The impact of spatial structure and sexual selection on a host/parasite interaction

Submitted by:

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I certify that all material in this dissertation which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

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ABSTRACT

It is only relatively recently that we have begun to focus on the impact of ecological and population level effects on the outcome of host-parasite interactions. This dissertation uses the *Plodia interpunctella* moth/granulosis virus system to examine two distinct questions (1) the effect of spatial structure on parasite transmission and (2) the effect of varying sexual selection on the evolution of resistance. I manipulate the host environment to influence the speed of larval movement in a virus-naive host population in order to produce rare empirical data on the impact of spatial structure on transmission (Chapter 2). Contrary to the simple prediction that more movement would lead to more infection, increasing food viscosity (0-35%) was shown to significantly increase the infection prevalence. I argue that this is due to the extra effort of the host needed to move through higher viscosity foods that is traded off with immunity although it may also result from higher local densities promoting higher transmission rates. Next I explored how changes in the level of sexual selection in a population can influence the evolution of host defence. I found that the resistance to viral infection in the populations evolving under female bias mating sex ratio was significantly lower than that of the male bias populations. I argue that either there is a trade off between investment in reproduction (male mating frequency is higher in female biased populations) and immunity, or that sexual selection may have been more effective in the male bias treatments resulting in offspring with an improved immune system. Since the populations evolved in the absence of disease, my work emphasises

that viral resistance can be influenced indirectly by behavioural traits such as different mating strategies.

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Author's Declarations

All Chapters in this thesis were written by Stephen Richard Sharpe with comments from Mike Boots and Nina Wedell. All data used in this study was collected by Stephen Richard Sharpe in collaboration with members of Mike Boot's and Nina Wedell's laboratory groups at the University of Exeter.

Chapter 1: General Introduction

1.1. Introduction

Previous work has focused on answering questions surrounding spatial structure and its affect on the evolution of pathogen infectivity however it has not yet been determined whether spatial structure affects host movement rates and host infection prevalence directly. As such in Chapter 2 I address the question; how does spatial structure affect host movement rates and host infection prevalence?

Another cause of altered host infection prevalence can potentially be explained by changes in evolutionary sex ratios. Studies have previously focused on how it affects other traits associated with viral resistance however it has not yet focused on how this affects viral resistance between females and males. So In Chapter 3 I address the question; how does sexual selection alter immunity in a host/pathogen system.

1.2. The Host/Pathogen System

The host the Indian Meal moth P. *interpunctella* is a widely known agricultural pest (Simmons & Nelson 1975). Populations are found worldwide, often infesting stored products such as grain. The ability to spread is due partly to their capability to easily adapt to changing environmental pressures and the rapid growth of their populations under high quality food resource, and as such the wide use of grain stores, provides ideal conditions for the exponential growth of populations (Fontenot et al 2012). One of the fundamental reasons *P. interpunctella* is often used as a model system is their fast generational turnover; living ~28 days from egg to adult (Knell et al 1996). There are 5 larval

stages (instars) each identifiable by its size; more specifically the proportion of head to body size (Bjornstad et al 1998). Sex becomes distinguishable as larvae grow older; males are identifiable by a black mark (the males' testes) on their abdomen, which can be seen by eye through its partially transparent skin.

The pathogen *P. interpunctella* granulosis virus (PiGV), part of a subgroup of the baculoviruses, is an obligate killer specific to *P. interpunctella*. They convert host cells and release large amounts of occlusion bodies into the environment (Ebert & Weisser 1997). In *P. interpunctella*, the larvae present symptoms of an opaque white colour attributed to the replication of these occlusion bodies (Tidbury et al 2011). The infected larvae often rupture at the 5th instar releasing free-living infectious particles into the surrounding substrate (Knell et al 1998). Furthermore it has been shown that transmission mainly takes place due to the cannibalism of the infected host larva by an uninfected larva rather than contracting the infection from the environment/substrate, sexually, or from its parents (Knell et al 1998). Larvae are least resistant to infection at early instars (Sait et al 1994) and 3rd instar larvae that are still susceptible to infection combined with the ease of handling are ideal for use in dosing assays (Boots & Begon 1993; Sait et al 1994).

Due to its importance as a pest species, research on the evolution of transmission, virulence and defence in the P. *interpunctella*/granulosis virus model host-parasite system contributes not only directly to agricultural pest management, but also provides transferrable knowledge as a model system for the ecology and evolution of hosts/diseases more broadly, including interactions in humans.

1.3. Spatial Theory

Theory suggests that the frequency and locality of interactions between Infected and un-infected hosts underpin the evolutionary outcomes of viral infectivity (Rand et al 1995; Haraguchi & Sasaki 2000). There is also theory that shows how space can potentially influence the evolution of resistance in the host (Best et al 2011). It has also been suggested that interspecific interactions (that can be manipulated through changes in space) can trigger changes in immunity in response to the risk of infection (Lazzaro & Little 2009; Vale et al 2011). In addition there are a number of theoretical papers showing that spatial structure has implications for ecology in general and host pathogen interactions in particular (Rand et al 1995; Diekmann et al 2000,2005; Law et al 2001; Boots & Sasaki 2002). There is also considerable spatial ecological theory on the importance of space for the spread of disease (Tilman & Kareiva 1997; Keeling 1999). This theory shows the potential importance of spatial structure, but we lack empirical tests. The *P. interpunctella PiGV* system has demonstrated its usefulness in carrying out these tests (Boots & Mealor 2007; Boots et al 2009).

The outcome of transmission also partially depends on the transmission route that the virus takes. PiGV has been known to transmit vertically in small proportions, however the majority of infection occurs by horizontal means (Burden et al 2002). In this case, due to the nature of the virus with its release of occlusion bodies into the surrounding environment, transmission occurs when an uninfected larva consumes the contaminated substrate or through means of cannibalism. When transmission is through direct contact, the transmission is likely to be density-dependent (May & Anderson 1979; Levin &

Pimentel 1981; Bremermann & Pickering 1983; Bremermann & Thieme 1989). Therefore we would expect to see a change in overall transmission as density changes. Whereas sexually transmitted diseases are more likely to be considered frequency-dependent where the level of transmission would change with the number of matings (Bremermann & Pickering 1983; Antonovics et al 1995; Rudolf & Antonovics 2005; Ryder et al 2007), in *P. interpunctella* the main route of PiGV transmission is likely to be through density dependent interactions. This means that by changing host environment/substrate we can influence density and therefore the transmission of virus. Moreover if we can alter the spatial structure of the environment we influence local densities and again as the spatial theory suggests, we would expect to influence transmission. The simplest expectation is that with more local interactions there will be less global contacts and therefore lower transmission, but at the same time less movement may increase local density and increase transmission. It is therefore necessary to carry out experimental studies that directly address the question does altering spatial structure/ host movement rates influence host infection prevalence. I predict that in more viscous food resource, there will be lower host infection prevalence due to fewer contact events, than in less viscous food resource where I expect it to be higher due to more contact events.

