The Ecology Within:
Health Implications of Within-host Ecology

by

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ABSTRACT

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Many of the medical problems that continue to elude us involve complex networks of interacting cells, biochemicals, and microorganisms. These within-host dynamical systems constitute the ecology that happens inside of us, and we are only beginning to explore these systems as ecological systems. In this thesis, I investigate how untangling these webs of interactions and their corresponding dynamics can help us mechanistically understand many diseases, particularly those caused by infections. I address how to further apply ecological theory to within-host systems and demonstrate how deeper ecological understanding of an infection can reveal unexpected behaviours that may hinder the long-term effectiveness of the vaccines we develop to combat such infections. I begin by proposing that some lessons from ecology, specifically from community ecology, have not been fully harnessed in our studies of the dynamics of infections. For instance, within-host trade-offs are poorly understood and rarely sought after empirically. And yet, with a simple mathematical model of viral dynamics I showed that within-host trade-offs strongly determine the outcomes of chronic and acute infections. For instance, I showed how simple human behaviours that affect immunity, like smoking, can alter these within-host trade-offs and favour infections dominated by more virulent strains.

Unfortunately, studies to identify what evolutionary and ecological processes shape infectious diseases are not usually undertaken before we develop control methods against them. I consider the specific example of Human Papillomavirus (HPV), where discussions about the ecology and evolution of HPV types did not seriously begin until after the launch of a new type-specific vaccine. After developing a novel viral dynamics model of HPV co-infections that incorporated the essential patchiness of lesion dynamics, I investigated competing hypotheses of HPV type interactions. I found that ‘neutral’ interactions, the prevailing assumption in the literature, do not best explain the (limited) within-host co-infection data that we have. This suggests that neutrality between HPV types could be a fallacy, and the consequence could be that the vaccine is releasing types not targeted by the vaccine from competitive pressures. Similarly, with another within-host model I found that the vaccine within-host environment also releases HPV from important selection pressures which could consequently allow the virulence level of HPV to increase. I demonstrate, then, that understanding ecological conditions inside hosts allows us to make predictions about the potential evolutionary responses of pathogens against control methods such as vaccines. Overall, this dissertation highlights that more studies into the ecology happening within the body are needed in order to improve our confidence in the long-term successes of our medical strategies.
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Dedication

To my loving parents, Mónica and Luis, who instilled in me a love of learning, and who have always encouraged me to surpass barriers by working hard and to dream big.
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b) $R_0$ with respect to oncogene expression for various sexual behaviours (immuno-competent only). Including the sexual behaviour model with the transmission function in the unvaccinated case does not noticeably change the $\varepsilon^*$ away from the within-host optimal, thus all three groups select for the same $\varepsilon^*$.

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Chapter 1

Introduction
The Ecology and Evolution of Pathogens

The disciplines of infectious disease research, parasitology, evolutionary biology and ecology all budded from the same rich history of the naturalists. The observations of both naturalists and physicians alike built up the important concepts and methods needed for our modern understanding of host-pathogen interactions. The first empirically grounded and well-reasoned work on the contagiousness of diseases is accredited to Girolamo Fracastoro from the 1500s [1]. His writings tell of how syphilis was brought to Europe by Columbus’ sailors. He identifies three modes of transmission of contagious diseases: direct contact with a sick person, contact with a contaminated object, or through the air at a reasonable distance from the sick person. Around the same time other physicians and naturalists were busy identifying a vast number of insects, worms and parasites, even though they lacked microscopes, which were not developed until the 1600s [1]. The concept that the contagious agents were living organisms was not convincingly demonstrated until van Leeuwenhoek showed the Royal Society his numerous microscope findings of “animalcules” (microorganisms) and argued against the idea of spontaneous generation [2].

The great explorers of the 18th and 19th centuries not only helped catalogue the vast biodiversity of earth but also made some very important observations about infectious diseases, though at the time they were referred to as contagion, fevers, and spirits. For example, von Humboldt described how species interacted with their environment and argued that physical characteristics of the environment, like climate, affect species distributions [3]. His writings on how the great epidemics of Central America were brought by ships from Chile were used by Darwin as additional evidence of what he was seeing himself on his voyage in the Beagle. In a short passage in his journal, Darwin draws together evidence from various explorers and observations from livestock transportation that some diseases are contagious and that these diseases are strong regulators of population sizes. In Australia he writes,

*The number of aborigines is rapidly decreasing...This decrease, no doubt, must be partly owing to the introduction of spirits, to European diseases (even the milder ones of which, such as the measles, prove very destructive)...We may look to the wide extent of the Americas, Polynesia, the Cape of Good Hope, and Australia, and we find the same result. Nor is the white man alone that thus acts the destroyer... The varieties of man seem to act on each other in the same way as different species of animals – the stronger always extirpating the weaker.*

*Darwin, 1839*

*The Voyage of the H.M.S. Beagle*

Following this period of observation of global contagious disease patterns, came the golden age of bacteriology and the concept of spirits became a thing of the past. Thanks to Pasteur and Koch’s postulates there was a burst of discoveries of the biological agents that caused many of the diseases that had plagued humans since antiquity. Indeed the late 1800s was a golden age for scientific advancement in general and from it sprang the modern disciplines we are now familiar with, namely microbiology, evolutionary biology, and ecology. These as disciplines expanded in
their own right and it was not until about 100 years later that evolutionary ecologists would focus their attention onto human pathogens and diseases. What led to their renewed interest was the quest to answer a simple question: why do some pathogens harm their hosts and others not?

Dating back to Pasteur and Koch were the first mentions that virulence of pathogens may change over time but the first hypothesis to explain changes in virulence appeared in the early 20th century [4]. Once a pathogen first infects and circulates in a new host species, it was believed that the pathogen evolves towards avirulence because it benefited the pathogen to not harm the host. This ‘avirulence hypothesis’ became conventional wisdom [4,5], and even today, this idea can still be found in medical circles. The idea was historically well-rooted, because if the knowledge of the epidemics caused by colonialism is coupled with the observation that many pathogens do not harm or kill their host, then this hypothesis seems reasonable. Yet, the existence of pathogens that have inflicted humans with considerable virulence for centuries, e.g. malaria, contradicts this hypothesis. In the early 1980s a series of works challenged this avirulence hypothesis. An important criticism of the avirulence hypothesis is that arguments like “From the standpoint of its survival and species perpetuation, it would be deleterious to the parasite to cause the death of the host” (Palmieri 1982, in [5]) are rooted in group selection, an intensely critiqued evolutionary theory. As an alternative, a new hypothesis based on life history theory was proposed, which suggested that, due to a trade-off between virulence and transmission, intermediate levels of virulence should be selected for given the constraints created by recovery and transmission [6,7] and that virulence is affected by the mechanism of transmission [8]. A simple equation [9] captures the relationship between features of a pathogen’s life history traits and the basic reproductive ratio, $R_0$, the number of new infections produced when an infected individual is introduced to a wholly susceptible host population$^1$,

$$R_0 = \frac{\beta N}{\alpha + b + \nu}$$  

(1.1)

where $\beta$ is the rate of transmission, $N$ is the density of hosts, $\alpha$ is the mortality induced by the pathogen, $b$ is mortality for other reasons, and $\nu$ is the rate of recovery of infected hosts. Here, infected individuals produce newly infected hosts at a rate $\beta N$, and the average duration of infectiousness is the period ending with recovery or death, $1/(\alpha + b + \nu)$. Assuming that natural selection would act to maximize the pathogen’s $R_0$, to do so would require either increasing transmission, $\beta$, or decreasing recovery or pathogen-induced mortality, $\alpha$ [10]. In the 1990s, this virulence-transmission trade-off hypothesis was debated considerably particularly with theoretical studies [11–16]. Critics of this trade-off hypothesis argue that if this relationship between transmission and virulence is really so simple and general, then, more empirical evidence should exist [17]. Proponents of the hypothesis point to several examples of empirical studies and argue that generally, it is not easy to clearly demonstrate a trade-off of this nature [4]. Overall, however, more studies are needed better understand this trade-off in nature or in the lab.

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$^1$ $R_0$ comes from a rich history in demographics and ecology, where in the latter the meaning of $R_0$ is the mean lifetime reproductive success of a typical member of a species (circa 1911 Alfred Lotka and Ronald Ross) [197]. However, in infectious disease research, $R_0$ has come to represent the expected number of secondary infected hosts produced by an infected individual over their lifetime (Ross 1911, [198]). See Heffernan et al. 2005 for a history of $R_0$ and its calculation.
Asking evolutionary questions about pathogens led to an appreciation that these evolutionary processes happen within the context of the ecology of the system. Not surprisingly, ecological processes, such as competition, mutualism, and trade-offs, were found to matter to pathogens. As in the virulence-transmission trade-off, life-history theory and trade-offs were used in evolutionary studies to understand parasite strategies [18]. Furthermore, ecological theory has also been applied to host-pathogen interactions. The best example of this is the application of predator-prey theory to host-immunity interactions with the simple analogy that the immune system cells are ‘predators’ that kill pathogens which are their ‘prey’ [19,20]. Studies using this framework, found that the interactions with the immune system, that is within-host processes, could also select for intermediate levels of virulence [10]. More recent work in immune system functioning has challenged this ‘predator-prey’ analogy, citing several differences in immunity cell behaviour from free-living predators [21]. Nevertheless, this simple analogy has inspired many studies, has underpinned new discoveries and has been the stepping stone to other more detailed investigations of host-parasite interactions, which is discussed in more detail in sections that follow.

Another important ecological process that has garnered considerable attention from those who study infectious diseases is competition. Both intra-species and inter-species competition between parasites are common, and competition, including exploitative and interference competition, is known to affect the adaptations of parasites [22]. A form of competition that is commonly investigated is indirect competition via the immune system, called ‘immune-mediated competition’ or often referred to as ‘cross-immunity’ or ‘cross-reactivity’. This competition is analogous to competition due to a shared predator called ‘apparent competition’ [23] in free-living ecological systems. It is unclear if the ability of the adaptive immune response to cross-react with various strains of a pathogen is deliberate or whether it is an accidental consequence of the antigen specificity process [24]. Regardless, it is clear that some degree of imprecision in antigen detection creates a process that strongly affects co-infecting strains, and has a profound effect on their evolution and the infection outcome.

Some ecologists and zoologists recognized that in order to understand virulence it is necessary to investigate the interactions between pathogens and their environment. Thus, the interaction between the host (namely a human) and the pathogen is not the sole contributor to a pathogen’s traits. This type of research investigates how interactions with other non-human hosts [25], with other microorganisms, and with abiotic factors in the environment shape pathogen virulence. For example, cholera’s virulence may be in part due to cholera’s interaction with other micro-organisms in water, for example cholera attaches to zooplankton and benthos [26]. Colwell et al. showed that filtering water with a cloth removed the zooplankton which consequently almost entirely eliminated the disease. While there were some exceptions, the mildness of the subsequent infection suggests that the more serious infections are caused by the concentrated amounts of cholera attached to zooplankton [27]. Indeed this study found the ecological mechanism for the reduced virulence of cholera due to changes in the mode of transmission that Paul Ewald had described a decade earlier [28], which had helped form the idea that transmission and virulence are linked. Overall, this type of systems thinking is an important contribution that ecologists are bringing to infectious disease research and will help contribute to our understanding of virulence.
The pioneering work in virulence evolution from the 1980s and 1990s led to a new discipline where evolutionary thinking was applied to what had been mainly microbiology and medical topics since the time of Koch and Pasteur. Proponents of this ‘evolutionary medicine’ research framework such as George C. Williams, Paul W. Ewald, and Stephen C. Stearns inspired a new generation of evolutionary ecologists to tackle new challenges and topics such as infectious diseases out of which emerged the sub-field of “evolutionary epidemiology”, while in tandem the medical and microbiology communities began adopting a more Darwinian perspective. Indeed, evolutionary and ecological concepts have finally begun to infiltrate epidemiology, microbiology and medicine.

1.2 Modern challenges

Thanks to the founding work of Pasteur in vaccination and Koch in experimental techniques [29], we now have a large repertoire of drugs and vaccines that we throw at infectious diseases, and whose success varies. Our modern control methods have increased survival rates of humans around the world [30]. Modern medicine has transformed many of the most deadly infectious diseases into preventable or curable diseases. However, with these new interventions we have created new and often unforeseen problems [31]. Tackling new challenges has driven growing interest in how ecological, evolutionary and epidemiological processes interact and affect disease severity and pathogen responses to our control methods [18].

One of the most serious problems in modern medicine is the emergence of antibiotic resistance. Not only do antibiotics prevent us from dying from common infections, they are also fundamental to complicated procedures such as surgeries. Antibiotic resistance, then, is threatening to become a global catastrophe [32,33]. After the invention of any antibiotic, resistance soon arises [34]. Thus resistance undermines the long term success of antibiotics and necessitates that we invent new antibiotics. Currently, our invention of new antibiotics is slower than the pace of resistance emergence and, in fact, the development of new antibiotic drugs in the last two decades is in a precipitous decline [35]. Not surprisingly then, microbes are currently winning this arms race. Unfortunately, similar resistance problems arise in other parasites and viruses. Resistance to anti-malaria drugs has appeared regularly for all the new drugs we have developed [36] and chloroquine resistance is becoming widespread, as the resistant malaria strain spreads [37].

Anti-viral drugs also drive rapid evolution of resistance in the viruses they target. Often resistance emerges so quickly that it prevents new drugs from ever being marketed and in single drug treatments, emergence of resistant strains can appear within a few weeks of starting therapy [38]. There are reported cases of resistance against most of the antiviral drugs used for treating Herpes simplex virus (HSV), Cytomegalovirus (CMV), Influenza A, HIV and other viruses [39]. Traditionally, antiviral drugs targeted viral replication, for example by interfering with the synthesis of viral nucleic acids, which readily selected for single nucleotide mutations that confer resistance to the specific effect of the antiviral drug [39]. Identifying this weakness prompted new approaches of targeting other parts of the virus replication cycle. On the whole, the most successful approach has been to combine antiviral drugs, in order to make resistance via a single mutation ineffective. Combination therapy for HIV patients has changed HIV infection from a
death sentence to a chronic illness [40]. A downside to these drug cocktails is that they can be quite toxic to the patient and so often patients do not comply with treatment regimens thus increasing the likelihood of the emergence of new mutants.

An unexpected and unwanted result of our most effective control method is evolution in response to vaccines. Though smallpox was successfully eradicated, there is a growing list of examples of vaccine-driven evolution that are hampering our attempts to control viruses, bacteria and macroparasites [41,42]. Vaccines have been shown to drive two forms of response by pathogens: ‘escape mutants’ and ‘virulence evolution’ [43]. Escape mutants are novel strains that are able to evade the immune response created by the vaccine by altering their antigenic properties [44]. Other pathogens evolve strains with different life-history traits, such as higher levels of virulence (e.g. via higher replication rates) that render the vaccine less effective at clearing the pathogens, and alarmingly, lead to the circulation of new more virulent pathogens. Seminal work by Gandon et al. 2001 showed that vaccines designed to reduce the growth rate of the pathogen would likely select for higher virulence, whereas vaccines that block infection should select for lower virulence. Further theoretical studies followed that examined the conditions that could lead to vaccine-driven evolution and that could select for increased virulence [43,45–48]. In the meantime, evidence of vaccine-driven virulence evolution is being observed empirically, with notable examples in Marek’s disease virus, MDV [49,50] and Poliovirus [51,52]. A novel feature of our modern vaccines is strain-specificity and this has led to ‘strain replacement’, the increase in prevalence of strains not targeted by the vaccine. Indeed, several vaccines, such as Streptococcus pneumoniae and Bordetella pertussis vaccines, have caused strain replacement, and more research is needed to understand the mechanisms [53].

Finally, after the emergence of HIV there has been a strong interest in what drives emergence [54]. Humans and their pathogens do not exist in isolation and thus researchers are looking to the animal kingdom to see what pathogens could jump to humans. Zoonoses (vector-borne diseases and infections in animals that are transmitted to humans) are often studied by zoologists, ecologists and veterinary researchers alongside epidemiologists and other medical researchers. Work that focuses on zoonoses and environmental interactions is now becoming a sub-discipline in its own right called “disease ecology”, which is gaining more attention after outbreaks like avian influenza and SARS [55]. Unfortunately, we are only beginning to understand the ecological conditions that allow for pathogens to adapt to new species [56], but what is clear is that global climate change and habitat destruction coupled with our increased global inter-connectedness are accelerating the chances of disease emergence [54,57].

Ideally, we would like to understand the evolution of pathogens well enough that we would be able to anticipate resistance evolution or manipulate the evolution of pathogens so that they become less virulent [42,58]. This idea of making our infectious disease control methods ‘evolution-proof’ is an important aim in the evolutionary studies of pathogens [42]. We unintentionally achieved this in Cholera, with water purification systems and the separation of water used for sewers and bathing and cooking we severely disrupted its transmission route and so inadvertently selected for less virulent strains to circulate [58]. Another example of virulence management is in Koella et al. (2009) where they propose several ways to manage insecticide resistance by malaria carrying mosquitoes using evolutionary arguments. Applying concepts from the evolutionary theories of aging and senescence they suggest using two insecticides,
larval-killing and late acting, to impose opposite selective pressures that should maintain the susceptibility of the mosquito population to insecticides [59]. In general, artificially selecting for decreased virulence and managing resistance may be more attainable for some pathogens than total eradication and is more sustainable than having to win a never ending arms race between our drugs and escape mechanisms [31]. Undeniably the idea of virulence management using evolutionary approaches has smitten many evolutionary biologists [60,61]. Yet, to attain this requires a deep understanding of the evolutionary and ecological processes that shape our infectious diseases, which, currently, still entails considerably more empirical studies and theories.

1.3 The battleground: inside the host

We have come a long way since the first microscope photographs of anthrax that Koch famously published. Modern advancements in molecular and clinical biology techniques have resulted in fascinating descriptions of how pathogens harm the host and how the immune system attacks the invader. These sophisticated techniques allow us to see what is for the most part invisible to the naked eye and allows us to build a picture of what happens during an infection with ever more detail. From this we have uncovered a very complex network of interacting microorganisms and host cells. In fact, the more we look, the more we find. Current estimates of the number of bacteria that live inside and on human bodies is 10 times that of our own cells [62]. Nevertheless, infectious disease research has traditionally and still is predominantly focused on descriptions, such as identifying which viral proteins interact with a host cytokine, and as a result this research generally lacks systems thinking. These incredibly detailed descriptions are really snapshots of dynamical processes happening in a web of interacting within-host cells and microorganisms.

As an analogy, once ecologists adopted a systems approach it became apparent that many dynamical processes were general to ecosystems and our understanding of ecosystem functioning deepened substantially. Similarly, untangling interactions and finding the dynamical processes of within-host communities should become fundamental to understanding disease burden and emergence. As an example, it is generally understood that the variations in humans implies that their responses to the same pathogens will vary, and so their within-host conditions can either promote or impede the emergence of resistant or more virulent strains. For instance, the immune system can in some cases impede the appearance of resistant strains by creating conditions that are less favourable for a resistant trait [34]. Yet, we are far from the day where we can predict a patient’s likelihood of promoting an emergent strain by taking particular measurements of their within-host system. For pathogens that are in the process of switching to a new host, there are many within-host challenges, such as less productivity, new interactions and trade-offs, chance effects, and other ecological obstacles that make switching to a new host a formidable task [63]. These within-host evolutionary ecology processes are still poorly understood but would be very helpful in explaining why some host switching or emergence events are able to succeed.

As our microbiology technologies advance we can begin to move beyond simply identifying the players of an infection and move towards measuring the dynamics of the infection
within the context of the within-host system. Because of these technological advances, within-host ecological communities and infections are poised to be some of the most tractable ecological systems that we can study. Consequently, mathematical, experimental and clinical biologists are teaming up in order to characterize and measure the dynamics of important infectious diseases that harm humans. The hope is that we can use this deeper systems knowledge in order to better design drugs and vaccines, and ultimately, outsmart the pathogens that currently kill us.

