UBI MEL, IBI APES:
On the evolutionary ecology of infectious diseases and intersections with apiculture.

Lewis James Bartlett

Submitted to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, November 2018.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

……

Dedication

“You’ll be ready once you’ve finished learning… although, I suppose no-one is ever done learning?” – *words given to me by Carola Morriën-Gokke, a good friend and mentor, when I was fourteen.*

Four years, three cities, two contininents, and one thesis have come and gone on the wavecrest of dedication, duty, passion, and favour shown to me by innumerable friends, mentors, colleagues, and family. There is no first and foremost among them, so I give thanks here in no special order. I am grateful to every person mentioned on this page and hundreds elsewhere otherwise.

Mike Boots and Lena Wilfert, who took me on as a student with enthusiasm and zeal from the first day to the last, who challenged me just as much as I was a challenge for them, and who offered me not just guidance but friendship and honesty. I could not have asked for better supervisors and mentors.

Alice Williams, Bethany Clark, Ian Skicko, and Paula Marjamäki, who made for a fellowship of support and comraderie without which we would all be poorer.

Elisa Visher, Cat Taylor, Trevor Ramsey, and Laura Alexander, for being kindred souls to me when I was thousands of miles from home.

Jennifer Berry, Keith Delaplane, Britt Koskella, Lucy Hawkes, Angus Buckling, Juliet Osborne, and Brendon Godley, for showing how small kind words in passing can make academia a warmer and brighter place.

Stephen Sharpe and Kat Roberts, for inducting me into the branches of science which have led me on such grand adventures.

Iain Stott and Jack Boyle, for building the brotherhood I had always needed.

Jess Knapp, who I walked this journey in spirited tandem with, for her candid wit, endless inspiration, and limitless talent for science and life.

My mum, dad, and brother, whose faith in me frankly borders on the fanatical.

Thesis Abstract

Infectious diseases shape almost every aspect of nature and society; understanding the multitude of factors influencing infectious diseases is a critical goal of modern evolutionary ecology. This thesis explores this broad topic using theoretical and empirical approaches to understand the forces at work in infectious disease ecology and evolution, with application to the specific system of managed honeybees (*Apis mellifera* L.).

I demonstrate that a well-documented evolutionary trade-off governing pathogen resistance is both constitutive and genetic – critical for supporting assumptions made by mathematical theory. I go further to demonstrate that this trade-off breaks down when the action of selection is reversed, in that when the 'cost of resistance' phenotype is selective for, we do not incidentally select for higher resistance too. This is important for understanding genetic linkage of traits and downstream evolutionary modelling. I undertake theoretical modelling on the topic of spatial structure and how it affects pathogen evolution. In doing so I interrogate a critical assumption made in much of the prior theoretical body, showing that the effect of spatial structure on virulence is quantitatively changed when a core assumption concerning reproduction is relaxed, but is otherwise qualitatively robust.

I continue on the theme of spatial structuring and pathogens by developing novel theoretical models on how changing apicultural management alters honeybee population spatial structure, surprisingly leading to only marginal changes in pathogen burden. I stay on this topic to examine empirical data on honeybee colony viriomes in an observation experiment showing that colonies from very intensively managed, migratory backgrounds show elevated viral titres – critical for management and wild bee conservation. I synthesise that the honeybee system is our most informative natural experiment in showing that vectored pathogens are more virulent than directly transmitted counterparts. I also show that outbreaking human epidemics (Zika virus) can threaten apiculture – and by extension livelihoods and agriculture.

Contents

List of Tables

List of Figures

List of Supplementary Tables and Figures

Content Reproduction

Parts of this thesis represent work which is now published elsewhere, and is reproduced here verbatim from the published record, excepting differences in formatting between the published typeset form and the style used in this thesis.

The parts of this thesis which are published or pre-print archived are cited throughout the thesis so as to refer to the published or archived work, rather than the chapter they correspond to.

Reproduced content constitutes the following:

Chapter 2 – 'A genotypic trade-off between constitutive resistance to viral infection and host growth rate' is reproduced from the following publication:

Lewis J Bartlett, Lena Wilfert, Michael Boots (2018) "A genotypic trade-off between constitutive resistance to viral infection and host growth rate." – Evolution (Early View) 10.1111/evo.13623

Chapter 8 – 'Identifying regions of risk to honeybees from Zika vector control in the U.S.' is reproduced from the following publication:

Lewis J Bartlett, Colin J Carlson, Michael Boots (2018) "Identifying regions of risk to honeybees from Zika vector control in the U.S." – Journal of Apicultural Research 57 (5) 10.1080/00218839.2018.1494914

Chapter 5 – 'Industrial bees: when agricultural intensification doesn't impact disease burden.' is reproduced from its posting on bioRxiv, following review by Proceedings of the National Academy of Sciences and PloS Biology, currently under review with Journal of Applied Ecology at the time of submission of this thesis.

Lewis J. Bartlett, Carly Rozins, Berry J. Brosi, Keith S. Delaplane, Jacobus C. de Roode, Andrew White, Lena Wilfert, Michael Boots (2018) "Industrial bees: when agricultural intensification doesn't impact disease burden." bioRxiv, 428656

Author's Declaration and Acknowledgements

Each chapter of this thesis represents a different degree of involvement and collaboration by a diverse set of co-authors and colleagues, and different funders and institutions were involved with each chapter. I detail this below in the style typical of published studies.

Chapter 1 is the sole work of Lewis J. Bartlett.
L.J.B. acknowledges funding from a NERC training grant (NE/L002434/1).

Chapter 2 was authored by: Lewis J. Bartlett, Lena Wilfert, Michael Boots.
L.J.B. and M.B. initiated the idea for the study. All authors contributed to designing methodology. L.J.B. executed the experiment, gathered data, and performed analysis with substantial input from L.W & M.B. L.J.B. drafted the paper with significant contributions from all authors. All authors critically revised the manuscript, and gave approval of the final version for submission.
L.J.B. acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1). M.B. was funded by the BBSRC (BB/L010879/1) and the NERC (NE/J009784/1), (NE/K014617/1).

Chapter 3 was authored by: Lewis J. Bartlett, Elisa Visher, Yazmin Haro, Lena Wilfert, Michael Boots.
L.J.B., L.W. & M.B. designed the study. L.J.B. & E.V. maintained the experiment and gathered data with Y.H. E.V. & Y.H. curated the data. L.J.B. analysed the data with help from M.B. L.J.B. drafted the work, with contributions from all authors.
L.J.B. acknowledges funding from a NERC training grant (NE/L002434/1). E.V. acknowledges support from an NSF GRFP DGE 1752814. Y.H. acknowledges support from the UC Berkeley NIH Bridges to Baccalaureate Programme R25GM095401. M.B. notes funding for this project from the BBSRC (BB/L010879/1) and the NERC (NE/J009784/1), (NE/K014617/1).

Chapter 4 was authored by: Lewis J. Bartlett, Michael Boots.
L.J.B. conceptualised the study, M.B. provided guidance for derivation of equations. Computational work was undertaken by L.J.B. L.J.B. drafted the first

manuscript with both authors contributing substantially to the final version. L.J.B. acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1).

Chapter 5 was authored by: Lewis J. Bartlett, Carly Rozins, Berry J. Brosi, Keith S. Delaplane, Jacobus C. de Roode, Andrew White, Lena Wilfert, Michael Boots. L.J.B. and C.R. contributed equally to this study. All authors contributed to conceptualisation and scope definition of the study. L.J.B., C.R., M.B. developed approach. Mathematical modelling was undertaken by C.R., A.W., and M.B. Computational modelling by L.J.B., K.D., and M.B. Model scope and parameterisation by L.J.B., K.D., J.C.d.R., B.J.B., L.W. L.J.B. and C.R. created figures, interpreted results and drafted manuscript with guidance and input from all authors. All authors contributed to further drafting, revision, and finalisation.
L.J.B. acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1). C.R., M.B., and A.W. acknowledge funding from a Biotechnology and Biological Sciences Research Council grant (BB/L010879/1). B.J.B., J.C.d.R. and K.S.D. acknowledge funding from National Institutes of Health (R01-109501); the content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Chapter 6 was authored by: Lewis J. Bartlett, Michael Boots, Berry J. Brosi, Keith S. Delaplane, Catherine A. Hernandez, Jacobus C. de Roode, Lena Wilfert. L.J.B., K.S.D. and M.B. conceptualised the study, with input from B.J.B., J.C.D., and L.W. Molecular work was undertaken principally by L.J.B. with guidance from L.W. and assistance from C.A.H. L.J.B. analysed data and drafted the manuscript, with contributions from all authors.
We would like to thank Norma Morello for advice and guidance with ddPCR, Jennifer Berry for insightful discussion on commercialised industrial beekeeping, and Jack Garrison, Nicholas Weaver, Westley Hester, and Katherine Hagan for assistance with colony management and sample collection. L.J.B. acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1). M.B. acknowledges funding from a Biotechnology and Biological

Sciences Research Council grant (BB/L010879/1). B.J.B., J.C.d.R. and K.S.D. acknowledge funding from National Institutes of Health (R01-109501); the content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Chapter 7 was authored by: Lewis J. Bartlett, Lena Wilfert, Michael Boots.
LJB conceptualised, undertook, and drafted the paper in its entirety with continual guidance and input from LW and MB. All authors contributed to editing of the paper.
L.J.B. acknowledges funding from a NERC training grant (NE/L002434/1).

Chapter 8 was authored by: Lewis J. Bartlett, Colin J. Carlson, Michael Boots.
L.J.B. and M.B. initiated the idea for the study. L.J.B. and C.J.C. designed methodology and gathered data. L.J.B. performed analysis and drafted the paper with significant contributions of specific sections from all authors. All authors critically revised the manuscript, and gave approval of the final version for submission.
L.J.B. acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1). C.J.C. acknowledges support by the National Socio-Environmental Synthesis Center (SESYNC) under funding received from the National Science Foundation DBI-1639145.

Chapter 9 is the sole work of Lewis J. Bartlett.
L.J.B. acknowledges funding from a NERC training grant (NE/L002434/1).

Chapter 1 – Overview & Introduction

Infectious diseases represent a poisoned chalice in evolutionary biology. They are a rapidly evolving, ubiquitous, diverse aspect of the natural world – a playground for evolutionary ecological research. However those same qualities highlight why infectious diseases constitute a perpetual battle for society at large, representing some of the most devastating and burdensome forces to shape human society both throughout history and into the modern day. This thesis explores a diverse set of questions relating to the evolution and ecology of infectious diseases – I bring to bear analytical and computational models, field data, molecular biology, and laboratory experiments to explore the underpinnings of resistance, virulence, vector adaptation, vector control, host biology and population structure in shaping parasite dynamics.

I intersect this with a biological field system of managed western honeybees (*Apis mellifera* L.). Keeping of honeybees (apiculture) is a critical component to many agricultural systems and has huge cultural value, yet is an embattled industry facing a plethora of threats – including emerging and re-emerging infectious diseases. Understanding the forces shaping the infectious disease burden in honeybees grants us a dual-benefit: insight into the fundaments of infectious disease evolutionary ecology, and applied understanding of how best to protect the future of our pollinator livestock and those whose livelihoods rely on them.

Managed western honeybees *Apis mellifera* have experienced emerging or re-emerging outbreaks of numerous pathogens (Martin et al. 2012; Mondet et al. 2014; McMahon et al. 2016, 2018; Wilfert et al. 2016), and elevated losses to infectious disease for a variety of reasons (vanEngelsdorp et al. 2009; Genersch et al. 2010; Pettis and Delaplane 2010; vanEngelsdorp and Meixner 2010). Invasive pests, poor forage, pesticide exposure, behavioural stress, and lack of genetic diversity are all proximate causes of increased vulnerability to pathogens (Yang and Cox-Foster 2005; Oldroyd 2007; Conte et al. 2010; Forsgren and Fries 2010; Neumann and Carreck 2010; Aronstein et al. 2012; van der Zee et al. 2012; Pasquale et al. 2013; Sánchez-Bayo and Goka 2014; Zee et al. 2014; Goulson et al. 2015; Dolezal et al. 2016; Rumkee et al. 2017; Bartlett et al. 2018a).

This multifaceted threat of many stressors all impacting honeybee immunonology and capability to cope with infectious pathogens and parasites has constituted something of a crises in beekeeping (Neumann and Carreck 2010; vanEngelsdorp and Meixner 2010). Host health in the face of infectious diseases is defined by their ability to tolerate or resist pathogens and parasites (Boots and Bowers 1999). Therefore these traits are candidates for selective breeding of either more resistant or more tolerant honeybee stock (Guzman et al., 2008; Dietemann et al., 2012; Toufailia et al., 2014; Brosi et al., 2017). Such selective breeding for resistance relies on organisms' potential to evolve, however in the case of the evolution of resistance to parasites, it is broadly understood that resistance comes at a cost (Schmid-Hempel 2003). This is an evolutionary trade-off, and understanding such trade-offs is critical to informing a selective breeding programme hoping to evolve new honeybee stocks. Accordingly, understanding host resistance evolution is the focus of chapters 2 and 3, using a laboratory model organism.

In chapters 2 and 3, we use experimental evolution in a laboratory system to better understand a previously demonstrated trade-off between development time and resistance to pathogens in the moth *Plodia interpunctella* (Boots and Begon 1993; Oppert et al. 2000; Boots 2011). Prior work has shown that exposing successive populations of *Plodia* to an infectious agent – the baculovirus Plodia interpunctella Granulosis Virus (PiGV) – selects for evolution of resistance to this virus, but at the cost of longer development times. In chapter 2 I evolve populations of *Plodia* not through selection, but through drift using a strict inbreeding regime. This evolution, without any exposure to the pathogen, tests whether this widely cited resistance trade-off is definitively genetic and constitutive. This is a critical assumption made in much of the evolutionary theory on host evolution and host-parasite coevolution (Cressler et al. 2015).

Chapter 3 futher examines this trade-off through a more conventional selection experiment, however rather than selecting *Plodia* by exposing them to a lethal pathogen, we artificially select for fast or slow development. Again, this is evolution in absence of the pathogen. We test the hypothesis that selecting for longer development time will indirectly select for increased resistance to PiGV. Testing

this hypothesis is informative for understanding whether phenotypes can move away from well-characterised trade-off relationships and is an indirect test of how genetically linked the two phenotypic traits are.

Moving away from host evolution and instead focussing on pathogen evolution, one critical determinant of pathogen virulence is host population structure; in particular, how local interactions select for lower pathogen virulence (Lion and Boots 2010). In the face of a 'shrinking world' (Janelle 1973; Hanski 2005) this link between local vs global interactions and pathogen virulence is a critical part of the evolutionary management of infectious disease burdens, including in apiculture (Brosi et al. 2017). However, as with all theory, it is important to interrogate established hypotheses to examine how sensitive they are to simplifying assumptions capturing real-world biology. This is the focus of chapter 4.

In chapter 4 I undertake theoretical work on the relationship between spatial structure and optimum virulence. One frequent assumption in the large body of theory demonstrating how local interactions select for lower virulence is that infected individuals do not reproduce. Such assumptions are made for improved analytical tractability and insight, and calls to better examine the role of demography in determining host-parasite evolutionary outcomes have been made before (Lion and Boots 2010; Messinger and Ostling 2013). In chapter 4 I relax the demographic assumption concerning reproduction from infected individuals. In doing so I test whether the critical finding that local interactions lead to lower virulence is robust to this assumption on castration of hosts.

Spatial structuring and infectious disease evolution is of critical concern to managed honeybees. Management practices of beekeepers have changed drastically in North America in recent decades, with moves toward much higher stocking densities (Seeley and Smith 2015) and cross-continental migratory beekeeping (Whynott 1991; Welch et al. 2009; vanEngelsdorp et al. 2013; Simone-Finstrom et al. 2016). These changes constitute a cross-continental mixing of the managed honeybee population, with abundant opportunities for transmission of parasites (Brosi et al. 2017; Nolan and Delaplane 2017). There is a clear hypothesis that these ecological changes may be selecting for more virulent

pathogens. Further, it is not yet clear which of the many different aspects of apicultural intensification are expected to meaningfully influence honeybee epidemiology. Examining the infectious disease outcomes of some aspects management intensification is the focus of chapter 5 (a modelling approach) and chapter 6 (an empirical approach).

In chapter 5 we undertake ecological modelling of honeybee pathogens at the scale of individual apiaries, attempting to capture some critical aspects of the industrialisation and intensification of apiculture. We test whether industrialisation at the scale of an individual apiary leads to meaningfully different infection dynamics and parasite burdens. For modelling tractability, we focus in this first endeavour on apiary size, layout, and rates of transmission (bee movement or certain beekeeper behaviours) between colonies. We do this by using both analytical and agent-based models and examining R_0 values under different ecological scenarios. This chapter constitutes the only analytical modelling of honeybee infectious disease at the scale of multiple colonies. Critically, the apiary scale is where actionable management recommendations are easiest to communicate and implement, as this is the scale at which individual beekeepers are primarily concerned with.

In chapter 6 I use an empirical approach to test how honeybee colony origin affects infectious disease burden. I use molecular ecology coupled with a ‘common garden’ transplantation experiment to test whether colonies from three different origins: an industrial supplier, a traditional supplier, and a feral population, show significant differences in their viriome after a year of identical management on a single site. In doing so, I test whether management history has a meaningful and non-ephemeral effect on colony epidemiology, and frame this in the potential for industrial management to increased parasite burdens of bee populations across the continent. Such hypotheses have been speculatively discussed before (Brosi et al. 2017). In the specific case of whether industrial management has long-term negative effects on parsite burden in managed honeybees, there are profound implications for how industrial migratory beekeeping practices may be defining

infectious disease dynamics in non-migratory honeybee populations and wild pollinator populations throughout North America.

One of the most widely-discussed threats to North American apiculture is the invasion of the ectoparasitic varroa mite. Mechanisms by which *Varroa* impact honeybee health have been discussed across the literature for decadees, although it was only recently that insights were made into their actual mechanical action when feeding on the honeybee (Ramsey et al. 2018). Early in the timeline of the invasion of *Varroa* into *Apis mellifera* stocks, it was posited that *Varroa* interact with viral infections (Chastel et al. 1991; Koch and Ritter 1991), either as vectors or by some kind of 'activiation' of latent viruses. Since then, abundant studies have been produced to help battle *Varroa* pestilience. This research attention on the *Varroa* mite and its associated honeybee viruses is the premise of chapter 7.

In chapter 7 I review and synthesise the large body of work describing the evidence that the invasion of varroa mites into *Apis mellifera* populations has selected for increased virulence in certain viruses. This is critical to understanding how *Varroa* control may help or may fail to reduce honeybee viral burdens back towards their historically lower titres. I also detail how the study of honeybee pathogens, currently a popular and well-studied system, may constitute a new and promising biological context for understanding fundamental evolutionary processes defining infectious diseases. Specifically, in chapter 7, I discuss how the *Varroa*-virus-vectoring system is our best current test of the hypothesis that vectored pathogens are selected to be more virulent than their directly transmitted counterparts (Ewald 1991). This is a critical hypothesis for the ecology and evolution of infectious disease, with clear ramifications for public health and agriculture.

An interacting factor driving part of the elevated pathogen burden experienced by honeybees, and their wild counterparts, is pesticide exposure. Pesticides are known to impair honeybee immunity (Sánchez-Bayo and Goka 2014; Sánchez-Bayo et al. 2016), leaving individuals and colonies more vulnerable to elevated pathogen titres and therefore lower productivity and greater mortality. This is one of the candidate stressors I discuss in chapter 6 related to migratory beekeeping and

management intensity. Further, pesticides and their insection with emerging pathogens as a threat to managed honeybees is featured as the core topic in chapter 8.

One route of pesticide exposure experienced by beekeepers and managed honeybees is from insecticide application to control mosquito vectors of human or livestock diseases. This is examined in chapter 8, where I discuss and attempt to quantify the risk to managed honeybees from spraying of insecticides to control Zika virus. I attempt this by using niche models, developed to try and forecast where autochthanous Zika transmission is most likely in the U.S.A. and overlaying these with estimates of managed honeybee desnities. In doing so, I present the highest resolution mapping of managed honeybees in the U.S.A. yet published. This study was prompted by the loss of apiaries to Zika control during the 2016 Zika pandemic, and the concerns raised by beekeepers over their perception that private contracted pesticide application was becoming more common because of Zika fears, leading to more colony losses.

Overall, the seven research chapters constituting this thesis are ultimately a diverse set of approaches and topics, either directly or indirectly relevant to infectious diseases and managed honeybees. Broadly, this thesis should be understood not as a linear set of studies which directly inform one another, but as many independent branches of academic investigation growing from a core intersection of the ecology and evolution of infectious diseases and apiculture. Each one provides new future research directions for this exciting intersection of apiculture and pathogen evolutionary ecology.

Chapter 2 – A genotypic trade-off between constitutive resistance to viral infection and host growth rate.

Abstract

Genotypic trade-offs are fundamental to the understanding of the evolution of life-history traits. In particular, the evolution of optimal host defence and the maintenance of variation in defence against infectious disease is thought to be underpinned by such evolutionary trade-offs. However, empirical demonstrations of these trade-offs that satisfy the strict assumptions made by theoretical models are rare. Additionally, none of these trade-offs have yet been shown to be robustly replicable using a variety of different experimental approaches to rule out confounding issues with particular experimental designs. Here, we use inbred isolines as a novel experimental approach to test whether a trade-off between viral resistance and growth rate in *Plodia interpunctella*, previously demonstrated by multiple selection experiments, is robust and meets the strict criteria required to underpin theoretical work in this field. Critically, we demonstrate that this trade-off is both genetic and constitutive. This finding helps support the large body of theory which relies on these assumptions, and makes this trade-off for resistance unique in being replicated through multiple experimental approaches and definitively shown to be genetic and constitutive.

Introduction

The understanding of trade-offs remains central to evolutionary ecology (Shoval et al. 2012; Acerenza 2016). They are fundamental to understanding life-histories in general (Roff and Fairbairn 2007), and more specifically in the context of the evolution of infectious disease, trade-offs are generally assumed to determine both the evolution of pathogen virulence (May and Anderson 1983; Alizon et al. 2009; Alizon and Michalakis 2015), and host resistance to pathogens (Gillespie 1975; Sheldon and Verhulst 1996; Gemmill and Read 1998; Boots and Haraguchi 1999). In particular, the idea that defence against infectious disease is costly underpins a large theoretical and empirical literature examining the determinants of optimal defence (Boots and Bowers 1999; Boots and Haraguchi 1999; Gandon et al. 2002,

2008; Miller et al. 2007; Best et al. 2011; Best and Hoyle 2013; Gandon and Vale 2013) . Overall, the costs of resistance to pathogens are a well-studied phenomenon (Antonovics and Thrall 1994; Brown 2002; Graham et al. 2005; Best et al. 2008; Schwenke et al. 2016). However there is ambiguity in the nature of many trade-offs demonstrated empirically and those modelled by theoreticians; specifically, whether trade-offs are facultative or constitutive, and genetic or plastic (Reznick 1985; Roff and Fairbairn 2007; Cressler et al. 2015). Facultative costs are costs paid only when confronted with a pathogen, whereas constitutive costs are paid regardless of whether a pathogen is encountered. Genetic costs are directly inherited, and associated phenotype prevalences change only by evolution. Plastic phenotypes (such as those determined by epigenetics or maternal signalling) can vary in their prevalence without evolutionary changes in the population.

Costs of resistance are often measured when there is exposure to the pathogen (Dallas et al. 2016) – however such measures represent activated facultative costs only, and cannot inform us about the costs paid by organisms which never encounter a pathogen. Such facultative costs of immune activation are now well demonstrated (Nordling et al. 1998; Moret and Schmid-Hempel 2000; Armitage et al. 2003; Bonneaud et al. 2003; Sadd and Siva-Jothy 2006); however, evolutionary costs of immunological maintenance in the absence of disease (constitutive costs), due to negative genetic correlations between immunocompetence and fitness, are typically assumed in the evolution of defence literature (for example: Boots and Bowers 1999; Boots and Haraguchi 1999; Gandon et al. 2002, 2008; Miller et al. 2007; Best et al. 2011; Best and Hoyle 2013; Gandon and Vale 2013). The absence of empirical demonstrations of genetic, constitutive trade-offs is a recognised problem (McKean et al. 2008; Cressler et al. 2015) as these are the costs to resistance are modelled by theoreticians; empirical studies are rarely designed to show a trade-off in such a way as to conclusively meet these strict criteria. Demonstrating trade-offs which meet the criteria of being both constitutive and genetic is therefore a critical goal of bridging empirical and theoretical work on the evolution of host resistance to pathogens. These costs are important since they not only define optimal resistance but also because they are essential in explaining the wide-spread variation apparent in the susceptibilities of hosts to pathogens

(Bowers et al. 1994; Henter and Via 1995; Schmid-Hempel 2003; Duffy and Forde 2009). Without clear evidence of whether a trade-off is definitively genetic or plastic, and constitutive or facultative, it remains unsuitable for incorporation into theoretical models. While one part of this solution is further work to build theoretical models on facultative and plastic costs, and to explore how these complementary routes to resistance interact, it is also critical to validate the current body of theory by showing trade-offs which are definitively genetic and constitutive. While in reality many different routes to resistance do all interact, theory tackling subsets of these route should be empirically integrated before being further developed.

Demonstrations of resistance costs in wild populations (Duffy et al. 2012; Auld et al. 2013; Susi and Laine 2015) and through selection experiments (Fuxa and Richter 1989; Boots and Begon 1993; Kraaijeveld and Godfray 1997; Luong and Polak 2007; Duncan et al. 2011) have provided evidence of constitutive costs of resistance in the absence of infection. However, these studies do not necessarily rule out the well documented phenomena of transgenerational immune-priming or epigenetic (plastic) effects (Roth et al. 2009; Tidbury et al. 2011, 2012; Best et al. 2013) as such populations are not historically naïve to their pathogens. Studies have shown that resistance rapidly disappears in the absence of the pathogen (Fuxa and Richter 1989; Duncan et al. 2011), suggesting some cost to maintaining resistance, even if it is a phenotypically plastic or epigenetic effect. Examining these apparent trade-offs with a plurality of experimental approaches will help to rule out some of the potential confounds of specific experimental designs and clarify whether trade-offs are indeed demonstrably constitutive and genetic.

Here we take a new approach to examine a previously documented, highly-cited evolutionary trade-off in the moth *Plodia interpunctella* (Hübner). A resistance cost in this system was first demonstrated by Boots and Begon (1993), and a further selection experiment confirmed this trade-off in a fully replicated experimental design (Boots 2011), making it one of the few evolutionary trade-offs confirmed by multiple independent selection experiments. However, such selection experiments cannot rule out intergenerational effects. Here we used inbred isolines rather than selection experiments to gain novel insights into this evolutionary trade-off and

prove it meets the criteria detailed in McKean *et al.* (2008) and Cressler, Graham & Day (2015) of being both genetic and constitutive. Genetically restricted lines have shown great value in their application to evolutionary biology in model species such as *Drosophila melanogaster* (David et al. 2005), and have been successfully used elsewhere to demonstrate variation in insect-virus systems, for example in vector competence (Jacobson and Kennedy 2013). Inbred isolines were established in this system to evolve to different genotypes via genetic drift during inbreeding, reflecting variation in the initial outbred stock population. When these genetically restricted isolines are assayed, this variation between isolines recovers the previously documented costs associated with resistance to a pathogen. This is despite all isoline populations being historically naïve to the pathogen (all evolution occurs in the absence of infection), providing the strongest evidence yet that the evolutionary trade-off is based on genetic, constitutive costs of resistance.

Materials and Methods

The host organism used was the pyralid moth *Plodia interpunctella*, the larvae of which are a common grain-feeding pest of cereals worldwide (Mohandass et al. 2007). It has been used as a study species for a variety of biological experiments due to its ease of population maintenance and its importance as a food pest (Silhacek and Miller 1972; Mohandass et al. 2007). It exhibits a simple life history, divided into larval and adult stages. Eggs are laid into cereal media by adults in a semelparous event, larvae then develop in the food media until pupation, and following pupation adult moths emerge, mate and a new generation of eggs are laid into the cereal medium (Gage 1995). Adults do not have functioning feeding physiology, and their reproductive success is broadly determined by their rate of development and pupal mass (Silhacek and Miller 1972; Boots and Begon 1993). *Plodia* larvae are susceptible to infection by the baculovirus Plodia interpunctella Granulosis Virus (PiGV) (Sait et al. 1994). PiGV infections are obligately lethal, and infection occurs via oral consumption of viral occlusion bodies. PiGV has strong effects on *Plodia* population dynamics (Sait et al. 1994), and *Plodia* may evolve resistance to the virus at the cost of increased development time (Boots and Begon 1993, 1994; Boots 2011). Intrahaemocoelic antiviral activity has been

demonstrated (Saejeng et al. 2011), although this resistance is not connected to the often-studied phenoloxidase pathway (Saejeng et al. 2010). Resistance is thought to occur mostly at the gut wall, through mechanical barriers such as the peritrophic membrane and apoptosis of infected gut wall cells (Begon et al. 1993; Tidbury 2012).

Plodia were raised following well-established protocols. All lines originated from an outbred laboratory stock population of *Plodia*. Male-female pairs were selected at their fifth larval instar, when males can be differentiated from females by the conspicuous gonads, visible as a dark spot on the back of the larva. To establish genetically isolated lines, single male-female pairs were then placed in 250ml straight-side wide-mouth Nalgene jars (ThermoFisher Scientific, U.K.) with 20g of food media. Food media was prepared in batches consisting of 250g 'Ready Brek' (Weetabix Ltd., U.K.), 150g wheat bran (Bob's Red Mill, U.S.A.), 100g rice flour (Bob's Red Mill, U.S.A.), 100g brewer's yeast (MP Biomedicals, U.S.A.), 125ml glycerol (VWR, U.S.A), 125ml clear organic honey (Dutch Gold Honey Inc., U.S.A.), 2.2g methyl paraben (VWR, U.S.A.), and 2.2g sorbic acid (Spectrum Chemicals, U.S.A.). Food media batches were homogenised with industrial mixers before being sealed and frozen for a minimum of 24 hours prior to thawing at ambient temperature for use as growth media.

Plodia pairs were incubated at constant conditions of 27±2^{o}C and 35±5% humidity, with 16:8hr light:dark cycles. After four weeks, *Plodia* pairs had typically undergone a full generation cycle, where the founding pair had successfully pupated, eclosed, mated, laid eggs, and died, with progeny developing to their fifth instar in the supplied food media. For each line of full-sibling progeny, full-sibling male-female pairs were placed as above in a new jar of food media. The subsequent generation represented one generation of full-sibling inbreeding. For each line, five replicates were set up each generation. This was done as some pairs invariably produce no offspring due to a failure, or mismatch in timing, of pupation, eclosure, or mating. In cases were multiple replicates produced successful subsequent generations, one jar of full-sibling larvae was randomly selected to found the next generation. This inbreeding regime was maintained for thirty generations to establish genetic

isolines with very high levels of homozygosity (Rumball et al. 1994). Of the initial twenty-five founding lines, twenty lines became extinct in the first eight generations of inbreeding, likely due to five mating-jar replicates being an inadequate insurance against unsuccessful mating during this period of intense inbreeding depression. The remaining five survived all thirty generations of inbreeding to establish the genetic isolines used in this study.

The growth rate of each isoline was estimated using two measures: time to pupation (development time) and pupal mass. For each isoline, sixty adult *Plodia*, known to have eclosed in the last 24 hours, were taken and placed on 200g of food media in 1000ml straight-side wide-mouth Nalgene jars (ThermoFisher Scientific, U.S.A.) and incubated as above. After 11 days, fifty larvae on the 1st day of their 3rd instar were taken from each isoline population and placed into individual compartments on 25-cell compartmentalised square petri dishes (ThermoFisher Scientific, U.S.A.) (two petri dishes per isoline). Each compartment contained ample food media. 1st-day 3rd-instar larvae were identified by examining the size of their head (which changes only during moulting and identifies different instars) compared to the size of their body (which if smaller in diameter than the head signifies their 1st day at that instar). Petri dishes were then incubated as above and checked daily to monitor larval development. All growth rate assays were set up simultaneously on the same day and incubated on the same shelf of the same incubator. The date of each larva's pupation was recorded, and two days later the pupa was extracted from its silk cocoon and weighed using a 1µg -precision microbalance. Not all larvae were recovered, as some inevitably die due to handling or other causes of stochastic mortality ($\bar{n}$ = 18 larvae recovered per line, Supplementary Material 1).

The susceptibility to pathogens of each isoline was characterised by comparing infection rates of larvae to PiGV. For each isoline, 300 1st-day 3rd-instar larvae were selected as described above. Larval cohorts of fifty larvae were placed in circular petri dishes (six cohorts per isoline) and starved for one hour. Droplets of virus solution were then pipetted into these petri dishes, with each cohort given one of six solutions. Virus solutions represented six doses: a control dose (no virus)

and five doses of virus solution each diluted by an order of magnitude (such that the strongest dose is 10^4 times stronger than the weakest). Solutions were diluted with distilled water, and otherwise made up to contain 0.1% Coomassie Brilliant Blue R-250 dye (ThermoFisher Scientific, U.S.A.), and 2% sucrose (ThermoFisher Scientific, U.S.A.). Larvae voluntarily fed on the solution droplets due to the sucrose content, and were considered dosed when 50% of their alimentary track was stained blue (visible due to the blue dye and translucent larval body) at which point they were removed from the petri dish. 25 suitably dosed larvae from each cohort were taken and placed individually in 25-cell compartmentalised square petri dishes and incubated for ten days as above. All susceptibility assays were set-up simultaneously on the same day and incubated on the same shelf of the same incubator. After ten days, petri dishes were frozen to kill all remaining live larvae, before being opened for counting. Infected larvae are easily recognised by their bright white cadavers, caused by the accumulation of viral occlusion bodies in the haemolymph. Uninfected larvae were easily identified as healthy larval cadavers or as developing pupae. Not all larvae were recovered to be categorised as either infected or uninfected, as some inevitably die due to handling or other causes of stochastic mortality ($\overline{n}$ = 18 larvae recovered per line per dose, Supplementary Material 1).

All statistical analyses were undertaken using R (v. 3.3.2 ‘Sincere Pumpkin Patch’). Differences between isoline growth rates were examined using ANOVAs, where tests were undertaken comparing mean pupal mass, mean development time, and mean growth rate (pupal mass / development time). Susceptibility was analysed using generalised linear models (glms), with a binomial error structure and logit link function, where the response variable was whether each recovered larva was infected (1) vs uninfected (0) for each dose and line. Doses were analysed after being transformed using a $\log_{10}(x + 1)$ function. We compared models using ANOVA (chi-squared) tests to eliminate model terms in the style of a backwards stepwise model simplification (Crawley 2012). The starting (most complicated) model included the following explanatory terms: isoline, viral dose strength, and an interaction between the two (to test for heterogeneity in dose response).

Significantly differing components of growth rate and susceptibility across the isolines were then tested for correlation against one another.

Results

Isolines differed in both their susceptibility to the pathogen and their development patterns (fig. 2.1 & fig. 2.2). Growth rate (pupal mass / development time) significantly differed across the five isolines ($F_{4,79} = 2.86$, $p = 0.029$; fig. 2.2a), however this was driven principally by a significant difference in development time between lines ($F_{4,79} = 2.86$, $p = 0.0054$; fig. 2.2c), with no detectable significant difference in pupal mass ($F_{4,79} = 2.86$, $p = 0.18$; fig. 2.2b). As expected, higher dose strengths lead to higher infection likelihoods ($p < 0.001$). There was no evidence for heterogeneity in dose-response between lines (fig. 2.1) when modelled as an interaction effect between dose and line ($p = 0.397$), however lines did differ in their susceptibility to infection overall ($p = 0.0057$).

Given the lack of heterogeneity in dose response between lines' susceptibilities, each line's susceptibility could be characterised using the line-effect values extracted from the binomial glm. These extracted values were directly tested (without transformation back to identity space) for correlation against the five lines' demonstrably different mean development times using a Pearson's product moment correlation. The two isoline characteristics showed a significant correlation ($r_3 = -0.976$, $p = 0.004$), where a faster development time correlated with greater susceptibility to the pathogen (fig. 2.3).

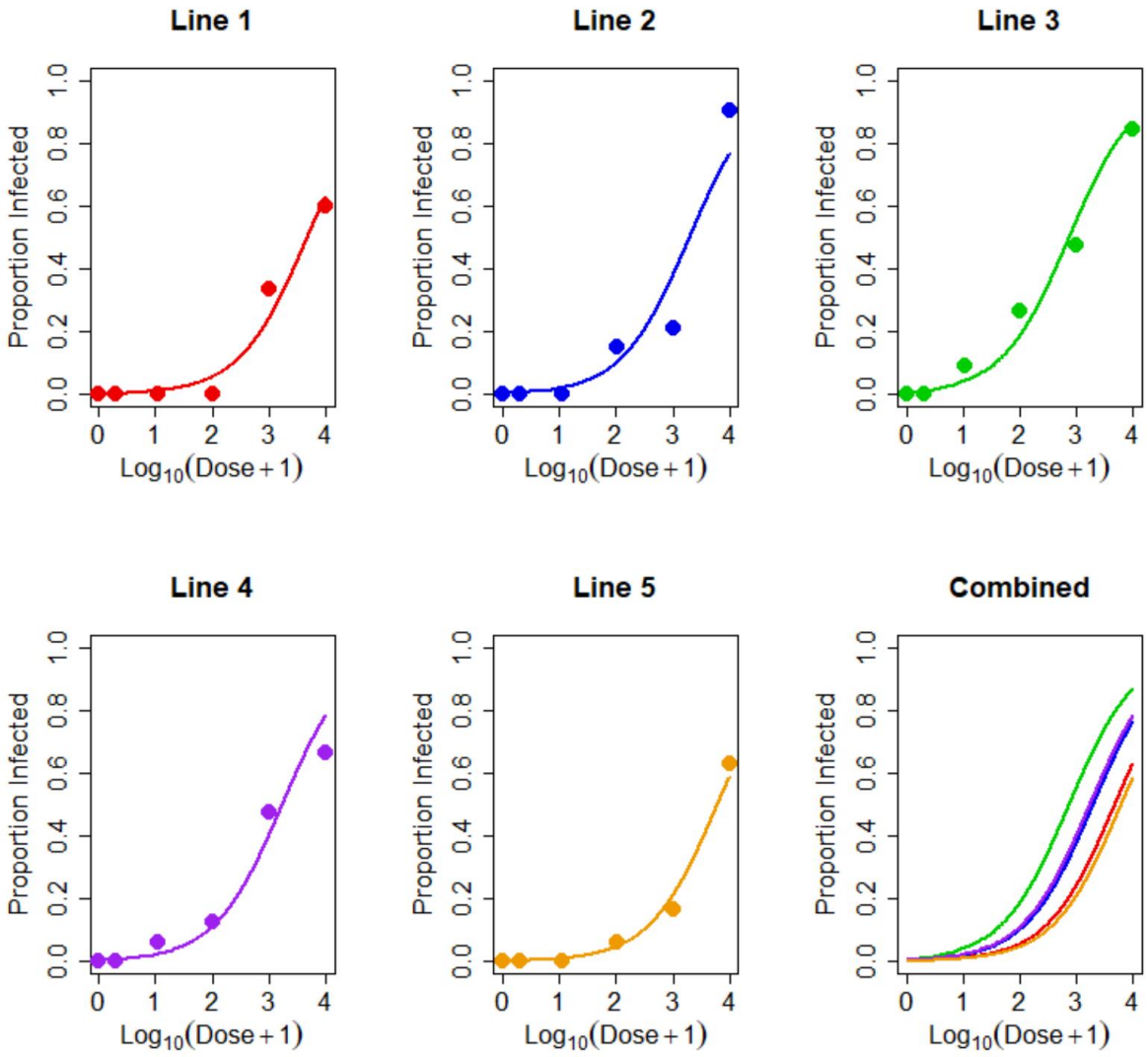


Figure 2.1 - Plots showing susceptibility data and best-model fit for each of the five isolines. Data are shown as the proportion of larvae which succumb to infection across the six doses, with doses plotted in logit space. Data and model fits for each line are shown in single panels and model fits are compared in the 'Combined' panel. Differences between lines' susceptibilities to the pathogen are clear from the distance between the plotted lines, with line 3 the most susceptible and line 5 the least. No fitted lines cross when plotted on the same axes, as would be expected when no significant interaction effect was apparent.

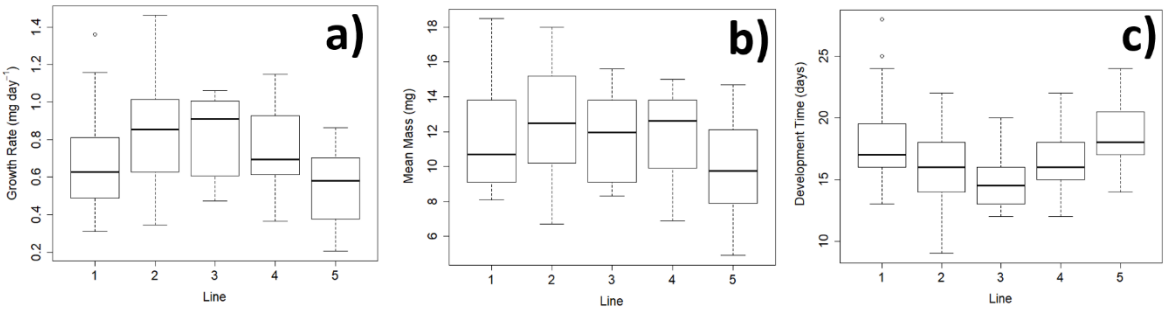


Figure 2.2 - Plots comparing growth rate, mean mass, and development time of each of the five isolines. Growth rate (a) is calculated for each individual larva as the pupal mass (b) divided by the development time (c). Significant differences between isoline growth rates (a) and development time (c) were apparent, however no significant difference between lines was found for mean mass (b). Line 3 is the fastest growing, owing to its shorter development time, while line 5 appears to be the slowest growing.

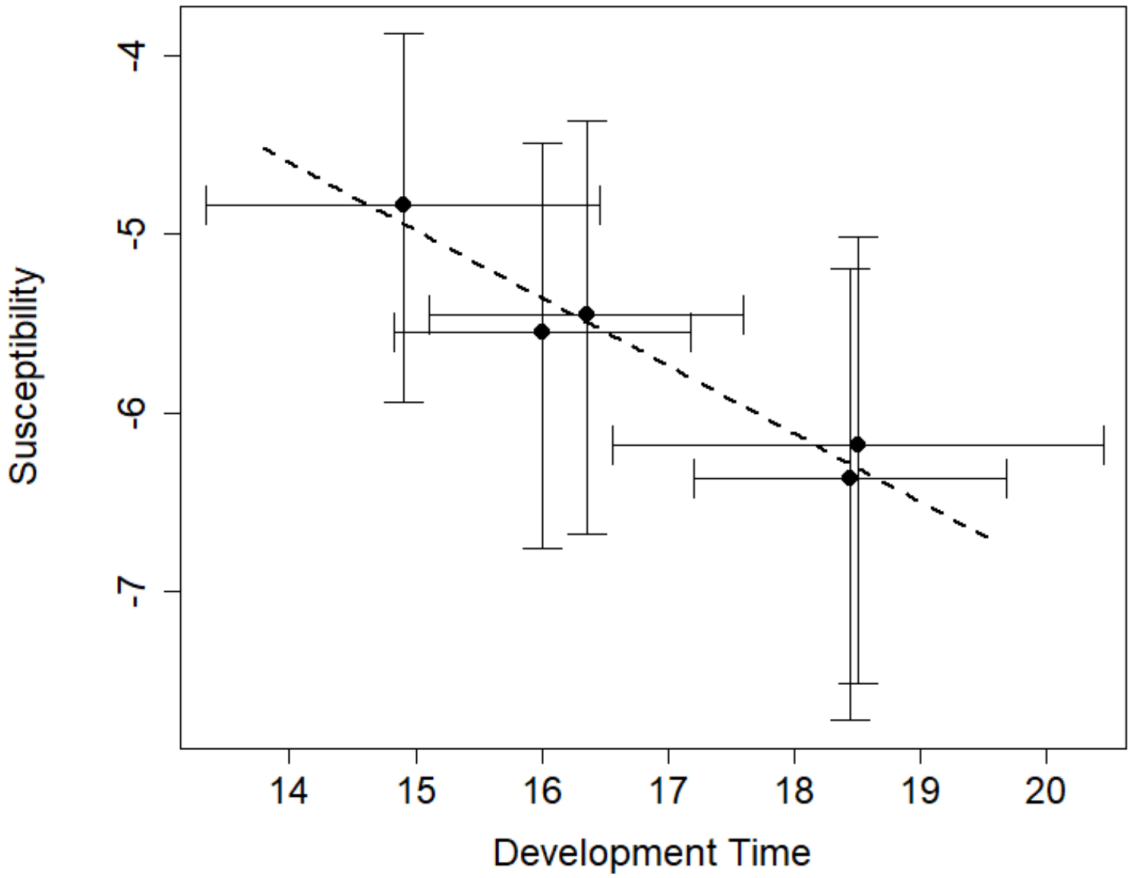


Figure 2.3 - Plot showing correlation between susceptibility (y axis) and development time (x axis) for the five isolines. 95% confidence intervals are plotted around the point estimates for both susceptibility and development time. Susceptibility estimates were extracted from the binomial glm used to analyse the infection data (fig. 2.1). Development time (fig. 2.2c) was directly measured as described in the manuscript. The correlation was found to be significant using a Pearson's product moment correlation test. Line 3 is the most susceptible and has the shortest development time, the least susceptible lines with the longest development times are lines 1 and 5.

Discussion

Our results demonstrate a trade-off between development time and resistance to a viral pathogen (fig. 2.3). This is not the first time such a trade-off has been shown in this system. Previous experiments (Boots and Begon 1993; Boots 2011) have shown that populations of *Plodia* can be selected to evolve increased resistance to

PiGV at the cost of longer developmental times. We confirm the existence of this trade-off between resistance and development time using an alternative method, without selection for specific traits. For practical reasons it is often the case that key findings in evolutionary ecology are never replicated. Given the multiple methods now used to demonstrate this evolutionary trade-off in at least three separate experimental instances, the cost of resistance in *Plodia* is arguably one of the most robust and well-evidenced evolutionary trade-offs in evolutionary ecology.

This study's use of inbred isolines provides novel insights into this trade-off, with important ecological and evolutionary implications. Unlike in previous selection experiments, all sampling in this study was carried out using host populations that were not historically exposed to the pathogen. Evolution of each isoline to a genotype with either higher or lower resistances was achieved through inbreeding and genetic drift, rather than selection for particular traits, and all evolution occurred in absence of any infection. Whilst previous experiments have taken steps to try and rule out intergenerational effects by using larvae two generations removed from the selective pressure, it remained possible that epigenetic effects were underpinning the apparent trade-off. This study rules out such possible confounds, and confirms that this widely-cited evolutionary trade-off is genetic in its basis.

Similarly, this study also demonstrates that longer development time is a constitutive cost of host resistance, rather than facultative. Interestingly, resistance to *Bacillus thuringiensis* (Bt) toxin has also been linked to longer development times in *Plodia interpunctella* (Oppert et al. 2000). Constitutive costs of resistance are understood to be more difficult to detect empirically (Armitage et al. 2003; Cressler et al. 2015), but have now been demonstrated in multiple ways in this system. This experiment shows that the cost of resistance (longer development time) manifests even in larvae which are evolutionarily naïve to, and have not been exposed to, the pathogen. This differentiates the cost of resistance in this system from cases where resistance costs manifest only after exposure to a pathogen (Dallas et al. 2016). The importance of demonstrating that this evolutionary trade-off is underpinned by a genetic, constitutive cost begins to help validate the rich

body of theory on the evolution of host resistance to pathogens. As an evolutionary force, genetic resistance to pathogens is critical in theories from the active evolutionary management of our agricultural food supply (Brown 2002; Seifi et al. 2013), to the evolution of sexual reproduction (Hamilton et al. 1990; Ashby and King 2015) especially regarding the well-supported Red Queen hypothesis (Morran et al. 2011), which relies specifically on genetically underpinned resistance. Ongoing research in this field reveals many nuances concerning costs of resistance such as how they vary with diverse environmental factors (Boots and Begon 1994; Lazzaro and Little 2009; Boots 2011; Ferris and Best 2018), or how differences in types of resistance or immunity may have different evolutionary outcomes, interacting further with the consequences of infection (for example, morbidity or sterility) (Ferguson et al. 2001; Best et al. 2009; Donnelly et al. 2015). Still, the evolutionary importance of these resistance-costs may be even greater than previously thought, as resistance to multiple pathogens may carry increasingly accelerating costs to the host (Koskella et al. 2011). Empirically validating model assumptions, as is done in this study, is therefore critical to the advancement of this field of evolutionary research.

Methodologically, this study confirms that strict inbreeding regimes are capable of yielding experimentally useful isolines in *Plodia*. Beginning with randomly selected individuals of an outbred laboratory stock population, highly homozygous isolines can be established with genotypes that differ in competitively relevant traits. Indirectly, this also validates that ‘outbred’ laboratory stock populations of *P. interpunctella* do maintain appreciable amounts of standing genetic variation. There is potential for this technique to be used widely in this system, including to improve on the results shown here. In particular, beyond the simple existence of evolutionary trade-offs between host resistance and other competitive traits, the shape of such trade-offs is under important scrutiny. This shape of resistance-cost trade-offs has crucial evolutionary relevance (Boots and Haraguchi 1999; de Mazancourt and Dieckmann 2004; Bowers et al. 2005; Hoyle et al. 2008; Boots et al. 2009), with qualitatively different evolutionary outcomes depending on trade-off shape. Inferences have been made about the shape of the trade-off in this *Plodia* - PiGV system before, implying a decreasing-costs trade-off shape (Mealor and

Boots 2005), where increasing investment in resistance yields increasingly higher gains per unit of investment, rather than a trade-off shape where investment in resistance yields large benefits at low resistance levels but much smaller increases in resistance when resistance is already high. Despite the importance of these differences, direct characterisation of resistance-cost trade-offs has yet to be achieved in any comparable system. With a larger number of inbred isolines examined, it may be possible to use this technique to examine directly what the shape of the resistance-cost trade-off is in this system. In this study, too few isolines survived the inbreeding regime, and confidence intervals around susceptibility and development time were too large, for such precise analysis of the apparent trade-off. We speculate that with a greater capacity for mating-jar replicates, more lines would have survived the initial period of intense inbreeding depression, and that the loss of most of these lines was principally stochastic due to overall very low mating success during this stage of the experiment.

Plastic adjustment of development time has been demonstrated in *Plodia* (Gage 1995), a phenomenon which may play a part in other aspects of host defense. By using highly homozygous isoline genotypes in this study to examine a genetic, constitutive component of host resistance we eliminate the possibility of plastic effects. But applications in understanding plasticity of developmental and susceptibility traits is also equally possible, where eliminating genetic ‘noise’ in data (via the use of homozygous isolines) is a powerful study tool. Given the aforementioned need to work towards understanding the interacting, complementary routes of resistance organisms exploit, use of such isolines to study both genetic and plastic, and facultative and constitutive, costs to resistance may prove useful for building future theoretical syntheses. Additionally, such inbred isolines can be used as a standardised host background for studies seeking to better understand pathogen evolution. This system has been used to demonstrate selective pressures on the adaptation of PiGV before (Boots and Mealor 2007); similar experiments may benefit from the use of inbred host isolines when characterising viral traits.

Our approach of using inbred lines has yielded clearly beneficial insights into this evolutionary trade-off, however the very high rates of population loss during severe inbreeding (80% lost during first 8 generations, with no further losses after this initial stage) is worth some consideration. Following the period of severe inbreeding depression, the purging of strongly deleterious recessive alleles, the surviving inbred lines should be highly homozygous with no lethal or sterility-inducing gene variants. The inbred lines here therefore should represent populations with almost no recessive-lethal or recessive-sterile alleles. Additionally, these lines are more likely to be highly viable, high-fitness populations with lower prevalences of mildly deleterious genetic material. As to how this relates to nature is open to interpretation. Natural populations will likely contain far more recessive-deleterious alleles evading selection than the inbred lines here, although the effect of this absence on our results isn't clear. Outbred laboratory stocks are by design exposed to very benign environments with minimal selective pressure, which contrasts populations in nature. Laboratory stocks are therefore likely to accumulate far more deleterious alleles than are present in wild populations, and thusly be of lower fitness if reintroduced to a 'natural' environment (Bryant and Reed 1999). Inbreeding may help reverse this by favouring highly viable, fit lines, which would better reflect natural populations under constant selection. Detailed discussion of the benefits and caveats of studying equivalent *Drosophila melanogaster* isofemale (inbred) lines is given in David et al. (2005).

In summary, this study confirms the previously evidenced trade-off between resistance to a virus and development time in *Plodia interpunctella,* employing a method which avoids the confounds of selection experiments and proves that this trade-off is genetic and constitutive. Additionally, this study demonstrates that inbred isolines of *Plodia interpunctella* can be established such that isoline genotypes differ in their traits due to genetic drift during inbreeding. These isolines may be a powerful tool for this system in further work examining the shape of the resistance-cost trade-off, and may also prove useful in experiments examining viral evolution in this system.

Chapter 3 – Selection asymmetry in a resistance development-time trade-off.

Abstract

Trade-offs are a critical aspect of eco-evolutionary theory. In particular, trade-offs governing resistance to parasites are understood to be crucial in accounting for the variation in resistance to parasites observed in nature. As trade-offs governing parasite resistance have been used widely in both applied and fundamental science, empirical interrogation of established trade-offs is important. For example, how phenotypes deviate from a defined trade-off relationship, and the genetic underpinnings of correlations between resistance and other phenotype aspects. Here, we examine a well-characterised trade-off between development time and resistance to viral infection in the moth *Plodia interpunctella* to test whether selection for a phenotype known to be a cost of resistance (longer development time) indirectly selects for increased resistance. We show that, opposite to our expected hypothesis, selecting for longer development time in this system also apparently selects for reduced resistance when compared to selection for shorter development time. This implies that phenotypes typically charactised by a trade-off can deviate from that trade-off relationship, and suggests little genetic linkage between the genes governing viral resistance and life-history in this species. Such observations are important for both selection strategies in applied biological systems and for evolutionary modelling of host-parasite interactions.

Introduction

Trade-offs are fundamental to modern ecological and evolutionary thinking (Shoval et al. 2012; Acerenza 2016). They are central to our understanding of the evolution and ecology of infectious diseases (May and Anderson 1983; Alizon et al. 2009; Alizon and Michalakis 2015) and in particular to the evolution of resistance to both chemical and biological agents (Gillespie 1975; Sheldon and Verhulst 1996; Gemmill and Read 1998; Boots and Bowers 1999; Boots and Haraguchi 1999; Shirley and Sibly 1999; Gandon et al. 2002, 2008; Gwynn et al. 2005; Miller et al. 2007; Best et al. 2011; Foster et al. 2011; Best and Hoyle 2013; Gandon and Vale 2013). Large bodies of both theoretical work and empirical studies have made

strides in our fundamental understanding of evolutionary trade-offs, however we need work to better integrate theory and empirical findings (Schmid-Hempel 2003; McKean et al. 2008; Cressler et al. 2015). A better understanding of fundamental evolutionary processes is crucial to the evolutionary management of many aspects of modern biological policy (e.g. Yan et al. 1997; Brown 2002; Seifi et al. 2013; Brosi et al. 2017), and therefore investigating the fundamental nature of trade-offs with both empirical and theoretical bodies remains of considerable importance.

The rules selection and trade-offs are governed by is still a topic of significant research. One notable question relates to whether the manifestation of trade-offs depends on the direction of selection. That is to say, whether selection for A at the cost of B operates equivalently to selection on B also changing A. Most theoretical work implicitly assumes this to be the case, both in adaptive dynamics eco-evolutionary modelling and in population genetics (Geritz et al. 1998; Kisdi and Geritz 1999; Abrams 2001; Waxman and Gavrilets 2005). This assumption of symmetrical behaviour depending on which phenotype axis selection acts on is a powerful simplification for making progress in evolutionary theory, however, it may not be as reasonable an assumption as it first appears. For example, when one trait in a trade-off is highly polygenic in its underpinning, it may be able to evolve in ways outside an otherwise well-defined trade-off. Testing such theoretical assumptions on many trade-offs is often difficult, partly given that detecting trade-offs is already challenging (McKean et al. 2008; Cressler et al. 2015, 2016). However, regardless of the practical difficulty, it remains an important part of better synthesising evolutionary research; while theory must be built to better accommodate reality, empirical work must also make effort to test which assumptions theory can work with.

One particularly well evidenced trade-off is that of *Plodia interpunctella* (Hübner) and resistance to the baculovirus, Plodia interpunctella Granulosis Virus (PiGV). Two notable selection experiments have shown that *Plodia* experiencing selection through exposure to the pathogen evolve resistance to it, at the cost of increased development time (Boots and Begon 1993; Boots 2011). This apparent trade-off was confirmed to be both genetic and constitutive, as a test of assumptions made

in evolutionary theory, in a third experiment where evolution happened via inbreeding and genetic drift (Bartlett et al. 2018c). As such this trade-off has rather unusually been shown in replicated selection experiments, under different resource conditions and using inbred lines. This same system has been used to infer trade-off shape from population level patterns of resistance (Mealor and Boots 2005), another critical aspect of theoretical work. Additionally, similar developmental trade-offs in *Plodia* have been demonstrated in the context of bacterial and parasitoid resistance (Oppert et al. 2000; Niogret et al. 2009). Overall this trade-off is one of most robustly demonstrated in the evolutionary literature, where resistance to a parasite consistently evolves at the cost of increased development time. Here, we use this system to test whether selection in the opposite direction along this trade-off – that is, selecting for specific development time phenotypes rather than resistance phenotypes, would behave in a comparable way.

Our main hypothesis is that selecting for faster or slower development time would also indirectly select for differences in immunocompetence, specifically that fast-developing selected lines would be less resistant than slow-developing selected lines. Previous work has noted no significant change in mass at pupation in *Plodia* selection experiments on resistance; our leading hypothesis relies on this being the case during selection on development as well. Selection may however act to alter mass at pupation alongside development time, possibly 'balancing' growth rate to remain similar regardless of development time differences. This may have consequences to resistance. Our leading hypothesis also relies on close genetic linkage or pleiotropic effects underpinning the previously established genetic constitutive trade-off between development time and susceptibility to pathogens (Bartlett et al. 2018c). Without tight genetic links between these two phenotype axes, we cannot assume that selection acting on one will always inadvertently lead to effects on the other.

Materials and Methods

STUDY ORGANISMS

We used the host organism *Plodia interpunctella*, a pyralid moth, and the baculovirus Plodia interpunctella Granulosis Virus. The larvae of *P. interpunctella* are a widespread grain-feeding pest (Mohandass et al. 2007), used as an experimental study species for its ease of population maintenance and its agricultural importance (Silhacek and Miller 1972; Mohandass et al. 2007). It exhibits a simple ecology, divided into larval and adult stages. Eggs are laid into cereal media by adults in a semelparous event, larvae then develop in the food media until pupation, and following pupation adult moths emerge, mate and a new generation of eggs are laid into the cereal medium (Gage 1995). Adults do not have functional feeding physiology; their reproductive success is broadly determined by how quickly they can develop and their pupal mass (Silhacek and Miller 1972; Boots and Begon 1993). *Plodia* larvae can be infected by the baculovirus Plodia interpunctella Granulosis Virus (PiGV) (Sait et al. 1994). PiGV infections are obligately lethal, following infection via consumption of viral occlusion bodies. PiGV infection likelihood can differ between *Plodia* populations (Vail and Tebbets 1990; Boots and Begon 1995; Bartlett et al. 2018c), and *Plodia* may evolve resistance to the virus, with a cost of increased development time (Boots and Begon 1993, 1994; Boots 2011; Bartlett et al. 2018c). Intrahaemocoellic antiviral activity against PiGV infection is documented (Saejeng et al. 2011), although is apparently separate from the phenoloxidase pathway (Saejeng et al. 2010). Resistance is thought to occur mostly at the gut wall, through mechanical barriers such as the peritrophic membrane and apoptosis of infected gut wall cells (Begon et al. 1993; Tidbury 2012).

POPULATION MAINTAINANCE AND ARTIFCIAL SELECTION

We maintained *Plodia* populations following well-established protocols. Our selection lines all originated from the same outbred laboratory stock population, which we have shown in previous studies to maintain appreciable amounts of genetic variation in life-history characteristics and and resistance to PiGV (Bartlett et al. 2018c). We originated each line as a starting cohort of 60 randomly selected, recently emerged *Plodia* adults, placed on 200g of fresh food media inside 1000ml straight-side wide-mouth Nalgene jars (ThermoFisher Scientific, U.K.). We

prepared food media in batches consisting of 250g ‘Ready Brek’ (Weetabix Ltd., U.K.), 150g wheat bran (Bob’s Red Mill, U.S.A.), 100g rice flour (Bob’s Red Mill, U.S.A.), 100g brewer’s yeast (MP Biomedicals, U.S.A.), 125ml glycerol (VWR, U.S.A), 125ml clear organic honey (Dutch Gold Honey Inc., U.S.A.), 2.2g methyl paraben (VWR, U.S.A.), and 2.2g sorbic acid (Spectrum Chemicals, U.S.A.). We homogenised the media with industrial mixers before it was sealed and frozen for a minimum of 24 hours prior to thawing at ambient temperature for use.

Adult moths mate, lay eggs into the food media, and die in a single semelparous event with no overlap of generations. Eggs then hatch to develop in the food media through five larval instars, before pupation and eclosure as adult moths. We select sixty of these next-generation adult moths to be placed in a new jar of food media to found the next generation. How we select these sixty moths to found the next generation is how we differentiate our two selection regimes, dubbed ‘early-‘ or ‘late-‘ selected.

For the early-selected lines, each population was checked daily. Any newly emerged adults were counted. When we could see at least sixty next-generation adults in a jar, they were collected to found the next population, and the rest of the larvae or pupae frozen. Under this regime, only the very fastest developing larvae (relative to the rest of their population) were allowed to reproduce. For the late-selected lines, populations were checked daily. Once adult moths had emerged, the population was monitored for the presence of 5^{th} instar larvae. So long as abundant 5^{th}-instar larvae were present, any adults moths were removed from the population and frozen. Only once no 5^{th} instar larvae were visible would sixty adults then be randomly selected from the remaining population and transferred to a new jar of food media. In this way, we allowed only slower developing larvae to found the next generation for that line, although we could not guarantee these were the absolutely most slowly developing of their generation.

We maintained these selection regimes for approximately four years, however the number of generations this time period represents is different for each line (Table 3.1). We maintained all selection lines on the same level of the same incubator throughout the experiment, where they experienced a constant climate of 27±2^{o}C

and 35±5% humidity, with 16:8hr light:dark cycles. Following this period of maintenance and selection, we assayed the lines' life history and resistance phenotypes.

We did not maintain and assay any 'non-selected' lines. This decision was made on the basis that we would expect a much greater amount of genetic variation to persist in such lines, by virtue of not experiencing any directional selection, compared to our lines experiencing a selection regime. Such anticipated heteroskedasticity would pose a serious problem for our intended statistical approaches, potentially mandating the exclusion of such data. As maintaining these population lines represents a large investment of resources, we opted not to maintain lines which were likely to be uninformative come analysis.

RESISTANCE AND LIFE-HISTORY ASSAYS

We followed nearly identical protocols to those detailed in Bartlett et al. (2018c) for characterising lines' life history and resistance phenotypes, with two exceptions. We used fewer viral doses (three in lieu of six) due to a limited number of larval cohorts we could dose in a single reproductive bout. Secondly, we undertook assaying in two blocks: four lines were assayed per block, with two early-selected and two-late selected lines in each block. The two blocks were separated by approximately one calendar month. This was a limitation of asynchronous generation timings between lines, and a limit on the human-resource intensive nature of setting up assays leading us to not attempt to eight lines in a single day, which would require what we the authors regard as irresponsible and discriminatory working hours.

We characterised the life-history traits of each line using two measures: time to pupation (development time) and pupal mass. For each line, we took sixty adult *Plodia*, known to have eclosed in the last 24 hours, placed them in jars of new food media and incubated them as above. After 11 days, we selected fifty larvae on the 1st day of their 3rd instar from each line and placed them in individual compartments on 25-cell compartmentalised square petri dishes (ThermoFisher Scientific, U.S.A.) (two petri dishes per isoline), with ample food media. We can

identify 1st-day 3rd-instar larvae based on the size of their head (which changes only during moulting and identifies different instars) and the size of their body (which if smaller in diameter than the head signifies their 1st day at that instar). Petri dishes were then incubated as above and checked daily to monitor larval development. We recorded the date of each larva's pupation, and two days later we extracted the pupa from its silk cocoon and weighed it using a 1µg -precision microbalance. Not all larvae were recovered, as some inevitably die due to handling or other causes of stochastic mortality; in particular, losses of pupa where the pupation date is known but pupa were damaged during extraction mean we had larger sample sizes for the development time measure ($\bar{n}$ = 27.5 pupae recovered per line, Supplementary Material 2) compared to the weight at pupation measure ($\bar{n}$ = 22.375 pupae recovered per line, Supplementary Material 2).