1.4. Sexual Selection Theory

There is the potential for behavioral and physiological changes in the host to influence viral resistance. In particular, mating is an important trait that can influence survivorship and immunity such as in the 'immuno-handicap' hypothesis. This hypothesis suggests that females may choose parasite

resistant males as revealed by the quality of males' secondary sexual characteristics such as testosterone and that these signals must be costly handicaps in order to signal male quality (Folstad & Karter 1992). Reproduction and immunity are closely linked also in invertebrates and are associated with high costs and therefore could be traded off with one another (Schmid-Hempel & Ebert 2003). For example, an increased risk of sperm competition may be responsible for changes in sperm production and therefore increased ejaculate quality (Koudelova & Cook 2001). This in turn suggests that there could be a trade off between sperm quality and investment in immunity (Simmons 2012). It has been theorized that the concentration of phenol oxidase (PO) enzyme in the haemolymph can be used as a measure of the ability to mount an immune response, as it is involved in the melanisation reaction; a common response to parasite entry in invertebrates (Söderhäll & Cerenius 1998). However in order to test the relationship between PO and immunity, due to the complexity of the immune system, it requires us to support measures of PO in addition to other measures of the immune response such as lysosome activity, but empirical data linking these measured to reproductive activity has largely been lacking (Adamo 2004). There have been examples that address the effect of mating structure on immunity that have used PO as an immune measure (Zuk et al 2004), but little experimental examination involving challenges to the immune system in relation to varying potential cost of reproduction has been carried out to date. This is particularly true for immune challenges involving naturally occurring pathogens. However, due to the transmission of virus in the *P. interpunctella* PiGV system being mainly through the means of cannibalism of infected individuals, it is possible to examine resistance directly by dosing using virus made of homogenised infected individuals (Boots 1998). The question

how does sexual selection alter immunity in a host/parasite system will be tested by subjecting populations to sexual selection and measuring immunity after subjecting to them to virus. I predict that there will be a higher amount of infection prevalence in female bias populations due to increased male-male competition trading off with immunity.

1.5. Aims and objectives

The aim of Chapter 2 is to determine how spatial structure affects host movement rates and host infection prevalence. To achieve this, our objectives are to find a means of manipulating the spatial structure of the hosts' food resource. Then administer a standardized dose of virus to challenge the larvae, counting the number of infected individuals and using this as a measure of infection prevalence to compare between varying degrees of spatial structure.

The aim of Chapter 3 is to determine how sexual selection affects immunity in a host/pathogen system. To achieve this, populations of moths should be subject to varying sex ratios to influence sexual selection. Several doses of virus should be used to dose the larvae after a sufficient number of generations and then count the proportion of infected individuals to use as a measure of the changing immunity in response to sexual selection.

Chapter 2: Spatial Transmission – Altering host infection by manipulating movement rates in a model host/parasite system

2.1. Abstract

There is little empirical data on the influence of spatial structure on parasite transmission. The aim of this chapter was to identify the impact spatial structure had upon the transmission of a density dependent horizontally transmitted baculovirus (PiGV). I measured the proportion of larvae infected that have been maintained on virus-contaminated substrate of varying viscosities. I expected that the proportion of infected individuals would decrease as the percentage of viscosity was increased due to slower movement of larvae leading to fewer interaction events. In contrast, it was found that infection increases as viscosity increases. I suggest that the most likely explanation is a trade off between immunity and the cost associated with infection risk that can explain this result.

2.2. Introduction

Theory suggests that spatial structure has important implications for ecology and evolution (Diekmann et al 2005). Both spatial and general host parasite theory is particularly well developed in part due to its application to the transmission and evolution of diseases in humans and to agricultural pest control processes (May & Anderson 1979; Bremermann & Thieme 1989). In parasites, theory has shown that spatial structure can influence the parasite evolutionarily stable transmission rates and virulence (Boots & Sasaki 1999; Haraguchi & Sasaki 2000). Spatial theory has also shown that there is selection for increased resistance to parasites as dispersal becomes more localized (Best et al 2011). Combined there is selection of hosts with high parasite defence and parasites with low transmissibility and virulence when transmission is more local (Best et al 2011). The mechanisms that underlie the effect of space on evolutionary outcomes are a combination of host and parasite genetic correlations and spatial ecological processes (Lion & Boots 2010). As a whole this theory has highlighted the need for the collection of more empirical data and in particular manipulative experiments where spatial structure is varied. This will help answer the question does altering spatial structure/ host movement rates influence host infection prevalence.

Transmissibility relies heavily upon the frequency of interactions between individuals (Boots et al 2009) and therefore the number of infected individuals in a population depends on the influence of spatial structure on transmission, transmission type, and genetic correlations. Spatial structure is likely to alter individual movement rates and local population density and thereby impact on parasite transmission by causing varying rates of larval interaction events. It is

clear that environmental conditions may also alter the spatial structure of a host's environment (viscosity and homogeneity) and thereby impact on local population densities and structure. When density is variable, the investment in host immune response mechanisms is increased in response to increasing densities. This is known as density-dependent prophylaxis (Wilson et al 2001). So where possible the density of a population has to be controlled for to allow us to examine the effects of spatial structure on transmission (Knell et al 1998).

While the theory is well developed, there is however a distinct lack of empirical tests to support how spatial structure affects transmission, especially between extremes of social interaction (Boots & Sasaki 1999; Best et al 2011). However, previous experiments have altered the spatial structure of resource/viscosity (soft, intermediate, and hard food; least viscous to most viscous) influencing movement rates and therefore dispersal distance resulting in an increased dispersal distance and viral infectivity in 'soft' (least viscous) food (Boots & Mealor 2007). We can use these protocols to investigate to what degree varying spatial structure influences transmission. Here I use a manipulative experiment examining how varying levels of spatial structure affects transmission. I use a host/parasite system whereby the host (P. *interpunctella*) larva moves through a food resource and a virus (PiGV) is transferred horizontally via cannibalism of infected cadavers. This structure is easily manipulated by altering the amount of glycerol in the food medium to change its viscosity. This in turn alters the host movement rates influencing interaction events between healthy and infected larvae and therefore viral transmission. Once larvae are infected with PiGV, the host turns an opaque

white colour due to internal replication of occlusion bodies, allowing us to easily distinguish healthy and infected larvae during assays.

2.3. Methods

Summary

- Prepare the virus using Extraction and Purification techniques.
- Dose healthy individuals, incubate and freeze to produce the infected cadavers for the introduction into populations.
- Prepare the food resource according to the host environment design.
- Rear the healthy larvae on pre prepared food.
- Carry out the spatial assay as outlined in the methods below.
- Carry out the appropriate statistical tests to see if spatial structure affects transmission of virus.

Virus Preparation - Extraction and Purification

Sucrose Gradient

Sucrose solutions (100g of each) were made of concentrations spanning from 40% to 60% at 5% intervals. This was done by measuring amounts of sucrose (65, 60, 55, 50, 45, 40g each) into 6 petri dishes ready for use later. Sodium Dodecyl Sulphate (SDS) 1% solution was made by boiling 300ml of distilled water in a single beaker then removing the distilled water from the heat, adding 3g SDS by stirring in slowly to avoid the forming of bubbles. SDS solution was then decanted into 6 separate conical flasks in the following order; 35ml for the 65% sucrose solution, 40ml for the 60% solution, 45ml for the 55% solution, 50ml for the 50% solution, 55ml for the 45% solution, 60ml for the 40% solution. Then the sucrose was added in the following order; 65g of sucrose to 35ml of SDS to make the 65% sucrose solution, 60g of sucrose to 40ml of SDS to make the 60% solution, 55g of sucrose to 45ml of SDS to make the 55% solution, 50g of sucrose to 50ml of SDS to make the 50% solution, 45g of sucrose to 55ml of

SDS to make the 45% solution, 40g of sucrose to 60ml of SDS to make the 40% solution. These were stirred carefully to dissolve the sucrose without bubbles forming, and then layered into ultracentrifuge tubes using a pipette to dispense 1 ml of each solution onto the surface of each previous solution starting with the highest concentration (65%) at the bottom of the tube and finishing with the lowest concentration (40%) at the top. These were then left overnight to allow the sucrose to diffuse into a continuous sucrose gradient ranging from 65% - 40%.

Virus Extraction

Frozen infected larvae (1g) were deposited into the lower section of a glass tissue grinder and distilled water (~2ml) was added. The larvae and water was repeatedly compressed for 2-3 minutes to release the occlusion bodies through rupturing the larvae. The resulting white opaque liquid was pipetted into 1.5ml Eppendorfs. This solution was then centrifuged to make a pellet (larval tissue) and the resulting supernatant pipetted into new 1.5ml Eppendorfs and the pellets discarded. The supernatant was then ultracentrifuged at 9464 RCF for 3 minutes, this time the resulting supernatant was discarded without disturbing the pellet. The pellet was then resuspended by adding 0.5ml distilled water and disturbed to mix. From two extractions, 1g of frozen infected larvae should produce approximately 3ml non-purified extracted virus solution which can then be frozen in a -20°C freezer. This is then ready to be purified.