1.4 Theoretical studies and Mathematical Immunology

The use of mathematical models to capture the dynamics of infections is increasingly recognized by the wider infectious disease community. Mathematical modeling of within-host infection dynamics is relatively new compared to epidemiological or ecological modeling, and today the young field called ‘mathematical immunology’ [64] aims to understand the complex dynamical processes in which the immune system engages. These studies focus on either how the immune system works or on the pathogen and how it interacts with the immune system. The latter entails working with data from ever more sophisticated methods of probing the immune system cells and, at times, these methods work with mathematical models to make sense of the data. For example, mathematical models help to extract quantitative information, for instance, interpreting which processes are contributing to the rise or drop of labelled cells. Models can also be used to extract parameters such as cell death rates from labelling experiments such as a bromodeoxyuridine (BrdU) cell-labeling system, that aim to characterize the kinetics of immune cells [65].

Mathematical methods such as dynamical systems theory, network theory, information theory [66] and other methods from physics, such as statistical mechanics, are used to understand immunity processes, such as clonal expansion and non-self-discrimination [64]. This type of research investigates how immune cells communicate with each other via cell-to-cell contact and chemical signaling, and how the immune system learns and develops memory. The rest of this introduction, however, will focus on the types of models that aim to understand the interactions between the pathogen and the immune system. The bulk of these studies have been done to understand viral and host cell dynamics, with many studies of HIV, hepatitis C virus (HCV), Influenza, and to a lesser extent hepatitis B virus (HBV), cytomegalovirus (CMV), and lymphocytic choriomeningitis virus (LMCV) [65].

Soon after the first mathematical immunology models were published, models of host-parasite dynamics appeared that used an ecological analogy [10,20,67]. This new application of predator-prey theory, where the immune system is the predator and the parasite is the prey, allowed for simple conceptual models to be used for determining causal links between processes. For example, it was demonstrated how the dynamics of drug resistance emergence in HIV is oscillatory and non-linear in nature, similar to predator prey dynamics, where the increase in abundance of the target cells due to treatment facilitates the emergence of resistant strains [38,68,69]. The simplicity of these models also allows them to be connected to epidemiological models in order to study how within-host processes affect epidemiological processes and vice
versa. Linking these two scales is particularly important for understanding how the different selection pressures at different scales interact with each other, the result of which shapes the virulence of the pathogen and its ability to spread in a population [70–72].

Currently, mathematical models of pathogen within-host dynamics are used in mainly three ways [73]. First, mathematical models are used for estimating parameters relating to the infection, for example, the replication rate of the pathogen, infected cell life-spans or the attack rates of cytotoxic T-cells (e.g. in [74–77]). In the virology literature this type of research that joins mathematical models and experimental data is coined ‘viral kinetics’, where the aim is to characterize and quantify the dynamics of viruses in vivo and in vitro.

Another use of mathematical models that is less appreciated, particularly in empirical circles, is using models to compare hypotheses. By fitting several models that represent different competing hypotheses to clinical data, and then comparing the likelihood of each model, one can ascertain which hypothesis is most likely to be true [73,78]. For example, in malaria this technique has been used to compare various competing hypotheses for why some strains cause more anemia than others. A differential infection preference for the age of red blood cells by viral strains was found to lead to higher or lower parasite load and so different levels of disease burden, therefore, strains that preferred younger red blood cells caused more anemia [79,80].

Finally, mathematical models called ‘conceptual models’ are used to deepen the understanding of infections by finding the causative processes for empirical observations [38,73,81]. Conceptual models have advanced the theory of within-host dynamics and its ecology and evolution, and thus have aided in our overall understanding of infectious diseases. Conceptual models play a role in addressing important questions. An important example of this is the model by Nowak, Anderson and May (1990) that argued that evolutionary dynamics could explain how the HIV progresses to AIDS [73,82,83]. Though this model had only a few assumptions it matched empirical observations well and illustrated that continual emergence of mutant strains could trigger a collapse of the immune system and thus cause AIDS [73]. Another important example of conceptual models that advanced our thinking was a model by Gandon et al. on vaccine-driven evolution [84]. This paper spurred debate and investigations and spread the term ‘imperfect vaccines’ [45,85–88].

1.5 Model organism: Human Papillomavirus (HPV)

One of the most recent examples of an imperfect vaccine that has been developed and marketed is the HPV vaccine, which is sold under the names Cervarix® and Gardasil®. Though cervical and penile cancer has been known since antiquity, the causal link to HPV infection was only recently demonstrated. The experiments of Herald zur Hausen, now a Nobel laureate for his work, and others showed how HPV infection can lead to the transformation of a cell into a malignant cell [89]. Widespread public awareness of HPV and their diseases is a very recent event and is largely due to the recent commercialization of the vaccines.
HPV has several characteristics that make it an excellent model for studying the ecology and evolution happening inside hosts. First, as a double-stranded DNA virus HPV makes fewer mistakes in viral replication, and thus the quasi-species effect seen in RNA viruses does not readily apply [90]. More stable and conserved traits allow for a more clear understanding of how traits are selected for given different ecological scenarios. In addition, infections by HPV are for the most part acute infections (of very long duration), but HPV infections can also become chronic. Manifesting both acute and chronic infections creates a window into what ecological conditions allow for this change in stability of the system. Also, HPV exists as several strains (called types) which regularly co-infect the same host, thus permitting for investigations into whether interactions between these types lead to higher or lower virulence. Finally, the new HPV vaccine is strain-specific and only targets two of the cancer causing types. Therefore this imperfect coverage raises questions as to whether the vaccine will cause changes to the within-host ecology and evolution of HPV and whether these could lead to unwanted consequences. For the aforementioned reasons, investigation into the ecology and evolution of HPV is a recurring theme in this thesis.

1.6 Health implications of within-host ecological communities

The research discussed so far, for the most part, has focused on one-parasite-one-host interactions. However, like free-living ecological systems, host systems are home to a large diversity of microorganisms, most benign to the host. A community-level understanding of these systems is currently in its infancy. Early indications, demonstrate that how these species interact with each other and with the host’s cells can profoundly affect disease progression and the overall health of the host. For example, co-infected children with both malaria and helminthes experience high malaria virulence. This is because the immune system is unable to mount an effective response to the malaria infection since the helminth infection elicits a response that compromises the cell-associated response needed to tackle malaria [91]. Situations like these call for a community approach, one similar to that used in community ecology. With new technologies and international collaborations like the Human Biome Project, we are at the cusp of elucidating the ecology that happens inside us.

1.7 Objectives

In this thesis, I begin by applying some community ecology concepts to viral dynamics (chapter 2). I set out to demonstrate that within-host ecology will help us deepen our understanding of infections (their severity, virulence, disease progression). I investigate how changing the ecological conditions inside hosts affects infection outcomes (chapter 2), co-infections (chapter 2 and 3), and virulence evolution (chapter 4). Following the work on the virulence-transmission trade-offs, I focus on trade-offs which have received little attention, namely within-host trade-offs (chapter 2) and the recovery-transmission trade-off (chapter 4).
investigate how trade-offs affect infection outcomes and how vaccines interact with them. On the whole, I address how this knowledge should also help explain how artificially changing within-host ecology can drive evolutionary responses to our control mechanisms. I believe that unfolding the dynamics of within-host communities using a systems approach will help us tackle the tough challenges imposed on us by the infectious diseases we fight.
Chapter 2

Food Webs in the Human Body: linking ecological theory to viral dynamics

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Abstract

The dynamics of in-host infections are central to predicting the progression of natural infections and the effectiveness of drugs or vaccines; however, they are not well understood. Here, we apply food web theory to in-host disease networks of the human body that are structured similarly to food web models that treat both predation and competition simultaneously. We show that in-host trade-offs, an under-studied aspect of disease ecology, are fundamental to understanding the outcomes of competing viral strains under differential immune responses. Further, and importantly, our analysis shows that the outcome of competition between virulent and non-virulent strains can be highly contingent on the abiotic conditions prevailing in the human body. These results suggest the alarming idea that even subtle behavioral changes that alter the human body (e.g. weight gain, smoking), may switch the environmental conditions in a manner that suddenly allows a virulent strain to dominate and replace less virulent strains. These ecological results therefore cast new light on the control of disease in the human body, and highlight the importance of longitudinal empirical studies across host variation gradients, as well as, of studies focused on delineating life history trade-offs within hosts.
2.1 Introduction

The future of infectious disease control is threatened by the growing frequency of evolutionary responses of pathogens to antibiotics, antiviral therapies, and vaccines [61,92]. Some `imperfect' or `leaky' vaccines [46] allow the pathogen the opportunity to evolve, potentially leading to either increased virulence (e.g. Marek's disease, [49]) or increased prevalence of non-target strains, known as strain replacement (e.g. 7-valent pneumococcal conjugated vaccine [93]). These challenges have led researchers to adopt new approaches that move beyond the basic biology of infections. Most notable are the studies of the kinetics [65,94] and of the evolutionary biology of infections [95,96]. These approaches distinguish themselves by describing interacting strains and immune cells as dynamical systems, and are used to understand persistence and virulence. This research, therefore, is closely aligned with classical food web research, which seeks to understand the persistence of whole ecological communities.

Though the need to untangle in-host ecological interactions is increasingly recognized [63,80,97], ecologically inspired empirical studies are not yet common, particularly for infectious diseases that affect humans. In contrast, ecology is part of the backbone of theoretical approaches to in-host studies because the original in-host models sprang from classic population ecology [38]. The analogy is that the immune system effector cells (e.g. cytotoxic T-cells, CTL, or B cells) target the invading parasite population in a similar fashion to predators consuming prey (e.g. CTL destroy infected cells). Consequently, most in-host models in the literature are Lotka-Volterra-like systems (for a review see [4]).

The analogy can be furthered to view the entire body as an ecological environment in which pathogens, host resources and immunity form networks of interacting populations that are analogous to food-webs [98–100]. In a rare example of this extended analogy, Pedersen and Fenton [99] broke down the entire in-host parasite-host system into community networks according to regions of the body, and argued cogently that further understanding in disease control would require the development of a strong multi-species approach. Similarly, Smith and Holt [101] emphasized the idea of the host as the environment for pathogen growth and competition. One approach to unifying these earlier efforts, and one taken in ecology, is to derive a theory for important sub-systems (modules) as a function of changing environmental conditions.

Much recent food web research uses food web modules, multi-species extensions of pairwise interactions between consumers and resources (Fig. 2.1). These modules, or “motifs” in network theory, are sub-networks used to probe the dynamical behavior of larger ecological communities [102] (see Box A1 for review of modular theory). Though modules (Fig. 2.1 i - iv) are regularly found in infectious disease studies (e.g. immune-mediated apparent competition, [22,23], Fig. 2.1 ii, and model 2.3), surprisingly, a modular theory of in-host systems is not yet developed. Modular food web theory has found that species persistence depends on the topology of the web, as well as the strength of these interactions [103]. This suggests that to understand persistence, variation in disease burden, or strain dynamics in the human body, we need to identify the main players in the ecological network (e.g. which cells or cytokines fight a particular infection) as well as the relative strengths of the interactions.

Life histories of species in a community play an important role in food web dynamics. Interestingly, life history trade-offs often mediate the strength of interactions between species.
As an example, let us consider species interacting in the diamond food web module (Fig. 2.1 iv), where the intermediate consumers (C₁ and C₂) are under both predation and competition pressure (Fig. 2.1 iv). Ecologists have regularly found trade-offs between competitive strength (i.e., growth) and the ability to avoid predation, such that, fast growing, highly competitive organisms put little energy into defenses, while slow growing, weak competitors heavily invest energy into defense mechanisms. It is well known that this trade-off can yield coexistence [23]. Like their ecological counterparts, “in-host trade-offs” should arise because energy and time limit the replication and development of the parasite, and limit the production of immunity defense strategies.

While an important trade-off between virulence and transmission is thoroughly explored in disease research [4], examples of empirical in-host trade-off studies are surprisingly few, and we know of only one such virus study. De Paepe and Taddei (2006) found that the mortality rates of bacteriophages were positively correlated with multiplication rates, thus showing that the reproduction-and-survival trade-off also acts on viruses. They argued that the cost of having higher multiplication rates led to the production of more unstable virions because the thickness of the capsids and the density of the packed genomes were compromised [104]. Clearly, other in-host trade-offs ought to exist, and below we make theoretical arguments that suggest focused empirical research on in-host trade-offs can importantly delineate the abiotic conditions within the human body that drive either the dominance of virulent, or non-virulent, strains.

Finally, the application of drugs and vaccines clearly resonates with a major area of interest in food webs which is concerned with the implications of press (continuous) and pulse (discrete) perturbations on whole ecosystems [105,106]. Drugs and vaccines effectively alter the strength of in-host web interactions by decreasing resource use or increasing pathogen visibility [107] and by boosting the ability of the immunity effector cells to quickly attack the infection, respectively. In an ecological sense, these control methods are strong perturbations, and it is interesting to note that they are often introduced without consideration of the environmental conditions and food web structure inside the host. A timely example of the introduction of vaccines without a clear picture of strain interactions and in-host ecology occurs in Human Papillomavirus (HPV). The strain-specific HPV vaccines target the two most virulent types (synonymous to ‘strain’), and they provide some cross-protection against a few antigenically similar types [108]. We considered how ecological understanding from the framework presented here could help explain HPV vaccine efficacy.

In what follows, we first revisit food web theory to show that changing environmental conditions modify the outcome of competition in predictable ways. We then extend this theory by considering the diamond food web module (Fig. 2.1 iv and model 2.2) within a disease framework. The diamond module ought to be common in hosts given that in localized regions of the body pathogens regularly share resources (cells) and often share a common predator (adaptive or innate immune responses [109]). With this viral dynamics food web module (model 2.2), we then look at how in-host life history trade-offs mediate competition and the disease burden between virulent and non-virulent strains across a gradient in host conditions. We end by analyzing the diamond and apparent competition modules (models 2.2 & 2.3) in different in-host environmental conditions, each constrained by empirically-estimated parameter sets and find that the results are remarkably consistent, which emphasizes the generality and plausibility of these results.
Figure 2.1. Common community modules in both free-living and in-host systems. $P = \text{predator (e.g. carnivore), } C = \text{competitor (e.g. herbivore) and } R = \text{resource (e.g. plant species).}$ Modules: (i) Single-chain (ii) Apparent competition (iii) Resource competition (iv) Diamond (v) Intraguild predation. (vi) Modules are sub-webs of a larger web of all interacting host cells and coinfecting parasites.

2.2 Results

*Historical Results: Food Webs across Changing Environmental Conditions*

Species coexistence, or dominance, depends greatly on environmental context. To illustrate this, we review a well-known result from ecological theory that considers a trade-off between growth and predation defense across a range of environmental productivity (Fig. 2.2 A). For low productivity, the high growth species, $C_1$, is able to suppress the common resource, $R$, to a point where the slow growing competitor, $C_2$, has negative growth rates (i.e. it has lower $R^*$, sensu [110], see Box A1). This leads to the dominance of the species with the fast growing strategy and the exclusion of the slow growing species, because, at low productivity, there is only enough energy to maintain a low density of predators (Fig. 2.2 A.i). However, at intermediate productivity, both species coexist as predator densities are now elevated enough that the predator consumes the faster growing species $C_1$, to an extent that allows the slower growing, well-defended species, $C_2$, entry into the community (Fig. 2.2 A.ii). At higher productivity still, the well-defended strategy dominates as predation increases to such an extent that the fast growing, highly consumed, species is decimated (Fig. 2.2 A.iii).

In summary, life history trade-offs mediate the strength of the interactions between populations of the community. Here, the slower growing competitor is a weaker consumer of the
common resource but is also weakly consumed by the predator (i.e. the weak chain; Fig. 2.2 A.ii, R-C$_2$-P chain), while the faster growing competitor is a stronger consumer of the common resource but also strongly consumed by the predator (i.e. the strong chain; Fig. 2.2 A.ii, R-C$_1$-P chain). This growth-defense trade-off, then, couples a weak food chain and a strong food chain. Therefore, under changing environmental conditions, trade-offs like these, mediate competitive outcomes in predictable ways (i.e. context dependence). This context dependent result pushes us to ask an interesting, if simple, question for disease ecology. Can we expect the individual abiotic conditions of the human body to also play a fundamental role in mediating the dominance of virulent disease strains? We examined this within a viral disease food web framework, with the hope that this gradient idea leads to predictable dynamical outcomes (Fig. 2.2 B) as it does in free-living food webs (Fig. 2.2 A).

**Figure 2.2.** Variation between hosts as environmental gradients. The different weighted arrows represent the overall interaction strengths, and the various sized circles indicate relative densities of: shared resource, $R$, competitor species $i$, $C_i$, top predator, $P$, antibodies, $Abs$, macrophages, $MAC$, cytotoxic T cells, $CTL$, uninfected cells, $X$, and infected cells by strain or species $i$, $Y_i$. Similar to studies of abiotic environmental gradients studied in ecology (A), finding patterns of dynamic behaviours and outcomes across host heterogeneities (B) could greatly improve our understanding of variability in disease burden and what factors affect virulence and persistence.
Disease Food Webs and Life History Trade Offs

To explore how the strength of an in-host trade-off influences competition between strains, we performed simple numerical experiments by varying a trait involved in a given trade-off such as the mortality rate (decay rate) of the virulent strain and followed a given property of the dynamics (e.g. the equilibrium densities) of the strains as a response variable (see Fig. 2.3 A for a schematic). We focused on the reproduction-and-decay trade-off [104], though results of another in-host trade-off are in the Supplementary Material (Appendix A.1). In the parameter space considered, the full (1) and diamond models (2) gave nearly identical results (not shown) only the diamond model (2) does not output viral loads. However, a more formal analysis of these two models would be needed to see if and when the similarity in dynamical behaviour between models breaks down. This implies that the two-strain viral dynamics model behaves like a diamond module, therefore, consideration of the diamond module ecological literature should be of interest to those investigating viral strain competition. The plots presented here are of the full model (1) because discussion of viral loads, the main measurable quantity of infections, is best for empirical comparisons.

As expected from modular theory, the results suggest that trade-offs may be extremely powerful in mediating the dominance or coexistence of virulent and non-virulent strains (or less virulent), regardless of whether the infection was chronic (Fig. 2.3 B) or acute (Fig. 2.3 C). Clearly, there is little cost to higher reproduction rates until the decay rate of the virulent strain is greater than the less virulent strain (i.e. to the right of the dotted lines in Fig. 2.3 B and C). Varying the strength of the trade-off gave a common sequence of events: virulent dominance, coexistence, and non-virulent dominance. In the case of HIV, the dominance of the strains switches as the strength of the trade-off changes without passing through coexistence (Fig. 2.3 B; no grey region) but in the acute infection case, the coexistence region comes first and then enters into non-virulent dominance (Fig. 2.3 C). See Supplementary Material for conditions that give coexistence (Appendix A.1, Fig. A.1.1) and for another example of this sequence of outcome events as the strength of the reproduction-and-lytic-effect trade-off is increased (Fig. A.1.3). These results, then, are consistent across different viral empirical parameter sets. Also, given that the strength of life history trade-offs alters coexistence, it becomes interesting to also consider how life history trade-offs interact with changing host conditions to alter coexistence.
Figure 2.3. Effects of in-host trade-offs on dynamical outcomes. Viral loads of the virulent strain (strain 1; blue), and of the less virulent strain (strain 2; red). **A: Example bifurcation plot.** Replication-defense trade-off. As the cost of higher replication increases, transient coexistence becomes possible (area near the edge of shaded region). If this cost is raised even higher (less investment in immune defenses; blue link becomes stronger), then coexistence or dominance of the less virulent strain are possible. Mapped to each dynamical outcome is the corresponding module with its respective interaction strengths and relative densities. **Replication and decay trade-off in viral dynamics model: Chronic and transient infections.** HIV is plotted in B and Influenza A in C. The dashed lines indicate where both strains have identical decay rates ($u_1 = u_2$), which is where most conventional in-host models fall. However, without strain-specific measurements of viral decay rates we do not know where the system actually lies along these plots.
We considered how smoking, a host behavior that changes abiotic in-host environments, can be a potential environmental gradient across which in-host disease ecology can vary. Figure 2.4 A.i shows how smoking can affect the outcome of hosts infected with two HPV types. The non-smoker line indicates where the virion decay rate of HPV-16, \( u_1 \), is within a non-smoker. Because smoking impairs the ability of antibodies to neutralize free virions [111], then the smoker host is necessarily to the left of the non-smoker (the exact quantity is not known only the direction) and so the smoker experiences higher viral loads because more virions are able to infect cells (Fig. 2.4. A.i). The result in Fig. 2.4 A.i was fairly robust because the qualitative results were not affected by including either impaired CTL attack or suppression of LC, but differential CTL killing rates did. For example, if the CTL killing rate of HPV-16 infected cells is more impaired by smoking (i.e. \( p_1 < p_2 \)), then the bifurcation plot is shifted to the right (not shown). Biologically this means that HPV-16 will dominate at a higher viral load because as the difference between the CTL killing rates against each strain increases, the biologically reasonable region is closer to the steepest region of the viral load of HPV-16 (i.e. closer to the origin). This implies that smokers will have a much higher viral load than is demonstrated in Fig. 2.4 A.i.