We measured the resistance of each line to PiGV by comparing infection rates of larvae to different PiGV doses. We took 150 1st-day 3rd-instar larvae from each line, following the same protocol as described above. We placed larval cohorts of fifty larvae into circular petri dishes (three cohorts per line) and starved the larvae for one hour. We then pipetted droplets of virus solution into these petri dishes, with each cohort given one of three solutions. Virus solutions represented three doses of each diluted by an order of magnitude (such that the strongest dose is 100 times stronger than the weakest). We diluted solutions with distilled water, all solutions contained 2% sucrose (ThermoFisher Scientific, U.S.A.) and 0.1% Coomassie Brilliant Blue R-250 dye (ThermoFisher Scientific, U.S.A.). Larvae voluntarily feed on the solution droplets due to their brief starvation and the solution sucrose content. We considered an individual larva dosed when 50% of their alimentary track was stained blue (visible due to the blue dye and translucent larval body) at which point we removed them from the petri dish and placed them individually in 25-cell compartmentalised square petri dishes, before incubating for twenty days as above. After twenty days, we froze the petri dishes to kill all remaining live larvae, before opening them for counting. Infected larvae are apparent due to their bright white cadavers, a consequence of the accumulation of viral occlusion bodies in the haemolymph. Uninfected larvae were distinguishable as healthy larval cadavers or as developing pupae. Not all larvae were recovered

to be categorised as either infected or uninfected, as some inevitably die due to handling or other causes of stochastic mortality ($\bar{n}$ = 16.75 larvae recovered per line per dose, Supplementary Material 2).

STATISTICAL APPROACH

All analyses were undertaken in R (v.3.4.4 - "Someone to Lean On") (R Core Team 2018). We analysed life history traits (development times and pupal masses) without transformation using analyses of variance (ANOVAs) to test for differences amongst lines, and Welch's t-tests to test for differences between selection regimes. A Bonferroni correction for multiple testing of these life-history traits would place our significance threshold at $p < 0.0125$.

We analysed infection data using generalised linear models in the style of a backward stepwise model selection (Crawley 2012), and in some instances compared against generalised linear mixed effects models (Schielzeth and Nakagawa 2013) using the package 'lme4' (Bolker et al. 2009; Bates et al. 2015). In both cases, we used binomial models with a logit link function, and fit models to infection data based on combinations of dose, line, selection regime, and interactions between these predictors. Dose was provided in $\log(x + 1)$ rather than identity space. We first tested for significant differences in infection likelihood amongst lines, subsequently testing for an effect of treatment only if between-line differences were found. Significant interactions between the predictors 'line' and 'dose' would indicate heterogeneity of host response. We checked our final binomial GLMs for overdispersion using the equivalent quasibinomial GLMs and examining whether the dispersion parameters were > 1.

Table 3.1 - Number of generations of selection experienced by each line at the point of assaying, and which assay block each line was assigned to.

Line	Selection Regime	Generations of Selection	Assay Block
E1	Early	52	A
E2	Early	55	A
E3	Early	48	B
E4	Early	51	B
L1	Late	37	A
L2	Late	38	B
L3	Late	40	B
L4	Late	37	A

Results

Our selection regimes lead to significant differences in life history patterns amongst lines, in the direction anticipated for the experimental design. Lines showed significant differences in their development time (ANOVA, $F_{7,212} = 6.61$, $p < 0.001$), which when grouped by late- or early- selection showed that early-selected lines developed significantly faster (Welch's t-test, $t_{217.18} = -4.27$, $p < 0.001$) than late-selected lines (fig. 3.1). We found possible marginal evidence that lines differed in their mean pupal masses (ANOVA, $F_{7,171} = 2.65$, $p = 0.0125$), however when grouped by selection regime (fig. 3.1), no difference was observed in mean pupal mass between early- and late- selected lines (Welch's t-test, $t_{172.2} = 0.580$, $p = 0.563$). Note that if we were to undertake a Bonferroni correction for multiple testing of life-history traits, the evidence that lines differ in their mean pupal weight sits exactly at this corrected significance threshold.

Lines also showed significant variation in their resistances to PiGV (fig. 3.2). We used a binomial GLM with a logit link function to analyse whether lines differed in their resistance to PiGV and if so, if they showed heterogeneity of dose response. We found no evidence ($p = 0.326$) of an interaction effect between 'line' and 'dose',

indicating no heterogeneity of dose response (in agreement with previous work in this system (Boots and Begon 1994; Boots 2011; Bartlett et al. 2018c)). However lines did differ in their overall resistance to the pathogen ($p < 0.001$), illustrated in fig. 3.2. We undertook further testing to see if these differences corresponded to our two selection regimes.

When grouped by selection regime, we found that resistance to the pathogen did differ based on whether larvae were from late-selected or early-selected populations (fig. 3.3). We found selection regime to be a significant predictor of infection ($p < 0.001$), where early-selected lines were more resistant to infection than late-selected lines (fig. 3.3), counter to our initial hypothesis. We confirmed this finding with a binomial generalised mixed-effects model with both 'line' and 'block' (Table 3.1) as random effects to account for our nested experimental structure (Schielzeth and Nakagawa 2013), and recovered the same finding (Supplementary Material 2 fig. S2.1). We present the results of the GLM as we can more easily extract confidence intervals (fig. 3.3) from this model compared to the mixed-effects model. Neither the binomial GLM used to show differences amongst lines nor the GLM used to show differences between treatments showed evidence of overdispersion (equivalent-model quasibinomial model dispersion parameters = 0.866 and = 0.847 respectively).

We provide an annotated R script for reproducibility of all analyses (Supplementary Material 2).

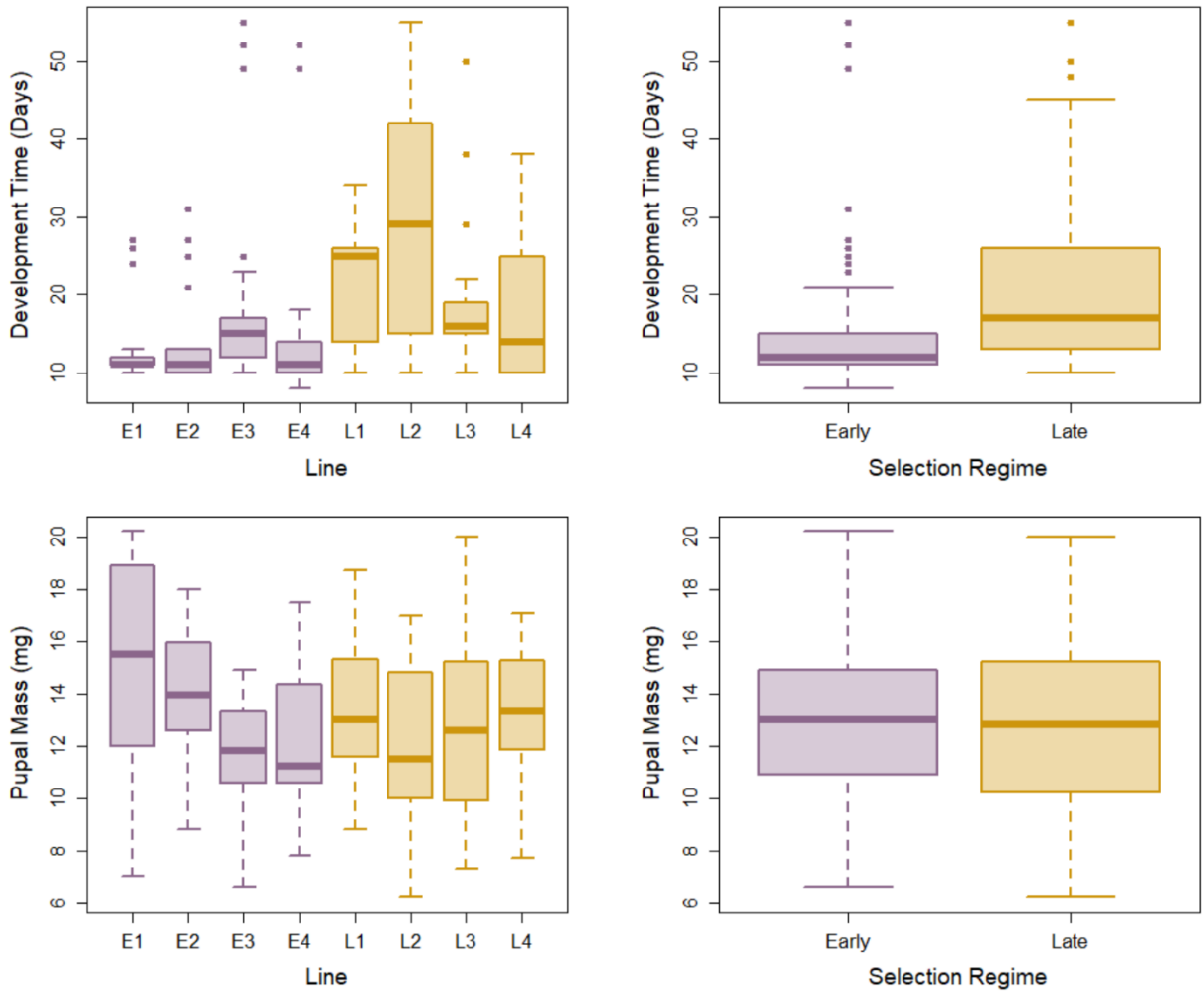


Figure 3.1 – Panelled box plots illustrating life history traits of each line (left) and lines grouped by treatment (right). Significant differences amongst lines and between treatment are observed for development time (upper row), with the early-selected lines developing more quickly than the late-selected lines. No appreciable difference in pupal masses is observed between treatments (lower right) or amongst lines (lower left). Early-selected lines are shown in purple and left-aligned on each subplot, late-selected lines are shown in orange and right-aligned on each subplot.

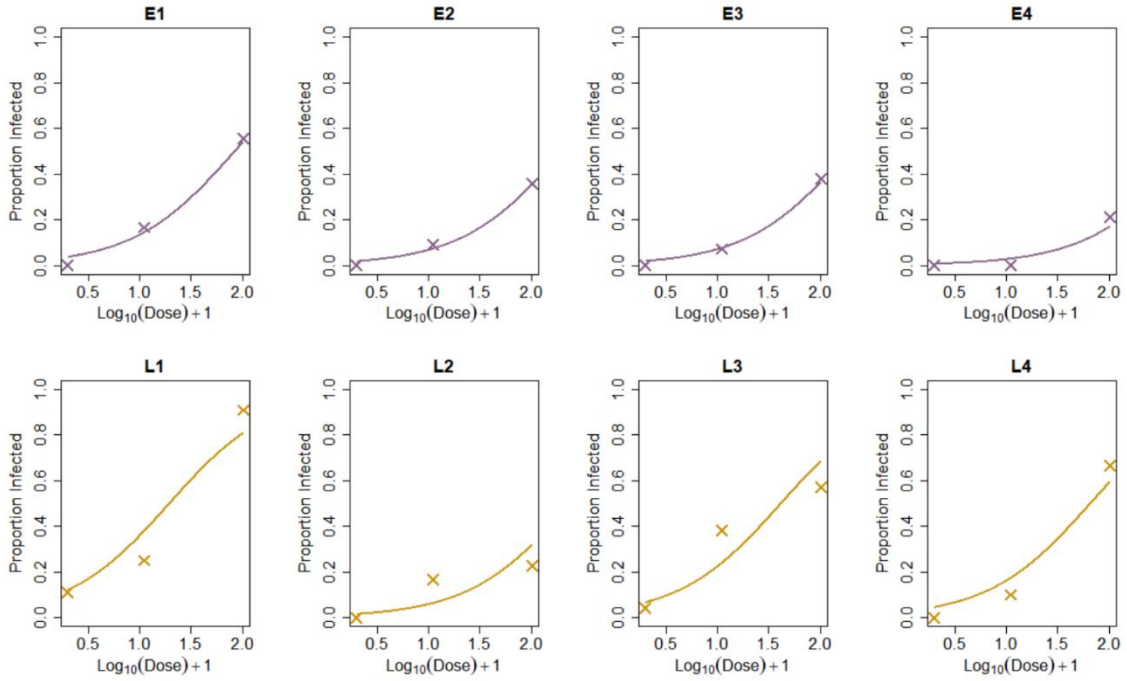


Figure 3.2 – Panelled plots showing resistance of each line to PiGV. Plotted crosses represent proportion of larvae infected across the three administered viral doses, where dose strength is plotted in logit space. Plotted curves represent the predicted proportion of infected larvae with changing dose extracted from the binomial GLMs used to analyse the infection data. The four early-selected lines are plotted on the upper row in purple; the late-selected lines are plotted on the lower row in orange. Lines do not show heterogeneity of dose response, but do significantly differ in their resistance to PiGV.

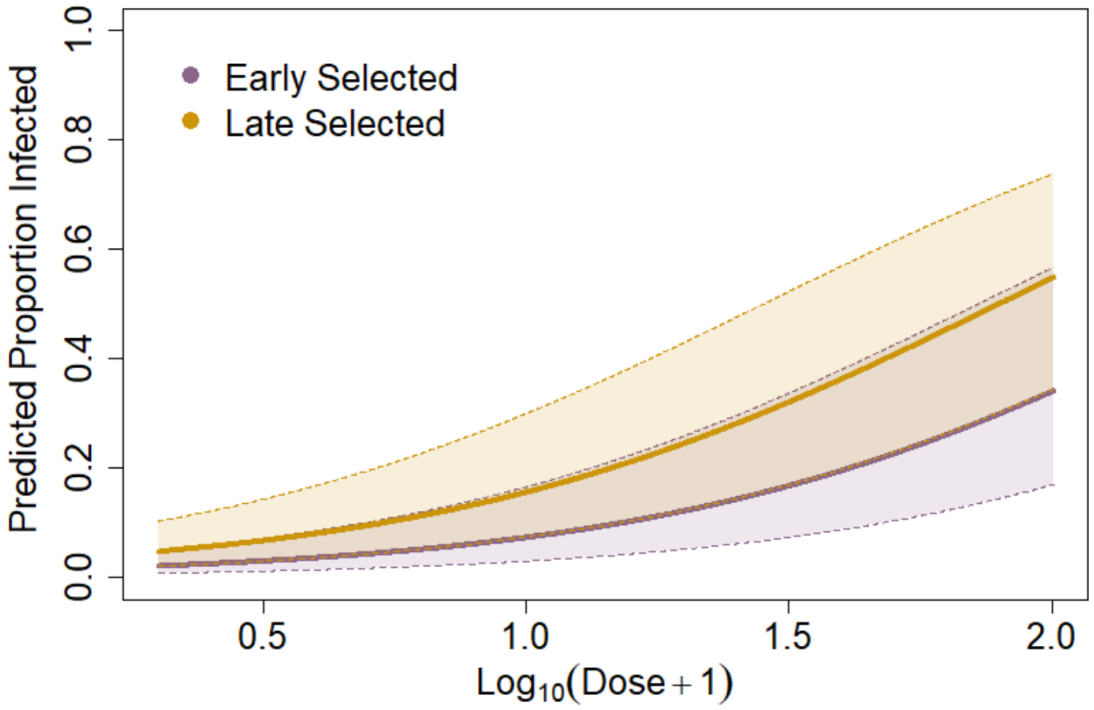


Figure 3.3 – Plot illustrating predicted proportion of larvae infected across the dose range for average early-selected or late-selected lines, taken from the binomial GLMs used to analyse the infection data. Shading and dashed lines represent 95% confidence intervals with solid lines representing the best fit. Late-selected lines were significantly less resistant to PiGV than early-selected lines, counter to our hypothesis.

Discussion

We successfully selected for differences in development time (fig. 3.1), without changes in pupal mass, which means that there are differentiated growth rates between our two selection treatments. No change in pupal mass and a change in development time is in agreement with the previous work on this system demonstrating a resistance development time trade-off (Boots and Begon 1993; Oppert et al. 2000; Boots 2011; Bartlett et al. 2018c). However, the consequence of these selection regimes ran opposite to our initial hypothesis. Selection lines which had evolved faster development times were less susceptible to infection than lines which had evolved slower development times (Figs. 3.2 & 3.3), counter to the

repeated demonstration of this trade-off documented elsewhere (Boots and Begon 1993; Oppert et al. 2000; Boots 2011; Bartlett et al. 2018c). Therefore our experimental approach was methodologically successful, however our results run counter to our initial hypothesis.

This finding is not necessarily evidence against the existence of an otherwise robustly supported trade-off. Rather, our findings offer some insight into the likely genetic underpinnings of this trade-off, the mechanisms of immunity in this system, as well the potential caveats of selection experiments. There are multiple plausible explanations for our finding, including the mechanistic underpinnings of the differential immunity that we observed. Additionally, we believe these insights are better discussed by separating our results into two linked parts: 1) a failure to see the same correlation between development time and resistance that has been found in the established trade-off; 2) significant differences in resistance running counter to the previously established trade-off.

We failed to recover the previously established trade-off and found the opposite correlation between resistance and development time when selection occurred on development time. While it has been demonstrated that the trade-off in question is constitutive and genetic (Niogret et al. 2009; Bartlett et al. 2018c), currently no published information exists from which to infer how many genes may be responsible or involved in links between immunity and development time. Variation in insect life-history phenotypes are often highly polygenic (Comeault et al. 2014; Jha et al. 2015), with many interacting genes responsible for determining fecundity, growth rates, or size at maturation. Our failure to recover the development time resistance trade-off may be a consequence of such polygenic underpinnings. Our leading hypothesis that the trade-off would be recovered relies on strong genetic linkage between genes governing development time and genes governing viral immune response. This is a bold assumption to make, given that as stated above, developmental traits are typically understood to be highly polygenic. Our failure to recover the previously established trade-off constitutes reasonable evidence that there is indeed low genetic linkage between most genes governing life-history phenotype and most genes governing viral immunity.

As molecular genetic and sequencing methods become increasingly widespread and affordable, efforts to link specific genes to selection experiments or evolutionary trade-offs have become more common (Korte and Farlow 2013). However, such endeavours remain effort-intensive and comparably expensive, and can rely on a few highly deterministic pleiotropic or linked genes. Such endeavours in other systems have had some success in identifying pathogen-resistant quantitative trait loci (Zhong et al. 2005); however, our findings demonstrate that the trade-off established in *Plodia* is perhaps unlikely to be suitable for such genomics approaches and as a consequence population-genetics modelling (Gassmann et al. 2009). We anticipate that *Plodia* are not unique in this regard, and that even replicably-demonstrable trade-offs proven to be genetic in basis may not necessarily be easily detected using GWAS-like approaches.

The significant differences in resistance that we observed, running counter to the established trade-off, have multiple plausible explanations with potential insight for related future experiments. One possible hypothesis is that haemocoellic immunity, which has been shown to vary between populations in this system (Saejeng et al. 2011) is unaltered between our selection lines, whilst midgut immunity is inherently greater in the fast-developing lines due to greater likelihood of shedding viral occlusion bodies, before infection occurs, through accelerated ecdysis. Engelhard and Volkman (1995) showed in a similar lepidoptera-baculovirus system that a development time difference between larval instars of just a few hours significantly affects infection likelihood and that larvae were able to fully clear early infections from the midgut epithelium during ecdysis. Interestingly, similar work in mosquitoes has shown that increased resistance to an ingested pathogen correlates with faster development (Yan et al. 1997; Koella and Agnew 1999), yet some of those same authors show that late-selected mosquito lines exhibit higher haemocoellic immune activity than early-selected lines (Koella and Boëte 2002), counter to their previous findings. Furthermore, there is fundamental theoretical work exploring how accelerated development can be an adaptive response to age-structured infection (Hochberg et al. 1992).

It is therefore plausible that this study has indirectly increased resistance in the faster-developing lines by allowing larvae to escape infection more quickly, potentially through more rapid ecdysis after inoculation. We speculate this is likely at the expense of some other competitive axis which doesn't manifest in these experiments. If this is indeed the case, such midgut ecdysis mediated immune measures can be inferred to grant only small degrees of resistance compared to other routes (such as haemocoellic immune activity), as the absence of this correlation in previously cited trade-off work (Bartlett et al. 2018c) suggests it is overshadowed by other aspects of differential immunity. Speculation on the comparative roles of midgut (primary) and haemocoellic (secondary) pathogen defense in this system (Begon et al. 1993; Saejeng et al. 2010, 2011; Tidbury 2012) and other lepidoptera-baculovirus systems (Shikano and Cory 2014; Iwata et al. 2017; Nakai et al. 2017) has been made elsewhere, and may be further informed by this hypothesis of escaping infection through rapid ecdysis.

Conversely, it may not be that the early-selected lines are benefitting from increased immunity, but that nuances of our experimental design have led to late-selected lines being particularly vulnerable to infection. It is well established that many mutations are purely detrimental yet aren't easily purged by large populations, constituting 'genetic load' (Whitlock and Bourguet 2000; Crnokrak and Barrett 2002; Wielgoss et al. 2013); captive managed (laboratory) populations may harbour much larger genetic loads than their wild counterparts (Bryant and Reed 1999) on the basis of relaxed selection. Our late-selected lines favoured long development time, in isolation a disadvantageous trait (Silhacek and Miller 1972; Boots and Begon 1993; Bowers et al. 1994), and saw no corresponding increase in pupal mass (which may have otherwise counterbalanced the apparent loss of fitness). By selecting for a broadly less-fit phenotype, we may have simply inadvertently selected for individuals of broadly low quality – harbouring large numbers of deleterious alleles and significantly elevated genetic loads across the population. This confound is arguably a predictable outcome of such selection experiments. Our 'late-selected' treatment may have manifested as selection for poor performing, low-fitness phenotypes. In this case, it may be no surprise these populations show elevated susceptibility to infection. Without characterising the

ancestral phenotypes alongside our two selection lines, we cannot determine if both our early-selected and late-selected lines show increased susceptibility compared to their ancestral state. In this case, the early-selected lines may indeed have conformed to the established trade-off, whereas the late-selected lines move away from regions of phenotypic space where trade-offs can manifest – instead showing entirely reduced fitness and poor genetic stock quality.

The latter case may be an unfortunate peril of selection experiment design, highlighting the need to maintain non-selected 'ancestral' populations for comparison. Such designs do also pose significant statistical challenges however. Ancestral populations assayed at the start of a four year experiment such as this may not be comparable to final selected-population assays given different assaying conditions (including reagents, viral stock (which is known to degrade with time), researchers, locations). Additionally, we would anticipate populations undergoing no selection to show greatly elevated within-population variance compared to populations subjected to four years of directional selection. Such heteroskedasticity can significantly impede meaningful comparisons between populations.

Such caveats also act as a warning for comparing our results in this study with those we have generated elsewhere, for example in Bartlett et al. (2018c), where populations originating from the same stock were assayed, but using different PiGV batches. We can tentatively compare life-history assays, which do not fall foul of being confounded by different viral stocks, and we can see that the selected lines here occupy the same approximate range of development times and pupal masses as those in Bartlett at al. (2018c), although are somewhat more extreme in their range as might be expected given the differences in evolutionary strength of selection compared to drift via inbreeding. We therefore see some degree of reproducibility between experiments, despite the fundamental difference in a reversal of immunocompetence – development correlation between these two studies.

Future investigations of these selection lines may provide fruitful if assayed in comparison to those detailed in Bartlett et al. (2018c) under one single experiment. Doing so, we may be able to differentiate between whether our early-selected lines

fall on the same trade-off as previously characterised inbred lines while our late-selected lines appear as an ill-performing outlier (see above), or whether the selection experiment here does deviate entirely from correlations elsewhere. In this latter case, the apparent ‘costless gain’ in fitness here shown by the early-selected lines would require investigation – it may be that our early-selected lines show reduced fecundity (deviating from the reproductive predictors laid out in Boots and Begon (1993)), or perhaps greatly increased sensitivity to environmental fluctuations such as desiccation or temperature stress. There are numerous insect-resistance studies which similarly seem to identify ‘costless resistance’ (Milks et al. 2002; Undorf-Spahn et al. 2012; Faria et al. 2015). However these findings are difficult to reconcile with theory and widespread observation in variation of resistance (Schmid-Hempel 2003; Susi and Laine 2015; Koskella 2018), and it is acknowledged that trade-offs are difficult to tractably characterise experimentally (Cressler et al. 2015). Whether unknown axes of fitness may easily explain our findings here would need further investigation.

In conclusion our results suggest a likelihood of a polygenic underpinning of the established trade-off in *Plodia* and its immunity to PiGV. Consequently, pursuing population-genetics approaches in this system may not be fruitful. Additionally, these results highlight potential mechanisms of immunity which may be worth further investigation; notably, accelerated growth to escape infection, and in this case rapid ecdysis clearing midgut epithelial infection. Finally, our possible myopia in experimental design highlights some of the challenges associated with selection experiments investigating trade-offs, evolution from ancestral states, as well as the pitfall of inadvertently selecting for entirely deleterious alleles which do not constitute any aspect of a phenotypic trade-off, but rather represent purely disadvantageous genetic load. We hope that explicit acknowledgement of these experimental limitations informs future experiments in the field.

Chapter 4 – The role of infected reproduction in the evolution of virulence in spatially structured populations.

Abstract

Spatial structure within populations due to local interactions has fundamental implications for evolutionary outcomes due to ecological and genetic correlations. In particular, local infection has been predicted to have major implications for the evolution of parasite virulence, with spatial structure selecting for less harmful parasites. However, demographic assumptions can have major implications to selection in spatially structured populations and the existing theory has typically assumed that infected individuals do not reproduce. With an approximate analysis we show that the key result that virulence is predicted to be higher in mixed populations is robust to the assumption of infected reproduction. However the magnitude of the effect on virulence is reduced. Furthermore, we show that local infection always reduces virulence, and that the prediction that a small proportion of local infection can select for higher virulence only occurs for highly castrating pathogens. The results empahsise the importance of demographic details to evolutionary outcomes in spatially structured populations, but also show that the importance of spatial structure is robust to a frequently used assumption.

Introduction

Parasites rely on their hosts to survive and transmit, but by definition damage their host typically leading to an increased rate of mortality in the infected hosts (virulence). Since virulence defined this way typically shortens the infectious period of a classic micro-parasite, it reduces transmission opportunities and hence parasite fitness. The persistence of ‘pathogens’ causing severe host mortality is therefore a key question for evolutionary biologists, particularly because of the burden infectious diseases cause for society (Jones et al. 2008; Lambrechts and Scott 2009; Froissart et al. 2010). One fundamental idea is that increasing virulence increases the transmission rate of the parasite, as without any correlated benefits, virulence itself is maladaptive (Anderson and May 1982; Ewald 1987, 1993; Read 1994; Alizon et al. 2009; Alizon and Michalakis 2015). This trade-off

hypothesis between virulence and transmission predicts an optimal level of virulence and underpins theoretical work understanding how ecology shapes parasite evolution (Cressler et al. 2016), and has considerable empirical support (Fraser et al. 2007; de Roode et al. 2008; Atkins et al. 2013).

Classic theory assumes that populations are homogenous and assume the 'mean-field' such that they are completely mixed and interactions are essentially random. However, in reality, typically there is population structure within populations, most simply due to individuals closer in space interacting more often. Local interactions leading to strong spatial structuring within populations can impact evolutionary outcomes due to a combination of ecological clustering (infected tend to be next to each other) and genetic clustering (more interactions with related individuals leading to kin selection) (Lion and Boots 2010). There is a particularly rich theory on how spatial structure impacts the evolution of parasite virulence (Rand et al. 1995; Boots and Sasaki 1999, 2000; Haraguchi and Sasaki 2000; Kamo et al. 2007; Webb et al. 2007a, 2013a; Lion and Boots 2010; Lion and Gandon 2015) with the most compelling result that highly local interactions lead to lower optimum virulence evolution than in well-mixed systems with 'global' infection; this prediction also has empirical support (Kerr et al. 2006; Boots and Mealor 2007; Szilágyi et al. 2009). However the interaction of selective pressures from ecological and genetic correlations have different selective outcomes when infection occurs both locally and globally with a small proportion of local infections selecting for higher virulence (Kamo et al. 2007).

Much of this theoretical work has successfully used a coupling of pair-approximations (Matsuda et al. 1992) and adaptive dynamics (Geritz et al. 1998; Mágori et al. 2005) approaches to develop an approximate spatial analytical prediction of evolutionary outcomes. However, in order to increase analytical tractability, this body of theory utilises numerous simplifying assumptions concerning the biology it attempts to model. One prevalent assumption across this body of studies is that infected individuals do not reproduce – infection by a parasite is fully castrating. While in some theoretical studies this assumption is relaxed, these studies are either purely ecological (Webb et al. 2007b) rather than

evolutionary, or they are non-spatial (Best et al. 2009, 2017). In spatial evolutionary work on parasite or host evolution, this assumption is almost universal (Boots and Sasaki 1999, 2000; Haraguchi and Sasaki 2000; Kamo and Boots 2006; Kamo et al. 2007; Webb et al. 2007a, 2013a,b; Best et al. 2011; Lion and Gandon 2015). This assumption improves analytical tractability, but a key characteristic of parasites as opposed to predators is that infected individuals can reproduce or recover and then reproduce. One exception is Débarre et al. (2012), who do consider variable castration in an spatial host-parasite evolutionary context, but only examined host evolution.

Castration by parasites clearly occurs, however is typically understood to be an alternative strategy where infected host mortality is very low while overall host fitness costs still remain very high, often to the parasite's overall benefit (O'Keefe and Antonovics 2002). However full castration does not represent the majority of parasitic infections in nature, although it is likely that infection reduces host reproduction to some proportion, possibly in correlation with virulence due to resource theft (Heins et al. 2010). The prevalence of this assumption in the spatial parasite evolution theory therefore needs to be interrogated, and calls to better account for host demography (including reproduction) have been previously made (Lion and Boots 2010; Messinger and Ostling 2013). Given the likely impact of local reproduction from infected individuals on both ecological and genetic correlations, there are potentially important implications for the evolutionary outcome.

Here we develop approximate theory to better understand how relaxing this castration assumption impacts the evolutionary outcomes of spatial models of parasite virulence. We adapt established theory to allow infected individuals to reproduction, sacrificing some analytical tractability and numerically solving our pair equations.

Methods and Model Description

We construct two models: one to describe the dynamics of a resident pathogen strain and one to describe the local dynamics of a rare mutant pathogen strain. We

approximate a regular lattice where each site is in state σ, and pairs of sites are in state σσ', where σ ∈ { 0, S, I, J }; '0' represents an empty site, 'S' a site with a susceptible host, 'I' a site with a resident-strain infected and infectious host, and 'J' a site with a mutant-strain infected and infectious host. We vary independently the proportion of reproduction and infection occurring locally (between neighbouring sites only) and globally (between any two sites). We vary the amount of reproductive potential an infected host represents compared to a susceptible host (we vary degree of castration caused by infection). Parameters are detailed in Table 4.1.

Table 4.1. Variables used in ODE system models, where Z ∈ { I , j } and σ ∈ { 0, S, I, J }

Variable	Description
A	Reproduction rate
B	Natural mortality (natural death rate)
C	Degree of castration ($0 \leq C \leq 1$)
α_Z	Virulence of strain z (additional mortality due to infection by strain z)
β_Z	Transmission rate of strain z
θ	Inverse of number of neighbours (= ¼ for a regular lattice)
G_R	Proportion of global reproduction
G_T	Proportion of global transmission
P_σ	Proportion of sites in state σ
$P_{\sigma\sigma'}$	Proportion of pairs of sites in state σσ', where $P_{\sigma\sigma'} \equiv P_{\sigma'\sigma}$
$Q_{\sigma\|\sigma'}$	Conditional probability that for a site in state σ' a neighbouring site will be in state σ

As P_σ and $P_{\sigma\sigma'}$ represent proportions of sites and proportions of pairs of sites, and $P_{\sigma\sigma'} \equiv P_{\sigma'\sigma}$, the following definitions hold where σ ∈ { 0, S, I, J }

$$\sum_{\sigma} \boldsymbol{P_\sigma = 1}$$

$$\sum_{\sigma} \boldsymbol{P_{\sigma\sigma}} + \sum_{\sigma \neq \sigma'} \boldsymbol{2P_{\sigma\sigma'} = 1}$$

$$\sum_{\sigma} \boldsymbol{q_{\sigma|\sigma'} = 1 \quad for\ \sigma' \in \{0, S, I, J\}}$$

$$\boldsymbol{q_{\sigma|\sigma'} = P_{\sigma\sigma'}/P_{\sigma'}}$$

In constructing the models, we approximate conditional probabilities, where $q_{\sigma|\sigma'\sigma''}$ represents the conditional probability of a site being in state σ in the neighbourhood of the σ′ site of a σ′σ″ pair. We use an ordinary pair approximation following Matsuda et al. (1992), where $q_{\sigma|\sigma'\sigma''} \approx q_{\sigma|\sigma'}$. This conditional probability that given a focal site is in state σ' it has a neighbor in state σ is defined as $q_{\sigma|\sigma'} = P_{\sigma\sigma'}/P_{\sigma'}$. An illustrative example of this approximation is as follows:

$$\dot{P}_{II} = 2\left[\ \beta G_T P_I P_{SI} + \beta(1-G_T)(\theta + (1-\theta)q_{I|SI})P_{SI} - (b+\alpha)P_I\ \right]$$

becomes:

$$\dot{P}_{II} = 2\left[\ \beta G_T P_I P_{SI} + \beta(1-G_T)(\theta + (1-\theta)P_{SI}/P_S)P_{SI} - (b+\alpha)P_I\ \right]$$

Accordingly, the following system of ordinary differential equations describes the endemic state (see Table 4.1) of a single resident strain, where $\dot{P}_X$ denotes a time derivative of P_X.

$$\dot{P}_0 = (b+\alpha)P_I + bP_S - aG_R P_S P_0 - CaG_R P_I P_0 - a(1-G_R)(P_{S0}/P_0)P_0 - Ca(1-G_R)(P_{I0}/P_0)P_0$$

$$\dot{P}_S = aG_R P_S P_0 + CaG_R P_I P_0 + a(1-G_R)(P_{S0}/P_0)P_0 + Ca(1-G_R)(P_{I0}/P_0)P_0 - bP_S - \beta G_T P_I P_S - \beta(1-G_T)(P_{S0}/P_S)P_S$$

$$\dot{P}_I = \beta G_T P_I P_S + \beta(1-G_T)(P_{SI}/P_S)P_S - (b+\alpha)P_I$$

$$\dot{P}_{00} = 2\left[\ bP_{S0} + (b+\alpha)P_{I0} - aG_R P_S P_{00} - CaG_R P_I P_{00} - a(1-G_R)(1-\theta)(P_{S0}/P_0)P_{00} - Ca(1-G_R)(1-\theta)(P_{I0}/P_0)P_{00}\ \right]$$

$$\dot{P}_{S0} = aG_R P_S P_{00} + CaG_R P_I P_{00} + a(1-G_R)(1-\theta)(P_{S0}/P_0)P_{00} + Ca(1-G_R)(1-\theta)(P_{I0}/P_0)P_{00} + (b+\alpha)P_{SI} + bP_{SS} - bP_{S0} - aG_R P_S P_{S0} - CaG_R P_I P_{S0} - a(1-G_R)(\theta + (1-\theta)P_{S0}/P_0)P_{S0} - Ca(1-G_R)(1-\theta)(P_{I0}/P_0)P_{S0} - \beta G_T P_I P_{S0} - \beta(1-G_T)(1-\theta)(P_{SI}/P_S)P_{S0}$$

$$\dot{P}_{I0} = \beta(1-G_T)(1-\theta)(P_{SI}/P_S)P_{S0} + \beta G_T P_I P_{S0} + bP_{SI} + (b+\alpha)P_{II} - (b + \alpha)P_{I0} - aG_R P_S P_{I0} - a(1-G_R)(1-\theta)(P_{S0}/P_0)P_{I0} - CaG_R P_I P_{I0} - Ca(1-G_R)(\theta + (1-\theta)P_{I0}/P_0)P_{I0}$$

$$\dot{P}_{SS} = 2[\ aG_R P_S P_{S0} + CaG_R P_I P_{S0} + a(1-G_R)(\theta + (1-\theta)P_{S0}/P_0)P_{S0} + Ca(1 - G_R)(1-\theta)(P_{I0}/P_0)P_{S0} - bP_{SS} - \beta G_T P_I P_{SS} - \beta(1-G_T)(1 - \theta)(P_{SI}/P_S)P_{SS}\ ]$$

$$\dot{P}_{SI} = aG_R P_S P_{I0} + CaG_R P_I P_{I0} + a(1-G_R)(1-\theta)(P_{S0}/P_0)P_{I0} + Ca(1-G_R)(\theta + (1-\theta)P_{I0}/P_0)P_{I0} + \beta G_T P_I P_{SS} + \beta(1-G_T)(1-\theta)(P_{SI}/P_S)P_{SS} - bP_{SI} - (b+\alpha)P_{SI} - \beta G_T P_I P_{SI} - \beta(1-G_T)(\theta + (1 - \theta)P_{SI}/P_S)P_{SI}$$

$$\dot{P}_{II} = 2[\ \beta G_T P_I P_{SI} + \beta(1-G_T)(\theta + (1-\theta)P_{SI}/P_S)P_{SI} - (b+\alpha)P_I\ ]$$

We assess whether a mutant strain J can invade a resident strain I. We numerically solve the single-resident strain ODE system (above) to establish an endemic equilibrium. We then model a mutant strain J which has virulence (α_J) and transmission (β_J), where virulence and transmission are governed by a relationship of the form:

$$\beta = D \cdot ln(\alpha + 1)$$

where *D* is a positive scalar. Accordingly, β is a saturating function of α, used elsewhere in equivalent theoretical modelling (Hoyle et al. 2008). As has been previously established (Boots and Sasaki 1999; Lion and Gandon 2015), strain J is able to invade if $\lambda(J|I) > 0$ where:

$$\lambda(J|I) = \left(\frac{b + \alpha_I}{\beta_I} - \frac{b + \alpha_J}{\beta_J}\right) + (1 - G_T)\left(\hat{q}^*_{S|J} - q^*_{S|I}\right)$$

$q^*_{S|I}$ is the endemic equilibrium density of susceptible sites in the local neighbourhood of an infected individual for the single resident strain. $\hat{q}^*_{S|J}$ is the quasi-equilibrium density of susceptibles in the local neighbourhood of the invading mutant early on in its invasion. The justification for this quasi-equilibrium

approximation has been discussed in previous studies (Boots and Sasaki 1999; Lion and Gandon 2015), and justified by the generalisation that early on in a mutant's invasion it remains globally rare, and so global dynamics change much more slowly from the single-strain endemic equilibrium compared to the dynamics in the local neighbourhood of the invading rare mutant.

Accordingly, we asses the quasi-equilibrium state by approximating all pair densities and conditional probabilities which do not include the rare mutant J as constant values taken from the endemic equilibrium (denoted by a *). We examine the rates of change of $q_{\sigma/J}$ for $\sigma \in \{ 0, S, I, J \}$ and approximate $P_J \approx 0$. This yields the following system of ordinary differential equations:

$$\begin{aligned} q_{0|J} = {} & b q_{S|J} + (b + \alpha_I) q_{I|J} + (b + \alpha_J) q_{J|J} - a G_R P_S^* q_{0|J} - Ca G_R P_I^* q_{0|J} - a(1 - G_R)(1 \\ & - \theta)(P_{S0}^*/P_0^*) q_{0|J} - Ca(1 - G_R)(1 - \theta)(SPI0/P_0^*) q_{0|J} - Ca(1 \\ & - G_R)\theta q_{0|J} + \beta_J(1 - G_T)(1 - \theta)(P_{S0}^*/P_S^*) q_{S|J} + \beta_J G_T P_{S0}^* - \beta_J(1 \\ & - G_T) q_{S|J} q_{0|J} - \beta_J G_T P_S^* q_{0|J} \end{aligned}$$

$$\begin{aligned} q_{S|J} = {} & a G_R P_S^* q_{0|J} + Ca G_R P_I^* q_{0|J} + a(1 - G_R)(1 - \theta)(P_{S0}^*/P_0^*) q_{0|J} + Ca(1 - G_R)(1 \\ & - \theta)(P_{I0}^*/P_0^*) q_{0|J} + Ca(1 - G_R)\theta q_{0|J} - b q_{S|J} - \beta_I G_T SPI q_{S|J} - \beta_I(1 \\ & - G_T)(1 - \theta)(P_{SI}^*/P_S^*) q_{S|J} + \beta_J(1 - G_T)(1 - \theta)(P_{SS}^*/P_S^*) q_{S|J} \\ & + \beta_J GT P_{SS}^* - \beta_J(1 - G_T)\theta q_{S|J} - \beta_J(1 - G_T) q_{S|J} q_{S|J} - \beta_J G_T P_S^* q_{S|J} \end{aligned}$$

$$\begin{aligned} q_{I|J} = {} & \beta_I G_T P_I^* q_{S|J} + \beta_I(1 - G_T)(1 - \theta)(P_{SI}^*/P_S^*) q_{S|J} - (b + \alpha_I) q_{I|J} + \beta_J(1 - GT)(1 \\ & - \theta)(P_{SI}^*/P_S^*) q_{S|J} + \beta_J G_T P_{SI}^* - \beta_J(1 - G_T) q_{S|J} q_{I|J} - \beta_J G_T P_S^* q_{I|J} \end{aligned}$$

$$q_{J|J} = 2\beta_J(1 - G_T)\theta q_{S|J} - (b + \alpha_J) q_{J|J} - \beta_J(1 - G_T) q_{S|J} q_{J|J} - \beta_J G_T P_S^* q_{J|J}$$

Having numerically solved this ODE system, we can substitute $\hat{q}^*_{S|J}$ into the invasion criteria $\lambda(J|I) > 0$ (see above). Using this approach across all values of α we can generate pairwise invasibility plots (PIPs) to assess if any value of α yields an evolutionary singular strategy (ESS) (Geritz et al. 1998) for a given set of parameters. An example is shown in fig. 4.1. We computationally inspect these pairwise invasibility plots to extract the apparent approximate ESS values for many parameter combinations. This allows us to compare how changes in ecological

parameters affect the optimal virulence (α) for an evolving pathogen. We investigate how altering two spatial parameters (global transmission G_T and global reproduction G_R) and altering degree of castration (C) affects the ESS virulence of the pathogen, where $0 \leq \{G_T, G_R, C\} \leq 1$.

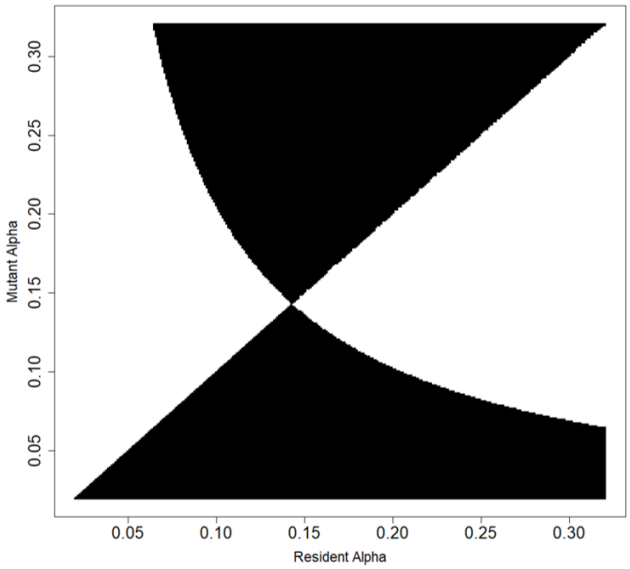


Figure 4.1 - Example pairwise invasibility plot (PIP) with ESS virulence $\alpha = 0.0143$. Parameters values used to generate this plot are $G_T = 0.7$, $G_R = 0.3$, $C = 0.9$, with remaining parameters taken used unvaried universally elsewhere in this study (a = 5, b = 0.01, $\theta = ¼$, D = 5). For this plot, alpha (α) is approximated to a precision of 0.001.

Results

We find that across our range of varied parameters ($0 \leq \{G_T, G_R, C\} \leq 1$), we recover evolutionary singular strategies in every case, allowing us to characterise how these changing ecological parameters affect optimum virulence. We present here example plots for a small subset of parameter value combinations which capture our main findings.

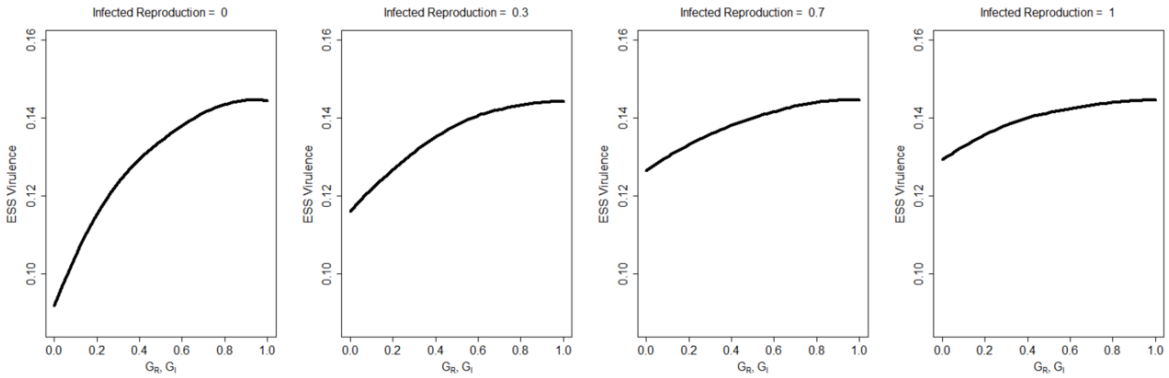


Figure 4.2 – Example panels showing relationship between degree of spatial structure (x-axis) and optimum parasite virulence (α) (y-axis) for four different levels of parasitic castration. Curves are interpolated between many numerically solved virulence ESS values. In this figure, proportion of global reproduction (G_R) and proportion of global infection (G_I) are fixed as equal to one another and vary from 0 (fully local) to 1 (fully global or 'mean field'). Non-varied parameters used unvaried universally elsewhere in this study (a = 5, b = 0.01, θ = ¼, D = 5). For this plot, alpha (α) is approximated to a precision of 0.00015.

In fig. 4.2 we recover the well-established result that reducing local interactions (increased mixing) increases optimum virulence. However we show that effect of reduced spatial structure is strongest when a pathogen is fully castrating, and the effect weakens as we permit infected individuals to reproduce. This difference is driven by the effect of castration when interactions are principally local. When the system is fully mixed with no spatial structure, castration has no affect on optimum virulence. We more clearly show this in fig. 4.3, castration (infected reproduction) is plotted along the x-axis.

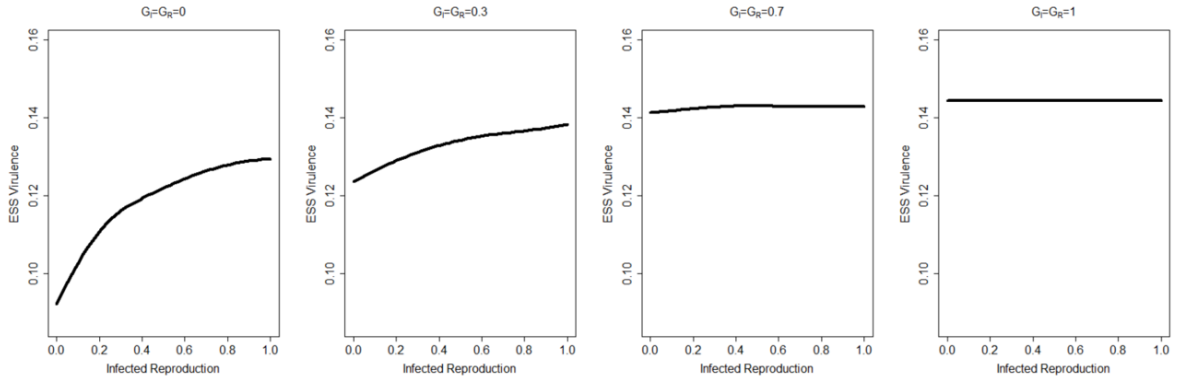


Figure 4.3 - Example panels showing relationship between degree of castration (x-axis) and optimum parasite virulence (α) (y-axis) for four different levels of population mixing. Curves are interpolated between many numerically solved virulence ESS values. In this figure, proportion of global reproduction (G_R) and proportion of global infection (G_I) are fixed as equal to one another for each panel. Non-varied parameters used unvaried universally elsewhere in this study (a = 5, b = 0.01, θ = ¼, D = 5). For this plot, alpha (α) is approximated to a precision of 0.00015.

We show in fig. 4.3 that castration limits virulence, in that higher rates of castration select for lower optimum virulence ESS values. However this virulence limitation only manifests when interactions are mainly local. Spatial structure is required for varying castration to affect optimum virulence. The magnitude of this effect in the fully local system (fig. 4.3) is comparable to that of increased global infection rates (fig. 4.4), however very quickly becomes negligible with only moderate increases in mixing of the system, and any effects of castration on virulence disappear entirely when the system is fully mixed.

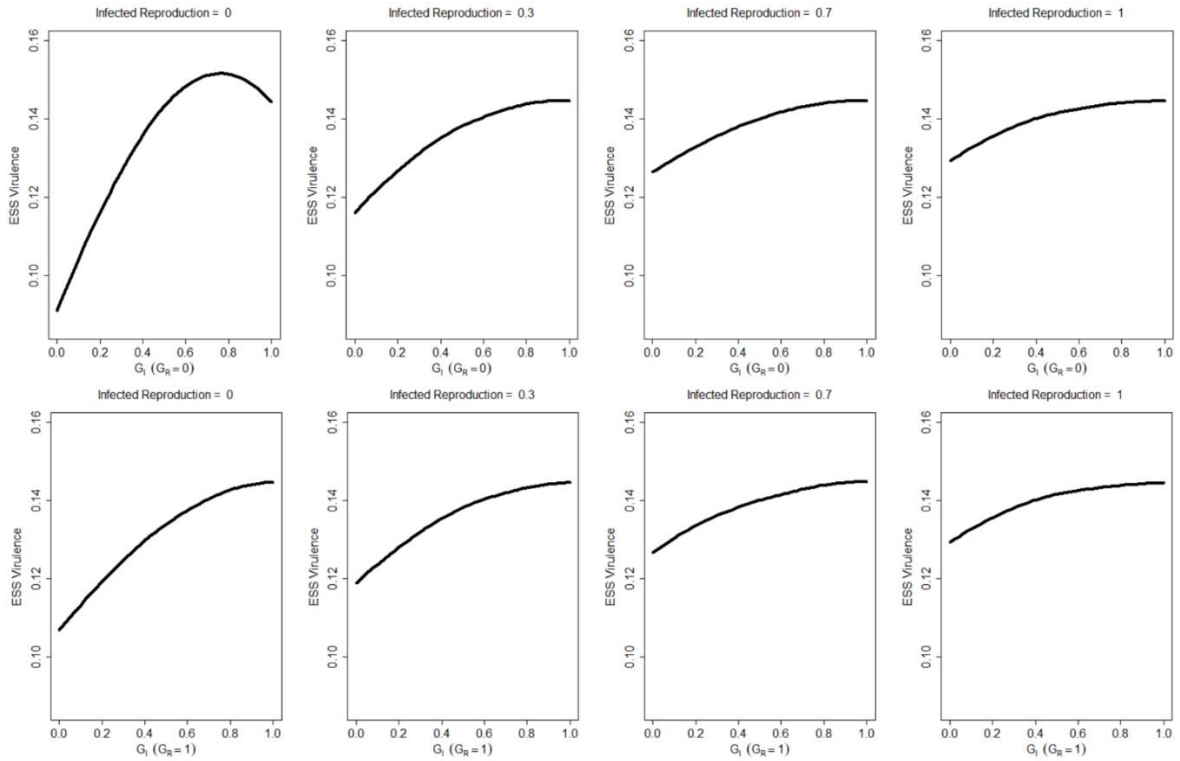


Figure 4.4 - Example panels showing relationship between global infection proportion (G_I) (x-axis) and optimum parasite virulence (α) (y-axis) for four different levels of parasitic castration and two different proportions of global reproduction (G_R). Curves are interpolated between many numerically solved virulence ESS values. Upper row of panels shows global-infection virulence relationship for four different castration levels in a system where reproduction is entirely local ($G_R = 0$). Lower row of panels shows the same relationships but for a system where reproduction is entirely global ($G_R = 1$). Non-varied parameters used unvaried universally elsewhere in this study ($a = 5$, $b = 0.01$, $\theta = ¼$, $D = 5$). For this plot, alpha (α) is approximated to a precision of 0.00015.

We show that in specific cases, optimum virulence is highest for well-mixed, but not entirely global, infection (fig. 4.4). However, this phenomenon manifests only when reproduction is almost entirely local and infected individuals almost never reproduce. Comparing between fully local and fully global reproduction in fig. 4.4, we observe a similar effect to that of infected reproduction. Global reproduction leads to higher optimum virulence but only when infection is predominantly a local process. Additionally, we see that this difference in virulence is only notable when

infected individuals do not reproduce. That is to say, the effects of spatial structuring of reproduction only affect virulence optima if infection is primarily local and infected individuals are strongly castrated.

Discussion

We show that reproduction from infected individuals has important implications to the predictions of pair approximated evolutionary models of parasite virulence. In particular the 'virulence hump' (Kamo et al 2007) – where virulence first increases before decreasing as infection becomes more local – only manifests when reproduction is local and only susceptible (rather than infected) individuals reproduce. Furthermore, we find that reduced fecundity in infected individuals selects for lower virulence in spatially structured systems, but in moderately- or well- mixed systems has little or no effect on optimum virulence for a pathogen, as expected from classic mean-field theory (Jaenike 1996; O'Keefe and Antonovics 2002). In general we find that a lack of castration reduces the magnitude of the effect of spatial structure on virulence.

The phenomena of a virulence 'hump' (fig. 4.4), where populations with some small amount of local infection select for more virulent pathogens, has been found in multiple theoretical studies (Kamo et al. 2007; Lion and Boots 2010; Webb et al. 2013a). We recover this effect, but show it occurs when reproduction is a predominantly local process mostly from uninfected individuals. This is in line with earlier work showing that 'mostly local' reproduction is an important determinant in these evolutionary outcomes (Webb et al. 2007a) but also highlighting the importance of demography in critically determining the evolutionary outcomes in spatial models (Lion and Boots 2010; Messinger and Ostling 2013). In the non-spatial model the evolutionary outcomes are not affected by the assumption of infected castration, but in spatial models demography is critical to the outcome.

Similarly, we find that the overall importance of spatial structuring of infection, in terms of its effect on optimum virulence, is sensitive to both how local reproduction is and whether infected individuals can reproduce (fig. 4.4). The largest impacts on optimum virulence are observed under scenarios with entirely local reproduction by

uninfected individuals (with the castration of infected). One interpretation of this could be that theory has overestimated the magnitude of the effect of spatial structing in shaping pathogen evolution. The strong empirical evidence demonstrating the phenomenon that reduced spatial structure selected for more virulent pathogens (Kerr et al. 2006; Boots and Mealor 2007; Szilágyi et al. 2009) was also found in castrating parasites, although reproduction was not necessarily local. It may also be that the pair-approximation approach used here captures an insufficient, or conservative, degree of spatial structuring, and so is predisposed to underestimate the effect of local vs global dynamics on evolution. Full spatial simulations will be carried out to test this question.

Ultimately however these are points of discussion concerning quantitative differences in a highly abstracted, arguably arbitrary theoretical system. We recover the same qualitative finding that increasing rates of global transmission selects for higher pathogen virulence even when the assumptions around infected reproduction are relaxed. In this sense, we find that the core statement that reduced spatial structure selects for increased virulence is robust. This is crucial for informing evolutionary management of infectious diseases, as in a ‘shrinking world’ of reduced spatial structure (Janelle 1973; Hanski 2005) we may select for hypervirulent pathogen strains with obvious risks for human health, agriculture, and wildlife.

Further, it is important to note that the effect of castration is most profound in entirely, or very local, systems (fig. 4.3). Once a moderate degree of mixing occurs, assumptions around castration have little (or, in the case of the mean field, no) effect on optimum virulence. The classic ‘mean-field’ framework is therefore more robust to assumptions concerning processes of reproduction. For some systems, close to well-mixed populations are realistic; for example, infectious agents existing in the environment in aquatic systems, such as the well studied *Daphnia* (Ebert et al. 2004; Jensen et al. 2006) system where its pathogen is indeed castrating, or for increasingly studied marine viruses (Middelboe and Brussaard 2017). Hopefully, this nuance of when assumptions around infection and castration are most likely to

seriously affect outcomes will help inform more tailored theory for specific infectious disease modelling.

A related understanding of our findings is that local reproduction only appears to matter to virulence evolution when infected individuals do not, or rarely, reproduce (fig. 4.4). In previous theory (Boots and Sasaki 2000), where castration is assumed, even with fully local infection, increased rates of global reproduction select for higher virulence. We do not recover this finding when infected individuals are capable of reproduction (fig. 4.4). Biological analogues of such a 'local infection, global dispersal' system include holometabolous insects, and sessile organisms with dispersing progeny such as many plants, or corals. Notably, both corals and plants have been highlighted as areas of interest for the study of the evolution of castrating pathogens (Hartikainen and Okamura 2012; Vijayan et al. 2017). Overall however, this change in understanding that reproduction distance only matters when the pathogen is castrating arguably simplifies our understanding of 'small worlds' and virulence evolution, in that for most systems where castration is not apparent, only changing the rates of global infection matters to the outcome.

For a full analysis, we need to confirm our results by stochastic simulation (Boots and Sasaki 1999; Kamo et al. 2007; Webb et al. 2013a). Never-the-less, as this study uses the same framework and parameter combinations as other, simulation-confirmed studies (Boots and Sasaki 1999; Kamo et al. 2007; Webb et al. 2013a), and we recover the same finding for analogous reproduction, the pair approximations results are likely to hold. A further improvement on this study would be to examine the impact of ecological parameters such as natural mortality and birth rate. In this case, in order to maintain equivalence to previous studies, a 'long-lived' host is modelled. Speculatively, this long-lived host may be driving some of the critical importance of the assumptions around castration. A clustering of long-lived hosts all infected with a castrating pathogen 'choke out' opportunity for new infections and therefore without a high mortality, no space becomes empty for new susceptible individuals to be born into. This 'self-shading' (Boots and Sasaki 2000; Messinger and Ostling 2013) could be perhaps be more important for long-lived hosts as they inherently limit demographic turnover. Linked to this is the evidence

that parasistic castration selects for more rapid (shorter lived) host life-history strategies (Lafferty and Kuris 2009), which would increase demographic turnover, making our long-lived castrated ‘host’ potentially rare in nature.

Here, castration is modelled as a phenomenon separate to pathogen phenotype, interpretably determined by different host biology. However, other understandings of castration due to infection should be pursued in future. Maintaining the idea that castration is a host-driven phenomenon, it would be perhaps insightful to positively link castration to virulence. In its simplest form, this link can be understood from resource budgeting (Bonds 2006) and nutrient theft – increased parastemia sequesters more nutrients, reducing fecundity and increasingly mortality (Heins et al. 2010). More nuanced interpretations can also be argued, for example the biological phenomena of pariahship (Cremer et al. 2007) where the most visibly infected individuals, exhibiting more virulent symptoms, are avoided by other individuals including mates. While mating is not explicitly modelled here, where asexual reproduction is assumed, there are abundant hermaphroditic species which require sexual reproduction, but do not have individuals categorisable into distinct sexual phenotypes, and are indeed infected by castrating pathogens (Lafferty and Kuris 2009). We would not therefore forgo biological realism by pursuing a question relating to higher virulence leading to greater castration.

It is equally interesting to consider castration as negatively correlated with virulence if we assume that castration and additional mortality are different pathogen strategies (Jaenike 1996; O’Keefe and Antonovics 2002; Abbate et al. 2015). Expanding this work to link transmission to both castration and additional mortality would provide insight into when castration is more costly to parasites compared to additional mortality, and may raise questions of why we do not appear to observe infection-driven castration more commonly.

One clear explanation already for why castration may be rare is due to host evolution. For host evolution, virulence is instead more broadly defined (and correctly so) as a loss of fitness resulting from infection (Abbate et al. 2015). Using this frame of thought, a completely castrating pathogen represents a total loss of fitness to the host, akin to an obligately lethal pathogen. The selection pressure on

hosts to resist or tolerate this infection without succumbing to castration is therefore extremely strong. This may account for how infrequent cases of castrating pathogens are. The pair-approximation, adaptive dynamics framework we study here has been used extensively also for host evolution and indeed parasite-host coevolution, and a clear next goal of this theoretical body should be to interrogate assumptions around reproduction and castration from the standpoint of host evolution. It is tempting to speculate that the castration assumption discussed throughout manuscript may be much more important in determining evolutionary outcomes for host evolution and host-parasite co-evolution, an idea which has been highlighted specifically in the context of spatial structuring (O'Keefe and Antonovics 2002).

Chapter 5 – Industrial bees: when agricultural intensification doesn't impact local disease prevalence.

Abstract

Although it is generally thought that the intensification of farming will result in higher disease prevalences there is little specific modelling testing this idea. We build multi-colony models to inform how 'apicultural intensification' is predicted to impact honeybee pathogen epidemiology at the apiary scale. Counter to the prevailing view, our models predict that intensification, captured though increased population sizes, changes in population network structure, and increased between-colony transmission, is likely to have little effect on disease prevalence within an apiary. The greatest impacts of intensification are found for diseases with relatively low R_0 (basic reproduction number), however, such diseases cause little overall disease prevalence and therefore the impacts of intensification are minor. Furthermore, the smallest impacts of intensification are found for diseases with high R_0 values, which we argue are typical of important honeybee diseases, principally because these diseases appear very prevalent regardless of intensification. Our findings highlight a lack of support for the hypothesis that larger apiaries lead to notably higher disease prevalences. More broadly, our work demonstrates the need for informative models of agricultural systems and management practices in order to understand the implications of management changes on diseases.

Introduction

Infectious diseases exact tolls on agricultural sustainability (Brijnath et al. 2014) and profitability (James 1981). A key question is how agricultural intensification and novel agricultural practices impact the emergence and epidemiology of infectious disease (Gandon et al. 2013; Cressler et al. 2016). A general assumption is that intensification increases vulnerability to severe disease outbreaks (Mennerat et al. 2010; Jones et al. 2013; Kennedy et al. 2016), but there is relatively little empirical data we can use to understand how different agricultural approaches influence infectious disease prevalences, epidemiological theory is therefore a useful

alternative (Atkins et al. 2013; Rozins and Day 2016). Here we build specific models of apiary-level intensification in commercially farmed honeybees to examine the impact of industrial-scale management practices on honeybee infectious disease prevalence.

Honeybee health and the apicultural industry are under threat from a variety of pressures (Ghazoul 2005; vanEngelsdorp and Meixner 2010), including parasites and pathogens (De la Rúa et al. 2009; Potts et al. 2010; Budge et al. 2015). There is a growing body of literature documenting the damage that emerging or re-emerging diseases (Wilfert et al. 2016) are causing in apiculture (Kielmanowicz et al. 2015; Jacques et al. 2017) and native pollinators (Fürst et al. 2014; Manley et al. 2015; McMahon et al. 2015, 2018; Graystock et al. 2016; Cohen et al. 2017). Evidence exists supporting a link between the risk of these diseases and specific apicultural practices (Giacobino et al. 2014; Mõtus et al. 2016; Pacini et al. 2016). However, the evidence is geographically limited, lacking in mechanistic underpinning, or contradictory even within this small collection of studies. It is therefore critical that we learn how different apicultural practices impact disease outcomes (Brosi et al. 2017). The need for an epidemiological framing of honeybee diseases has been frequently discussed (Fries and Camazine 2001; Brosi et al. 2017) in both empirical (van Engelsdorp et al. 2013) and modelling (Becher et al. 2013) studies, but we lack a modelling framework for disease ecology in honeybees at a scale larger than a single colony. The complexity of factors interacting in the process of apicultural intensification mandates selecting a subset of scales and factors to focus modelling efforts upon, as we do here.