Virus Purification

The non-purified virus solution was removed from the -20°C freezer and thawed

at room temperature. These Eppendorfs were then placed in the sonic bath for 1 minute and placed on a whirlimixer for 15 seconds to assure solution homogeneity. The sucrose gradients (made previously for extraction) were placed in centrifuge tubes in the Beckman Ultracentrifuge sample rack. Then 2ml of non-purified virus solution was pipetted slowly onto the surface of the sucrose solution. This made a total of 8ml of liquid (2ml non-purified virus solution and 6ml sucrose solution) per tube. These were then pair balanced by weighing each tube using the OHAUS Explorer Pro scale in mg then matching similar weighted tubes, extra non-purified virus solution was added to the lightest tubes so that they then weighed the same as the heaviest (+/-10-20mg). These were reweighed and the new weights recorded in mg for reference. The ultracentrifuge was then turned on and opened, the red Beckman Ultracentrifuge 8-hole rotor fitted into the rotor base in the ultracentrifuge compartment. The pair balanced centrifuge tubes were then placed into the rotor holes in opposing positions and the rotor cover placed over the top. The ultracentrifuge door was then closed and the vacuum button pressed triggering the vacuum process that took approximately 30 minutes to reach <75microns. The settings used were 22400 RCF for 1 hour at 20°C with no brake selected for deceleration and once the vacuum had fully formed the start button was pressed. When the program had finished the vacuum button was pressed to pressurize the compartment and door opened. The white band produced at approximately 55% was the band of purified virus. So the solution above was removed carefully using a pipette, the tip replaced and then the virus band extracted into a new 5ml tube. For every 1ml of purified virus, 4ml of distilled water was added, shaken thoroughly and then distributed equally into pairs of centrifuge tubes. These were then pair balanced by adding water to

compensate for the difference in weights. The ultracentrifuge was prepared again and set to 12600 RCF for 3 minutes at 20°C and the brake set to on. The start button was pressed after the vacuum had formed. Once the program had finished, the vacuum was released and the centrifuge tubes removed using forceps to place them in the rack. The supernatant was carefully removed (containing the water, SDS, Sucrose solution). The centrifuge tubes were weighed and virus pellets calculated from this. Water was then added to make a 50% concentration purified virus solution and was mixed thoroughly with the distilled water in the centrifuge tube. The 50% purified virus solutions were then mixed in a suitable container and deposited into Eppendorfs that were then frozen at -20°C.

Infected Cadaver Preparation

To replicate a natural transmission pathway, 1000 3rd instar larvae were collected and dosed in 90mm petri dishes with a 0.25% virus solution. Droplets (~2µl) of solution were pipetted using a µl micro-injector into the petri dish of pre collected third instar larvae covering the base and sides. A double-layered square of blue roll big enough to cover the lid of the petri dish was cut for each dish and these were soaked in water. They were then used to cover the top of each petri dish and the petri dish lid placed on top to seal the edges. They were then left for 20-30 minutes or until all larvae had consumed half of their body's length of solution indicated by the blue dye. The remaining blue dye was soaked up with blue roll and then 5g of normal quality food (enough not to limit growth) was placed into each 90mm petri dish and resealed. These were then left for 10 days incubated at 27°C with 16:8 hour light/dark cycle to allow for larvae to develop symptoms of infection. Each of the infected larvae were weighed and ~12mg of larvae distributed into each of the 300 Eppendorfs and

frozen at -20°C.

Host Environment Design

To manipulate host movement rates of P. *interpunctella* larvae through the environment that they have inhabited, it is necessary to alter the viscosity of the food. Previous research has focused on changing viscosity by altering amounts of glycerol in proportion to dry cereal mix (Boots & Mealor 2007). The food mix consists of a cereal mix; bran, ground rice, and ready brek, a source of protein; brewers yeast, a fast release energy source; honey, a fungicide and antibacterial; Sorbic Acid and Methyl Paraben, and glycerol to alter viscosity. These ingredients are homogenously mixed to ensure that the only limiting factor on larval movement is the viscosity of resource. This ensures that each healthy individual has an equal probability of making contact with infected cadaver only varying between treatments. Ten food types of varying viscosities were made covering a range of between 5% to 50% at 5% intervals. These were then frozen at -20°C to kill any eggs or larvae that may have infested the food during production.

Host Rearing

Host larvae are particularly susceptible to infection at 3rd instar of their development but also have a partially developed immune system (Sait et al 1994). This is ideal for assays using PiGV as it allows for variation in the number of infected individuals. Host larvae are reared on a high quality food mix designed to reduce the probability of infection occurring in populations from the quality of the food influencing immune response. Larvae are reared in this environment from eggs for ten days under a consistent temperature of 27°C and 16:8 hour light/dark cycle allowing them to grow to the 3rd instar of their life

cycle.

Spatial Assay

A total of 10 batches of food were made, 1 batch of each food treatment, 5%-50% and then were used to fill 10 60ml pots per treatment with 20g of the treatment food. 2000 3rd instar larvae were collected and then 20 larvae were placed in each pot, covered with muslin and lid, and left for 48 hours to disperse through the food (equal dispersal allows us to measure the transmission rate) at 27°C and 16:8 hour light/dark cycle. The contents of the pre prepared Eppendorfs of infected cadavers were transferred using forceps onto the surface of the pots of food (1 Eppendorf = 12mg infected cadavers per pot) and the pots resealed. These were left for 48 hours under the same conditions as before and then the infected cadavers were removed using forceps and the pots placed back into the incubator until larvae show symptoms of infection (~7-10 days later). The pots were then frozen and the number of healthy and symptomatic larvae were counted and recorded.

Statistical analysis

Statistical analyses were carried out using R (3.1.1 GUI 1.65 Mavericks build (6784)) for Mac OS X GUI. I used a general linear model (GLM) with binomial error distribution for the analysis of proportion of infected larvae. The independent variables in the model were treatment (viscosity) and block, with block treated as a random effect. Model simplification (using analysis of variance with Chi square test) was then used to reduce the model (2.6. Appendix – Model A) by removing non-significant variables one by one.

2.4. Results

Observation

Through observation of larval movement between viscosities, there was a noticeable change in the way larvae moved. Larvae in food types 5% - 35% were able to move through the resource and larvae in food types 40% and above moved only on and around the surface of the food resource, or were not able to move at all due to the food being too sticky. Therefore, as my question looks to investigate the effects of larvae movement through the food resource, I have split the data into two and analysed separately with particular focus upon 5%-35% Resource Viscosity (where Larvae move through the food) to answer the question.

Proportion Infected

5%-35% Resource Viscosity

Following model simplification of our model (2.6. Appendix – Model A) encorporating treatments 1-7 (5%-35%), using analysis of variance between models with the Chi-square test to remove non-significant variables from the model. When removed from the model, overall viscosity (5%-35%) had a significant effect on the proportion of infected individuals (χ^2=3.9,df=1,p=0.05) (Figure 1.1).

40%-50% Resource Viscosity

Following model simplification of our full model (2.6. Appendix) this time encorporating just treatments 8-10 (40%-50%), using analysis of variance between models with the Chi-square test to remove non-significant variables from the model. Here the overall viscosity (40%-50%) had no significant effect on the proportion of infected individuals (χ^2=0.73,df=2,p=0.4) (Figure 1.2).