We considered another informative example, where we assumed that the conditions in healthy hosts favored the less virulent strain. Not surprisingly, the less virulent strain dominates the non-smoker (Fig. 2.4 A.ii), however, if the same level of smoking impairment is included we find that the system is now shifted to a completely different equilibrium, where the more virulent strain dominates the infection. Consequently, in such a case, smokers can experience a dramatically different outcome in that they have infections dominated by virulent strains. Alarmingly, the result of a simple human behavior pushes the host to an entirely different equilibrium that is potentially life threatening.

Finally, we considered another example of immunosuppression: HIV infection. Here, the depletion of CTL leads to higher HPV viral loads (a consequence of a larger number of infected cells; Fig. 2.4 B), which corresponds to what is known about HIV-positive HPV infected hosts [112]. This high viral load can be increased further with the trade-off (Fig. 2.4 B.i), again demonstrating that dynamics and in-host trade-offs could help explain clinical results and variation of disease burden across hosts. Finally, this immune suppression by CTL depletion gave longer transients (Fig. 2.4 B.ii dotted vs. solid line). In the parameter range considered, on average the HIV-positive environment took 92 days longer for the exclusion of one of the types (range was 9-350 days longer to exclusion, where the longer transients were near the bifurcation). Therefore, ecologically HPV types are transiently coexisting for longer and so, clinically, more HPV types are more likely to be detected than in immunocompetent infections where exclusion happens faster.
Figure 2.4. Immune deficiency and vaccination in HPV. Viral load of HPV-16 (blue), viral load of non-vaccine HPV type (red). A: Immunocompetent CTL response. (i) Smoking impairs the humoral response which shifts the system away from the bifurcation, resulting in higher viral load and thus higher disease burden. (ii) Hypothetical scenario: Smoking changes the strain dominance structure, by weakening the strength of the natural trade-off, so that the more virulent strain is dominant in the smoker. Epidemiologically, some strains would be more prevalent in smokers than in non-smokers, and their viral loads would be significantly higher. B: HIV-positive hosts with HPV infection. Over the entire trade-off axis the viral loads are higher (than in...
A) due to the depletion of the CTL population by HIV. (i) Similar to A.i, the effect of the simultaneous suppression of the humoral system is augmented by the in-host trade-off. (ii) Example time series at $u_1 = 0.83$ and $u_2 = 1.2$. Another dynamical consequence of CTL depletion by HIV is that HPV types coexist longer inside the host (differences in time till exclusion of one type). Compare transients of dotted (immunocompetent) vs. solid (immunocompromised) curves. **Vaccination. C:** If stochasticity near zero is considered, then vaccination in the immune-mediated apparent competition module leads to the clearance of both strains, i.e. cross-protection (here the curves represent infected cells of the HPV types). **D:** In contrast, the diamond module, with shared resources, behaves differently. By increasing the strength of the trade-off, the vaccine changes the conditions to favor the less virulent strain.

### Disease Food Webs and Disturbances: Drugs and Vaccines

Currently cross-reactivity is the only known form of HPV type-type interaction. The HPV community then is assuming that the underlying in-host web is an apparent competition module (Fig. 2.1 ii). When we considered vaccination in this apparent competition module (model 2.3), the in-host trade-off (varying $u_1$) had a similar qualitative effect, i.e. switching from $Y_1$-wins to $Y_2$-wins equilibrium with the same bifurcation. Nevertheless, the infected cell equilibria are close to zero ($< 1$) so the oscillations of the transients drive them to crash, and subsequently the very large CTL population also crashes. Therefore, if you consider stochastic effects close to zero and very strong overshoot properties of the transients, the vaccine case in the apparent competition module corresponds to vaccine clearance of both types via cross-protection (Fig. 2.4 C). However, should this cross-reactivity only assumption be incorrect and instead the web structure is the diamond model (which we suspect it might be), then the vaccine CTL do not suppress the two types as much (Fig. 2.4 D). Thus increasing the antibody neutralization rates to vaccine levels against HPV-16 does change the outcome of the system, where now the non-vaccine type has a higher viral load than in the unvaccinated host (Fig. 2.4 D). Therefore, on account of resource competition, the slower reproducing strain is not completely cleared by the cross-reactive vaccine and, though at low levels, now dominates the system (a phenomenon called “type replacement”). This demonstrates that the underlying web matters and that the unwanted outcome of competitive release could be amplified because the vaccine changes the in-host trade-off. It is imperative to look for evidence of resource competition between high-risk HPV types, regardless of their antigenic similarities.

### 2.3 Discussion

Here, we have extended ecological theory to show that host abiotic environment coupled to life history trade-offs may play a fundamental, but underappreciated, role in the dynamics of infectious diseases. Specifically, we have shown that even modest differences in host environment can significantly change disease burden. Similarly, human behavior that alters environmental conditions (e.g. smoking) has the potential to flip the in-host ecosystem from non-virulent viral strain dominance to one dominated by virulent strains. Interestingly, patients are asked to stop smoking to help clear HPV infections, and this in-host community dynamics and trade-offs framework helps interpret why this may often work. Similarly, the results of the HIV-positive environment captured empirically known features of higher viral loads and more coinfection with non-HPV-16 types [113]. Our environmental gradient analogy, then, could aid in building a more mechanistic understanding of variation in disease burden and clearance across different patients.

The application of food web theory to the in-host environment can be further justified when the role of pathogens in classical ecological food webs is considered. The parasitic strategy
is fundamentally a consumer strategy, whereby fluxes of energy and biomass flow from the host to the parasite [114]. The in-host environment, then, is not a completely separate system from the larger food web. Consequently, we believe, viruses also participate in this biomass and energy loss, though they themselves are not cellular organisms. Because viruses cannot perform their own metabolic processes, they hijack the host cell’s metabolic products in order to replicate themselves. This is a redirection of energy that the virus now uses, not the host. The immune systems’ role is to break the link between parasite and host and stop the energy loss. The immune system does not directly get energy or biomass from the parasite but it indirectly benefits by the depletion of the parasite population because the host has more resources to contribute to the immune system.

Ultimately, however, what food web theory offers most to infectious disease studies is a community approach that complements the conventional reductionist approach. For instance, the concept of meta-populations has aided developing epidemiological theories and models of infectious diseases [115]. A true extension of this work will be to consider populations of hosts as meta-communities. This attempt to connect in-host ecology and between-host ecology should help us understand how these two ecological stages affect the evolution of infectious diseases [70].

We show here that if HPV coinfection modules are more complex than currently expected, then the cross-protection of the vaccines may not be as strong as expected for some types. Thus the underlying module of the in-host community could affect vaccination outcome, and indeed, a couple of types have been identified as types that could potentially benefit from the vaccine, namely HPV-33 [116], and HPV-52 [117]. Note that our results are tempered by the fact that trade-offs experienced by HPV have not been looked for or found. We also found that a dynamical consequence of HIV infection was longer transient coexistence of HPV types. This ecological result could help explain why HIV-positive patients have more multiple infections [113]. Since HPV types regularly coexist in the same hosts, this virus is an interesting model to study the mechanisms of strain coexistence and in-host trade-offs experienced by viruses.

For simplicity, we assumed that the strains do not evolve or change phenotypes (e.g. shedding of an antigenic coat) during the course of the infection. Nonetheless, fast evolving infectious diseases still fit this framework. In fact, their rapid evolution exploits life history trade-offs by changing the relative costs of advantageous traits. One can envision that the evolutionary change allows the system to move across these plots during the course of an infection. Since rapid evolutionary changes affect the important rates of the system which determine the interaction strengths, they effectively alter dynamical outcomes. Life history trade-offs, then, serve as a link between evolutionary changes and the ecology of the infection.

Strains can be distinguished by a suite of traits, yet strains in most current models usually differ only by their replication or infection rates. Thus, a priori, these models tend to exclude cases in which the life histories of the strains differ. As shown here, differences in traits other than replication rates can have measurable effects. Consequently, reasonable estimates of strain-specific mortality rates, burst sizes, immunity evasion rates, etc., in conjunction with knowing what in-host trade-offs exist, should allow us to better predict and understand the outcomes of coinfections.
Taxonomic studies distinguish strains by their genetic differences, and attempt to piece together the evolutionary history of their genes (example [118]). A potentially fruitful avenue of study would be to find the ecological context in which traits are favored by tying these genetic variations to their role in life history and in-host trade-offs, while also searching for the costs of traits and not just focusing on their advantages.

For example, HPV types are either of high- or low-oncogenic risk. This depends on their host cell transformation properties, where their early proteins E6 and E7 work together to extend the life of the host cell (prevent apoptosis) and stimulate cell cycle progression [119]. This strategy appears to be advantageous for high-risk types, because, they increase: a) the number of infected cells without having to burst from one cell and then find another cell to infect, and b) the viral production per infected cell. However, to be clear, the malignization of the host cell (which usually happens several years later) is not what increases viral fitness. Even though these traits appear advantageous, we should also ask: what are the low-risk types good at that would explain why they can coexist with high-risk types? Also, is there a cost to these cell transformative traits of high-risk types? This exemplifies that our current focus on virulent forms limits our understanding of in-host trade-offs and dynamics.

Future in-host models of HPV should include several features ours did not. For example, considering the spatial nature of HPV infection could be used to address other interesting questions, such as, why is the distribution of HPV types in normal mucosa different than in cancer tissue? Can ecological aspects of the in-host environment play a role (e.g. are types being competitively excluded over time)? Though the spatial ecology of HPV was not considered here, the importance of space cannot be understated. Host resources might be clumped or sparse, and the distribution of different cell types is also heterogeneous, therefore, other modeling methods, such as partial differential equations or individual-based-models, may be more appropriate for capturing these interactions and local spatial effects [120]. Spatial heterogeneity can change the strengths of the interactions that in well-mixed environments are strong, and the spatial coupling of more weak interactions can lead to more stable dynamics and less oscillations [121]. Studies of HPV in-host ecology in future models could also help elucidate other HPV debates that could be related to resource competition between types (e.g. do types cluster together? [122] Or are lesions formed by only one HPV type? [123]). Our model was a first and course attempt at addressing some of the many interesting questions in HPV that could be tackled using this in-host community ecology approach.

The models used here were kept simple in order to maintain explanatory power for conceptual development. Changes to our assumptions would lead to interesting extensions of this work. For example, we assume the in-host environment is held constant over the course of the infection, and yet, non-constant environments would be very interesting to consider. Issues of drug non-compliance, self-medication or habits that cycle could impact in-host dynamics, much like environmental perturbations or seasonal/periodic changes ecologists study. Another example of an assumption change, would be to include coinfection of cells, which not only would affect strain-interactions [124] but could also be considered a change in community module, one akin to the intraguild predation module [125] found in free-living systems (Fig. 2.1 v). Finally, an important change to these assumptions is finding more realistic and appropriate forms for the rates presented here. For instance, in ecology, modes of predation have been well characterized (functional and numerical responses) but, in-host dynamic studies are yet to fully capture and synthesize the immune cell-pathogen interaction types. Excitingly, the field of mathematical
immunology is growing rapidly and, they are finding features that depart from classic predator-prey interactions [21].

We propose two in-host trade-offs to be sought after in future work. First, the reproduction-and-predation-resistance trade-off found in free living organisms, can be envisioned as a trade-off between reproduction-and-immune-evasion, where there is a choice between allocating energy and resources to efficient replication or to immune evasion methods (e.g. anti-interferon or down-regulation of TLR) or to other mechanisms that interfere with the immune system. Second, a trade-off between resource-use-and-immune-evasion would be analogous to the predation risk of foraging. In viruses, the more time spent inside host cells (even if reproducing at low levels) increases the likelihood of being detected by the immune system. These trade-offs might help us understand the context under which immune evasion strategies evolve.

Though there are features unique to in-host environments [21], it is exciting to explore how they fit within the ecological stage in which these systems unfold. Studies using evolutionary ecology methods that look for in-host trade-offs in viral families infecting humans could lead to a powerful understanding of how strains interact. This type of viral ecology research could also help motivate our decisions as to which interactions to strengthen or weaken artificially using drugs or vaccines, as well as, inform as to why some attempts fail.

With the development of more sophisticated molecular techniques, probing in-host dynamics is increasingly more feasible and studying the in-host environment as a web of interacting populations is imminent. Studies that measure multiple populations longitudinally are crucial for grounding in-host theory. We suggest that comparisons of in-host dynamics across a range of hosts of a potential gradient (Fig. 2.2 B) will accelerate our understanding of disease burden and clearance, and will be fundamental in our development of an ecological theory of the human body. This in-host web theory coupled to a more static molecular biology view of these infections will enrich and bring together the studies of kinetics and evolutionary biology of infections, while proving to be of enormous power in disease control.

2.4 Methods

Viral Food Web Models
The two-strain viral dynamics model with cross-reactive CTL immunity[38,67] is

\[
\frac{dX}{dt} = \lambda - dX - \beta_1 XV_1 - \beta_2 XV_2 \\
\frac{dY_1}{dt} = \beta_1 XV_1 - a_1 Y_1 - p_1 Y_1 Z \\
\frac{dY_2}{dt} = \beta_2 XV_2 - a_2 Y_2 - p_2 Y_2 Z \\
\frac{dV_1}{dt} = k_1 Y_1 - u_1 V_1 \\
\frac{dV_2}{dt} = k_2 Y_2 - u_2 V_2 \\
\frac{dZ}{dt} = c_1 Y_1 Z + c_2 Y_2 Z - mZ
\] (2.1)
The uninfected cells, $X$, become infected cells, $Y_i$, by contact with free virions of strain $i$, $V_i$, and infected cells are killed by CTLs, $Z$, at a rate of $p_i$. The uninfected cells are born and die at constant rates, $\lambda$ and $d$ respectively. Infected cells become infected, $\beta_i$, and die at a rate caused by the virus, $a_i$ (this equals $d$ if the virus is non-lytic). Free virions are produced by the infected cells by rate $k_i$ and are cleared at a rate $u_i$ either due to decay, mucosal flushing or antibody neutralization. The CTL population grows at a rate proportional to the infected cell population, $c_i Y_i Z$, and the CTLs have a constant death rate, $m$.

In order to reduce this model to be more manageable we assumed the free virion variables, $V_i$ and $V_2$, reach steady state because their dynamics are much more rapid than the other variables [124]. This reduction gives a viral dynamics model that is analogous to the diamond module in food webs

$$
\begin{align*}
\frac{dX}{dt} &= \lambda - d X - \beta_1 Y_1 - \beta_2 Y_2 \\
\frac{dY_1}{dt} &= \beta_1 Y_1 - a_1 Y_1 - p_1 Y_1 Z \\
\frac{dY_2}{dt} &= \beta_2 Y_2 - a_2 Y_2 - p_2 Y_2 Z \\
\frac{dZ}{dt} &= c_1 Y_1 Z + c_2 Y_2 Z - m Z
\end{align*}
$$

(2.2)

where $\beta_i = k_i \beta_i / u_i$. Also, model 2.2 can be modified to represent the apparent competition module [23] by assuming the resource, $X$, is constant. Therefore the infected cells, $Y_i$, grow at a rate, $\phi \beta_i$, which is affected by the free virus population parameters. This gives,

$$
\begin{align*}
\frac{dY_1}{dt} &= \phi \beta_1 Y_1 - a_1 Y_1 - p_1 Y_1 Z \\
\frac{dY_2}{dt} &= \phi \beta_2 Y_2 - a_2 Y_2 - p_2 Y_2 Z \\
\frac{dZ}{dt} &= c_1 Y_1 Z + c_2 Y_2 Z - m Z
\end{align*}
$$

(2.3)

where $\beta_i = k_i \beta_i / u_i$. We used this immune-mediated apparent competition module for comparison to the diamond.

**Parameters**

Parameter estimates were taken from the literature. HIV: $\lambda = 397 \text{ cells} \cdot \text{day}^{-1}$, $d = 0.3 \text{ day}^{-1}$, $\beta_1 = \beta_2 = 0.001 \text{ day}^{-1}$, $k_1 = 1114 \text{ virions} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$, $k_2 = 600 \text{ virions} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$, $u_2 = 3.12 \text{ day}^{-1}$, $a_1 = a_2 = 0.064 \text{ hr}^{-1}$, $u_2 = 0.105 \text{ hr}^{-1}$, $a_1 = a_2 = 0.066 \text{ hr}^{-1}$ all from [94]. Influenza A: $\beta_1 = \beta_2 = 7.5 \times 10^7 \text{ cells} \cdot \text{hr}^{-1}$, $k_1 = 0.098 \text{ hr}^{-1}$, $k_2 = 0.064 \text{ hr}^{-1}$, $u_2 = 0.105 \text{ hr}^{-1}$, $a_1 = a_2 = 0.066 \text{ hr}^{-1}$ all from [94]. HPV: $\lambda = 36000 \text{ cells} \cdot \text{day}^{-1}$ [127], $d = 0.048 \text{ day}^{-1}$ [128], $\beta_1 = \beta_1 = 0.0067 \text{ day}^{-1}$ [129], $k_1 = 100 \text{ virions} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$, $k_2 = 50 \text{ virions} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$ [130], $u_2 = 0.52 \text{ day}^{-1}$ [104], and since HPV is a non-lytic virus $d = a_1 = a_2 = 0.048 \text{ day}^{-1}$ [128].

Immunity against HIV: $p_1 = p_2 = 1 \text{ day}^{-1}$ [131], $c_1 = c_2 = 0.3 \text{ day}^{-1}$ [132], $m = 0.5 \text{ day}^{-1}$ [133].

Immunity against HPV: (i) Immunocompetent: $p_1 = p_2 = 1 \text{ day}^{-1}$ [131], $m = 0.5 \text{ day}^{-1}$ [133], and because HPV is a poor natural immunogen and immunity against HPV types is cross-reactive at best $c_1 = 0.1$ and $c_2 = 0.05 \text{ day}^{-1}$ [134]. (ii) Immunodeficient (HIV-positive): $p_1$ and $c_1$ decreased 100 fold, while $m = 5$. (iii) Vaccine: $p_1$ and $c_1$ increased 100 fold, while $m$ is same as in (i).

**Numerical Experiments**

In order to unfold a trade-off, we assumed that the two strains were identical, except that the virulent strain had a higher reproduction rate. Thus, our experiment varies the cost (e.g. less
investment in defenses or higher decay rate of the virulent strain) associated with having a higher reproduction rate (schematic Fig. 2.3 A). Note that we start this experiment with the virulent form experiencing a low cost (to the left of the dotted line, \( a \), in Fig. 2.3 A) and end with a significantly higher cost (past \( b \) and \( c \)) where now the immunity attack rate (or decay rate) is much higher than its competitor. We ran this analysis on the two-strain viral dynamics model [38] (model 2.1 and 2.2) with the replication and decay trade-off for HIV and for HPV, using parameter estimates from in-host data in the literature. To our knowledge, we are the first to apply this model to HPV and to compile an in-host parameter set. For chronic infections, the stable viral loads (that are reached after some transient time) were plotted for various values of the decay rate of the virulent strain (Fig. 2.3 B). To consider transient acute infections that do not reach equilibrium, we used a model specific for Influenza [94], and instead plotted the maximum peaks in viral load as a response variable (Fig. 2.3 C).