Honeybees are typically managed in apiaries, which are associated colonies placed together for beekeeping convenience at a single site. Pathogen dynamics at the apiary level are determined both by pathogen transmission within and between colonies. Intensification of apiculture changes apiary ecology in a number of ways, all potentially relevant to disease (Brosi et al. 2017). In this study, we focus on how increasing the number of colonies and changing the arrangement of those colonies influences epidemiology through changes in both the size and network structure of the population. We also examine increased transmission opportunities between

colonies, such as beekeepers transplanting infection material unknowingly, or via more frequent ‘drifting’ of honeybees (Free 1958; Neumann et al. 2003). Drift is a key mechanism of between-colony pathogen transmission (Goodwin et al. 1994; Roetschi et al. 2008) and has been invoked as an explanatory mechanism accounting for higher disease prevalences in larger apiaries (Mõtus et al. 2016).

The intensification of agricultural systems generally means larger, denser population sizes and greater pathogen transmissibility at the local and landscape scale. This paper addresses some of these aspects at the local scale. To understand these effects in honeybees we build multi-colony models to examine how apicultural intensification is predicted to impact honeybee pathogen epidemiology. We examine the epidemiological consequences of increasing the number of colonies within an apiary, changing colony configurations, and increasing between-colony pathogen transmission.

Methods

We combine mathematical models and agent-based model (ABM) simulations to make predictions on how intensification affects disease risk, spread, and endemic prevalence within an apiary. The key to our approach is that we capture pathogen transmission both within and between colonies.

We generalise colony arrangements to three unique configurations: array, circular and lattice (fig. 5.1). We restrict between-colony pathogen transmission to nearest neighbours (see discussion), those in closest proximity to each other (connected by an arrow in fig. 5.2). Between-colony transmission is always assumed to be at a lower rate than within colony transmission. The mathematical model allows us to obtain tractable analytical results while the ABM simulations allow us to model disease at the level of the individual bee and consider stochastic effects.

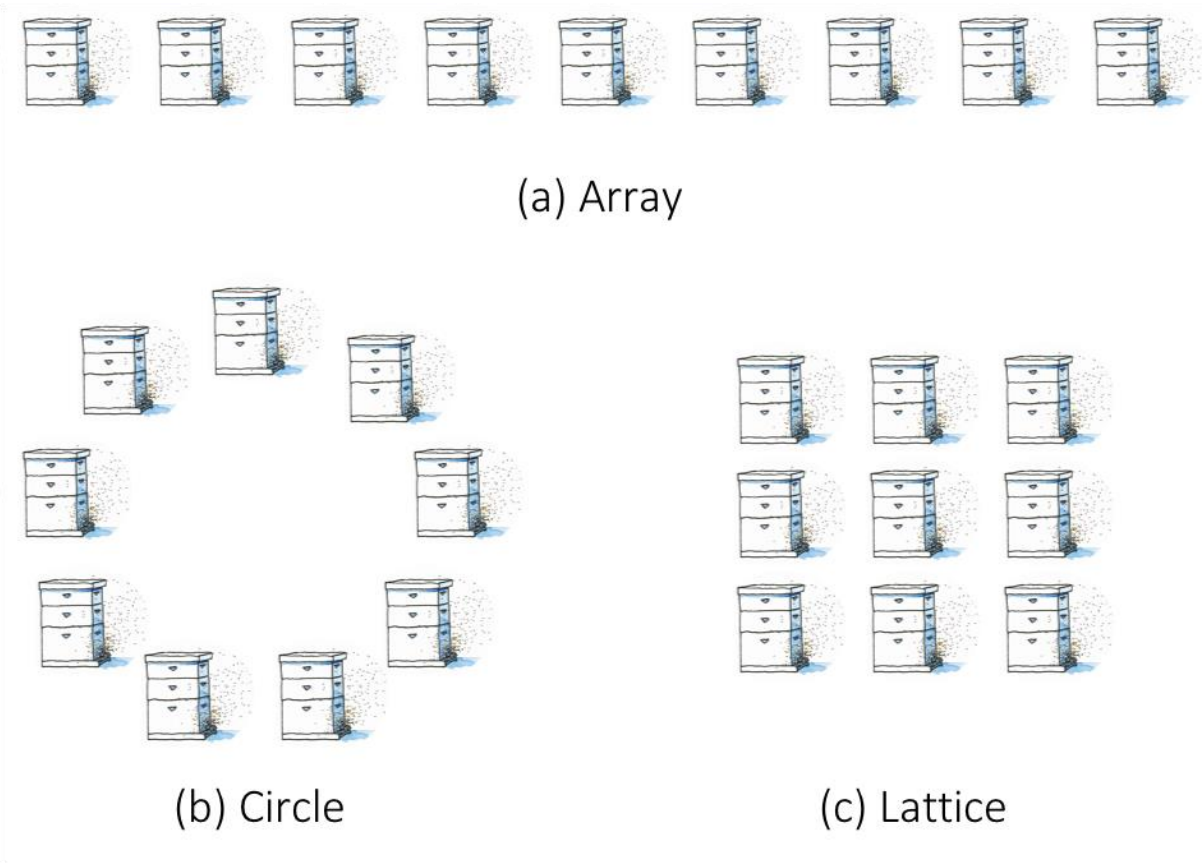


Figure 5.1 - Colony configurations demonstrated for apiaries with nine colonies.

We first derive a compartmental SI (Susceptible, Infected) model for pathogen transmission within an apiary. The model treats each colony as an individual population and allows for within colony as well as between-colony transmission (for nearest neighbours). Within a colony, honeybees are either susceptible to infection or infected (and infectious). We denote the number of susceptible honeybees in colony i at time t as $S_i(t)$. Likewise, we denote the number of honeybees in colony i infected with the pathogen at time t as $I_i(t)$. Susceptible honeybees in colony i become infected at rate β_{ij} following contact with an infected bee that resides in colony j. We assume that honeybees do not recover from infection. Honeybees are born at rate ϕ, have a natural mortality rate of m, and an additional mortality rate of v if infected. The following $2n$ differential equations, [1], model disease transmission within and between n colonies in an apiary.

$$\frac{dS_i}{dt} = -\sum_{j=1}^{n} \beta_{ij} S_i I_j - mS_i + \phi$$

$$\frac{dI_i}{dt} = \sum_{j=1}^{n} \beta_{ij} S_i I_j - (m+v) I_i \qquad [1]$$

The matrix $\beta=[\beta_{ij}]$ will depend on the colony arrangement (see fig. 5.1; and Supplementary Material 3 Section 1). The transmission rate between a susceptible and infected honeybee within the colony is *a*, and transmission between neighbouring colonies is *b*. We assume that honeybees are much more likely to become infected by a honeybee that resides within its home colony than by a honeybee from a neighbouring colony (i.e. *a*>>*b*). Note that for each apiary configuration to be possible and unique, the number of colonies (n) must be a perfect square, $n=L^2$ where $L\geq3$ (see fig. 5.1). Therefore, the minimum number of colonies per apiary is 9, which has been observed to be a representative size of the smallest class of beekeeping operations typically studied across the literature, for example Pocol et al. (2012) and Mõtus et al. (2016).

We complement our mathematical model [1] with the ABM; our ABMs are simulations of pathogen spread, through individual bee movements, across an apiary. Apiaries are differentiated by the same characteristics as in the mathematical model; a description of the ABM is available in Supplementary Material 3 Section 2. We use the ABM to make standalone predictions on the effects of different aspects of intensification on pathogen epidemiology (Supplementary Material 3 Figs. S3 & S4). We use the ABM to simulate disease dynamics for both different pathogen phenotypes (varying both pathogen virulence and transmissibility) and different apiary ecologies (varied as previously described in the number of colonies per apiary, layout, and likelihood of bees moving between colonies).

We can understand the dynamics presented by our models by focussing on the basic reproduction number, R_0. R_0 is a fundamental concept in infectious disease ecology, defined as the average number of secondary infections caused by one

infectious individual in an otherwise entirely susceptible population (Anderson and May 1992). We derive R_0 expressions, using model [1], for each of the apiary configurations. R_0 derivations using model [1] allow us to characterise the relationship between R_0 and pathogen prevalence, defined as the proportion of honeybees within an apiary that are infected at the endemic equilibrium. For the ABM we calculate R_0 values for particular parameter combinations by treating simulation outputs as ideal empirical data (Keeling and Rohani 2008) and track the number of infections following the index case. The term 'base R_0' is used throughout the remainder of this paper and refers to a value of R_0 for a specific pathogen phenotype in a least intensified apiary (see fig. 5.2). We determine how intensification affects R_0 by separating R_0 into a 'base R_0' and an 'additional R_0'. The term 'additional R_0' refers to the observed difference in R_0 for a given pathogen phenotype when comparing a 'lower intensity' apiary to a 'high intensity' one (fig. 5.2)

The most extreme plausible examples of intensification are used in these comparisons. Specifically, these are increases in colonies per apiary from 9 to 225 colonies, a change to a lattice configuration, and/or a tenfold increase in honeybee movement likelihood between colonies (0.015 to 0.15 per bee per day), demonstrated in fig. 5.2. Each is examined individually but we focus on the combined effect (reflected in fig. 5.2). The difference in the R_0 before and after intensification is how we calculate 'additional R_0'. This permits the interaction (non-additive) effects of our three aspects of intensification. The 'additional R_0' can then be used in combination with the analytically derived relationship between R_0 and prevalence (see model [1] & Results) to characterise how intensification affects disease prevalence. We focus on disease prevalence as both models show rapid pathogen spread across apiaries, such that infection prevalence at the endemic equilibrium was the major result differentiating modelling scenarios (Supplementary Material 3 Figs. S4 & S5).

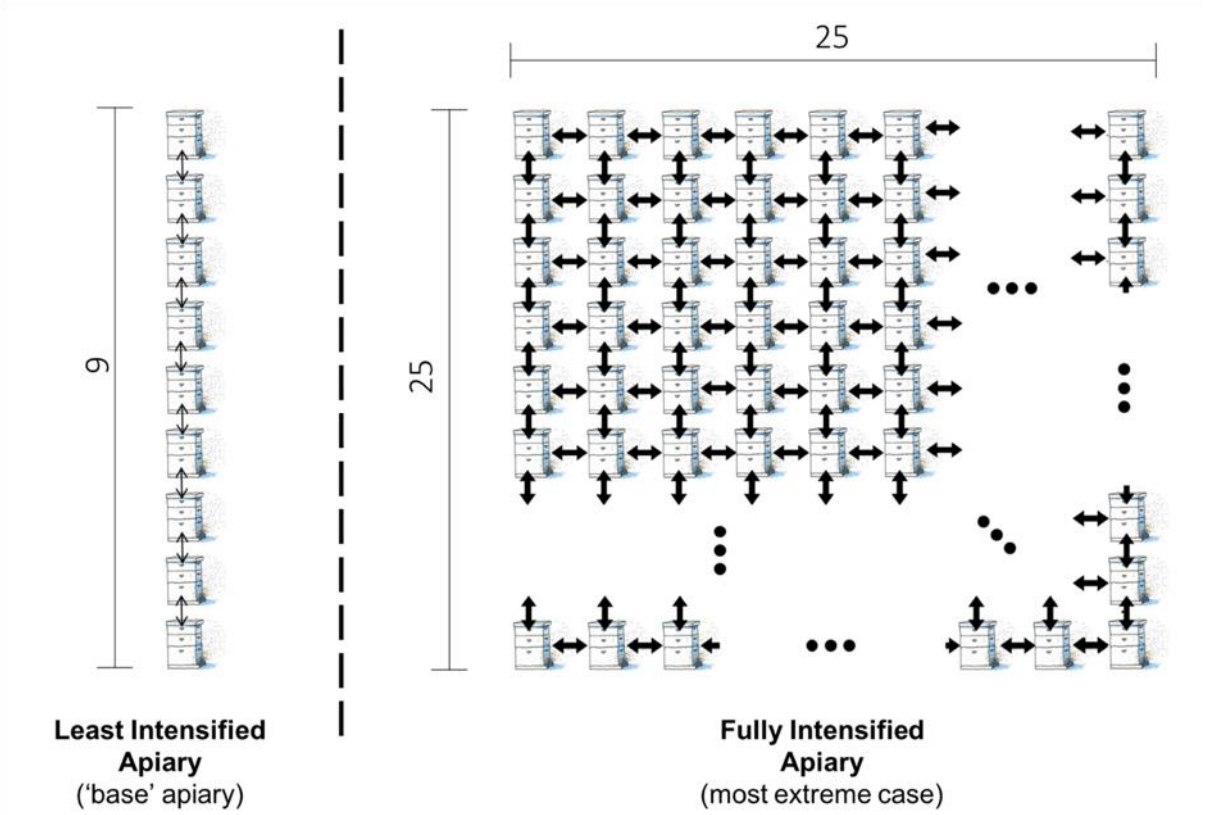


Figure 5.2 - Illustrative schematic of 'intensification' as it is used in parts of this manuscript. We show the apiary used to calculate 'base R_0' (left) compared to the intensified apiary (right) reflecting an increase in number of colonies from 9 to 225, a change from an array to a lattice, and a tenfold increase in movement of honeybees between colonies (illustrated using arrow weight) from a likelihood of 0.015 per bee per day to 0.15. Note that for the intensified apiary, not all 225 colonies are shown, with missing colonies denoted by ellipses (...).

Results

The R_0 expressions for apiaries with *n>1* colonies were calculated using the next generation method (van den Driessche and Watmough 2002), (see Supplementary Material 3 Section 1).

$$R0_{Array} = \frac{\phi}{m(m+v)}(a - 2b\cos\frac{n\pi}{n+1}) \quad [2a]$$

$$R0_{Circle} = \frac{\phi}{m(m+v)}(a + 2b) \quad [2b]$$

$$R0_{Lattice} = \frac{\phi}{m(m+v)}(a - 4b\cos\frac{\sqrt{n}\pi}{\sqrt{n}+1}) \quad [2c]$$

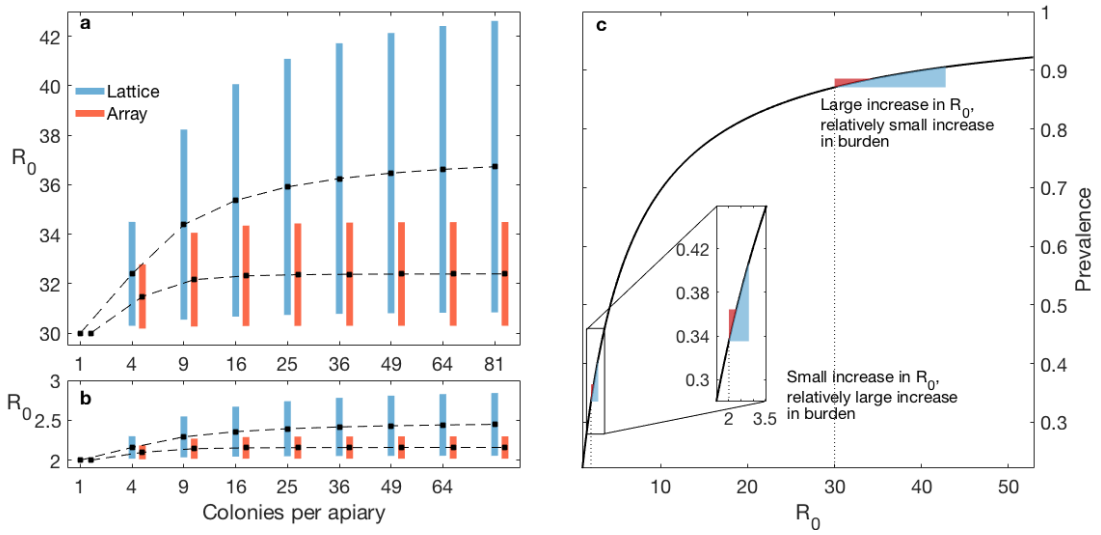


Figure 5.3 - Relationships between number of colonies, R_0, and prevalence. a) When R_0=30 for a single colony-apiary, the addition of colonies yields a maximum increase in R_0 of 12.7 for the lattice and 4.5 for the array. b) When R_0=2 for a single colony, there is a maximum increase in R_0 of 0.85 for the lattice and 0.29 for the array, when colonies are added. Recall that the R_0 for the circle is independent of *n* (see [2b]), and hence absent from the figure. Black dots are values where between-colony transmission is held at 10% of total transmission, with the bottom and top of the bars representing 1% and 20% of the total transmission respectively. c) The relationship between R_0 and disease prevalence. The range of R_0 values is generated by varying the overall transmission rate (i.e. *a*+*b*) from 2.143×10^{-6} to 1.178×10^{-4} as reported by Roberts & Hughes (2015) for *Nosema ceranae.*

Both model [1] and the ABM simulations show that, for a given number of colonies per apiary, R_0 is always greatest for the lattice arrangement — the most highly connected configuration. As the number of colonies per apiary increases

(increasing *n*), the values of R_0 in both the array and lattice configurations increase (fig. 5.3a & 5.3b), while the R_0 for the circular configuration remains unchanged (see R_0 equations). The increase in R_0 from the addition of colonies asymptotes quickly due to convergence in the mean number of neighbours across the apiary; this is also why the R_0 for the circular apiary is independent of number of colonies as the number of neighbours per colony remains two.

If $R_0>1$, the pathogen will rapidly invade (see Supplementary Material 3 Section 1 & Supplementary Material 3 fig. S5) and each colony will reach a stable population size and infection prevalence, called the endemic equilibrium (See Supplementary Material 3 Section 1). Mathematically the disease prevalence at equilibrium for colony *j* is $I_j^*/(I_j^*+S_j^*)$, where S_j^* is the number of susceptible honeybees and I_j^* is the number of infectious honeybees in colony *j* at equilibrium. The endemic equilibrium for the circular configuration model can be solved explicitly (see Supplementary Material 3 Section 1). Due to symmetry, all colonies within the circular apiary have disease prevalence at the endemic equilibrium of:

$$\frac{\phi(a+2b)-m(m+v)}{\phi(a+2b)+v(m+v)}$$

We can approximate the endemic equilibrium for the lattice and array configured models using perturbation theory, assuming $0<b\ll 1$ (See Supplementary Material 3 Section 1). The approximate disease prevalence in colony *j* at equilibrium for a colony in the array or lattice configurations is:

$$\frac{\phi a^2+lbm(m+v)}{\phi a^2+a(m+v)^2-blv(m+v)}$$

where *l* is the number of neighbours that colony *j* has. For any given set of parameters, we can therefore formulate both R_0 and prevalence, allowing us to characterise the relationship shown in fig. 5.3c.

We show analytically, and in the ABM (Supplementary Material 3 Section 3) that intensification in the form of an increase in colonies or an increase in movement between colonies increases R_0 (fig. 5.3a & 5.3b). Figure 4 shows the additional R_0

caused by our most extreme plausible changes in apiary management. The change in R_0 caused by increasing apiary size rapidly asymptotes (fig. 5.3a & 5.3b).

Increasing movement between colonies has the strongest effect on R_0 (fig. 5.4). However, there are clear interaction effects present; the combined effect of all three aspects of intensification is greater than their additive sum. The effect of intensification is dependent on the base R_0 – for small base R_0, intensification causes little additional R_0, but at intermediate or high base R_0, intensification leads to large additional R_0 (fig. 5.4). The relationship shows a strong nonlinearity when examining all three aspects of intensification in combination.

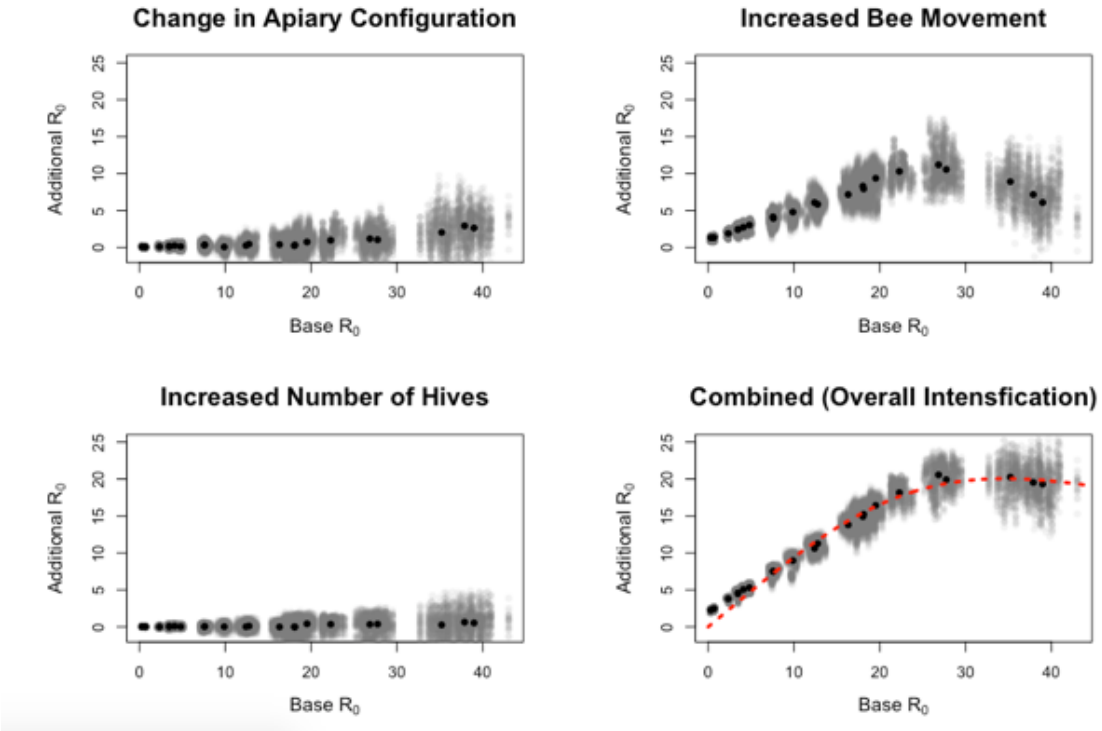


Figure 5.4 - Simulation results from the ABM. The change in R_0 caused by plausibly extreme increases in colonies per apiary, bee movement, and a change in configuration, across a range of different 'base R_0' values determined by pathogen phenotype. Grey points represent individual simulation comparisons, black points represent mean values. Base R_0 values are unevenly distributed across the range due to R_0 being an emergent property of the system. We derive a non-linear relationship between 'base R_0' and 'additional R_0' for the 'Combined' treatment (represented in fig. 5.2), plotted as a dashed red line.

By understanding the effect of intensification on R_0 (fig. 5.4) and by characterising the relationship between R_0 and disease prevalence (fig. 5.3c), we can show how intensification impacts disease prevalences. We approximate the non-linear relationship between 'base R_0' (pathogen phenotype) and the 'additional R_0' (effect of intensification) for the 'Combined' treatment (fig. 5.4). We use a bootstrapping approach to create 1000 subsamples (10% of full sample with replacement). Each subsample is used to generate a non-linear model of the form $y = ax / (b + x^c)$, where y is 'additional R_0' and x is 'base R_0', using a least squares approach. The relationship generated using the full sample is plotted in fig. 5.4.

We combine this relationship characterising how base R_0 affects intensified additional R_0 (fig. 5.4) with the derived relationship between R_0 and pathogen prevalence shown in fig. 5.3c, allowing us to predict how intensification impacts prevalences (fig. 5.5). fig. 5.5a shows the proportion of bees infected by a given (base R_0) pathogen for the apiaries in fig. 5.2. The difference in disease prevalence between these lines is the impact of intensification and is plotted in fig. 5.5b. fig. 5.5b shows a distinctly non-linear relationship between base R_0 and the impact of intensification, with the impact of intensification peaking around base $R_0 = 3.3$, and then rapidly declining. Even at its peak, the effect of intensification (which is as extreme as plausible), leads to an additional ~18% of bees infected at equilibrium.

We contextualize these results by calculating an estimate of the lower-bound of R_0 value for a honeybee pathogen (see highlighted regions in fig. 5.5). We identified this region based on empirical data for the microsporidian pathogen *Nosema ceranae*; this was the only pathogen for which experimentally derived transmission rates as well as robust information on mortality due to infection is available (Martín-Hernández et al., 2011; Paxton, Klee, Korpela, & Fries, 2007; Roberts & Hughes, 2015). To estimate the plausible R_0 boundary in our model for this pathogen, we parameterised our mathematical model using the lowest empirically supported transmission value with the highest supported additional mortality, and fixed movement of honeybees between colonies at its lowest supported natural rate (Currie and Jay 1991). We then calculated the R_0 for a circular apiary due to its scale independence.

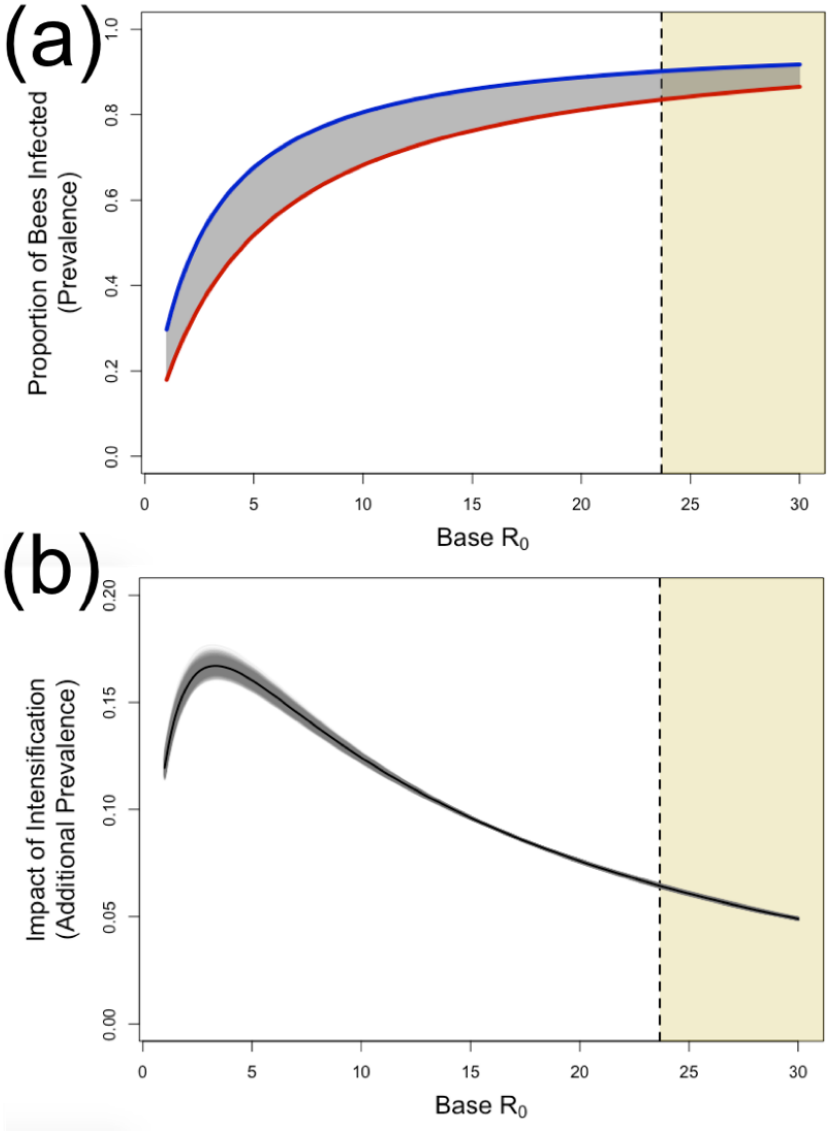


Figure 5.5 - Consequences of intensification on disease prevalence for pathogens with different base R_0 values, starting at R_0 = 1.0008. Panel (a) shows the proportion of bees infected (prevalence) in non-intensified apiaries (lower red line) compared to intensified apiaries (upper blue line), calculated from the mean values derived in fig. 5.4 and the relationship shown in fig. 5.3c. The shaded grey area between these curves is the additional prevalence caused by intensification – the 'impact of intensification'. This is plotted in panel (b) where the black line represents the mean relationship, and the grey lines represent 1000 bootstrapped samples. The vertical dashed line and yellow-shaded region of the graphs to the right of the dashed line show a lowest estimated value of R_0 for *Nosema ceranae*.

Discussion

Our results present a counterintuitive picture of the consequences of increasing apiary size and within-apiary transmission on disease prevalence. Even in their most plausibly extreme cases, changes in the number of colonies, their spatial arrangement, and the transmission rate (for example, movement of individual bees between colonies) had only a small effect on the severity of disease at the apiary level. Intensification leads to large gains in R_0 when R_0 is initially high and small gains in R_0 when R_0 is initially low (fig. 5.4). However, increases in R_0 cause large increases in prevalence only when R_0 is initially low (fig. 5.3c), principally because an already high R_0 means the pathogen in question is already very prevalent, with little 'room' for increase. Pathogens with a base $R_0 \approx 3$ benefit most from intensification in terms of increased prevalence (fig. 5.5); however, the magnitude of this is moderate. As discussed below, we argue that there is likely to be a high base R_0 in important honeybee diseases and therefore our models suggest that there is likely to be little effect of apiary-scale intensification on disease prevalence. A key testable prediction of this is whether small apiaries with seemingly little likelihood of between-colony transmission still exhibit high pathogen prevalence.

Our models most closely resemble the ecology of a directly transmitted microparasite able to infect individual honeybees at any life stage, conceptually similar to the microsporidian pathogens *Nosema* spp. (Fantham and Porter 1912). *Nosema* is a major concern to beekeepers worldwide (Higes et al. 2008, 2009; Paxton 2010), and has a minimum estimated base R_0 of 23 (fig. 5.5) when modelled here. We found that apicultural intensification, in the context of a pathogen with an initial R_0 of 23, leads to a maximum 6.6% increase in disease prevalence, from ~80% to ~86%. Our models predicted disease prevalences of up to 90% (fig. 5.3, fig. 5.5; Supplementary Material 3 Section 3), which while high, are empirically supported for the honeybee system (Higes et al. 2008; Kielmanowicz et al. 2015), and feature in other modelling studies that use similar transmission parameters to ours (Betti et al. 2014). *Nosema* was the only pathogen for which there are direct empirical studies characterising its transmissibility, however, other honeybee pathogens such as deformed wing virus are also well

studied. While estimating an R_0 for DWV is difficult due to active management by beekeepers, maximum reported prevalences that may be indicative of its true 'unmanaged' R_0 are high, for example 73% in Natsopoulou et al. (2017), 80% in Budge et al. (2015), and 100% in Stamets et al. (2018). These high prevalences are consistent with high R_0 values (fig. 5.3c & Supplementary Material 3 (Section 3)).

We additionally explored the behaviour of a more specific model, using an age-structured approach to infection dynamics, where only larvae are vulnerable to infection and develop into infectious adults with a high pathogen-associated mortality (as might be appropriate for pathogens such as the acute paralysis virus complex (Martin 2001)), presented in Supplementary Material 3 Section 3. Convergence to equilibrium happens more slowly than the main model presented here, but still occurs quickly (within a single beekeeping season; see Supplementary Material 3 fig. S6). However adult-bee infection prevalence is far lower than seen in our SI model (Supplementary Material 3 fig. S6) – this is in agreement with observations of lower prevalence of paralysis viruses (Budge et al. 2015). Notably, the endemic equilibrium prevalence increases only by small magnitudes as movement between colonies or apiary sizes are drastically increased (Supplementary Material 3 fig. S6), in agreement with our main general result. This equivalence in behaviour between different models reflecting large disparities in infection mechanics, with empirically-supported different endemic prevalences, provides evidence that these results are likely generalisable to many honeybee pathogens.

We find rapid spread of a given pathogen across an apiary, which quickly reaches endemic equilibrium (Supplementary Material 3 Figs. S4 & S5). While pathogens with a higher R_0 reach this equilibrium more quickly, there is universally rapid spread. Given this result, we focussed throughout this manuscript on the disease prevalence experienced at endemic equilibrium. This is important for our assumption that pathogen transmission (driven by movement of bees between colonies) only occurs between nearest neighbours. This assumption is conservative as rates of pathogen spread would be faster by virtue of not being

limited to nearest-neighbour transmission. However, as we already observe rapid pathogen spread across apiaries, the effect of this conservative assumption should be negligible. The rate at which epidemics are established in our model is also in agreement with other honeybee pathogen models. For example, Jatulan et al. (2015) show a single infectious adult causes an American Foulbrood (*Paenibacillus larvae*) epidemic that peaks within 50 days. Whilst they do not explicitly find an R_0 for *P. larvae*, the short timescales characterising their epidemics are in line with ours (Supplementary Material 3 Section 3), suggesting high R_0 values and that their model would behave similarly to ours at an apiary scale.

Changes in rates of bees moving between colonies emerged as a determining component of apicultural intensification (fig. 5.4). One cause of this movement is honeybee drift (Jay 1965) which can be managed through changes in the number of colonies and apiary configuration (Jay 1966, 1968). Links between drift-mediated pathogen transmission and colony numbers have been documented for a variety of pathogens (Seeley and Smith 2015) – including brood specialised and non-specialised, micro- and macro- parasites (Belloy et al. 2007; Budge et al. 2010; Dynes et al. 2017; Nolan and Delaplane 2017). Larger numbers of colonies per apiary are a driver of higher drift (Currie and Jay 1991), as are changes in apiary arrangement (Jay 1966). This is why we focus on a 'combined' interpretation of intensification in this study (illustrated in fig. 5.2), supported by our observation that changes in colonies per apiary and apiary size matter most when movement between colonies is high (fig. 5.4; Supplementary Material 3 fig. S4). However it should be noted that in its most basic sense, our analytical model simplifies 'drift' as a generic 'between-colony transmission', which could represent a host of bee or beekeeper behaviours pertinent to apicultural intensification at the apiary scale.

Our results contradict some empirical findings that larger apiaries are at higher risk of notably greater disease prevalences (Mõtus et al. 2016). However, our models do not account for landscape-scale movement of pathogens between apiaries. This is a phenomenon which has been well documented (Lindström et al. 2008; Nolan and Delaplane 2017). Given our results, and empirical studies that did not find an association between colonies per apiary and disease risk (Giacobino et al. 2014),

we argue that increasing the number of colonies in an apiary does not meaningfully alter within-apiary ecology to cause of increased disease prevalence. Larger apiaries may instead be more likely to import pathogens from other apiaries. Additionally, overstocking of colonies may lead to resource limitation and consequently impaired immune function (Pasquale et al. 2013; Al-Ghamdi et al. 2016). These effects are important for a broader understanding of honeybee epidemiology, but should be separated from the within-apiary processes studied here. Additionally, most honeybee infectious diseases are caused by multi-host pathogens shared with other wild bees (Fürst et al. 2014; Manley et al. 2015; McMahon et al. 2015, 2018). Honeybee colony density across a landscape therefore has implications for wild pollinator health (Graystock et al. 2016; Cohen et al. 2017), however our results suggest that increased stocking of honeybees may have smaller impacts on local pollinator infectious disease dynamics than may have been previously thought.

Two clear candidates for future development of this model include seasonality and demography, which are closely linked. Honeybee demography within a colony influences epidemiology (Betti, Wahl, & Zamir, 2016) due in part to the temporal polyethism of task allocation influencing exposure and immunity (Calderone and Page 1996), as well as the flexible ability of honeybees to regain immune function when they revert roles (Robinson et al. 1992; Amdam et al. 2005). However, patterns in how age and immunosenescence in honeybees relates to survival and infectiousness remain complicated (Roberts & Hughes, 2014). Analytically tractable models accounting for the role of this complex demography in understanding stress in a colony have only recently been developed (Booton et al. 2017), and extending these models to incorporate diseases at the apiary scale is challenging. However, notable phenomena worth pursuing include the role of male bees, which are known to be more easily infected, more infectious, and more likely to drift between colonies (Currie & Jay, 1991; Roberts & Hughes, 2015), as well as the role of robbing – where honeybees invade other colonies to steal food (Fries and Camazine 2001).

Other industrialised agricultural livestock systems reflect extreme host densities similar to those in this study. However, the R_0 for honeybee diseases may exceed that of other livestock diseases. We compare our lower threshold estimate for the R_0 of *N. ceranae* to all available R_0 values for livestock diseases that we could readily find in the literature (Supplementary Material 3 fig. S8, see Supplementary Material 3 Section 4). Notably, all other livestock diseases for which R_0 estimates exist show minimum R_0 values far below our honeybee estimate, however examples of agricultural R_0 values as high or higher than those we present for honeybees do also exist. There is therefore a clear need to develop explicit models of agricultural intensification scenarios for important agricultural disease.

Overall, our findings represent the first stage in developing robust epidemiological models for studying honeybee pathogens at an apiary scale. In the face of increasing challenges to global apiculture, our models predict that the size of apiaries *per se* is not causing notable increases in disease prevalence for important bee pathogens, principally because even in small apiaries with low transmission rates, prevalences remain high. Future work may rather better focus on how apicultural practices affect bee immunity, perhaps leading to higher infection loads per individual, rather than higher proportions infected. Finally, this study demonstrates that conventional thought on how agricultural intensification influences disease may not be robust in the face of specific system modelling.

Chapter 6 – Origin and management intensity leave lasting effects on honeybee colony viriomes.

Abstract

Infectious disease is now recognised as a major threat to both managed and wild pollinators. A key question is whether management and systemic intensification of managed honeybees determines their disease epidemiology. Here we examined this question by examining the viriome of populations of differently managed honey bees that had been kept in a common garden. Specifically, we asked: if (1) feral bees were clearly differentiated from managed bees, (2) whether bees from a highly-intensified background show elevated or reduced viral titres, and (3) if particular viruses can be associated with certain management backgrounds. Despite a year in a common garden, we show that there are clear associations between specific viruses and viral titre and colonies from specific backgrounds. Colonies from a feral origin showed qualitatively different viral abundance patterns to our managed bees. Amongst the managed bees, colonies from the high intensity background exhibited higher viral abundances for all viruses. Remarkably, the difference between high and low intensity management is on the same scale as that between feral and managed bees. Our results therefore show that not only does management have long lasting impacts on honeybee disease epidemiology, but moreover, the style of management is critical, implicating apicultural intensification as having major impacts on pollinator health.

Introduction

The loss of pollinators, both managed and wild, is a growing concern for both agriculture (Brosi et al. 2008; Aizen and Harder 2009; Gallai et al. 2009) and conservation (Williams and Osborne 2009; Potts et al. 2010, 2016; Kleijn et al. 2015). Bee pollinators are crucial for ecosystem function (Corbet et al. 1991; Brosi and Briggs 2013), agricultural fruit set (Klein et al. 2007; Garibaldi et al. 2013) and quality (Knapp et al. 2017), and are also culturally highly valued (Bingham 2006; Watson et al. 2011; Mace et al. 2012). One key driver of bee declines is infectious disease (Potts et al. 2010; Becher et al. 2013; Manley et al. 2015; Kent et al.

2018). Managed bees, especially the western honeybee *Apis mellifera* L., have experienced emerging or re-emerging outbreaks of numerous pathogens (Martin et al. 2012; Mondet et al. 2014; McMahon et al. 2016, 2018; Wilfert et al. 2016), and elevated losses to infectious disease for a variety of reasons (vanEngelsdorp et al. 2009; Genersch et al. 2010; Pettis and Delaplane 2010; vanEngelsdorp and Meixner 2010). Whilst invasive pests, poor forage, pesticide exposure, behavioural stress, and lack of genetic diversity are all proximate causes of increased vulnerability to pathogens (Yang and Cox-Foster 2005; Oldroyd 2007; Conte et al. 2010; Forsgren and Fries 2010; Neumann and Carreck 2010; Aronstein et al. 2012; van der Zee et al. 2012; Pasquale et al. 2013; Sánchez-Bayo and Goka 2014; Zee et al. 2014; Goulson et al. 2015; Dolezal et al. 2016; Rumkee et al. 2017; Bartlett et al. 2018a), a more ultimate eco-evolutionary perspective is that changing pollinator management fosters increased abundances of, and potentially more virulent, pathogens (Graystock et al. 2016; Brosi et al. 2017). As evidence mounts showing how managed pollinator pathogens spill over into their wild counterpart populations (Graystock et al. 2013, 2015, 2016; Fürst et al. 2014; Manley et al. 2015; McMahon et al. 2015; Cohen et al. 2017) understanding the epidemiology of managed pollinators becomes increasingly important.

The management of pollinator livestock has changed in multiple ways in different parts of the world in recent years (Delaplane et al. 2000; Aizen and Harder 2009; vanEngelsdorp and Meixner 2010; Aebi et al. 2012; Graystock et al. 2013, 2016; Moritz and Erler 2016). Here, we focus on the managed pollinator, *A. mellifera*, in the context of the United States of America (USA). Beekeeping in the USA has undergone a recent shift towards large-scale, intensified, industrial beekeeping (Corbet et al. 1991; Brosi et al. 2017) – reflecting changes in the wider agricultural environment experienced by beekeepers throughout the 20th century (Odoux et al. 2014; Otto et al. 2016). This counterpart to agricultural intensification – apicultural intensification – represents profound potential changes in the population-level underpinnings of honeybee epidemiology. Critical aspects include much higher stocking densities (Seeley and Smith 2015), cross-continental migratory beekeeping (Whynott 1991; Welch et al. 2009; vanEngelsdorp et al. 2013; Simone-Finstrom et al. 2016), treatment for candidate pathogen vectors (Delaplane 2001;

Dietemann et al. 2012), all partially driven by moves away from honey production towards pollination services (Whynott 1991; Southwick and Southwick 1992; Hodges et al. 2001; Gallai et al. 2009; USDA - NASS 2012b; Bartlett et al. 2018a) as a source of income.

There have been a number of predictions made about which aspects of intensified beekeeping are likely to have significant effects on pathogens (Brosi et al. 2017; Bartlett et al. 2018b), with scales discussed spanning global (Wilfert et al. 2016) to landscape (Lindström et al. 2008; Giacobino et al. 2014; Nolan and Delaplane 2017) to colonies and individuals (Simone-Finstrom et al. 2016; Booton et al. 2017). Brosi et al. (2017) predicted that intensively or industrially managed *A. mellifera* populations will sustain higher pathogen burdens compared to their counterparts, on the basis that wild colonies are smaller and densities of wild colonies across a landscape much lower (Seeley 2007), leading to lower disease burdens (Loftus et al. 2016). As a conceptual extension of this, hypotheses have been put forward that traditional beekeeping may sustain lower pathogen burdens than modern high-intensity operations (Mõtus et al. 2016; Dynes et al. 2017; Nolan and Delaplane 2017). However, as described in chapter 5, aspects of this hypothesis have been questioned from a theoretical standpoint (Bartlett et al. 2018b). Emerging from these discussions are clear questions of if and how wild (in the case of the USA, feral) honeybee epidemiology differs from that of managed honeybees, and how industrial high-intensity management affects honeybee epidemiology across multiple spatial scales. Especially in the context of migratory beekeeping (Welch et al. 2009; Simone-Finstrom et al. 2016), an open question remains as to the ephemerality of changes in viriome dynamics, and whether management effects on viruses are temporary and transient. In this study, we examine how honeybee colony origin and management history affects colony viriomes in the long term.

To investigate these questions, and better inform the growing work on bee landscape epidemiology, we undertook a ‘common garden’ experiment. We took honeybee colonies from three different origins: a feral population, a ‘low intensity’ operation, and a ‘high intensity’ operation, and maintained them for a year at the

same site, subject to the same environment and management. We then characterised their viriomes. Our principal question was whether origin would have a demonstrable long-term effect on colony viriome. A persistent effect of colony origin would inform us that the ecological history of a colony has a meaningful and lasting effect on its viral dynamics, and therefore potentially its role in spill-over into other colonies or bee populations. Specifically, we also asked: if (1) feral bees were clearly differentiated from managed bees, (2) whether bees from a highly-intensified background show elevated or reduced viral titres, and (3) if certain viruses can be associated with certain origins / management backgrounds.

Materials and Methods

HONEYBEE COLONY SOURCING AND MAINTAINANCE

Colonies were sourced in 2013 from three different origins, represented by 14 colonies from each origin (42 colonies total in this study). Two origins were managed backgrounds (beekeeping operations), which we refer to as ‘high’ and ‘low’ intensity. Note that this study therefore constitutes three populations: one feral population from a single location, and two managed populations each from one beekeeping operation. One operation was a honeybee supplier of industrial scale, with a large emphasis on profit through pollination services, where colonies are maintained in extremely large, dense apiaries, migrating annually across the USA as part of a modern, high-intensity apiculture regime (Welch et al. 2009; Brosi et al. 2017). Bees are exposed to mostly monoculture environments, and are treated with pesticides routinely outside of an integrated pest management (IPM) framework. The second managed operation was a supplier reflecting traditional, lower-intensity beekeeping practices where colonies are maintained at reduced densities in smaller apiaries, and do not undertake migrations (although note that such professional operations still practice active management and should not be conflated with economically inviable “natural” or “organic” treatment-free beekeeping). Colonies were treated with pharmaceuticals / chemical control agents as part of an IPM framework, and profit emphasis of the operation was on colony sales and honey production (with no routine payment for pollination services). We cannot name our suppliers due to data protection and commercial interest

concerns. Our third origin was a source of feral honeybees, where we captured colonies in the centre of the federally designated wilderness area constituting part of the Okefenokee Swamp. Such wilderness areas preclude any agricultural activity, and the size of the Okefenokee swamp (in addition to its broadly inaccessible nature) makes it likely such feral swarms are not 'recently feral' but truly sustained feral populations with potentially little immigration from the managed honeybee population, in line with other such populations identified in the USA (Schiff et al. 1994; Seeley 2007). Collections were undertaken with approval and in line with federal and state laws governing the use of designated wilderness areas for scientific research.

All our sourced colonies were then maintained in a 'common garden' at a single location (University of Georgia Horticultural Farm – Watkinsville, GA, USA), by a team of professional apicultural technicians. Colonies were maintained as though they were ordinary hobbyist colonies under beekeeper care, following standard practise for the region (see Georgia Master Beekeeping Programme – University of Georgia College of Agricultural and Environmental Sciences). Colonies were managed from the summer of 2013 onwards, with samples for this study then collected in May 2014, meaning approximately one year of common garden management for all colonies, varying by one or two months depending on supply dates and dates of capture in Okefenokee.

SAMPLE COLLECTION AND VIRAL CHARACTERISATION

Each colony represents an independent sample assigned to one of the three origins ('treatments'). To compare the viriomes of colonies, we randomly selected 30 adult bees from the brood frames of each colony. Samples from all colonies were gathered within three days to eliminate potential seasonal effects of viral dynamics (Sumpter and Martin 2004; Tentcheva et al. 2004b). For each sample, the thirty live bees were sealed in 50ml centrifuge tubes (VWR, USA) and immediately placed on dry ice. All samples were then transferred for storage at -80^{o} within two hours of collection.

Samples were processed on-site at the UGA Horticulture Farm for RNA extraction and conversion of RNA to cDNA using the following protocol. RNA extractions were undertaken inside a class II biological safety cabinet. Thirty frozen bees were transferred to one side of a fine-meshed universal long tissue extraction bag (Bioreba, Switzerland) with 15ml RiboZol^{TM} RNA extraction reagent (Amresco, USA) before being thoroughly crushed using an ultralow-chilled solid marble rolling pin. 1.5ml aliquots of sample extract were then pipetted from the opposite side of the extraction bag mesh and stored again at -80 ^{o}. Batches of aliquots were then subsequently thawed for RNA purification. Samples were centrifuged at 12000r.c.f. at 4^{o} for 2 minutes before 900µl of supernatant was pipetted into 140µl chloroform, thoroughly mixed, and incubated at 20^{o} for 5 minutes. Following this, solutions were centrifuged at 12000r.c.f. at 4^{o} for 10 minutes after which the aqueous phase supernatant was pipetted into 450 µl isopropanol and thoroughly mixed. Samples were centrifuged at 12000r.c.f. for 10 minutes to pellet out RNA, and all supernatant was drawn off and discarded. RNA was then subjected to two rounds of ethanol washes using 75% ethanol and DEPC-treated water, before being supplemented with 100µl DEPC-treated water and frozen at -80^{o} for storage.

Batches of RNA were converted to first-strand cDNA using an M-MLV reverse transcription protocol. At this step, we introduced three ‘no-sample’ controls of molecular water to check for likely contamination in downstream analysis. RNA was thawed at 4^{o} and resuspended into solution, then 2µl of sample solution was combined with 2µl of molecular water and 1µl of random hexamers (Promega, USA) at 50µM concentration, and incubated at 70^{o} for 5 minutes before submersion in an ice-bath for a further 5 minutes. Next, 3µl of this solution was combined with 1.2µl molecular water, 3.8µl of $MgCl_2$ solution at 25mM concentration (Promega, USA), 5µl DNTPs at 10mM concentration (Amresco, USA), 1µl M-MLV enzyme (Amresco, USA), and 4µl reverse-transcription reaction buffer (Promega, USA). RT-PCR first-strand cDNA synthesis used the following thermocycler conditions: 25^{o} for 5 minutes, 37^{o} for 1 hour, 70^{o} for 15 minutes, then chilled and held at 4^{o}. cDNA solutions were then split onto multiple replicate plates and diluted down with molecular water using a five-fold dilution; cDNA was then

stored at -20º before a subset of plates were shipped to U.C. Berkeley on ice for target sequence quantification.

We sought to quantify eight viral targets: the ABPV/KBV/IAPV 'acute paralysis virus complex' (de Miranda et al. 2010a), chronic bee paralysis virus ('CBPV'), slow bee paralysis virus ('SBPV'), sacbrood virus ('SBV'), black queen cell virus ('BQCV'), two deformed wing virus ('DWV') variants, DWV-A and DWV-B ('VDV-1') (McMahon et al. 2015, 2016; Wilfert et al. 2016), and four strains of Lake Sinai virus ('LSV1-4') (Daughenbaugh et al. 2015; Ravoet et al. 2015). We also sought to quantify a housekeeping gene, in our case *Apis mellifera* β-actin. We chose this housekeeping gene because it has a characterised counterpart in the parasitic mite, *Varroa destructor* β-actin. Originally we had planned to also examine the viriome within the mite (data not presented in this study).

We diluted our cDNA down by a 20-fold dilution to ensure enough sample volume for all forward work and to create separate sealed plates of backup samples. We then used BioRad's QX200TM Droplet Digital^{TM} PCR system to attempt to quantify target sequences specific to a housekeeping gene and eight viral sequence targets – see Table 6.1 for targets and references. All primer sequences have been previously tested and used in the honeybee virus literature for equivalent qPCR virus quantification studies. Lyophilized primers were resuspended to 100µM using molecular water (VWR, USA) following the manufacturer's specifications (Integrated DNA Technologies, USA) as supplied with each oligonucleotide set.

For each sample and target combination, we used a reaction mixture of 2µl sample solution, 0.22µl forward primer at 10µM, 0.22µl reverse primer at 10µM, 8.56µl molecular water (VWR, USA), and 11µl BioRad (USA) QX200^{TM} EvaGreen Supermix. Droplets of this reaction mixture were then formed using up to 70µl of EvaGreen droplet-forming oil and according to manufacturer instructions. 42µl of reaction-droplet oil emulsion was then used for the subsequent PCR and quantification steps.

PCR cycling conditions were as follows, with only reaction temperature varying between targets (plates); 95°C for 10 min, 95°C for 30 s, T_R for 30 s (see Table

6.1), 72°C for 2 min, repeat steps 2–4 40 times total, 4°C for 5 min and 90°C for 5 min. Target sequences were grouped such that DWV-A and DWV-B were done on the same plate simultaneously, as were ABPV/KBV/IAPV and SBPV (see Table 6.1). All other target sequences were subject to separate reactions owing to different reaction temperatures. Following PCR, plates were then held in dark conditions at 4°C for 12 – 24hrs for droplets to stabilise, before being transferred to the BioRad droplet reader for droplet fluorescence measurement. Raw fluorescence data was then exported for further handling and statistical analysis (see below).

NOTES ON MOLECULAR DATA APPROACH

Our original intention was to use the ddPCR approach to quantify target sequences in absolute terms in an approach that has been successfully deployed elsewhere. However, the large variability between samples in positive droplet amplitudes of the housekeeping gene and in viral sequence targets (Supplementary Material 4 fig. S4.1) would have introduced insurmountable biases if a 'threshold' positive / negative approach had been taken following additional sample diluting. Our no-template controls (NTCs) showed clear, tight bands of negative droplets, which matched with our true negative controls (originating from the step of sample RNA extraction). However clear positive / negative bands were not apparent for our 'true' samples, although samples clearly clustered around median positive fluorescences (Supplementary Material 4 fig. S4.1).

This phenomenon has multiple related possible explanations. Field conditions around initial sample freezing and storage may have compromised RNA quality in the adult honeybees differently across samples, reducing final cDNA quality. However, this effect should affect all RNA within a sample approximately equally (such that for a more compromised sample, both viral and β-actin are equivalently reduced). Additionally, and perhaps more likely, are differences in the amount of RNA extracted or number of inhibitors present in each sample due to different extraction qualities. Differences in inhibitor or total RNA concentrations would have resulted in differences in downstream cDNA. More importantly for these results, increasing concentration of inhibitors also reduces droplet fluorescence in

otherwise identically-treated samples, and additionally increases the variability of positive droplet amplitudes (Dingle et al. 2013). Apparent negative droplets therefore may not reliably represent droplets lacking the target sequence, but that stochastically contain elevated concentrations of inhibitors, pushing them below the user-defined ‘positive’ fluorescence threshold.

Due to this suspected action of unequal inhibitor concentrations, further dilution to pursue the intended ‘positive/negative’ digital PCR approach would have simply resulted in samples with the lowest positive-droplet amplitudes (most inhibited) showing the lowest target sequence concentrations, and consequently being classified as false negatives. Instead, we exploit the high target-sequence concentrations in our samples (all non-control sample well droplets test positive for β-actin) to analyse this data as though each droplet is a repeat of the same qPCR.

Following this approach, each droplet will vary in amplitude based on 1) inhibitor concentrations (Dingle et al. 2013) 2) number of copies of the target sequence in the droplet (the principal of which underpins copy-variant detection using ddPCR (Wu et al. 2017). As a consequence of high target sequence concentrations and variable inhibitor levels, we do not expect clear ‘grouping’ of negative droplets for any given sample. Instead, we pursue a relative (rather than absolute) analysis of target sequence abundance (droplet fluorescence) as would be done for traditional qPCR; for each sample, viral target sequence abundance is expressed relative to β-actin target sequence abundance (both inferred by droplet fluorescence). Differences in inter-sample total cDNA and inhibitor concentrations are thus controlled for.

We do not compare relative fluorescence values between any two different viral targets, as we have no measure of comparative primer efficiency for our different targets. However, any differences amongst samples of within-sample ratios of a given viral target to β-actin fluorescence should reflect differences in colony viral titres, so long as the assumption that β-actin expression is the same across each set of 30 adult honeybees holds. We can then control for the multiple-testing of each virus-colony combination statistically, and additionally check our assumptions regarding this approach in the context of our statistical results.

DATA PROCESSING AND STATISTICAL ANALYSIS

All data handling and analysis was undertaken in R (v 3.4.4.'Someone to Lean On') (R Core Team 2018). We used a bootstrapping approach to estimate the relative fluorescence of each viral-target compared to β-actin for each sample, before testing for an effect of colony origin. We generated 1000 bootstrapped datasets. Each dataset included *n* relative fluorescence values for each viral target for each colony, where *n* was equal to the smallest number of droplet reads across all target-sample combinations. We used the same *n* across the entire bootstrapping approach to balance sampling effort in order to better meet assumptions of downstream modelling (Schielzeth and Nakagawa 2013). For each viral target and colony combination, we sampled the absolute fluorescence values for that virus-colony read *n* times with replacement, and similarly sampled the absolute fluorescence values of β-actin reads for that colony *n* times with replacement. This gave us *n* pairs of absolute fluorescence values for each virus-colony combination. Relative fluorescence values were then taken for each pair as the difference in fluorescence between the viral-target and β-actin droplets, as a proportion of the fluorescence of the β-actin droplet.

Each of the 1000 bootstrapped datasets was then used for fitting linear mixed-effects models (package 'lme4' (Bates et al. 2015)). We fit to maximum-likelihood, rather than residual maximum-likelihood, to permit model comparison. In each case we fit three models, where fixed effects included target (viral sequence), target and treatment (colony origin), or target and treatment and an interaction effect between the two. We included each colony as a random effect above each measurement and nested under treatment (origin) to reflect the nested structure of our sampling (Schielzeth and Nakagawa 2013). Our response variable was relative fluorescence as described above. Our models used a Gaussian (normal) error structure. We separately assessed which model was best-performing using both the Bayesian Information Criterion (BIC) and by comparing models using ANOVAs (where we compared 'target' only to 'target + treatment', and 'target + treatment' to 'target * treatment'). For each of the 1000 bootstrapped datasets, we recorded which models were selected as best-performing. Separate to model selection, we

took the most complex model (always allowing for interaction effects between target and treatment) and predicted from it expected average relative fluorescence values for each virus / colony-origin combination. These 1000 predictions for each virus-origin combination were then plotted, on separate graphs and axes for each virus, for comparing the effect of colony origin in the context of a specific virus. We did not plot predictions in such a way as to compare between different viruses, for the reasons detailed above.

Table 6.1 – Primers used in this study to target specific cDNA sequences for amplification and quantification using ddPCR.

Target	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Length	T_R - Reaction Temp. (°C)	Reference	Primer Name (Forward)	Primer Name (Reverse)
ABPV/KBV/IAPV	GGCGAGCCACTATGTGCTAT	ATCTTCAGCCCACTT	401	50.0	(Evans 2001; de Miranda et al. 2010a)	AKIF8140	AKIFR8507
CBPV	CAACCTGCCTCAACACAG	AATCTGGCAAGGTTGACTGG	276	53.0	(Ryabov et al. 2017)	CBPV1FqF1818	CBPV1FqB2077
SBPV	GCGCTTTAGTTCAATTGCC	ATTATAGGACGTGAAAATATAC	226	50.0	(de Miranda et al. 2010b)	SPV-F3177	SPV-B3363
SBV	TTGGAACTACGCATTCTCTG	GCTCTAACCTCGCATCAAC	335	54.0	(Locke et al. 2012)	SBV-F3164	SBV-B3461
BQCV	AGTGGCGGAGATGTATGC	GGAGGTGAAGTGGCTATATC	294	53.0	(Locke et al. 2012)	BQCV-F7893	BQCV-B8150
DWV-A	TGTCTTCATTAAAGCCACCTGGAA	TTTCCTCATTAACTGTGTCGTTGAT	140	57.3	(McMahon et al. 2015)	DWV-F2	DWV-R2a
DWV-B (VDV-1)	TATCTTCATTAAAACCGCCAGGCT	CTTCCTCATTAACTGAGTTGTTGTC	140	57.3	(McMahon et al. 2015)	VDV-F2	VDV-R2a
LSV 1-4	CGTGCGGACCTCATTTCTTCATGT	CTGCGAAGCACTAAAGCGTT	152	59.5	(Daughenbaugh et al. 2015)	LSV1-4-F-2157	LSV1-4-R-2309
Beta-Actin (*A. mellifera*)	CGTGCCGATAGTATTCTTG	CTTCGTCACCAACATAGG	271	52.0	(Locke et al. 2012)	Am-actin2-qF	Am-actin2-qB

Outside of our bootstrapping approach, we also calculated relative fluorescence values using the median values of each virus-sample combination and median β-actin fluorescence for each sample. These median relative fluorescence values were used to see if there was any clear grouping of colonies by origin using dimensionality reduction analyses. We plotted the first two dimensions of a principal component analysis (PCA) and eight replicates of a t-sne analysis (Van

Der Maaten and Hinton 2008; Van Der Maaten 2014) using package ‘Rtsne’ (Krijthe 2015). We then visually inspected plots for any grouping of samples by origin.

Results

Our mixed-effects models proved highly consistent; across all 1000 bootstrapped iterations, the GLMM including an interaction effect between target (virus) and treatment (colony origin) was always the best performing model (fig. 6.1) both using ANOVA ($\overline{p} < 0.001$) and BIC model comparisons (see Supplementary Material 4 Table S4.1). This informs us that colony origin does have a significant effect on the relative abundance of viruses in a colony, and that the direction of this effect depends on which virus is being considered; this is apparent in fig. 6.1, where origins switch ranks in showing lowest to highest abundance depending on the virus at hand. For comparison, we show simplified median-averages of the data used to generate these bootstraps in fig. 6.2, where the differences highlighted in fig. 6.1 are also apparent in the context of overall inter-colony variation.

Notably, during our GLMM selection, it emerged that treatment (colony origin) alone was never informative ($\overline{p} = 0.138$), with a ‘null model’ where viral target was the only fixed effect always outperforming a model which included viral target and colony origin (but no interaction) – see Supplementary Material 4 Table S4.1. We can infer from this there is no colony origin which has significant universally higher relative viral abundances; instead, as can be seen in fig. 6.1, the colony origin is informative in the context of which virus is being discussed.

Colonies from our ‘low intensity’ origin typically showed the lowest relative abundance of viruses, except for the AKIV-complex and possibly CBPV (fig. 6.1). Colonies from our ‘high intensity’ background had the highest relative abundances of most of the viruses, except for LSV and SBV targets, where feral-origin colonies exhibited the highest relative abundances. Feral-origin was the most changeable effect in that for some viruses it was the origin with the least relative abundance and for the others the most (fig. 6.1). Comparing between our two managed-

population origins, the ‘low intensity’ origin showed lower relative abundance of all viruses compared the ‘high intensity’ origin.

We found no apparent grouping based on origin when attempting dimensionality reduction analyses using only median-averaged relative abundances (see data summarised in fig. 6.2) for all colonies. We used two unsupervised machine-learning approaches, a PCA and multiple t-sne replicates (fig. 6.3). No clear clustering of any description was evidently emergent, and notably not by colony origin. From this, it is apparent that looking at the viriome of a colony in our cohort is not easily predictive of its origin, despite origin being informative in understanding the relative abundance of a given virus compared between colonies (fig. 6.1, Fig 6.2), reflecting the degree of inter-colony variation in the data (see Supplementary Material 4 fig. S4.2).

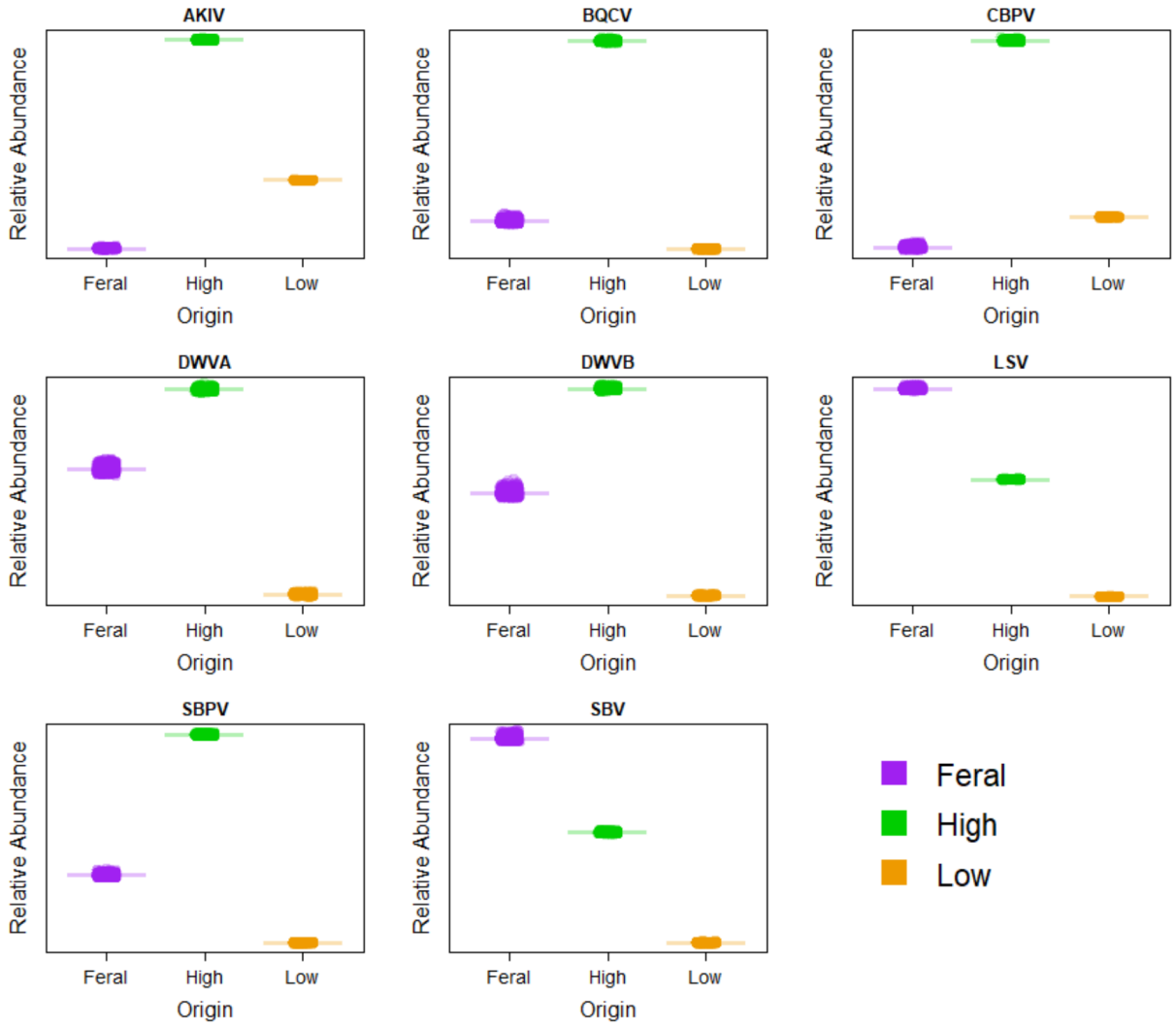


Figure 6.1 – Predicted relative abundances of each viral target for a 'typical' colony of each origin according to our 1000 bootstrapped data sets. Individual points are model predictions from a single bootstrap data set, whilst horizontal bars are median values across all 1000 predicted relative abundances. Y-axes are not labelled as the relative abundance units are arbitrary, and y-axes scales are not conserved between each viral target panel (no comparisons between viruses should be made – the feature of interest is in the difference between origins for each viral target). AKIV: acute/Kashmir/Israeli paralysis virus complex; BQCV – black queen cell virus; CBPV – chronic bee paralysis virus; DWVA – deformed wing virus (A strain); DWVB – deformed wing virus (B strain, ' VDV-1'); LSV – Lake Sinai virus complex, Lake Sinai viruses 1 – 4; SBPV – slow bee paralysis virus; SBV – sacbrood virus.