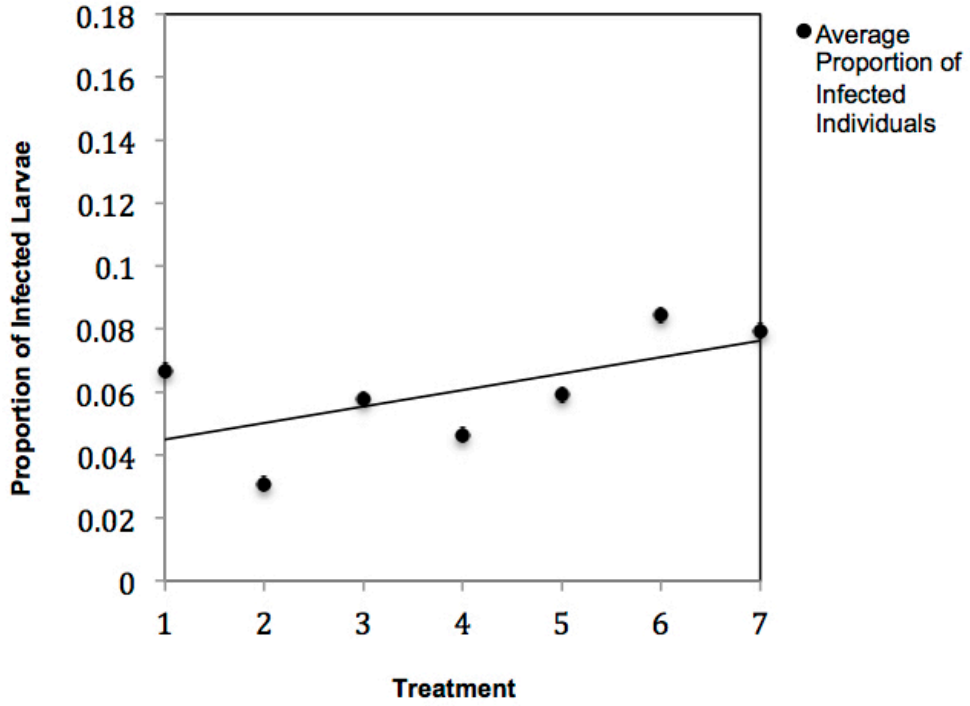

Figure 1.1: Scatterplot of mean proportion of infected larvae at each treatment. Treatments are: 1=5%, 2=10%, 3=15%, 4=20%, 5=25%, 6=30%, 7=35% viscosity (glycerol to cereal mix ratio).

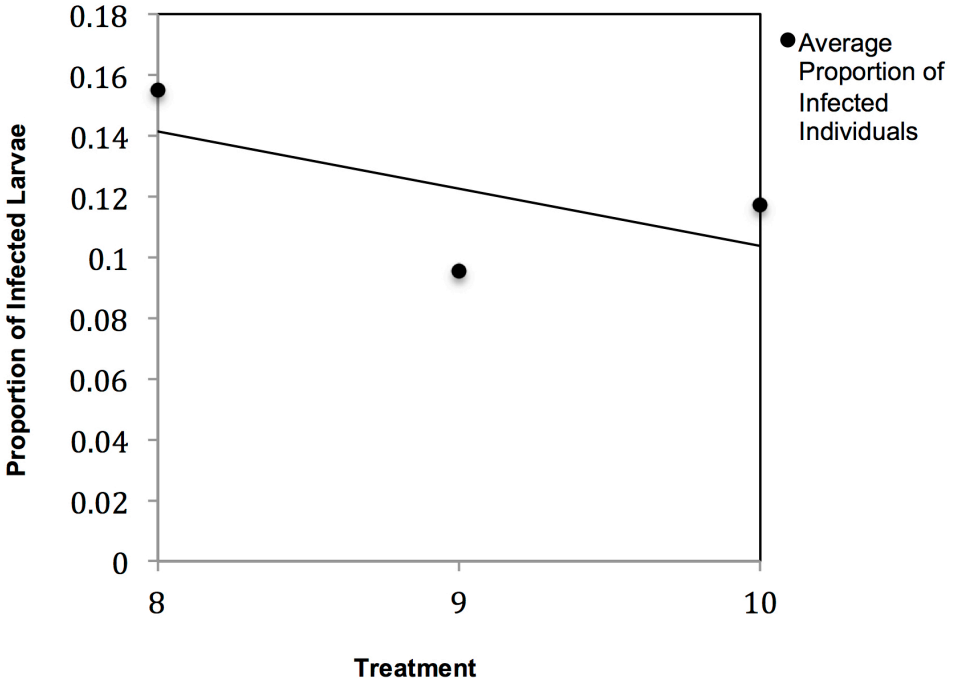

Figure 1.2: Scatterplot of mean proportion of infected larvae at each treatment. Treatments are: 8=40%, 9=45%, 10=50% viscosity (glycerol to cereal mix ratio).

2.5. Discussion

I found that as viscosity increases between 5% and 35% so does the proportion of infected individuals (Figure 1.1). As such the key result is that populations in more viscose media with less movement, show more infection (Figure 1.1). This is counter to what was expected since naively, more movement is expected to produce more contact events and therefore opportunities for infection. We therefore need to understand how our manipulation led to more infection when there was less movement. This effect saturated at higher viscosities, but was nevertheless a clear insight from our results.

A possible cause of our results is that in lower viscosity resource, there is a higher risk of per contact interactions and larvae prioritize investment in better immune defence. Transmission increases until the viscosity of the food resource directly limits the ability of larvae to disperse through the food (40%-50% viscosity). This level of transmission is then sustained for the remainder of the higher viscosities measured, the dispersal rates staying relatively the same as larvae are moving on the surface of food resource (Figure 1.2). Here I suggest that the physical exertion of moving across the food is very high and consequently costly and trades off with immunity. The result is that it overrides the effects of costs associated with the risk of per contact interaction, shown by the elevation in the proportion of infected individuals.

Stress has long been theorized to have a role in influencing immunity in both vertebrates and invertebrates (Ottaviani & Franceschi 1996). This has previously been shown in model systems such as *Mytilus edulis (*the Blue mussel) showing that hemocytes are produced in response to stress (Stefano et

al 1990) and similarly in *Anodonta cygnea* (the Swan mussel) where heat stress increased levels of serotonin (Stefano et al 1979). Both haemocytes and serotonin are involved in the immune/stress response (Stefano et al 1979,1990). So it is plausible that stress (the risk of per contact interaction) is acting upon the immune system of *P. interpunctella* through a similar mechanism (Schmid-Hempel & Ebert 2003). So transmission is the product of both the number of contacts and the per-contact risk of an infection occurring. Here I am suggesting that the per-contact risk of infection increases in less viscose foods. In this situation larvae prioritise investment in the immune system. My results suggest that the increase in per-contact risk is such that it overrides the higher number of contacts, potentially explaining the higher infection frequency in more viscose populations.

Another possibility is that local movement leads to higher local densities with all of the individuals clumped together. In this context there is the chance of higher secondary infection occurring. However in our experimental system we would expect that the infections often occurred through virus contacted in the food rather than secondly from other infected hosts. As such we would have expected more contacts with virus in the less viscose populations with more movement. It is possible that the virus concentration was so high that it reduced the impact of movement on contact with the pathogen, thus reducing the difference between the treatments of this primary route of infection.

As a whole our results provide rare empirical data on the impact of spatial structure on virus transmission. In the end the approach may not have been able to successfully manipulate the transmission dynamics in a way that mirrors the classic assumptions of the theory. We found a counterintuitive result that

may result from our experiment simply stressing the individuals rather than manipulating their spatial structure in the way in which we initially set out to do. These findings emphasize the difficulty in designing appropriate spatial experiments, but given the lack of empirical data, there is a real need for such experiments.

2.6. Appendix

Model A

Proportion infected~Viscosity+(1|Block)

Chapter 3: Sex Bias – How does sexual selection alter immunity in a host/parasite system?