To investigate the potential role of individual host conditions, we explored how the behavior of smoking can affect HPV type interactions. In HPV, smoking is known to decrease the strength of the CTL response [135], decrease the antibody response [111,136], and is associated with lower numbers of intraepithelial Langerhans’ cells (LC) in the cervix [137]. These biological implications of smoking are specifically embedded in the two-strain diamond model (model 2.2) by reducing the parameters, \( p_i \) and \( u_i \) which mimic impaired CTL and antibodies responses in the model [38]. We also considered the compound effect of impaired CTL attack (lower \( p_i \)) and the suppression of LC (lower \( c_i \)). The effect of HIV coinfection with HPV was considered by similarly lowering CTL response and increasing its natural death rate (higher \( m \)) due to HIV’s lytic activity.

We investigated how the introduction of the HPV vaccine would affect a coinfection in-host community that experiences a reproduction-decay trade-off. The vaccine targeted HPV-16 (strain 1) and weakly cross-protected against a related non-targeted HPV type (strain 2). Since the vaccine Gardasil© boosts antibody response by 100 fold [138], we considered this antibody increase in the free virus decay rate of HPV-16 (antibody neutralization is implicit in \( u_i \)) in two modules, the diamond (with shared resource; model 2.2) and in the immune-mediated apparent competition module (without shared resource; model 2.3).

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Chapter 3

Revising ecological assumptions about Human Papillomavirus interactions and type replacement

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Abstract

The controversy over whether oncogenic, non-vaccine HPV types will replace vaccine types remains unresolved. This is in part because little is known about the ecology of HPV types. Patient data has been interpreted to suggest neutral or facilitative interactions between types and therefore replacement is believed to be unlikely. With a novel mathematical model, we investigated which HPV type interactions and their immune responses gave qualitatively similar patterns to those frequently observed in patients. To assess the possibility of type replacement, vaccination was added to see if non-vaccine types increased their ‘niche’.

Our model predicts that neutrality and facilitation are not necessary for the coexistence of types inside hosts, especially given the patchy nature of HPV infection. In fact, neutrality and strong facilitation inadequately represented co-infected patients. We found weak-to-moderate in-host competition as likely in natural co-infections. Hence, non-cross-reactive non-vaccine types would spread to more patches and would increase in viral load in vaccinated hosts. The degree to which this happens will depend on replication and patch colonization rates. Our results suggest that true neutrality between types could be a fallacy, and so without conclusively untangling HPV in-host ecology, type replacement remains theoretically viable. More ecological thinking is needed in future studies.
3.1 Introduction

Infection by Human Papillomavirus (HPV) is responsible for approximately 270,000 cervical cancer deaths and roughly 97,000 cases of other cancers (e.g. anal, oropharyngeal) globally every year [139]. The significance of finding a virus as a causal agent to cancer cannot be understated since it permits us to prevent cancers with vaccines. Nevertheless, controversy has surfaced around the type specificity of the HPV vaccine. The vaccine targets two of the many oncogenic high-risk (HR) HPV types and therefore their removal could lead to an increase of other HR types. Recently, an increase in prevalence of non-vaccine HPV types was measured in vaccinated young women and in the study population [140] -- a potential first warning that type replacement in HPV is occurring.

Whether a population will expand its niche once another population is removed from a shared environment is fundamentally an ecological question. Here, we present the first analysis of this problem using an ecological framework. Predicting the outcome of removing HPV-16 and -18 requires filling the primary knowledge gap: how HPV types interact during co-infections.

Little is known about HPV type interactions. Of all HPV infections 30-50% are multiple infections and joint transmission of various types is common [141]. How such a large diversity of HPV types can regularly coexist inside hosts is not understood and has led to speculations of facilitative interactions between types [142] or that types are neutral [143]. In contrast, there is some evidence that HPV types may compete for resources [144], either by co-infecting the same cells and competing for intra-cellular resources or by competing for cells via blocking cell entry.

The only interaction that has been clearly demonstrated is that some types may interact via the immune response. Types phylogenetically related to HPV-16 (i.e. α-9 types) have a negative effect on its viral load [145,146]. Likewise, similar L1 epitopes to HPV-16 and -18 [108] allow for a handful of related types to experience some cross-protection by the vaccine (HPV-31, -33, -45, -51 [147]). Together these studies demonstrate “immune-mediated apparent competition” [22,42] between some related types.

The possibility of type replacement has been tackled by a couple of mathematical transmission models [142,148]. However in-host interactions are a black-box in these models. They find that the occurrence of type replacement will depend on whether types compete or facilitate, and so, their results hinge on the assumptions about in-host interactions. Their predictive power, then, is limited, considering that we still do not know how types interact inside hosts.

The most common interpretations of clinical data in the literature include: (1) random type distribution, at the epidemiological level, which implies underlying neutrality between types; (2) facilitative interactions are needed for coexistence inside hosts; and (3) the vaccine will not affect non-vaccine types. Here we test which hypothesized interaction scenarios are most like HPV co-infections by using an in-host model. This approach allows us to look inside the black-box by explicitly considering the behaviour of different possible interactions inside unvaccinated and vaccinated hosts.

We found that in-host ecological dynamics that are contrary to the most common interpretations (listed above) can readily give rise to observed co-infection patterns. Hence, we caution that these interpretations require more support.
3.2 Methods

Model
HPV needs abrasions in the skin to reach and infect basal epithelial cells (Fig. 3.1 a), and new virions are not released until the cells die at the surface of the skin [119]. This spatial restriction implies that HPV infections are localized, which leads to characteristic lesions, or warts [119]. HPV infection should thus be conceptualized as occurring in various “patches” distributed across space (Fig. 3.1 a).

![Figure. 3.1 HPV infection. a) An illustration of layered squamous cell infections; a top-down and cross-section view of the epidermis. Free virus particles are released at the surface and need an abrasion in the epidermis to reach basal cells to start a new patch. b) A schematic of the model. See methods for description of symbols.](image)

We developed a novel patch model that represents HPV co-infections. The model, represented in Fig. 3.1 b, is

\[
\frac{dZ}{d\tau} = \gamma Z \left( P_{16} + P_{hr} + P_{co} \right) - \mu Z
\]

\[
\frac{dP_{16}}{d\tau} = (f_{16} P_{16} + f_{16co} P_{co})P_0 - \epsilon (f_{hr} P_{hr} + f_{hrco} P_{co})P_{16} - \alpha_{16}P_{16}Z
\]

\[
\frac{dP_{hr}}{d\tau} = (f_{hr} P_{hr} + f_{hrco} P_{co})P_0 - (f_{16} P_{16} + f_{16co} P_{co})P_{hr} - \alpha_{hr}P_{hr}Z
\]
\[
\frac{dP_{co}}{dt} = (f_{16}P_{16} + f_{16co}P_{co})P_{hr} + \varepsilon (f_{hr}P_{hr} + f_{hrco}P_{co})P_{16} - (\alpha_{16} + \alpha_{hr})P_{co}Z
\]

\[P_0 = 1 - P_{16} - P_{hr} - P_{co}\]

(3.1)

The equations represent the population of cytotoxic T-cells (CTL), \(Z\), the patches infected with HPV-16, \(P_{16}\), patches with another HR non-vaccine type, \(P_{hr}\), co-infected patches, \(P_{co}\), and the proportion of empty patches, \(P_0\). CTL proliferate at rate \(\gamma\), die at rate \(\mu\), and clear a patch at rate \(\alpha_i\). Singly infected patches produce virions at rate \(f_i\), while co-infected patches do so at a rate \(f_{ico}\).

The establishment rate of the non-vaccine type into patches with HPV-16 is \(\varepsilon\). See Appendix A.2 for derivations and other details.

**Model Parameterization**

We obtained estimates for CTL parameters from the biomedical literature (\(\mu\) and \(\gamma\) from [133] and [134] respectively). Given the novelty of this model and the lack of HPV kinetics studies, we could not obtain point estimates for other parameters, so we created plausible ranges consistent with the known natural history of HPV (appendix Table A.2.1).

**Analysis: Interaction scenarios**

To investigate competing hypotheses, parameters were varied to represent possible combinations of interactions. We considered: (a) Neutrality. We used model A.2.2 a (Appendix A.2) which does not have competition for patches, intra-patch competition or facilitation; (b) Resource competition. We considered no intra-patch competition \((f_{16co} = f_{16} \text{ and } f_{hrco} = f_{hr})\) or competition such that the presence of one type affects the replication rate of the other type \((f_{16co} < f_{16} \text{ and/or } f_{hrco} < f_{hr})\); (c) Facilitation. To include facilitation we varied \(\varepsilon\) from 0 (none) to 100 (strong); and finally, we considered both competition and facilitation together.

**Analysis: Immunity scenarios**

Cross-reactivity was varied from none \((\alpha_{hr} = 0 \text{ or } \alpha_{16} = 0)\) to full cross-immunity, in both natural and vaccine immunity conditions. (a) Natural. HPV is a very poor immunogen, so we assumed the adaptive response was absent during the first 10 months of infection [149], and then attacked either HPV-16 \((\alpha_{16} > \alpha_{hr})\) or the other type first \((\alpha_{hr} > \alpha_{16})\). We asked: which ecological scenario best represented natural co-infections? (b) Vaccine. The vaccine induces a strong humoral immunity response (100 fold to natural), with a subsequent cell-mediated response to L1 proteins [108], which we assumed invaded 10 days post infection (no empirical estimate was available). We asked: Which scenario gave competitive release, and how does the vaccine change the in-host ecology?

For more on parameterization and ecological scenarios see Appendix A.2.
3.3 Results

**Natural Immunity**

Figure 3.2 summarizes the outcome of a wide range of co-infections for different combinations of types that experience no to full cross-reactivity ($0 < \alpha_{16 \text{ or } hr} < 0.5$) and that experience immunity mounting first against HPV-16 (i) or against the other type (ii). Notice that in all these ecological scenarios coexistence is possible for some combinations of types (see ‘all coexist’ regions in Fig. 3.2), demonstrating that coexistence does not solely arise from neutrality.

In neutrality (Fig. 3.2 a) the outcomes of co-infections with types of various replication rates (from none, $f_{hr} = 0$, to double HPV-16’s replication rate, 0.8), suggest that related types ($0.25 < \alpha_{16 \text{ or } hr} < 0.5$) would be pressured to have similar replication rates to HPV-16 in order to avoid clearance (see arrow Fig. 3.2 a.i). However, less cross-reactive types ($\alpha_{hr} < 0.25$) can have lower replication rates ($f_{hr} < 0.4$), and still persist in co-infections (Fig. 3.2 a.i: ‘all coexist’ region), or even outlast HPV-16 (‘$P_{hr}$ wins’ region). Usually, non-HPV-16 types are found at lower abundances, and to get this discrepancy in viral load under neutrality requires non-HPV-16 types to have different intrinsic replication rates and burst sizes, which, we believe, has not been quantified.

Strong facilitation is unrealistic because it allows the non-HPV-16 type to dominate all natural infections (Fig. 3.2 c, d, $\varepsilon > 34$, in both i and ii). Strong intra-patch competition results were similar because if competition was very strong ($f_{heco} < 0.02$, Fig. 3.2 b), then most types would always be excluded by HPV-16 (larger ‘$P_{16}$ wins’ region with increased competition, Fig. 3.2 b; plots i or ii). Yet, it has not been demonstrated that HPV-16 consistently outlasts all other co-infecting types.

When neutral, both types infect the same fraction of patches ($< 300$ days in Fig. 3.3 a.i and appendix Fig. A.2.1 i) if colonization rates of patches is medium-high. Clinically, this would lead to finding co-infecting types just as often as HPV-16 by randomly sampling from different spots of the cervix, vagina or vulva, which is not the case for all types, and does not coincide with the clustering of types that is often found in patients (e.g. some types are more common on the vaginal wall or in mucosal cells [150,151]). However, heterogeneous patch use is more characteristic of low colonization rates (Fig. 3.3 b) and competition (Fig. 3.3 b.ii). In fact, weak-to-moderate competition, generally, slows down the rate at which patches fill with both types (Fig. 3.3 ii).

Recent data showed a significant decrease in HPV-16 viral load but not exclusion when in co-infection with another HR $\alpha$-9 type [146]. Here, this drop happened across both immunity conditions (i and ii) and in all competitive and facilitative scenarios (Table 3.1 B-D under $\alpha_{hr} = 0.2$ and appendix Fig. A.2.2). With facilitation, however, HPV-16’s viral load could be driven to negligible levels after immunity, which is inconsistent with [146], thus, weak-to-moderate competition best represented these findings.

In all natural scenarios, HPV-16 did not exclude the other type before CTL invasion (0 to 300 days in Fig. 3.3 a, b and in Fig. A.2.1) even if replication rates were very different, e.g. $f_{hr} = 1$ and $f_{16} = 0.01$. This is because the non-HPV-16 type can exist within co-infected patches, unless intra-patch competition is so high that it cannot reproduce at all $f_{heco} = 0$. This suggests that in all
considered interaction scenarios, types can coexist before the adaptive immune system invades to clear the infection. Once again, this shows that neutrality is not required for coexistence.

Figure 3.2. Parameter plots: infection outcomes for various ecological scenarios. a) Neutrality. At $f_{hr} = 0.4$ (dotted line), the two types reproduce at the same rate. In the bottom-left quarter of these two plots (below $f_{hr} = 0.4$ and below $\alpha_{16} = 0.25$) the non-vaccine type has a lower reproductive rate than HPV-16 and the types are weakly cross-reactive. The two completely neutral scenarios are at $\alpha_{hr} = 0$ (for all $f_{hr}$) in (i) and $\alpha_{16} = 0$ (for all $f_{hr}$) in (ii), because types do not share patches (no resource competition) and nor experience cross-reactivity. The coexistence regions exist because of symmetry; while one type is a faster
replicator, the other is less affected by the CTL attack. Parameters: $f_{\alpha} = 0.4, f_{\beta} = 0.2, \alpha_{16} = 0.5, \alpha_{16} \text{ or } \beta_{16} \text{ vary, } \mu = 0.2$ and $\gamma = 0.6$. b) Intra-patch competition. i. When cross-reactivity is weak ($\alpha_{hr} < 0.25$) then the strength of the intra-patch competition affects the outcome. At $f_{hrco} = 0$, the intra-patch competition is so strong that the other type is excluded from patches altogether. ii. Since the immune system targets the HR type, then $P_{hr}$ wins always and, therefore, the other type only exists inside the host before the CTL invade. Parameters: $f_{hr} = f_{hrco} = 0.4, f_{\beta} = 0.2, f_{hrco} \text{ varies, } \alpha_{16} \text{ or } \beta_{16} = 0.5, \alpha_{16} \text{ or } \beta_{16} \text{ vary, } \mu = 0.2, \gamma = 0.6$, and no facilitation $\varepsilon = 1$. c) Facilitation. i. Facilitation allows for large regions of $P_{16}$ wins and coexistence, even with a small amount of facilitation ($1 < \varepsilon < 12$). Just above $\varepsilon = 1$, the $P_{hr}$ wins region completely disappears. Therefore, even a bit of facilitation, allows for coexistence ii. If the immune response mounts against the other type first, then facilitation also helps avoid clearance ($\varepsilon > 12$). Parameters: $f_{hr} = f_{hrco} = 0.4, f_{16} = f_{hrco} = 0.2$ (i.e. $f_{hr} > f_{16}$ by 2:1 and no competition inside $P_{hrco}$), $\alpha_{16} \text{ or } \beta_{16} = 0.5, \alpha_{16} \text{ or } \beta_{16} \text{ vary, } \mu = 0.2, \gamma = 0.6$, and facilitation none, $\varepsilon = 1$, or varies. d) Facilitation and competition. The inclusion of intra-patch competition considerably shrinks the coexistence region, and competition decreases the ability of facilitation to release the other type from clearance by the immune system. Parameters: $f_{hr} = 0.4, f_{16} = 0.2$ (i.e. $f_{hr} > f_{16}$ by 2:1), competition inside $P_{hrco}$ where $f_{hrco} = 0.2, f_{hrco} = 0.05$ (i.e. $f_{hrco} > f_{hrco}$ by 4:1), $\alpha_{16} \text{ or } \beta_{16} = 0.5, \alpha_{16} \text{ or } \beta_{16} \text{ vary, } \mu = 0.2, \gamma = 0.6$, and facilitation none, $\varepsilon = 1$, or varies.

**Vaccine Conditions**

Under vaccine conditions, all non- and most weakly cross-reactive types were not cleared by the vaccine (the large ‘$P_{hr}$ wins’ regions in Fig. 3.4 compared to Fig. 3.2) which corresponds with vaccine trials that showed limited cross-protection [147]. Underlying neutrality or facilitation had an effect on the size of the ‘$P_{hr}$ wins’ region but intra-patch competition did not (Fig 3.4 a and d vs. c), rather the strength of the cross-reactivity determined whether a type was cleared ($\alpha_{hr} > 22$). The non-vaccine type was able to infect newly available patches, instead of residing in co-infected patches (Fig. 3.3 before 300 days: compare a and b vs. c). How quickly the non-vaccine type filled all available patches depended on the non-vaccine type’s own replication rate (appendix Fig. A.2.1 C) and the strength of cross-reactivity (appendix Fig. A.2.1 c in A vs. B).

Whether the non-vaccine type’s new dominance translated to noticeably higher viral loads depended on which natural ecological scenario existed before vaccination: under neutrality, vaccine and natural viral loads were almost identical for the first 300 days (A in Table 3.1); under facilitation, the vaccine loads were lower though the decrease depended on the strength of the cross-immunity (C in Table 3.1); and under competition, the vaccine viral loads were higher (B in Table 3.1). The degree of competitive release depended on the strength of the intra-patch competition and the strength of the cross-reactivity. For example, at moderate competition ($f_{hrco} = 0.1$) non-cross-reactive types doubled their viral load (B in Table 3.1).

Since our natural immunity results point to weak-to-moderate levels of competition as being the most likely underlying ecological scenario, then the vaccine would increase viral loads of the non-vaccine types in co-infected vaccine patients, particularly for those that are not cross-reactive with the vaccine.
Figure 3.3. Example time-series of natural and vaccine cases (neutral, competition, facilitation). Used model A.1b. Types are not cross-reactive. a) natural immunity and high establishment rate, \( e = 1 \). b) natural immunity and low establishment rate, \( e = 0.1 \). c) solid \( e = 1 \) and dashed \( e = 0.1 \). In all natural scenarios (a and b) both types coexist until the immune invasion (before day 300), i.e. co-infecting patches prevents exclusion of less replicative type. Intra-patch competition (ii) gives more heterogeneous patch use and HPV-16 dominance, whereas facilitation (iii) implies dominance by the less replicative type, which is unrealistic.

c) Under vaccination, non-vaccine types are able to infect all patches once the vaccine type is cleared. How quickly this happens depends on replication rate of the non-vaccine type (see also Fig. A.2.1 C) and its establishment rate, \( e \). Dotted line in (ii) implies no competition, and solid line for competition. Parameters natural: \( f_{16} = 0.4, f_{hr} = 0.2 \) (i.e. \( f_{16} > f_{hr} \) by 2:1), if intra-patch competition \( f_{16,co} = 0.2 \) and \( f_{hr,co} = 0.05 \), no cross-reactivity \( \alpha_{16} = 0.5, \alpha_{hr} = 0, \gamma = 0.6, \mu = 0.2 \) and no facilitation \( e_{hr,co} = 1 \) or 0.1, while strong facilitation was \( e_{hr,co} = 34 \). Parameters vaccine: same as above except \( \alpha_{16} = 50 \) and \( \alpha_{hr} = 0, \gamma = 60, \mu = 0.2 \).
Figure 3.4. Parameter plots of vaccine conditions: outcomes for various ecological scenarios.  