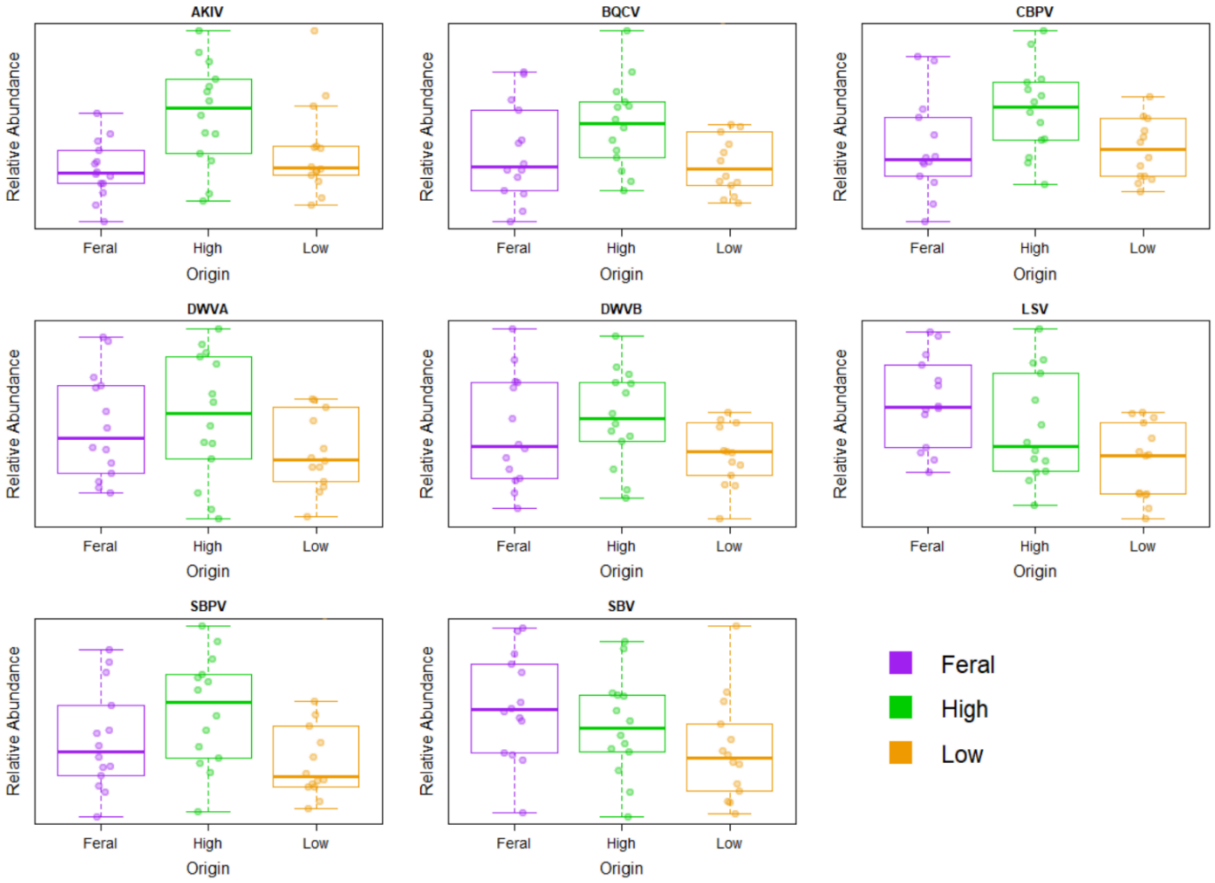


Figure 6.2 – Median relative abundances of each virus for each colony, plotted according to viral target (panel) and colony origin (x-axes and colour) comparable to fig. 6.1. Y-axes scales differ between panels. This median data was used as the data set for the dimensionality reduction analyses presented in fig. 6.3 (also see fig. S2). This representation using only median data points for colony targets is coarsely similar to the bootstrapped datasets generated for GLMM analysis, the results of which are presented in fig. 6.1. The clear pattern correspondence of the two figures acts as 'sanity check' that our bootstrapping and mixed-models were reflections of real effects observable even in this coarse presentation. See fig. 6.1 for expansion of virus abbreviations.

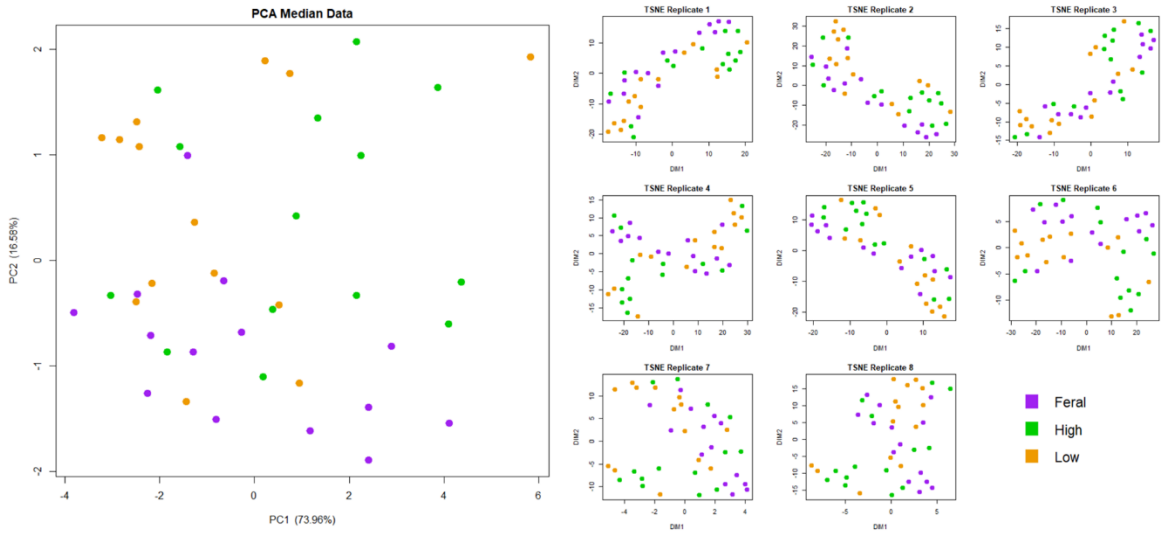


Figure 6.3 – Dimensionality reduction analyses on median relative abundance data for each virus and each colony. Points are plotted in each case according to the two orthogonal axes with the most explanatory power, and colour coded by known origin. The large panel on the left represents a principal component analysis (PCA) with explanatory power labelled on each axis. The eight smaller panels on the right represent t-sne replicates, plotted similarly. We deemed there to be no meaningful evidence of grouping or clustering in these analyses. A restructured plot of the data used for these analyses (see fig. 6.2) is presented in the Supplementary Material 4 (fig. S4.2).

Discussion

We show that a honeybee colony's history, specifically its ecology of origin, has a lasting and meaningful effect on its viriome. Despite a year in a common garden, we show that there are tractable differences in viriomes of colonies from our three sampled origins. Notably, these differences are virus specific, rather than generalisable across all viruses (fig. 6.1). It is not simply that one origin had elevated viral titres across all viruses, but rather that colonies from a feral origin showed qualitatively different viral abundance patterns to our managed bees. Amongst colonies from managed backgrounds, there was a clear finding – colonies from the high intensity background exhibited higher viral abundances for all viruses compared to those from the low intensity background.

Lasting elevated viral titres in colonies from the ‘high-intensity’ background is evidence that moves towards industrial beekeeping is negatively impacting honeybee health. As industrial high-intensity operations become necessary tactics for beekeepers (Whynott 1991; Odoux et al. 2014), this effect becomes increasingly relevant to the industry and elsewhere. We present evidence that a history of experiencing such high-intensity management leads to colonies either inheriting, or gaining, elevated viral titres. We cannot distinguish if these colonies were already exhibiting elevated titres at the time of acquisition from a ‘high-intensity’ background, or whether these titres developed once imported into our common garden. Despite this, the result remains that our low-intensity origin bees harbour less honeybee viruses in the long term. However, it is crucial to acknowledge that each of our origins represents only a single population. Larger scale studies where beekeeping operations (or populations) are the sampling level will be the necessary next step to lend strength to these early threads of evidence.

The scale of this management effect, between low- and high- intensity, is worth comparing to the effect of a feral origin. For half of our target viruses, the lasting effect of management regime is greater than the effect of originating from a feral population (fig. 6.1). That is, for half of the target viruses, the two managed backgrounds differ more to each other than either of them do to the feral origin. This effect-size observation should not be understated. Feral honeybee populations exhibit population ecologies which are profoundly different from their managed counterparts, including colony densities up to thousands of times lower, much more frequent swarming and reduced colony sizes, as well as archaic genotypes and differentiated genetic stock (Schiff et al. 1994; Loper et al. 2006; Seeley 2007; Loftus et al. 2016; Brosi et al. 2017). These profound ecological differences leave a lasting effect on colony viriomes of equivalent scale to comparing a traditional, low-intensity management regime to a modern, industrial, high-intensity management regime. Whilst speculation on the effects of such industrialisation have been made (Oldroyd 2007; Seeley and Smith 2015; Brosi et al. 2017; Nolan and Delaplane 2017) our evidence that it is ecologically comparable to the difference in feral and domesticated bee ecology is surprising. While many scientists and practitioners may have expected industrialised

management of honeybees to have negative consequences, we are surprised that the scale of such consequences to be readily comparable to the effect of a feral 'wild' ecology.

Despite these differences, the variation amongst colonies in their viriomes is large enough that no clear grouping of colonies can be identified in our dimensionality reduction analyses (fig. 6.3). Whilst colony origin does have a meaningful effect on relative viral abundance (fig. 6.1), the effect of origin after a year in a common garden is moderate relative to other sources of inter-colony variation (fig. 6.3). We do not have access to these colonies' 'imported' viriomes, and so it is not clear what degree of change occurred in their viral dynamics after being placed in the shared common garden. Comparison of C-14 isotope signatures showed that differentiation between feral and managed colonies rapidly disappeared following a common-garden nutritional regime (Anderson et al. 2014), in contrast to our findings examining the colony viriome. Future work will be needed to establish the dynamics underpinning these differences, revealing why these effects manifest and persist; for example: differences at the point of origin, genetic differentiation of either honeybee or pathogen populations, differences in queen quality, or lasting effects of stress from management regimes. Given our significant findings, these effects of origin need to be considered in a wider agroecological context.

Whilst migratory beekeeping is a clear potential driver of the elevated viral titres high-intensity origin colonies show, it also has critical ramifications for continental-scale bee viral dynamics outside of just *Apis mellifera*. There are many candidate mechanisms for how such migration may foster elevated viral abundances (Goulson et al. 2015). Such colonies may be more likely to be nutritionally stressed due to experiencing principally monocultured crops (Potts et al. 2010; Becher et al. 2013; Pasquale et al. 2013; Odoux et al. 2014; Otto et al. 2016), exposed to more pesticides (Sánchez-Bayo and Goka 2014; Sánchez-Bayo et al. 2016; Bartlett et al. 2018a), and are exposed to a wider variety of pathogens as they contact colonies from elsewhere across the continent at high densities with elevated opportunity for transmission (vanEngelsdorp and Meixner 2010; Brosi et al. 2017). It is also possible that these practices, such as reduced spatial structuring of the

honeybee (host) populations, have recently selected for more virulent viral variants (Boots and Sasaki 1999; Boots et al. 2004; Kamo and Boots 2006; Boots and Mealor 2007; McMahon et al. 2016), leading to elevated viral titres. It may be possible that the results we observe here are driven not by heterogeneity of honeybee biology, but in differences in pathogen biologies, which have evolved to specialise on the host ecologies in each of our three origins. Such evolutionary selection may be driving the elevated viral titres in migratory bees – a hypothesis which is not mutually exclusive with the more direct effects such management may have on honeybees (detailed above).

After the experience of migratory beekeeping has potentially established elevated viral titres in colonies, such colonies continue to migrate, both elsewhere in the USA and eventually to their home counties or states (Whynott 1991). We now have a large and growing body of literature documenting how honeybee viruses spill over into native bee populations (Choi et al. 2010; Singh et al. 2010; Li et al. 2011; Zhang et al. 2012; Graystock et al. 2013, 2016; Reynaldi et al. 2013; Mazzei et al. 2014; Forsgren et al. 2015; Guzman-Novoa et al. 2015; Manley et al. 2015; Cohen et al. 2017; Forzan et al. 2017; Santamaria et al. 2017), a phenomenon which is likely elevated if higher viral abundances are observed in these migratory colonies. Our observation that high-intensity origin bees show the most elevated viral abundances establishes them as potential super-spreaders (Stein 2011) in that they are more infectious and are exposed to far more native pollinator populations, potentially infecting many more threatened species or populations. This double-driver of risk to native bees (and also non-migratory beekeeping operations) is of potentially critical conservation concern (Williams and Osborne 2009; Kleijn et al. 2015; Potts et al. 2016), practitioner interest (Brosi et al. 2008; Pettis and Delaplane 2010; Connell et al. 2012), and may be informative for policy both in the US (FWS 2016) and elsewhere, where migratory beekeeping is becoming more common (Odoux et al. 2014).

The role of feral honeybees in the bee virus landscape is also worth considering. Honeybees are not native to the Americas, and while feral honeybees are hypothesised to foster far lower viral abundances, and possibly less virulent

strains, compared to managed honeybees (Loftus et al. 2016; Brosi et al. 2017) our evidence does not corroborate those speculations. Whilst our common-garden approach cannot give direct insight into viral dynamics of feral populations, our results suggest it is possible that feral populations of honeybees sustain circulation of the well-characterised viruses examined here, and in some cases (such as sacbrood virus and the Lake Sinai viruses) possibly at higher per-colony abundances than in managed populations. Sacbrood virus has been implicated with *Varroa*-mite mediated losses (Nielsen et al. 2008), whilst Lake Sinai viruses are fairly understudied (Daughenbaugh et al. 2015; McMahon et al. 2018). It is possible that even in protected areas, honeybees may be sustaining viral circulation with the capacity to spill over into native bee populations. From an apicultural perspective, pursuing eradication of various honeybee pathogens may also prove difficult if feral populations act as reservoirs for *Apis* pathogens to continue circulating in.

Unlike some previous approaches to honeybee virology, we do not seek to focus in this study on whether any colonies are ‘negative’ for a particular virus. We believe this to be a valid approach, as reports of ‘negative’ colonies may be reflections of low viral abundances or titres, and consequent detection failure. As molecular methods in this system become more developed (Tentcheva et al. 2004a; de Miranda et al. 2010a; Škubník et al. 2017), and honeybee epidemiology is better characterised from a theory standpoint (Betti et al. 2014, 2016; Jatulan et al. 2015; Bartlett et al. 2018b), it is becoming increasingly apparent that most honeybee colonies may not be truly negative for almost all honeybee pathogens (Budge et al. 2010, 2015; Ryabov et al. 2016). In work elsewhere, we show that honeybee pathogens are theoretically predicted to establish in all colonies in a given apiary within a year (Bartlett et al. 2018b), we would therefore expect in this common garden study system that all viruses are present in all colonies (as we appear to observe). Additionally, recent efforts to test individual honeybees, such as those by Stamets et al. (2018) studying sacbrood virus and deformed wing virus, find that 100% of adult honeybees tested as positive for a target virus (however vary significantly in titre). Given each of our colony samples constitutes 30 adult bees, we do not expect any of our sampled colonies to necessarily test negative for any

target virus. We propose that honeybee virology, especially at the colony scale, moves away from a paradigm of discussing positive/negative colonies, and potentially positive-negative individuals, and instead towards relative viral titres or abundances. Such measures have been informative in survivorship studies looking at specific viruses elsewhere (McMahon et al. 2016; Natsopoulou et al. 2017), and may be more robust to failures to detect low-level pathogens.

Clear testable hypotheses can be established for this approach; colonies with elevated relative abundances of viruses should be more infectious to neighbouring, or experimentally inoculated, colonies. However counter-predictions can also be made, as theoretical work informs us that most honeybee pathogens are likely to easily transmit between honeybee colonies regardless of management regime (Bartlett et al. 2018b). It is therefore plausible that the effect of elevated viral titres in certain colonies may not be meaningful in field experiments. Establishing the relative importance of viral load for further transmission may be a critical step for continued research in managed and native bee health.

Overall, our results indicate that colony history, origin, and management have lasting effects on the relative abundance of circulating viruses in those colonies. Notably, comparing different management regimes reveals effects of similar magnitude to comparing managed and feral bees, suggesting these practices represent extremely different population ecologies. Colonies from high-intensity, industrial backgrounds show higher viral titres for all studied viruses compared to those from low-intensity, traditional backgrounds. This finding is of critical relevance to ongoing efforts to control managed pollinator diseases and understanding how migratory beekeeping practices may be affecting already embattled bee populations. Additionally, our evidence is counter to hypotheses examining the expected pathogen burden of feral colonies, which in some cases showed the highest abundances of certain viruses. The implication of this for evolutionary understanding of insect viruses across landscapes prompts further investigation.

Chapter 7 – When vectoring leads to more virulent pathogens: deformed wing virus in honeybees.

Abstract

A longstanding hypothesis concerning the evolution of pathogen virulence is that vectored pathogens are typically more virulent than their directly transmitted counterparts. Testing this hypothesis however has proven difficult, despite its importance for society in its application to public health and agriculture. Here, we review the evidence concerning a candidate vector (the varroa mite) in the important system of the western honeybee, *Apis mellifera*, and associated viruses, which may have switched from a purely directly transmitted ecology to a partially, or perhaps fully, vectored strategy. The evidence at hand suggests one virus, deformed wing virus, has adapted to exploit this novel vector and has switched from a directly transmitted strategy to a vectored one. In adapting to this new transmission mode, deformed wing virus appears to have evolved to be much more virulent. As such, this review demonstrates that the *Varroa*-virus-vector system in honeybees is a well-documented test of the hypothesis that vectored pathogens are more virulent than directly transmitted counterparts. We find that evidence favours the hypothesis. More broadly, this review also synthesies decades of research on emerging or re-emerging honeybees viruses following invasion by *Varroa*, providing critical understanding for this important system. We also argue that managed honeybees are a favourable potential system for future research in the fundemntal evolutionary processes shaping parasites and pathogens.

Understanding the Vector-Virulence Hypothesis

Vectored pathogens represent some of the most important and burdensome infectious diseases for both human health and agriculture (Jones et al. 2008; Lambrechts and Scott 2009; Froissart et al. 2010). One proposed reason for this is the hypothesis that vectorborne transmission selects for higher pathogen virulence compared to direct transmission. This hypothesis was extensively discussed by Ewald (Ewald, 1987, 1991, 1993), and originally focussed principally on how

vectors circumvent some of the costs of virulence by allowing transmission from an otherwise immobile definitive host (Ewald, 1996). Relatively little empirical evidence in human infectious diseases supports this overall hypothesis however (Ewald, 1993), and the idea remains disputed theoretically (Cressler et al. 2016). Nevertheless, the idea that vector transmission may negate the costs to direct transmission associated with virulence and morbidity remains an interesting, and mostly untested, evolutionary question.

Ewald's verbal arguments rely on the concept of 'optimum virulence' (Bull, 1994; Ewald, 1996; Ewald, 1993), a now well-explored and formally-developed theory explaining why pathogens evolve to damage their host (Alizon, Hurford, Mideo, & Van Baalen, 2009; Levin & Pimentel, 1981; May & Anderson, 1983; Read, 1994). Ewald's initial verbal arguments focussed on the immobilisation of host, and can be easily expanded to encompass pariahship (social exclusion) or other biological phenomena which reduce frequencies of direct transmission (Cremer et al. 2007). While appealing, this initial mechanistic account of why vectored pathogens may be more virulent is not easily generalisable when formalised mathematically (Day 2002).

While the initial mechanistic reasoning for the vector-virulence hypothesis has not fully held up to scrutiny (Day 2002; Cressler et al. 2016), a number of other possible mechanisms have been proposed which also provide a basis for the same hypothesis. Arguments have been put forward that vectorborne pathogens may be predisposed to increased likelihoods of coinfection, which leads to selection for higher virulence in competing pathogens (Alizon, de Roode, & Michalakis, 2013; Alizon & van Baalen, 2008). Another compelling line of reasoning focusses on the phenomenon that vectorborne pathogens typically infect both their definitive host and their vector. As such, they are multi-host pathogens and must balance their virulence in two different hosts (Gandon 2004). Evidence suggests that lower virulence in one host may trade off against higher virulence in another, especially if hosts are not too distantly related (Gower et al. 2004; Rigaud et al. 2010). Both theory (Ewald, 1987, 1991) and some empirical evidence (Elliot, Adler, & Sabelis, 2003) exists to suggest that pathogens should prioritise lower virulence in their

vector than their definitive host, leading to a generalisation of elevated virulence in the definitive host.

Despite the importance of vectored pathogens, and the undoubted interest in the hypothesis that they are typically of higher virulence, there is a lack of empirical work testing this hypothesis. This is in part due to the considerable difficulty in measuring a pathogen's virulence in vectors, and very few candidate diseases for which good measures of virulence can be compared (Cressler et al. 2016). However, as pathogen research continues to broaden, in part thanks to developments in molecular methods, new opportunities to scrutinise this hypothesis may appear. Furthermore, as new diseases emerge there is the potential for 'natural' experiments that allow us to address hypotheses such as this. One such opportunity has occurred in the western honeybee, *Apis mellifera*.

A Brief History of Honeybees, Varroa, and Deformed Wing Virus

A pathogen gaining or losing the vector transmission mode, and the subsequent evolutionary outcomes, may provide a direct test of the hypothesis that vectored pathogens are more virulent than their directly transmitted counterparts. Such evolutionary events are likely to be rare, and the chance of scientists being in a position to document such a shift is also likely slim. However, one well documented example of such a phenomenon is the virological study of honeybees *(Apis* spp.). Commercially managed western honeybees (*A. mellifera*) have recently experienced widespread losses, at least in part due to infectious diseases (Potts et al. 2010; Brosi et al. 2017). Due to the commercial, agricultural, and cultural value of this species, an abundance of research now exists seeking to understand their pathogens (Genersch 2010; vanEngelsdorp and Meixner 2010; Potts et al. 2016; Brosi et al. 2017). A critical pathogen in this system is deformed wing virus (DWV), which has undergone a partial shift in transmission mode: from being exclusively directly transmitted, to vectored by an ectoparasite (the acarid mite *Varroa destructor*). The corresponding change in pathogen ecology and increase in virulence associated with this shift in transmission mode constitutes some of the best evidence currently available for assessing whether vectored pathogens are indeed more virulent than their directly transmitted counterparts.

Varroa destructor is a recent parasite of *A. mellifera;* its association with western honeybees is a result of a host shift from its ancestral host, the Asian honeybee *A. cerana* (Oldroyd 1999; Conte, Ellis & Ritter 2010). DWV however has a long association with *A. mellifera*, and was circulating in western honeybees prior to the arrival of *V. destructor* (Allen and Ball 1996; Shutler et al. 2014). In this pre-*Varroa* state, overt clinical symptoms of DWV, which manifest as the conspicuous presence of worker honeybees with malformed crippled wings, were only very rarely observed (Allen and Ball 1996; Wilfert et al. 2016; Locke et al. 2017). Evidence points to pre-*Varroa* DWV being present at low titres, and likely low prevalence (Martin, Ball, & Carreck, 2013; Martin et al., 2012; Mondet, de Miranda, Kretzschmar, Conte, & Mercer, 2014). In the earliest studies examining DWV (notably in the United Kingdom (Bowen-Walker et al. 1999)), evidence confirming this to be the case is compromised somewhat by the then lack of molecular genetic methods to detect and quantify viruses; prior to these methods, detection relied on seropositive or seronegative immunoassays with higher detection thresholds. However, more recent invasions of *Varroa*, for example into Hawai'i and New Zealand (Martin et al., 2012; Mondet et al., 2014) monitored using molecular genetic methods, also support earlier assertions that DWV was present in *A. mellifera* prior to *Varroa* at both lower titres and prevalence.

Strong evidence has been accumulated documenting the capability of DWV to be directly transmitted through a variety of routes – presumably the ancestral routes associated with its pre-*Varroa* epidemiology. Oral transmission of DWV is possible via contaminated flowers, and through honeybee trophallaxis, including adult-adult transmission and adult-larval transmission (Möckel et al. 2011). Queen honeybees have been shown to frequently test positive for DWV (Chen, Pettis, & Feldlaufer, 2005; Francis, Nielsen, & Kryger, 2013; Gregorc & Bakonyi, 2012; Muz & Muz, 2009) and provide a vertical transmission route directly to their offspring (Chen, Evans, & Feldlaufer, 2006; Chen, Higgins, & Feldlaufer, 2005; Chen, Smith, Collins, Pettis, & Feldlaufer, 2004). Both of these mechanisms establish how DWV infections may persist in a colony, and as colonies split via fission, propagate vertically at the colony-scale. Transmission between colonies may have been principally through a venereal route, as it is also well demonstrated that infected

drones produce DWV positive semen (Fievet et al. 2006; Yue et al. 2006), and that this can infect gynes via mating during their conjugal flights (Amiri, Meixner, & Kryger, 2016; de Miranda & Fries, 2008). As suggested by Ewald and others (Ewald 1993, 1996; Bull 1994), these direct transmission routes (especially in the case of vertical transmission) can be expected to select for low pathogen virulence. Given the rarity of overt clinical pathology, low titres, and low prevalences of DWV prior to *Varroa*, virulence theory agrees with the ancestral case.

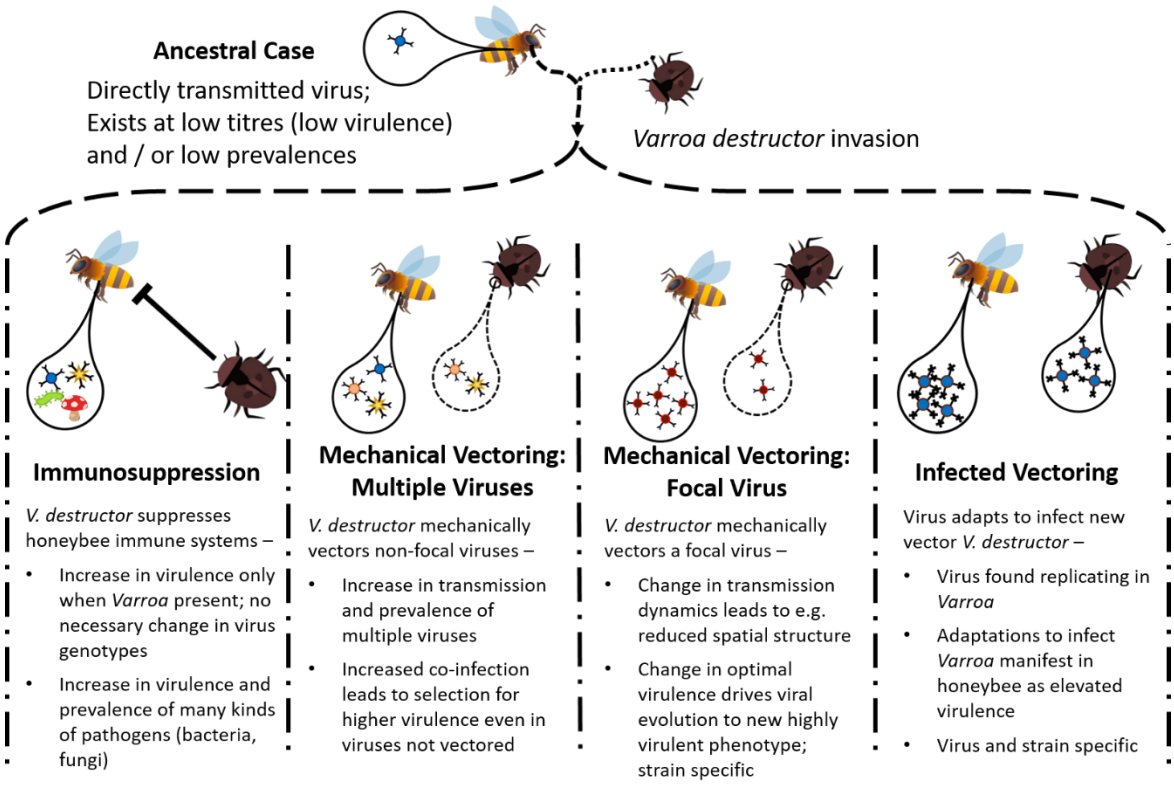


Figure 7.1 - Annotated cartoon describing four of the main hypotheses which may explain observed increases in pathogen virulence following the invasion of *Varroa*. Predicted observations attached to each hypothesis are detailed in each column. These predicted observations provide a testable framework to distinguish these different mechanisms of heightened *Varroa*-implicated virulence.

Following the invasion of *Varroa* into *A. mellifera* populations, greatly increased mortality of both individuals and colonies has been observed almost worldwide (Koch & Ritter 1991; vanEngelsdorp & Meixner 2010; Conte, Ellis & Ritter 2010). Interaction of *Varroa* with other pathogens (and viruses in particular) has long been suspected as a cause of this mortality (Chastel, Robaux, & le Goff, 1991; Martin, Hogarth, Van Breda, & Perrett, 1998; Nordström, 1999; Nordström, 2003), and is

now well studied, including the necessity for *Varroa* control to treat viral infections and the increased transmission opportunities afforded by pathogens experiencing coinfection (Rosenkranz et al. 2010; Forfert et al. 2015). However a variety of viruses have been implicated (Genersch 2010), and whether such viruses are truly vectored by *Varroa*, mechanically vectored, or activated from latent infection due to *Varroa*-driven immunosuppression (Nazzi et al., 2012; Yang & Cox-Foster, 2005), has been a topic of debate. We illustrate these various, and not mutually-exclusive, hypotheses in Figure 7.1.

We present in this review the evidence which, as a whole, indicates that: DWV has evolved to uniquely specialise in being vectored by the mite; that this specialisation to vector transmission leads to increased pathogen virulence; and that this highly-virulent specialisation in being vectored by *Varroa* has been selected for following *Varroa* mite invasion. This mostly closely corresponds to the 'Infected Vectoring' hypothesis outlined in fig. 7.1, although we stress that no hypotheses are necessarily mutually exclusive.

Assessing the Evidence Around Deformed Wing Virus Virulence Evolution

Many arthropod-vectored pathogens rely on being able to infect both their definitive host and their vector (Elliot, Sabelis, & Adler, 2004). Whilst mechanical vectoring of pathogens without infection of the vector is also possible, there may be advantages to being able to infect – and therefore amplify or persist long term – in a candidate vector. In the case of DWV and *Varroa*, there has been mixed evidence regarding whether DWV can indeed infect *Varroa*; for example compare Santillán-Galicia *et al.* (2008) and Boncristiani *et al.* (2009), where the former failed to find any evidence of immunological response to DWV in any *Varroa* tissue, whilst the latter assert they identify replicating DWV in *Varroa* by use of RT-PCR. Further evidence now suggests that some strains, including candidate recombinant strains, of DWV are capable of infecting *Varroa*. Particularly in Europe there is a recent focus on recombinant 'Varroa destructor virus -1' or 'DWV-B' strains (Ongus et al. 2004; Wang et al. 2013; Mordecai et al. 2015; McMahon et al. 2018), for which there is now cumulative and fairly strong evidence of *Varroa* infection (Campbell et al. 2016).

Initial work provided good evidence, experimental vectoring and molecular detection of DWV RNA, that DWV (or certain strains) could infect *Varroa* (Bowen-Walker et al. 1999; Yue and Genersch 2005; Gisder et al. 2009). However this has been questioned on the basis that the virus may be replicating only in the honeybee haemocytes ingested by the mite, rather than replicating within *Varroa* cells. This possibility creates a narrative where some *Varroa*-implicated DWV strains may better replicate in the mite and be preferentially vectored, without 'infecting' the vector itself in a manner traditionally studied in virology; there would in this case be no selection for competence in entering or replicating in *Varroa* cells. Still, the expectation exists that the depletion of honeybee haemocytes in the *Varroa* gut by the replicating virus is interpretable as competition for a shared 'food source' and may still carry a negative consequence for the mite; in this case DWV could be understood to be a gut parasite of the mite, and while atypical of a virus still an infection in the broader parasitological sense. Critically, further work on this topic by Campbell et al. (2016) circumvented this methodological criticism by dissecting out neural synganglia of *Varroa* mites; Campbell et al. (2016) found replicating DWV-B in these neural cells, confirming the previously evidenced hypothesis that at least some strains of DWV are indeed capable of infecting the mites. The same methodology has not yet been used however to differentiate the ability to infect *Varroa* between competing DWV strains; notably Campbell et al. (2016) cannot discern if their honeybees and *Varroa* were ever exposed to strains other than the one they found replicating in the *Varroa* neural synganglia, for example diverse DWV-A strains. Still, they crucially confirm that the well-studied DWV-B strain does infect *Varroa*.

Our ability to easily differentiate DWV strains is a recent benefit of developments in molecular genetics techniques (Tentcheva et al. 2004a; Lanzi et al. 2006; Baker and Schroeder 2008; Bradford et al. 2017), and possibly explains why disagreements existed between earlier studies on the capabilities of DWV to infect *Varroa*. It is possible only some strains of DWV are likely capable of competently replicating in the mite (either in gut haemocytes or in *Varroa* cells), and some evidence points towards these *Varroa*-implicated strains being much more effectively vectored by *Varroa* than other strains (Yue and Genersch 2005). Whilst

work is still being done in resolving the exact (likely multiple) origins and identities of these readily recombining strains (Wilfert et al. 2016; Cornman 2017), there is a history of insightful work looking at the pathology of these *Varroa*-implicated strains.

Notably, critical work by Yue & Genersch (2005) demonstrated that there are powerfully informative correlations between whether a DWV infection is *Varroa*-implicated and its clinical manifestation in *A. mellifera*. Yue & Genersch (2005) found that overt crippling of honeybee individuals exactly correlated with the presence of replicating DWV in the honeybee head (and vice-versa). Yue & Genersch (2005) also show that this specific pathology (overt crippling and replication in the head) is positively correlated with whether DWV appears able to replicate in *Varroa*. These correlations were an early demonstration of differential pathologies of DWV infections, which we can relate to whether the infection appears *Varroa*-associated or not. Further work has since corroborated these earlier findings. Gisder, Aumeier & Genersch (2009) show that mites which induce overt crippling have between two and four orders of magnitude more DWV genome equivalents per mite than those which don't induce crippling. That is, Gisder, Aumeier & Genersch (2009) confirm that differential replication in the mite is tied to how DWV infection pathology manifests in the honeybee host. Strain-specific confirmation of this phenomenon was shown by Zioni, Soroker & Chejanovsky (2011), who demonstrated that specific strains of DWV are more likely to show replication in infected honeybee heads and manifest a more severe pathology. Whilst further work has shown that covertly infected bees may also test positive for DWV in the head, the prevalence of such infection still predicts the likelihood of overt symptoms and colony-level mortality (Genersch et al. 2010). Taken as a single body, the above work shows that different DWV strains or recombinants manifest in different infection pathologies, and that those differential pathologies are correlated with apparent *Varroa* infection and vectoring.

There is abundant work linking the pathologies of DWV to its overall virulence at both individual and colony scales. Overtly infected (crippled) honeybees, characterised by DWV replication in the head (Chen, Pettis, Collins, & Feldlaufer,

2006; Yue & Genersch, 2005; Zioni et al., 2011), have significantly elevated viral titres (Brettell et al., 2017; Chen et al., 2004; de Miranda & Genersch, 2010) compared to typical covert infections. Whilst covert infection still reduces foraging and survival rates of individual honeybees (Benaets et al. 2017), overt infections are linked to near-total loss of worker honeybee performance and extremely elevated mortality rates (Brosi et al., 2017; de Miranda & Genersch, 2010). The link between these two infection pathologies and their principal transmission routes has been made before (Möckel et al. 2011), but bears further discussion in the context of potential selection on DWV.

Varroa-implicated DWV infection, due to its correlation with overt crippling and higher titres, is clearly more virulent than its ancestrally-transmitted infection counterpart. True at the individual level (de Miranda & Genersch, 2010), this elevated virulence translates to the colony level as well (McMahon et al. 2016). Given that honeybee colonies are often described as existing as a 'superorganism' including in the context of their infectious diseases (Evans and Schwarz 2011), it is important to examine pathogen virulence and transmission in honeybees at both the individual and colony level. Unsurprisingly, given the profound impacts of *Varroa*-implicated DWV on individual honeybee performance and longevity, the presence and intra-colony prevalence of *Varroa*-implicated DWV (regardless of whether overt crippling is apparent) is strongly linked to future colony mortality (Dainat & Neumann, 2013; Francis, Nielsen, & Kryger, 2013; Natsopoulou et al., 2017). The higher virulence of *Varroa*-implicated DWV infections therefore manifests at both the individual and colony level.

A critical question is if these *Varroa*-implicated DWV infections represent the adoption of a more specialised, vectored, virulent strategy by certain DWV strains, and if so, whether this appears to provide a selective advantage when the vector is present. From a predictive theory standpoint, there are clear expectations; increased virulence in and of itself is typically maladaptive, and so must be selected for in light of it being a product of competition or gains in transmission (Alizon et al., 2009). Worldwide, there is strong evidence that this new transmission route has led to a pandemic of re-emerging DWV driven by its new association with

the *Varroa* mite (Wilfert et al. 2016). The proliferation of *Varroa*-implicated DWV infections suggests that if specific strains are responsible for re-emerging DWV pandemic, the highly virulent strains are being selected for. Insightful work by McMahon *et al.* (2016) shows how across Europe, certain DWV strains are displacing others, a finding which has since been more recently corroborated (Manley, 2017). This is clearly selection in action, although *Varroa* are not yet proven to be responsible for this specific strain displacement. However, work elsewhere on more recent *Varroa* invasions yields further insights on selection on DWV.

Work by Martin *et al.* (2012) captured selection on DWV following the staggered arrival and invasion of *Varroa* mites in Hawai'i (Martin, 2010). Martin *et al.* (2012) show that prior to *Varroa*, DWV persists at low prevalences and low titres (in line with previous work on pre-*Varroa* landscapes) but despite low prevalences, a diverse variety of strains coexist. In the years following *Varroa* colonisation, DWV prevalence greatly increases, and infections are on average much more severe (higher viral titres). Simultaneously, most of the previously persisting strains are driven to extinction, such that only one highly virulent, prevalent, *Varroa*-implicated strain remains. This change from a highly-diverse DWV population to a viral landscape dominated by one strain demonstrates the incredibly strong purifying selection caused by *Varroa*. Notably however this specific *Varroa*-implicated strain was of the DWV-A type, not the well-studied DWV-B type that is found to be *Varroa*-implicated in Europe. Whilst still exemplifying the adaptive ability of DWV strains to specialise on a new *Varroa*-dominated epidemiological landscape, it presents the possibility that multiple different *Varroa*-implicated strains have independently arisen in different geographic regions. It is possible that different *Varroa*-implicated strains have different underpinning phenotypes, such as the aforementioned 'gut haemocyte replication' hypothesis compared to infection of and replication in *Varroa* cells.

Building on the observation study by Martin et al. (2012), further work showed that the shift between two states, from one where DWV is highly diverse but shows low titres and low prevalence, to one where DWV is dominated by a single strain with

high titres and high prevalence, can be readily demonstrated in laboratory experiments. Ryabov *et al.* (2014) showed that DWV strains (including those known as VDV-1 / DWV-B) readily recombine, an observation also detailed by other works looking at recombination in the field rather than the lab (Cornman, 2017; Moore et al., 2011; Wang et al., 2013; Zioni et al., 2011). Ryabov *et al.* (2014) go further to show that when DWV infects a honeybee through an ancestral (oral) transmission route, the subsequent infection is low level (low titres) but highly diverse. When *Varroa* are used to infect honeybees with DWV, the resulting infections are very severe (high titres) and dominated by a single, recombinant, *Varroa*-implicated strain. Again, this demonstrates strong purifying selection in favour of a *Varroa*-implicated strain, in this case at the scale of an individual infection rather than the colony or landscape scale.

As well as observing the competitive replacement of avirulent strains by virulent *Varroa*-implicated strains, studies have also looked for genetic evidence of selection on DWV. Cornman *et al.* (2013) present clear evidence of either a genetic bottleneck or recent strong selection on DWV in continental North America, however consistent with the results in Hawai'i this evidence for selection is amongst DWV-A type strains. More recent work (Dalmon et al. 2017) reports strong signatures of positive selection on the recombinant regions of the DWV-B genome associated with the ability to infect *Varroa*. Such evidence begins to present a clear 'smoking gun' of recent selection in multiple regions, which when coupled with the observational and experimental work previously discussed, points to the rapid, recent, and likely repeated evolution of *Varroa*-exposed DWV away from an avirulent, directly transmitted strategy to a highly virulent, vectored strategy, both in Europe and North America.

DWV isn't the only virus which has been implicated in honeybee colony losses following *Varroa* invasion, however there is currently scant evidence that any other notable viruses are capable of replicating in *Varroa*. Some evidence exists suggesting other viruses may be purely mechanically vectored by the mite (Prisco et al., 2011; Genersch & Aubert, 2010; Santillán-Galicia, Ball, Clark, & Alderson, 2010; Santillán-Galicia, Ball, Clark, & Alderson, 2014), an observation which may

also be informative for assessing hypotheses on vectoring and virulence. As outlined in fig. 7.1, these viruses may still adapt to increase their virulence in response to their altered ecology, such as reduced spatial structuring of infection or increased likelihood of coinfection, resulting in competition between viruses. In this latter case, we may expect an adaptive increase in virulence in viruses, and indeed other pathogens, even if they are not mechanically vectored by *Varroa*. Genetic evidence for or against such selection has yet to be gathered.

Across all honeybee pathogens, some influence of *Varroa* parasitism may manifest due to the immunosuppressive effect that *Varroa* mites have on their host honeybee (Shen, Yang, Cox-Foster, & Cui, 2005; Yang & Cox-Foster, 2007). This immunosuppression was at times argued as the sole reason behind DWV-driven colony loss associated with *Varroa* (Chastel et al., 1991; Genersch & Aubert, 2010; Nazzi et al., 2012; Yang & Cox-Foster, 2005), however given the extensive evidence discussed above it is now clear that virulent *Varroa*-implicated strains of DWV are vectored by the mite and have adapted accordingly, counter to the immunosuppression hypothesis (fig. 7.1). Additionally, negative correlations between DWV titres and *Nosema* infection burden run somewhat counter to immunosuppression arguments (Costa et al. 2011).

In the cases of some of these other implicated viruses, mixed evidence exists for their role in colony losses and interaction with *Varroa*. Some studies have reported *Varroa* and / or colony loss correlations with the acute bee paralysis, Israeli acute paralysis, Kashmir bee virus complex (ABPV-IAPV-KBV) (Calderón et al. 2003; Berényi et al. 2006; Berthoud et al. 2010; Genersch and Aubert 2010; Di Prisco et al. 2011), as well as sacbrood virus (SBV) (Nielsen et al. 2008), and slow bee paralysis virus (SBPV) (Carreck, Ball, & Wilson, 2002; Moore, Wilson, & Skinner, 2015; Santillán-Galicia et al., 2010). Whilst the acute paralysis viruses were initially thought to play a major role in interacting with *Varroa* to drive colony losses, that focus has since been questioned. Early modelling work predicted that DWV would be in a position to benefit from *Varroa* while ABPV would not (Martin, 2001). And while many studies found correlations with ABPV and *Varroa*, numerous others have shown no association (Budge et al., 2015; Carreck, Ball, & Martin, 2010;

Dainat, Evans, Chen, Gauthier, & Neumann, 2012; Highfield et al., 2009; Martin et al., 1998). Very different viral pathologies make it clear that viruses such as SBV and SBPV are unlikely to behave in a similar manner to DWV (Santillán-Galicia et al. 2014; Ryabov et al. 2016), both of which are already highly virulent and will rapidly kill a colony (and consequently the *Varroa* in that colony) at high prevalences; currently the evidence for an association between SBV and *Varroa* remains mixed.

Across the literature cited above, the only virus which appears to be consistently associated with *Varroa* infestation and colony loss is DWV (Dainat and Neumann 2013; Kielmanowicz et al. 2015; McMahon et al. 2018). As such, evidence points towards DWV being the only honeybee virus to have made a switch from direct transmission to specialisation in vectored infection via replication in *Varroa*. Other cases of viruses associating with *Varroa* may instead be driven by the mite's immunosuppressive nature, and/or its incidental mechanical vectoring of virus particles (fig. 7.1) or simply a symptom of poor colony health brought about by *Varroa* and DWV. Currently, no compelling evidence exists for other honeybee viruses being able to infect *Varroa*, or similarly to have undergone selection for higher virulence; for example, no such studies are featured in the recent review by McMahon *et al.* (2018). This may however be a simple absence of evidence, with much work currently undertaken on DWV to the exclusion of many other honeybee viruses. Future examination of whether and why these viruses have seemingly not exploited a potential vector may prove informative for better understanding evolutionary predispositions of viruses to adopting a vectored strategy.

One particularly insightful study differentiating DWV and its interaction with *Varroa* from other, potentially associated viruses was undertaken by Mondet *et al.* (2014). The key strength of Mondet *et al.* (2014) is the length of time for which detailed sampling and observation was undertaken. This 12-year study shows, in agreement with shorter-term studies, that following *Varroa* invasion in New Zealand, increasing *Varroa* populations correlate with increasing prevalence and titres of multiple viruses, including DWV, KBV (an acute paralysis virus (de Miranda, Cordoni, & Budge, 2010; Genersch & Aubert, 2010)), SBV, and BQCV.

However, as *Varroa* populations slightly fall towards a stable equilibrium, all viruses apart from DWV also decrease in prevalence and titre. Critically, the length of this study shows that once the initial invasion of *Varroa* has equilibrated, the only virus which shows a long-term change in prevalence following *Varroa* invasion is DWV. This data runs counter to the predictions we would expect from immunosuppression by the mite or mechanical vectoring of multiple viruses (fig. 7.1). Mondet *et al.* (2014) observe that DWV is also the only virus more prevalent in *Varroa* mites than honeybees, which is also suggestive of the unique ability of DWV to adapt to replicate in *Varroa* mites. Indeed, the authors of the study speculate that the decrease in *Varroa* populations after their initial invasion is driven by *Varroa*-implicated DWV controlling the mite population (but see evidence discussing the potential mutualism of DWV and *Varroa* (Prisco et al. 2016)). Taken alongside the previously discussed literature on virus-*Varroa* associations, evidence here too definitively indicates DWV is unique in its newly-acquired specialisation of being vectored by *Varroa* mites and begins to challenge some of the hypotheses around immunosuppression or mechanical vectoring of multiple viruses.

The overall case presented in this review can be further assessed by examining other pieces of circumstantial evidence. For example, following the invasion of *Varroa* into many honeybee populations, reports of outbreaks of overt crippling can occasionally be observed in close-by, but *Varroa*-free bee populations. For example, a case in north Sweden (Forsgren et al. 2012) is anecdotal, but may represent an occurrence of a highly virulent *Varroa*-implicated strain being imported into a *Varroa*-free honeybee population, causing a severe epidemic, but then being unable to persist due to its maladaptively high virulence in the absence of its vector. If this is the case, it again definitively supports an adaptation by DWV due to either mechanical or infected vectoring (fig. 7.1). Similar observations of overt crippling of non-*Apis* bees have also recently been documented, including in bumblebee species (Genersch et al. 2006) and carpenter bees (Lucia et al. 2014). Again, overt crippling events such as these were not reported prior to *Varroa* arrival into honeybee populations, although awareness of DWV creates an obvious sampling bias. Still, the extensive and growing body of work demonstrating that

honeybee viruses spill over into wild pollinators (Choi et al. 2010; Singh et al. 2010; Li et al. 2011; Zhang et al. 2012; Reynaldi et al. 2013; Mazzei et al. 2014; Forsgren et al. 2015; Guzman-Novoa et al. 2015; Cohen et al. 2017; Forzan et al. 2017; Santamaria et al. 2017) further underlines the threat of selection for more prevalent and highly virulent DWV strains to repeatedly cause outbreaks in already embattled native pollinator species (Manley, Boots, & Wilfert, 2015; McMahon et al., 2018; Potts et al., 2010, 2016).

Future Tests

Open questions remain as to how these emerging high virulence *Varroa*-implicated strains will behave in less studied *Apis* populations (Chantawannakul et al. 2016). *Varroa* mites were ancestrally parasitic on *A. cerana*, where they cause minimal colony-level virulence by parasitizing only drones (male honeybees) (Rath 1999; Brosi et al. 2017). As such, *Varroa* and DWV both are present in *A. cerana*, but aren't known to manifest clinical symptoms (Sumpter and Martin 2004; Fries 2011; Yañez et al. 2015). The highly-virulent forms of DWV seemingly emerging in *A. mellifera* populations could plausibly spill over into *A. cerana* and cause overt but unsustainable epidemics, or could potentially lead to sustained circulation in *A. cerana* by exploiting drones as a reservoir from which to directly infect workers. Whether this is observed could be an insightful opportunity for better understanding how virulence and vectoring relates to a pathogen's likelihood of spill-over into a closely related species. Similarly, many of the landraces of *Apis mellifera* which are found outside of Europe and the Americas show potential for resistance to DWV, *Varroa*, or both (Locke et al. 2014; Toufailia et al. 2014). Differential vulnerability to the vector, or higher immune competence leading to differential virulence of the pathogen, may also provide insightful research avenues from which evolutionary virology can benefit. Clear candidates include 'Russian bees' (Khongphinitbunjong et al. 2015, 2016), as well as various generally understudied African landraces (Hamiduzzaman et al. 2015; Adjlane et al. 2016) that have already yielded important insights on this system such as molecular tools for a variety of honeybee viruses (Davison et al. 2003).

Recent studies on the topic of *Varroa* and DWV also provide future opportunities to test and better understand the case presented in this review. One opportune case is that documented by Mattos, Jong & Soares (2016), who detail an island population of managed honeybees exhibiting extensive *Varroa* parasitism without any associated colony mortality. Brettell & Martin (2017) followed up on this observation to propose that the isolated population lacks the required DWV diversity necessary for *Varroa*-implicated strains to occur and be selected for. In the absence of a *Varroa*-implicated (vector-suitable) DWV strain, *Varroa* populations remain high (possibly unconstrained the virus) but without any associated colony mortality. Brettell & Martin (2017) describe this as a 'ticking time-bomb', and the eventual arrival or emergence of such strains provides another clear test of the case presented in this review. Alternatively, the population of *Varroa* on this island may be a different genotype to the globally distributed *Varroa* and poor vectors of DWV, yielding a potential candidate for DWV-resistant *Varroa*, which may again provide evidence for whether resistance centres around competition for consumed bee haemocytes or resistance on the part of the *Varroa* mite to direct infection. In this case, it is possible these *Varroa* do not immunosuppress their host bees (fig. 7.1), however this island-genotype hypothesis aligns more closely with 'infected vectoring' than any mechanical vectoring hypotheses (fig. 7.1).

At a larger scale, Ryabov *et al.* (2017) have recently documented the emergence in the United States of the well-studied European *Varroa*-implicated DWV strain (DWV-B). As the US beekeeping industry is under close scientific scrutiny (Pettis and Delaplane 2010) there are clear opportunities to explicitly test hypotheses concerning this system. For example, whether there are minimum vector population sizes required for the European *Varroa*-implicated strain to invade into honeybee populations, or whether the arrival of *Varroa*-implicated strains will lead to a reduction in the *Varroa* population as speculated by Mondet *et al.* (2014). Alternatively, there may have already been adaptation within the standing DWV-A population in the U.S., filling the *Varroa*-implicated niche, as appears to have happened in Hawai'i (Martin et al., 2012), in which case DWV-B will not necessarily displace 'native' strains. The samples or data necessary to answer such pertinent

questions likely already exist in laboratories across the U.S. Another study opportunity is that of Australia, which remains as one of the few last bastions of *Varroa*-free *A. mellifera* in the world (Iwasaki et al. 2015).

One critical insight into vectored-pathogen virulence which this system may also still yield differentiates whether the apparently large increase in *Varroa*-implicated DWV virulence is an indication of a large shift in the optimum virulence of the pathogen (Alizon, Hurford, Mideo, & Van Baalen, 2009; Levin & Pimentel, 1981; May & Anderson, 1983; Read, 1994) or whether this high virulence represents a new evolutionary constraint imposed by the biological basis of infecting the *Varroa*. In the case of the former, higher virulence has rapidly evolved to a new optimum value due to change in transmission opportunities afforded by *Varroa*, via either mechanical or infected vectoring (fig. 7.1). This *Varroa*-induced change in DWV transmissibility reflects a variety of different biological processes, for example assisted ability to spread between colonies (Forfert *et al.* 2015, Brosi *et al.* 2017) reducing spatial structuring. If this is the case, then experimental manipulation of transmission opportunity, or observation of different honeybee management regimes with different transmission opportunities, should yield corresponding changes in DWV virulence. This contrasts with the alternative hypothesis, related only to infected vectoring (fig. 7.1), that the ability to infect *Varroa* comes at the cost of greatly elevated virulence but is still a competitive phenotype by benefitting from a superior transmission route. In this case, it may be that the current virulence observed in honeybees of *Varroa*-adaptive strains is higher than the hypothetical optimum, and that if a strain could be equally replication-competent in *Varroa* but replicate to much lower titres in the honeybee, it would be evolutionarily favourable. In the case of this hypothesis, the current *Varroa*-implicated strains have hit a ‘glass floor’, where the advantageous molecular adaptation through which they can infect *Varroa* causes greatly elevated, disadvantageous virulence in the honeybee. In this case, the previously proposed experiments or observational studies of different transmission potential for the virus would not yield corresponding changes in DWV virulence, as the changes in optimum replication in the honeybee are irrelevant when the virus is already biologically constrained to a phenotypically distant, higher than optimal replication rate in the honeybee.

Discerning between these two hypotheses is therefore empirically viable and would potentially align with the concepts first introduced in this review. For example, the first hypothesis of changing optimum virulence, afforded by greater ease of transmission, reflects the initial arguments first laid out by Ewald and others (Bull, 1994; Ewald, 1996; Ewald, 1993) which have since between shown to be theoretically unlikely (Day 2002; Cressler et al. 2016). However, arguments around reduced spatial structure do remain theoretically sound (Boots and Sasaki 1999). The second hypothesis, that the high virulence exhibited by the vectored pathogen is far higher than a hypothetical optimum, and reflects instead a biological constraint, aligns with work examining the balancing act of virulence between definitive host and vector (Elliot et al., 2004; Elliot et al., 2003; Gandon, 2004; Gower et al., 2004; Rigaud et al., 2010), and would provide great insight into this virulence-balancing phenomenon.

Summary

Overall, the substantial research investment undertaken to understand honeybee losses to infectious diseases offers not only applied solutions for the system (Sumpter and Martin 2004; Neumann et al. 2012) but a multitude of opportunities for studying the ecology and evolution of infectious diseases (Chantawannakul & Cutler, 2010; Chen et al., 2006; Möckel et al., 2011). This review details one of those opportunities: a demonstration of the consequences of a pathogen evolving to exploit an indirect, vectored transmission route from an ancestral state of direct transmission, and in doing so manifesting as profoundly more virulent. This phenomenon, captured by honeybee researchers across the last three decades, provides in our view the best evidence we have to date supporting the hypothesis that transmission via vectors can lead to, or select for, higher virulence. More specifically, the system provides opportunities to test the mechanistic underpinning of this observation; either that adapting to infect a second host (in this case the *Varroa* mite) comes at the cost of above-optimum virulence in the definitive host leading to an evolutionary constraint, or that the large change in transmission leads to a higher optimum virulence which may respond to further changes in transmission likelihood.

As research into this system continues it may be possible to better measure the virulence manifested by *Varroa*-implicated strains in the mite, providing avenues to investigate how vectored pathogens balance virulence between their vector and definitive host. Should such research endeavours prove fruitful, and given the recent developments in DWV research such as characterisation of its surface molecular structure (Škubník et al. 2017), the *Varroa*-DWV-honeybee system stands to become a uniquely valuable asset for the ecology and evolution of infectious disease. Similarly, further work into the potential mechanical vectoring of a suite of honeybee viruses may provide their own critical insights, both independently and as comparisons to the candidate adaptations shown by DWV. Understanding the evolutionary drivers underpinning the wide virulence range observed across infectious diseases remains a key research goal with the ultimate aim of virulence management. Systems, such as DWV, that have undergone natural experiments can, alongside theory, help us to determine the general principles that determine virulence in nature. In this case, it provides excellent evidence that vectors can select for highly virulent diseases.

Chapter 8 – Identifying regions of risk to honeybees from Zika vector control in the U.S.

Abstract

Managed honeybees are a crucial component of many countries' agricultural systems. Critically, it is now well established that honeybees are declining in the face of multiple threats, and therefore, it is important that we determine and mitigate new threats. The emergence of Zika virus has introduced the new threat of insecticidal mosquito control leading to honeybee losses, with demand from beekeepers for a comprehensive risk assessment to help mitigate losses. Here, we present novel estimates of county-level honeybee colony densities across the United States, and combine this new data with different projections of Zika virus suitability to assess the magnitude of this risk. We find that up to 13% of colonies can reasonably be expected to experience elevated risk of damaging pesticide exposure, according to interpretation of current Zika virus projections. We show a significant positive correlation between areas of Zika suitability and honeybee colony density. Increased risk of colony loss to pesticides are found in the South-East, Gulf Coast, Florida, and the California Central Valley. We highlight certain states which are better placed to mitigate threats, recommending other states look towards these schemes to protect apiculture from both government and commercial pesticide application.

Introduction

Threats to pollinators are of serious and growing concern for global agricultural systems (Potts et al. 2016) which rely on robust and diverse pollinator assemblages to provide pollination services (Hoehn et al. 2008; Garibaldi et al. 2013), especially in the context of global change (Klein et al. 2007; Brittain et al. 2013a; Rader et al. 2013). Pollination services are important for both fruit quantity (Garibaldi et al. 2013) and quality (Knapp et al. 2017). However a small subset of bee species are the majority providers of these necessary pollination services (Kleijn et al. 2015), with honeybees frequently and successfully employed as supplementary pollinators (Rader et al. 2012). In the United Sates (U.S.), managed

pollinating bees (principally *Apis mellifera)* are estimated to be worth $15bn, principally due to the demand for managed honeybee colonies to provide temporary pollination services (Levin 1983; Calderone 2012). However both wild (Potts et al. 2010; Koh et al. 2016) and managed (vanEngelsdorp and Meixner 2010) pollinating bees are declining in the U.S., leading to large increases in costs of honeybee colony rental for farmers (Burgett et al. 2010). There is a complex set of ecological literature on the variety of drivers behind these declines including disease, landscape change, and pesticide exposure (Potts et al. 2010; Becher et al. 2013; Sánchez-Bayo et al. 2016). Although most insecticide exposure to managed honeybees is experienced via crop treatments (Johnson et al. 2010), an important route of exposure is insecticidal spraying to control nuisance or disease vector insects – especially mosquitos (Harriott 2016).

Emphasis on mosquito control in the U.S. has increased rapidly in response to the recent emergence and rapid expansion of Zika viral disease (Schmidt 2016). Whilst mosquito controls are already exercised for viruses such as West-Nile or Dengue (Rose 2001; Petersen and Hayes 2008; Hadler et al. 2015), public concern has traditionally been low in the U.S. (Ho et al. 2007), with mosquitos viewed as a nuisance rather than a disease vector (Dickinson and Paskewitz 2012). However, the association of Zika with severe birth defects (Mlakar et al. 2016) has led to major public health concerns (Fauci and Morens 2016; Gulland 2016; Petersen et al. 2016) and widespread media attention, for example the extreme concern surrounding the 2016 Olympics in Brazil (Codeco et al. 2016). Consequently, mosquito control measures may increase at both the local and regional level in response to the emergence of Zika virus, driven by both official public health measures and private contracting. This poses a risk to U.S. apiculture and native pollinator health (Harriott 2016).

The proximate risk to apiculture comes from adulticidal mosquito controls (Schmidt 2016); however, the severity of the threat depends on control approach. Control may be decided upon as in the public benefit at the county or state level, but may also be undertaken on a private basis by home or land owners soliciting commercial mosquito control. In the case of county or state controls, the Centers

for Disease Control and Prevention (CDC) first-line recommendation for adulticidal spraying is the organophosphate Naled (Dimethyl-1,2-dibromo-2,2-dichlorethyl phosphate) (CDC 2016a), applied as an 'ultra-low volume' spray (Mount et al. 1996; Breidenbaugh and Szalay 2010); however, the effectiveness of Naled spraying in controlling *Aedes* has been called into question following recent mixed evidence (Bouzid et al. 2016). Various studies have shown low risk quotients (Davis et al. 2007), and a generally low impact on most terrestrial non-target species (Schleier and Peterson 2010) and invertebrate biodiversity (Breidenbaugh and Szalay 2010), partly due to its rapid degradation (Schleier and Peterson 2009). However an acute risk remains for some insects exposed to Naled during its application (Hoang et al. 2011), with high mortality in exposed honeybees leading to demonstrable impacts on colonies (Zhong et al. 2004). Furthermore, there is evidence that bees are comparatively more sensitive to stressors such as pesticides compared to other insects (Claudianos et al. 2006; Klein et al. 2017).

Additionally, the CDC lists a variety of alternative insecticides, some of which operate as residual sprays (CDC 2016b). A number of these control agents have been demonstrated to have negative impacts on individual honeybees or honeybee colony performance at sub-lethal levels (Desneux et al. 2007), including the CDC-listed residual sprays imidacloprid, deltamethrin, bifenthrin, and lambda-cyhalothrin (Decourtye et al. 2004; Dai et al. 2010; Ingram et al. 2015; Dolezal et al. 2016). While night-time spraying or notification of beekeepers to allow the covering of colonies can prevent exposure to rapidly degradable insecticides (Harriott 2016), exposure to residual sprays is much harder to prevent due to their permanence in the landscape (Sánchez-Bayo and Goka 2014). Other mosquito control approaches such as source reduction, biocontrol, larvicides (for example, hormone mimics), and the use of toxins from *Bacillus thuringiensis israelensis* are all viable in reducing mosquito prevalence (Floore 2006) and do not threaten honeybees. Where adulticidal spraying is mandated, aerial, backpack, or truck-mounted may all be employed based on the scale of the operation. Of these, aerial spraying is of the greatest threat to honeybees, and is routinely employed as a prevalent method of pesticide delivery (Matthews 2011). Honeybee colony losses in response to

county-mandated Zika control measures using aerial spraying have already been confirmed (Clemson University 2016).

There is a lack of information on the prevalence of, and mitigation techniques employed by, commercial pesticide services when solicited by private home- or land- owners. Minimum standards require that all applicators follow pesticide labels, but no further legal requirements exist at the federal level. States issue pesticide applicator licenses for private or commercial mosquito control, but information on what pesticides or approaches are used by commercial agents is difficult to obtain. Additionally, mitigation methods used by commercial control agents are likely very variable and another unknown. Night-time spraying may be much less likely, and information on local apiaries is not immediately accessible to private operators. Understanding the role of commercial mosquito control may be critical in protecting beekeeper's livelihoods – with beekeeping organisations already expressing concern over colony losses to commercial mosquito control operations (MABA 2016). As efforts to verify and quantify these reports are undertaken, understanding of the prevalence of honeybee colony loss due to privately contracted mosquito control activities will improve. Until then, as we lack information on the prevalence, timing, and target areas of much of the spraying undertaken in the U.S., a formal risk assessment remains outside this paper's scope.