3.1. Abstract

Although there is considerable theoretical interest in the role that parasites may play in sexual selection, little is known about how different mating strategies impact on the evolution of disease resistance. Here I aim to explore the impact of varying levels of sexual selection, created by manipulating mating frequencies, have upon virus resistance. I found that resistance to viral infection is significantly lower in populations evolving under a female biased adult sex ratio where all males are able to reproduce at least once. In contrast, viral resistance is higher in populations evolving under a male biased mating sex ratio where there is more male-male competition over females (not all males get to mate), meaning there is higher sexual selection potentially resulting in higher quality and more resistant individuals. I argue that either there is a trade off between investment in reproduction and immunity and/or that sexual selection may have been more effective in the male biased populations, resulting in offspring with a more effective immune system. Since the populations evolved in the absence of disease, my work emphasises that viral resistance can be influenced indirectly by behavioural traits such as different mating strategies.

3.2. Introduction

Theory suggests that mating and immunity are closely linked and that each plays an important role in influencing the evolution of the other (Lawniczak et al 2007). Both mating and immunity are important fitness traits and are likely to be associated with different costs (Fedorka et al 2004). Furthermore, these costs may contribute to a trade off between investment in reproductive traits and immunity, potentially explaining changes in immune response with varying reproductive strategies (Simmons 2012). Investment in reproductive traits (e.g. number of sperm) could be responsible for altered resource distribution that influences the changes in immunity between populations (Ingleby et al 2010). It has been shown that varying levels of promiscuity and sperm competition in particular are responsible for some of these changes (Parker & Ball 1998). For example, a trade off between immunity and the number of matings has been previously identified in *Gryllus texensis* (the Texas field cricket) (Adamo et al 2001). Little work has been carried out to date to examine whether different mating systems corresponding to varying levels of sexual selection and/or sexual conflict can influence virus resistance. To address the question how does sexual selection alter immunity in a host/parasite system? Empirical measures of immune response are required to explore the relationship between varying levels of sexual selection and sexual conflict and determine how they affect host immunity.

Previous research has focused largely on exploring the pressures of varying sexual selection by manipulating mating frequencies to see how this influences behavioural and physiological responses of individuals and populations (Ingleby et al 2010). By varying the adult sex ratio, mating patterns can easily be

manipulated, whereby the number of successful copulations will vary depending on the ratio of males to females. In female biased populations females mate fewer times compared to females in male-biased populations due to intense male courtship attempts. In contrast, males mate more often in female biased than in male bias populations as they endure less male-male competition and have an excess of females to mate with (Ingleby et al 2010). This in turn means there is greater sexual selection in male-biased populations due to greater variance in male mating success than in female biased populations (Willis 2015). The higher frequency of female matings in male-biased populations will also result in increased sperm competition (Simmons et al 1999) and these males have increased their investment in sperm production (Ingleby et al 2010). An increased investment in sperm production can trade off with the fitness and immunity of offspring (Lawniczak et al 2007; Triggs & Knell 2012).

To empirically test the theory that sexual selection will impact on disease resistance, I subjected *P. interpunctella* populations that have been evolving under different adult sex ratios to a species-specific virus in order to obtain a direct measure of virus resistance. This will directly test whether sexual selection influence immunity in this moth species and allow me to compare my results with previous research that has discovered higher PO concentrations in the same male-biased populations of (McNamara et al 2013) as the ones examined here.

Our understanding of insect host defence is becoming increasingly complete as new components of the host immune system are identified. These defences

comprise phagocytosis, melanisation, synthesis of extracellular matrix, adhesion cells, recognition molecules, reactive intermediates of oxygen and nitrogen, proapoptotic molecules, pro-inflammatory cytokines, and antimicrobial peptides (Nappi & Vass 2001; Tunaz et al 2003; Bulet 2004; Nappi & Christensen 2005). The melanisation reaction is recognised as a common response to parasite entry in invertebrates (Söderhäll & Cerenius 1998). It involves the deposition of melanin around a damaged tissue or foreign object (Nappi & Christensen 2005). Phenoloxidase (PO) an activating enzyme plays a key part in this reaction assisting in the production of quinines that are then polymerized to obtain melanin (González-Santoyo & Córdoba-Aguilar 2012). The products of this reaction are thought to play an important role in the immune response of individuals (Marmaras et al 1996). As such the concentration of circulating PO has been used as a measure for pathogen resistance in *P. interpunctella* moths (McNamara et al 2013). These authors found that in female-biased populations circulating PO concentration is lower than in male-biased populations when challenged with a bacterium. They speculated that the difference in PO levels may possibly be due to a trade-off between immune investment and reproduction due to increased male mating demands in female-biased populations.

The concentration of PO corresponds with varying immune response to bacteria in *P. interpunctella* (McNamara et al 2013). However it has been shown that measures of PO concentration are not correlated with direct measures of immune response to the DNA virus in our system and therefore is not involved in virus defence in *P. interpunctella* (Saejeng et al 2010). Therefore in order to determine the potential for sexual selection to affect virus resistance a direct

measurement of resistance needs to be used. This can be achieved by counting the number of infected individuals after dosing with PiGV (we can use this as it is an obligate killer; once it has infected the host it kills it. Therefore we can be sure that individuals are either infected or healthy rather than confusing them with infected and recovered individuals) (Miller et al 1983; Boots & Begon 1993; Ebert & Weisser 1997). As such my study directly addresses the question of how sexual selection impacts on resistance to a natural pathogen. It therefore builds on the previous studies that used a single measure of the immune system: PO (McNamara et al 2013).

3.3. Methods

The host *P. interpunctella* is a widespread agricultural stored product pest (Simmons & Nelson 1975). Populations are often found worldwide, infesting stored products such as grain. The ability to spread is due partly to their capability to easily adapt to changing environments and the rapid growth of their populations under a high quality food resource. The wide use of grain stores provides ideal conditions for the exponential growth of *P. interpunctella* populations (Fontenot et al 2012). Their biology allows us to use this system to raise evolving population and selection lines over a large number of generations to test evolutionary hypotheses. Theoretical work has focused on investigating the evolution of resistance to the species-specific virus PiGV in its host, *P. interpunctella* (Miller et al 2006). This virus, a subgroup of the baculoviruses, is an obligate killer meaning that they grow inside the host, converting host cells and releasing large amounts of occlusion bodies into the environment (Ebert & Weisser 1997). In *P. interpunctella*, the larvae present symptoms of an opaque white colour attributed to the replication of these occlusion bodies (Tidbury et al 2011). However the virus has been shown to transmit mainly through the means of cannibalism of infected individuals, rather than from consuming free living infected particles that have contaminated the food resource (Knell et al 1998). Therefore the homogenisation of infected individuals made into a palatable solution can be used to dose other individuals. This allows for a direct measurement of immune response to the virus. This measurement has been used to identify that resource limitation has a significant effect on resistance to virus (Boots & Begon 1994). Varying spatial structure of the food resource that the larvae live within has also been shown to influence the transmission of virus and social structure of the population that subsequently influences the evolution

of viral resistance (Boots & Mealor 2007). Hence this is why it is important to keep food quality and population structure homogenous when setting up experiments to quantify the impact of reproduction on immunity.

Summary

- Prepare the selection lines according to the food resource design, host rearing, and the relaxing the selection lines sections.
- Prepare a purified virus stock as per the Virus preparation – extraction and purification section on pages 18,19,20,21 (Chapter 2).
- Carry out serial dilutions to obtain a range of virus doses.
- Prepare larvae for dosing, dose, then use destructive sampling ~15 days after when infected larvae would become symptomatic.
- Follow the life history section to take life history trait measurements.
- Follow the statistical analysis section to see if there is a difference of infection prevalence between female and male bias selection lines.

Virus Preparation - Extraction and purification

For Sucrose Gradient, Virus Extraction and Virus Purification methods please refer to pages 19 and 20 (Chapter 2).