(a) Neutrality. For non-cross-reactivity types the vaccine allows for the non-vaccine type to infect all patches, regardless of its replication rate. This is no different from the natural case when HPV-16 is targeted (Fig. 3.2 i at $a_{\alpha_{16}} = 0$), however, compared to Fig. 3.2 (ii) this is the opposite result. Therefore, vaccinated hosts will always give the same outcome as Fig. 3.2 (i) for unrelated types, i.e. in vaccinated hosts the non-vaccine type always wins. This is also true for weakly cross-reactive types (within the range $0 < a_{16_0} < 0.25$). The neutral case where $f_{16} = 0.2$ (between the dotted lines) is equivalent to the no competition ($f_{hr} = 0.2$) and the no facilitation ($\epsilon = 1$) regions of plots b and c. Therefore, the neutral vaccine case gives similar results to the full model without intra-patch competition or facilitation.  

(b) Compared to the neutral parameter plot (a) the clearance region here is smaller. Increasing the replication rate of the non-vaccine type is more effective at allowing the type to avoid clearance by the vaccine even if it experiences strong cross-reactivity (arrow up). Types that are more cross-reactive could evolve to increase its replication rate to similar or even higher replication rates than HPV-16 to avoid being wiped out by the vaccine.  

(c) Intra-patch competition. The vaccine results are independent of intra-patch competition.  

(d) Facilitation (only and with competition) vs. cross-reactivity. For both cases facilitation only and facilitation with intra-patch competition cases, the parameter plots are identical. As facilitation increases the size of the P wins region grows. Parameters: $f_{16} = 0.4$; $f_{hr} = 0.2$ (i.e. $f_{16_0} > f_{hr}$ by 2:1), for intra-patch competition $f_{hr_0} = 0.2$, $f_{hrco} = 0.05$, $a_{16_0} = 50$, $a_{hr} = \text{varies}$, $\gamma = 60$, $\mu = 0.2$; no facilitation $\epsilon = 1$ or varies.
Table 3.1. Mean viral loads. The mean quantities of free virions produced by all patches that contain a particular type per day. These are not meant to represent real measurements of viral titers but rather are simply a method of measuring the relative viral loads of various hosts. No cross-reactivity $\alpha_{hr} = 0$ or $\alpha_{16} = 0$; weak cross-reactivity $\alpha_{hr} = \alpha_{16} = 0.2$ (natural) or $\alpha_{16} = 20$ (vaccine).

3.4 Discussion

The most common hypothesis for HPV type interactions is that they do not interact [117,152], and so far, most vaccine trials have not seen significant increases in prevalence of non-vaccine types [147] which seems to support the neutrality hypothesis. Similarly, studies using odds ratios have concluded that type replacement is not likely because HPV types were found to occur randomly and to lead to cervical disease independently [139,153–155]. However, two types have been flagged as potentially having a competitive advantage [116,117] and a
recent study found that the prevalence of non-vaccine types, including high-risk types, was indeed higher in vaccinated patients [140]. Our study helps reconcile these different findings by illustrating that complex in-host dynamics can give rise to observed clinical patterns without invoking neutrality or facilitation. We propose that, by adopting an ecological perspective, capturing complex nonlinear interactions and being mindful of scale can help explain surprising results.

Consider three scales: the tissue or patch level, individual hosts, and the host population. At the tissue level, there are some suggestions of type interactions. HPV-40 and HPV-11 can separate regionally [156], and recently a study showed that lesions are caused by only one HPV type [123]. These results could be explained by spatial ecology concepts such as “local founder control” (the first in a patch blocks the other from entering) or “hierarchical competition” (the superior local competitor always, quickly or slowly, outcompetes the other) [157], and thus, should be considered. HPV’s highly spatial infection cycle suggests that more complex interactions are at work than is appreciated. Common clinical methods (e.g. swabs) are too coarse to see these differences in HPV tissue distribution [158,159] and thus are unlikely to find exclusion at the patch level.

As we have shown, various patch-level interactions can lead to coexistence at the host level. Hence, coexistence of types inside a patient does not necessarily imply that types are neutral to one another. Ecology shows that a myriad of complex non-neutral interactions involving trade-offs [160] can combine to result in coexistence similar to that observed under pure neutrality. Our model shows how this may occur in HPV, because adding the heterogeneity of patches allows types to coexist. It is fallacious, then, to infer neutral interactions from approximately uniform occurrence at the population level. Since species distributions do not always hint at the underlying local interactions, more direct empirical studies at lower scales are needed and finding competitive interactions will require manipulative experiments. Untangling these interactions is very important because the response to a perturbation, such as vaccination, will vary widely depending on the underlying mechanism, i.e. whether or not the types really are neutral.

Inadvertently, the issue of HPV neutrality or competition has landed in the centre of an ecological debate on whether niche or neutrality theory best describes ecological interactions [161–165]. Its resolution should, therefore, be of interest to both the medical and ecological communities. Since closer examination of ecological systems often uncovers underlying non-neutral interactions [163,165], and since most medically important viruses are highly competitive (e.g. HIV), conclusively finding neutrality in HPV would be very interesting indeed.

Since our model suggests that some competition is likely, non-vaccine types in vaccinated hosts should increase patch use and viral load. What is needed to see this are clinical studies that compare viral loads (preferably longitudinally) of non-vaccine types in vaccinated and unvaccinated hosts, in order to capture any niche expansion (or contraction). Our model shows that niche expansion could be slight and so we suspect this in-host signature will be detectable before there are significant changes in prevalence at the population level.

There are some limitations and caveats to consider. First, this model is not explicitly spatial. However, because HPV interact either within or between patches, and disperse to either close or far new patches, then this kind of setup is most appropriately modeled by patch models [157]. Second, we did not model antibody response, which is the main response stimulated by
the vaccine. Despite these caveats, mathematical models are often used to help untangle complex interactions in ecology and in disease dynamics [80], and are particularly useful when there are knowledge gaps. Although our model is a simplification of reality, it is grounded in the biology of HPV infections, which allowed us to explore the competing hypotheses from the literature.

Though we do not directly address the evolutionary potential of HPV, our results suggest that vaccinated hosts could set the stage for some non-vaccine cross-reactive types to escape the vaccine response. Vaccine trials have demonstrated that cross-protection is partially effective (e.g. 44.8% effective against HPV-33 6-month persistent infection [147]), and so this “leaky” vaccine might select for variants to escape this new vaccine-induced immune response. Our results point to two traits that could allow variants of non-vaccine types to escape: decreased epitope similarity, or more alarmingly, increased replication rates. In time then, vaccine cross-protection could wane or non-vaccine types could become more aggressive. Rapid ecological changes drive evolutionary changes and not enough is known about the differences between the natural and vaccine in-host ecology to believe the vaccine will not select for trait changes in non-vaccine types.

Currently, our model does not lend itself to model fitting since enumerating infected patches from samples is not done at present, however, with new methods (e.g. [123]) it is possible, and we hope future work will link models with patch data. As we move to using more ultrasensitive HPV genotyping assays [166], we can more frequently sample co-infections, in order to better quantify the dynamics of all types in the infection. This will help to measure natural vs. vaccine in-host differences; fit models to data and hence better tease apart the in-host ecology; see if non-vaccine types in co-infections are reciprocally affected by the vaccine types; and, finally, get direct evidence for the duration over which vaccinated hosts produce and shed virus relative to unvaccinated hosts. Instead of the ‘wait and see’ approach of long-term monitoring of type prevalence in vaccinated populations, we hope this work will incite new proactive studies.

More ecologically cognisant HPV studies will help explain why the vaccine drives or avoids an evolutionary ecological response. They will either lead us to remedy the problem of type replacement if it appears, or help us understand more mechanistically why the vaccine worked. This knowledge can then help us avoid type replacement in future vaccination programs.

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Chapter 4

Could the Human Papillomavirus vaccines drive virulence evolution?

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Abstract

The Human Papillomavirus (HPV) vaccines hold great promise for the prevention of several cancers that are caused by HPV infections. However, little attention has been given to whether HPV could respond evolutionarily to the new selection pressures imposed on it by the novel immunity response created by the vaccine. Here we feedforward couple a within-host model of HPV infection into between-host transmission equations and consider how naïve and vaccinated hosts differ. We find that the vaccine lifts the constraint on higher oncogene expression by altering the recovery-transmission trade-off that most likely maintains HPV’s oncogene expression low in natural infections. The immune response of the vaccinated host is able to effectively clear low oncogene expression strategies. However, if the virus uses a high oncogene expression strategy, it is able to increase its infected cell population and viral load before clearance by the vaccine, and thus, improve its chances of transmission. This new higher oncogene strategy is able to circulate between hosts with higher turnover rates of sexual partners, however, individuals with longer partnerships (> 12 weeks) do not contribute to circulation. We discuss how vaccine immunity might select for HPV to become a more aggressive oncovirus. We also discuss the importance of better quantifying how long challenge infections last and quantifying the degree to which a vaccinated host can shed virus.
4.1 Introduction

The development of the Human Papillomavirus (HPV) vaccine brought excitement due to its innovative virus-like-particles (VLP) technology and the very high efficacy rates found in clinical trials [167,168]. The HPV vaccine was hailed as a new preventative measure against the several cancers (cervical, penile, anal, head-and-neck) that are caused by this very common sexually transmitted virus. Since HPV is a double-stranded DNA (dsDNA) virus, it was argued that the virus would be unlikely to evolve escape mutants that evade the VLP-induced immune response against the virus’s L1 surface protein, the way that other RNA viruses can [90,168]. However, lacking in these discussions of whether HPV has the evolutionary ability to respond to the vaccine is the idea that viruses can respond to vaccines by increasing their virulence [42,84]. An important example for HPV researchers to take heed of is the vaccine-induced evolution of Marek’s Disease virus (MDV), particularly since this virus is also a dsDNA oncovirus. Indeed, MDV has evolved increased virulence and escape mutants in response to vaccination [49,50]. Here we use a mathematical model to investigate the potential of HPV to evolve higher virulence in response to the vaccine-induced immune response.

Defining virulence in HPV

In many infections, the quantity of the infectious agent is the appropriate measure of virulence. For example, Antia et al. define a lethal quantity of a parasite when studying what level of virulence is selected for by within-host dynamics [10]. However, this virulence definition does not apply to HPV because most people carry the virus for 1-2 years asymptomatically and, for the most part, the virus is avirulent. Indeed, HPV infections become deadly only after several years of persistence, and death is due to malignant host cells [169,170]. Thus, the classic definition of virulence as being related to pathogen load does not apply to HPV.

HPV exists as dozens of different types with differing pathologies; high risk (HR) types have oncogenes, E5/6/7, that interfere with the cell’s growth cycle. Despite the name, these genes are not for causing cancer but rather they are needed for the replication cycle of the virus. The main function of the oncogenes, E6 and E7, is to stimulate cell cycle re-entry in the mid-epithelial layers in order to allow genome amplification [171]. As a result, without the oncogenes, the virus cannot amplify its genome. There are two main additional beneficial functions of these genes in HR types: 1) the oncogenes interfere with the innate immune system (including inhibition of interferon response [171]), thus they delay the activation of the adaptive immune response, and 2) a beneficial consequence of the oncogenes inactivating the host’s regulators of the cell cycle (proteins p53 and pPB) in order to stimulate cell proliferation [119], is that this increases the number of infected cells without having to infect new cells or without having to increase the intrinsic replication rate of the virus. Both these functions improve the chances of transmission by increasing the duration of the infection, and by increasing the amount transmitted per contact. However, it has been found that these oncogenes are not expressed at high levels during acute infections, since the early viral protein E2 down-regulates oncogene production. But if the oncogenes are so beneficial then why are they not expressed in higher quantities? It is believed that the cost to increased numbers of infected cell is that it triggers the immune response. This is the fate of infections by low-risk, LR, types that create genital warts, since they are cleared faster than HR types [171,172]. Most HR lesions begin flat and
inconspicuous but with time the cell proliferation they induce becomes noticeable [171], and we propose this same cost eventually also leads to the clearance of HR types. Immune detection and clearance appears to be a major factor affecting HPV’s life history and therefore, we and Orlando et al. believe that the main trade-off that affects this virus is the Recovery-Transmission trade-off [7,173], and not the classic Virulence-Transmission trade-off that affects more virulent pathogens [172].

**The vaccinated host environment**

Vaccinated hosts are a new environment that targeted HPV types need to adapt to or they will be eradicated. A unique feature of HPV vaccine-induced immunity is that it triggers a large antibody response, one that is at least two orders of magnitude larger than the natural response. Also distinct from natural immune responses to HPV infections is the duration of infection. Vaccine efficacy trials have shown that 99% of vaccinated hosts clear challenge infections with targeted types within 6 months [174]. Since the immune response in vaccinated hosts will always be triggered by memory cells and will always be mounted within 6 months of infection, then the current “lay low” strategy that HPV uses to stay inside a host longer is no longer effective. Regardless of whether there are few or many infected cells, the adaptive immune response will always occur and clear the infection. We propose, then, that in vaccinated hosts the cost of oncogene expression is lifted, and potentially, targeted HR types may change their oncogene production, which would change their virulence. Using mathematical models we investigate whether this can happen.

**4.2 Methods**

We developed a within-host model to represent an HPV infection in an unvaccinated host, which was then modified to represent a vaccinated host. These models were then linked to epidemiological functions (similar to [10,173,175]) because selection pressures happen at both the within and between host levels.

**Within-host models**

Let $X$ represent the population of uninfected basal epithelial cells that HPV target. The population of free virions, $V$, come into contact with uninfected cells, $X$, and infect them at a rate $\psi$ making infected cells, $Y_1$. These infected cells can either continue their life cycle (while producing virions) or they can become self-replicating cells, which we will call $Y_2$. These cells have a higher expression of the oncogenes, E6/7. Let $\varepsilon$ represent oncogene expression. The equations for HPV growth inside an unvaccinated host are,
\[
\begin{align*}
\frac{dX}{dt} &= \lambda(t) - \mu X - \psi V \left( \frac{X}{\phi + X} \right) \\
\frac{dY_1}{dt} &= \psi V \left( \frac{X}{\phi + X} \right) - \varepsilon Y_1 - \mu Y_1 - aY_1Z \\
\frac{dY_2}{dt} &= \varepsilon Y_1 + r \varepsilon Y_2 - \mu Y_2 - aY_2Z \\
\frac{dV}{dt} &= \mu (k_1 Y_1 + k_2 Y_2) - \delta V \\
\frac{dZ}{dt} &= \omega Y_2Z
\end{align*}
\]

(4.1)

Infection of new uninfected cells is limited by the fact that most cells are hidden under the epithelium and so abrasions are needed in order for HPV virions to reach them. For this reason we have slowed down the interaction between \( V \) and \( X \) by making their relationship grow hyperbolically (using a type-II functional response). The rate of oncogene expression controls the conversion of \( Y_1 \) cells becoming self-proliferating cells, and both types of infected cells die at the same rate, \( \mu \). Self-proliferating infected cells grow at a rate \( r \varepsilon \), proportional to their own density, and is dependent on oncogene expression (i.e. the higher the oncogene expression the more cell division). Both types of infected cells contribute to the overall population of free virions, \( V \). Note that virion production rates, \( k_i \), are adjusted by the infected cell death rate, \( \mu \), because HPV is a non-lytic virus. Free virions are cleared at a rate \( \delta \) and the antibody response is captured by this viral clearance rate. Finally, we assume that the CTL response, \( Z \), is only initiated by the growth of \( Y_2 \). The reason for this is two-fold: i) HPV infection is exclusively intraepithelial and so no viremia occurs and so antigen is well hidden [171], thus extra cell growth is a sign that something is wrong [171,172], and ii) the cell-mediated response needed for clearance is predominantly against E6, an oncogene and E2. Note that the CTLs should kill both types of infected cells with equally efficiency, so the killing rate \( a \) is the same.

Two assumptions can be made that allow for a reduction of this model. First, assume that birth rate of the uninfected cells, \( \lambda(t) \), maintains the total population size of epithelial cells at a constant population size of \( N \) and \( \frac{d(X + Y_1)}{dt} = 0 \), thus \( X \) can be replaced by \( X = N - Y_1 \). Second, assume that the virion dynamics are faster than the cellular dynamics and thus a quasi-steady-state assumption, \( dV/dt \approx 0 \), can be made. Altogether, then, the \textbf{unvaccinated host model} becomes,

\[
\begin{align*}
\frac{dY_1}{dt} &= \frac{\psi \mu}{\delta} \left( \frac{N - Y_1}{\phi + (N - Y_1)} \right) (k_1 Y_1 + k_2 Y_2) - \varepsilon Y_1 - \mu Y_1 - aY_2Z \\
\frac{dY_2}{dt} &= \varepsilon Y_1 + r \varepsilon Y_2 - \mu Y_2 - aY_2Z \\
\frac{dZ}{dt} &= \omega Y_2Z
\end{align*}
\]

(4.2)
In order to represent vaccinated hosts, several changes were made to this model: (i) The vaccine causes a strong antibody response, therefore, $\delta$ is increased to $\delta_{\text{vac}}$; (ii) proliferation of the CTLs is now initiated by the vaccine-created memory response, not the innate response, so only a very small amount of virus present (in $Y_1$, $Y_2$ or $V$) will trigger the memory response to activate the adaptive response to invade, thus this changes the $Z$ equation and $Z$'s initial conditions; (iii) the antibodies that flood the infection site help prevent newly produced free virions from infecting new cells, thus $\delta_{\text{vac}}$ scales down the infection rate of new cells, $\psi$. Together, this gives the model for a vaccinated host,

$$\frac{dY_1}{dt} = \frac{\psi \mu}{\delta_{\text{vac}}} \left( \frac{N - Y_1}{\phi + (N - Y_1)} \right) (k_1 Y_1 + k_2 Y_2) - \epsilon Y_1 - \mu Y_1 - a Y_2 Z$$
$$\frac{dY_2}{dt} = \epsilon Y_1 + r \epsilon Y_2 - \mu Y_2 - a Y_2 Z$$
$$\frac{dZ}{dt} = \omega_{\text{vac}} Z$$

(4.3)

where, now $Z_0$ is set to a value that initiates the $Z$ equation once the infection is started. This is equivalent to having a very low threshold, such that a very small amount of the virus triggers the response (equivalent to being triggered by the mere presence of the virus, not viral growth dependent as it is in the unvaccinated host).