Instead, this paper seeks to establish the more basic question of where and at what magnitudes Zika responsive mosquito control poses a risk to apiculture, and by extension pollination service provision and therefore wider agriculture. The threat of emerging agricultural diseases is recognised (Anderson et al. 2004; Fisher et al. 2012), however the potential indirect impact of human disease on agriculture seems unaddressed. Our identification of areas of high Zika suitability coinciding with high levels of apicultural activity will help mitigate potential conflict between apiculture and Zika control. In doing so, we hope to inform beekeepers, commercial mosquito control agents, and county or regional officials in finding the most responsible approaches to vector control whilst minimising damage to apiculture.

Materials and Methods

ZIKA SUITABILITY

For prediction of where Zika is likely to be a problem in the U.S., we used published projections from independent predictions of where Zika may endogenously transmit. These published projections of Zika virus extent employ ecological niche modelling approaches, an approach to mapping disease transmission risk that has recently become highly popular in disease ecology. While some diseases are globally cosmopolitan, most vector-borne diseases do not occupy the entire range of their vectors, and ecological niche models have been increasingly employed to make these geographic delineations. We examine three separate projections generated from independent studies which sought to predict Zika-suitable regions using Zika-specific ecological niche models (others adapting dengue or *Aedes* data have been omitted here): Carlson et al. (2016), Messina et al. (2016), and Samy et al. (2016); hereafter we refer to these projections by the name of the first author ('Carlson', 'Messina', 'Samy' respectively). The differences between these studies are subtle and often technical, but the disagreement between their results is in some regions profound (for a detailed analysis of their disagreement, see Carlson, Dougherty, Boots, Getz, & Ryan, (2018)), with Carlson (the most restrictive) suggesting Zika will be confined to the southernmost tip of Florida and highly limited areas of Los Angeles county while Samy suggests isolated outbreaks of Zika are possible throughout the entire U.S. For each projection, we projected U.S. counties onto mapped suitability, and calculated the proportions of county areas which a given projection predicts to be suitable for Zika. These values are detailed in Supplementary Material 5, and presented as choropleth maps in fig. 8.2.

HONEYBEE COLONY NUMBERS

In order to approximate the commercial honeybee colony numbers for each county across the U.S. we use the most recently available 2012 Agricultural Census data (USDA - NASS 2012a). This census data presents the number of honeybee farms (hereafter referred to as 'beekeeping operations') and number of honeybee colonies for most counties in the U.S. For some counties, no information on the

number of beekeeping operations and honeybee colonies was available (Supplementary Material 5) due to no active beekeeping operations voluntary contributing to the census. We expect the numbers of commercial colonies in these counties to be small, as the total number of colonies captured in the census is in line with the total number of recognised colonies in the U.S.

Some counties had the number of colonies withheld to protect the commercial interest of beekeepers which could be identified (USDA - NASS 2012a). A bootstrapping approach was used to estimate the number of colonies present in these counties. For each state, we generated a distribution of the mean number of colonies per beekeeping operation from counties with known colony counts. In addition, we calculated the total number of unaccounted for colonies in the state by comparing the given state total against the total number of colonies in counties with known counts. We then sampled this state-wide distribution for all beekeeping operations with unknown numbers of colonies, and scaled the sampled numbers to match the number of unaccounted for colonies. This was repeated 10,000 times to obtain mean and standard deviation estimates of colony numbers in these counties. Once bootstrapping had been completed for all counties with withheld numbers of colonies, we scaled our total number of colonies in the U.S. to match the published number of bee colonies as of January 2016 (USDA - NASS 2017a), which includes all operations of five or more colonies as defined by the USDA. We assume the distribution of these commercial colonies across the U.S. is represented by the estimates we generate from the 2012 census. Estimates of commercial colony numbers for each U.S. county are found in Supplementary Material 5.

ASSESSING RISK TO HONEYBEES

We examined the risk of honeybee colonies being exposed to Zika prevention measures through choropleth maps (using ‘choroplethr’ - (Lamstein and Johnson 2015)) and U.S.-wide summaries. We multiplied the density of colonies in each county by the proportion of the county area predicted to be suitable for Zika in each projection. This approach yielded density-based maps of where and total estimates of how many colonies are likely to be at risk from preventative Zika measures,

according to each projection. Additionally, we assembled a county-by-county table detailing estimated number and density of colonies, and proportion of county area suitable for Zika according to each projection (Supplementary Material 5).

Finally, we examined whether there was any correlation between where a given projection predicted high Zika suitability and where high densities of honeybees could be found. We used non-parametric tests for correlation (Spearman's rank) to establish whether, amongst counties with a non-zero area of Zika suitability, counties with larger or smaller proportions of their area predicted as Zika suitable were also more likely to have higher or lower densities of honeybee colonies. Non-parametric testing was necessary due to the highly irregular distributions of the data, which rendered any parametric testing unsuitable.

Results

Our estimates of colony density across the U.S. revealed considerable variation, even between neighbouring counties, with cross-continental densities spanning two orders of magnitude (fig. 8.1). Notable regions of extremely high colony density included the Central Valley of California, Florida, and the Dakotas (fig. 8.1), with expansive areas of moderately high colony density across much of the eastern United States. This pattern is in line with traditional rhetoric on where the major beekeeping regions of the U.S. are (Caron and Connor 2013). The conspicuous band of counties with no information reflects counties where no beekeepers replied to the census, and very clearly matches a notable north-sound band of the U.S. with extremely low population density and high rates of population emigration (US Census Bureau 2015), suggesting that indeed very few beekeeping operations exist in these counties.

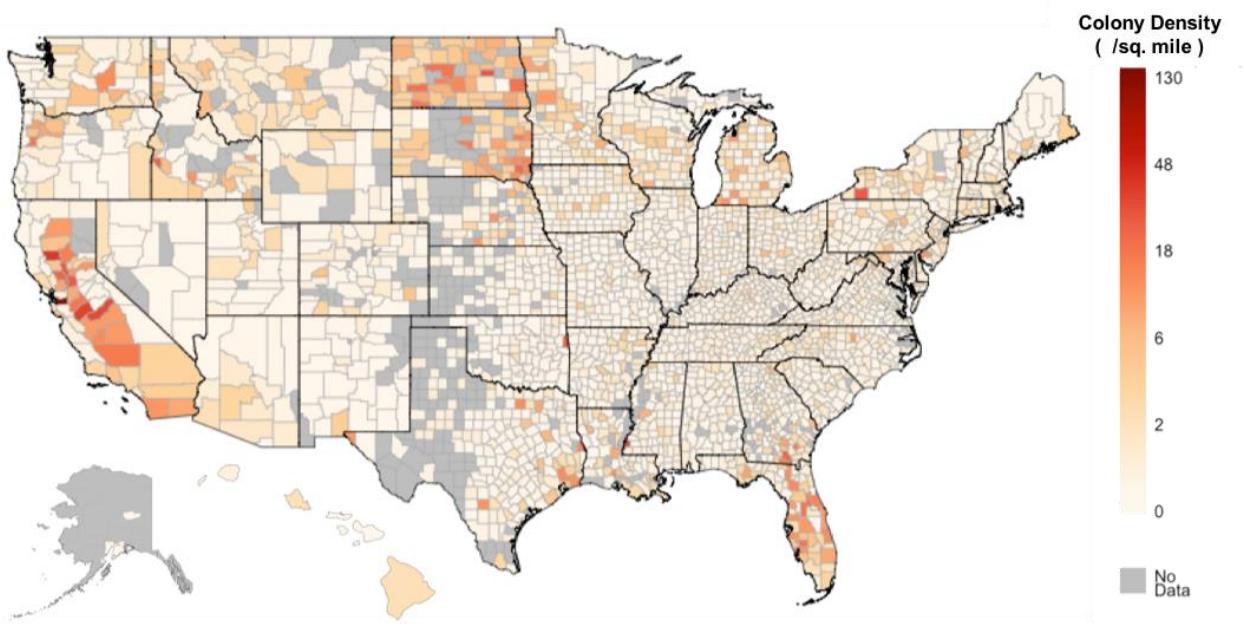


Figure 8.1 - Choropleth map of estimated colony density for beekeeping operations with 5 or more hives. Calculated using data taken from USDA Agricultural Census (USDA – NASS, 2012), with withheld values estimated by bootstrapping.

We found some significant correlations of county area suitability for Zika and density of honeybee colonies. Due to the large number of counties with no projected Zika suitability, we limited this rank correlation analysis to include only counties with some area predicted suitable for Zika. For each projection, we assessed whether counties with higher proportional areas of predicted Zika suitability also had higher honeybee colony densities. We found no correlation for the Carlson projection ($\rho = -0.316$, $p = 0.216$), which may be limited by the low number of counties included. However significant correlations were found for the Messina ($\rho = 0.138$, $p = 0.004$), Samy ($\rho = 0.168$, $p < 0.001$) projections.

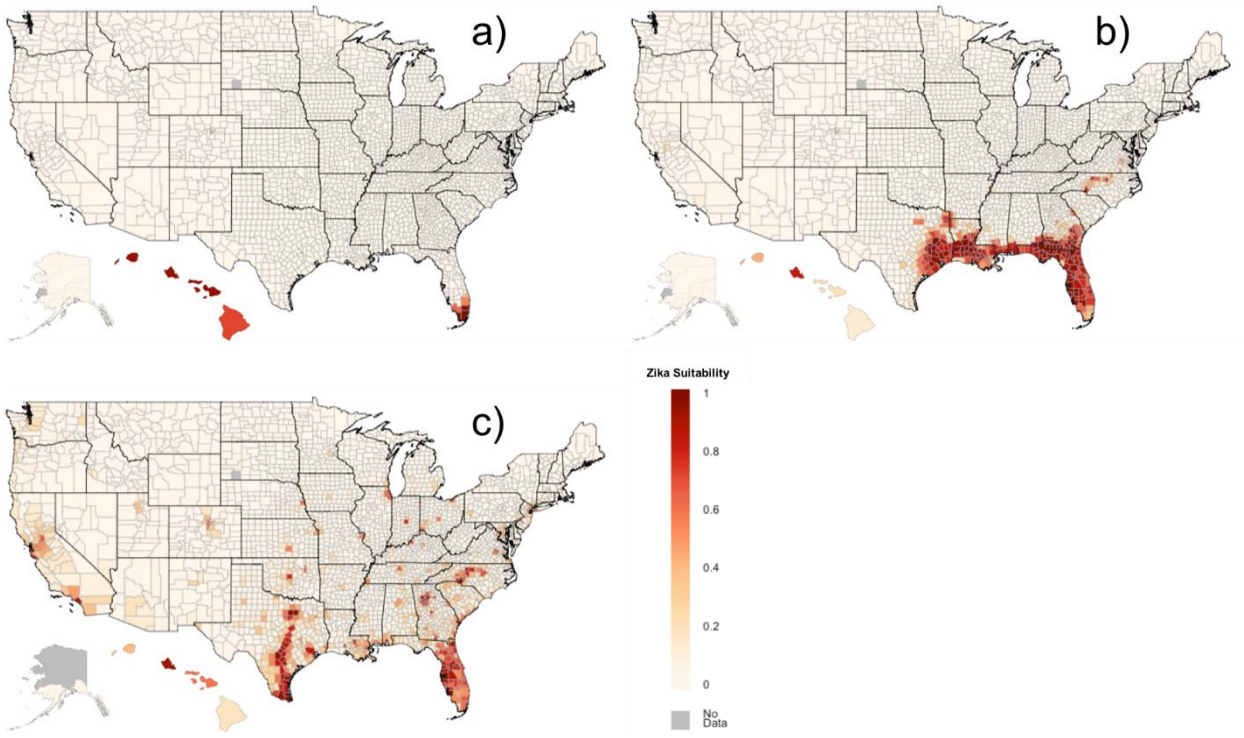


Figure 8.2 - Choropleth maps showing proportion of County area suitable for Zika across the United States. a) shows data from Carlson's projection, b) shows data from Messina's projection, c) shows data from Samy's projection.

Our risk maps (fig. 8.3) quantitatively account for the area of a county suitable for Zika, and colony density, and show large difference between projections as would be expected (see fig. 8.2 and Carlson et al., (2018)). Carlson's projection shows the least worrying projection. Overall, we expect less than 1% of colonies will coincide with autochthonous Zika transmission according to Carlson's datatset (Table 8.1). The Messina projection shows a pattern of coincidence with colonies now confined principally to Florida, South Georgia, and the Gulf Coast (fig. 8.3b). However, the high density of colonies in these regions combined with expansive areas of Zika suitability leads to an expectation that around 9.4% of colonies in the U.S. may coincide with established Zika, over ten-times the estimate from the Carlson projection (Table 8.1). For the Samy projection (fig. 8.3c) the Californian Central Valley appears as a large area of high colony density and extensive Zika suitability, coupled with Florida and parts of the Gulf Coast. Under the Samy

projection, 13% of colonies in the U.S. may be expected to coincide with potential for Zika outbreaks (Table 8.1).

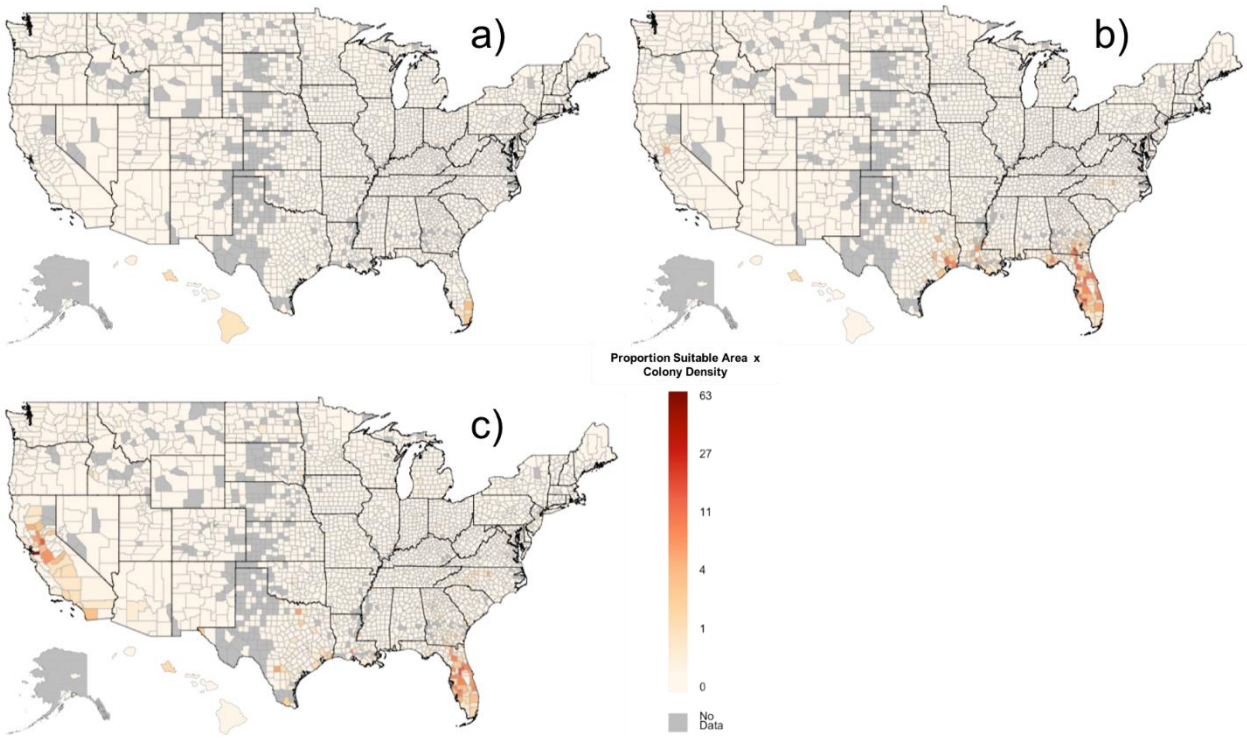


Figure 8.3 - Choropleth maps of estimated colony density for beekeeping operations with 5 or more colonies across the United States multiplied by proportion of county area suitable for Zika. a) uses suitabilities from Carlson's projection, b) uses suitabilities from Messina's projection, c) uses suitabilities from Samy's projection.

The data underpinning the choropleth maps can be examined in full detail in Supplementary Material 5, and should be consulted for information about individual counties.

Table 8.1 - Percent of colonies in the United States estimated to geographically coincide with Zika preventative or responsive measures under different published projections. Uncertainties are standard errors associated from the bootstrapping used when gauging honeybee colony densities.

Zika Suitability Projection	Percent of colonies in U.S. Geographically Coinciding with Response
Carlson	0.75 ± 0.01
Messina	9.44 ± 0.30
Samy	13.03 ± 0.20

Discussion

Our analysis shows the potential for regional exposure of honeybee colonies to mosquito-controlling insecticides, as well as scope for mitigating this threat. The risk of exposure could be most pronounced in areas of the U.S. where agriculture heavily relies on pollination services for good yields and profitable farming, potentially exacerbating the overall risk posed to apiculture. However, the degree of uncertainty between our analyses illustrates a difficult challenge for officials to navigate. The magnitude of differences between projections, and therefore uncertainty in the numbers of colonies which may be exposed to insecticidal spraying, is a problem which must be addressed if responsible Zika control is to be achieved. Additionally, the challenges of interpreting these projection differences is as profound if our analysis is to be extended to native pollinators.

Should spraying be confined only to the specific areas within counties which are considered Zika suitable, differences in colony exposure between published projections span over an order of magnitude (Table 8.1), reflected by the regions identified as potential hotspots of colony losses in the choropleth maps (fig. 8.3). For the Messina and Samy projections, proportions of colonies at risk across the U.S. are of magnitudes relevant to or comparable with summer losses of colonies

across the entire U.S. (Steinhauer et al. 2014). Whilst it is difficult to say what the proportional loss or reduction in productivity of colonies would be following spraying, pesticide kills are cited as the 3rd most common cause of colony loss by American beekeepers (Bee Informed Partnership (Connell et al. 2012), accessed May 2017). This magnitude of exposure highlights that the potential economic loss to agriculture discussed here is of industry relevance even if only very targeted spraying is carried out.

We therefore stress our capability to reduce impact on colonies to insignificant levels through thorough warning procedures, and through mosquito control approaches which do not pose an acute threat to honeybees. As previously discussed, the principal routes of impact on honeybees from Zika control are from adulticidal approaches. Non-residual 'space sprays' (CDC 2016b) are only a threat to adult honeybees outside the hive, and are therefore preferably applied at night time (when no bees are outside the hive), eliminating risk of exposure. The greatest threat occurs during day time hot weather when most adult bees are 'bearding' outside the entrance to their hive in order to thermoregulate (Caron and Connor 2013). Notably, warm weather in much the U.S. aligns with when mosquito vectors are most abundant. Notifying beekeepers in such conditions (day time spraying in warm weather), and reducing space spray drift, is critical in preventing further losses. In the case of residual sprays, permanence in the landscape inevitably means some exposure to honeybees, as pesticides will be brought into the hives in both flower pollen and nectar, where honeybee larvae are then exposed (Rumkee et al. 2017). In these cases, reducing the quantities of pesticide used is paramount. In both of these instances (reducing pesticide volumes and reducing drift during application) ensuring the use of effective modern application technologies is of great benefit (Matthews and Thomas 2000; Matthews and Hamey 2003; Matthews 2008)

It is immediately apparent from our choropleth maps (fig. 8.2) that there is currently limited consensus on where in the U.S. we might expect areas of autochthanous Zika transmission (Carlson et al. 2018). This, in part, reflects the different approaches used by these studies, and the challenges posed by a recently

emerging infectious disease. For example, Carlson uses exclusively Old World Zika occurrence across the last half century (Carlson et al. 2016) so as to avoid the confound of the outbreaks coinciding with an El Niño year (Paz and Semenza 2016). This, however, presents a much more limited projection and may miss ecological differences between Old World and New World strains. Messina does include the much more expansive New World projection, but parameterises much of their ecological niche model with assumptions taken from knowledge of dengue virus (Messina et al. 2016). Samy differs from both by including socioeconomic factors as a semi-separate driver of autochthonous Zika transmission, additional to environmental suitability (Samy et al. 2016). This understandably leads to a much more expansive Zika range and highlights areas of high population density, which if not interpreted correctly could incite alarm. These critical but nuanced differences between projections are impractical for consideration by most officials, but as our analysis demonstrates, could lead to very different economic impacts as Zika responses mount.

We believe that some of the differences between projections may however be useful in assessing the more challenging question of where mosquito control poses most threat to honeybee colonies. For example, in the case of colonies being lost to spraying in Dorchester County, South Carolina in September 2016 (Clemson University 2016), our county-level data shows that two of the three projections predict no areas of suitability for Zika in this region, with the third projection (Samy) showing about 20% of the county area is classified as suitable. This case of seemingly low Zika suitability demonstrates how many counties across the U.S. may be sites of future spraying.

The Samy projection's inclusion of socioeconomic factors – including population density – is a likely driver of the strong statistical correlation between Zika suitability and colony density for this projection (Carlson et al. 2018). Honeybee colonies are unsurprisingly associated with higher population densities (fig. 8.1), and it is likely that many of the colonies missed by this analysis (operations with < 5 colonies) are found in urban or sub-urban areas. Pressure on officials to take action against Zika will be influenced by population densities and likelihood of

travel cases – and therefore could be considered partly accounted for in the Samy projection, regardless of the veracity of its autochthonous Zika transmission predictions. Additionally, solicitation of commercial mosquito control agents will pose threat to honeybee colonies only where homes are close enough together for substantial pesticide drift or exposure to foragers – again tightly aligned with population density. We therefore take the opinion that combining our colony density map with the Samy projection may inherently capture additional factors contributing to the likelihood of conflict between Zika abatement and managed honeybees.

The correlative association between apparent Zika suitability and honeybee colony density is cause for concern. Whilst this correlation is not apparent for the limited area of the Carlson projection, for the Messina and Samy projections, counties with more Zika suitable area have higher densities of colonies. The potential population-density driver behind this for the Samy projection is discussed above. However in the case of the Messina projection, Zika suitability is evaluated on purely environmental (principally climatic) grounds. The significant correlation in this case supports a hypothesis that, at smaller scales, environmental conditions which attract high colony densities (areas which are good for beekeeping) are also environmental conditions associated with supporting Zika transmission. Speculatively, one potential environmental driver behind this is that neither Zika vectors nor honeybees fare well in very arid environments.

Some specific regions of the U.S. stand out as areas of concern in this analysis. Two regions are consistently identifiable across all projections: southern Florida and parts of Hawaii (fig. 8.3). Whilst Zika risk in Hawaii is high (fig. 8.2), and apiculture prevalent at moderate densities on the islands (fig. 8,1), insecticidal responses for mosquito control are more likely to be assessed based on threats to native fauna. This is especially likely given the recent listing of several endemic Hawaiian bees on the U.S. endangered species listing (FWS 2016). Our presentation of projected environmental suitability estimates for Zika virus (Supplementary Material 5) may be relevant when considering the threat to endemic insects.

In the case of Florida, the South-East, and the Gulf Coast, Carlson's projection limits Zika to southernmost Florida where there are only moderate estimated colony densities (fig. 8.3a); but both Messina and Samy predict greater environmental suitability in northern Florida where colony densities are higher (fig. 8.3b – 8.3d). Similarly, both projections predict an appreciable degree of Zika suitability coinciding with moderate to high colony densities across parts of the gulf and South-East. The need for immediate review of *Aedes* control processes and protection of apiculture likely varies across this region. Florida, for example, already exercises considerable mosquito control programs (Duprey et al. 2008), which are already implicated in the successful control of Zika (Dinh et al. 2016), and therefore may be better equipped with processes and policies to protect apiculture, agriculture, and the environment. Additionally, beekeepers in this region may already have measures in place to mitigate losses due to aerial drift from commercial mosquito control agents. However other states or counties across this region, and beekeepers operating in them, may not have robust processes in place. States such as Louisiana legally require registration of every apiary in the state; however, this information is not publicly available, in order to protect commercial interests and to prevent opportunistic theft or vandalism of apiaries. Requiring commercial or county-mandated pesticide application to account for nearby apiaries in mitigating unintended pesticide damage would be very beneficial, and could be a sensible model for other states in the region to adopt. The prevalence of commercial mosquito control in this region makes due process particularly important as public concern over Zika grows: for example in Georgia, there are 994 active mosquito control licences as of May 2017.

The Californian Central Valley is another important region our analysis highlights. There is stark disagreement between suitability projections for Zika across California (fig. 8.2). Considering the large area, high agricultural value (Schoups et al. 2005), high colony density (fig. 8.1), and large population (US Census Bureau 2015) present across California's Central Valley, these mixed predictions are liable to pose a considerable challenge. However, California law already specifies the requirement of licensed mosquito control agents to notify beekeepers, with exceptions only granted for commercial control agents who are part of the

Department of Health Services ‘Cooperative Agreement’. This model may be a useful example for other states when navigating conflict between mosquito control and the apicultural industry.

There is additional concern that Florida and the Central Valley of California are areas of identifiable risk. Our estimation of colony densities in these areas is likely conservative due to the number of transient colonies which pass into these areas as part of migratory beekeeping operations. Both Florida and the Californian Central Valley draw large numbers of migratory colonies (Hodges et al. 2001; Simone-Finstrom et al. 2016), either for overwintering, or due to the high demand of pollination services required for the agricultural industries in these areas (Potts et al. 2016). Of particular note are the almond orchards in the Central Californian Valley (Brittain et al. 2013b) and the citrus industry in Florida (Russ 1999; Albrigo and Russ 2002). The potential risk of new or heightened mosquito control measures in these regions may pose a threat to migratory colonies, however the phenology of major pollination demand periods may not necessarily overlap with periods requiring abundant mosquito control.

It however worth framing the risk of Zika-associated pesticide exposure in these intensive agricultural environments to the agricultural-use insecticides likely already present. Areas of agriculture intense enough to warrant supplemetentary pollination (provided by migratory beekeeping) are also likely to see widespread pesticide application for agricultural purposes. Efforts have been made to map U.S. pesticide use at a county-by-county resolution before using the common herbicide atrazine as a proxy for pesticide use (Nakagaki and Wolock 2005), resulting in similar maps to those presented in this study. Nakagaki and Wolock (2005) highlight that whilst the mid-west of the U.S. sees the most intensive pesticide use, where Zika is unlikely (fig. 8.2), honeybees are widespread and abundant (fig. 8.1). Notably, regions of concerned highlighted in thus study such as the Californian central valley, Southeast, and Florida, also show evidence of widespread agricultural pesticide (Nakagaki and Wolock 2005). It may therefore be likely that, excepting aerial spraying in hot weather, the presence of additional pesticide in

landscape in Zika-prone areas will be dwarfed by the abundance of agricultural pesticides.

This study presents what we believe to be the first honeybee colony density map of the U.S. resolved to county level, and the limitations of this require some appraisal. The patterns presented in fig. 8.1, as described in the results, are in good agreement with population densities and areas traditionally understood to be important for U.S. apiculture. One caveat of our approach is the difficulty in accounting for migratory beekeeping, which underpins much of U.S. apiculture (Rucker et al. 2012; Brosi et al. 2017). To our knowledge, no suitable quantitative data on seasonal variation in honeybee colony densities due to migratory practices is available, and so could not be included in the analysis. However as discussed above, regions known to be destinations of large numbers of migratory operations can still be assessed. Additionally, beekeeping operations (regardless of their location) can refer to the data presented here to make their own assessments of how likely they are to encounter Zika preventative measures during their migratory movements.

Our approach is based on the 2012 Agricultural Census data, which is part of a voluntary program; it is therefore difficult to establish what the uptake amongst beekeepers is. For some states, beekeeper registers are maintained, and can therefore be tested against. In Louisiana, as of 2016 there were 679 registered beekeepers in the state – more than twice the 323 beekeeping operations accounted for by our analysis. Whilst the census may therefore miss many beekeepers, the scale of these missed operations appears small. The agricultural census, and most USDA records, exclude operations with fewer than five colonies. Across the country the census accounts for 3,282,570 colonies in the U.S. in 2012. The most recently available total for the U.S. is annual peak honeybee colony number for July 2015 - 3,132,880 colonies (USDA - NASS 2017a). Unfortunately, equivalent data does not exist for 2012. Instead, records for specifically honey producing colonies do exist. Honey producing colonies in 2015 peaked at 2,660,000 colonies (USDA - NASS 2017b), 85% of the previously stated total. The equivalent figure in 2012 is 2,624,000 (USDA - NASS 2013); if we assume that

85% of U.S. colonies are listed as honey producing, as previously derived, we can estimate that in 2012 colony number peaked at approximately 3,080,000 colonies. This is fewer colonies than accounted for in the census, suggesting that whilst many beekeepers may not take part in the census, it accounts for a large majority of colonies in the U.S., captures more colonies than other quoted sources, and that uncounted beekeepers represent small operations. We therefore consider the density map of honeybee colonies across the U.S. to be defensible and accurate in its portrayal of U.S. apiculture.

In addition to identifying broad regions where conflict between Zika control measures and apiculture is likely, we present county-level data for practitioners to consult (Supplementary Material 5). We hope this data, showing estimated colony numbers and Zika suitability, will allow officials to more easily assess the case for protecting apiculture. Additionally, beekeepers who are in counties with few colonies may not fall in an identified ‘high risk’ region, but still require knowledge of likelihood of insecticidal spraying. We therefore present the county-level data for all beekeepers to assess the case for their own county, and hope that it will help beekeepers in preventing losses. It is apparent that beekeepers are already engaging with mosquito control following the Zika virus pandemic and providing this information should assist in these efforts.

In summary, we conclude that the greatest risk to apiculture from Zika abatement mosquito controls is likely to be in the South-East and the Gulf Coast. Notably, we provide evidence that environmental conditions thought to be conducive to Zika virus are also associated with higher densities of honeybee colonies. California, Florida, and Hawaii appear as other notable regions but appear to have schemes already in place to mitigate impacts from necessary pesticidal control. We believe there is potential for effective preventative action in the South-East and Gulf, noting Louisiana as an example where registration of apiaries with the state may allow for easy preventative measures to be introduced. We strongly encourage officials and beekeepers to use the data available and address protecting honeybee colonies. Cases can be made for wider mandatory registering of beekeeping activities if commercial mosquito control agents must consult this information via official

channels. Additionally, these regions of the U.S. should be targeted for increased monitoring of colony losses due to pesticides, and should be considered for initiatives to encourage beekeepers to report such losses to the authorities.

Chapter 9 – Synthesis & Future Directions

As we work to protect pollinators from the emerging and re-emerging infectious diseases threatening pollinator populations (Manley et al. 2015; McMahon et al. 2018), we must bring a diverse variety of approaches and tools to the challenge. This thesis has contributed to that goal by better understanding some of the ecological and evolutionary forces determining the dynamics of infectious diseases, especially in honeybees and possibly by extension, many wild bee populations.

As briefly mentioned in chapter 7, breeding of *Varroa*-resistant or otherwise pathogen-resistant honeybee stocks is a current goal amongst apiculturalists in the fight against honeybee pathogens (Khongphinitbunjong et al. 2015, 2016). In some cases, this is understood as an attempt at reversing the accidental selection for less resistant honeybee stocks when previous breeders have foscussed on phenotypes such as lower aggression (Rittschof et al. 2015). In chapters 2 and 3, I examined how selection for viral resistance in a model insect (*Plodia interpunctella*) behaves. I showed in chapter 2 that at least one aspect of viral resistance is a genetic and constitutive trait – if the same is true of hygienic or resistance genes in honeybees, current efforts to breed better bee stock may be more successful knowing that such traits are potentially genetic. However as shown in chaper 3, simply selecting for other allegedly correlated traits (hypothetically for example, selecting for increased honeybee aggression) will not necessarily also select for increased resistance. Such caveats to selection will be critical in honeybee breeding programmes.

Future work on this topic would do well to establish whether there are tractable trade-offs in honeybee immunity and other phenotypic traits. A potential approach to this would be to exploit studies examining honeybee colony patrilines and polyandry, whereby worker bees of specific patrilines may exhibit greater degrees of resistance to certain pathogens or higher levels of hygienic behaviour, at some cost. The role of polyandry in ensuring colony health, and tying this to genetic variation amongst patrilines, is an already established area of research (Tarpy et al. 2011, 2012). Having shown that pathogen resistance is likely to have at least

some genetic element, I propose as one avenue of future research that individuals varying by patriline have their lifespan measured in both in the presence and absence of a pathogen. The proposed hypothesis being that ‘low resistance’ patrilines will live longer than ‘high resistance’ patrilines in the absence of infection, but that they will be vulnerable to much higher mortality in the face of a pathogen, and high resistance patrilines will show longer lifespans than low resistance patrilines when infected with a cadidiate pathogen. Such work has clear implications for the evolutionary management of honeybee diseases, and is justified based on well-demonstrated resistance trade-offs such as the one I interrogate and better understand in chapters 2 and 3.

Further, modelling work on the evolutionary outcomes of emerging or re-emerging bee diseases is being currently undertaken. If models are used to understand potential host evolutionary responses of wild pollinators to the spillover of honeybee pathogens, we require the assumptions of such models to be either robust or true to reality. Having confirmed in chapter 2 that the common evolutionary assumption that parasite resistance is a genetic and constitutive trait has some biological support, we can rely with greater sureity on the application of evolutionary models to modern conservation challenges. As ecological modelling of wild pollinators becomes further established (Henry et al. 2017), evolutionary modelling may be a fruiful and justifiable next step.

Evolutionary modelling in the context of pollinator pathogens is not only relevant to hosts, but critically important for pathogen evolution as well. As chapter 7 details, we have good reason to believe that selection upon specific bee pathogens (namely deformed wing virus, DWV) is currently occurring. Broadly, it is accepted that candidate examples of selection on bee viruses point towards increases in virulence (Brosi et al. 2017), although as mentioned in chapter 4, understanding why parasites are highly virulent has been a critical question for much of evolutionary biology (Anderson and May 1982; Ewald 1987, 1993; Read 1994; Alizon et al. 2009; Alizon and Michalakis 2015; Cressler et al. 2016). Three chapters of this thesis discuss ecological forces determining honeybee epidemiology, and one frames this as an evolutionary question. In both the cases

of *Varroa*-vectoring discussed in chapter 7, and industrailisation and intensification of apiculture discussed in chapters 5 and 6, selection for increased virulence in part relies on reduced spatial structure of the host (honeybee). Invoking this hypothesis is done on the basis of the abundant modelling and notable empirical demonstrations that reduced spatial structure selects for more virulent parasites as discussed in chapter 4. However, as with all modelling, assumptions underpinning the models might be critically determining outcomes, and such assumptions must be interrogated if they are to inform actionable recommendations in real systems.

In chapter 4 I examined a critical assumption present in much of the work on spatially explicit evolutionary modelling of pathogen virulence. Specifically, I relaxed a demographic assumption by allowing reproduction from infected hosts. Critically, despite some nuanced differences in outcomes, the core finding remains that reducing spatial structure of the infection process selects for more virulent pathogens. This confirmation of a core finding highlights the robustness of the link between spatial structure and virulence; returning to applying that to the pollinator-pathogen system, this further highlights why changes in beekeeping such as cross-continental migration (see references in chapter 6) or novel vectors (chapter 7) may pose such a threat. Having taken a step to validate the theory linking spatial structure to virulence evolution (chapter 4) and empirically demonstrated a link between colony background and long term pathogen dynamics (chapter 6) future work on honeybee pathogens should focus on modelling virulence evolution in light of changing apiculture.

We make this future direction of research even more attainable by establishing the first ecological model to describe honeybee pathogen dyanmics at the multi-colony scale (chapter 5), based on which evolutionary work is currently being undertaken. Chapter 5 was an initially surprising result, highlighting that intensification at the apiary scale has little meaningful effect on pathogen burden. Future empirical work is planned to test this result. This is being done by examining viral titres in colonies experiencing different levels of honeybee drift due to manipulated densities and apiary structuring. Preliminary data (see Travis Dynes PhD thesis – Emory University, quoted here from personal communication) shows results in line with

our model prediction – that apiary structure matters little for pathogen ecology. However, as chapter 6 shows, there are determining factors which significantly alter viral burden in honeybee colonies – in this case, origin or background. Synthesising these results, we can narrow down which aspects of modern apiculture in the U.S.A. are likely drivers of increased viral abundance and potential drivers of selection for higher virulence. Rather than focussing on the scale of individual apiaries, instead it appears worthwhile to focus on landscape – and indeed continental-level – population mixing and structure. Additionally, this larger scale will allow levels of replication to compare pathogen dynamics amongst different management regimes.

Available data on the population structure of honeybees in the U.S.A. is not as precise as would be necessary for such landscape-level questions, as partially demonstrated and described in chapter 8. As part of chapter 8, I present the first and most highly resolved map of where honeybees in the U.S.A. are. Such data will be critical in future research understanding landscape level epidemiology of honeybees, and by extension, potentially many wild bee populations. Hopefully, the application of such data as is showcased in chapter 8 will build momentum for beekeepers to consider making their operations more transparent. Fostering such practices should be a goal of applied research on honeybees in the U.S.A. Being able to estimate, to any reliable degree, the annual movement patterns of honeybees across the U.S.A. (rather than the single snapshot presented in chapter 8) would be an immensely powerful tool for building on the work of chapters 5 and 6.

Overall, this thesis demonstrated the aforementioned dual benefit of better establishing honeybees as a system for the study of infectious disease ecology and evolution. Applying ecological and evolutionary techniques, knowledge, and modelling paradigms to honeybees will be of increasingly important benefit for their management, which is a critical interest for many stakeholders – including beekeepers, wider agriculture, the public, and those focussed on conserving wild pollinators. To understand the disease burden wild pollinators are suffering, we will

need to understand the epidemiology of honeybees, who are likely drivers of spillover defining parasitic threats to their wild counterparts.

It is an ambition following this thesis that honeybees be further developed as a study system for pathogen research. In particular, I think chapter 7 best highlights the gains which can be made in fundamental understanding of pathogen biology by exploiting the interest in, and importance of, the uniquely well-understood honeybee system. I believe this thesis makes some of the first steps in demonstrating that by working to protect bees we will gain fundamental knowledge beneficial to many critical aspects of public health, agriculture, and the eco-evolutionary management of society's infectious disease burdens.

Appendices

Supplementary Material 1 - A genotypic trade-off between constitutive resistance to viral infection and host growth rate

Raw data and analysis script for this chapter is not suitable for presentation in this format.

Associated analysis script and raw data files for this study are available for download from both https://onlinelibrary.wiley.com/doi/full/10.1111/evo.13623 and https://datadryad.org/resource/doi:10.5061/dryad.5g86200.

Supplementary Material 2 – Selection asymmetry in a resistance development-time trade-off.

Raw data and analysis script for this chapter is not suitable for presentation in this format.

Associated analysis script and raw data files for this study are available for download from https://github.com/LBartlett/ThesisChapter3.

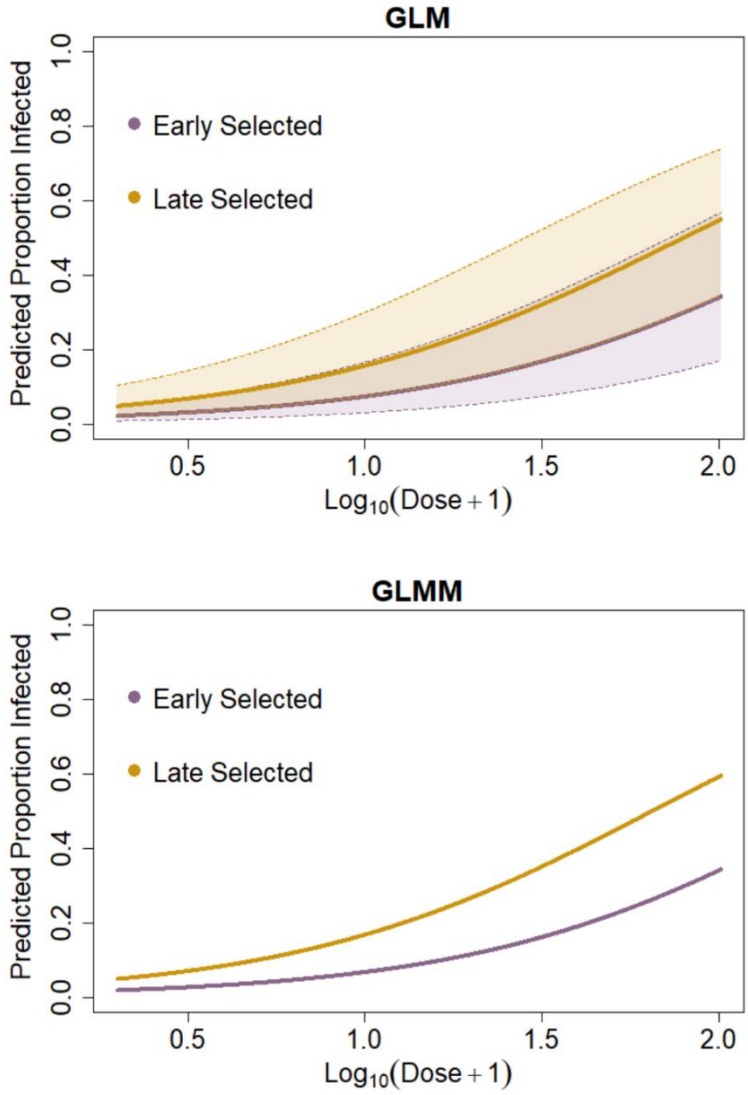


Figure S2.1. Comparison of fig. 3.3 to alternative analysis using mixed-effects model to account for blocked experimental design, as detailed in Chapter 3.

Supplementary Material 3 – Industrial bees: when agricultural intensification doesn't impact local disease prevalence.

Due to the constraints of generating this supplementary material and to preserve mathematical typesetting, it is inserted in the following pages as inset images taken from the version currently under review at Journal of Applied Ecology, and can be downloaded in standalone PDF format from https://github.com/LBartlett/ThesisChapterFive.

Please note that Supplementary Material 3 (following) was co-authored by Carly Rozins, who contributed to this work equally with myself.

Supplementary Information Section 1

Mathematical Model

In this appendix we reintroduce the mathematical model, solve for the basic reproduction numbers, R_0 and approximate expressions for the endemic equilibrium. Additionally we show that R_0 can be used to determine the global stability of the system. When $R_0 > 1$ there is global asymptotic convergence to the endemic equilibrium and when $R_0 < 1$ there is global asymptotic convergence to the disease free equilibrium.

The Model

The following $2n$ differential equations, [1], model the transmission of disease within and between n subpopulations.

$$\begin{aligned} \frac{dS_i}{dt} &= -\sum_{j=1}^{n} S_i I_j \beta_{ij} - mS_i + \phi \\ \frac{dI_i}{dt} &= \sum_{j=1}^{n} I_j S_i \beta_{ij} - I_i(m+v) \end{aligned} \qquad \text{[S1]}$$

All parameter values are assumed to be nonnegative. The matrix $\beta = [\beta_{ij}]$ will depend on the arrangement of colonies within the apiary (see Fig. S1). It can be easily verified that each β (for each colony arrangement) is irreducible through the construction of the associated directional graphs (see Fig.1 and (1)). The feasible region for [S1]:

$$\Gamma = \left\{ (S_1, I_1, \ldots, S_n, I_n) \in \mathbb{R}_+^{2n} \middle| S_i + I_i \leq \frac{\phi}{m} \right\}, i = 1, 2, \ldots, n$$

is positively invariance with respect to [S1]. Let $\mathring{\Gamma}$ denote the interior of Γ. If there is no disease-causing pathogen then there exists a disease free equilibrium (DFE) $P_0 = (S_1^0, 0, S_2^0, 0, \ldots, S_n^0, 0)$ where, $S_i^0 = S^0 = \phi/m$, for $i = 1, 2, \ldots, n$ regardless of configuration (lattice, array, circle) or

population size (n). Below are examples for a 9-hive apiary, also see Fig. S1.

array

$$\begin{bmatrix} a & b & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ b & a & b & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & b & a & b & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & b & a & b & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & b & a & b & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & b & a & b & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & b & a & b & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & b & a & b \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & b & a \end{bmatrix}$$

circular

$$\begin{bmatrix} a & b & 0 & 0 & 0 & 0 & 0 & 0 & b \\ b & a & b & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & b & a & b & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & b & a & b & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & b & a & b & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & b & a & b & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & b & a & b & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & b & a & b \\ b & 0 & 0 & 0 & 0 & 0 & 0 & b & a \end{bmatrix}$$

lattice

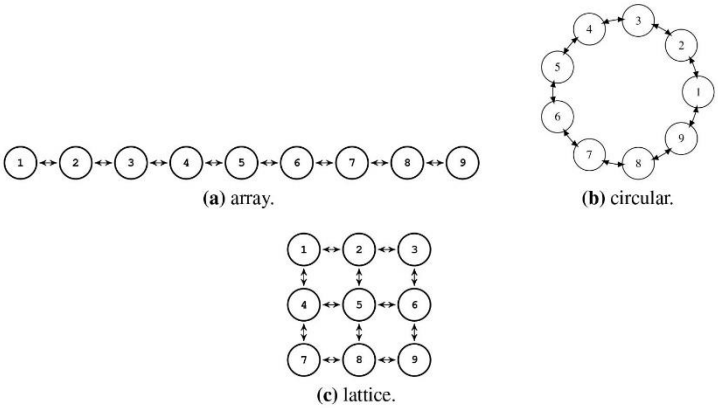
$$\begin{bmatrix} a & b & 0 & b & 0 & 0 & 0 & 0 & 0 \\ b & a & b & 0 & b & 0 & 0 & 0 & 0 \\ 0 & b & a & 0 & 0 & b & 0 & 0 & 0 \\ b & 0 & 0 & a & b & 0 & b & 0 & 0 \\ 0 & b & 0 & b & a & b & 0 & b & 0 \\ 0 & 0 & b & 0 & b & a & 0 & 0 & b \\ 0 & 0 & 0 & b & 0 & 0 & a & b & 0 \\ 0 & 0 & 0 & 0 & b & 0 & b & a & b \\ 0 & 0 & 0 & 0 & 0 & b & 0 & b & a \end{bmatrix}$$

Invasion - R_0 and local stability of the DFE

We use the *next generation matrix* (NGM) method (2, 3) to solve for the basic reproduction number, R_0. Let

$$V = \text{diag}(m+v, m+v, \ldots, m+v) \quad \text{and} \quad F(S) = \left(S_i \beta_{ij}\right)_{n \times n}, \qquad \text{[S2]}$$

be the $n \times n$ matrices for disease transition and new infections. Let:



(a) array. **(b)** circular. **(c)** lattice.

Figure S1. Schematics for sub-population configurations. Nodes represent sub-populations and edges represent possible modes for disease transmission

$$M(S) = F(S)V^{-1} = \left(\frac{\beta_{ij}S_i}{m+v}\right)_{n\times n} \quad \text{and} \quad M_0 = F(S^0)V^{-1} = \left(\frac{\beta_{ij}S^0}{m+v}\right)_{n\times n},$$

where M_0 is the NGM and the basic reproduction number, R_0, is defined as its spectral radius ($R_0 = \rho(M_0)$). Since β is irreducible, so too is $M(S)$ and M_0 as well as $M(S) + M_0$ and for $S^0 \neq S_i$, $M(S) < M_0$ and $\rho(M(S)) < \rho(M_0)$ (see (1)). Recall that the transmission matrix $\beta = [\beta_{ij}]$ differs for each population structure (array, circle, lattice), and thus so too will the spectral radius (R_0) of each unique NGM. Eigenvalues for the NGM are found in *Additional Information-Eigenvalues of the NGM* (at the end of this Supplementary Section).

$$R_0^{\text{Array}} = \frac{\phi}{m(m+v)}\Big[a - 2b\cos(\frac{n\pi}{n+1})\Big] \quad \text{[S3a]}$$

$$R_0^{\text{Circular}} = \frac{\phi}{m(m+v)}(a+2b) \quad \text{[S3b]}$$

$$R_0^{\text{Lattice}} = \frac{\phi}{m(m+v)}\Big[a - 4b\cos(\frac{\sqrt{n}\pi}{\sqrt{n}+1})\Big] \quad \text{[S3c]}$$

Global Dynamics of the DFE

In this section the global asymptotic stability of the DFE is established by constructing a suitable Lyapunov function. We use the matrix-theoretic approach, which is based on the Perron eigenvector of the NGM (see (1, 4, 5)).

Theorem 1. *The following holds for system [S1]:*

1. *If $R_0 \leq 1$ then the disease-free equilibrium is globally asymptotically stable in Γ.*
2. *If $R_0 > 1$ then the disease-free equilibrium is unstable and there exists an endemic equilibrium (EE), $P^* = \{S_1^*, I_1^*, S_2^*, I_2^*, \ldots, S_n^*, I_n^*\}$*

Proof. We proceed as in Proposition 3.1 in (1) and Theorem 4.1 in (5). Let $x = (I_1, I_2, \ldots I_n)^T$ and $F(S^0)$ and V are defined as in [S2]. Since $S^0 > S_i$, we have $x' < (F(S^0) - V)x$ and from Theorem 2.1 in (4) we have $L = w^T V^{-1} x$ is a global Lyapunov function for [S1], where w^T is

the left Perron eigenvector of the matrix $V^{-1}F(S^0)$ (the NGM).

$$\begin{aligned} L' &= w^T V^{-1} x' \\ &\leq w^T V^{-1}(F(S^0) - V)x \\ &= (w^T V^{-1} F(S^0) - w^T V^{-1} V)x \\ &= (R_0 - 1) w^T x \\ &\leq 0 \quad \text{if} \quad R_0 \leq 1 \end{aligned} \tag{S4}$$

The only compact invariant subset, with respect to [S1], where $L' = 0$, is the singleton $\{P_0\}$. By LaSalle's Invariance Principle (6) P_0 is globally asymptotically stable in Γ if $R_0 \leq 1$.
If $R_0 > 1$ and $x > 0$, then $(R_0 - 1)w^T x > 0$ and

$$L' = w^T V^{-1} x' = w^T V^{-1}(F(S) - V)x = w^T(M(S)x - x) > 0$$

in a neighbourhood of P_0 in $\mathring{\Gamma}$ by continuity. Thus P_0 is unstable. It can be shown that when $R_0 > 1$, the instability of P_0 implies the uniform persistence of [S1] (7), thus concluding the proof (see (1) for example). The existence of P^* follows from the uniform persistence and the positive invariance of the compact set (see Theorem 2.2 in (4) and Theorem 4.1 in (5)). □

Existence and Global Dynamics of the EE

In this section we show, with the use of a Lyapunov function, that when $R_0 > 1$ the EE, $P^* = \{S_1^*, I_1^*, S_2^*, I_2^*, \ldots, S_n^*, I_n^*\}$, is globally asymptotically stable. We will proceed as others have (1) using the graph theoretic approach.

Theorem 2. *If $\beta = [\beta_{ij}]$ is irreducible and $R_0 > 1$ then there exists a unique endemic equilibrium P^* that is globally asymptotically stable in $\mathring{\Gamma}$*

Proof. Set $\bar{\beta_{ij}} = \beta_{ij} S_i^* I_j^*$, for $1 \leq i, j, \leq n$, and $n \geq 2$ and let:

$$\overline{B} = \begin{bmatrix} \sum_{l \neq 1} \bar{\beta_{1l}} & -\bar{\beta_{21}} & \dots & -\bar{\beta_{n1}} \\ \bar{\beta_{12}} & \sum_{l \neq 2} \bar{\beta_{2l}} & \dots & -\bar{\beta_{n2}} \\ \vdots & \vdots & \ddots & \vdots \\ -\bar{\beta_{1n}} & -\bar{\beta_{2n}} & \dots & \sum_{l \neq 2} \bar{\beta_{nl}} \end{bmatrix}.$$

Where $\overline{B}$ is the transpose of the Laplacian matrix of the directional graph $\bar{\beta}$. Then by Lemma 2.1 in (1), a basis for the solution space for $\overline{B}u = 0$, where $u = (u_1, u_2, \dots, u_n)$, can be written as

$$(u_1, u_2, \dots, u_n) = (C_{11}, C_{22}, \dots, C_{nn}), \qquad \text{[S5]}$$

and

$$C_{ii} = \sum_{T \in \mathbb{T}_i} \prod_{(i,j) \in E(T)} \bar{\beta_{ij}},$$

where $\mathbb{T}_i$ is the set of all directed trees rooted at vertex i and $E(T)$ is the set of edge weights of the directed tree, T. By Kirchhoffs theorem C_{ii} is also the cofactor of the i-th diagonal entry of $\overline{B}$.

Set

$$L = \sum_{i=1}^{n} u_i (S_i - S_i^* \ln S_i + I_k - I_k^* \ln I_k). \qquad \text{[S6]}$$

Differentiating L and making use of right hand side of [S1] and the equilibrium conditions:

$$\phi = m S_i^* + \sum_{j=1}^{n} S_i^* I_j^* \beta_{ij} \text{ and } (m + v) I_i^* = \sum_{j=1}^{n} S_i^* I_j^* \beta_{ij}$$

we obtain,

$$
\begin{aligned}
L' &= \sum_{i=1}^{n} u_i \Big(S_i' - \frac{S_i^*}{S_i} S_i' + I_i' - \frac{I_i^*}{I_i} I_i' \Big) \\
&= \sum_{i=1}^{n} u_i \Big[\phi - m S_i - \sum_{j=1}^{n} S_i I_j \beta_{ij} - \frac{\phi S_i^*}{S_i} + S_i^* m + \sum_{j=1}^{n} S_i^* I_j \beta_{ij} \\
&+ \Big(\sum_{j=1}^{n} S_i I_j \beta_{ij} \Big) - (m+v) I_i + (m+v) I_i^* - \sum_{j=1}^{n} \frac{S_i I_j I_i^* \beta_{ij}}{I_i} \Big] \\
&= \sum_{i=1}^{n} u_i \Big[- S_i^* m \Big(\frac{S_i^*}{S_i} + \frac{S_i}{S_i^*} - 2 \Big) + \Big(\sum_{j=1}^{n} S_i^* I_j \beta_{ij} - (m+v) I_i \Big) \\
&+ \Big(2 \sum_{j=1}^{n} S_i^* I_j^* \beta_{ij} - \sum_{j=1}^{n} \frac{(S_i^*)^2 I_j^* \beta_{ij}}{S_i} - \sum_{j=1}^{n} \frac{S_i I_j I_i^* \beta_{ij}}{I_i} \Big) \Big]
\end{aligned}
$$

Note that $\left(\frac{S_i^*}{S_i} + \frac{S_i}{S_i^*} - 2 \right) \geq 0$, thus $-S_i^* m \left(\frac{S_i^*}{S_i} + \frac{S_i}{S_i^*} - 2 \right) \leq 0$. Also

$$\sum_{i=1}^{n} u_i \Big(\sum_{j=1}^{n} S_i^* I_j \beta_{ij} - (m+v) I_i \Big) = 0$$

since $\sum_{i=1}^{n} u_i \sum_{j=1}^{n} \beta_{ij} S_i^* I_j = \sum_{j=1}^{n} u_j \sum_{i=1}^{n} \beta_{ji} S_j^* I_i = \sum_{i=1}^{n} \Big(\sum_{j=1}^{n} \beta_{ji} S_j^* u_j \Big) I_i$ and we can show that $\sum_{j=1}^{n} \beta_{ji} S_j^* u_j = u_i (m+v)$ using the equilibrium condition $(m+v) I_i^* = \sum_{j=1}^{n} S_i^* I_j^* \beta_{ij}$ and the following equality:

$$\begin{bmatrix} S_1^* I_1^* \beta_{11} + S_1^* I_2^* \beta_{12} + \cdots + S_1^* I_n^* \beta_{1n} \\ S_2^* I_1^* \beta_{21} + S_2^* I_2^* \beta_{22} + \cdots + S_2^* I_n^* \beta_{2n} \\ \vdots \\ S_n^* I_1^* \beta_{n1} + S_n^* I_2^* \beta_{n2} + \cdots + S_n^* I_n^* \beta_{nn} \end{bmatrix} = \begin{bmatrix} (m+v) I_1^* \\ (m+v) I_2^* \\ \vdots \\ (m+v) I_n^* \end{bmatrix}$$

$$\begin{bmatrix} S_1^* \beta_{11} & S_1^* \beta_{12} & \dots & S_1^* \beta_{1n} \\ S_2^* \beta_{21} & S_2^* \beta_{22} & \dots & S_2^* \beta_{2n} \\ \vdots & \vdots & & \vdots \\ S_n^* \beta_{n1} & S_n^* \beta_{n2} & \dots & S_n^* \beta_{nn} \end{bmatrix} \begin{bmatrix} I_1^* \\ I_2^* \\ \vdots \\ I_n^* \end{bmatrix} = \begin{bmatrix} (m+v) & 0 & \dots & \\ 0 & (m+v) & 0 & \dots \\ \vdots & & \ddots & \\ & & & (m+v) \end{bmatrix} \begin{bmatrix} I_1^* \\ I_2^* \\ \vdots \\ I_n^* \end{bmatrix}$$

If we left multiply by u^T then we can show:

$$\begin{bmatrix} S_1^* u_1^* \beta_{11} + S_2^* u_2^* \beta_{21} + \cdots + S_n^* u_n^* \beta_{n1} \\ S_1^* u_1^* \beta_{12} + S_2^* u_2^* \beta_{22} + \cdots + S_n^* u_n^* \beta_{n2} \\ \vdots \\ S_1^* u_1^* \beta_{1n} + S_2^* u_2^* \beta_{2n} + \cdots + S_n^* u_n^* \beta_{nn} \end{bmatrix} = \begin{bmatrix} u_1 (m+v) \\ u_2 (m+v) \\ \vdots \\ u_n (m+v) \end{bmatrix}$$

and thus,

$$
\begin{aligned}
L' &\leq \sum_{i=1}^{n} u_i \Big(2 \sum_{j=1}^{n} S_i^* I_j^* \beta_{ij} - \sum_{j=1}^{n} \frac{(S_i^*)^2 I_j^* \beta_{ij}}{S_i} - \sum_{j=1}^{n} \frac{S_i I_j I_i^* \beta_{ij}}{I_i} \Big) \\
&= \sum_{i=1}^{n} u_i \Big(\sum_{j=1}^{n} 2\bar{\beta_{ij}} - \frac{s_i^* \hat{\beta_{ij}}}{S_i} - \frac{S_i I_j I_i^* \bar{\beta_{ij}}}{I_i S_i^* I_j^*} \Big) \\
&= \sum_{i=1}^{n} \sum_{j=1}^{n} u_i \bar{\beta_{ij}} \Big(2 - \frac{S_i^*}{S_i} - \frac{S_i I_j I_i^*}{I_i S_i^*} I_j^* \Big)
\end{aligned}
$$

It remains to show that $H_n \leq 0$ for all $(S_1, I_1, \ldots, S_n, I_n) \in \mathring{\Gamma}$. While explicit expressions for cofactors, u_i can be derived by computing the number of matrix trees for the diagonal entries in $\bar{\beta}$, it is difficult to do so for the lattice structure. Therefore we will direct the reader to (1, 8) and give a sketch of the remainder of their proof for a general irreducible transmission matrix β and associated matrix $\bar{\beta_{ij}} = \beta_{ij} S_i^* I_j^*$. Guo et al. (2006, 2008) show that $u_i = C_{ii}$ is the sum of n^{n-2} terms, each of which can be expressed as the product of $(n-1)$, $\bar{\beta_{ij}}$'s. Importantly the subindices of $\bar{\beta_{ij}}$ can be represented by all arcs in a directed tree T rooted at the $i-$th vertex. The product $u_i \bar{\beta_{ij}}$ can be interpreted as the weight of the unicycle graph, Q, obtained from the tree T, by adding an edge from node i to j. Each unicycle graph Q has a unique cycle CQ of length $1 \leq l \leq n$. Guo et al. show that there are l terms in H_n each with coefficients correspond to all $l-$rotations of the same $l-$cycle and are thus the same, and can be grouped together. Furthermore, all of the terms of H_n can be grouped based on corresponding cycle

lengths. Thus $H_n = \sum_Q H_{n,Q}$, where,

$$H_{n,Q} = \prod_{(r,m)\in E(Q)} \bar{\beta_{rm}} \sum_{(i,j)\in E(CQ)} \left(2 - \frac{S_i^*}{S_i} - \frac{S_i I_j I_i^*}{I_i S_i^* I_j^*}\right)$$
$$= \prod_{(r,m)\in E(Q)} \bar{\beta_{rm}} \left(2l - \sum_{(i,j)\in E(CQ)} \left(\frac{S_i^*}{S_i} - \frac{S_i I_j I_i^*}{I_i S_i^* I_j^*}\right)\right)$$

where $E(CQ)$ is the edge weight of the cycle CQ and l denotes the number of edges in CQ. Note that

$$\prod_{(i,j)\in E(CQ)} \left(\frac{S_i^*}{S_i} \frac{S_i I_j I_i^*}{I_i S_i^* I_j^*}\right) = \prod_{(i,j)\in E(CQ)} \frac{I_j I_i^*}{I_j^* I_i} = 1$$

for each unicycle in Q. Therefore

$$\sum_{(i,j)\in E(CQ)} \left(\frac{S_i^*}{S_i} - \frac{S_i I_j I_i^*}{I_i S_i^* I_j^*}\right) \geq 2l$$

and thus $H_{n,Q} \leq 0$ for each Q and $H_{n,Q} = 0$ when $\frac{S_i^*}{S_j} = \frac{S_i I_j I_i^*}{I_i S_i^* I_j^*}$. Thus $H, \leq 0$ and $L' \leq 0$ for all $(S_1, I_1, \ldots, S_n, I_n) \in \mathring{\Gamma}$ and $L' = 0$ iff $S_i = S_i^*$ and $H_n = 0$. Guo et al. (2006,2008) show that $H_n = 0 \Leftrightarrow I_i = aI_j^*$ where a is some arbitrary positive number. If we substitute $S_i = S^*$ and $I_j = aI_j^*$ into (S1), then

$$0 = \phi - mS_i^* - a\sum_{j=1}^{n} S_i^* I_j^* \beta_{ij},$$

holds true if $a = 1$ (i.e. at P^*), and otherwise the right hand side is strictly decreasing in a. Therefore the only compact invariant subset of the set where $L' = 0$ is the singleton $\{P^*\}$ and therefore by LaSalle Invariance Principle, P^* is globally stable in $\mathring{\Gamma}$ when $R_0 > 1$.