Serial dilutions

A series of dilutions were necessary to be made to cover the lethal dose values from 0 to 100 according to previous dosings of *P. interpunctella* stock populations. The stock virus which had been diluted to a 1:1 ratio with distilled H2O was further diluted to make a 2.5% solution by adding 19m of distilled water per 1ml of stock virus (1:1). 1ml of 2.5% was then diluted with 9ml of

distilled water to make a 0.25% solution, that was then diluted in the same ratio for 3 more dilutions making in total 5 doses of virus solution excluding the stock virus; 2.5%, 0.25%, 0.025%, 0.0025% and a control solution (just distilled H^20, sucrose, dye). These 5 doses were then aliquoted into 0.5ml Eppendorffs and subsequently were ready to be used for dosing the sex bias selected lines.

Food Resource Design

The sex biased selection lines were raised on a similar food resource to that in Chapter 1. This time the cereal mix consisted of just bran to make it easier for identification of individuals and again used glycerol to maintain a consistent viscosity, however it followed this design whereby protein (Brewers yeast), fungicide (Sorbic Acid), antibacterial (Methyl Paraben) and a simple carbohydrate (Honey) were all provided in a plethora to maintain a high quality of food resource to keep larvae healthy and prevent limitation of life history traits. The bran was cooked in 200g batches to kill any mites that may have infested it. It was cooked for 3 minutes at high power on an 800W microwave, then stirred thoroughly (to prevent burning), and was then cooked again for a further 2 minutes. The bran was added to a large Tupperware container, 400g of bran was added per container. The rest of the dry ingredients were then added; 6g of methyl paraben, 6g sorbic acid and 160g of brewers yeast. A further 200g batch of bran was added, before measuring out 200ml glycerol and 200ml of honey in a measuring cylinder. The wet ingredients were then added to the dry mix and stirred thoroughly. A final batch of 200g of bran was cooked, and added to the mix, again constantly stirred to ensure a homogenous mixture of all of the ingredients. This is key to ensure all larvae have access to the same ratio of ingredients for consumption of the same nutrients. It was then left

to cool down before being stored in a fridge at 2C-5C ready for rearing the host larvae.

Host Rearing

To maintain the sex-biased populations, we ensure that the only difference between all of the populations is the adult mating sex ratio. Three replicates of two different treatment populations were established from a stock population and raised for ~100+-5 generations. These treatments were female-biased (FB, N=3) and male-biased (MB, N=3) populations that were maintained by manipulating the adult mating sex ratio at 3:1 females:males and 1:3 females:males respectively. For the FB populations, from each of the three replicates we collected 120 female and 40 male 5^{th} instar larvae at random and housed in sexed pots (1L) until eclosion. Upon eclosion, adult moths were introduced together in an egg collector (modified stock pot with black mesh across the bottom, taped into a funnel which allows eggs to be collected in a conical flask underneath). They were left to interact, mate freely and lay eggs for 3 days. Eggs were collected during this 72 hour period, transferred to new medium in stock pots and allowed to develop for the next generation. MB lines were managed in the same way, except larvae were collected at a ratio of 1:3 females:males (i.e. 40 females and 120 males). As we are only changing one factor in the experiment, over time it allows for experimental evolution to occur in response to varying mating sex ratios. Larvae are all fed *ad libitum* to ensure no larval competition or stunted growth arises from any food shortage in each of the evolving populations.

Relaxing the Selection Lines

To relax the selection on sex ratio for the next generation of moths, we began by collecting 90 larvae of each sex from each of the 6 experimental populations, to make an equal sex ratio population that were then housed in single sex pots. Upon eclosion into adults, the moths were placed into an egg collector for 3 days to produce enough eggs to move onto the next generation as described above. The resulting (F1) eggs were then placed onto larval medium so that they can develop into 5th instar larvae and again 90 larvae of each sex for each population were identified, collected and placed into single sex pots. Eggs (F2 generation) were then collected in the same fashion for 3 days, however larvae were collected every 24 hours so that we could more accurately ascertain larval age at the time of dosing. The resulting F2 eggs were then placed within a 500ml pot with larval medium and grown to third instar (11-14 days) ready to dose.

Dosing Preparation

Square petri dishes (25 well) were prepared by sprinkling larval food medium into every well *Ad libitum*. A third full will provide plenty of food for the 3rd instar larvae to develop into 5th instar larvae without limiting development. Then squares of blue roll were cut, at double thickness and larger than the 90mm petri dishes and the square petri dishes. These were then to be used during dosing and after dose rearing.

Dosing

In order to produce a value which will constitute a direct measurement of resistance between populations as previously trialed, we must then dose the

sex ratio populations with the virus solutions and compare the resulting ratio (infected:healthy) between pots within lines. To do this we thawed the control and virus solutions at room temperature ready for use during dosing. I then pipetted drops of solution ~2µl into a petri dish (with larvae in) using a micropipette, then soaked a prepared square piece of blue roll in water. This was then used to cover the top of the dish placing the lid on top to seal the edges. This was left for 20-30 minutes or until larvae had consumed half of their body's length of solution (to standardize virus consumption), then the individuals were transferred using forceps, into separate wells in the prepared square petri dishes for growth to 5th instar.

Destructive Sampling

F2 larvae were checked ~15 days after dosing for symptoms of infection by PiGV. Both the number of healthy individuals (5th instar and pupae) and the number of infected individuals were counted and recorded ready to convert to a proportion for statistical analysis. Infected moth larvae at 5th instar appear white and are noticeably different to a healthy 5th instar larva.

Life History

50 Larvae were collected from each replicate (3 replicates per selection regime) and transferred to 25 well square petri dishes (1 larva per well) pre filled with normal recipe food (see food resource design). These were checked for pupation once a day until each individual had either died or pupated, and the time to pupation recorded to record development time. Once pupated the pupae were then weighed with the OHAUS Explorer Pro scale in mg two days after to allow for hardening and easier handling to provide a measure of body

size.

Statistical analysis

Statistical analyses were done using R (3.1.1 GUI 1.65 Mavericks build (6784)) for Mac OS X GUI. I used a general linear model (GLM) with binomial error distribution for the analysis of proportion of infected larvae. The independent variables in the model were dose (treated as a factor), selection, dose*selection, and replicate. Model simplification (using analysis of variance with Chi square test) was then used to reduce the full model (3.6. Appendix – Model B) by removing insignificant variables one by one.

This was used to determine whether these variables had a significant effect on the proportion of infected larvae (when dosing 50 individuals per dose per selection regime) between the 2 selection regimes (FB and MB), 4 doses (0.0025%, 0.025%, 0.25%, 2.5%), and 3 replicates per selection regime.

Life history data variables were the time to pupation and the weight (2 days) after pupation. These were analysed to see if they significantly varied between selection regimes. This was carried out using linear models and model simplification (using analysis of variance with Chi square test) to remove variables one by one. Two models were used including these independent variables: Selection line and replicate as a random effect on the weight (2 days) after pupation (3.6. Appendix - Model C); and selection line and replicate as a random effect on the time to pupation (3.6. Appendix - Model D). Here I determined whether weight (2 days) after pupation or time to pupation varied significantly between selection regimes.

3.4. Results

1. Proportion infected (immune response assay)

Following regression of our general linear model, selection regime was shown to have a significant effect on the proportion of infected larvae (χ^2=-38.485,df=21,p<0.001). Larvae from the female bias selection regime showed a higher overall proportion of infection than larvae from the male bias selection regime (Figure 2.1). There was also a significant interaction between dose and selection regime (χ^2=-8.833,df=16,p<0.01). There was no significant effect of replicate (within Selection regimes) on the proportion of infected larvae (χ^2=-8.3701,df=16,p<0.1).