**Within-host viral fitness**

Viral load is a measure of the virus’ reproductive output inside a particular host environment. The total amount of virus it is able to produce during the course of the infection represents the fitness of the virus for that particular within-host environment. We are interested to see how oncogene production changes viral output, so we want to determine the optimal oncogene strategy, $\epsilon^*$, which is defined as the oncogene expression that maximizes the total viral output of a host. To determine this we first find the total viral output, $V_{\text{Total}}$, of an a host, by finding the integral of the viral load curve, $V$,

$$V_{\text{Total}}(\epsilon) = \int_{0}^{\infty} V(\epsilon, t) \, dt$$

(4.4)

then, we find the maximum with respect to $\epsilon$, which gives $\epsilon^*$. We can then compare the $\epsilon^*$ selected for in distinct within-host environments (vaccinated vs. unvaccinated). Note that because the model cannot be solved analytically then equation 4 was computed numerically, which is also true for the equations that follow. The maxima were computed numerically using the function *NMaximize* in *Mathematica*. 
Transmission and between-host fitness

Next we consider the effects of transmission. An optimal strategy at the within-host level might not be optimal for between-host transmission [72]. We consider, then, how linking these within-host models to a different transmission function that represent the relationship between viral load and transmission (similar to [10,175]). We considered a linear but scaled down rate of transmission, where \(\alpha\) is \(0 < \alpha < 1\).

\[
\beta(V) = \alpha V
\]  

(4.5)

Since HPV is for the most part avirulent (virus produces almost no mortality), we equate the reproductive number, \(R_0\), to the number of new infections caused by an infected host before recovering (similar to [173]). To find an expression for \(R_0\) we consider an equation that represents the number of hosts infected by the focal infected individual,

\[
I(t+dt) = I(t) + mg(t)\beta(t)dt
\]  

(4.6)

where \(m\) is the rate of sexual acts, \(g(t)\) is the probability that the partner is susceptible, given a sex act, and \(\beta(t)\) is the probability of transmission given a sex act with a susceptible partner. From this equation we get an expression for the total number of infected hosts an individual can cause, such that

\[
R_0(\varepsilon) = \int_0^\infty m \cdot g(t) \cdot \beta(V(\varepsilon, t)) \, dt
\]  

(4.7)

We include \(\varepsilon\) since we are interested in how oncogene expression can affect the \(R_0\) of the infected host. It is important to consider \(g(t)\) because humans are fairly monogamous, so transmission to a new host happens only after switching to a new sexual partner, the chance of which goes up in time. This changes the value of each contact event putting more weight on later sexual contacts. Thus the state of partnership affects transmission of a sexually transmitted pathogen like HPV. We modeled \(g(t)\) explicitly using a model of three different states that the infected individual can be in with respect to sexual partnerships,

\[
\begin{align*}
\dot{g} &= \rho s - \sigma g - m\beta(t)g \\
\dot{s} &= \sigma g - \rho s + \sigma b \\
\dot{b} &= m\beta(t)g - \sigma b
\end{align*}
\]  

(4.8)

where \(\rho\) is the rate of new partner acquisition and \(\sigma\) is the rate of partner break up. Here \(g(t)\) is the probability the individual is in a partnership with a susceptible, \(s(t)\) is the probability of them being single, and \(b(t)\) is the probability that their partner is also infected. Note that a host can only be in one of these states and thus at any given time \(g(t) + s(t) + b(t) = 1\). An analytic solution for \(g(t)\) is not easily found because \(\dot{g}\) is non-autonomous and so \(g(t)\) was calculated numerically.
Host Heterogeneity: Immune status

HPV vaccine efficacy in immuno-competent patients is very high, where most vaccinated individuals clear challenge infections within 6 months [176]. The effect of the HPV vaccine in immuno-compromised patients should be diminished and overall, the strength of the immune response will vary among individuals. It is believed that immuno-compromised patients can build a vaccine-induced humoral response because the HPV VLPs used in the vaccine are highly immunogenic [177]. For instance, HIV-positive men without low CD4+ counts have shown to successfully seroconvert after vaccination [178] though at lower titres than HIV-negative patients [179,180]. Immuno-compromised individuals with low CD4+ counts or B-cell deficiencies will have trouble building the adaptive response needed to clear the HPV infection and so, at the very least, vaccinated immuno-compromised patients should clear a challenge HPV infection slower than vaccinated immuno-competent patients. Unfortunately, HPV vaccine efficacy and immunological studies in immuno-compromised patients are few [179]. Here, we considered how impairment to the adaptive response affects the results.

Host Heterogeneity: Sexual behaviour

Sexual behaviour varies between hosts and the host’s sexual partnership switching behaviour is important to the transmission of the virus. Hosts that are celibate or do not change sexual partners within the duration of the infection are dead ends for the virus. Indeed if partnerships are very long then the $R_0$ of that individual is less than 1; only the formation of a new partnership can lead to transmission [181]. We grouped sexual behaviours into three groups of people, see Table 1. Partner switching rates, break-up rates, and other sexual behaviour parameters were obtained from the literature (Table 4.1). Note that the per-partnership transmission probability for HPV is 0.6 [182], and its $R_0$ is 2, though higher for core-group individuals (e.g. super-spreaders) [183].
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of partnerships/yr</th>
<th>Rates (in years)</th>
<th>Parameter set (in days)</th>
<th>References</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long partnerships</td>
<td>average 1 partners/year</td>
<td>( \rho = 1 )</td>
<td>( \rho = 0.0027 )</td>
<td>( \sigma = 0.17^* )</td>
<td>( \sigma = 0.0004 )</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Short partnerships</td>
<td>average 2-5 partners/year</td>
<td>( \rho = 3.5 )</td>
<td>( \rho = 0.0096 )</td>
<td>( \sigma = 18.25 )</td>
<td>( \sigma = 0.05 )</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Casual relationships</td>
<td>average 6-8 partners/year</td>
<td>( \rho = 7 )</td>
<td>( \rho = 0.019 )</td>
<td>( \sigma = 36.5 )</td>
<td>( \sigma = 0.1 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Super-spreader</td>
<td>20 + partners /year</td>
<td>( \rho = 25 )</td>
<td>( \rho = 0.068 )</td>
<td>( \sigma = 162 )</td>
<td>( \sigma = 0.44 )</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 4.1. Sexual behaviour groups and between-host parameters from literature

4.3 Results

Unvaccinated host results

For various values of oncogene expression, the viral load time-series of the unvaccinated host show that the model captures the recovery constraint that we expect to see, such that if the virus drives many infected cells to divide too quickly then the immune response detects the infection and the CTL invasion happens quickly, thus shortening the duration of the infection (Fig. 4.1). We calculated the amount of oncogene expression that is favoured under this constraint as that for which \( V_{total} \) (equation 4.4) is maximized. We find that this optimal oncogene expression, \( \varepsilon^* \), is low (Fig. 4.2 a). This model thus captures the feature of HR HPV types that produce few extra infected cells through virus-induced cell division because their lesions are fairly flat for most of an acute infection [172].
The unvaccinated host $\varepsilon^*$ gives a duration of infection of 1.4 years, which is within the HPV-16 range of clearance times (0.5 - 4.9 years for HPV-16 [193] and the average HPV-16 infection is cleared before two years).

The HPV $R_0$ for an unvaccinated host is $R_0 = 2$ [183] and the free parameter $\alpha$ was determined to be $3 \times 10^{-6}$ in order for the short partnership group to have an $R_0$ of 2. The $R_0$ of the long partnership group was below one ($R_0 = 0.11$) because the partnerships are generally much longer than the duration of the infection, and the $R_0$ of casual partnerships was 2.8 (Fig. 4.2 b). The resulting $R_0$ of super-spreaders was 8.8 which is still realistic though a bit low considering their high partnership turnover rates.

Figure 4.1. Time-series of unvaccinated within-host model for various oncogene expression levels. Lower $\varepsilon$ gives slower growth of $Y_1$ and $Y_2$. Note that $Y_1$ and $Y_2$ infected cells produce the $V$ curves. The invasion of $Z$ is delayed at lower levels of $\varepsilon$, thus faster growth of $Y_2$, due to higher $\varepsilon$, leads to faster clearance. Unvaccinated within-host parameters: $\psi = 0.1$, $n = 5000$, $\phi = 10^6$, $\delta = 0.05$, $\mu = 0.2$, $r = 0.01$, $k_1 = 100$, $k_2 = 150$, $\alpha = 0.01$, $\omega = 0.001$, $\varepsilon$ varies from 0 to 1.
Figure 4.2. Unvaccinated host plots. a) $V_{total}$ of both immuno-competent and immunodeficient hosts. The $\varepsilon^*$ that is selected for by within-host processes is low, which demonstrates that recovery is the cost to rapid growth inside the host. Immunodeficient hosts can select for a slightly higher optimal oncogene expression, $\varepsilon_d^* > \varepsilon_c^*$. Immunodeficient parameters: $\omega = 0.0001$, $Z_0 = 10^{-5}$. b) $R_0$ with respect to oncogene expression for various sexual behaviours (immuno-competent only). Including the sexual behaviour model with the transmission function in the unvaccinated case does not noticeably change the $\varepsilon^*$ away from the within-host optimal, thus all three groups select for the same $\varepsilon^*$.

**Vaccinated host results**

The vaccinated within-host environment does not select for low oncogene expression like the unvaccinated host. Instead, oncogene expression can be very high, $\varepsilon_{vac}^*$, given that the $V_{total}$ curve increases with higher $\varepsilon$ values (Fig. 4.4 a). This suggests that the cost of growth via cell division is removed in vaccinated hosts. For low $\varepsilon$ ($\varepsilon < 15$) the total viral output is low for strains with this low oncogene expression strategy (see Fig. 4.3 where $Y_2$ and $V$ decay for $\varepsilon$ values below 15). For strains with this low $\varepsilon$ strategy the vaccine is able to clear them effectively. For the higher $\varepsilon$ case shown in Figure 4.4 a, the exponential growth of $V_{total}$ can be explained by Figure 4.3 where the $Y_2$ and $V$ curves grow before clearance. Therefore, switching to $\varepsilon$-driven growth allows the virus to produce a high viral load before the inevitable clearance by the vaccine. Note also that vaccinated immuno-deficient hosts with high $\varepsilon$ ($\varepsilon > 15$) will produce higher viral loads than vaccinated immuno-competent hosts with the same $\varepsilon$ (Fig. 4.4 a). Another measure of virulence is comparing the populations of $Y_2$ cells in the host environments. Note that vaccinated hosts have less $Y_2$ cells than the unvaccinated host for $\varepsilon < 25$, but for $\varepsilon$ values that could allow for persistence, $\varepsilon > 25$, the $Y_2$ populations are higher than the unvaccinated host.
Figure 4.3. Time-series of vaccinated within-host model for various oncogene expression levels. At lower levels of oncogene expression the virus is effectively cleared by the CTL (decay of $Y_1$, $Y_2$, and $V$ for $\varepsilon < 15$) but if higher, then viral load increases due to an increase in self-dividing infected cells. Note that $Z$ appears at the same time regardless of oncogene expression: CTL invade after 60 days of infection. Vaccinated within-host parameters: $\delta_{vac} = 5$, $\omega_{vac} = 0.1$.

Using equation 4.7, we can determine if the viral loads produced by these higher $\varepsilon$ values are high enough for transmission and whether they can persist in a population. Since there is no longer a maximum in the vaccinated host that defines the optimal oncogene expression, we instead find where $R_0 = 1$ and define $\varepsilon_{vac}^*$ as the oncogene expression necessary for a strain to persist in a population (Fig. 4.4 b). We find that the $R_0(\varepsilon)$ curve of the long partnership group does not reach $R_0 = 1$ within any reasonable $\varepsilon$ value. This implies that even with very high viral loads, there is not enough partner-switching happening to allow for transmission within the window of the infection in a vaccinated host (in this analysis the vaccine CTL invade within 60 days of the infection). However, the other three groups, short, casual and super-spreaders, do reach $R_0 = 1$ when $\varepsilon = 49.5, 32.3, \text{ and } 29.0$ respectively (Figure 4.4 b). If compared to Figure 4.4 a, this shows that super-spreaders require a lower minimum viral load, of around $10^7$, for persistent transmission.

Since the main response of the vaccine is through a high antibody response, we considered how increasing the strength of the antibody response affected $\varepsilon_{vac}^*$. In Figure 4.5 a, we see that as $\delta_{vac}$ is increased to 100 times the natural antibody clearance rate, a higher $\varepsilon_{vac}^*$ is needed for a strain to persist. Thus, the vaccine response selects for high oncogene expression. The shaded regions above the curves represent strains with $\varepsilon$ values above $\varepsilon_{vac}^*$. These strains can also persist, and could potentially out-compete a strain expressing $\varepsilon_{vac}^*$, especially since $\varepsilon$ higher than both curves can circulate in both types of host. In Figure 4.5 b, we plotted the derivative at $\varepsilon_{vac}^*$ for different strengths of the humoral response (for increasing $\delta_{vac}$) as a measure of the strength of the selection for $\varepsilon_{vac}^*$. Selection for $\varepsilon_{vac}^*$ is faster when the humoral response is weaker and it is also faster in immunodeficient hosts (Fig. 4.5 b).
Figure 4.4. Vaccinated host plots. a) $V_{total}$ of both immuno-competent and immunodeficient hosts. No maximum is achieved, instead higher the oncogene expression allows for higher viral loads. Immunodeficient hosts have steeper curves implying they reach higher viral loads with lower $\varepsilon$ values. b) $R_0$ with respect to oncogene expression for various sexual behaviours (immuno-competent only). The slow rise in $V_{total}$ (a) implies that the $R_0(e)$ has a shallow incline such that it does not cross 1 until higher $\varepsilon$. Only causal and super-spreader groups rise fast enough to cross 1.

Figure 4.5. The effect of the strength of the vaccine humoral response on optimal epsilon. a) The oncogene expression needed for persistence, $\varepsilon_{vac}^*$, with respect to the strength of the antibody response, $\delta_{vac}$. Generally, $\varepsilon_{vac}^*$ increases with a stronger humoral response. Note that above each line are $\varepsilon$ values that can also persist (at $R_0$ values > 1). b) The derivative at $\varepsilon_{vac}^*$ for various $\delta_{vac}$. The strength of selection for higher epsilon is stronger in immune deficient hosts in both casual and super-spreader groups. Higher $\delta_{vac}$ implies slower selection towards $\varepsilon_{vac}^*$. 
Note that the long partnership group is not included in the analysis in Figure 4.5 and 4.6 because this group does not reach $R_0 = 1$ (as explained above). This implies that hosts with longer partnerships coupled with a duration of a challenge infection between 60 to 150 days results in a $R_0 < 1$. These hosts, then, do not contribute to the persistent circulation of strains with higher oncogene expression.

Finally, in Figure 4.6, we investigate how the duration of infection in a vaccinated host affects the $\varepsilon_{\text{vac}}^*$. The higher the initial $Z$ value, $Z_0$, equates to faster invasion by the adaptive response. As the duration of the infection shrinks, i.e. the CTL clear the infection quickly, the higher $\varepsilon_{\text{vac}}^*$ is need for persistence. Note, however, that if the invasion happens within less than 50 days ($Z_0 > 1$), then the vaccine is able to clear all infections in all groups, regardless of the level of oncogene expression.

Figure 4.6. The effect of the timing of vaccine-induced clearance on optimal epsilon. Each line represents the oncogene expression needed for persistence, $\varepsilon_{\text{vac}}^*$, in a particular sex group, thus the shaded region above are $\varepsilon$ values that have $R_0$ values higher than 1. The oncogene expression needed for persistence, $\varepsilon_{\text{vac}}^*$, that is selected for in the vaccinated host depends on how quickly vaccine-induced clearance happens. At $Z_0 = 10^{-4}$ the vaccinated host sheds virus for about 150 days, and at $Z_0 = 1$ the vaccinated host shed the virus for 50 days. For all three sexual behaviour groups, if the challenge infection is cleared quickly (high $Z_0$) then a higher $\varepsilon_{\text{vac}}^*$ is favoured, but if the infection is cleared in under 50 days then even high oncogene expression cannot escape the vaccine.
4.4 Discussion

The evolutionary responses of viruses to vaccines are of serious concern, and they may appear several years after the introduction of the vaccine. Lacking in the HPV literature is a discussion as to whether the vaccine could create the conditions in which more virulent HPV types have an advantage. Here we show that virulence evolution is a method that targeted HR types could use in order to increase their chances of transmission during the short window of time before vaccine-induced clearance.

The within-host ecology that targeted types now encounter in vaccinated hosts is novel in three main ways: (i) the adaptive response now targets exclusively epitopes of the surface protein L1 \[194]\; (ii) targeted types now experience a strong antibody response that is unnaturally high \[194]\; and (iii) the effector cells used to clear the infection invade faster, and this invasion can no longer be delayed using slow viral replication and signalling interference. We investigated the latter two changes.

First, we found that removing the ability of the virus to delay effector cell invasion allowed types with higher oncogene expression to have \( R_0 \) values higher than 1, which is not possible in unvaccinated hosts because of the Transmission-Recovery trade-off (compare Figure 4.2 and Figure 4.4). Consequently, the vaccine is lifting the constraint that is most likely keeping HPV virulence low. Second, we found that increasing the antibody response drove the oncogene expression necessary for persistence to be increasingly higher (Figure 4.5 a). Selection, however, for higher oncogene expression was faster when antibody responses were lower (\( \tilde{\epsilon}_{\text{vac}}^* \) was reached faster if the antibody response is weaker, Figure 4.5 b) suggesting that the hosts with lower antibody titers might be better hosts for the emergence of types with higher oncogene expression.

Our findings that the new immunity imposed by the vaccine can create the conditions for higher oncogene expression coincide with prior studies into vaccine-induced evolution. In a review, Read and Mackinnon contrast old successful vaccines that stimulated natural immunity (which were already sterilizing and naturally very effective) to novel vaccines created from technical breakthroughs that stimulate new immunity responses that differ considerably from natural immunity. They warn then that by imposing new effector mechanisms these vaccines create very different selection pressures, with potentially unwanted consequences \[42\].

The very strong vaccine-induced humoral response is believed to be effective, in part, due to how antibodies can attach to the L1 epitopes on the virus’ surface while the virus is docked onto the cell during its slow entry \[194\]. Our model shows how a high antibody response is an effective method in decreasing the number of infected cells by reducing infection of an uninfected host by free virions (Figure 4.3, decaying \( Y_f \)). Because this new vaccine environment drastically reduces free virions, the strength of the interaction between free virions and uninfected cells is weakened significantly, and this method of creating new infected cells becomes less viable. Since HPV has an alternate way of increasing infected cell populations, variants of the target types that can drive higher than average cell proliferation may have an advantage in this new environment. Indeed, oncoviruses in general have two ways of creating new infected cells, either by free virions entering new target cells or by driving already infected cells into cell division. With the HPV vaccine strongly selecting against one strategy the virus might switch to depending more on the other.
In discussions of HPV’s potential evolutionary responses, the main form of vaccine “leakiness” that has been addressed is that of type-specificity and whether or not this could lead to type replacement [148,195]. A type of vaccine leak that has not been considered is whether or not the vaccine blocks viral shedding. Gandon et al. 2001 showed that vaccines that block transmission lower pathogen prevalence and select for lower levels of virulence [84]. Given that in HPV vaccine trials challenge infections by vaccine-targeted types were detectable in vaccinated women [176], we argue then that the vaccine does not fully block viral shedding. Bear in mind that HPV is transmitted mechanically with the transfer of both free virions and dead infected keratinocytes that are shed from the surface of the epithelium. Thus, even if the antibody response lowers the free virion population significantly, a vaccinated host could still transmit the virus by shedding infected keratinocytes. We once again consider the MDV example, an oncavirus that is also transmitted by the shedding of epithelial cells. The MDV vaccines are leaky in that they do not block viral shedding, and this has played an important role in the subsequent virulence evolution of MDV [49,50]. In light of this, we strongly encourage studies of how long challenge infections last in vaccinated hosts and to what degree they shed infected cells.

It is important to find estimates of the degree to which free virions and infected cell populations are reduced by the vaccine, as they are currently unknown. Our model shows that viral load and infected cell populations are driven down quickly with a strong antibody response (Figure 2, ε < 20) but our model assumes that the high antibody response is instantaneous (δ vac is a constant). However, in reality there will be a lag from the time of first infection until the time the vaccine-induced antibody response invades at full force. We have not seen empirical estimates of how many days this takes, but it could have considerable consequences on the evolution of the virus and its transmission.

In a recent and interesting study, Orlando et al. address the evolutionary and ecological reasons for the two main HPV strategies, LR and HR [172]. They find that HR types are best suited for transmission in longer and more stable partnerships (because HR infections last longer) while shorter partnerships with higher turnover rates allow for the persistence of LR types (because LR types are cleared faster). We show here that by artificially shortening the infection duration by vaccination, targeted HR types can now adopt the strategy of increasing their cell proliferation, a strategy that was costly in natural conditions. By doing so they increase their chance of persistence in hosts with high partner turnover rates, thus adopting a similar strategy to how LR types persist now. The important difference, however, is that these HR types have oncogenes with stronger cell transforming abilities, and expression at higher levels should more readily cause cellular genetic instabilities and potentially lead to faster progression towards cancer.