The Endemic Equilibrium

Equilibrium values for model [S1] are found by setting $dS_i/dt = dI_i/dt = 0$.

$$0 = -\sum_{j=1}^{n} S_i I_j \beta_{ij} - mS_i + \phi$$
$$0 = \sum_{j=1}^{n} I_j S_i \beta_{ij} - I_i(m + v)$$

For the circular configured model we can solve for the endemic equilibrium explicitly by solving the above for an apiary comprised of three colonies. This is done without loss of generality. The endemic equilibrium for the circular hive is:

$$(S^*, I^*) = \Big(\frac{m+v}{a+2b}, \frac{\phi}{m+v} - \frac{m}{a+2b}\Big)$$

If we assume that $0 < b << 1$, then we can express the solutions of the system of $2n$ nonlinear equations (above) each as a power series in b. This technique is refereed to as *perturbation theory*. For example, the power series solution for S_1 would be: $S_1(t) \approx S_1^0(t) + bS_1^1(t) + b^2S_1^2(t) + b^3S_1^3(t) + \ldots$. We proceed by substituting the first two terms of each power series (i.e. $S_1 = S_1^0 + bS_1^1$, $S_2 = S_2^0 + bS_2^1$,...,$I_1 = I_1^0 + bI_1^1$,$I_2 = I_2^0 + bI_2^1$,... etc) into the the system of equations. We then collect equal powers of b, while neglecting higher powers (greater than 2). This results in two systems of of equations (one system for the variables with 0 as subscripts, and one system for the variables with both 0 and 1 as subscripts). The first system of equations (with variable superscripts 0, i.e. S_1^0, S_2^0,...,I_1^0, I_2^0,...) is easily solvable.

$$S_1^0 = S_2^0 = S_3^0 = ... = \frac{m+v}{a}$$

and

$$I_1^0 = I_2^0 = I_3^0 = ... = \frac{\phi}{m+v} - \frac{m}{a}$$

By substituting the solution to the fist set of equations into the second system of equations, the second system of equations can be expressed as a nonhomogeneous linear system of equations $b = Ax$, where:

$$A = \begin{bmatrix} P & Q \\ R & 0 \end{bmatrix}, \qquad b = \begin{bmatrix} l_1 S^0 I^0 \\ l_2 S^0 I^0 \\ l_3 S^0 I^0 \\ \vdots \\ -l_1 S^0 I^0 \\ -l_2 S^0 I^0 \\ -l_3 S^0 I^0 \\ \vdots \end{bmatrix}, \qquad x = \begin{bmatrix} S_1^1 \\ S_2^1 \\ S_3^1 \\ \vdots \\ I_1^1 \\ I_2^1 \\ I_3^1 \\ \vdots \end{bmatrix}$$

The vector b is what differs between the array and the lattice models. The coefficients l_i are the number of neighbors that hive i has. For example, in the array configuration, hive one will have one neighbor, thus $l_1 = 1$ and hive two will have two neighbors $l_2 = 2$. The matrices P, Q, R and S are all $n \times n$ diagonal matrices, with zeros in all entries but the main diagonal. $P = \text{diag}(-aI^0 - m) = \frac{-a\phi}{m+v}$, $Q = \text{diag}(-aS^0) = -(m+v)$ and $R = \text{diag}(aI^0) = \frac{a\phi - m(m+v)}{m+v}$. The solution to the non-homogeneous linear system can be found by inverting the matrix A: $A^{-1}b = x$. The inverse of A is can be expressed in block form.

$$A^{-1} = \begin{bmatrix} 0 & C^{-1} \\ B^{-1} & AB^{-1}C^{-1} \end{bmatrix}$$

and

$$x = \begin{bmatrix} S_1^1 \\ S_2^1 \\ S_3^1 \\ \vdots \\ I_1^1 \\ I_2^1 \\ I_3^1 \\ \vdots \end{bmatrix} = \begin{bmatrix} \frac{-l_1(m+v)}{a^2} \\ \frac{-l_2(m+v)}{a^2} \\ \frac{-l_3(m+v)}{a^2} \\ \vdots \\ \frac{l_1 m}{a^2} \\ \frac{l_2 m}{a^2} \\ \frac{l_3 m}{a^2} \\ \vdots \end{bmatrix}$$

Therefore, the linear approximate endemic equilibrium is:

$$\begin{aligned} S_i^* &= \frac{m+v}{a} - lb\Big(\frac{m+v}{a^2}\Big) \\ I_i^* &= \frac{\phi}{m+v} + lb\Big(\frac{m}{a^2}\Big) \end{aligned} \qquad \text{[S7]}$$

where l is the number of neighbours that hive i has. Notice that the endemic equilibrium for a single colony is independent of the total number of colonies in the apiary. However, as the number of colonies increases, the average number of nearest neighbours any given colony has approaches a constant (2 for array and 4 for lattice). Thus, as the number of colonies increase, the populations-wide disease prevalence asymptotes.

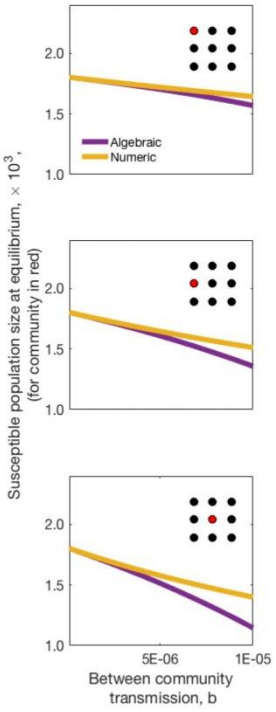


Figure S2. Analytic and numeric approximations for the susceptible endemic equilibrium population for three subpopulations and a range of between population transmission values, b. Other parameters are set to: $v = 0.16$, $m = 0.0275$, $\phi = 1600$ and finally the totoal transmission $T = a + b$ is held at $T = 1.04 \times 10^{-4}$

Robustness of EE approximations

The endemic equilibrium solutions found in the previous section rely on the between colony transmission being small. Therefore as the between colony transmission increases, the result [S7] will become less accurate (see Fig S2). Additionally, as the number of nearest neighbours

increases, so too does the discrepancy between the analytic and numeric results (Fig. S2). In Figure S2 we plot the endemic susceptible population size for particular colony within a nine-community lattice population. In yellow is the numeric solution and in purple is the algebraic solutions [S7]. You can see that as the parameter b, between colony transmission, increases, the two solutions diverge and the algebraic solution becomes an underestimate of the true susceptible populations size. Total transmission $a + b$ is held constant - when we increase b we also decreased a. As the between colony transmission proportion of the total transmission increases, the endemic susceptible populations size decreases (Fig S2) and the disease prevalence increases.

Additional Information- Eigenvalues of the NGM

Array Configuration

The next generation matrix for the array configuration model is:

$$FV^{-1} = \frac{\phi}{m(m+v)} \begin{bmatrix} a & b & & \\ b & a & \ddots & \\ & \ddots & \ddots & \\ & & & \end{bmatrix}$$

The matrix FV^{-1} is a tridiagonal Toeplitz matrix. Toeplitz matrices have been widely studied (9) and the eigenvalues of FV^{-1} are:

$$\lambda_k = \frac{\phi}{m(m+v)}\left[a - 2b\cos(\frac{k\pi}{n+1})\right] \qquad \text{[S8]}$$

where $k = 1, .., n$ (9).

Lemma 1. *The largest values of the sequence,*

$$f_k = a - 2bcos\Big(\frac{k\pi}{n+1}\Big)$$

where $k = 1, 2, \ldots, n$ *and* $0 < b << a$ *is when* $k = n$.

Proof. We will prove through contradiction. Suppose that the maximum element of f_k is not f_n (the last element in the sequence). Then there must exist a $m \in 1, 2, ...(n-1)$ such that $f_m > f_n$.

$$\begin{aligned} a - 2b\cos\Big(\frac{m\pi}{n+1}\Big) &> a - 2b\cos\Big(\frac{n\pi}{n+1}\Big) \\ \cos\Big(\frac{m\pi}{n+1}\Big) &< \cos\Big(\frac{n\pi}{n+1}\Big) \\ \Big(\frac{m\pi}{n+1}\Big) &> \Big(\frac{n\pi}{n+1}\Big) \\ m &> n \end{aligned}$$

which is a contradiction. Hence the largest element of f_k is f_n.

□

Therefore the dominant eigenvalue of FV^{-1}, and thus R_0 for the array model is:

$$R0^{\text{array}} = \frac{\phi}{m(m+v)}\Big[a - 2b\cos(\frac{n\pi}{n+1})\Big]$$

Circular Configuration

We proceed as we did above. For the circular model,

$$FV^{-1} = \frac{\phi}{m(m+v)} \begin{bmatrix} a & b & 0 & \cdots & b \\ b & a & b & \cdots & \\ & \cdots & \cdots & & \\ & & & & \end{bmatrix}$$

Again FV^{-1} is a Toeplitz matrix, but it is not tridiagonal. Unlike the matrix for the array model, the above matrix is a special class of Toeplitz matrices called a circular matrix where each row vector is rotated one element to the right relative to the preceding row vector. We denote the elements of the first row as $c_0, c_1, \ldots, c_{n-1}$, and note that regardless of what n is, $c_0 = a(\phi/(m(m+v)))$, $c_1 = b(\phi/(m(m+v)))$ and $c_{n-1} = b(\phi/(m(m+v)))$ while $c_j = 0$ where $j = (l \in \mathbb{N} | l < (n-1), l \neq 0, 1)$. The n eigenvalues of our circular matrix, FV^{-1}, are:

$$\lambda_k = \sum_{j=0}^{n-1} c_j e^{\frac{-2\pi i k j}{n}}$$

where c_j of the k^{th} element of the top row of the matrix FV^{-1}. Notice that the top row of FV^{-1} has only three nonzero elements $c_0 = a(\phi/(m(m+v)))$, $c_1 = b(\phi/(m(m+v)))$ and $c_{n-1} = b(\phi/(m(m+v)))$. Therefore we can rewrite the above:

$$\begin{aligned} \lambda_k &= \frac{\phi}{m(m+v)} \Big[a + be^{\frac{-2\pi i k}{n}} + be^{\frac{-2(n-1)\pi i k}{n}} \Big] \\ &= \frac{\phi}{m(m+v)} \Big[a + 2b \Big[\cos\Big(\frac{-2\pi k}{n}\Big) \Big] \Big] \end{aligned}$$

Note that $\sin\left(\frac{-2\pi k}{n}\right) + \sin\left(\frac{-2\pi k(n-1)}{n}\right) = 0$, since the function is periodic with period 2π and $\cos\left(\frac{-2\pi k}{n}\right) = \cos\left(\frac{-2\pi k(n-1)}{n}\right)$ since cos is periodic with period 2π and also a odd function.

We can proceed just as we did in the previous section to show that the dominant eigenvalue (and hence R_0) for the circular model is:

$$R0^{\text{Circular}} = \frac{\phi}{m(m+v)}(a+2b)$$

Lattice Configuration

To find R_0 for the lattice model we will make use of the Kronecker product and Kronecker sum. Consider the next generation matrix for the lattice model:

$$FV^{-1} = \frac{\phi}{m(m+v)} \begin{bmatrix} A & B & & \\ B & A & \ddots & \\ & \ddots & \ddots & \\ & & & \end{bmatrix}$$

$FV^{-1} \in \mathbb{M}^{n,n}$, and

$$A = \begin{bmatrix} a & b & & \\ b & a & \ddots & \\ & \ddots & \ddots & \\ & & & \end{bmatrix}, \qquad B = \begin{bmatrix} 0 & b & & \\ b & 0 & \ddots & \\ & \ddots & \ddots & \\ & & & \end{bmatrix}$$

where $A \in \mathbb{M}^{N,N}$ and $B \in \mathbb{M}^{N,N}$, $n = N^2$ and both A and B are tridiagonal Toeplitz matricies. Let

$$M = \begin{bmatrix} A & B & & \\ B & A & \ddots & \\ & \ddots & \ddots & \\ & & & \end{bmatrix}$$

The matrix M can be written as:

$$M = (B \otimes I) + (I \otimes A) = B \oplus A$$

Both A and B are Toeplitz with eigenvalues $-2b\cos(\frac{k\pi}{N+1})$ and $a - 2b\cos(\frac{k\pi}{N+1})$ for $k = 1, 2, ..., N$ respectively.

The n eigenvalues of M are:

$$\Big(-2b\cos(\frac{k\pi}{N+1})\Big) + \Big(a - 2b\cos(\frac{l\pi}{N+1})\Big)$$

for $k = 1, \ldots N$ and $l = 1, \ldots N$. The above is maximized when both $k = N = \sqrt{n}$ and $l = N = \sqrt{n}$ (See Lemma). Therefore R_0 for the lattice model is:

$$R0^{\text{Lattice}} = \frac{\phi}{m(m+v)}\Big[\Big(-2b\cos(\frac{\sqrt{n}\pi}{\sqrt{n}+1})\Big) + \Big(a - 2b\cos(\frac{\sqrt{n}\pi}{\sqrt{n}+1})\Big)\Big]$$

References - Section 1

1. H. Guo, M. Li, and Z. Shuai, "Global stability of the endemic equilibrium of multigroup sir epidemic models," *Canadian Applied Mathematics Quarterly*, vol. 14, no. 3, pp. 259–284, 2006.

2. P. Van den Driessche and J. Watmough, "Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission," *Mathematical biosciences*, vol. 180, no. 1, pp. 29–48, 2002.

3. O. Diekmann, J. Heesterbeek, and J. Metz, "On the definition and the computation of the basic reproduction ratio r0 in models for infectious diseases in heterogeneous populations," *Journal of mathematical biology*, vol. 28, no. 4, pp. 365–382, 1990.

4. Z. Shuai and P. van den Driessche, "Global stability of infectious disease models using lyapunov functions," *SIAM Journal on Applied Mathematics*, vol. 73, no. 4, pp. 1513–1532, 2013.

5. Z. Shuai and P. van den Driessche, "Modelling and control of cholera on networks with a common water source," *Journal of biological dynamics*, vol. 9, no. sup1, pp. 90–103, 2015.

6. J. LaSalle, *The stability of dynamical systems*, vol. 25. Siam, 1976.

7. H. Freedman, S. Ruan, and M. Tang, "Uniform persistence and flows near a closed positively invariant set," *Journal of Dynamics and Differential Equations*, vol. 6, no. 4, pp. 583–600, 1994.

8. H. Guo, M. Li, and Z. Shuai, "A graph-theoretic approach to the method of global lyapunov functions," *Proceedings of the American Mathematical Society*, vol. 136, no. 8, pp. 2793–2802, 2008.

9. D. Kulkarni, D. Schmidt, and S. Tsui, "Eigenvalues of tridiagonal pseudo-toeplitz matrices," *Linear Algebra and its Applications*, vol. 297, no. 1-3, pp. 63–80, 1999.

Supplementary Information Section 2

Description of our Agent-Based Model

We use a discrete time simulation with time-steps of 1 day. We define an apiary of size n where n is the number of colonies, arranged in one of three configurations following Fig. 1 in the main manuscript.

Within a colony, individuals are either susceptible (S) or infected (I). New susceptible individuals enter the colony at birth rate ϕ. Each colony has a constant birth rate ϕ, randomly drawn from a normal distribution with mean $\phi = 1500$ and $\sigma^2 = 150$ in line with quoted maximum laying rates (1). We fix mean maximum colony size in a disease free state as 40,000 individuals (2), and from this calculate a universal natural death rate m = 40000/1500 = 0.0375 (likelihood per individual per day). This death rate is in line with rates quoted for various honeybee life stages (1, 3, 4). Differences in birth rate cause colonies to reach different maximum sizes in their disease free state, meant to approximate differences in queen quality (5), but is likely conservative in this regard.

Our starting state at time t = 0 is intended to represent the beginning of a beekeeping season. Each colony has a starting number of susceptible individuals, randomly drawn from a normal distribution with mean $S_{t=0} = 13500$ and $\sigma^2 = 1687.5$. Notably this is well below the maximum colony size, as would be more realistic following overwintering (2). One colony in the apiary is randomly selected, and a single susceptible individual replaced with an infected individual.

Infected individuals inside a colony infect susceptible individuals at rate β. Infected individuals suffer an additional induced mortality rate v. We vary the values of β and v as part of this study. All individuals may additionally move into nearest-neighbouring colonies for a single time step at rate ρ – in the case of the lattice, we used a Von Neumann neighbourhood. We vary movement-rate ρ during this study, with minimum realistic rates derived from various other studies (4, 6, 7) and corrected for our lack of internal colony demography. Susceptible individuals which move into neighbouring colonies are not available to be infected within their own colony in that time step, but may become infected by infected individuals in the neighbouring colony to which they have temporarily moved. Likewise, infected individuals which have moved cannot contribute to infection within their own colony in this time step, but can infect susceptible individuals in the colony to which they have moved. Notably, the likelihood of movement into another colony ρ is per bee per day per neighbouring colony; a colony with four immediate neighbours will experience approximately 4x as much between-colony movement as a colony with a single immediate neighbour. Individuals which move between colonies remain residents of their 'home colony' and do not permanently become individuals in the colony to which they drifted for the day.

Each time step, the above described processes of birth, death, and infection are modelled to occur simultaneously within and across all colonies in the apiary. Notably, this means that

new susceptible individuals do not contribute to any other processes in the same time step in which they are born – they cannot die, move into another colony or become infected. Additionally, individuals can die and contribute to infection in the same time step.

For this study, the agent-based model was built and run using R (v. 3.3.0 "Supposedly Educational").

References – Section 2

1. Schmickl T, Crailsheim K (2007) HoPoMo: A model of honeybee intracolonial population dynamics and resource management. *Ecol Model* 204(1):219–245.

2. Caron DM, Connor LJ (2013) *Honey Bee Biology and Beekeeping, Revised Edition* eds Muir RG, Harman A (Wicwas Press, Kalamazoo, MI). Revised edition.

3. Martin SJ (2001) The role of Varroa and viral pathogens in the collapse of honeybee colonies: a modelling approach. *J Appl Ecol* 38(5):1082–1093.

4. Becher MA, et al. (2014) BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. *J Appl Ecol* 51(2):470–482.

5. Hatjina F, et al. (2014) A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. *J Apic Res* 53(3):337–363.

6. Allen MD (1963) Drone production in honey-bee colonies (Apis mellifera L.). *Nature* 199:789–790.

7. Wilkinson D, Smith GC (2002) A model of the mite parasite, Varroa destructor, on honeybees (Apis mellifera) to investigate parameters important to mite population growth. *Ecol Model* 148(3):263–275.

Supplementary Information Section 3

Additional Analysis

Model outputs were initially tested in mapping the effects of intensification to disease burden without consideration of R_0. This appendix shows select outputs confirming model function, supporting assertions made in the main text, and that the agent based models yield the same qualitative results as are derived from the mathematical model in the main text.

Figure S3 explores endemic equilibrium states for four pathogen phenotypes, for the which the R0 values have been retrospectively approximated. Of note is the confirmation that higher R0 yields higher pathogen burdens. By separately varying both virulence and transmission, we demonstrate the expected result that the highest prevalence is achieved by a low-virulence, high-transmission (i.e. 'well adapted') pathogen. The greatest degree of colony-size suppression results from a high-virulence, high-transmission pathogen, which is used as the pathogen in the subsequent figures.

Figure S4 shows how increasing movement between colonies (ρ) affects pathogen spread via mean colony sizes and pathogen prevalence for large or small apiaries in all configurations. As expected from the explanation and results presented in the main text, all aspects of intensification have little influence on the equilibrium disease burden (prevalence & colony size, which are positively related. Notably, for any given pathogen, it demonstrates that prevalence is directly relatable to colony size. We use this as justification for explicitly examining burden as the main focus of the manuscript. Additionally, it shows the very rapid rate at which the system reaches disease equilibrium for all apiaries in lattice configurations, and all small apiaries. The cases of spread in large circular or array apiaries are somewhat slower (and slightly influenced by higher rates of movement between colonies) but may be limited by our limitation of nearest-neighbour-only transmission. Again, we use this rapid rate of pathogen spread to disease equilibrium to justify our focus on disease endemic states in the main manuscript.

Figure S5 demonstrates the behaviour of the mathematical model in reaching disease equilibrium. Even in a large apiary of 100 colonies arranged in an array, endemic equilibrium is quickly established. This is in broad agreement with the results presented in Fig S4, with some minor differences potentially due to the agent based simulation being restricted to 1 day time steps (a constraint absent from the mathematical model). Our rate of spread present here (time taken to reach endemic equilibrium) may be a conservative estimate of reality, as we restrict inter-colony transmission to be between nearest neighbour colonies only.

Figure S6 Demonstrates that the model reaches the equilibrium rapidly. This is the case for the SI model presented in the main document, as well as for an alternative model where only larvae are vulnerable to infection by infectious adult bees.

Figure S7 uses purely the analytical mathematical model to examine the impact of aspects of intensification on burden in a similar approach to the results shown in main manuscript Fig. 5b. However it does not examine 'intensification' as one combined process and instead shows different combinations of numbers of colonies and configurations, with bee movement

between colonies held constant. This figure (like Figure S5) does not involve the agent-based model results and should be understood as a test of consistency of results when comparing between modelling approaches.

Alternative Model

The alternative model has two age classes, Larvae (L) and Adults (A). Larvae develop into adults at rate g and die are rate m'. Larvae can become infected through contact with infected adults (carrying mites), A_I, with transmission rate of beta. Infected Adults have an additional death rate of v and all adults have a natural mortality of and infected adults can recover from infection at rate gamma.

$$\frac{dL_S}{dt} = \phi - m'L_S - gL_S - \beta A_I L_S$$

$$\frac{dL_I}{dt} = -m'L_I - gL_I + \beta A_I L_S$$

$$\frac{dA_S}{dt} = gL_S - mA_S + \gamma A_I$$

$$\frac{dA_I}{dt} = gL_I - (m + v)A_I - \gamma A_I$$

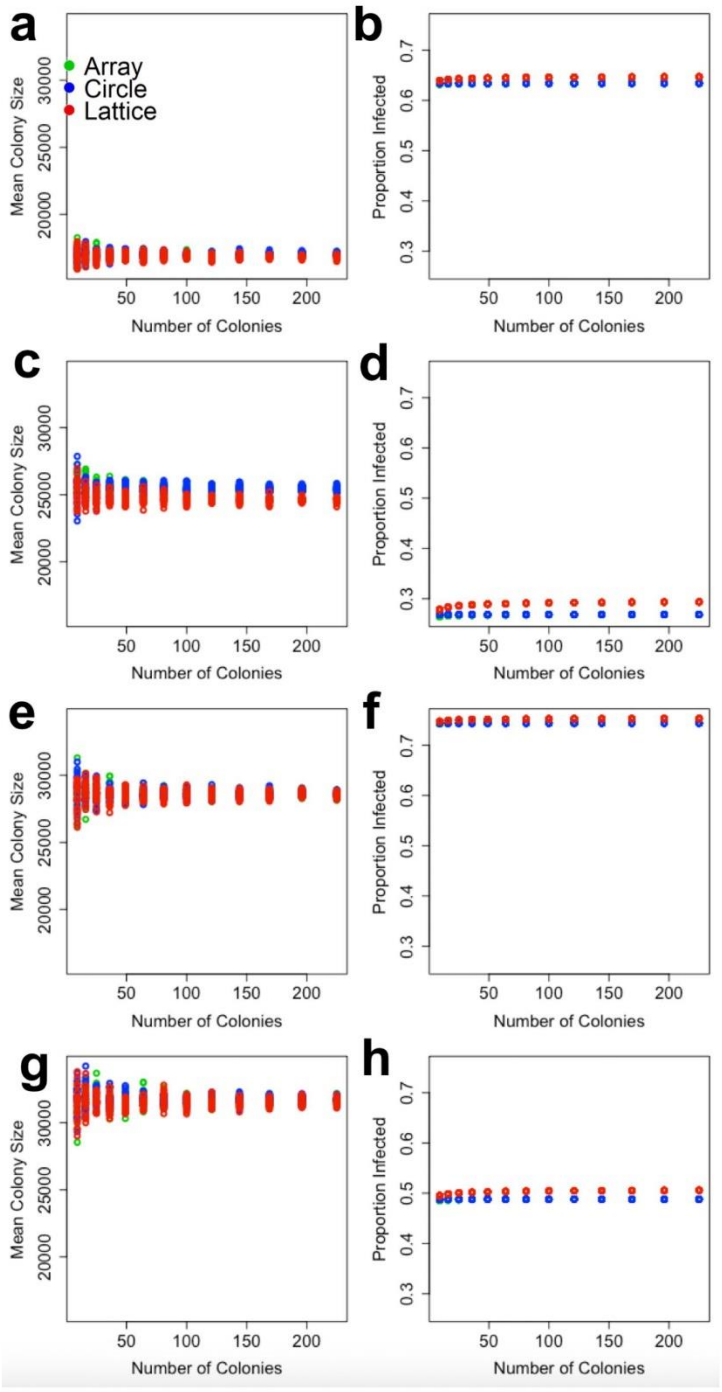


Figure S3. Graphs showing approximate endemic disease equilibria states expressed as mean colony size (left – a, c, e, g) and proportion of individuals infected (right – b, d, g, h) across a apiaries of varying sizes after 2000 days of simulation. Each row represents a different pathogen phenotype, and therefore R_0. Figs. a & b show equilibria for a high-mortality high-transmission pathogen ($R_0 \simeq 18$). Figs. c & d show a high-mortality low-transmission pathogen ($R_0 \simeq 2.5$); figs. e & f represent a low-mortality high-transmission pathogen ($R_0 \simeq 36$); and a low-mortality low- transmission pathogen ($R_0 \simeq 7.5$) is represented in figs. g & h. These prevalences can be compared to the relationship derived by the purely analytical model (Fig. 3c, main manuscript) between R_0 and prevalence, demonstrating the close agreement of the agent based model and its mathematical counterpart.

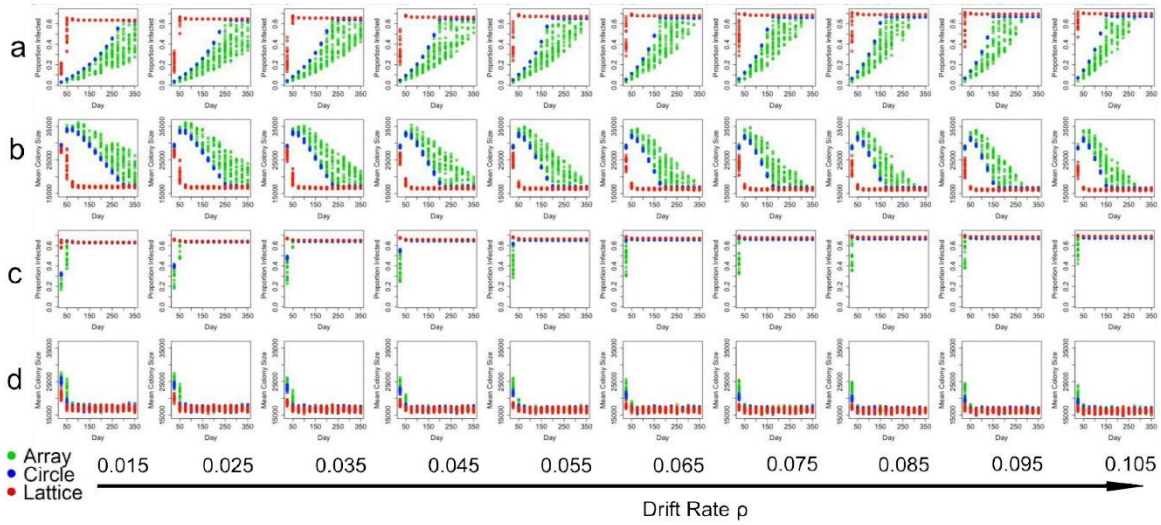


Figure S4. Graphs show change in mean colony size (rows b & d) and proportion of bees infected (rows a & c) across an apiary over the first 300 days of simulation, representing pathogen spread from a single colony. Rows a & b (top) show a large apiary (144 colonies), rows c & d (bottom) show a small apiary (16 colonies). Movement rate ρ increases from left to right. Corresponding R_0 values for these scenarios range from $R_0 \approx 18$ at $\rho = 0.015$ in the small array apiary to $R_0 \approx 30$ at $\rho = 0.0105$ in the large lattice apiary.

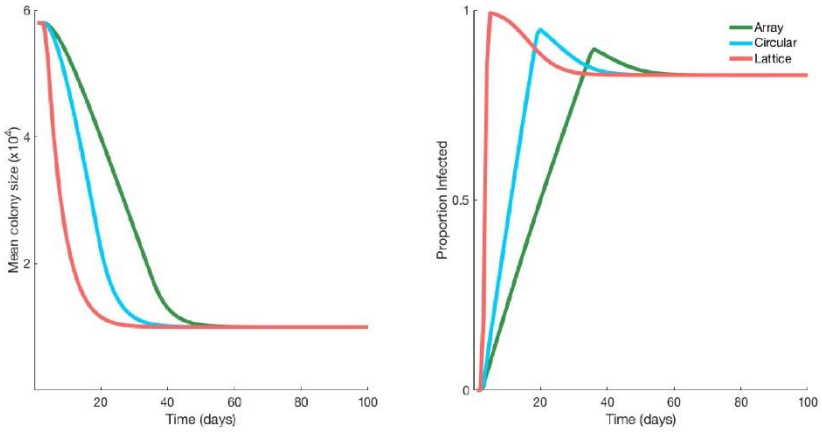


Figure S5. Graphs obtained from the mathematical model showing the rapid rate of spread throughout an apiary, where endemic disease equilibrium is quickly reached. Graphs show the case for each apiary arrangement (Array, Circular, Lattice) for an apiary of 100 colonies and movement rate between colonies of 0.02. The model starts with a single bee infected in one colony. The left panel shows the mean colony size, which closely matches the right panel showing proportion infected (compare also to the agent based simulation outputs Figs. S3 and S4.

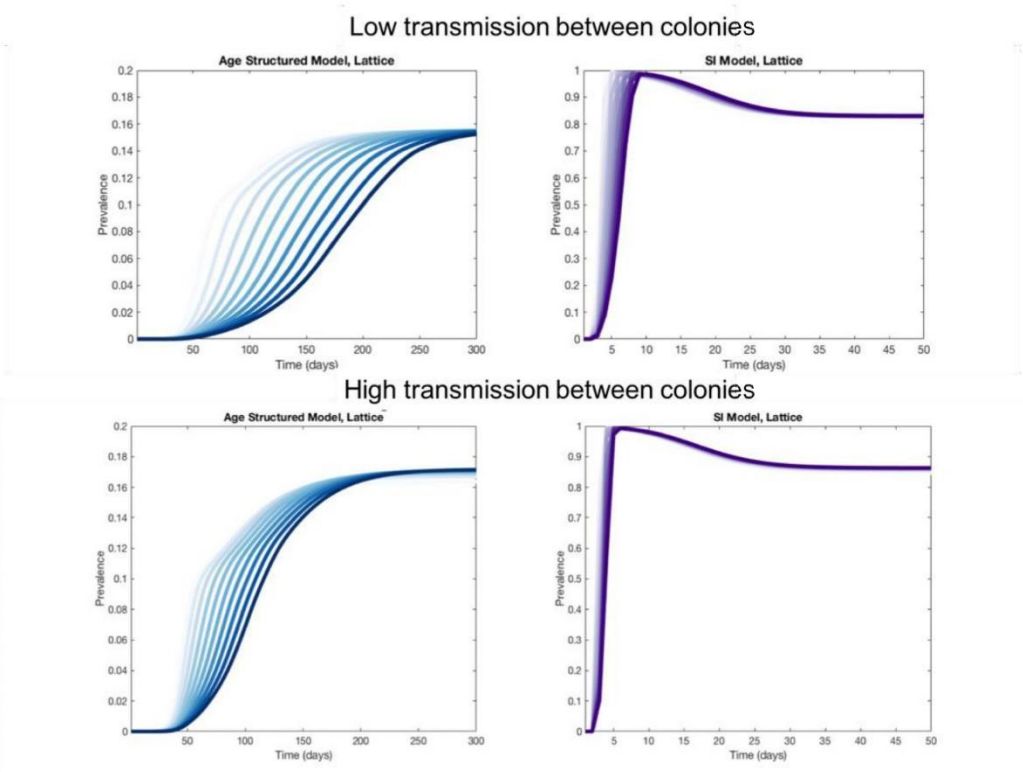


Figure S6. Comparison of the alternative (age-structured) model (left) behaviour and main (SI) model (right). **Note both axes differ in scales.** Plotted is the proportion of adult bees infected over time for the age structured model and proportion of all bees infected for the SI model, for ten apiary sizes (number of colonies) and two different inter-colony transmission rates (bee movement between colonies). The ten lines of different hues in each colour represent a unique number of colonies per apiary. Lightest colour represents an apiary with four hives and the darkest line represents an apiary with 121 hives. All simulations are for a lattice structure. The age-structured model shows much slower convergence than the SI model, however still converges in all cases in a single-season timescale. The largest apiaries in all cases take the longest amount of time to converge. Faster convergence is seen for the higher transmission rate between colonies, and slightly higher prevalences are apparent in the age-structured model for larger apiaries and higher inter-colony transmission rates.
Alternative model: phi=1600, a= a=1.04×10^{-4}, b=0.1*a or b=0.018*a, v=0.16, m=0.033, m'=0.01,gamma=0.1,g=0.0476 .
SI model: phi=1600, a=1.04×10^{-4}, b=0.1*a or b=0.018*a, v=0.16, m=0.0275.

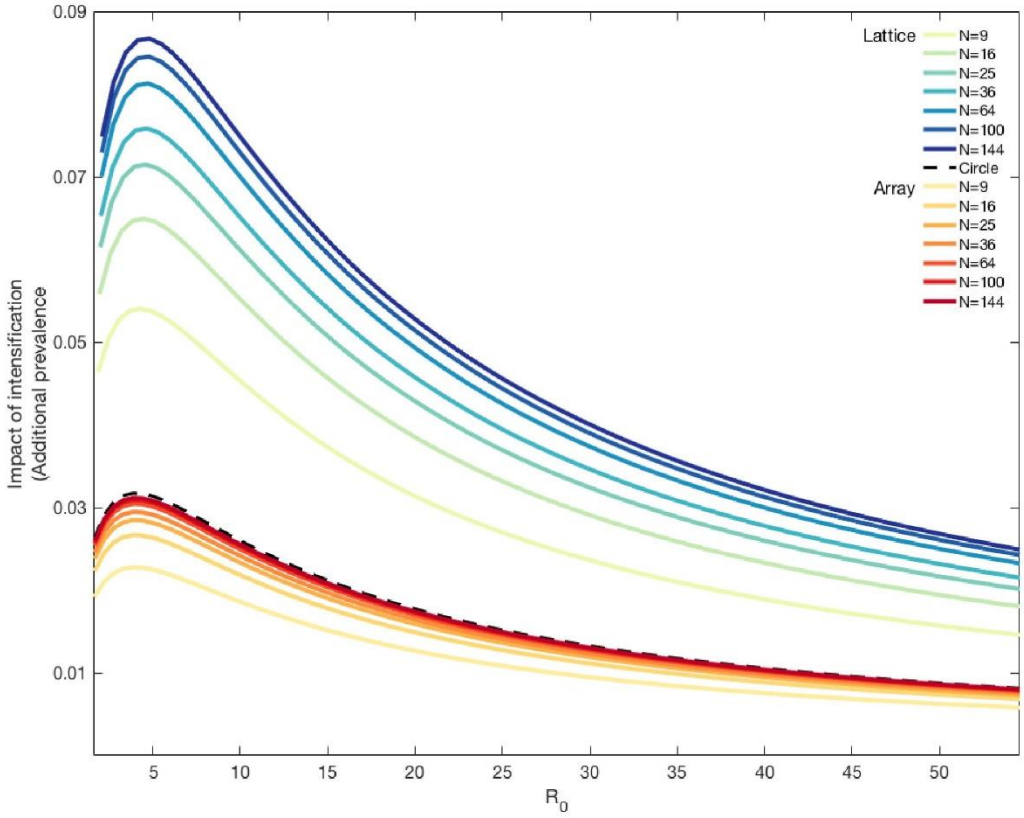


Figure S7. Impact of intensification on disease burden analytically derived from the mathematical model, showing only the effect of increasing apiary size under different spatial configurations; movement rate between colonies is held constant. The circular apiary dynamics are independent of intensification, thus remaining constant through intensification (i.e. increasing n). The impact of intensification for the circular apiary (of any size) is in black.

Supplementary Information Section 4

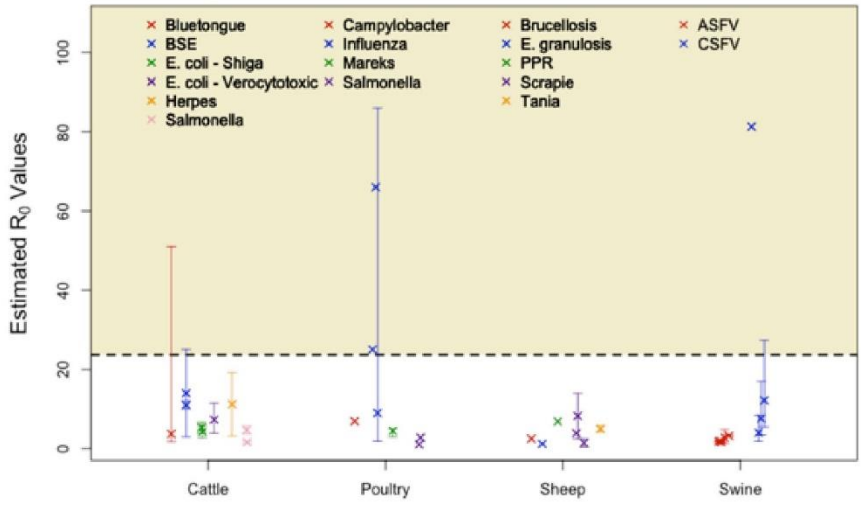


Figure S8: Examples of R_0 values for other agricultural livestock diseases (see Supplementary Information Section 4 for references), spanning a range of different farming stages and practices. This figure is not intended as an exhaustive or representative summary of agricultural disease R_0 values, but represents what is readily available in the literature. We highlight the lower boundary shown in Fig. 5, which is our best estimate of the lower R_0 value for *N. ceranae* in honeybees.

Table S1

Estimates for R_0 values for agricultural livestock diseases available across the literature.

R_0 Value	Lower	Upper	Host	Disease	Ref
4.5	3	5	Poultry	Mareks	Atkins et al 2013
3.24	3.21	3.27	Swine	ASFV	Barongo et al 2015
1.64	1.56	1.72	Swine	ASFV	Barongo et al 2015
1.91	1.87	1.94	Swine	ASFV	Barongo et al 2015
1.78	1.74	1.81	Swine	ASFV	Barongo et al 2015
66	66	66	Poultry	Influenza	Bos et al 2007
25	25	25	Poultry	Influenza	Bos et al 2007
5	4.2	5.8	Sheep	Tania	Cabrera et al 1995
1.2	1.2	1.2	Sheep	E. granulosis	Cabrera et al 1995
4	1.9	8.4	Swine	CSFV	Durand et al 2009
7.6	3.4	17	Swine	CSFV	Durand et al 2009
12.2	5.5	27.3	Swine	CSFV	Durand et al 2009
11	10	12	Cattle	BSE	Ferguson et al 1999
2.8	1.3	4.8	Swine	ASFV	Guinat et al 2016
18	6.9	46.9	Swine	ASFV	Sanches-Vizcaino et al 2015
9.8	3.9	15.6	Swine	ASFV	Gulenkin et al 2011
8.25	2.5	14	Sheep	Scrapie	Hagenaars et al 2003
2.5	2.5	2.5	Sheep	Brucellosis	Hou et al 2013
14	3	25	Cattle	BSE	Koeijer et al 2004
4.3	2.8	5.9	Cattle	E. coli - Shiga	Laegried and Keen 2004
5.3	3.9	6.6	Cattle	E. coli - Shiga	Laegried and Keen 2004
1.62	1.34	1.9	Cattle	Salmonella	Lanzas et al 2008
4.7	3.65	5.75	Cattle	Salmonella	Lanzas et al 2008
81.3	81.3	81.3	Swine	CSFV	Leavens et al 1998
3.9	3.9	3.9	Sheep	Scrapie	Matthews et al 1999
11.2	3.2	19.2	Cattle	Herpes	Mollema et al 2005
2.8	2.8	2.8	Poultry	Salmonella	Rabsch et al 2000
1.05	1.05	1.05	Poultry	Salmonella	Rabsch et al 2000
3.7	1.8	51	Cattle	Bluetongue	Santman-Berends et al 2013
7.3	3.92	11.51	Cattle	E. coli - Verocytotoxic	Schouten et al 2009
1.43	0.42	2.43	Sheep	Scrapie	Truscott and Ferguson 2009
6.85	6.85	6.85	Sheep	PPR	Zahur et al 2009
9	1.9	86	Poultry	Influenza	Bouma et al 2009
6.92	6.92	6.92	Poultry	Campylobacter	Goddard et al 2014

References – Section 4

Atkins, K.E., Read, A.F., Savill, N.J., Renz, K.G., Islam, A.F., Walkden-Brown, S.W. & Woolhouse, M.E.J. (2013) Vaccination and Reduced Cohort Duration Can Drive Virulence Evolution: Marek's Disease Virus and Industrialized Agriculture. *Evolution*, **67**, 851–860.

Barongo, M.B., Ståhl, K., Bett, B., Bishop, R.P., Fèvre, E.M., Aliro, T., Okoth, E., Masembe, C., Knobel, D. & Ssematimba, A. (2015) Estimating the Basic Reproductive Number (R0) for African Swine Fever Virus (ASFV) Transmission between Pig Herds in Uganda. *PLOS ONE*, **10**, e0125842.

Bos, M.E.H., Boven, M.V., Nielen, M., Bouma, A., Elbers, A.R.W., Nodelijk, G., Koch, G., Stegeman, A. & Jong, M.C.M.D. (2007) Estimating the day of highly pathogenic avian influenza (H7N7) virus introduction into a poultry flock based on mortality data. *Veterinary Research*, **38**, 493–504.

Bouma, A., Claassen, I., Natih, K., Klinkenberg, D., Donnelly, C.A., Koch, G. & Boven, M. van. (2009) Estimation of Transmission Parameters of H5N1 Avian Influenza Virus in Chickens. *PLOS Pathogens*, **5**, e1000281.

Cabrera, P.A., Haran, G., Benavidez, U., Valledor, S., Perera, G., Lloyd, S., Gemmell, M.A., Baraibar, M., Morana, A., Maissonave, J. & Carballo, M. (1995) Transmission dynamics of Echinococcus granulosus, Taenia hydatigena and Taenia ovis in sheep in uruguay. *International Journal for Parasitology*, **25**, 807–813.

Durand, B., Davila, S., Cariolet, R., Mesplède, A. & Le Potier, M.-F. (2009) Comparison of viraemia- and clinical-based estimates of within- and between-pen transmission of classical swine fever virus from three transmission experiments. *Veterinary Microbiology*, **135**, 196–204.

Ferguson, N.M., Donnelly, C.A., Woolhouse, M.E.J. & Anderson, R.M. (1999) Estimation of the basic reproduction number of BSE: the intensity of transmission in British cattle. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 23–32.

Goddard, A.D., Arnold, M.E., Allen, V.M. & Snary, E.L. (2014) Estimating the time at which commercial broiler flocks in Great Britain become infected with Campylobacter: a Bayesian approach. *Epidemiology & Infection*, **142**, 1884–1892.

Guinat, C., Gubbins, S., Vergne, T., Gonzales, J.L., Dixon, L. & Pfeiffer, D.U. (2016) Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. *Epidemiology & Infection*, **144**, 25–34.

Gulenkin, V.M., Korennoy, F.I., Karaulov, A.K. & Dudnikov, S.A. (2011) Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. *Preventive Veterinary Medicine*, **102**, 167–174.

Hagenaars, T.J., Donnelly, C.A., Ferguson, N.M. & Anderson, R.M. (2003) Dynamics of a scrapie outbreak in a flock of Romanov sheep – estimation of transmission parameters. *Epidemiology & Infection*, **131**, 1015–1022.

Hou, Q., Sun, X., Zhang, J., Liu, Y., Wang, Y. & Jin, Z. (2013) Modeling the transmission dynamics of sheep brucellosis in Inner Mongolia Autonomous Region, China. *Mathematical Biosciences*, **242**, 51–58.

Koeijer, A. de, Heesterbeek, H., Schreuder, B., Oberthür, R., Wilesmith, J., Roermund, H. van & Jong, M. de. (2004) Quantifying BSE control by calculating the basic reproduction ratio R0 for the infection among cattle. *Journal of Mathematical Biology*, **48**, 1–22.

Laegreid, W.W. & Keen, J.E. (2004) Estimation of the basic reproduction ratio (R0) for Shiga toxin-producing Escherichia coli O157:H7 (STEC O157) in beef calves. *Epidemiology & Infection*, **132**, 291–295.

Lanzas, C., Brien, S., Ivanek, R., Lo, Y., Chapagain, P.P., Ray, K.A., Ayscue, P., Warnick, L.D. & Gröhn, Y.T. (2008) The effect of heterogeneous infectious period and contagiousness on the dynamics of Salmonella transmission in dairy cattle. *Epidemiology & Infection*, **136**, 1496–1510.

Leavens, H., Koenen, F., Deluyker, H., Berkvens, D. & Kruif, A. de. (1998) An experimental infection with classical swine fever virus in weaner pigs. *Veterinary Quarterly*, **20**, 41–45.

Matthews, L., Woolhouse, M.E.J. & Hunter, N. (1999) The basic reproduction number for scrapie. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 1085–1090.

Mollema, L., Jong, M.C.M.D. & Boven, M.V. (2005) Prolonged persistence of bovine herpesvirus in small cattle herds: a model-based analysis. *Epidemiology & Infection*, **133**, 137–148.

Rabsch, W., Hargis, B.M., Tsolis, R.M., Kingsley, R.A., Hinz, K.H., Tschäpe, H. & Bäumler, A.J. (2000) Competitive exclusion of Salmonella enteritidis by Salmonella gallinarum in poultry. *Emerging Infectious Diseases*, **6**, 443–448.

Sánchez-Vizcaíno, J.M., Mur, L., Gomez-Villamandos, J.C. & Carrasco, L. (2015) An Update on the Epidemiology and Pathology of African Swine Fever. *Journal of Comparative Pathology*, **152**, 9–21.

Santman-Berends, I.M.G.A., Stegeman, J.A., Vellema, P. & van Schaik, G. (2013) Estimation of the reproduction ratio (R0) of bluetongue based on serological field data and comparison with other BTV transmission models. *Preventive Veterinary Medicine*, **108**, 276–284.

Schouten, J.M., Graat, E. a. M., Frankena, K., Zijderveld, F.V. & Jong, M.C.M.D. (2009) Transmission and quantification of verocytotoxin-producing Escherichia coli O157 in dairy cattle and calves. *Epidemiology & Infection*, **137**, 114–123.

Truscott, J.E. & Ferguson, N.M. (2009) Control of scrapie in the UK sheep population. *Epidemiology & Infection*, **137**, 775–786.

Zahur, A.B., Ullah, A., Irshad, H., Farooq, M.S., Hussain, M., Jahangir, M. & others. (2009) Epidemiological investigations of a peste des petits ruminants (PPR) outbreak in Afghan sheep in Pakistan. *Pakistan Veterinary Journal*, **29**, 174–178.

Supplementary Material 4 – Origin and management intensity leave lasting effects on honeybee colony viriomes.

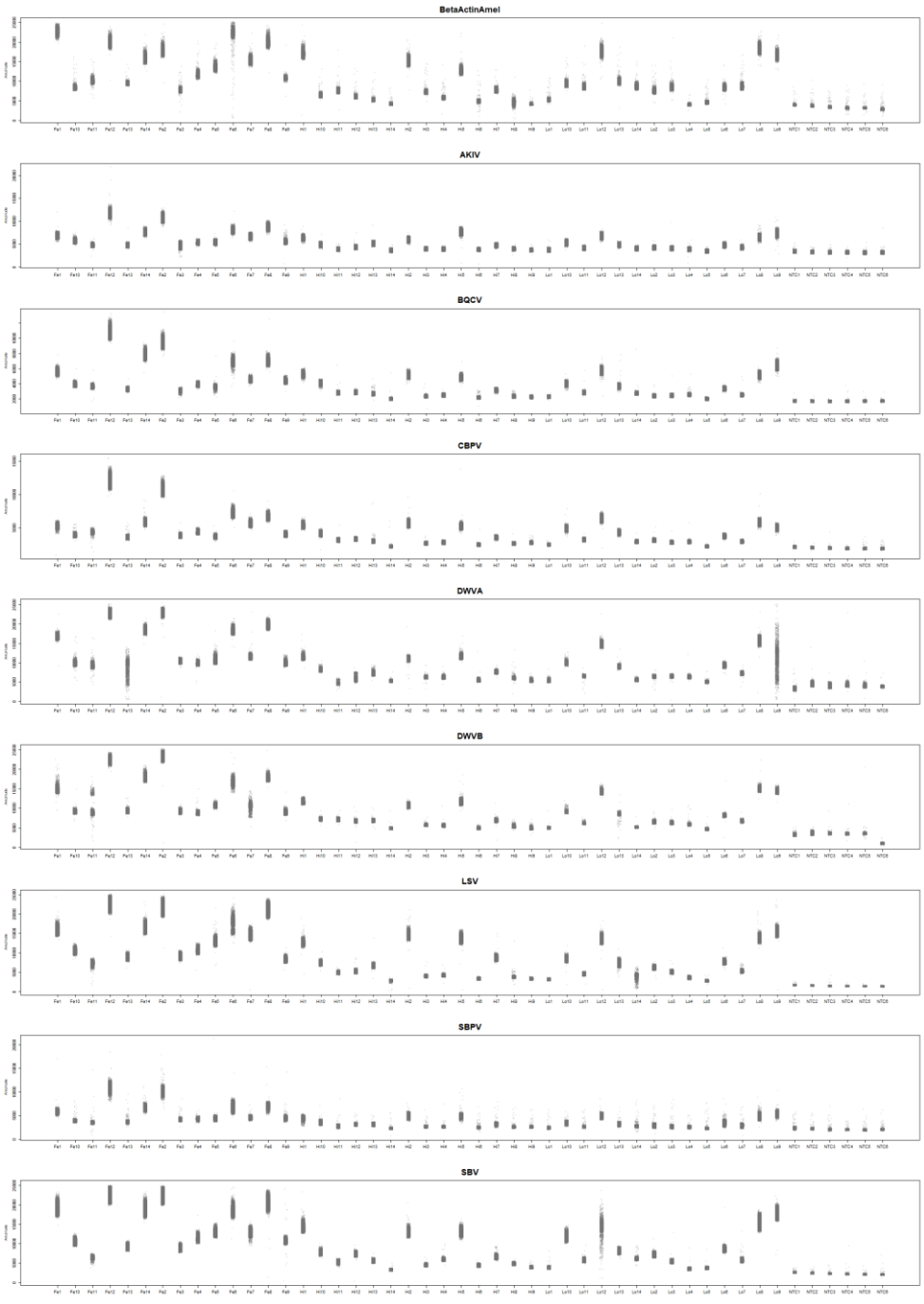


Figure S4.1 – Raw fluorescence reads for all targets including the housekeeping gene (top, 'BetaActinAmel'). 'No-template-controls' (NTCs) are plotted for the final 6, rightmost x-axis points on each panel. Colonies correspond vertically such that order along each panel's x-axis is maintained.

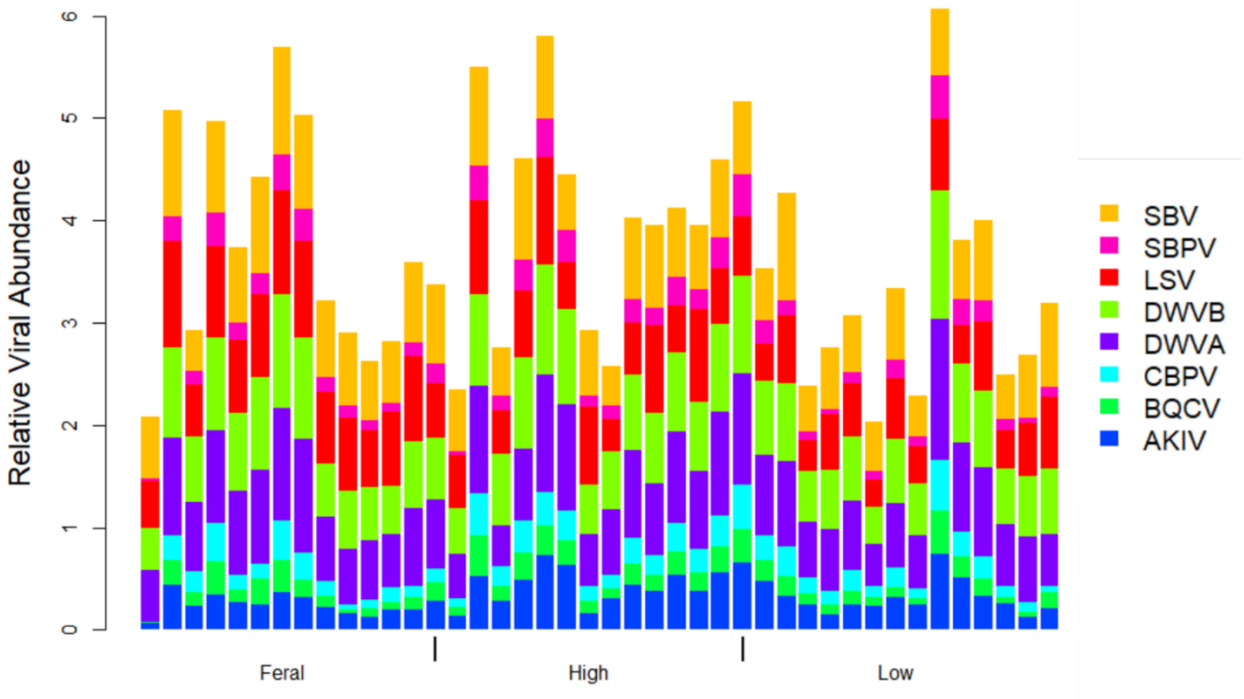


Figure S4.2 - Alternative visualisation of the relative abundance data plotted in fig. 4.2, calculated from median-average data and used for the dimensionality reduction analyses in fig. 4.3. Relative viral abundance is scaled against the housekeeping gene, and colonies are group by origin on the x-axis. Colonies clearly vary in their total viral relative abundance as well as their relative abundance for each virus.

Table S4.1 **–** Model selection results across all 1000 bootstrapped samples used for GLMM fitting. Leftmost column shows the best performing model according to the Bayesian information criterion (BIC). Centre column shows comparison between the GLMM include an interaction effect between origin and viral target and the GLMM including viral target and origin as non-interacting, additive fixed effects. Rightmost column shows the comparison between the GLMM with non-interacting, additive fixed effects of viral target and a null model including only viral target. Across all 1000 iterations the interaction model was always the best performing.

Best Model (BIC)	Model Comparison p-value: Interaction Model - Additive Model	Model Comparison p-value: Additive Model - Null Model
Interaction	< 0.00001	0.135658383
Interaction	< 0.00001	0.137328093
Interaction	< 0.00001	0.135685433
Interaction	< 0.00001	0.136553847
Interaction	< 0.00001	0.138143244
Interaction	< 0.00001	0.137239037
Interaction	< 0.00001	0.139891378
Interaction	< 0.00001	0.138427531
Interaction	< 0.00001	0.136303994
Interaction	< 0.00001	0.13823584
Interaction	< 0.00001	0.137265553
Interaction	< 0.00001	0.135826458
Interaction	< 0.00001	0.135295978
Interaction	< 0.00001	0.138250376
Interaction	< 0.00001	0.139737914
Interaction	< 0.00001	0.138952676
Interaction	< 0.00001	0.139718599
Interaction	< 0.00001	0.136554682
Interaction	< 0.00001	0.133382937
Interaction	< 0.00001	0.138587663
Interaction	< 0.00001	0.138056536
Interaction	< 0.00001	0.136657796
Interaction	< 0.00001	0.139251294
Interaction	< 0.00001	0.135362549
Interaction	< 0.00001	0.137326709
Interaction	< 0.00001	0.137485684
Interaction	< 0.00001	0.136981937
Interaction	< 0.00001	0.135738407
Interaction	< 0.00001	0.138294767
Interaction	< 0.00001	0.138997221
Interaction	< 0.00001	0.135414491
Interaction	< 0.00001	0.138005852
Interaction	< 0.00001	0.139048942
Interaction	< 0.00001	0.138639772
Interaction	< 0.00001	0.138663551
Interaction	< 0.00001	0.135209356
Interaction	< 0.00001	0.138581021
Interaction	< 0.00001	0.136933051
Interaction	< 0.00001	0.138201352
Interaction	< 0.00001	0.139850428
Interaction	< 0.00001	0.138552661
Interaction	< 0.00001	0.139606425

Interaction	< 0.00001	0.136746886
Interaction	< 0.00001	0.136627678
Interaction	< 0.00001	0.140519881
Interaction	< 0.00001	0.137691802
Interaction	< 0.00001	0.136855133
Interaction	< 0.00001	0.137042451
Interaction	< 0.00001	0.138050102
Interaction	< 0.00001	0.138190096
Interaction	< 0.00001	0.138292914
Interaction	< 0.00001	0.137864432
Interaction	< 0.00001	0.138248171
Interaction	< 0.00001	0.138085966
Interaction	< 0.00001	0.135312823
Interaction	< 0.00001	0.138351836
Interaction	< 0.00001	0.13706965
Interaction	< 0.00001	0.134288684
Interaction	< 0.00001	0.139167655
Interaction	< 0.00001	0.138637988
Interaction	< 0.00001	0.141549856
Interaction	< 0.00001	0.138336151
Interaction	< 0.00001	0.137099021
Interaction	< 0.00001	0.135873927
Interaction	< 0.00001	0.13821601
Interaction	< 0.00001	0.138710308
Interaction	< 0.00001	0.13908637
Interaction	< 0.00001	0.138818481
Interaction	< 0.00001	0.139791322
Interaction	< 0.00001	0.134330098
Interaction	< 0.00001	0.138406305
Interaction	< 0.00001	0.137821836
Interaction	< 0.00001	0.140537154
Interaction	< 0.00001	0.136352387
Interaction	< 0.00001	0.139693639
Interaction	< 0.00001	0.139651256
Interaction	< 0.00001	0.136767998
Interaction	< 0.00001	0.13712084
Interaction	< 0.00001	0.136807235
Interaction	< 0.00001	0.137485704
Interaction	< 0.00001	0.137789999
Interaction	< 0.00001	0.139430192
Interaction	< 0.00001	0.139356175
Interaction	< 0.00001	0.139545061
Interaction	< 0.00001	0.136915276
Interaction	< 0.00001	0.136398719
Interaction	< 0.00001	0.138708475
Interaction	< 0.00001	0.13629569
Interaction	< 0.00001	0.139137517
Interaction	< 0.00001	0.13836038
Interaction	< 0.00001	0.136624907
Interaction	< 0.00001	0.138439421
Interaction	< 0.00001	0.137727009
Interaction	< 0.00001	0.137338685
Interaction	< 0.00001	0.135638077
Interaction	< 0.00001	0.138868773
Interaction	< 0.00001	0.134969463
Interaction	< 0.00001	0.140218315
Interaction	< 0.00001	0.135868682
Interaction	< 0.00001	0.139332636
Interaction	< 0.00001	0.137355103

Interaction	< 0.00001	0.138265567
Interaction	< 0.00001	0.135134634
Interaction	< 0.00001	0.138504472
Interaction	< 0.00001	0.13973659
Interaction	< 0.00001	0.136081334
Interaction	< 0.00001	0.138759496
Interaction	< 0.00001	0.137823271
Interaction	< 0.00001	0.137040247
Interaction	< 0.00001	0.141533121
Interaction	< 0.00001	0.138998456
Interaction	< 0.00001	0.139974639
Interaction	< 0.00001	0.135543135
Interaction	< 0.00001	0.138984393
Interaction	< 0.00001	0.135854914
Interaction	< 0.00001	0.138176406
Interaction	< 0.00001	0.137326227
Interaction	< 0.00001	0.138117433
Interaction	< 0.00001	0.139602402
Interaction	< 0.00001	0.138277407
Interaction	< 0.00001	0.137101395
Interaction	< 0.00001	0.135102884
Interaction	< 0.00001	0.138071682
Interaction	< 0.00001	0.137126475
Interaction	< 0.00001	0.138172764
Interaction	< 0.00001	0.137456806
Interaction	< 0.00001	0.138391609
Interaction	< 0.00001	0.136346704
Interaction	< 0.00001	0.13757595
Interaction	< 0.00001	0.136187502
Interaction	< 0.00001	0.137223414
Interaction	< 0.00001	0.138515636
Interaction	< 0.00001	0.1386019
Interaction	< 0.00001	0.136464724
Interaction	< 0.00001	0.138153319
Interaction	< 0.00001	0.138948379
Interaction	< 0.00001	0.138592302
Interaction	< 0.00001	0.138343195
Interaction	< 0.00001	0.138916355
Interaction	< 0.00001	0.138159952
Interaction	< 0.00001	0.139538301
Interaction	< 0.00001	0.135902709
Interaction	< 0.00001	0.138380091
Interaction	< 0.00001	0.138694747
Interaction	< 0.00001	0.137241149
Interaction	< 0.00001	0.139927807
Interaction	< 0.00001	0.136735166
Interaction	< 0.00001	0.138349657
Interaction	< 0.00001	0.137217983
Interaction	< 0.00001	0.138568232
Interaction	< 0.00001	0.139896799
Interaction	< 0.00001	0.137892811
Interaction	< 0.00001	0.13625929
Interaction	< 0.00001	0.134971899
Interaction	< 0.00001	0.137518522
Interaction	< 0.00001	0.139411224
Interaction	< 0.00001	0.137894633
Interaction	< 0.00001	0.139399836
Interaction	< 0.00001	0.136993765
Interaction	< 0.00001	0.13771898

Interaction	< 0.00001	0.136889526
Interaction	< 0.00001	0.138849172
Interaction	< 0.00001	0.13857746
Interaction	< 0.00001	0.137507001
Interaction	< 0.00001	0.13837759
Interaction	< 0.00001	0.135665121
Interaction	< 0.00001	0.136343561
Interaction	< 0.00001	0.136635757
Interaction	< 0.00001	0.136616685
Interaction	< 0.00001	0.137884617
Interaction	< 0.00001	0.137660506
Interaction	< 0.00001	0.139273382
Interaction	< 0.00001	0.140832829
Interaction	< 0.00001	0.138820977
Interaction	< 0.00001	0.138116019
Interaction	< 0.00001	0.137980601
Interaction	< 0.00001	0.138457704
Interaction	< 0.00001	0.141310875
Interaction	< 0.00001	0.13995824
Interaction	< 0.00001	0.137465306
Interaction	< 0.00001	0.1380728
Interaction	< 0.00001	0.13624365
Interaction	< 0.00001	0.138165139
Interaction	< 0.00001	0.136114178
Interaction	< 0.00001	0.139052225
Interaction	< 0.00001	0.137660484
Interaction	< 0.00001	0.139174022
Interaction	< 0.00001	0.138169761
Interaction	< 0.00001	0.13825807
Interaction	< 0.00001	0.134656361
Interaction	< 0.00001	0.136917877
Interaction	< 0.00001	0.140377295
Interaction	< 0.00001	0.13734707
Interaction	< 0.00001	0.137799823
Interaction	< 0.00001	0.136437116
Interaction	< 0.00001	0.137694317
Interaction	< 0.00001	0.139565987
Interaction	< 0.00001	0.137989472
Interaction	< 0.00001	0.138638968
Interaction	< 0.00001	0.136766526
Interaction	< 0.00001	0.13882953
Interaction	< 0.00001	0.139166064
Interaction	< 0.00001	0.135967596
Interaction	< 0.00001	0.136545327
Interaction	< 0.00001	0.137766784
Interaction	< 0.00001	0.135880468
Interaction	< 0.00001	0.139177168
Interaction	< 0.00001	0.136715733
Interaction	< 0.00001	0.136975121
Interaction	< 0.00001	0.137792272
Interaction	< 0.00001	0.138208248
Interaction	< 0.00001	0.138425787
Interaction	< 0.00001	0.135127881
Interaction	< 0.00001	0.136675555
Interaction	< 0.00001	0.137998841
Interaction	< 0.00001	0.135083922
Interaction	< 0.00001	0.1361597
Interaction	< 0.00001	0.138114379
Interaction	< 0.00001	0.139457049

Interaction	< 0.00001	0.136617547
Interaction	< 0.00001	0.134806755
Interaction	< 0.00001	0.138469865
Interaction	< 0.00001	0.136511915
Interaction	< 0.00001	0.137108204
Interaction	< 0.00001	0.135746668
Interaction	< 0.00001	0.136468423
Interaction	< 0.00001	0.136892347
Interaction	< 0.00001	0.137021613
Interaction	< 0.00001	0.139975708
Interaction	< 0.00001	0.137379398
Interaction	< 0.00001	0.138175997
Interaction	< 0.00001	0.140261266
Interaction	< 0.00001	0.136510132
Interaction	< 0.00001	0.137021697
Interaction	< 0.00001	0.13581923
Interaction	< 0.00001	0.135255807
Interaction	< 0.00001	0.137712795
Interaction	< 0.00001	0.137385552
Interaction	< 0.00001	0.138377234
Interaction	< 0.00001	0.139025186
Interaction	< 0.00001	0.13576828
Interaction	< 0.00001	0.139760159
Interaction	< 0.00001	0.137882297
Interaction	< 0.00001	0.138906332
Interaction	< 0.00001	0.139067071
Interaction	< 0.00001	0.13751389
Interaction	< 0.00001	0.137464511
Interaction	< 0.00001	0.138515978
Interaction	< 0.00001	0.139317825
Interaction	< 0.00001	0.137755699
Interaction	< 0.00001	0.13926038
Interaction	< 0.00001	0.136908867
Interaction	< 0.00001	0.138103286
Interaction	< 0.00001	0.138441952
Interaction	< 0.00001	0.138892541
Interaction	< 0.00001	0.141736402
Interaction	< 0.00001	0.136950011
Interaction	< 0.00001	0.139496325
Interaction	< 0.00001	0.134537556
Interaction	< 0.00001	0.139432582
Interaction	< 0.00001	0.138189705
Interaction	< 0.00001	0.138825778
Interaction	< 0.00001	0.137845569
Interaction	< 0.00001	0.139946976
Interaction	< 0.00001	0.137154754
Interaction	< 0.00001	0.135918263
Interaction	< 0.00001	0.138052261
Interaction	< 0.00001	0.139630034
Interaction	< 0.00001	0.139107428
Interaction	< 0.00001	0.139018107
Interaction	< 0.00001	0.140641145
Interaction	< 0.00001	0.137819945
Interaction	< 0.00001	0.136474271
Interaction	< 0.00001	0.139072177
Interaction	< 0.00001	0.138256746
Interaction	< 0.00001	0.137077297
Interaction	< 0.00001	0.136849047
Interaction	< 0.00001	0.137648543

