E+09
2	1.46E+09	9.72E+09

The results of the two methods are similar, although more work is required in the future before the output from FACS can be said to be equivalent to standard electron microscopy.

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