Our model does not contain a full population model of interacting hosts, thus we cannot investigate the conditions needed for a host population to maintain an emergent vaccine-adapted type. Heterogeneity of hosts plays an important role in the emergence of strains [56], and indeed we found variation in the optimal oncogene expression needed for persistence between different sexual activity groups. For instance, we found that super-spreaders required lower viral loads for persistent transmission, and in a closed population, that is highly sexually active, this could favour the emergence of a variant with higher oncogene expression. Future studies may want to consider how pockets of core-group individuals (the causal and super-spreaders in this
study) or of immune-deficient individuals may contribute to the emergence and circulation of new variants.

Though we show that virulence evolution is possible, we cannot determine with this study whether it is probable. The levels of high oncogene production that are needed to escape the vaccine-induced immune response were significantly higher than those in the unvaccinated hosts, however, it is not clear if the virus can increase its oncogene expression so significantly. However, this model is a simple conceptual model that does not include more details about the viral replication processes. Therefore, another more detailed model or experiments would be needed to assess the degree to which oncogene expression needs to rise in order to evade the vaccine and whether or not it is probable (e.g. what mutations or recombination events would be needed).

In conclusion, the HPV vaccine has a unique feature that may give us pause; it targets a virus that is for the majority of hosts avirulent. Other childhood vaccines target much more virulent pathogens with considerable morbidity and mortality. Should we, then, use the same vaccination policies (such as mass vaccination campaigns to create herd immunity) that were successfully used against more virulent pathogens on avirulent pathogens? Given that virulence is not a fixed trait in any pathogen, should we be drastically changing the ecological landscape of a virus that is, for the most part, avirulent? Is it worth creating strong selection pressures we do not understand? Maybe in the future when personalized preventative medical care is better realized, we might choose to use vaccines against less virulent pathogens on only sub-populations of people who are at risk of developing these diseases. Maintaining a majority of hosts unvaccinated, who ecologically select against virulence, might buffer the emergence of a novel more virulent strain that is adapted to vaccinated hosts. Of course, identifying which patients will have persistent infections with any certainty is currently not possible but as we move in that direction, we may want to reconsider if the smallpox “eradication” strategy is really the best approach for all infectious diseases.

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Chapter 5

Discussion
5.1 Discussion and Conclusions

The aim of this thesis was to determine how deeper investigations into within-host ecology could help improve our understanding of disease. In particular, I focused on infections and how knowledge about within-host ecology could increase our confidence in the long-term efficacy of our control methods, such as vaccines. I began by examining how community ecology concepts could help explain disease progression or severity. Important processes that affect food webs, such as trade-offs and interaction strengths, are also shown to be important to within-host viral dynamics. Indeed, all the chapters in this thesis had examples of how an improved understanding of the ecology of a within-host system could help explain disease progression or could help explain successes or failures of medical interventions.

Generally, ecological thinking is one that promotes system-level thinking, whereas, currently, infectious disease research is overwhelmingly pathogen specific. One of the most important community ecology concepts that have been applied, though only sparingly, to within-host systems is that of community modules. I show that incorrectly identifying underlying community modules (the fundamental structure of the community) and disregarding trade-offs (that determine the balancing of the strengths of the interactions) can lead to false expectations of how disturbances, such as vaccines, will affect a system. In both chapter 2 and 3, I discuss how the impact of the vaccine on HPV co-infections depends on which underlying community modules are present (e.g. if strains experience only one form of competition or two forms; resource competition and apparent competition). Similarly, in chapters 2 and 4, I show how altering trade-offs either with life-style choices or with vaccines, can change which strains of a virus have an advantage, potentially resulting in conditions that now favour strains with higher virulence.

HPV and its vaccine are a timely and unique example of how a medical community is openly discussing how a vaccine may or may not fail over the long-term due to the ecological changes that the vaccine will produce. Though many of the inferences about the within-host ecology of HPV lack empirical evidence, as I find in chapter 3, it is encouraging to see medical communities engaging in discussions about the ecology and evolution of an infectious disease pre-emptively, i.e. not just after resistance or after strain replacement has occurred. The HPV investigations in this thesis demonstrate the importance of finding the underlying natural within-host ecological interactions before we launch vaccines.

As we deepen our understanding of how ecosystems are structured and function, we are also in parallel uncovering the complex networks of interactions inside us. Remarkably, the more we uncover of these systems within, the more we find similar properties and behaviours as those in the ecosystems around us. Together then, these two bodies of research can elegantly unite the biological sciences within an overarching context of the evolution and ecology of biological systems. As humans impact both the ecosystems within us and around us, this work becomes paramount. Thus, uncovering the general patterns and developing a general theory of the ecology within us holds great promise.
5.2 Future Research

The field of infectious disease ecology is blossoming. This thesis contributes to this body of work with ideas and techniques. The future research that extends this work should attempt techniques not used here. For example, future within-host mathematical studies of HPV should consider using spatial models to represent the patches created by HPV infection of host cells via abrasions. One approach in this regard is to use dispersion models. However, unlike the dispersal of influenza across epithelial cells [196], HPV does not readily infect new cells horizontally in the bottom layers of the epithelial but rather HPV infections grow via the cell proliferation caused by HPV. Thus, mathematical descriptions of how lesions or warts grow need to take cell division into account. Also, it would be interesting to see how spatial models of HPV co-infections may validate or differentiate from the discussions here on competition and co-existence.

Another important future direction is towards data collection to test predictions of these models. Combined with mathematical modeling, these emerging techniques provide better parameterization of viral traits and immunity cell functions, which is particularly needed in infections that have characteristics that are distinct from HIV or influenza, the two mostly studied viruses. Indeed, it was the lack of available and useful data from HPV within-host dynamics that focussed the approach in this thesis to strictly mathematical modelling. HPV longitudinal studies are based on sampling taken once every 6 months or once a year, which is much too infrequent to make time-series that reflect the viral and immunity dynamics happening within these hosts. This is an example of how protocols or techniques used by medical researchers are not always well suited for investigating ecological or evolutionary questions. Frequent measurements of infections along with immunity counts are fundamental to improving our understanding of ecological within-host processes, and this is particularly lacking for medically important infectious diseases that are not HIV or Influenza.

Investigating pathogens that are less virulent would also help create a broader understanding of the processes in viral kinetics, pathogen-pathogen interactions and immunity dynamics. Ideally, this work would help us better understand why some pathogens are benign to their hosts. Our current research into within-host ecology and viral dynamics is, with good reason, predominantly of highly virulent pathogens. If we better understand what conditions maintain a pathogen to be avirulent then that might help us in our studies of the more virulent viruses by potentially leading to novel ideas on how to create the conditions needed to drive them to be less virulent.

For a more community ecology perspective, there is need for more data on co-infecting species of pathogens, e.g. HPV and chlamydia, particularly those that infect the same regions in the body. This of course should include avirulent microorganisms that may not harm the host directly but their interactions with invading pathogens might completely change the outcomes of infections. If we wish to understand host variation in disease progression and variation in severity of morbidity, we need to move away from one-disease only approaches, and if sampling is to be undertaken then measurements of other key players in the community would give us a better view of the within-host community and its dynamics.

By and large, infectious disease communities would benefit from more pro-actively investigations of the ecology and evolution of infectious diseases. This would build a more complete picture of the diseases we are trying to understand and manage. As in the HPV
example, medical researchers such as epidemiologists and virologists would benefit from collaborating more with ecologists and evolutionary biologists in order to address questions such as strain-strain competition, community composition and dynamics, the trade-offs that constrain pathogen traits, etc. Insight into these issues has the potential to affect the long-term effectiveness of the HPV vaccine. Since many of the current medical techniques or types of studies may not be suited for uncovering interactions as they are currently used, they might be tweaked or other techniques might be needed to address ecological and evolutionary questions. Beyond assisting the improvement of probing techniques, the systems-level thinking that ecologists can bring to collaborations can be of great assistance to medical researchers hoping to approach infectious diseases in a novel way.

In the future, it will be of service to young researchers to have virology and bacteriology textbooks with sections on the ecological and evolutionary processes that shape the pathogens they study, and with descriptions of the human body as an ecological system. This way vaccine and drug developers of the future will have these ecological and evolutionary perspectives in mind during research and development phases of their products and not as an afterthought.
Bibliography


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Appendices
A.1 Appendix for Chapter 2

Conditions for Coexistence inside a Host

Some pathogens are rarely found to coinfect a single host, while others are regularly found together. Here we see that mutualism or facilitation is not necessary to explain the coexistence of strains inside the same host. Instead we show that community ecology concepts such as in-host trade-offs and enrichment greatly influence whether coexistence is possible inside a host.

We investigated the conditions necessary for coexistence in a two strain community with asymmetric immunity attack (i.e. cross-reactivity) and asymmetric resource competition. Following Holt et al. (1994), we developed graphical invasion criteria for the model system (model 2.2). Specifically, we looked at the conditions that allow either strain (starting from a low density) to invade the X- Yi-Z sub-system containing the other strain at equilibrium (e.g. whether Y2 can invade X-Y1-Z). To accomplish this and to simultaneously create graphical conditions for coexistence consistent with the approach of Holt et al. (1994) [1], we looked at informative two-dimensional reduced nullsurfaces for the cases X-Y1-Z and X-Y2-Z.

To reduce the nullsurfaces, first recognize that for either X-Yi-Z sub-system, its \( dZ/dt = 0 \) isocline completely determines the \( Y_i^* \) equilibrium value (i.e. when \( dZ/dt = 0 \), then \( Y_i^* = (m/c_i) \)). Substituting this value into \( dX/dt = 0 \) and solving yields;

\[
X_{yi}^* = \frac{\lambda \ c_i \ u_i \ d \ c_i + \beta_i \ k_i \ m}{u_i \ d \ c_i + \beta_i \ k_i \ m},
\]  

(A.1.1)

which are the invariant reduced nullsurface (Fig. S.1). Next we solved for the \( dY/dt = 0 \) nullsurfaces;

\[
Z_{yi}^* = X_{yi}^* \ (k_i \ \beta_i \ u_i \ p_i) - a_i \ / \ p_i,
\]  

(A.1.2)

which are identified graphically in Fig. A.1.1. Taken together, this produces a two-dimensional plot of reduced nullsurfaces that represent the underlying three-dimensional nullsurfaces for each sub-system (X-Yi-Z). The intersection of the two reduced nullsurfaces (\( dX/dt = 0 \) and \( dY/dt = 0 \)) give us the \( X \)-equilibrium value and the \( Z \)-equilibrium value for the specific three-dimensional sub-system. In a sense, the substitution of the \( Y_i^* \)-equilibrium allows us to lose a dimension at the cost of not showing the exact value of the \( Y_i^* \) (although we know it is \( Y_i^* = (m/c_i) \)).
Figure A.1.1 Phase-plane cases. A: Competitive exclusion. (i and ii) The black dots represent the equilibrium solution of each subsystem (i.e. where X, Y, Z can exist together). The winner is determined by invasion criteria, such that if the isocline of strain \( i \) is above the equilibrium of the subsystem with strain \( j \), then strain \( i \) can invade but strain \( j \) cannot. Strain \( i \) then, is the winner and its subsystem equilibrium is an attractor. B: Coexistence. Here the isoclines cross inside the two subsystem curves and both strains can invade, thus the interior equilibrium is stable. Finally, C: Priority Effects. The interior equilibrium is unstable therefore the initial conditions determine who wins.

To complete the invasion analysis, we need to consider the dynamics of the two possible invasion cases. Can \( Y_j \) invade (allowing \( Y_i \) to be an arbitrarily small number, \( \epsilon \)) when the system is at the \( X-Y_i-Z \) sub-system equilibrium (for \( j = 1,2 \) and \( i = 1,2 \) ? Graphically, the invasion criteria for \( Y_j \) occurs when the \( dY_j/dt = 0 \) reduced nullsurface lies above the intersection of the \( dX/dt = 0 \) (eq. S.1; for \( X-Y_i-Z \) sub-system) and the \( dY_j/dt = 0 \) (i.e. lies above the equilibrium for the \( X-Y_i-Z \) sub-system). The above conditions ensure that the growth of \( Y_j \) is positive.
We can classify the fate of the invading $Y_j$, given $X-Y_i$-$Z$, into the four possible cases:

1. strain $j$ invades and excludes strain $i$ (Fig. A.1.1 A and ii) if $Z_{Y_i}^* < Z_{Y_j}$ and $Z_{Y_j}^* \geq Z_{Y_i}$

2. both strains can invade, i.e. coexistence (Fig. A.1.1 B) if $Z_{Y_i}^* < Z_{Y_j}$ and $Z_{Y_j}^* < Z_{Y_i}$

3. neither can invade the other, i.e. priority effects (Fig. A.1.1 C) if $Z_{Y_i}^* > Z_{Y_j}$ and $Z_{Y_j}^* > Z_{Y_i}$

4. neutral, i.e. coexistence is not possible (Fig. A.1.2 ii) if $Z_{Y_i}^* = Z_{Y_j}$ and $Z_{Y_j}^* = Z_{Y_i}$

where $Z_{Y_i}^*$ is the intercept of $dY/dt = 0$ (eq. S.2) of the $X-Y_i$-$Z$ sub-system with $dX/dt = 0$ (eq. A.1.1) of the same subsystem system,

$$Z_{Y_i}^* = \lambda c_i k_i \beta_i / (p_i (u_i d c_i + \beta_i k_i m)) - a_i / p_i,$$

and where $Z_{Y_i}$ is the intercept of $dY/dt = 0$ (eq. S.2) of the $X-Y_i$-$Z$ sub-system with $dX/dt = 0$ (eq. S.1) of the other subsystem, $X-Y_j$-$Z$,

$$Z_{Y_i} = \lambda c_j u_j k_i \beta_i / (u_j p_i (u_j d c_j + \beta_j k_j m)) - a_i / p_i.$$

Note that $Z_{Y_j}^*$ and $Z_{Y_j}$ are found in a likewise fashion.

Case (2) above depicts coexistence since both strains can invade when rare, and the boundary sub-system repels into the four-dimensional system yielding an interior solution (Fig. A 1.1 B).

To illustrate these ideas more clearly, we show how these nullsurfaces match specific regions of their corresponding bifurcation plot (Fig. A.1.2 and A.1.3). For example, the reproduction and decay trade-off falls into case (4) above, which drives an instantaneous switching from one exclusion region to the other (see Fig. A.1.2). This can also be appreciated in the equations (model 2.2) because, from looking at the $dY/dt$ equations, we can see that changes to either the replication rate, $k_i$, or to the decay rate, $u_i$, only affect $\beta_i'$ (they are both inside $\beta_i'$) therefore the strains can only differ from one another by $\beta_i'$, while their $dY/dt = 0$ Z-axis intercepts remain identical, $a_i/p_i = a_j/p_j$ (eq. A.1.2). This implies, for instance, that increasing $u_j$ only changes the slope of $dY_j/dt = 0$ until, and past, the point where the two lines completely overlap, driving a degenerate bifurcation (Fig.A.1.2 ii). Biologically then, this trade-off changes which strain excludes the other and does not allow for coexistence (technically a coexistence region exists but it is infinitesimally small).
Figure A.1.2. The reproduction and decay trade-off in HPV with matching phase-planes. No coexistence. Plots (i) and (iii) represent the phase-planes before and after the bifurcation, respectively, and plot (ii) is at the bifurcation. Parameter estimates. HPV: $\lambda = 36000 \text{ cells/day}^{-1}$ [2], $d = 0.048 \text{ day}^{-1}$ [3], $\beta_1 = \beta_2 = 0.0067 \text{ day}^{-1}$ [4], $k_1 = 100 \text{ virions/cell}^{-1} \text{ day}^{-1}$, $k_2 = 50 \text{ virions/cell}^{-1} \text{ day}^{-1}$ [5], $u_2 = 0.52 \text{ day}^{-1}$ [6], and since HPV is a non-lytic virus $d = a_1 = a_2 = 0.048 \text{ day}^{-1}$ [3]. Immunity: $p_1 = p_2 = 1 \text{ day}^{-1}$ [7], $m = 0.01 \text{ day}^{-1}$ [8], $c_1 = c_2 = 0.1 \text{ day}^{-1}$ [9]

In Figure A.1.3 we give an example of a trade-off that can give coexistence. Consider that increased replication rates of the virus, high $k$, could have a negative effect on the lifespan of its host cell (causing an increase in $a$). Though viruses can be lytic (virus kills the host cell) or non-lytic (the average life span of the host cell is not affected by viral infection), there should still be some variation in lytic effect (the rate at which the virus kills the host cell) and it would be interesting to study whether rapidly replicating strains, on average, kill their host cells faster than other strains. We considered this hypothetical in-host trade-off and found that it allowed for coexistence, albeit for parameter values of the infected cell death rate, $a$, that were not biologically feasible. This lack of biological realism, however, depends on the parameter estimates of the attack rates of the CTL, $p$, which, to our knowledge, is currently not well measured. Once again, better estimates of the timing of vital processes happening during
infections could help improve our ability to understand when these in-host trade-offs could be affecting the community dynamics of the infection.

There are other factors that can affect whether or not coexistence is possible. For example, we know from ecology that resource availability and resource use strategies are important. Here, we assumed that the resource, the uninfected cell population, grows at a constant rate, $\lambda$. This may not be true, depending on the cell type. Also, not all cells are necessarily accessible at all times and, the growth rates of the cells could range considerably, again, depending on the target cell type. In reference to the latter, we found that changing $\lambda$ from low to high values, had the singular effect of moving $X_{Yi}^*$ along the $X$-axis either towards the origin when small or away from the origin when $\lambda$ was large (not shown). Thus, depending on the movement of the $dY/dt = 0$ nullsurfaces caused by changing the bifurcation parameter of interest, the change in $\lambda$ affects the likelihood of coexistence. If the movement of the nullsurfaces is such that they cross more easily close to the origin, then if the $X_{Yi}^*$ are near the origin then it is more likely that small changes in the bifurcation parameter will lead to them crossing within the bounded region. Also note, that lower $\lambda$ values shrank these bounded regions and could also change (i.e. decrease) the value of the bifurcation parameter. These changes can be particularly important for keeping the values of the bifurcation parameter within a biologically feasible range. This highlights that the concept of “enrichment” from ecology should be investigated further in these systems. Whether the “paradox of enrichment” is found or not, infectious disease dynamics studies could benefit from developing their own version of enrichment theory.

Finally, the linear rates used in these equations are the simplest assumptions possible and thus give simple straight nullsurfaces. With the inclusion of carrying capacities or more realistic functional responses the shapes of the nullsurfaces would be more interesting and could make coexistence more probable.
Figure A.1.3. The reproduction and lytic effect trade-off in HPV allows for coexistence. Plots (i) and (v) are before and after the bifurcations, (ii) and (iv) are at the bifurcations, and (iii) is stable coexistence. Parameter estimates. HPV: $\lambda = 36000$ cells/day$^{-1}$ [2], $d = 0.048$ day$^{-1}$ [3], $\beta_1 = \beta_1 = 0.0067$ day$^{-1}$ [4], $k_1 = 100$ virions/cell$^{-1}$ day$^{-1}$, $k_2 = 50$ virions/cell$^{-1}$ day$^{-1}$ [5], $u_2 = 0.52$ day$^{-1}$ [6], and since HPV is a non-lytic virus $d = a_1 = a_2 = 0.048$ day$^{-1}$ [3]. Immunity: $p_1 = p_2 = 1$ day$^{-1}$ [7], $c_1 = c_2 = 0.1$ day$^{-1}$ [9], $m = 0.5$ day$^{-1}$ [10].
Box A.1. Using modules to understand community dynamics

The theory of food web modules allows us to determine the conditions that lead to ecological outcomes such as dominance, coexistence and exclusion. For example, competitive exclusion during resource competition is determined by the $R^*$ rule: the species that can maintain a viable population (birth rate is equivalent to death rate; A below) at the lowest density of the shared resource will drive its competitor to extinction [14]. Competitive exclusion can also happen due to indirect competition via a shared predator, as in apparent competition. A similar rule, the $P^*$ rule, governs the outcome of this module and it states that the species that can withstand the highest levels of predation will exclude its competitor [15]. Importantly, exclusion is not the only outcome of these communities. Understanding how species coexist has been a focus of ecologists for decades [34, 35], and modular theory has helped decipher this as well.