Interaction	< 0.00001	0.137352688
Interaction	< 0.00001	0.138784722
Interaction	< 0.00001	0.138033693
Interaction	< 0.00001	0.139131674
Interaction	< 0.00001	0.138657158
Interaction	< 0.00001	0.136233753
Interaction	< 0.00001	0.137148827
Interaction	< 0.00001	0.135371007
Interaction	< 0.00001	0.137171198
Interaction	< 0.00001	0.139239138
Interaction	< 0.00001	0.136226386
Interaction	< 0.00001	0.136802766
Interaction	< 0.00001	0.134068889
Interaction	< 0.00001	0.138042002
Interaction	< 0.00001	0.138819985
Interaction	< 0.00001	0.136481545
Interaction	< 0.00001	0.138707769
Interaction	< 0.00001	0.139456208
Interaction	< 0.00001	0.13964001
Interaction	< 0.00001	0.139096946
Interaction	< 0.00001	0.134322103
Interaction	< 0.00001	0.137185235
Interaction	< 0.00001	0.137007193
Interaction	< 0.00001	0.136625873
Interaction	< 0.00001	0.137694766
Interaction	< 0.00001	0.137626812
Interaction	< 0.00001	0.134490008
Interaction	< 0.00001	0.1395876
Interaction	< 0.00001	0.138862813
Interaction	< 0.00001	0.137929093
Interaction	< 0.00001	0.138828936
Interaction	< 0.00001	0.139651914
Interaction	< 0.00001	0.136028462
Interaction	< 0.00001	0.138943465
Interaction	< 0.00001	0.136090507
Interaction	< 0.00001	0.140389715
Interaction	< 0.00001	0.136765498
Interaction	< 0.00001	0.136897537
Interaction	< 0.00001	0.139143812
Interaction	< 0.00001	0.139066768
Interaction	< 0.00001	0.137897288
Interaction	< 0.00001	0.139103624
Interaction	< 0.00001	0.138408338
Interaction	< 0.00001	0.13677574
Interaction	< 0.00001	0.140710352
Interaction	< 0.00001	0.136737908
Interaction	< 0.00001	0.137517598
Interaction	< 0.00001	0.140572024
Interaction	< 0.00001	0.13888414
Interaction	< 0.00001	0.136559996
Interaction	< 0.00001	0.135794646
Interaction	< 0.00001	0.139098314
Interaction	< 0.00001	0.137055206
Interaction	< 0.00001	0.137939854
Interaction	< 0.00001	0.135622505
Interaction	< 0.00001	0.139176549
Interaction	< 0.00001	0.137791116
Interaction	< 0.00001	0.138766609
Interaction	< 0.00001	0.137353109

Interaction	< 0.00001	0.137969769
Interaction	< 0.00001	0.140065191
Interaction	< 0.00001	0.13875108
Interaction	< 0.00001	0.139012156
Interaction	< 0.00001	0.137856885
Interaction	< 0.00001	0.137615137
Interaction	< 0.00001	0.136870064
Interaction	< 0.00001	0.136575378
Interaction	< 0.00001	0.135576223
Interaction	< 0.00001	0.139845719
Interaction	< 0.00001	0.137405674
Interaction	< 0.00001	0.14070838
Interaction	< 0.00001	0.139174851
Interaction	< 0.00001	0.138389982
Interaction	< 0.00001	0.136353482
Interaction	< 0.00001	0.13958038
Interaction	< 0.00001	0.138077609
Interaction	< 0.00001	0.135981871
Interaction	< 0.00001	0.138692945
Interaction	< 0.00001	0.140399897
Interaction	< 0.00001	0.138975771
Interaction	< 0.00001	0.139085691
Interaction	< 0.00001	0.136844186
Interaction	< 0.00001	0.136133583
Interaction	< 0.00001	0.138930834
Interaction	< 0.00001	0.138537129
Interaction	< 0.00001	0.136456892
Interaction	< 0.00001	0.135114284
Interaction	< 0.00001	0.136966404
Interaction	< 0.00001	0.135861632
Interaction	< 0.00001	0.136271187
Interaction	< 0.00001	0.138348506
Interaction	< 0.00001	0.1381281
Interaction	< 0.00001	0.139371196
Interaction	< 0.00001	0.137991952
Interaction	< 0.00001	0.137983651
Interaction	< 0.00001	0.13748818
Interaction	< 0.00001	0.138999423
Interaction	< 0.00001	0.137438133
Interaction	< 0.00001	0.137876428
Interaction	< 0.00001	0.137929355
Interaction	< 0.00001	0.138181248
Interaction	< 0.00001	0.136894185
Interaction	< 0.00001	0.13799729
Interaction	< 0.00001	0.138959206
Interaction	< 0.00001	0.138907909
Interaction	< 0.00001	0.140184181
Interaction	< 0.00001	0.139471501
Interaction	< 0.00001	0.139398916
Interaction	< 0.00001	0.137977688
Interaction	< 0.00001	0.138159653
Interaction	< 0.00001	0.137173401
Interaction	< 0.00001	0.139568689
Interaction	< 0.00001	0.134678524
Interaction	< 0.00001	0.138833025
Interaction	< 0.00001	0.138426842
Interaction	< 0.00001	0.137623691
Interaction	< 0.00001	0.138935359
Interaction	< 0.00001	0.135543491

Interaction	< 0.00001	0.137919422
Interaction	< 0.00001	0.136717748
Interaction	< 0.00001	0.137621358
Interaction	< 0.00001	0.137270529
Interaction	< 0.00001	0.137243027
Interaction	< 0.00001	0.138671487
Interaction	< 0.00001	0.137809495
Interaction	< 0.00001	0.137546747
Interaction	< 0.00001	0.138900616
Interaction	< 0.00001	0.139121146
Interaction	< 0.00001	0.137707583
Interaction	< 0.00001	0.140020828
Interaction	< 0.00001	0.13862581
Interaction	< 0.00001	0.139871485
Interaction	< 0.00001	0.133697402
Interaction	< 0.00001	0.140491981
Interaction	< 0.00001	0.137991074
Interaction	< 0.00001	0.138853983
Interaction	< 0.00001	0.13720786
Interaction	< 0.00001	0.138460109
Interaction	< 0.00001	0.139134723
Interaction	< 0.00001	0.139731195
Interaction	< 0.00001	0.137628412
Interaction	< 0.00001	0.139178975
Interaction	< 0.00001	0.138909326
Interaction	< 0.00001	0.136964638
Interaction	< 0.00001	0.139014661
Interaction	< 0.00001	0.139389319
Interaction	< 0.00001	0.137110621
Interaction	< 0.00001	0.137896244
Interaction	< 0.00001	0.140922589
Interaction	< 0.00001	0.136240048
Interaction	< 0.00001	0.137313023
Interaction	< 0.00001	0.13811238
Interaction	< 0.00001	0.139335961
Interaction	< 0.00001	0.138432651
Interaction	< 0.00001	0.137937478
Interaction	< 0.00001	0.139040874
Interaction	< 0.00001	0.137332462
Interaction	< 0.00001	0.135579054
Interaction	< 0.00001	0.137932414
Interaction	< 0.00001	0.138973036
Interaction	< 0.00001	0.138972446
Interaction	< 0.00001	0.135376383
Interaction	< 0.00001	0.136921932
Interaction	< 0.00001	0.136690232
Interaction	< 0.00001	0.13848557
Interaction	< 0.00001	0.138750862
Interaction	< 0.00001	0.139479847
Interaction	< 0.00001	0.13930067
Interaction	< 0.00001	0.136909701
Interaction	< 0.00001	0.136613317
Interaction	< 0.00001	0.136541162
Interaction	< 0.00001	0.137394703
Interaction	< 0.00001	0.137058531
Interaction	< 0.00001	0.139482262
Interaction	< 0.00001	0.139106021
Interaction	< 0.00001	0.137290623
Interaction	< 0.00001	0.138651442

Interaction	< 0.00001	0.137265644
Interaction	< 0.00001	0.138202844
Interaction	< 0.00001	0.136382157
Interaction	< 0.00001	0.138487687
Interaction	< 0.00001	0.138649538
Interaction	< 0.00001	0.135807062
Interaction	< 0.00001	0.135595748
Interaction	< 0.00001	0.136542181
Interaction	< 0.00001	0.137536942
Interaction	< 0.00001	0.13643177
Interaction	< 0.00001	0.136224796
Interaction	< 0.00001	0.136431791
Interaction	< 0.00001	0.137214278
Interaction	< 0.00001	0.137870214
Interaction	< 0.00001	0.141782437
Interaction	< 0.00001	0.135818922
Interaction	< 0.00001	0.137412479
Interaction	< 0.00001	0.137938259
Interaction	< 0.00001	0.138816625
Interaction	< 0.00001	0.138286101
Interaction	< 0.00001	0.136866349
Interaction	< 0.00001	0.138592509
Interaction	< 0.00001	0.135223056
Interaction	< 0.00001	0.137849151
Interaction	< 0.00001	0.136032334
Interaction	< 0.00001	0.136073407
Interaction	< 0.00001	0.140401066
Interaction	< 0.00001	0.139050698
Interaction	< 0.00001	0.136271576
Interaction	< 0.00001	0.135563421
Interaction	< 0.00001	0.136470535
Interaction	< 0.00001	0.13844039
Interaction	< 0.00001	0.135492664
Interaction	< 0.00001	0.137327497
Interaction	< 0.00001	0.138185495
Interaction	< 0.00001	0.139088631
Interaction	< 0.00001	0.137643837
Interaction	< 0.00001	0.1384817
Interaction	< 0.00001	0.137180068
Interaction	< 0.00001	0.13718976
Interaction	< 0.00001	0.135923336
Interaction	< 0.00001	0.139142431
Interaction	< 0.00001	0.137248136
Interaction	< 0.00001	0.136434734
Interaction	< 0.00001	0.13956622
Interaction	< 0.00001	0.137463449
Interaction	< 0.00001	0.137553675
Interaction	< 0.00001	0.13862903
Interaction	< 0.00001	0.13814827
Interaction	< 0.00001	0.138503232
Interaction	< 0.00001	0.138093999
Interaction	< 0.00001	0.136242126
Interaction	< 0.00001	0.135180292
Interaction	< 0.00001	0.137774381
Interaction	< 0.00001	0.138368171
Interaction	< 0.00001	0.13754125
Interaction	< 0.00001	0.137739739
Interaction	< 0.00001	0.136683907
Interaction	< 0.00001	0.137989591

Interaction	< 0.00001	0.138145946
Interaction	< 0.00001	0.136816726
Interaction	< 0.00001	0.138581431
Interaction	< 0.00001	0.137553914
Interaction	< 0.00001	0.13538748
Interaction	< 0.00001	0.140415321
Interaction	< 0.00001	0.138884398
Interaction	< 0.00001	0.137581341
Interaction	< 0.00001	0.138192761
Interaction	< 0.00001	0.135305079
Interaction	< 0.00001	0.13834333
Interaction	< 0.00001	0.138502561
Interaction	< 0.00001	0.134893362
Interaction	< 0.00001	0.137089198
Interaction	< 0.00001	0.137750371
Interaction	< 0.00001	0.136648664
Interaction	< 0.00001	0.139805097
Interaction	< 0.00001	0.138805168
Interaction	< 0.00001	0.136360925
Interaction	< 0.00001	0.139861385
Interaction	< 0.00001	0.139756705
Interaction	< 0.00001	0.137757377
Interaction	< 0.00001	0.138195364
Interaction	< 0.00001	0.137780379
Interaction	< 0.00001	0.136655123
Interaction	< 0.00001	0.138281663
Interaction	< 0.00001	0.133776606
Interaction	< 0.00001	0.13953377
Interaction	< 0.00001	0.138413034
Interaction	< 0.00001	0.136347981
Interaction	< 0.00001	0.138309655
Interaction	< 0.00001	0.138232215
Interaction	< 0.00001	0.13790678
Interaction	< 0.00001	0.139870763
Interaction	< 0.00001	0.138375311
Interaction	< 0.00001	0.140233653
Interaction	< 0.00001	0.139995449
Interaction	< 0.00001	0.137441692
Interaction	< 0.00001	0.136740587
Interaction	< 0.00001	0.138966362
Interaction	< 0.00001	0.139225354
Interaction	< 0.00001	0.135843684
Interaction	< 0.00001	0.137970993
Interaction	< 0.00001	0.139665179
Interaction	< 0.00001	0.135594765
Interaction	< 0.00001	0.136115882
Interaction	< 0.00001	0.138684939
Interaction	< 0.00001	0.137084357
Interaction	< 0.00001	0.137767717
Interaction	< 0.00001	0.138056777
Interaction	< 0.00001	0.137695461
Interaction	< 0.00001	0.13830448
Interaction	< 0.00001	0.138405771
Interaction	< 0.00001	0.137486434
Interaction	< 0.00001	0.140172262
Interaction	< 0.00001	0.137864128
Interaction	< 0.00001	0.138594044
Interaction	< 0.00001	0.136251879
Interaction	< 0.00001	0.139393954

Interaction	< 0.00001	0.140625504
Interaction	< 0.00001	0.137443597
Interaction	< 0.00001	0.137471174
Interaction	< 0.00001	0.139144516
Interaction	< 0.00001	0.136611402
Interaction	< 0.00001	0.139052685
Interaction	< 0.00001	0.137062273
Interaction	< 0.00001	0.136318564
Interaction	< 0.00001	0.13921202
Interaction	< 0.00001	0.13969889
Interaction	< 0.00001	0.138436195
Interaction	< 0.00001	0.133472804
Interaction	< 0.00001	0.137320923
Interaction	< 0.00001	0.135677719
Interaction	< 0.00001	0.13594961
Interaction	< 0.00001	0.139894432
Interaction	< 0.00001	0.139862756
Interaction	< 0.00001	0.139225645
Interaction	< 0.00001	0.138602703
Interaction	< 0.00001	0.134909012
Interaction	< 0.00001	0.137928673
Interaction	< 0.00001	0.139795543
Interaction	< 0.00001	0.137751971
Interaction	< 0.00001	0.138292005
Interaction	< 0.00001	0.13570015
Interaction	< 0.00001	0.139908318
Interaction	< 0.00001	0.139903349
Interaction	< 0.00001	0.139652832
Interaction	< 0.00001	0.135834946
Interaction	< 0.00001	0.139401385
Interaction	< 0.00001	0.135680775
Interaction	< 0.00001	0.138611871
Interaction	< 0.00001	0.13677191
Interaction	< 0.00001	0.13895399
Interaction	< 0.00001	0.13881762
Interaction	< 0.00001	0.138543074
Interaction	< 0.00001	0.1387813
Interaction	< 0.00001	0.136750322
Interaction	< 0.00001	0.138858051
Interaction	< 0.00001	0.138229614
Interaction	< 0.00001	0.135818735
Interaction	< 0.00001	0.138851985
Interaction	< 0.00001	0.137163174
Interaction	< 0.00001	0.138871256
Interaction	< 0.00001	0.136182725
Interaction	< 0.00001	0.140086924
Interaction	< 0.00001	0.137703802
Interaction	< 0.00001	0.138214962
Interaction	< 0.00001	0.138290234
Interaction	< 0.00001	0.135977554
Interaction	< 0.00001	0.138825456
Interaction	< 0.00001	0.137729956
Interaction	< 0.00001	0.136933181
Interaction	< 0.00001	0.136769892
Interaction	< 0.00001	0.140020484
Interaction	< 0.00001	0.139001279
Interaction	< 0.00001	0.138904545
Interaction	< 0.00001	0.137099985
Interaction	< 0.00001	0.139018528

Interaction	< 0.00001	0.135056952
Interaction	< 0.00001	0.138164316
Interaction	< 0.00001	0.139395109
Interaction	< 0.00001	0.136259479
Interaction	< 0.00001	0.138401789
Interaction	< 0.00001	0.13806975
Interaction	< 0.00001	0.136037141
Interaction	< 0.00001	0.137772514
Interaction	< 0.00001	0.138206528
Interaction	< 0.00001	0.137980082
Interaction	< 0.00001	0.134948726
Interaction	< 0.00001	0.136099776
Interaction	< 0.00001	0.139453604
Interaction	< 0.00001	0.138707693
Interaction	< 0.00001	0.138590795
Interaction	< 0.00001	0.136553956
Interaction	< 0.00001	0.136095392
Interaction	< 0.00001	0.140766781
Interaction	< 0.00001	0.136864962
Interaction	< 0.00001	0.138262127
Interaction	< 0.00001	0.135454888
Interaction	< 0.00001	0.138748749
Interaction	< 0.00001	0.136836866
Interaction	< 0.00001	0.136579788
Interaction	< 0.00001	0.137205332
Interaction	< 0.00001	0.139513044
Interaction	< 0.00001	0.141400345
Interaction	< 0.00001	0.138726777
Interaction	< 0.00001	0.135920285
Interaction	< 0.00001	0.137069844
Interaction	< 0.00001	0.136605388
Interaction	< 0.00001	0.136357976
Interaction	< 0.00001	0.137877485
Interaction	< 0.00001	0.136795386
Interaction	< 0.00001	0.137125233
Interaction	< 0.00001	0.138123554
Interaction	< 0.00001	0.13889483
Interaction	< 0.00001	0.139108891
Interaction	< 0.00001	0.139820901
Interaction	< 0.00001	0.137548802
Interaction	< 0.00001	0.137691373
Interaction	< 0.00001	0.136893858
Interaction	< 0.00001	0.135705552
Interaction	< 0.00001	0.135137899
Interaction	< 0.00001	0.13975085
Interaction	< 0.00001	0.133909422
Interaction	< 0.00001	0.136350365
Interaction	< 0.00001	0.137127115
Interaction	< 0.00001	0.136352587
Interaction	< 0.00001	0.137182478
Interaction	< 0.00001	0.137004771
Interaction	< 0.00001	0.13826713
Interaction	< 0.00001	0.136407636
Interaction	< 0.00001	0.138009538
Interaction	< 0.00001	0.136825985
Interaction	< 0.00001	0.136676032
Interaction	< 0.00001	0.136590851
Interaction	< 0.00001	0.138724553
Interaction	< 0.00001	0.136544222

Interaction	< 0.00001	0.138615387
Interaction	< 0.00001	0.140237361
Interaction	< 0.00001	0.136605128
Interaction	< 0.00001	0.139801686
Interaction	< 0.00001	0.138964398
Interaction	< 0.00001	0.141044145
Interaction	< 0.00001	0.138949153
Interaction	< 0.00001	0.137668125
Interaction	< 0.00001	0.138820043
Interaction	< 0.00001	0.137337841
Interaction	< 0.00001	0.137494029
Interaction	< 0.00001	0.13975096
Interaction	< 0.00001	0.137217142
Interaction	< 0.00001	0.138288613
Interaction	< 0.00001	0.139668825
Interaction	< 0.00001	0.136778234
Interaction	< 0.00001	0.139087914
Interaction	< 0.00001	0.139971098
Interaction	< 0.00001	0.136883539
Interaction	< 0.00001	0.138420002
Interaction	< 0.00001	0.13982622
Interaction	< 0.00001	0.136969282
Interaction	< 0.00001	0.137936206
Interaction	< 0.00001	0.137162739
Interaction	< 0.00001	0.139807753
Interaction	< 0.00001	0.139796942
Interaction	< 0.00001	0.140701091
Interaction	< 0.00001	0.136799324
Interaction	< 0.00001	0.137522779
Interaction	< 0.00001	0.137427455
Interaction	< 0.00001	0.135616359
Interaction	< 0.00001	0.138821606
Interaction	< 0.00001	0.138785085
Interaction	< 0.00001	0.136135636
Interaction	< 0.00001	0.135389887
Interaction	< 0.00001	0.137115917
Interaction	< 0.00001	0.139136154
Interaction	< 0.00001	0.138369475
Interaction	< 0.00001	0.138868389
Interaction	< 0.00001	0.13767554
Interaction	< 0.00001	0.138905235
Interaction	< 0.00001	0.137198494
Interaction	< 0.00001	0.137976328
Interaction	< 0.00001	0.138172401
Interaction	< 0.00001	0.136343769
Interaction	< 0.00001	0.1388382
Interaction	< 0.00001	0.139640657
Interaction	< 0.00001	0.136711617
Interaction	< 0.00001	0.133725908
Interaction	< 0.00001	0.140932007
Interaction	< 0.00001	0.136634211
Interaction	< 0.00001	0.139849842
Interaction	< 0.00001	0.138097638
Interaction	< 0.00001	0.138976869
Interaction	< 0.00001	0.140343261
Interaction	< 0.00001	0.138553376
Interaction	< 0.00001	0.138098902
Interaction	< 0.00001	0.136139856
Interaction	< 0.00001	0.137151675

Interaction	< 0.00001	0.136406989
Interaction	< 0.00001	0.136188547
Interaction	< 0.00001	0.137442685
Interaction	< 0.00001	0.139191406
Interaction	< 0.00001	0.137054338
Interaction	< 0.00001	0.136967286
Interaction	< 0.00001	0.136977689
Interaction	< 0.00001	0.139406777
Interaction	< 0.00001	0.135629964
Interaction	< 0.00001	0.136370758
Interaction	< 0.00001	0.137257537
Interaction	< 0.00001	0.136126504
Interaction	< 0.00001	0.13831089
Interaction	< 0.00001	0.138000298
Interaction	< 0.00001	0.137638016
Interaction	< 0.00001	0.138133858
Interaction	< 0.00001	0.133580135
Interaction	< 0.00001	0.137464329
Interaction	< 0.00001	0.137635672
Interaction	< 0.00001	0.139792724
Interaction	< 0.00001	0.134163225
Interaction	< 0.00001	0.135333898
Interaction	< 0.00001	0.138046543
Interaction	< 0.00001	0.138188692
Interaction	< 0.00001	0.135959068
Interaction	< 0.00001	0.138654399
Interaction	< 0.00001	0.137351792
Interaction	< 0.00001	0.139027271
Interaction	< 0.00001	0.139115409
Interaction	< 0.00001	0.138845525
Interaction	< 0.00001	0.139339659
Interaction	< 0.00001	0.136100224
Interaction	< 0.00001	0.134964694
Interaction	< 0.00001	0.139052061
Interaction	< 0.00001	0.140506047
Interaction	< 0.00001	0.138511766
Interaction	< 0.00001	0.140249765
Interaction	< 0.00001	0.138338482
Interaction	< 0.00001	0.136850164
Interaction	< 0.00001	0.138947559
Interaction	< 0.00001	0.138283504
Interaction	< 0.00001	0.138025262
Interaction	< 0.00001	0.13797072
Interaction	< 0.00001	0.13817739
Interaction	< 0.00001	0.136594931
Interaction	< 0.00001	0.137753608
Interaction	< 0.00001	0.135203827
Interaction	< 0.00001	0.138326077
Interaction	< 0.00001	0.137469718
Interaction	< 0.00001	0.13976172
Interaction	< 0.00001	0.137089267
Interaction	< 0.00001	0.135939764
Interaction	< 0.00001	0.137312315
Interaction	< 0.00001	0.137250938
Interaction	< 0.00001	0.139984083
Interaction	< 0.00001	0.138819918
Interaction	< 0.00001	0.137575047
Interaction	< 0.00001	0.138097098
Interaction	< 0.00001	0.139079859

Interaction	< 0.00001	0.134481184
Interaction	< 0.00001	0.139024249
Interaction	< 0.00001	0.137384277
Interaction	< 0.00001	0.14042444
Interaction	< 0.00001	0.139031814
Interaction	< 0.00001	0.139629397
Interaction	< 0.00001	0.139719274
Interaction	< 0.00001	0.138881136
Interaction	< 0.00001	0.139056891
Interaction	< 0.00001	0.135434185
Interaction	< 0.00001	0.138857209
Interaction	< 0.00001	0.138873278
Interaction	< 0.00001	0.139387089
Interaction	< 0.00001	0.138694063
Interaction	< 0.00001	0.139059431
Interaction	< 0.00001	0.136798879
Interaction	< 0.00001	0.13679178
Interaction	< 0.00001	0.136917436
Interaction	< 0.00001	0.138042167
Interaction	< 0.00001	0.138373548
Interaction	< 0.00001	0.136323048
Interaction	< 0.00001	0.136278353
Interaction	< 0.00001	0.139028404
Interaction	< 0.00001	0.138440744
Interaction	< 0.00001	0.139093239
Interaction	< 0.00001	0.136325321
Interaction	< 0.00001	0.139836659
Interaction	< 0.00001	0.137548334
Interaction	< 0.00001	0.136022922
Interaction	< 0.00001	0.137841384
Interaction	< 0.00001	0.137072235
Interaction	< 0.00001	0.138827581
Interaction	< 0.00001	0.13716783
Interaction	< 0.00001	0.135749338
Interaction	< 0.00001	0.137112163
Interaction	< 0.00001	0.136121497
Interaction	< 0.00001	0.138652859
Interaction	< 0.00001	0.136597174
Interaction	< 0.00001	0.136558353
Interaction	< 0.00001	0.13635937
Interaction	< 0.00001	0.137629261
Interaction	< 0.00001	0.137966587
Interaction	< 0.00001	0.136338104
Interaction	< 0.00001	0.137129308
Interaction	< 0.00001	0.137166572
Interaction	< 0.00001	0.137627885
Interaction	< 0.00001	0.136868757
Interaction	< 0.00001	0.136885056
Interaction	< 0.00001	0.137182709
Interaction	< 0.00001	0.134961687
Interaction	< 0.00001	0.13539683
Interaction	< 0.00001	0.138815329
Interaction	< 0.00001	0.137977277
Interaction	< 0.00001	0.135652071
Interaction	< 0.00001	0.137696403
Interaction	< 0.00001	0.139094516
Interaction	< 0.00001	0.13700962
Interaction	< 0.00001	0.140841264
Interaction	< 0.00001	0.135643161

Interaction	< 0.00001	0.137260835
Interaction	< 0.00001	0.138893294
Interaction	< 0.00001	0.136278418
Interaction	< 0.00001	0.137603339
Interaction	< 0.00001	0.140278896
Interaction	< 0.00001	0.138843111
Interaction	< 0.00001	0.138205538
Interaction	< 0.00001	0.139146036
Interaction	< 0.00001	0.137787989
Interaction	< 0.00001	0.136863881
Interaction	< 0.00001	0.140144946
Interaction	< 0.00001	0.13956114
Interaction	< 0.00001	0.137615136
Interaction	< 0.00001	0.13669251
Interaction	< 0.00001	0.137245033
Interaction	< 0.00001	0.13659351
Interaction	< 0.00001	0.138204077
Interaction	< 0.00001	0.137557916
Interaction	< 0.00001	0.139247857
Interaction	< 0.00001	0.138058526
Interaction	< 0.00001	0.138064355
Interaction	< 0.00001	0.138738825
Interaction	< 0.00001	0.135790897
Interaction	< 0.00001	0.14057641
Interaction	< 0.00001	0.137172979
Interaction	< 0.00001	0.1400792
Interaction	< 0.00001	0.138891085
Interaction	< 0.00001	0.136822582
Interaction	< 0.00001	0.136805983
Interaction	< 0.00001	0.136729995
Interaction	< 0.00001	0.137214903
Interaction	< 0.00001	0.138147591
Interaction	< 0.00001	0.138974751
Interaction	< 0.00001	0.13813208
Interaction	< 0.00001	0.138061487
Interaction	< 0.00001	0.137166356
Interaction	< 0.00001	0.135746721
Interaction	< 0.00001	0.13928469
Interaction	< 0.00001	0.136557289
Interaction	< 0.00001	0.137322896
Interaction	< 0.00001	0.137258805
Interaction	< 0.00001	0.13765733
Interaction	< 0.00001	0.136585924
Interaction	< 0.00001	0.140765699
Interaction	< 0.00001	0.139783531
Interaction	< 0.00001	0.138000824
Interaction	< 0.00001	0.135214229
Interaction	< 0.00001	0.138679933
Interaction	< 0.00001	0.138836593
Interaction	< 0.00001	0.138167602
Interaction	< 0.00001	0.137758392
Interaction	< 0.00001	0.138305962
Interaction	< 0.00001	0.138721153
Interaction	< 0.00001	0.137366318
Interaction	< 0.00001	0.141852992
Interaction	< 0.00001	0.137653372
Interaction	< 0.00001	0.13972265
Interaction	< 0.00001	0.137978414
Interaction	< 0.00001	0.135649259

Interaction	< 0.00001	0.139278639
Interaction	< 0.00001	0.139638727
Interaction	< 0.00001	0.139539883
Interaction	< 0.00001	0.137727616
Interaction	< 0.00001	0.138807899
Interaction	< 0.00001	0.135571838
Interaction	< 0.00001	0.138035197
Interaction	< 0.00001	0.138896002
Interaction	< 0.00001	0.137003603
Interaction	< 0.00001	0.135185695
Interaction	< 0.00001	0.135471292
Interaction	< 0.00001	0.137844102
Interaction	< 0.00001	0.137344065
Interaction	< 0.00001	0.137638198
Interaction	< 0.00001	0.139938444
Interaction	< 0.00001	0.140849199
Interaction	< 0.00001	0.138423317
Interaction	< 0.00001	0.13549288
Interaction	< 0.00001	0.13714747
Interaction	< 0.00001	0.136808043
Interaction	< 0.00001	0.136168092
Interaction	< 0.00001	0.137333601
Interaction	< 0.00001	0.139310222
Interaction	< 0.00001	0.136545472
Interaction	< 0.00001	0.137219176
Interaction	< 0.00001	0.136505742
Interaction	< 0.00001	0.138550155
Interaction	< 0.00001	0.138303762
Interaction	< 0.00001	0.137204115
Interaction	< 0.00001	0.137788051
Interaction	< 0.00001	0.139757083
Interaction	< 0.00001	0.135473383
Interaction	< 0.00001	0.139362605
Interaction	< 0.00001	0.135626764
Interaction	< 0.00001	0.139943381
Interaction	< 0.00001	0.13834826
Interaction	< 0.00001	0.140200993
Interaction	< 0.00001	0.137322182
Interaction	< 0.00001	0.136876407
Interaction	< 0.00001	0.139819367
Interaction	< 0.00001	0.136961365
Interaction	< 0.00001	0.141489207
Interaction	< 0.00001	0.139808163
Interaction	< 0.00001	0.137111906
Interaction	< 0.00001	0.138800966
Interaction	< 0.00001	0.137253183
Interaction	< 0.00001	0.138143127
Interaction	< 0.00001	0.139066478
Interaction	< 0.00001	0.136959033
Interaction	< 0.00001	0.139252133
Interaction	< 0.00001	0.137055546
Interaction	< 0.00001	0.138064998
Interaction	< 0.00001	0.136408201
Interaction	< 0.00001	0.136692436
Interaction	< 0.00001	0.139352518
Interaction	< 0.00001	0.139538661
Interaction	< 0.00001	0.135019775
Interaction	< 0.00001	0.139002175
Interaction	< 0.00001	0.139290585

Interaction	< 0.00001	0.139428366
Interaction	< 0.00001	0.138352945
Interaction	< 0.00001	0.137473838
Interaction	< 0.00001	0.135947949
Interaction	< 0.00001	0.134457304
Interaction	< 0.00001	0.138199561
Interaction	< 0.00001	0.139757438
Interaction	< 0.00001	0.138291299
Interaction	< 0.00001	0.138902025
Interaction	< 0.00001	0.137962454
Interaction	< 0.00001	0.140042901
Interaction	< 0.00001	0.137128653
Interaction	< 0.00001	0.137903058

Supplementary Material 5 – Identifying regions of risk to honeybees from Zika vector control in the U.S.

Supplementary material for this chapter constitutes a table of 3144 lines, representing each US county as described in chapter 8. It is unsuitable for print format. A digital version is freely available.

Supplementary material for this chapter is freely available at https://www.tandfonline.com/doi/full/10.1080/00218839.2018.1494914.

Bibliography

Abbate, J. L., S. Kada, and S. Lion. 2015. Beyond Mortality: Sterility As a Neglected Component of Parasite Virulence. PLOS Pathog. 11:e1005229.

Abrams. 2001. Modelling the adaptive dynamics of traits involved in inter- and intraspecific interactions: An assessment of three methods. Ecol. Lett. 4:166–175.

Acerenza, L. 2016. Constraints, Trade-offs and the Currency of Fitness. J. Mol. Evol. 82:117–127.

Adjlane, N., B. Dainat, L. Gauthier, and V. Dietemann. 2016. Atypical viral and parasitic pattern in Algerian honey bee subspecies *Apis mellifera intermissa* and *A. m. sahariensis*. Apidologie 47:631–641.

Aebi, A., B. E. Vaissière, D. vanEngelsdorp, K. S. Delaplane, D. W. Roubik, and P. Neumann. 2012. Back to the future: Apis versus non-Apis pollination—a response to Ollerton et al. Trends Ecol. Evol. 27:142–143.

Aizen, M. A., and L. D. Harder. 2009. The Global Stock of Domesticated Honey Bees Is Growing Slower Than Agricultural Demand for Pollination. Curr. Biol. 19:915–918.

Albrigo, L. G., and R. V. Russ. 2002. Considerations for improving honeybee pollination of citrus hybrids in Florida. Pp. 27–31 *in* Proceedings of the Florida State Horticultural Society.

Al-Ghamdi, A., N. Adgaba, A. Getachew, and Y. Tadesse. 2016. New approach for determination of an optimum honeybee colony’s carrying capacity based on productivity and nectar secretion potential of bee forage species. Saudi J. Biol. Sci. 23:92–100.

Alizon, S., J. C. de Roode, and Y. Michalakis. 2013. Multiple infections and the evolution of virulence. Ecol. Lett. 16:556–567.

Alizon, S., A. Hurford, N. Mideo, and M. Van Baalen. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. J. Evol. Biol. 22:245–259.

Alizon, S., and Y. Michalakis. 2015. Adaptive virulence evolution: the good old fitness-based approach. Trends Ecol. Evol. 30:248–254.

Alizon, S., and M. van Baalen. 2008. Transmission–virulence trade-offs in vector-borne diseases. Theor. Popul. Biol. 74:6–15.

Allen, M., and B. Ball. 1996. The incidence and world distribution of honey bee viruses. Bee World 77:141–162.

Amdam, G. V., A. L. T. O. Aase, S.-C. Seehuus, M. Kim Fondrk, K. Norberg, and K. Hartfelder. 2005. Social reversal of immunosenescence in honey bee workers. Exp. Gerontol. 40:939–947.

Amiri, E., M. D. Meixner, and P. Kryger. 2016. Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. Sci. Rep. 6:33065.

Anderson, L. M., T. M. Dynes, J. A. Berry, K. S. Delaplane, L. L. McCormick, and B. J. Brosi. 2014. Distinguishing feral and managed honeybees (Apis mellifera) using stable carbon isotopes. Apidologie 45:653–663.

Anderson, P. K., A. A. Cunningham, N. G. Patel, F. J. Morales, P. R. Epstein, and P. Daszak. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol. Evol. 19:535–544.

Anderson, R. M., and R. M. May. 1982. Coevolution of hosts and parasites. Parasitology 85:411–426.

Anderson, R. M., and R. M. May. 1992. Infectious diseases of humans: dynamics and control. Wiley Online Library.

Antonovics, J., and P. H. Thrall. 1994. The cost of resistance and the maintenance of genetic polymorphism in host—pathogen systems. Proc R Soc Lond B 257:105–110.

Armitage, S. A. O., J. J. W. Thompson, J. Rolff, and M. T. Siva-Jothy. 2003. Examining costs of induced and constitutive immune investment in Tenebrio molitor. J. Evol. Biol. 16:1038–1044.

Aronstein, K. A., E. Saldivar, R. Vega, S. Westmiller, and A. E. Douglas. 2012. How Varroa Parasitism Affects the Immunological and Nutritional Status of the Honey Bee, Apis mellifera. Insects 3:601–615.

Ashby, B., and K. C. King. 2015. Diversity and the maintenance of sex by parasites. J. Evol. Biol. 28:511–520.

Atkins, K. E., A. F. Read, N. J. Savill, K. G. Renz, A. F. Islam, S. W. Walkden-Brown, and M. E. J. Woolhouse. 2013. Vaccination and Reduced Cohort Duration Can Drive Virulence Evolution: Marek's Disease Virus and Industrialized Agriculture. Evolution 67:851–860.

Auld, S., R. M. Penczykowski, J. Housley Ochs, D. C. Grippi, S. R. Hall, and M. A. Duffy. 2013. Variation in costs of parasite resistance among natural host populations. J. Evol. Biol. 26:2479–2486.

Baker, A. C., and D. C. Schroeder. 2008. The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (order Picornavirales) infecting Apis mellifera L. populations. Virol. J. 5:10.

Bartlett, L. J., C. J. Carlson, and M. Boots. 2018a. Identifying regions of risk to honey bees from Zika vector control in the USA. J. Apic. Res. 57:709–719.

Bartlett, L. J., C. Rozins, B. J. Brosi, K. S. Delaplane, J. C. de Roode, A. White, L. Wilfert, and M. Boots. 2018b. Industrial bees: when agricultural intensification doesn't impact local disease prevalence. bioRxiv 428656.

Bartlett, L. J., L. Wilfert, and M. Boots. 2018c. A genotypic trade-off between constitutive resistance to viral infection and host growth rate. Evolution, doi: 10.1111/evo.13623.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models using lme4. J. Stat. Softw. 67:1–48.

Becher, M. A., J. L. Osborne, P. Thorbek, P. J. Kennedy, and V. Grimm. 2013. REVIEW: Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. J. Appl. Ecol. 50:868–880.

Begon, M., K. B. H. Daud, P. Young, and R. E. Howells. 1993. The Invasion and Replication of a Granulosis Virus in the Indian Meal Moth, Plodia interpunctella: An Electron Microscope Study. J. Invertebr. Pathol. 61:281–295.

Belloy, L., A. Imdorf, I. Fries, E. Forsgren, H. Berthoud, R. Kuhn, and J.-D. Charrière. 2007. Spatial distribution of Melissococcus plutonius in adult honey bees collected from apiaries and colonies with and without symptoms of European foulbrood. Apidologie 38:136–140.

Benaets, K., A. V. Geystelen, D. Cardoen, L. D. Smet, D. C. de Graaf, L. Schoofs, M. H. D. Larmuseau, L. E. Brettell, S. J. Martin, and T. Wenseleers. 2017.

Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. Proc R Soc B 284:20162149.

Berényi, O., T. Bakonyi, I. Derakhshifar, H. Köglberger, and N. Nowotny. 2006. Occurrence of Six Honeybee Viruses in Diseased Austrian Apiaries. Appl. Environ. Microbiol. 72:2414–2420.

Berthoud, H., A. Imdorf, M. Haueter, S. Radloff, and P. Neumann. 2010. Virus infections and winter losses of honey bee colonies (Apis mellifera). J. Apic. Res. 49:60–65.

Best, A., and A. Hoyle. 2013. The evolution of costly acquired immune memory. Ecol. Evol. 3:2223–2232.

Best, A., H. Tidbury, A. White, and M. Boots. 2013. The evolutionary dynamics of within-generation immune priming in invertebrate hosts. J. R. Soc. Interface 10:20120887.

Best, A., S. Webb, A. White, and M. Boots. 2011. Host resistance and coevolution in spatially structured populations. Proc. R. Soc. Lond. B Biol. Sci. 278:2216–2222.

Best, A., A. White, and M. Boots. 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proc. Natl. Acad. Sci. 105:20786–20791.

Best, A., A. White, and M. Boots. 2009. Resistance is futile but tolerance can explain why parasites do not always castrate their hosts. Evolution 64:348–357.

Best, A., A. White, and M. Boots. 2017. The evolution of host defence when parasites impact reproduction. Evol. Ecol. Res. 18:393–409.

Betti, M. I., L. M. Wahl, and M. Zamir. 2016. Age structure is critical to the population dynamics and survival of honeybee colonies. R. Soc. Open Sci. 3:160444.

Betti, M. I., L. M. Wahl, and M. Zamir. 2014. Effects of Infection on Honey Bee Population Dynamics: A Model. PLOS ONE 9:e110237.

Bingham, N. 2006. Bees, Butterflies, and Bacteria: Biotechnology and the Politics of Nonhuman Friendship. Environ. Plan. Econ. Space 38:483–498.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135.

Boncristiani, H. F., G. Di Prisco, J. S. Pettis, M. Hamilton, and Y. P. Chen. 2009. Molecular approaches to the analysis of deformed wing virus replication and pathogenesis in the honey bee, Apis mellifera. Virol. J. 6:221.

Bonds, M. H. 2006. Host Life-History Strategy Explains Pathogen-Induced Sterility. Am. Nat. 168:281–293.

Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the Cost of Mounting an Immune Response. Am. Nat. 161:367–379.

Booton, R. D., Y. Iwasa, J. A. R. Marshall, and D. Z. Childs. 2017. Stress-mediated Allee effects can cause the sudden collapse of honey bee colonies. J. Theor. Biol. 420:213–219.

Boots, M. 2011. The Evolution of Resistance to a Parasite Is Determined by Resources. Am. Nat. 178:214–220.

Boots, M., and M. Begon. 1994. Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth, Plodia interpunctella. Ecol. Entomol. 19:319–326.

Boots, M., and M. Begon. 1995. Strain differences in the indian meal moth,Plodia interpunctella, in response to a granulosis virus. Res. Popul. Ecol. 37:37–42.

Boots, M., and M. Begon. 1993. Trade-Offs with Resistance to a Granulosis Virus in the Indian Meal Moth, Examined by a Laboratory Evolution Experiment. Funct. Ecol. 7:528.

Boots, M., A. Best, M. R. Miller, and A. White. 2009. The role of ecological feedbacks in the evolution of host defence: what does theory tell us? Philos. Trans. R. Soc. B Biol. Sci. 364:27–36.

Boots, M., and R. G. Bowers. 1999. Three Mechanisms of Host Resistance to Microparasites—Avoidance, Recovery and Tolerance—Show Different Evolutionary Dynamics. J. Theor. Biol. 201:13–23.

Boots, M., and Y. Haraguchi. 1999. The Evolution of Costly Resistance in Host-Parasite Systems. Am. Nat. 153:359–370.

Boots, M., P. J. Hudson, and A. Sasaki. 2004. Large Shifts in Pathogen Virulence Relate to Host Population Structure. Science 303:842–844.

Boots, M., and M. Mealor. 2007. Local Interactions Select for Lower Pathogen Infectivity. Science 315:1284–1286.

Boots, M., and A. Sasaki. 1999. ‘Small worlds’ and the evolution of virulence: infection occurs locally and at a distance. Proc. R. Soc. Lond. B Biol. Sci. 266:1933–1938.

Boots, and Sasaki. 2000. The evolutionary dynamics of local infection and global reproduction in host-parasite interactions. Ecol. Lett. 3:181–185.

Bouzid, M., J. Brainard, L. Hooper, and P. R. Hunter. 2016. Public Health Interventions for Aedes Control in the Time of Zikavirus– A Meta-Review on Effectiveness of Vector Control Strategies. PLoS Negl. Trop. Dis. 10:e0005176.

Bowen-Walker, P. L., S. J. Martin, and A. Gunn. 1999. The Transmission of Deformed Wing Virus between Honeybees (Apis melliferaL.) by the Ectoparasitic MiteVarroa jacobsoniOud. J. Invertebr. Pathol. 73:101–106.

Bowers, R. G., M. Boots, and M. Begon. 1994. Life-history trade-offs and the evolution of pathogen resistance: competition between host strains. Proc R Soc Lond B 257:247–253.

Bowers, R. G., A. Hoyle, A. White, and M. Boots. 2005. The geometric theory of adaptive evolution: trade-off and invasion plots. J. Theor. Biol. 233:363–377.

Bradford, E. L., C. R. Christie, E. M. Campbell, and A. S. Bowman. 2017. A real-time PCR method for quantification of the total and major variant strains of the deformed wing virus. PLOS ONE 12:e0190017.

Breidenbaugh, M. S., and F. A. de Szalay. 2010. Effects of Aerial Applications of Naled on Nontarget Insects at Parris Island, South Carolina. Environ. Entomol. 39:591–599.

Brettell, L. E., and S. J. Martin. 2017. Oldest *Varroa* tolerant honey bee population provides insight into the origins of the global decline of honey bees. Sci. Rep. 7:45953.

Brettell, L. E., G. J. Mordecai, D. C. Schroeder, I. M. Jones, J. R. da Silva, M. Vicente-Rubiano, and S. J. Martin. 2017. A Comparison of Deformed Wing Virus in Deformed and Asymptomatic Honey Bees. Insects 8:28.

Brijnath, B., C. D. Butler, and A. J. McMichael. 2014. In an interconnected world: joint research priorities for the environment, agriculture and infectious disease. Infect. Dis. Poverty 3:2.

Brittain, C., C. Kremen, and A.-M. Klein. 2013a. Biodiversity buffers pollination from changes in environmental conditions. Glob. Change Biol. 19:540–547.

Brittain, C., N. Williams, C. Kremen, and A.-M. Klein. 2013b. Synergistic effects of non-Apis bees and honey bees for pollination services. Proc. R. Soc. Lond. B Biol. Sci. 280:20122767.

Brosi, B. J., P. R. Armsworth, and G. C. Daily. 2008. Optimal design of agricultural landscapes for pollination services. Conserv. Lett. 1:27–36.

Brosi, B. J., and H. M. Briggs. 2013. Single pollinator species losses reduce floral fidelity and plant reproductive function. Proc. Natl. Acad. Sci. 110:13044–13048.

Brosi, B. J., K. S. Delaplane, M. Boots, and J. C. de Roode. 2017. Ecological and evolutionary approaches to managing honeybee disease. Nat. Ecol. Evol. 1:1250.

Brown, J. K. M. 2002. Yield penalties of disease resistance in crops. Curr. Opin. Plant Biol. 5:339–344.

Bryant, E. H., and D. H. Reed. 1999. Fitness Decline under Relaxed Selection in Captive Populations. Conserv. Biol. 13:665–669.

Budge, G. E., B. Barrett, B. Jones, S. Pietravalle, G. Marris, P. Chantawannakul, R. Thwaites, J. Hall, A. G. S. Cuthbertson, and M. A. Brown. 2010. The occurrence of Melissococcus plutonius in healthy colonies of Apis mellifera and the efficacy of European foulbrood control measures. J. Invertebr. Pathol. 105:164–170.

Budge, G. E., S. Pietravalle, M. Brown, L. Laurenson, B. Jones, V. Tomkies, and K. S. Delaplane. 2015. Pathogens as Predictors of Honey Bee Colony Strength in England and Wales. PLOS ONE 10:e0133228.

Bull, J. J. 1994. Perspective: Virulence. Evolution 48:1423.

Burgett, M., S. Daberkow, R. Rucker, and W. Thurman. 2010. US pollination markets: Recent changes and historical perspective. Am. Bee J.

Calderón, R. A., J. van Veen, H. G. Arce, and M. E. Esquivel. 2003. Presence of deformed wing virus and Kashmir bee virus in Africanized honey bee colonies in Costa Rica infested with Varroa destructor. Bee World 84:112–116.

Calderone, N. W. 2012. Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009. PLOS ONE 7:e37235.

Calderone, N. W., and R. E. Page. 1996. Temporal polyethism and behavioural canalization in the honey bee,Apis mellifera. Anim. Behav. 51:631–643.

Campbell, E. M., G. E. Budge, M. Watkins, and A. S. Bowman. 2016. Transcriptome analysis of the synganglion from the honey bee mite, Varroa destructor and RNAi knockdown of neural peptide targets. Insect Biochem. Mol. Biol. 70:116–126.

Carlson, C. J., E. Dougherty, M. Boots, W. Getz, and S. Ryan. 2018. Consensus and conflict among ecological forecasts of Zika virus outbreaks in the United States. Sci. Rep. 8:41598.

Carlson, C. J., E. R. Dougherty, and W. Getz. 2016. An Ecological Assessment of the Pandemic Threat of Zika Virus. PLoS Negl. Trop. Dis. 10:e0004968.

Caron, D. M., and L. J. Connor. 2013. Honey Bee Biology and Beekeeping, Revised Edition. Revised edition. Wicwas Press, Kalamazoo, MI.

Carreck, N. L., B. V. Ball, and S. J. Martin. 2010. Honey bee colony collapse and changes in viral prevalence associated with Varroa destructor. J. Apic. Res. 49:93–94.

Carreck, N. L., B. V. Ball, and J. K. Wilson. 2002. Virus succession in honeybee colonies infested with Varroa destructor. Apiacta 37:33–38.

CDC. 2016a. Aerial Spraying and Mosquito Control.

CDC. 2016b. Interim CDC Recommendations for Zika Vector Control in the Continental United States.

Chantawannakul, P., and R. W. Cutler. 2010. Viral Host Specific Evolution: A Case of Honeybee Virus. Nova Science Publishers, Inc, Hauppauge.

Chantawannakul, P., L. I. de Guzman, J. Li, and G. R. Williams. 2016. Parasites, pathogens, and pests of honeybees in Asia. Apidologie 47:301–324.

Chastel, C., P. Robaux, and F. le Goff. 1991. New virus from honey bee colonies. Is Varroa a vector or does he amplify viruses?[Egypt Bee Virus (EBV), Deformed Wing Virus (DWV) in France]. P. *in* Recent research on bee pathology. International symposium of the International Federation of Beekeepers Associations. Gent (Belgium). 5-7 Sep 1990.

Chen, Y., J. Evans, and M. Feldlaufer. 2006a. Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. J. Invertebr. Pathol. 92:152–159.

Chen, Y. P., J. A. Higgins, and M. F. Feldlaufer. 2005a. Quantitative Real-Time Reverse Transcription-PCR Analysis of Deformed Wing Virus Infection in the Honeybee (Apis mellifera L.). Appl. Environ. Microbiol. 71:436–441.

Chen, Y. P., J. S. Pettis, A. Collins, and M. F. Feldlaufer. 2006b. Prevalence and Transmission of Honeybee Viruses. Appl. Environ. Microbiol. 72:606–611.

Chen, Y., J. S. Pettis, and M. F. Feldlaufer. 2005b. Detection of multiple viruses in queens of the honey bee Apis mellifera L. J. Invertebr. Pathol. 90:118–121.

Chen, Y., I. B. Smith, A. M. Collins, J. S. Pettis, and M. F. Feldlaufer. 2004. Detection of deformed wing virus infection in honey bees, Apis mellifera L., in the United States. Am. Bee J. 144:557–559.

Choi, Y. S., M. Y. Lee, I. P. Hong, N. S. Kim, H. K. Kim, K. H. Byeon, and H. J. Yoon. 2010. Detection of Honeybee Virus from Bumblebee (Bombus terrestris L. and Bombus ignitus). J. Apic.

Claudianos, C., H. Ranson, R. M. Johnson, S. Biswas, M. A. Schuler, M. R. Berenbaum, R. Feyereisen, and J. G. Oakeshott. 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Mol. Biol. 15:615–636.

Clemson University. 2016. Non-Agricultural follow-up pesticide use investigation. Report of case file number 083016-47-02. Published 3rd Oct 2016, available from Dorchester County (South Carolina) Administrative Office, Document ID 11980; accessed on 11th November 2016.

Codeco, C., D. Villela, M. F. Gomes, L. Bastos, O. Cruz, C. Struchiner, L. M. Carvalho, and F. Coelho. 2016. Zika is not a reason for missing the Olympic Games in Rio de Janeiro: response to the open letter of Dr Attaran and colleagues to Dr Margaret Chan, Director - General, WHO, on the Zika threat to the Olympic and Paralympic Games. Mem. Inst. Oswaldo Cruz 111:414–415.

Cohen, H., R. D. Quistberg, S. M. Philpott, and G. DeGrandi-Hoffman. 2017. Vegetation Management and Host Density Influence Bee–Parasite Interactions in Urban Gardens. Environ. Entomol., doi: 10.1093/ee/nvx155.

Comeault, A. A., V. Soria-Carrasco, Z. Gompert, T. E. Farkas, C. A. Buerkle, T. L. Parchman, and P. Nosil. 2014. Genome-Wide Association Mapping of Phenotypic Traits Subject to a Range of Intensities of Natural Selection in Timema cristinae. Am. Nat. 183:711–727.

Connell, J., K. S. Delaplane, S. Donohue, W. Esaias, B. Gross, J. Hayes Jr, E. J. Lengerich, J. Pettis, K. Rennich, R. Underwood, and others. 2012. The Bee Informed Partnership: using beekeeper's real-world experience to solve beekeepers' real-world problems. Am. Entomol. 58.

Conte, Y. L., M. Ellis, and W. Ritter. 2010. Varroa mites and honey bee health: can Varroa explain part of the colony losses? Apidologie 41:353–363.

Corbet, S. A., I. H. Williams, and J. L. Osborne. 1991. Bees and the Pollination of Crops and Wild Flowers in the European Community. Bee World 72:47–59.

Cornman, R. S. 2017. Relative abundance of deformed wing virus, Varroa destructor virus 1, and their recombinants in honey bees (Apis mellifera) assessed by kmer analysis of public RNA-Seq data. J. Invertebr. Pathol. 149:44–50.

Cornman, R. S., H. Boncristiani, B. Dainat, Y. Chen, D. vanEngelsdorp, D. Weaver, and J. D. Evans. 2013. Population-genomic variation within RNA viruses of the Western honey bee, Apis mellifera, inferred from deep sequencing. BMC Genomics 14:154.

Costa, C., G. Tanner, M. Lodesani, L. Maistrello, and P. Neumann. 2011. Negative correlation between Nosema ceranae spore loads and deformed wing virus

infection levels in adult honey bee workers. J. Invertebr. Pathol. 108:224–225.

Crawley, M. J. 2012. The R Book. John Wiley & Sons.

Cremer, S., S. A. O. Armitage, and P. Schmid-Hempel. 2007. Social Immunity. Curr. Biol. 17:R693–R702.

Cressler, C. E., A. L. Graham, and T. Day. 2015. Evolution of hosts paying manifold costs of defence. Proc. R. Soc. Lond. B Biol. Sci. 282:20150065.

Cressler, C. E., D. V. McLeod, C. Rozins, J. V. D. Hoogen, and T. Day. 2016. The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology 143:915–930.

Crnokrak, P., and S. C. H. Barrett. 2002. Perspective: Purging the Genetic Load: A Review of the Experimental Evidence. Evolution 56:2347–2358.

Currie, R. W., and S. C. Jay. 1991. Drifting behaviour of drone honey bees (Apis mellifera L.) in commercial apiaries. J. Apic. Res. 30:61–68.

Dai, P.-L., Q. Wang, J.-H. Sun, F. Liu, X. Wang, Y.-Y. Wu, and T. Zhou. 2010. Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligustica. Environ. Toxicol. Chem. 29:644–649.

Dainat, B., J. D. Evans, Y. P. Chen, L. Gauthier, and P. Neumann. 2012. Dead or Alive: Deformed Wing Virus and Varroa destructor Reduce the Life Span of Winter Honeybees. Appl. Environ. Microbiol. 78:981–987.

Dainat, B., and P. Neumann. 2013. Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. J. Invertebr. Pathol. 112:278–280.

Dallas, T., M. Holtackers, and J. M. Drake. 2016. Costs of resistance and infection by a generalist pathogen. Ecol. Evol. 6:1737–1744.

Dalmon, A., C. Desbiez, M. Coulon, M. Thomasson, Y. L. Conte, C. Alaux, J. Vallon, and B. Moury. 2017. Evidence for positive selection and recombination hotspots in *Deformed wing virus* (DWV). Sci. Rep. 7:41045.

Daughenbaugh, K., M. Martin, L. Brutscher, I. Cavigli, E. Garcia, M. Lavin, M. Flenniken, K. F. Daughenbaugh, M. Martin, L. M. Brutscher, I. Cavigli, E. Garcia, M. Lavin, and M. L. Flenniken. 2015. Honey Bee Infecting Lake Sinai Viruses. Viruses 7:3285–3309.

David, J. R., P. Gibert, H. Legout, G. Pétavy, P. Capy, and B. Moreteau. 2005. Isofemale lines in Drosophila: an empirical approach to quantitative trait analysis in natural populations. Heredity 94:3–12.

Davis, R. S., R. K. Peterson, and P. A. Macedo. 2007. An ecological risk assessment for insecticides used in adult mosquito management. Integr. Environ. Assess. Manag. 3:373–382.

Davison, S., N. Leat, and M. Benjeddou. 2003. Development of molecular tools for honeybee virus research: the South African contribution. Afr. J. Biotechnol. 2:698–713.

Day, T. 2002. The Evolution of Virulence in Vector-Borne and Directly Transmitted Parasites. Theor. Popul. Biol. 62:199–213.

De la Rúa, P., R. Jaffé, R. Dall'Olio, I. Muñoz, and J. Serrano. 2009. Biodiversity, conservation and current threats to European honeybees. Apidologie 40:263–284.

de Mazancourt, C., and U. Dieckmann. 2004. Trade-Off Geometries and Frequency-Dependent Selection. Am. Nat. 164:765–778.

de Miranda, J. R., G. Cordoni, and G. Budge. 2010a. The Acute bee paralysis virus–Kashmir bee virus–Israeli acute paralysis virus complex. J. Invertebr. Pathol. 103, Supplement:S30–S47.

de Miranda, J. R., B. Dainat, B. Locke, G. Cordoni, H. Berthoud, L. Gauthier, P. Neumann, G. E. Budge, B. V. Ball, and D. B. Stoltz. 2010b. Genetic characterization of slow bee paralysis virus of the honeybee (Apis mellifera L.). J. Gen. Virol. 91:2524–2530.

de Miranda, J. R., and I. Fries. 2008. Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). J. Invertebr. Pathol. 98:184–189.

de Miranda, J. R., and E. Genersch. 2010. Deformed wing virus. J. Invertebr. Pathol. 103:S48–S61.

de Roode, J. C., A. J. Yates, and S. Altizer. 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. Proc. Natl. Acad. Sci. 105:7489–7494.

Débarre, F., S. Lion, M. van Baalen, and S. Gandon. 2012. Evolution of Host Life-History Traits in a Spatially Structured Host-Parasite System. Am. Nat. 179:52–63.

Decourtye, A., J. Devillers, S. Cluzeau, M. Charreton, and M.-H. Pham-Delègue. 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol. Environ. Saf. 57:410–419.

Delaplane, K. S. 2001. Varroa destructor: revolution in the making. Bee World 82:157–159.

Delaplane, K. S., D. R. Mayer, and D. F. Mayer. 2000. Crop pollination by bees. Cabi.

Desneux, N., A. Decourtye, and J.-M. Delpuech. 2007. The Sublethal Effects of Pesticides on Beneficial Arthropods. Annu. Rev. Entomol. 52:81–106.

Di Prisco, G., F. Pennacchio, E. Caprio, H. F. Boncristiani, J. D. Evans, and Y. Chen. 2011. Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, Apis mellifera. J. Gen. Virol. 92:151–155.

Dickinson, K., and S. Paskewitz. 2012. Willingness to pay for mosquito control: how important is West Nile virus risk compared to the nuisance of mosquitoes? Vector Borne Zoonotic Dis. Larchmt. N 12:886–892.

Dietemann, V., J. Pflugfelder, D. Anderson, J.-D. Charrière, N. Chejanovsky, B. Dainat, J. de Miranda, K. Delaplane, F.-X. Dillier, S. Fuch, P. Gallmann, L. Gauthier, A. Imdorf, N. Koeniger, J. Kralj, W. Meikle, J. Pettis, P. Rosenkranz, D. Sammataro, D. Smith, O. Yañez, and P. Neumann. 2012. Varroa destructor: research avenues towards sustainable control. J. Apic. Res. 51:125–132.

Dingle, T. C., R. H. Sedlak, L. Cook, and K. R. Jerome. 2013. Tolerance of Droplet-Digital PCR vs Real-Time Quantitative PCR to Inhibitory Substances. Clin. Chem. 59:1670–1672.

Dinh, L., G. Chowell, K. Mizumoto, and H. Nishiura. 2016. Estimating the subcritical transmissibility of the Zika outbreak in the State of Florida, USA, 2016. Theor. Biol. Med. Model. 13:20.

Dolezal, A. G., J. Carrillo-Tripp, W. A. Miller, B. C. Bonning, and A. L. Toth. 2016. Pollen Contaminated With Field-Relevant Levels of Cyhalothrin Affects

Honey Bee Survival, Nutritional Physiology, and Pollen Consumption Behavior. J. Econ. Entomol. 109:41–48.

Donnelly, R., A. White, and M. Boots. 2015. The epidemiological feedbacks critical to the evolution of host immunity. J. Evol. Biol. 28:2042–2053.

Duffy, M. A., and S. E. Forde. 2009. Ecological feedbacks and the evolution of resistance. J. Anim. Ecol. 78:1106–1112.

Duffy, M. A., J. H. Ochs, R. M. Penczykowski, D. J. Civitello, C. A. Klausmeier, and S. R. Hall. 2012. Ecological Context Influences Epidemic Size and Parasite-Driven Evolution. Science 335:1636–1638.

Duncan, A. B., S. Fellous, and O. Kaltz. 2011. Reverse evolution: selection against costly resistance in disease-free microcosm populations of paramecium caudatum. Evolution 65:3462–3474.

Duprey, Z., S. Rivers, G. Luber, A. Becker, C. Blackmore, D. Barr, G. Weerasekera, S. Kieszak, W. D. Flanders, and C. Rubin. 2008. Community Aerial Mosquito Control and Naled Exposure. J. Am. Mosq. Control Assoc. 24:42–46.

Dynes, T. L., J. C. D. Roode, J. I. Lyons, J. A. Berry, K. S. Delaplane, and B. J. Brosi. 2017. Fine scale population genetic structure of Varroa destructor, an ectoparasitic mite of the honey bee (Apis mellifera). Apidologie 48:93–101.

Ebert, D., H. Joachim Carius, T. Little, and E. Decaestecker. 2004. The Evolution of Virulence When Parasites Cause Host Castration and Gigantism. Am. Nat. 164:S19–S32.

Elliot, S. L., F. R. Adler, and M. W. Sabelis. 2003. How Virulent Should a Parasite Be to Its Vector? Ecology 84:2568–2574.

Elliot, S. L., M. W. Sabelis, and F. R. Adler. 2004. Evolution of parasite virulence to vectors. Frontis 163–171.

Engelhard, E. K., and L. E. Volkman. 1995. Developmental Resistance in Fourth Instar Trichoplusia ni Orally Inoculated with Autographa californica M Nuclear Polyhedrosis Virus. Virology 209:384–389.

Evans, J. D. 2001. Genetic Evidence for Coinfection of Honey Bees by Acute Bee Paralysis and Kashmir Bee Viruses. J. Invertebr. Pathol. 78:189–193.

Evans, J. D., and R. S. Schwarz. 2011. Bees brought to their knees: microbes affecting honey bee health. Trends Microbiol. 19:614–620.

Ewald, P. W. 1996. Guarding against the most dangerous emerging pathogens. Emerg. Infect. Dis. 2:245–257.

Ewald, P. W. 1993. The Evolution of Virulence. Sci. Am. 268:86–93.

Ewald, P. W. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. Ann. N. Y. Acad. Sci. 503:295–306.

Ewald, P. W. 1991. Transmission modes and the evolution of virulence. Hum. Nat. 2:1–30.

Fantham, H. B., and A. Porter. 1912. The Morphology and Life History of Nosema Apis and the Significance of its Various Stages in the so-called ‘Isle of Wight’ Disease in Bees (Microsporidiosis). Ann. Trop. Med. Parasitol. 6:163–195.

Faria, V. G., N. E. Martins, T. Paulo, L. Teixeira, É. Sucena, and S. Magalhães. 2015. Evolution of Drosophila resistance against different pathogens and infection routes entails no detectable maintenance costs. Evolution 69:2799–2809.

Fauci, A. S., and D. M. Morens. 2016. Zika Virus in the Americas — Yet Another Arbovirus Threat. N. Engl. J. Med. 374:601–604.

Ferguson, N. M., C. A. Donnelly, and R. M. Anderson. 2001. Transmission intensity and impact of control policies on the foot and mouth epidemic in Great Britain. Nat. Lond. 413:542–8.

Ferris, C., and A. Best. 2018. The evolution of host defence to parasitism in fluctuating environments. J. Theor. Biol. 440:58–65.

Fievet, J., D. Tentcheva, L. Gauthier, J. de Miranda, F. Cousserans, M. E. Colin, and M. Bergoin. 2006. Localization of deformed wing virus infection in queen and drone Apis mellifera L. Virol. J. 3:16.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194.

Floore, T. G. 2006. Mosquito Larval Control Practices: Past and Present. J. Am. Mosq. Control Assoc. 22:527–533.