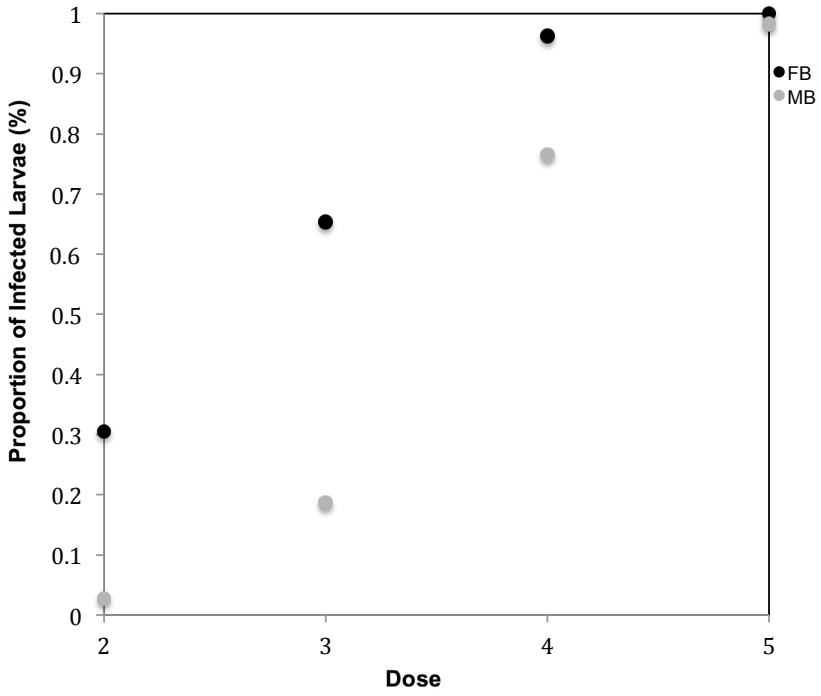

Figure 2.1: Scatter graph showing the mean proportion of infected larvae at each dose for treatments: Female (FB; grey circle) and Male (MB; black circle) bias lines. Doses are: 2=0.0025%, 3=0.025%, 4=0.25%, 5=2.5% (percentage of virus to distilled water). FB populations have an overall higher proportion of infected larvae than MB populations.

2. Life history traits

ANOVA results on linear models with replicate as a random effect show that there was no significant difference in weight between male and female bias populations (χ^2=2.6, df=3, p>0.1) (Figure 2.2).

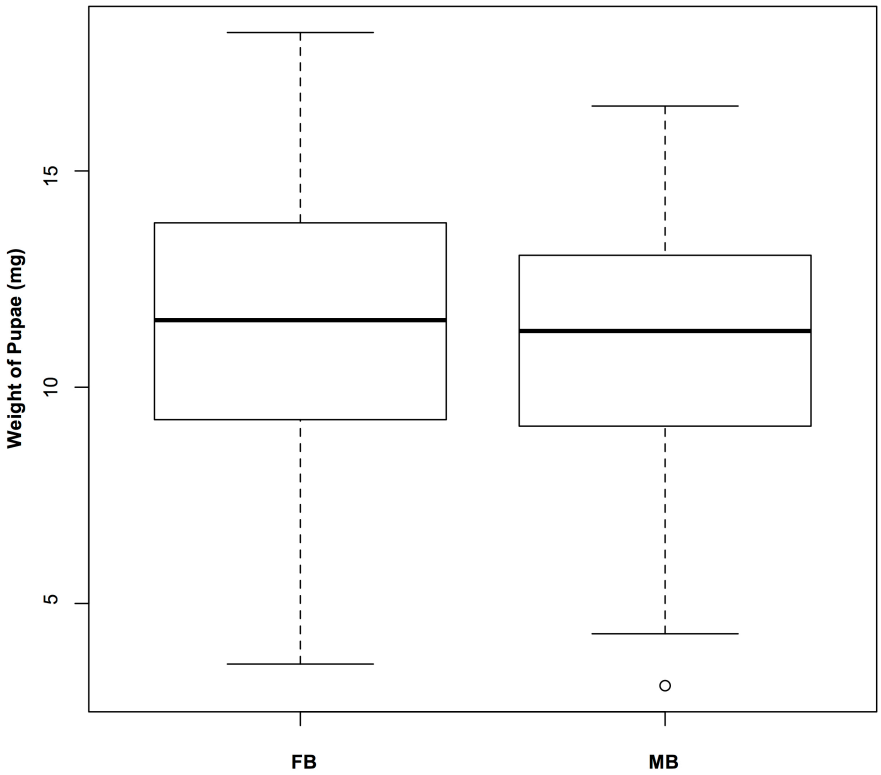

Figure 2.2: Box plot showing the mean weight of larvae in Female Bias (FB) and Male Bias (MB) treatments. Pupa weight does not differ significantly between FB and MB treatments.

There was also no significant difference in time to pupation between the male or female sex biased populations (χ^2=0.677, df=3, p=0.410) (Figure 2.3).

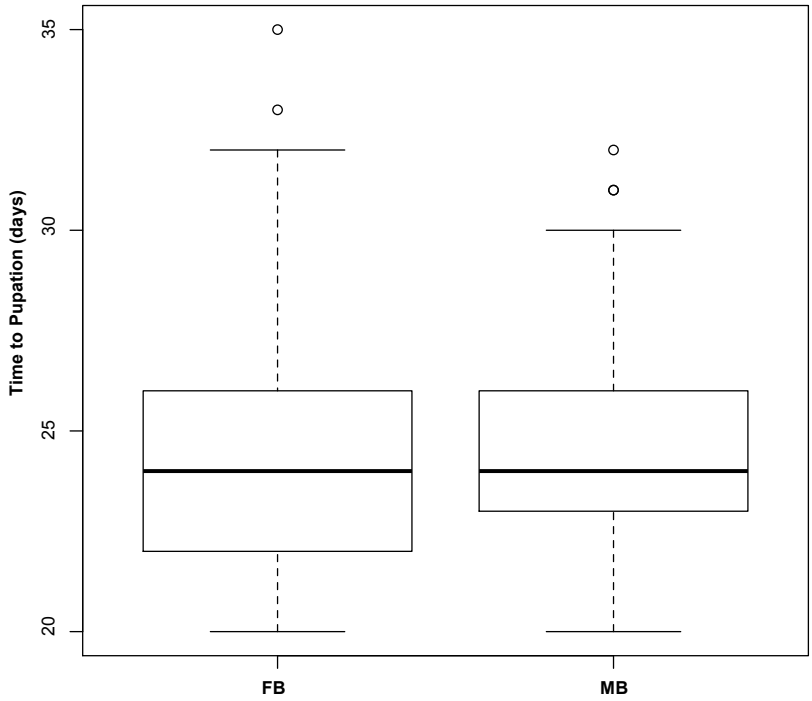

Figure 2.3: Box plot showing the mean time to pupation (days) of larvae in Female Bias (FB) and Male Bias (MB) treatments. Pupa time to pupation does not differ significantly between FB and MB treatments.

3.5. Discussion

I found that virus resistance in populations evolving under a female biased adult sex ratio is significantly lower than populations evolving under adult male sex ratio bias. This difference may be caused by a resulting increase in male mating demands triggering a trade-off with immunity in the female-biased treatment. Furthermore, this difference was true for the female biased populations across all virus doses. This cannot be explained by size differences that may reflect variation in total amount of available resources for investment in reproduction and immunity, because the weight of individuals in the female and male bias populations was not significantly different. This finding suggests that there is no apparent trade off between this life history trait and virus resistance and that there are no large differences in amount of resources available between treatments. Likewise the time to pupation did not differ significantly between female and male bias populations. If the life history traits do not differ between sex bias treatments and therefore are not the responsible factors driving evolutionary divergence in virus resistance, then there must be a yet to be identified underlying factor that is responsible for this change in evolved resistance. I cannot rule out whether there are differences in the response between males and females as the sex of the larvae were not identified due to infected individuals being too small to allow sexing.

By assaying resistance directly by challenging larvae with a natural pathogen I have shown that evolved viral resistance has diverged between the sex-biased populations (Figure 2.1). Female biased populations are significantly less resistant to virus than male biased populations. It seems that the manipulation of the ratio of adult female to males has triggered either a trade off

compromising viral resistance and/or generally affected the overall fitness of populations. The life history results suggest that body size or development time are not the underlying factor causing this evolved change in viral susceptibility as they did not differ between populations. This makes it likely that another factor is causing this change in viral susceptibility.