Adapted from Chase and Leibold (2003) Fig. 2.3[18]. A: $R^*$ rule; species 2 wins (lowest $R^*$). B: $P^*$ rule; species 1 wins (highest $P^*$).

The relative strengths of the interactions in communities determines whether coexistence or exclusion will result and thus if these rule apply. For example, in the diamond module, where apparent and resource competition exist jointly, whether there is symmetric predation (strength of predation has equal effect on both species) or symmetric resource competition, then the outcomes are determined by the $R^*$ or the $P^*$ rule respectively [1]. For example, if predation on one competitor is stronger than on the other but resource competition is symmetric in strength, then the system dynamically collapses into the apparent competition module, and the qualitative outcome depends on the $P^*$ rule. Thus in this case, the outcome of this diamond module is determined by a simple rule that comes from a simpler imbedded module (apparent competition).

The mathematical forms of consumer-resource models and in-host dynamic models are very similar, and not surprisingly the $P^*$ and $R^*$ rules have been found to also apply to strain interactions, when strains are simultaneously influenced by competition (e.g. for cells) and immunity attack [19]. However, new rules specific to in-host systems should be sought after. Also, finding relative interactions strengths is lacking attention in in-host studies, but given their explanatory power in ecological theory their potential to elucidate in-host dynamical outcomes they are an exciting avenue for in-host ecology and should be explored further.
A.2 Appendix for Chapter 3

Patch model derivation
To build the model we started with a variation of the two-species Levins’ model [11–13] which is

\[
\frac{dP_1}{dt} = e_1(f_1P_1 + f_{12}P_{12})P_0 + m_{21}P_{12} - e_{21}(f_2P_2 + f_{21}P_{12})P_1 - m_1P_1 \\
\frac{dP_2}{dt} = e_2(f_2P_2 + f_{21}P_{12})P_0 + m_{12}P_{12} - e_{12}(f_1P_1 + f_{12}P_{12})P_2 - m_2P_2 \\
\frac{dP_{12}}{dt} = e_{12}(f_1P_1 + f_{12}P_{12})P_2 + e_{21}(f_2P_2 + f_{21}P_{12})P_1 - (m_{12} + m_{21})P_{12} \\
P_0 = 1 - P_1 - P_2 - P_{12}
\] (A.2.1)

Here \(P_i\) is the fraction of patches with only species \(i\), \(P_{12}\) is the fraction of patches co-inhabited by both species, and \(P_0\) is the remaining patches that are empty. Also, \(e_i\) is the establishment rate of species \(i\), and \(e_{ij}\) is the establishment rate of species \(i\) of a patch already with species \(j\). The mortality rate of patches occupied by species \(i\) is \(m_i\), while \(m_{ij}\) is the mortality rate of species \(i\) in a patch also occupied by species \(j\). A species alone in a patch produces propagules (e.g. seeds) at a rate \(f_i\) and at a different rate when in a patch with the other species, \(f_{ij}\).

We made a series of assumptions in order to best capture the co-infection dynamics of HPV types inside a host. Co-infected patches cannot become singly infected. CTL cannot remove a single type from co-infected patches because a patch can contain co-infected cells. This removes the parameters \(m_{ij}\) and a couple of terms.

Co-infected patches cannot be created by a single instantaneous event by both type 1 and type 2 (because it is fairly unlikely that one cell in an empty patch be infected by two different types at exactly the same time), i.e. \(P_0\) cannot be come \(P_{12}\) without first being \(P_1\) or \(P_2\). Therefore, there is no need to add any more growth terms to \(P_{12}\).

Also, since clearance of HPV infection requires CTL invasion, patches are only cleared by CTL. Since CTL invasion is a dynamic process, then a variable that represents the CTL population, \(Z\), is included. Together these assumptions give,

\[
\frac{dZ}{dt} = c_1P_1Z + c_2P_2Z + (c_1 + c_2)P_{12}Z - mZ \\
\frac{dP_1}{dt} = e_1(f_1P_1 + f_{12}P_{12})P_0 - e_{21}(f_2P_2 + f_{21}P_{12})P_1 - a_1P_1Z \\
\frac{dP_2}{dt} = e_{12}(f_1P_1 + f_{12}P_{12})P_2 + e_{21}(f_2P_2 + f_{21}P_{12})P_1 - (a_1 + a_2)P_{12}Z \\
P_0 = 1 - P_1 - P_2 - P_{12}
\] (A.2.1a)
Note that type 1 is HPV-16 (denoted ‘16’ in the paper), type 2 is a HR non-vaccine type (denoted ‘hr’), co-infected patches are $P_{co}$, and finally, $f_{i_{co}}$ and $f_{hrco}$ are the reproductive rates of HPV-16 in a co-infection and the other type in a co-infection respectively.

Here, $e_i$ and $e_{ij}$ are the establishment rate of type $i$ alone or with type $j$, respectively. The rate at which CTL are produced from the presence of infected patches with HPV type $i$ is $c_i$. Also, $m_i$ and $a_i$ are the mortality rate of CTL and the removal rate by CTL of patches occupied by type $i$ respectively. The abundance of CTL, $Z$, increases proportionally to the proportion of all infected patches. The proportion of patches occupied by only type $i$, grows as a product of “the total amount of free virus particle produced both $P_i$ and $P_{12}$” by “the total proportion of empty patches” by “the establishment rate of type $i$ into empty patches”. The patches with only type $i$, $P_i$, become co-infected patches, $P_{12}$, by the number of free viruses of type $j$ that where produced by both $P_j$ and $P_{12}$ at an establishment rate of type $j$ that is affected by the presence of type $i$, $e_{ij}$.

The proportion of patches occupied by only type $i$, $P_i$, are reduced by the attack of CTL, $a_i P_i Z$, and co-infected patches are be removed by CTL at the same rates as single infected patches, $a_i$ or $a_j$. Finally, co-infected patches, $P_{12}$, are created only when singly infected patches $P_i$ are infected by the other type $j$ at an establishment rate that is affected by the presence of type $i$, $e_{ij}$. Once activated we assume that CTL grow equally against either type $c = c_1 = c_2$. Also, we assumed that the positive effect of the presence of one type on the establishment rate of the second type is only unidirectional, i.e. only the presence of HPV-16 benefits the entry of other non-HPV-16 types not vice versa (as it is discussed in the literature), $e_1 = e_{12}$ and $e_2 \neq e_{21}$. Therefore, this is facilitation (positive effect in one direction) not a mutualism (reciprocal positive effect). This allows us to make the following reductions:

$$
\frac{dZ}{dt} = c Z (P_1 + P_2 + P_{12}) - mZ \\
\frac{dP_1}{dt} = e(f_1 P_1 + f_{12} P_{12})P_0 - e_{21}(f_2 P_2 + f_{21} P_{12})P_1 - a_1 P_1 Z \\
\frac{dP_2}{dt} = e(f_2 P_2 + f_{21} P_{12})P_0 - e(f_1 P_1 + f_{12} P_{12})P_2 - a_2 P_2 Z \\
\frac{dP_{12}}{dt} = e(f_1 P_1 + f_{12} P_{12})P_2 + e_{21}(f_2 P_2 + f_{21} P_{12})P_1 - (a_1 + a_2)P_{12} Z \\
P_0 = 1 - P_1 - P_2 - P_{12} 
$$

(A.2.1b)

We assumed both types have the same establishment rates, $e_1 = e_2 = e$. However, type 2 more readily enters patches already infected with type 1 which implies $e_{21} > e$.

In order to make a dimension reduction, let $t = \tau / \varepsilon$.

$$
\frac{dZ}{dt} = \gamma Z (P_1 + P_2 + P_{12}) - \mu Z \\
\frac{dP_1}{dt} = (f_1 P_1 + f_{12} P_{12})P_0 - \varepsilon (f_2 P_2 + f_{21} P_{12})P_1 - \alpha_1 P_1 Z \\
\frac{dP_2}{dt} = (f_2 P_2 + f_{21} P_{12})P_0 - (f_1 P_1 + f_{12} P_{12})P_2 - \alpha_2 P_2 Z \\
\frac{dP_{12}}{dt} = (f_1 P_1 + f_{12} P_{12})P_2 + \varepsilon (f_2 P_2 + f_{21} P_{12})P_1 - (\alpha_1 + \alpha_2)P_{12} Z \\
P_0 = 1 - P_1 - P_2 - P_{12} \\
$$

(A.2.2)

where $\gamma = \frac{c}{e}$, $\mu = \frac{m}{e}$, $\varepsilon = \frac{e_{21}}{e}$, and $\alpha_i = \frac{a_i}{e}$.
Note that the terms \((f_1P_1 + f_{12}P_{12})\) and \((f_2P_2 + f_{21}P_{12})\) are measures of viral load in these scenarios, because \((f_iP_i + f_{ij}P_{ij})\) is the total number of type \(i\) virions produced by patches \(P_i\) and \(P_{ij}\) at one time step. We used these terms to calculate viral loads for the various ecological scenarios.

To consider neutral HPV in-host communities, we altered model 2 to get a model that does not have resource competition. Here the two HPV types do not use the same patches, and are completely independent if they do not share the immune response. If there is some cross-reactivity then the \(Z\) equation will also impact \(P_2\).

\[
\frac{dZ}{d\tau} = \gamma Z (P_1 + P_2) - \mu Z \quad \text{or} \quad \frac{dZ}{d\tau} = \gamma Z P_i - \mu Z
\]

\[
\frac{dP_1}{d\tau} = f_1 P_1 P_{01} - \alpha_1 P_1 Z
\]

\[
\frac{dP_2}{d\tau} = f_2 P_2 P_{02} - \alpha_2 P_2 Z
\]

\[
P_{01} = 1 - P_1
\]

\[
P_{02} = 1 - P_2
\]

where \(i = 1\) or \(2\)

The second \(Z\) equation was used when there was no cross-reactivity so \(\alpha_i = 0\).

Since the vaccine only grows in response to HPV-16 then the growth term of \(Z\) should not depend on \(P_2\). Therefore, the vaccine equation is

\[
\frac{dZ}{d\tau} = \gamma Z (P_1 + P_{12}) - \mu Z
\]

When the vaccine does not elicit a cross-reactive response we set \(\alpha_2 = 0\), otherwise \(\alpha_2 < \alpha_1\). This change to the model ensured that when \(P_2\) reaches 1 and \(P_1\) and \(P_{12}\) go to zero \(Z\) stops growing.

For completeness, we also considered a model that was in between model 2.2 and 2.2a. The types could only infect separate empty patches, \(P_{01}, P_{02}\), but they could also infect patches already infected by the other type. In other words, this relaxed the assumption from model 2.2 that the types were competing for the same empty patches.

To see what happens to the neutrality model if co-infection of patches \((P_{12})\) is added, the model was modified to

\[
\frac{dZ}{d\tau} = \gamma Z (P_1 + P_2 + P_{12}) - \mu Z
\]

\[
\frac{dP_1}{d\tau} = (f_1P_1 + f_{12}P_{12})P_{01} - \varepsilon (f_2P_2 + f_{21}P_{12})P_1 - \alpha_1 P_1 Z
\]

\[
\frac{dP_2}{d\tau} = (f_2P_2 + f_{21}P_{12})P_{02} - (f_1P_1 + f_{12}P_{12})P_2 - \alpha_2 P_2 Z
\]

\[
\frac{dP_{12}}{d\tau} = (f_1P_1 + f_{12}P_{12})P_2 + \varepsilon (f_2P_2 + f_{21}P_{12})P_1 - (\alpha_1 + \alpha_2)P_{12} Z
\]

\[
P_{01} = 1 - P_1 - P_{12}
\]

\[
P_{02} = 1 - P_2 - P_{12}
\]
Again for the vaccine case, the $Z$ equations becomes \( \frac{dZ}{dt} = \gamma Z(P_1 + P_{12}) - \mu Z \).

The vaccine results of this model can be compared to both the neutral case and the full patch model (Fig. 2.3 B). Note that the time-series of this model behave the same as the full model does.

When comparing vaccine to natural immunity, we assumed the vaccine’s cross-reactivity scaled linearly in strength, e.g. if the natural cross-reactive attack rate against the non-vaccine type is \( \alpha_2 = 0.1 \), and if the vaccine is 100 times stronger, then the vaccine cross-reactive attack rate is \( \alpha_2 = 10 \). This then assumes that non-vaccine types in vaccinated hosts experience stronger cross-reactive immune response, and therefore, we considered the region \( 0 < \alpha_2 < 50 \) in the vaccine parameter plots. If we had not made this assumption then we would consider a very small region of the vaccine plots (\( \alpha_2 < 0.5 \)).
### Strain parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$e = \frac{e_{21}}{e}$</td>
<td>establishment rate</td>
<td>$e = 1$ implies that every time a virion encounters a patch it establishes successfully. However, since it is biologically impossible for this success to be greater than 100%, then $e$ cannot be &gt;1, so, $0 &lt; e \leq 1$ and $0 \leq e_{21} \leq 1$. When $e_{21} = e$, there is no facilitation, so $e = 1$. There is facilitation when $e_{21} &gt; e$ and entry into P1 is easier than into P0 for strain 2, thus $e &gt; 1$. There is no reason to believe that type 1 blocks entry of type 2 (i.e. $e_{21}$ cannot be less than $e$). Overall then $e \geq 1$.</td>
</tr>
</tbody>
</table>

### $f_i$ and $f_0$ rates of virion production by strains inside patches alone and together

<table>
<thead>
<tr>
<th>Rate</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>If $f_i = 1$, then one virion is made per patch/unit time</td>
<td></td>
</tr>
<tr>
<td>If $f_i &gt; 1$, then more than one virion is made per patch/unit time</td>
<td></td>
</tr>
<tr>
<td>And, if $0 \leq f_i &lt; 1$, then less than one virion is made per patch/unit time</td>
<td></td>
</tr>
<tr>
<td>Therefore, $f_i \geq 0$ and $f_0 \geq 0$ But given that there are many cells per patch at any given time at least one cell will burst, this implies that $f_i &gt; 1$ and $f_0 &gt; 1$</td>
<td></td>
</tr>
</tbody>
</table>

### Immunity parameters*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_i = \frac{a_i}{e}$</td>
<td>CTL killing rate</td>
<td>If $a_i = 1$, implies 100% successful killing. i.e. each CTL removes one patch per unit time If $0 \leq a_i &lt; 1$, implies that one CTL removes less than one patch per unit time If $a_i &gt; 1$, implies each CTL removes &gt;1 patch per unit time Assume CTL are not incredibly efficient i.e. $0 \leq a_i \leq 1$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rate</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0 \leq c \leq 1$ and $0 \leq m &lt; 1$ implies $0 \leq \gamma \leq 1$ and $0 \leq \mu &lt; 1$</td>
<td>CTL growth rate</td>
</tr>
</tbody>
</table>

**Table A.2.1:** Parameter restrictions and ranges  
*Estimates for the immunity proliferation and death rate parameters were drawn from the literature, $\mu$ and $\gamma$ from [10] and [8] respectively.
**Viral load Analysis**

Given the large parameter range the parameter plots cover, only some representative time-series were calculated within each of the hypothesised ecological scenarios (Figure A.2.1). Their corresponding viral loads are graphically represented in Figure A.2.2 and were recorded in Table A.2.1. We calculated a rough estimate of the “mean viral load” by calculating the area under the curves of the viral loads of each type (in Figure A.2.2) and then divided them by the total time. The vaccine mean viral loads were calculated at different time points, at 300 and 600 days.
A. No cross-reactivity (coinfection with HPV-16 and unrelated type)

i) Neutral
(a) Natural
(against HPV-16)

(b) Natural
(against other type)

(c) Vaccine
(against HPV-16)

B. Weak cross-reactivity (coinfection with HPV-16 and related type)

i) Neutral
(a) Natural
(against HPV-16)

(b) Natural
(against other type)

(c) Vaccine
(against HPV-16)

C. Vaccine conditions (different replication rates of the non-vaccine type)

i) Neutral
(a) No cross-reactivity

(b) Weak cross-reactivity

Figure A.2.1: Time-series of natural immunity. ($\epsilon = 1$) A. and B. (i) Neutrality. Without cross-reactivity, the non-targeted type reaches 100% use of its own patches and is unaffected by the CTL population. The speed at which it reaches total patch use depends on its own replication rate. Weak cross-reactivity only decreases the population of the non-targeted type for a period of time and then it bounces back once the CTL population drops off. (ii) intra-patch competition. Intra-patch resource competition...
(compare dotted with solid lines) has little effect on overall result; it only changes how fast results are reached. Before the CTL invade all four cases are similar, coinfected patches dominate. After the immune system invades (past \( t = 300 \)), one strain outcompetes the other, and which strain wins can be explained by a trade-off. When the immune system mounts against HPV-16 (a), a trade-off is created that allows the other HPV type to win, otherwise HPV-16 excludes it (b). Parameters: \( f_1 = 0.4; f_2 = 0.2 \) (i.e. \( f_1 > f_2 \) by 2:1), while \( f_{12} = 0.2; f_{21} = 0.05 \) (i.e. \( f_{12} > f_{21} \) by 4:1), no facilitation \( \varepsilon = 1 \), no cross-reactivity \( \alpha_1 = 0, \alpha_2 = 0.05 \), while weak cross-reactivity \( \alpha_1 = 0.5, \alpha_2 = 0.05 \); \( \gamma = 0.6 \) and \( \mu = 0.2 \). (iii) facilitation only. Notice that in all cases, the outcome is the same as the competitive scenarios. The major difference is that the coinfected patches grow very quickly and no HPV type exists on its own until the immune response invades (all four plots before 300 dpi). Parameters: \( f_1 = f_{12} = 0.4, f_2 = f_{21} = 0.2 \) (i.e. \( f_1 > f_2 \) by 2:1; no competition inside \( P_{12} \), weak facilitation \( \varepsilon = 6 \), and strong \( \varepsilon = 34 \). (iv) facilitation and competition. Very similar to facilitation only plots. Parameters: \( f_1 = 0.4, f_2 = 0.2 \) (i.e. \( f_1 > f_2 \) by 2:1; while \( f_{12} = 0.2; f_{21} = 0.05 \), weak facilitation \( \varepsilon = 6 \), and strong \( \varepsilon = 34 \). C. Vaccine conditions: varying the replication rates of the non-vaccine type. All scenarios behave the same (i-iv), which implies that the strength of the cross-reactivity and the intrinsic replication rates of the non-vaccine type matter most to the outcome. a) No cross-reactivity with the vaccine. If the non-vaccine type has a very low replication rate then it takes several months to fill all patches available (light green), but if it has a high replication rate then it fills the patches almost instantly (dark green). b) Weak cross-reactivity. If the non-vaccine type has a replication rate that is substantially lower than the vaccine type then the vaccine wipes out the weakly cross-reactive non-vaccine type (light green goes to zero almost instantly). However, if the non-vaccine type’s replication rate is higher than the vaccine type then it fills all patches very quickly (dark green). Parameters: \( f_1 = 0.4, f_2 = 0.02 \) for low, and \( f_2 = 0.6 \) for high (for competition \( f_{12} = 0.2 \) and \( f_{21} = 0.01 \) for low and \( f_{21} = 0.3 \) for high), no facilitation \( \varepsilon = 1 \), weak \( \varepsilon = 6 \) and strong \( \varepsilon = 34 \); vaccine attack on HPV-16 \( \alpha_1 = 50 \), while no cross-reactivity \( \alpha_2 = 0 \), and weak cross-reactivity with the vaccine \( \alpha_2 = 20 \).
Figure A.2.2: Natural viral loads
Table A.2.2: Mean viral loads under natural and vaccine immunity ($e < 1$)

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<tr>
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Table A.2.2: Mean viral loads under natural and vaccine immunity ($e < 1$)
References