Forfert, N., M. E. Natsopoulou, E. Frey, P. Rosenkranz, R. J. Paxton, and R. F. A. Moritz. 2015. Parasites and Pathogens of the Honeybee (Apis mellifera) and Their Influence on Inter-Colonial Transmission. PLOS ONE 10:e0140337.

Forsgren, E., and I. Fries. 2010. Comparative virulence of Nosema ceranae and Nosema apis in individual European honey bees. Vet. Parasitol. 170:212–217.

Forsgren, E., I. Fries, and J. R. de Miranda. 2012. Adult honey bees (Apis mellifera) with deformed wings discovered in confirmed varroa-free colonies. J. Apic. Res. 51:136–138.

Forsgren, E., S. Wei, D. Guiling, L. Zhiguang, T. V. Tran, P. T. Tang, T. A. Truong, T. Q. Dinh, and I. Fries. 2015. Preliminary observations on possible pathogen spill-over from *Apis mellifera* to *Apis cerana*. Apidologie 46:265–275.

Forzan, M., A. Felicioli, S. Sagona, P. Bandecchi, and M. Mazzei. 2017. Complete Genome Sequence of Deformed Wing Virus Isolated from Vespa crabro in Italy. Genome Announc. 5:e00961-17.

Foster, S. P., I. Denholm, G. M. Poppy, R. Thompson, and W. Powell. 2011. Fitness trade-off in peach-potato aphids (Myzus persicae) between insecticide resistance and vulnerability to parasitoid attack at several spatial scales. Bull. Entomol. Res. 101:659–666.

Francis, R. M., S. L. Nielsen, and P. Kryger. 2013a. Patterns of viral infection in honey bee queens. J. Gen. Virol. 94:668–676.

Francis, R. M., S. L. Nielsen, and P. Kryger. 2013b. Varroa-Virus Interaction in Collapsing Honey Bee Colonies. PLOS ONE 8:e57540.

Fraser, C., T. D. Hollingsworth, R. Chapman, F. de Wolf, and W. P. Hanage. 2007. Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. Proc. Natl. Acad. Sci. 104:17441–17446.

Free, J. B. 1958. The drifting of honey-bees. J. Agric. Sci. 51:294–306.

Fries, I. 2011. Diseases of Asian Honeybees. Pp. 333–345 *in* Honeybees of Asia. Springer, Berlin, Heidelberg.

Fries, I., and S. Camazine. 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie 32:199–214.

Froissart, R., J. Doumayrou, F. Vuillaume, S. Alizon, and Y. Michalakis. 2010. The virulence–transmission trade-off in vector-borne plant viruses: a review of

(non-)existing studies. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365:1907–1918.

Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364.

Fuxa, J. R., and A. R. Richter. 1989. Reversion of resistance by Spodoptera frugiperda to nuclear polyhedrosis virus. J. Invertebr. Pathol. 53:52–56.

FWS. 2016. Endangered and Threatened Wildlife and Plants; Endangered Status for 49 Species From the Hawaiian Islands.

Gage, M. J. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth Plodia interpunctella. Proc. R. Soc. Lond. B Biol. Sci. 261:25–30.

Gallai, N., J.-M. Salles, J. Settele, and B. E. Vaissière. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68:810–821.

Gandon, S. 2004. Evolution of multihost parasites. Evolution 58:455–469.

Gandon, S., A. Buckling, E. Decaestecker, and T. Day. 2008. Host–parasite coevolution and patterns of adaptation across time and space. J. Evol. Biol. 21:1861–1866.

Gandon, S., M. E. Hochberg, R. D. Holt, and T. Day. 2013. What limits the evolutionary emergence of pathogens? Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20120086.

Gandon, S., and P. F. Vale. 2013. The evolution of resistance against good and bad infections. J. Evol. Biol. 27:303–312.

Gandon, S., M. van Baalen, and V. A. A. Jansen. 2002. The Evolution of Parasite Virulence, Superinfection, and Host Resistance. Am. Nat. 159:658–669.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, O. Afik, I. Bartomeus, F. Benjamin, V. Boreux, D. Cariveau, N. P. Chacoff, J. H. Dudenhöffer, B. M. Freitas, J. Ghazoul, S. Greenleaf, J. Hipólito, A. Holzschuh, B. Howlett, R. Isaacs, S. K. Javorek, C. M. Kennedy, K. M. Krewenka, S. Krishnan, Y. Mandelik, M. M. Mayfield, I. Motzke, T. Munyuli, B. A. Nault, M. Otieno, J. Petersen, G. Pisanty, S. G. Potts, R. Rader, T. H. Ricketts, M. Rundlöf, C. L. Seymour, C. Schüepp, H. Szentgyörgyi, H. Taki, T. Tscharntke, C. H. Vergara, B. F. Viana, T. C. Wanger, C. Westphal, N. Williams, and A. M. Klein. 2013. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 339:1608–1611.

Gassmann, A. J., D. W. Onstad, and B. R. Pittendrigh. 2009. Evolutionary analysis of herbivorous insects in natural and agricultural environments. Pest Manag. Sci. 65:1174–1181.

Gemmill, A. W., and A. F. Read. 1998. Counting the cost of disease resistance. Trends Ecol. Evol. 13:8–9.

Genersch, E. 2010. Honey bee pathology: current threats to honey bees and beekeeping. Appl. Microbiol. Biotechnol. 87:87–97.

Genersch, E., and M. Aubert. 2010. Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). Vet. Res. 41:54.

Genersch, E., W. von der Ohe, H. Kaatz, A. Schroeder, C. Otten, R. Büchler, S. Berg, W. Ritter, W. Mühlen, S. Gisder, M. Meixner, G. Liebig, and P. Rosenkranz. 2010. The German bee monitoring project: a long term study to

understand periodically high winter losses of honey bee colonies. Apidologie 41:332–352.

Genersch, E., C. Yue, I. Fries, and J. R. de Miranda. 2006. Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities. J. Invertebr. Pathol. 91:61–63.

Geritz, S. a. H., E. Kisdi, G. Mesze´NA, and J. a. J. Metz. 1998. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol. Ecol. 12:35–57.

Ghazoul, J. 2005. Buzziness as usual? Questioning the global pollination crisis. Trends Ecol. Evol. 20:367–373.

Giacobino, A., N. B. Cagnolo, J. Merke, E. Orellano, E. Bertozzi, G. Masciangelo, H. Pietronave, C. Salto, and M. Signorini. 2014. Risk factors associated with the presence of Varroa destructor in honey bee colonies from east-central Argentina. Prev. Vet. Med. 115:280–287.

Gillespie, J. H. 1975. Natural Selection for Resistance to Epidemics. Ecology 56:493–495.

Gisder, S., P. Aumeier, and E. Genersch. 2009. Deformed wing virus: replication and viral load in mites (Varroa destructor). J. Gen. Virol. 90:463–467.

Goodwin, R. M., J. H. Perry, and A. T. Houten. 1994. The effect of drifting honey bees on the spread of American foulbrood infections. J. Apic. Res. 33:209–212.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Gower, C. M., J. P. Webster, and R. Poulin. 2004. Fitness of indirectly transmitted pathogens: restraint and constraint. Evolution 58:1178–1184.

Graham, A. L., J. E. Allen, and A. F. Read. 2005. Evolutionary Causes and Consequences of Immunopathology. Annu. Rev. Ecol. Evol. Syst. 36:373–397.

Graystock, P., E. J. Blane, Q. S. McFrederick, D. Goulson, and W. O. H. Hughes. 2016. Do managed bees drive parasite spread and emergence in wild bees? Int. J. Parasitol. Parasites Wildl. 5:64–75.

Graystock, P., D. Goulson, and W. O. H. Hughes. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proc R Soc B 282:20151371.

Graystock, P., K. Yates, S. E. F. Evison, B. Darvill, D. Goulson, and W. O. H. Hughes. 2013. The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. J. Appl. Ecol. 50:1207–1215.

Gregorc, A., and T. Bakonyi. 2012. Viral infections in queen bees (*Apis mellifera carnica*) from rearing apiaries. Acta Vet. Brno 81:15–19.

Gulland, A. 2016. Zika virus is a global public health emergency, declares WHO. BMJ 352:i657.

Guzman-Novoa, E., M. M. Hamiduzzaman, R. Anguiano-Baez, A. Correa-Benítez, E. Castañeda-Cervantes, and N. I. Arnold. 2015. First detection of honey bee viruses in stingless bees in North America. J. Apic. Res. 54:93–95.

Gwynn, D. M., A. Callaghan, J. Gorham, K. F. A. Walters, and M. D. E. Fellowes. 2005. Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. Proc. R. Soc. Lond. B Biol. Sci. 272:1803–1808.

Hadler, J. L., D. Patel, R. S. Nasci, L. R. Petersen, J. M. Hughes, K. Bradley, P. Etkind, L. Kan, and J. Engel. 2015. Assessment of Arbovirus Surveillance 13 Years after Introduction of West Nile Virus, United States1. Emerg. Infect. Dis. 21:1159–1166.

Hamiduzzaman, M. M., E. Guzman-Novoa, P. H. Goodwin, M. Reyes-Quintana, G. Koleoglu, A. Correa-Benítez, and T. Petukhova. 2015. Differential responses of Africanized and European honey bees (Apis mellifera) to viral replication following mechanical transmission or Varroa destructor parasitism. J. Invertebr. Pathol. 126:12–20.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proc. Natl. Acad. Sci. 87:3566–3573.

Hanski, I. 2005. The shrinking world: ecological consequences of habitat loss. International Ecology Institute Oldendorf/Luhe.

Haraguchi, Y., and A. Sasaki. 2000. The Evolution of Parasite Virulence and Transmission Rate in a Spatially Structured Population. J. Theor. Biol. 203:85–96.

Harriott, N. 2016. Protecting pollinators in the age of Zika and other emerging mosquito diseases. Pestic. You 36:9–16.

Hartikainen, H., and B. Okamura. 2012. Castrating parasites and colonial hosts. Parasitology 139:547–556.

Heins, D. C., J. A. Baker, M. A. Toups, and E. L. Birden. 2010. Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode Schistocephalus solidus. Biol. J. Linn. Soc. 100:835–846.

Henry, M., M. A. Becher, J. L. Osborne, P. J. Kennedy, P. Aupinel, V. Bretagnolle, F. Brun, V. Grimm, J. Horn, and F. Requier. 2017. Predictive systems models can help elucidate bee declines driven by multiple combined stressors. Apidologie 48:328–339.

Henter, H. J., and S. Via. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49:427–438.

Higes, M., R. Martín-Hernández, C. Botías, E. G. Bailón, A. V. González-Porto, L. Barrios, M. J. del Nozal, J. L. Bernal, J. J. Jiménez, P. G. Palencia, and A. Meana. 2008. How natural infection by Nosema ceranae causes honeybee colony collapse. Environ. Microbiol. 10:2659–2669.

Higes, M., R. Martín-Hernández, E. Garrido-Bailón, A. V. González-Porto, P. García-Palencia, A. Meana, M. J. Del Nozal, R. Mayo, and J. L. Bernal. 2009. Honeybee colony collapse due to Nosema ceranae in professional apiaries. Environ. Microbiol. Rep. 1:110–113.

Highfield, A. C., A. E. Nagar, L. C. M. Mackinder, L. M.-L. J. Noël, M. J. Hall, S. J. Martin, and D. C. Schroeder. 2009. Deformed Wing Virus Implicated in Overwintering Honeybee Colony Losses. Appl. Environ. Microbiol. 75:7212–7220.

Ho, S. S., D. Brossard, and D. A. Scheufele. 2007. The Polls—Trends Public Reactions to Global Health Threats and Infectious Diseases. Public Opin. Q. 71:671–692.

Hoang, T. C., R. L. Pryor, G. M. Rand, and R. A. Frakes. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. Environ. Toxicol. Chem. 30:997–1005.

Hochberg, M. E., Y. Michalakis, and T. D. Meeus. 1992. Parasitism as a constraint on the rate of life-history evolution. J. Evol. Biol. 5:491–504.

Hodges, A., D. Mulkey, E. Philippakos, and M. Sanford. 2001. Economic impact of the Florida apiculture industry. Am. Bee J. 141:361–363.

Hoehn, P., T. Tscharntke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. Proc. R. Soc. Lond. B Biol. Sci. 275:2283–2291.

Hoyle, A., R. G. Bowers, A. White, and M. Boots. 2008. The influence of trade-off shape on evolutionary behaviour in classical ecological scenarios. J. Theor. Biol. 250:498–511.

Ingram, E. M., J. Augustin, M. D. Ellis, and B. D. Siegfried. 2015. Evaluating sub-lethal effects of orchard-applied pyrethroids using video-tracking software to quantify honey bee behaviors. Chemosphere 135:272–277.

Iwasaki, J. M., B. I. P. Barratt, J. M. Lord, A. R. Mercer, and K. J. M. Dickinson. 2015. The New Zealand experience of varroa invasion highlights research opportunities for Australia. Ambio 44:694–704.

Iwata, K., E. Haas-Stapleton, Y. Kunimi, M. N. Inoue, and M. Nakai. 2017. Midgut-based resistance to oral infection by a nucleopolyhedrovirus in the laboratory-selected strain of the smaller tea tortrix, Adoxophyes honmai (Lepidoptera: Tortricidae). J. Gen. Virol. 98:296–304.

Jacobson, A. L., and G. G. Kennedy. 2013. Specific Insect-Virus Interactions Are Responsible for Variation in Competency of Different Thrips tabaci Isolines to Transmit Different Tomato Spotted Wilt Virus Isolates. PLOS ONE 8:e54567.

Jacques, A., M. Laurent, E. Consortium, M. Ribière-Chabert, M. Saussac, S. Bougeard, G. E. Budge, P. Hendrikx, and M.-P. Chauzat. 2017. A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. PLOS ONE 12:e0172591.

Jaenike, J. 1996. Suboptimal virulence of an insect-parasitic nematode. Evolution 50:2241–2247.

James, C. 1981. The cost of disease to world agriculture. Seed Sci. Technol. Neth.

Janelle, D. G. 1973. Measuring Human Extensibility in a Shrinking World. J. Geogr. 72:8–15.

Jatulan, E. O., J. F. Rabajante, C. G. B. Banaay, A. C. Fajardo, and E. C. Jose. 2015. A Mathematical Model of Intra-Colony Spread of American Foulbrood in European Honeybees (Apis mellifera L.). PLOS ONE 10:e0143805.

Jay, S. C. 1965. Drifting of Honeybees in Commercial Apiaries 1. Effect of Various Environmental Factors. J. Apic. Res. 4:167–175.

Jay, S. C. 1966. Drifting of Honeybees in Commercial Apiaries. III. Effect of Apiary Layout. J. Apic. Res. 5:137–148.

Jay, S. C. 1968. Drifting of Honeybees in Commercial Apiaries. IV. Further Studies of the Effect of Apiary Layout. J. Apic. Res. 7:37–44.

Jensen, K. H., T. Little, A. Skorping, and D. Ebert. 2006. Empirical support for optimal virulence in a castrating parasite.

Jha, A. R., C. M. Miles, N. R. Lippert, C. D. Brown, K. P. White, and M. Kreitman. 2015. Whole-Genome Resequencing of Experimental Populations Reveals Polygenic Basis of Egg-Size Variation in Drosophila melanogaster. Mol. Biol. Evol. 32:2616–2632.

Johnson, R. M., M. D. Ellis, C. A. Mullin, and M. Frazier. 2010. Pesticides and honey bee toxicity – USA. Apidologie 41:312–331.

Jones, B. A., D. Grace, R. Kock, S. Alonso, J. Rushton, M. Y. Said, D. McKeever, F. Mutua, J. Young, J. McDermott, and D. U. Pfeiffer. 2013. Zoonosis emergence linked to agricultural intensification and environmental change. Proc. Natl. Acad. Sci. 110:8399–8404.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Kamo, M., and M. Boots. 2006. The evolution of parasite dispersal, transmission, and virulence in spatial host populations. Evol. Ecol. Res. 8:1333–1347.

Kamo, M., A. Sasaki, and M. Boots. 2007. The role of trade-off shapes in the evolution of parasites in spatial host populations: An approximate analytical approach. J. Theor. Biol. 244:588–596.

Keeling, M. J., and P. Rohani. 2008. Modeling Infectious Diseases in Humans and Animals. Princeton University Press.

Kennedy, D. A., G. Kurath, I. L. Brito, M. K. Purcell, A. F. Read, J. R. Winton, and A. R. Wargo. 2016. Potential drivers of virulence evolution in aquaculture. Evol. Appl. 9:344–354.

Kent, C. F., A. Dey, H. Patel, N. Tsvetkov, T. Tiwari, V. J. MacPhail, Y. Gobeil, B. A. Harpur, J. Gurtowski, M. C. Schatz, S. R. Colla, and A. Zayed. 2018. Conservation Genomics of the Declining North American Bumblebee Bombus terricola Reveals Inbreeding and Selection on Immune Genes. Front. Genet. 9.

Kerr, B., C. Neuhauser, B. J. M. Bohannan, and A. M. Dean. 2006. Local migration promotes competitive restraint in a host–pathogen “tragedy of the commons.” Nature 442:75–78.

Khongphinitbunjong, K., L. I. de Guzman, T. E. Rinderer, M. R. Tarver, A. M. Frake, Y. Chen, and P. Chantawannakul. 2016. Responses of Varroa-resistant honey bees (Apis mellifera L.) to Deformed wing virus. J. Asia-Pac. Entomol. 19:921–927.

Khongphinitbunjong, K., L. I. de Guzman, M. R. Tarver, T. E. Rinderer, Y. Chen, and P. Chantawannakul. 2015. Differential viral levels and immune gene expression in three stocks of Apis mellifera induced by different numbers of Varroa destructor. J. Insect Physiol. 72:28–34.

Kielmanowicz, M. G., A. Inberg, I. M. Lerner, Y. Golani, N. Brown, C. L. Turner, G. J. R. Hayes, and J. M. Ballam. 2015. Prospective Large-Scale Field Study Generates Predictive Model Identifying Major Contributors to Colony Losses. PLOS Pathog. 11:e1004816.

Kisdi, É., and S. A. H. Geritz. 1999. ADAPTIVE DYNAMICS IN ALLELE SPACE: EVOLUTION OF GENETIC POLYMORPHISM BY SMALL MUTATIONS IN A HETEROGENEOUS ENVIRONMENT. Evolution 53:993–1008.

Kleijn, D., R. Winfree, I. Bartomeus, L. G. Carvalheiro, M. Henry, R. Isaacs, A.-M. Klein, C. Kremen, L. K. M’Gonigle, R. Rader, T. H. Ricketts, N. M. Williams, N. Lee Adamson, J. S. Ascher, A. Báldi, P. Batáry, F. Benjamin, J. C. Biesmeijer, E. J. Blitzer, R. Bommarco, M. R. Brand, V. Bretagnolle, L. Button, D. P. Cariveau, R. Chifflet, J. F. Colville, B. N. Danforth, E. Elle, M. P. D. Garratt, F. Herzog, A. Holzschuh, B. G. Howlett, F. Jauker, S. Jha, E. Knop, K. M. Krewenka, V. Le Féon, Y. Mandelik, E. A. May, M. G. Park, G.

Pisanty, M. Reemer, V. Riedinger, O. Rollin, M. Rundlöf, H. S. Sardiñas, J. Scheper, A. R. Sciligo, H. G. Smith, I. Steffan-Dewenter, R. Thorp, T. Tscharntke, J. Verhulst, B. F. Viana, B. E. Vaissière, R. Veldtman, K. L. Ward, C. Westphal, and S. G. Potts. 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6:7414.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond. B Biol. Sci. 274:303–313.

Klein, S., A. Cabirol, J.-M. Devaud, A. B. Barron, and M. Lihoreau. 2017. Why Bees Are So Vulnerable to Environmental Stressors. Trends Ecol. Evol. 0.

Knapp, J. L., L. J. Bartlett, and J. L. Osborne. 2017. Re-evaluating strategies for pollinator-dependent crops: How useful is parthenocarpy? J. Appl. Ecol. 54:1171–1179.

Koch, W., and W. Ritter. 1991. Experimental Examinations Concerning the Problem of Deformed Emerging Bees after Infestation with Varroa jacobsoni. J. Vet. Med. Ser. B 38:337–344.

Koella, J. C., and P. Agnew. 1999. A correlated response of a parasite's virulence and life cycle to selection on its host's life history. J. Evol. Biol. 12:70–79.

Koella, J. C., and C. Boëte. 2002. A Genetic Correlation Between Age at Pupation and Melanization Immune Response of the Yellow Fever Mosquito Aedes Aegypti. Evolution 56:1074–1079.

Koh, I., E. V. Lonsdorf, N. M. Williams, C. Brittain, R. Isaacs, J. Gibbs, and T. H. Ricketts. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proc. Natl. Acad. Sci. 113:140–145.

Korte, A., and A. Farlow. 2013. The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29.

Koskella, B. 2018. Resistance gained, resistance lost: An explanation for host–parasite coexistence. PLOS Biol. 16:e3000013.

Koskella, B., D. M. Lin, A. Buckling, and J. N. Thompson. 2011. The costs of evolving resistance in heterogeneous parasite environments. Proc. R. Soc. Lond. B Biol. Sci. rspb20112259.

Kraaijeveld, A. R., and H. C. J. Godfray. 1997. Trade-off between parasitoid resistance and larval competitive ability in Drosophila melanogaster. Nature 389:278–280.

Krijthe, J. H. 2015. Rtsne: T-Distributed Stochastic Neighbor Embedding using a Barnes-Hut Implementation,.

Lafferty, K. D., and A. M. Kuris. 2009. Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol. 25:564–572.

Lambrechts, L., and T. W. Scott. 2009. Mode of transmission and the evolution of arbovirus virulence in mosquito vectors. Proc. R. Soc. Lond. B Biol. Sci. rspb.2008.1709.

Lamstein, A., and B. P. Johnson. 2015. choroplethr: Simplify the Creation of Choropleth Maps in R. URL HttpsCRAN R-Proj. Orgpackage Choroplethr R Package Version 3:2.

Lanzi, G., J. R. de Miranda, M. B. Boniotti, C. E. Cameron, A. Lavazza, L. Capucci, S. M. Camazine, and C. Rossi. 2006. Molecular and Biological Characterization of Deformed Wing Virus of Honeybees (Apis mellifera L.). J. Virol. 80:4998–5009.

Lazzaro, B. P., and T. J. Little. 2009. Immunity in a variable world. Philos. Trans. R. Soc. B Biol. Sci. 364:15–26.

Levin, M. D. 1983. Value of Bee Pollination to U.S. Agriculture. Bull. Entomol. Soc. Am. 29:50–51.

Levin, S., and D. Pimentel. 1981. Selection of Intermediate Rates of Increase in Parasite-Host Systems. Am. Nat. 117:308–315.

Li, J., W. Peng, J. Wu, J. P. Strange, H. Boncristiani, and Y. Chen. 2011. Cross-Species Infection of Deformed Wing Virus Poses a New Threat to Pollinator Conservation. J. Econ. Entomol. 104:732–739.

Lindström, A., S. Korpela, and I. Fries. 2008. Horizontal transmission of Paenibacillus larvae spores between honey bee (Apis mellifera) colonies through robbing. Apidologie 39:515–522.

Lion, S., and M. Boots. 2010. Are parasites ‘“prudent”’ in space? Ecol. Lett. 13:1245–1255.

Lion, S., and S. Gandon. 2015. Evolution of spatially structured host-parasite interactions. J. Evol. Biol. 28:10–28.

Locke, B., E. Forsgren, I. Fries, and J. R. de Miranda. 2012. Acaricide Treatment Affects Viral Dynamics in Varroa destructor-Infested Honey Bee Colonies via both Host Physiology and Mite Control. Appl Env. Microbiol 78:227–235.

Locke, B., E. Forsgren, and J. R. de Miranda. 2014. Increased Tolerance and Resistance to Virus Infections: A Possible Factor in the Survival of Varroa destructor-Resistant Honey Bees (Apis mellifera). PLOS ONE 9:e99998.

Locke, B., E. Semberg, E. Forsgren, and J. R. de Miranda. 2017. Persistence of subclinical deformed wing virus infections in honeybees following Varroa mite removal and a bee population turnover. PLOS ONE 12:e0180910.

Loftus, J. C., M. L. Smith, and T. D. Seeley. 2016. How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming. PLOS ONE 11:e0150362.

Loper, G. M., D. Sammataro, J. Finley, and J. Cole. 2006. Feral honey bees in southern Arizona 10 years after varroa infestation. Am. Bee J.

Lucia, M., F. J. Reynaldi, G. H. Sguazza, and A. H. Abrahamovich. 2014. First detection of deformed wing virus in Xylocopa augusti larvae (Hymenoptera: Apidae) in Argentina. J. Apic. Res. 53:466–468.

Luong, L. T., and M. Polak. 2007. Costs of resistance in the Drosophila–Macrocheles system: a negative genetic correlation between ectoparasite resistance and reproduction. Evolution 61:1391–1402.

MABA. 2016. Metro Atlanta Beekeeper Association - Bee Kills, Zika, and Pesticides.

Mace, G. M., K. Norris, and A. H. Fitter. 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends Ecol. Evol. 27:19–26.

Mágori, K., * Péter Szabó, F. Mizera, and G. Meszéna. 2005. Adaptive dynamics on a lattice: role of spatiality in competition, co-existence and evolutionary branching. Evol. Ecol. Res. 7:1–21.

Manley, R. A. 2017. Emerging viral diseases of pollinating insects. University of Exeter.

Manley, R., M. Boots, and L. Wilfert. 2015. REVIEW: Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. J. Appl. Ecol. 52:331–340.

Martin, S., A. Hogarth, J. Van Breda, and J. Perrett. 1998. A scientific note on Varroa jacobsoni Oudemans and the collapse of Apis mellifera L. colonies in the United Kingdom. Apidologie 29:369–370.

Martin, S. J. 2001. The role of Varroa and viral pathogens in the collapse of honeybee colonies: a modelling approach. J. Appl. Ecol. 38:1082–1093.

Martin, S. J. 2010. Trouble in paradise: Varroa spreads across Hawaii. Am. Bee J.

Martin, S. J., B. V. Ball, and N. L. Carreck. 2013. The role of deformed wing virus in the initial collapse of varroa infested honey bee colonies in the UK. J. Apic. Res. 52:251–258.

Martin, S. J., A. C. Highfield, L. Brettell, E. M. Villalobos, G. E. Budge, M. Powell, S. Nikaido, and D. C. Schroeder. 2012. Global Honey Bee Viral Landscape Altered by a Parasitic Mite. Science 336:1304–1306.

Martín-Hernández, R., C. Botías, L. Barrios, A. Martínez-Salvador, A. Meana, C. Mayack, and M. Higes. 2011. Comparison of the energetic stress associated with experimental Nosema ceranae and Nosema apis infection of honeybees (Apis mellifera). Parasitol. Res. 109:605–612.

Matsuda, H., N. Ogita, A. Sasaki, and K. Satō. 1992. Statistical mechanics of population the lattice lotka-volterra model. Prog. Theor. Phys. 88:1035–1049.

Matthews, G. A. 2008. Developments in application technology. The Environmentalist 28:19–24.

Matthews, G. A. 2011. Integrated Vector Management: Controlling Vectors of Malaria and Other Insect Vector Borne Diseases. John Wiley & Sons.

Matthews, G. A., and P. Hamey. 2003. Exposure of bystanders to pesticides. Pestic. Outlook 14:210–212.

Matthews, G. A., and N. Thomas. 2000. Working towards more efficient application of pesticides. Pest Manag. Sci. 56:974–976.

Mattos, I. M. de, D. D. Jong, and A. E. E. Soares. 2016. Island population of European honey bees in Northeastern Brazil that have survived *Varroa* infestations for over 30 years. Apidologie 47:818–827.

May, R. M., and R. M. Anderson. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc R Soc Lond B 219:281–313.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV Associated to Flower Pollen: Experimental Evidence of a Horizontal Transmission Route. PLOS ONE 9:e113448.

McKean, K. A., C. P. Yourth, B. P. Lazzaro, and A. G. Clark. 2008. The evolutionary costs of immunological maintenance and deployment. BMC Evol. Biol. 8:76.

McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. J. Anim. Ecol. 84:615–624.

McMahon, D. P., M. E. Natsopoulou, V. Doublet, M. Fürst, S. Weging, M. J. F. Brown, A. Gogol-Döring, and R. J. Paxton. 2016. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. Proc R Soc B 283:20160811.

McMahon, D. P., L. Wilfert, R. J. Paxton, and M. J. F. Brown. 2018. Emerging Viruses in Bees: From Molecules to Ecology. Adv. Virus Res., doi: 10.1016/bs.aivir.2018.02.008.

Mealor, M. A., and M. Boots. 2005. An indirect approach to imply trade-off shapes: population level patterns in resistance suggest a decreasingly costly resistance mechanism in a model insect system. J. Evol. Biol. 19:326–330.

Mennerat, A., F. Nilsen, D. Ebert, and A. Skorping. 2010. Intensive Farming: Evolutionary Implications for Parasites and Pathogens. Evol. Biol. 37:59–67.

Messina, J. P., M. U. Kraemer, O. J. Brady, D. M. Pigott, F. M. Shearer, D. J. Weiss, N. Golding, C. W. Ruktanonchai, P. W. Gething, E. Cohn, J. S. Brownstein, K. Khan, A. J. Tatem, T. Jaenisch, C. J. Murray, F. Marinho, T. W. Scott, and S. I. Hay. 2016. Mapping global environmental suitability for Zika virus. eLife 5:e15272.

Messinger, S. M., and A. Ostling. 2013. The influence of host demography, pathogen virulence, and relationships with pathogen virulence on the evolution of pathogen transmission in a spatial context. Evol. Ecol. 27:353–380.

Middelboe, M., and C. P. D. Brussaard. 2017. Marine Viruses: Key Players in Marine Ecosystems. Viruses 9:302.

Milks, M. L., J. H. Myers, and M. K. Leptich. 2002. Costs and stability of cabbage looper resistance to a nucleopolyhedrovirus. Evol. Ecol. 16:369–385.

Miller, M. R., A. White, and M. Boots. 2007. Host Life Span and the Evolution of Resistance Characteristics. Evolution 61:2–14.

Mlakar, J., M. Korva, N. Tul, M. Popović, M. Poljšak-Prijatelj, J. Mraz, M. Kolenc, K. Resman Rus, T. Vesnaver Vipotnik, V. Fabjan Vodušek, A. Vizjak, J. Pižem, M. Petrovec, and T. Avšič Županc. 2016. Zika Virus Associated with Microcephaly. N. Engl. J. Med. 374:951–958.

Möckel, N., S. Gisder, and E. Genersch. 2011. Horizontal transmission of deformed wing virus: pathological consequences in adult bees (Apis mellifera) depend on the transmission route. J. Gen. Virol. 92:370–377.

Mohandass, S., F. H. Arthur, K. Y. Zhu, and J. E. Throne. 2007. Biology and management of Plodia interpunctella (Lepidoptera: Pyralidae) in stored products. J. Stored Prod. Res. 43:302–311.

Mondet, F., J. R. de Miranda, A. Kretzschmar, Y. L. Conte, and A. R. Mercer. 2014. On the Front Line: Quantitative Virus Dynamics in Honeybee (Apis mellifera L.) Colonies along a New Expansion Front of the Parasite Varroa destructor. PLOS Pathog. 10:e1004323.

Moore, J., A. Jironkin, D. Chandler, N. Burroughs, D. J. Evans, and E. V. Ryabov. 2011. Recombinants between Deformed wing virus and Varroa destructor virus-1 may prevail in Varroa destructor-infested honeybee colonies. J. Gen. Virol. 92:156–161.

Moore, P. A., M. E. Wilson, and J. A. Skinner. 2015. Honey bee viruses, The Deadly Varroa Mite Associates. Bee Health 19:2015.

Mordecai, G. J., L. Wilfert, S. J. Martin, I. M. Jones, and D. C. Schroeder. 2015. Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. ISME J. 10:1264.

Moret, Y., and P. Schmid-Hempel. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1166–1168.

Moritz, R. F. A., and S. Erler. 2016. Lost colonies found in a data mine: Global honey trade but not pests or pesticides as a major cause of regional honeybee colony declines. Agric. Ecosyst. Environ. 216:44–50.

Morran, L. T., O. G. Schmidt, I. A. Gelarden, R. C. Parrish, and C. M. Lively. 2011. Running with the Red Queen: Host-Parasite Coevolution Selects for Biparental Sex. Science 333:216–218.

Mõtus, K., A. Raie, T. Orro, M.-P. Chauzat, and A. Viltrop. 2016. Epidemiology, risk factors and varroa mite control in the Estonian honey bee population. J. Apic. Res. 55:396–412.

Mount, G. A., T. L. Biery, and D. G. Haile. 1996. A review of ultralow-volume aerial sprays of insecticide for mosquito control. J. Am. Mosq. Control Assoc.-Mosq. News 12:601–618.

Muz, D., and M. N. Muz. 2009. Survey of the occurrence of Deformed Wing Virus and multiple parasites of queens (Apis mellifera L.) in apiaries with collapsed colonies in Hatay, Turkey. J. Apic. Res. 48:204–208.

Nakagaki, N., and D. M. Wolock. 2005. Estimation of agricultural pesticide use in drainage basins using land cover maps and county pesticide data. USGS Numbered Series Open-File Report, Number 2005-1188. DOI 10.3133/ofr20051188

Nakai, M., K. Takahashi, K. Iwata, K. Tanaka, J. Koyanagi, A. Ookuma, J. Takatsuka, S. Okuno, and Y. Kunimi. 2017. Acquired resistance to a nucleopolyhedrovirus in the smaller tea tortrix Adoxophyes honmai (Lepidoptera: Tortricidae) after selection by serial viral administration. J. Invertebr. Pathol. 145:23–30.

Natsopoulou, M. E., D. P. McMahon, V. Doublet, E. Frey, P. Rosenkranz, and R. J. Paxton. 2017. The virulent, emerging genotype B of Deformed wing virus is closely linked to overwinter honeybee worker loss. Sci. Rep. 7:5242.

Nazzi, F., S. P. Brown, D. Annoscia, F. D. Piccolo, G. D. Prisco, P. Varricchio, G. D. Vedova, F. Cattonaro, E. Caprio, and F. Pennacchio. 2012. Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honeybee Colonies. PLOS Pathog. 8:e1002735.

Neumann, P., and N. L. Carreck. 2010. Honey bee colony losses. J. Apic. Res. 49:1–6.

Neumann, P., S. E. Radloff, C. W. W. Pirk, and R. Hepburn. 2003. The behaviour of drifted Cape honeybee workers (Apis mellifera capensis): predisposition for social parasitism? Apidologie 34:585–590.

Neumann, P., O. Yañez, I. Fries, and J. R. de Miranda. 2012. Varroa invasion and virus adaptation. Trends Parasitol. 28:353–354.

Nielsen, S. L., M. Nicolaisen, and P. Kryger. 2008. Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (Apis mellifera) in Denmark. Apidologie 39:310–314.

Niogret, J., S. M. Sait, and P. Rohani. 2009. Parasitism and constitutive defence costs to host life-history traits in a parasitoid–host interaction. Ecol. Entomol. 34:763–771.

Nolan, M. P., and K. S. Delaplane. 2017. Distance between honey bee Apis mellifera colonies regulates populations of Varroa destructor at a landscape scale. Apidologie 48:8–16.

Nordling, D., M. ans Andersson, S. Zohari, and G. Lars. 1998. Reproductive effort reduces specific immune response and parasite resistance. Proc. R. Soc. Lond. B Biol. Sci. 265:1291–1298.

Nordström, S. 2003. Distribution of deformed wing virus within honey bee (*Apis mellifera*) brood cells infested with the ectoparasitic mite *Varroa destructor*. Exp. Appl. Acarol. 29:293–302.

Nordström, S. 1999. Virus infections and varroa mite infestations in honey bee colonies. Acta Univ. Agric. Sueciae - Agrar. 83 pp.

Odoux, J.-F., P. Aupinel, S. Gateff, F. Requier, M. Henry, and V. Bretagnolle. 2014. ECOBEE: a tool for long-term honey bee colony monitoring at the landscape scale in West European intensive agroecosystems. J. Apic. Res. 53:57–66.

O'Keefe, K. J., and J. Antonovics. 2002. Playing by Different Rules: The Evolution of Virulence in Sterilizing Pathogens. Am. Nat. 159:597–605.

Oldroyd, B. P. 1999. Coevolution while you wait: Varroa jacobsoni, a new parasite of western honeybees. Trends Ecol. Evol. 14:312–315.

Oldroyd, B. P. 2007. What's Killing American Honey Bees? PLOS Biol. 5:e168.

Ongus, J. R., D. Peters, J.-M. Bonmatin, E. Bengsch, J. M. Vlak, and M. M. van Oers. 2004. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. J. Gen. Virol. 85:3747–3755.

Oppert, B., R. Hammel, J. E. Throne, and K. J. Kramer. 2000. Fitness costs of resistance to Bacillus thuringiensis in the Indianmeal moth, Plodia interpunctella. Entomol. Exp. Appl. 96:281–287.

Otto, C. R. V., C. L. Roth, B. L. Carlson, and M. D. Smart. 2016. Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains. Proc. Natl. Acad. Sci. 113:10430–10435.

Pacini, A., A. Giacobino, A. Molineri, N. Bulacio Cagnolo, A. Aignasse, L. Zago, A. Mira, M. Izaguirre, L. Schnittger, J. Merke, E. Orellano, E. Bertozzi, H. Pietronave, and M. Signorini. 2016. Risk factors associated with the

abundance of Nosema spp. in apiaries located in temperate and subtropical conditions after honey harvest. J. Apic. Res. 55:342–350.

Pasquale, G. D., M. Salignon, Y. L. Conte, L. P. Belzunces, A. Decourtye, A. Kretzschmar, S. Suchail, J.-L. Brunet, and C. Alaux. 2013. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? PLOS ONE 8:e72016.

Paxton, R. J. 2010. Does infection by Nosema ceranae cause “Colony Collapse Disorder” in honey bees (Apis mellifera)? J. Apic. Res. 49:80–84.

Paxton, R. J., J. Klee, S. Korpela, and I. Fries. 2007. Nosema ceranae has infected Apis mellifera in Europe since at least 1998 and may be more virulent than Nosema apis. Apidologie 38:558–565.

Paz, S., and J. C. Semenza. 2016. El Niño and climate change—contributing factors in the dispersal of Zika virus in the Americas? The Lancet 387:745.

Petersen, L. R., and E. B. Hayes. 2008. West Nile virus in the Americas. Med. Clin. North Am. 92:1307–1322, ix.

Petersen, L. R., D. J. Jamieson, A. M. Powers, and M. A. Honein. 2016. Zika Virus. N. Engl. J. Med. 374:1552–1563.

Pettis, J. S., and K. S. Delaplane. 2010. Coordinated responses to honey bee decline in the USA. Apidologie 41:256–263.

Pocol, C. B., L. A. Marghitas, and A. A. Popa. 2012. Evaluation of sustainability of the beekeeping sector in the North West Region of Romania. J Food Agric Env. 10:1132–1138.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25:345–353.

Potts, S. G., V. Imperatriz-Fonseca, H. T. Ngo, M. A. Aizen, J. C. Biesmeijer, T. D. Breeze, L. V. Dicks, L. A. Garibaldi, R. Hill, J. Settele, and A. J. Vanbergen. 2016. Safeguarding pollinators and their values to human well-being. Nature 540:220–229.

Prisco, G. D., D. Annoscia, M. Margiotta, R. Ferrara, P. Varricchio, V. Zanni, E. Caprio, F. Nazzi, and F. Pennacchio. 2016. A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. Proc. Natl. Acad. Sci. 113:3203–3208.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rader, R., B. G. Howlett, S. A. Cunningham, D. A. Westcott, and W. Edwards. 2012. Spatial and temporal variation in pollinator effectiveness: do unmanaged insects provide consistent pollination services to mass flowering crops? J. Appl. Ecol. 49:126–134.

Rader, R., J. Reilly, I. Bartomeus, and R. Winfree. 2013. Native bees buffer the negative impact of climate warming on honey bee pollination of watermelon crops. Glob. Change Biol. 19:3103–3110.

Ramsey, S., C. J. Gulbronson, J. Mowery, R. Ochoa, D. vanEngelsdorp, and G. Bauchan. 2018. A Multi-Microscopy Approach to Discover the Feeding Site and Host Tissue Consumed by Varroa destructor on Host Honey Bees. Microsc. Microanal. 24:1258–1259.

Rand, D. A., M. Keeling, and H. B. Wilson. 1995. Invasion, stability and evolution to criticality in spatially extended, artificial host-pathogen ecologies. Proc. R. Soc. Lond. B Biol. Sci. 259:55–63.

Rath, W. 1999. Co-adaptation of Apis cerana Fabr. and Varroa jacobsoni Oud. Apidologie 30:97–110.

Ravoet, J., L. De Smet, T. Wenseleers, and D. C. de Graaf. 2015. Genome sequence heterogeneity of Lake Sinai Virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host. Virus Res. 201:67–72.

Read, A. F. 1994. The evolution of virulence. Trends Microbiol. 2:73–76.

Reynaldi, F. J., G. H. Sguazza, F. J. Albicoro, M. R. Pecoraro, C. M. Galosi, F. J. Reynaldi, G. H. Sguazza, F. J. Albicoro, M. R. Pecoraro, and C. M. Galosi. 2013. First molecular detection of co-infection of honey bee viruses in asymptomatic Bombus atratus in South America. Braz. J. Biol. 73:797–800.

Reznick, D. 1985. Costs of Reproduction: An Evaluation of the Empirical Evidence. Oikos 44:257–267.

Rigaud, T., M.-J. Perrot-Minnot, and M. J. F. Brown. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proc. R. Soc. Lond. B Biol. Sci. 277:3693–3702.

Rittschof, C. C., C. B. Coombs, M. Frazier, C. M. Grozinger, and G. E. Robinson. 2015. Early-life experience affects honey bee aggression and resilience to immune challenge. Sci. Rep. 5:15572.

Roberts, K. E., and W. O. H. Hughes. 2015. Horizontal transmission of a parasite is influenced by infected host phenotype and density. Parasitology 142:395–405.

Roberts, K. E., and W. O. H. Hughes. 2014. Immunosenescence and resistance to parasite infection in the honey bee, Apis mellifera. J. Invertebr. Pathol. 121:1–6.

Robinson, G. E., R. E. Page, C. Strambi, and A. Strambi. 1992. Colony Integration in Honey Bees: Mechanisms of Behavioral Reversion. Ethology 90:336–348.

Roetschi, A., H. Berthoud, R. Kuhn, and A. Imdorf. 2008. Infection rate based on quantitative real-time PCR of Melissococcus plutonius, the causal agent of European foulbrood, in honeybee colonies before and after apiary sanitation. Apidologie 39:362–371.

Roff, D. A., and D. J. Fairbairn. 2007. The evolution of trade-offs: where are we? J. Evol. Biol. 20:433–447.

Rose, R. I. 2001. Pesticides and public health: integrated methods of mosquito management. Emerg. Infect. Dis. 7:17–23.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. 2010. Biology and control of Varroa destructor. J. Invertebr. Pathol. 103:S96–S119.

Roth, O., B. M. Sadd, P. Schmid-Hempel, and J. Kurtz. 2009. Strain-specific priming of resistance in the red flour beetle, Tribolium castaneum. Proc. R. Soc. Lond. B Biol. Sci. 276:145–151.

Rozins, C., and T. Day. 2016. Disease eradication on large industrial farms. J. Math. Biol. 73:885–902.

Rucker, R. R., W. N. Thurman, and M. Burgett. 2012. Honey Bee Pollination Markets and the Internalization of Reciprocal Benefits. Am. J. Agric. Econ. 94:956–977.

Rumball, W., I. R. Franklin, R. Frankham, and B. L. Sheldon. 1994. Decline in heterozygosity under full-sib and double first-cousin inbreeding in Drosophila melanogaster. Genetics 136:1039–1049.

Rumkee, J. C. O., M. A. Becher, P. Thorbek, and J. L. Osborne. 2017. Modeling Effects of Honeybee Behaviors on the Distribution of Pesticide in Nectar within a Hive and Resultant in-Hive Exposure. Environ. Sci. Technol. 51:6908–6917.

Russ, R. V. 1999. August - In the South. Am. Bee J. 139:607–608.

Ryabov, E. V., A. K. Childers, Y. Chen, S. Madella, A. Nessa, D. vanEngelsdorp, and J. D. Evans. 2017. Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. Sci. Rep. 7:17447.

Ryabov, E. V., J. M. Fannon, J. D. Moore, G. R. Wood, and D. J. Evans. 2016. The Iflaviruses Sacbrood virus and Deformed wing virus evoke different transcriptional responses in the honeybee which may facilitate their horizontal or vertical transmission. PeerJ 4:e1591.

Ryabov, E. V., G. R. Wood, J. M. Fannon, J. D. Moore, J. C. Bull, D. Chandler, A. Mead, N. Burroughs, and D. J. Evans. 2014. A Virulent Strain of Deformed Wing Virus (DWV) of Honeybees (Apis mellifera) Prevails after Varroa destructor-Mediated, or In Vitro, Transmission. PLOS Pathog. 10:e1004230.

Sadd, B. M., and M. T. Siva-Jothy. 2006. Self-harm caused by an insect's innate immunity. Proc. R. Soc. Lond. B Biol. Sci. 273:2571–2574.

Saejeng, A., M. T. Siva-Jothy, and M. Boots. 2011. Low cost antiviral activity of Plodia interpunctella haemolymph in vivo demonstrated by dose dependent infection. J. Insect Physiol. 57:246–250.

Saejeng, A., H. Tidbury, M. T. Siva-Jothy, and M. Boots. 2010. Examining the relationship between hemolymph phenoloxidase and resistance to a DNA virus, Plodia interpunctella granulosis virus (PiGV). J. Insect Physiol. 56:1232–1236.

Sait, S. M., M. Begon, and D. J. Thompson. 1994. Long-Term Population Dynamics of the Indian meal Moth Plodia interpunctella and its Granulosis Virus. J. Anim. Ecol. 63:861.

Samy, A. M., S. M. Thomas, A. A. E. Wahed, K. P. Cohoon, and A. T. Peterson. 2016. Mapping the global geographic potential of Zika virus spread. Mem. Inst. Oswaldo Cruz 111:559–560.

Sánchez-Bayo, F., and K. Goka. 2014. Pesticide Residues and Bees – A Risk Assessment. PLOS ONE 9:e94482.

Sánchez-Bayo, F., D. Goulson, F. Pennacchio, F. Nazzi, K. Goka, and N. Desneux. 2016. Are bee diseases linked to pesticides? — A brief review. Environ. Int. 89–90:7–11.

Santamaria, J., E. M. Villalobos, L. E. Brettell, S. Nikaido, J. R. Graham, and S. Martin. 2017. Evidence of Varroa-mediated deformed wing virus spillover in Hawaii. J. Invertebr. Pathol., doi: 10.1016/j.jip.2017.11.008.

Santillán-Galicia, M. T., B. V. Ball, S. J. Clark, and P. G. Alderson. 2014. Slow bee paralysis virus and its transmission in honey bee pupae by Varroa destructor. J. Apic. Res. 53:146–154.

Santillán-Galicia, M. T., B. V. Ball, S. J. Clark, and P. G. Alderson. 2010. Transmission of deformed wing virus and slow paralysis virus to adult bees (Apis mellifera L.) by Varroa destructor. J. Apic. Res. 49:141–148.

Santillán-Galicia, M. T., R. Carzaniga, B. V. Ball, and P. G. Alderson. 2008. Immunolocalization of deformed wing virus particles within the mite Varroa destructor. J. Gen. Virol. 89:1685–1689.

Schielzeth, H., and S. Nakagawa. 2013. Nested by design: model fitting and interpretation in a mixed model era. Methods Ecol. Evol. 4:14–24.

Schiff, N. M., W. S. Sheppard, G. M. Loper, and H. Shimanuki. 1994. Genetic Diversity of Feral Honey Bee (Hymenoptera: Apidae) Populations in the Southern United States. Ann. Entomol. Soc. Am. 87:842–848.

Schleier, J. J., and R. K. D. Peterson. 2009. Deposition and Air Concentrations of Permethrin and Naled Used for Adult Mosquito Management. Arch. Environ. Contam. Toxicol. 58:105–111.

Schleier, J. J., and R. K. D. Peterson. 2010. Toxicity and risk of permethrin and naled to non-target insects after adult mosquito management. Ecotoxicology 19:1140–1146.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. Proc. R. Soc. Lond. B Biol. Sci. 270:357–366.

Schmidt, C. W. 2016. Zika in the United States: How Are We Preparing? Environ. Health Perspect. 124:A157–A165.

Schoups, G., J. W. Hopmans, C. A. Young, J. A. Vrugt, W. W. Wallender, K. K. Tanji, and S. Panday. 2005. Sustainability of irrigated agriculture in the San Joaquin Valley, California. Proc. Natl. Acad. Sci. 102:15352–15356.

Schwenke, R. A., B. P. Lazzaro, and M. F. Wolfner. 2016. Reproduction–Immunity Trade-Offs in Insects. Annu. Rev. Entomol. 61:239–256.

Seeley, T. D. 2007. Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. Apidologie 38:19–29.

Seeley, T. D., and M. L. Smith. 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite Varroa destructor. Apidologie 46:716–727.

Seifi, A., R. G. F. Visser, and Y. Bai. 2013. How to effectively deploy plant resistances to pests and pathogens in crop breeding. Euphytica 190:321–334.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11:317–321.

Shen, M., X. Yang, D. Cox-Foster, and L. Cui. 2005. The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. Virology 342:141–149.

Shikano, I., and J. S. Cory. 2014. Dietary Mechanism behind the Costs Associated with Resistance to Bacillus thuringiensis in the Cabbage Looper, Trichoplusia ni. PLOS ONE 9:e105864.

Shirley, M. D. F., and R. M. Sibly. 1999. Genetic Basis of a Between-Environment Trade-Off Involving Resistance to Cadmium in Drosophila Melanogaster. Evolution 53:826–836.

Shoval, O., H. Sheftel, G. Shinar, Y. Hart, O. Ramote, A. Mayo, E. Dekel, K. Kavanagh, and U. Alon. 2012. Evolutionary Trade-Offs, Pareto Optimality, and the Geometry of Phenotype Space. Science 336:1157–1160.

Shutler, D., K. Head, K. L. Burgher-MacLellan, M. J. Colwell, A. L. Levitt, N. Ostiguy, and G. R. Williams. 2014. Honey Bee Apis mellifera Parasites in the Absence of Nosema ceranae Fungi and Varroa destructor Mites. PLOS ONE 9:e98599.

Silhacek, D. L., and G. L. Miller. 1972. Growth and Development of the Indian Meal Moth, Plodia interpunctella (Lepidoptera: Phycitidae), Under Laboratory Mass-Rearing Conditions. Ann. Entomol. Soc. Am. 65:1084–1087.

Simone-Finstrom, M., H. Li-Byarlay, M. H. Huang, M. K. Strand, O. Rueppell, and D. R. Tarpy. 2016. Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. Sci. Rep. 6.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. vanEngelsdorp, W. I. Lipkin, C. W. dePamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA Viruses in Hymenopteran Pollinators: Evidence of Inter-Taxa Virus Transmission via Pollen and Potential Impact on Non-Apis Hymenopteran Species. PLOS ONE 5:e14357.

Škubník, K., J. Nováček, T. Füzik, A. Přidal, R. J. Paxton, and P. Plevka. 2017. Structure of deformed wing virus, a major honey bee pathogen. Proc. Natl. Acad. Sci. 114:3210–3215.

Southwick, E. E., and L. Southwick. 1992. Estimating the Economic Value of Honey Bees (Hymenoptera: Apidae) as Agricultural Pollinators in the United States. J. Econ. Entomol. 85:621–633.

Stamets, P. E., N. L. Naeger, J. D. Evans, J. O. Han, B. K. Hopkins, D. Lopez, H. M. Moershel, R. Nally, D. Sumerlin, A. W. Taylor, L. M. Carris, and W. S. Sheppard. 2018. Extracts of Polypore Mushroom Mycelia Reduce Viruses in Honey Bees. Sci. Rep. 8:13936.

Stein, R. A. 2011. Super-spreaders in infectious diseases. Int. J. Infect. Dis. 15:e510–e513.

Steinhauer, N. A., K. Rennich, M. E. Wilson, D. M. Caron, E. J. Lengerich, J. S. Pettis, R. Rose, J. A. Skinner, D. R. Tarpy, J. T. Wilkes, D. vanEngelsdorp, and for the B. I. Partnership. 2014. A national survey of managed honey bee 2012–2013 annual colony losses in the USA: results from the Bee Informed Partnership. J. Apic. Res. 53:1–18.

Sumpter, D. J. T., and S. J. Martin. 2004. The dynamics of virus epidemics in Varroa-infested honey bee colonies. J. Anim. Ecol. 73:51–63.

Susi, H., and A.-L. Laine. 2015. The effectiveness and costs of pathogen resistance strategies in a perennial plant. J. Ecol. 103:303–315.

Szilágyi, A., I. Scheuring, D. P. Edwards, J. Orivel, and D. W. Yu. 2009. The evolution of intermediate castration virulence and ant coexistence in a spatially structured environment. Ecol. Lett. 12:1306–1316.

Tarpy, D. R., J. J. Keller, J. R. Caren, and D. A. Delaney. 2012. Assessing the Mating 'Health' of Commercial Honey Bee Queens. J. Econ. Entomol. 105:20–25.

Tarpy, D. R., J. J. Keller, J. R. Caren, and D. A. Delaney. 2011. Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. Insectes Sociaux 58:569–574.

Tentcheva, D., L. Gauthier, S. Jouve, L. Canabady-Rochelle, B. Dainat, F. Cousserans, M. E. Colin, B. V. Ball, and M. Bergoin. 2004a. Polymerase Chain Reaction detection of deformed wing virus (DWV) in Apis mellifera and Varroa destructor. Apidologie 35:431–439.

Tentcheva, D., L. Gauthier, N. Zappulla, B. Dainat, F. Cousserans, M. E. Colin, and M. Bergoin. 2004b. Prevalence and Seasonal Variations of Six Bee Viruses in Apis mellifera L. and Varroa destructor Mite Populations in France. Appl Env. Microbiol 70:7185–7191.

Tidbury, H. 2012. The Evolutionary Ecology of Antiviral Resistance in Insects. University of Sheffield.

Tidbury, H. J., A. Best, and M. Boots. 2012. The epidemiological consequences of immune priming. Proc. R. Soc. Lond. B Biol. Sci. rspb20121841.

Tidbury, H. J., A. B. Pedersen, and M. Boots. 2011. Within and transgenerational immune priming in an insect to a DNA virus. Proc. R. Soc. Lond. B Biol. Sci. 278:871–876.

Toufailia, H. M. A., E. Amiri, L. Scandian, P. Kryger, and F. L. W. Ratnieks. 2014. Towards integrated control of varroa: effect of variation in hygienic behaviour among honey bee colonies on mite population increase and deformed wing virus incidence. J. Apic. Res. 53:555–562.

Undorf-Spahn, K., E. Fritsch, J. Huber, J. Kienzle, C. P. W. Zebitz, and J. A. Jehle. 2012. High stability and no fitness costs of the resistance of codling moth to Cydia pomonella granulovirus (CpGV-M). J. Invertebr. Pathol. 111:136–142.

US Census Bureau, D. I. D. 2015. County Totals Dataset: Population, Population Change and Estimated Components of Population Change: April 1, 2010 to July 1, 2015.

USDA - NASS. 2017a. January 1 Honey Bee Colonies Down 8 Percent for Operations with Five or More Colonies.

USDA - NASS. 2013. United States Honey Production Down 1 Percent.

USDA - NASS. 2017b. United States Honey Production Up 3 Percent for Operations with Five or More Colonies in 2016.

USDA - NASS, C. of A. 2012a. USDA - NASS, Census of Agriculture - 2012 Census Volume 1, Chapter 2: County Level.

USDA - NASS, C. of A. 2012b. USDA - NASS, Census of Agriculture - 2012 Census Volume 1, Chapter 1: U.S. National Level Data.

Vail, P. V., and J. S. Tebbets. 1990. Comparative Biology and Susceptibility of Plodia interpunctella (Lepidoptera: Pyralidae) Populations to a Granulosis Virus. Environ. Entomol. 19:791–794.

van den Driessche, P., and J. Watmough. 2002. Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission. Math. Biosci. 180:29–48.

Van Der Maaten, L. 2014. Accelerating t-SNE using tree-based algorithms. J. Mach. Learn. Res. 15:3221–3245.

Van Der Maaten, L., and G. Hinton. 2008. Visualizing high-dimensional data using t-sne. journal of machine learning research. J. Mach. Learn. Res. 9:26.

van der Zee, R., L. Pisa, S. Andonov, R. Brodschneider, J.-D. Charrière, R. Chlebo, M. F. Coffey, K. Crailsheim, B. Dahle, A. Gajda, A. Gray, M. M. Drazic, M. Higes, L. Kauko, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, J. Kralj, P. Kristiansen, R. Martin Hernandez, F. Mutinelli, B. K. Nguyen, C. Otten, A. Özkırım, S. F. Pernal, M. Peterson, G. Ramsay, V. Santrac, V. Soroker, G. Topolska, A. Uzunov, F. Vejsnæs, S. Wei, and S. Wilkins. 2012. Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10. J. Apic. Res. 51:100–114.

van Engelsdorp, D., E. Lengerich, A. Spleen, B. Dainat, J. Cresswell, K. Baylis, B. K. Nguyen, V. Soroker, R. Underwood, H. Human, Y. L. Conte, and C. Saegerman. 2013. Standard epidemiological methods to understand and improve Apis mellifera health. J. Apic. Res. 52:1–16.

vanEngelsdorp, D., J. D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B. K. Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. Chen, R. Underwood, D. R. Tarpy, and J. S. Pettis. 2009. Colony Collapse Disorder: A Descriptive Study. PLoS ONE 4:e6481.

vanEngelsdorp, D., and M. D. Meixner. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invertebr. Pathol. 103:S80–S95.

vanEngelsdorp, D., D. R. Tarpy, E. J. Lengerich, and J. S. Pettis. 2013. Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. Prev. Vet. Med. 108:225–233.

Vijayan, V., S. López-González, F. Sánchez, F. Ponz, and I. Pagán. 2017. Virulence evolution of a sterilizing plant virus: Tuning multiplication and resource exploitation. Virus Evol. 3.

Wang, H., J. Xie, T. G. Shreeve, J. Ma, D. W. Pallett, L. A. King, and R. D. Possee. 2013. Sequence Recombination and Conservation of Varroa destructor Virus-1 and Deformed Wing Virus in Field Collected Honey Bees (Apis mellifera). PLOS ONE 8:e74508.

Watson, R., S. Albon, R. Aspinall, M. Austen, B. Bardgett, I. Bateman, P. Berry, W. Bird, R. Bradbury, C. Brown, J. Bullock, J. Burgess, A. Church, C. Christie, I. Crute, L. Davies, G. Edwards-Jones, B. Emmett, L. Firbank, A. Fitter, A. Gibson, R. Hails, R. Haines-Young, H. A. L., L. Heathwaite, J. Hopkins, M. Jenkins, L. Jones, G. Mace, S. Malcolm, E. Maltby, L. Maskell, K. Norris, S. Ormerod, J. Osborne, J. Pretty, C. Quine, S. Russell, L. Simpson, P. Smith, M. Tierney, T. K., R. Van der Wal, B. Vira, M. Walpole, A. Watkinson, A. Weighell, J. Winn, and M. Winter. 2011. UK National Ecosystem Assessment : understanding nature’s value to society. Synthesis of key findings. Information Press.

Waxman, D., and S. Gavrilets. 2005. 20 Questions on Adaptive Dynamics. J. Evol. Biol. 18:1139–1154.

Webb, S. D., M. J. Keeling, and M. Boots. 2013a. A theoretical study of the role of spatial population structure in the evolution of parasite virulence. Theor. Popul. Biol. 84:36–45.

Webb, S. D., M. J. Keeling, and M. Boots. 2007a. Host–parasite interactions between the local and the mean-field: How and when does spatial population structure matter? J. Theor. Biol. 249:140–152.

Webb, S. D., M. J. Keeling, and M. Boots. 2007b. Spatially extended host–parasite interactions: The role of recovery and immunity. Theor. Popul. Biol. 71:251–266.

Webb, S. D., M. J. Keeling, and M. Boots. 2013b. The role of spatial population structure on the evolution of parasites with acquired immunity and demography. J. Theor. Biol. 324:21–31.

Welch, A., F. Drummond, S. Tewari, A. Averill, and J. P. Burand. 2009. Presence and Prevalence of Viruses in Local and Migratory Honeybees (Apis mellifera) in Massachusetts. Appl. Environ. Microbiol. 75:7862–7865.

Whitlock, M. C., and D. Bourguet. 2000. Factors Affecting the Genetic Load in Drosophila: Synergistic Epistasis and Correlations Among Fitness Components. Evolution 54:1654–1660.

Whynott, D. 1991. Following the bloom: across America with the migratory beekeepers. Stackpole Books.

Wielgoss, S., J. E. Barrick, O. Tenaillon, M. J. Wiser, W. J. Dittmar, S. Cruveiller, B. Chane-Woon-Ming, C. Médigue, R. E. Lenski, and D. Schneider. 2013.

Mutation rate dynamics in a bacterial population reflect tension between adaptation and genetic load. Proc. Natl. Acad. Sci. 110:222–227.

Wilfert, L., G. Long, H. C. Leggett, P. Schmid-Hempel, R. Butlin, S. J. M. Martin, and M. Boots. 2016. Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. Science 351:594–597.

Williams, P. H., and J. L. Osborne. 2009. Bumblebee vulnerability and conservation world-wide. Apidologie 40:367–387.

Wu, Y., X. Dong, and T. Kadowaki. 2017. Characterization of the Copy Number and Variants of Deformed Wing Virus (DWV) in the Pairs of Honey Bee Pupa and Infesting Varroa destructor or Tropilaelaps mercedesae. Front. Microbiol. 8.

Yan, G., D. W. Severson, and B. M. Christensen. 1997. Costs and Benefits of Mosquito Refractoriness to Malaria Parasites: Implications for Genetic Variability of Mosquitoes and Genetic Control of Malaria. Evolution 51:441–450.

Yañez, O., H.-Q. Zheng, X.-L. Su, F.-L. Hu, P. Neumann, and V. Dietemann. 2015. Potential for virus transfer between the honey bees Apis mellifera and A. cerana. J. Apic. Res. 54:179–191.

Yang, X., and D. Cox-Foster. 2007. Effects of parasitization by Varroa destructor on survivorship and physiological traits of Apis mellifera in correlation with viral incidence and microbial challenge. Parasitology 134:405–412.

Yang, X., and D. L. Cox-Foster. 2005. Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. Proc. Natl. Acad. Sci. 102:7470–7475.

Yue, C., and E. Genersch. 2005. RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). J. Gen. Virol. 86:3419–3424.

Yue, C., M. Schröder, K. Bienefeld, and E. Genersch. 2006. Detection of viral sequences in semen of honeybees (Apis mellifera): Evidence for vertical transmission of viruses through drones. J. Invertebr. Pathol. 92:105–108.

Zee, R. van der, R. Brodschneider, V. Brusbardis, J.-D. Charrière, R. Chlebo, M. F. Coffey, B. Dahle, M. M. Drazic, L. Kauko, J. Kretavicius, P. Kristiansen, F. Mutinelli, C. Otten, M. Peterson, A. Raudmets, V. Santrac, A. Seppälä, V. Soroker, G. Topolska, F. Vejsnæs, and A. Gray. 2014. Results of international standardised beekeeper surveys of colony losses for winter 2012–2013: analysis of winter loss rates and mixed effects modelling of risk factors for winter loss. J. Apic. Res. 53:19–34.

Zhang, X., S. Y. He, J. D. Evans, J. S. Pettis, G. F. Yin, and Y. P. Chen. 2012. New evidence that deformed wing virus and black queen cell virus are multi-host pathogens. J. Invertebr. Pathol. 109:156–159.

Zhong, D., A. Pai, and G. Yan. 2005. Costly Resistance to Parasitism: Evidence From Simultaneous Quantitative Trait Loci Mapping for Resistance and Fitness in Tribolium castaneum. Genetics 169:2127–2135.

Zhong, H., M. Latham, P. G. Hester, R. L. Frommer, and C. Brock. 2004. Impact of Naled on Honey Bee Apis mellifera L. Survival and Productivity: Aerial ULV Application Using a Flat-Fan Nozzle System. Arch. Environ. Contam. Toxicol. 45:216–220.

Zioni, N., V. Soroker, and N. Chejanovsky. 2011. Replication of Varroa destructor virus 1 (VDV-1) and a Varroa destructor virus 1–deformed wing virus

recombinant (VDV-1–DWV) in the head of the honey bee. Virology 417:106–112.