The trade offs discussed above may act upon the concentration of PO in haemolymph samples that in turn can be responsible for viral and bacterial immuno-competence in invertebrates (Rantala et al 2002). The PO enzyme is involved in the encapsulation and melanisation processes as well as wound healing and cuticle sclerotization so are key to the immune system (Hartzer et al 2005). Both the concentration of haemolymph phenoloxidase activity and the encapsulation response are highly heritable (Wilson et al 2001). As such it has been identified as a costly trait where the production and maintenance of it may have fitness costs for the host (González-Santoyo & Córdoba-Aguilar 2012). This cost could be involved in trade-offs between immunity and resource availability, environmental temperature, population density and food quality (Triggs & Knell 2012). A decreased resistance to infection can be associated with reduced haemolymph PO activity (Eleftherianos et al 2006). This haemolymph PO activity can vary between the sexes and is found typically in lower concentrations in females. As found in previous research on these moth popuations PO levels were found to be different between the sexed and sex-bias regime (McNamara et al 2013). This suggests that we cannot rule out that sex is a possible variable causing the difference in immunity between populations.

Recent works suggest that the trade-off between immunity and reproduction is fundamental (Simmons 2012). For example, previous work on *Caenorhabditis elegans* nematodes have shown that immunity and reproduction are closely linked (Miyata et al 2008). Furthermore work with *P. interpunctella* has highlighted that immunity and reproduction are also linked with fitness (Lawniczak et al 2007). In particular the number of successful matings may account for a substantial share of the significant difference in proportion of infected individuals between the sex biased regimes (McNamara et al 2013). These evolved populations, reveal that female bias populations have an increased male mating demand that appears to be costly, and possibly trades off with immunity (McNamara et al 2013). Another trade off may occur between mating effort and immunity (McKean & Nunney 2001). For example, there is a negative genetic correlation between measures of immune function and the quality of male courtship song in *Teleogryllus oceanicus* (the Black field cricket) (Simmons et al 2010). The costs associated with male courtship behaviours are particularly high (Kotiaho & Simmons 2003). When male insects are put under increased threat of sperm competition, they put more effort into courtship and mate guarding behaviours to secure a mate (Gage & Baker 1991). Therefore an individual's immunity may also be traded off against investment in securing a mate through mate searching, courtship, mate guarding, and male-male competition (Scharf et al 2013). This means we can infer that in male biased populations, where there are more competing males and therefore an increased threat of sperm competition, males are predicted to invest more in mating effort than in immunity. However, in contrast I find that in the male biased populations, immunity is actually higher than in the female biased populations suggesting that we cannot rule out additional trade offs

compromising immunity in the female-biased populations. Moreover, this difference could also be due to stronger sexual selection in the male bias populations as there is greater variance in male mating success than in the female biased populations (Willis 2015). This potentially leads to higher quality males being favoured that may also have a better immune system and hence increased sexual selection resulting in populations with a better immune response. Previous work has shown that stronger sexual selection in male biased populations can result in individuals of better genetic quality and populations of higher viability (Michalczyk et al 2011; Lumley et al 2015). My research supports these findings in that in male biased populations there is stronger sexual selection favouring individuals of higher fitness that may also have a better immune system.

In summary, this discussion has demonstrated that empirical measures of infection are fundamental to accurately measure host immunity. The proportion of infected larvae is more reliable than using a measure of PO activity, as it is a direct measurement of symptomatic to non-symptomatic individuals. However due to the nature of the symptoms influencing larval opacity it became difficult to identify the sex of these moths. Future work should focus on determining larval sex alongside viral resistance to examine potential sex-specific immunity. A method that either involves infecting later instar larvae or using an alternative measure of immunity should suffice. This could also be part of an investigation into whether the evolutionary divergence of viral resistance in the sex biased populations changes when a viral selection pressure is present/absent and to identify potential sex specific effects if present. In conclusion, the results can be explained by both a trade-off between reproductive traits (male mating

demand, pre-copulative male courtship behavior, and male mating effort) and immunity, and/or higher sexual selection in male bias populations, leading to better genetic quality of offspring resulting in a more effective immune system.

3.6. Appendix

Model B

Proportion of infected larvae~Dose*Selection+replicate

Model C

Weight (2 days) after pupation~Selection regime+(1|replicate)

Model D

Time to pupation~Selection regime+(1|replicate)

Chapter 4: Summary

4.1. General Discussion

As discussed in the introduction there is a mismatch between the amount of theory on the role of spatial structure in host-parasite interactions and empirical data in its support. Model laboratory systems such as *P. interpunctella* and PiGV are important tools for gaining experimental data under controlled and simplified conditions. Here I directly manipulated spatial structure and as such I had the potential to directly examine spatial impacts. The spatial work threw up new questions due to a counterintuitive result. I explained this result through costs that may arise due to the manipulation of the spatial structure that were not intended. As such it highlights the problem of manipulating only the target process in experimental ecology. However, only by carrying out such experiments can we hope to develop model experimental systems that can address these important general questions.

In the second part of the thesis, I used a laboratory evolution approach to directly alter sexual selection. This is a good example of where laboratory model systems can be useful. Once this selection had been applied I was able to directly test whether resistance differed between male and female biased populations. Previous measures of a general immune trait PO had been used to estimate general immunocompetence (Adamo et al 2001; McNamara et al 2013). However, it is unclear whether this measure really relates to direct defence. Our direct measure with a natural parasite is a much more rigorous way of addressing this question. In this case the results from the PO study and our direct measure yielded the same. One important point that arises from this finding is that PO is less likely to be a good measure of immunity to our DNA

virus (Saejeng et al 2010) and therefore our results may imply that defence is altered to a wide range of infectious disease: ones where PO plays a key role and here a DNA virus where it does not. To test this idea further direct infection with other parasites is necessary.

By collecting empirical data I have supplemented the theory base explaining the effects of both spatial ecology on transmission and varying levels of sexual selection on immunity in *P. interpunctella*. My research has focused on where current lack of empirical data needed, and focuses on one model host/parasite model system; *P. interpunctella* and the PiGV. Previous research has suggested that it might be important to incorporate spatial ecology into model experiments when examining the effects on host/parasite dynamics (Tilman & Kareiva 1997). I have shown that it is important to incorporate both spatial and behavioural ecology in order to examine this relationship. I have also shown that by keeping resource quality homogeneous we can control for variation in life history traits. This also means that while testing for spatial and behavioural effects on host/parasite dynamics, we control for variation in life history traits that consequently will not trade off with immunity. It is crucial that we understand how these host/parasites interact in order to determine their effect on evolutionary outcomes.

This thesis has identified that spatial and behavioural ecology has an effect on transmission of virus and host resistance respectively. It reveals a trade off between either stress or the physical exertion of dispersal and immunity, as shown by the decrease in the proportion of infected individuals, as viscosity increases. The empirical data collected and analysed, fills the gap in the

previous absence of data between the extremes of ‘local’ and ‘global’ interactions, providing us with insights from more natural interactions (Boots & Sasaki 1999). It shows that virus transmission relies heavily on the spatial structure of a host population and therefore the frequency of interactions. By showing that changing the spatial structure can influence transmission I have shown its importance to host/parasite modelling and therefore spatial structure should be included in future models and experiments.

My research also reveals that varying levels of sexual selection by altering adult mating frequencies can influence the immunity of offspring. By altering the adult mating frequencies to increase levels of sexual selection in male bias populations, I have subsequently successfully ‘improved’ evolved immune response in these populations. However, as shown by previous research looking at PO concentration in these same populations, we cannot rule out sex differences as a potential factor as males and females may have differing immune responses. Therefore, direct measures of proportion of infected individuals need to be collected in conjunction with individuals’ sex to ascertain any sex specific effects.

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