Unpacking β: Within-Host Dynamics and the Evolutionary Ecology of Pathogen Transmission

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infectious disease, microbial genetics, pathogenesis, plague, virulence

Abstract

Rather than being fixed, pathogen transmission varies and is thus an object of natural selection. I examine how opportunities for selection on pathogen transmission depend on (*a*) pathogen fitness, (*b*) genetic variability, and (*c*) forces acting at within- and between-host levels. The transmission rate, β, influences processes such as epidemic spread, postepidemic fade-outs, and low-level persistence. Complexity of infection processes within hosts leads to different transmission rates among hosts and between types of pathogens (viruses, bacteria, eukaryotic Protozoa). Generality emerges, however, by "unpacking" β into within- and between-host opportunities for selection. This is illustrated by evolutionary biology of the bacterium *Yersinia pestis*, which causes plague in mammals, remains highly virulent and is transmitted by multiple routes, including fleas and direct contacts with infected hosts. The strength of within-host selection is manifested through infectivity, replication, pathogenicity, and dissemination from hosts. At the between-host level, responses to selection are less predictable because of environmental variation, whereas vector-borne transmission (usually by arthropods) provides additional opportunities for selection and trade-offs between vectors and hosts. In subdivided host populations, selection favors transmission before local pathogen extinction occurs, but key components (e.g. infectious periods of hosts) are determined by within-host dynamics. Pathogen transmission is often viewed in the context of transmission-virulence trade-offs, but within-host dynamics may cause host damage unrelated to transmission, and thus transmission-virulence trade-offs are not universal.

INTRODUCTION

Transmission: a product of dissemination, translocation, and infectivity; each term can vary theoretically and in practice

Tissue tropism: specificity of a pathogen to infect particular tissues within a host, for example nose and throat (naso-pharyngial) or intestinal tract (enteric)

Pathogen transmission can be an object of natural selection, given genetic variability for traits that influence transmission and differential pathogen fitness when transmitted by alternative routes. The implications are profound: Most pathogens are microbes with short life spans and rapid reproduction relative to their hosts. Pathogens greatly outnumber hosts in terms of infectious propagules, making exposure a near certainty, and the vast populations of microbes constantly give rise to novel genetic variation by mutations, homologous recombination and horizontal gene transfer, making evolution a virtual certainty. Thus, the potential for transmission rates and pathways to evolve, for pathogens to diversify, and for disease to emerge in previously naïve populations has few apparent limits (Andre & Day 2005, Antia et al. 2003, Grenfell et al. 2004, Shackelton et al. 2005). Here, I examine three ingredients of opportunities for selection (Crow 1958) and the possibility for pathogen transmission to evolve: (*a*) differing pathogen fitness, (*b*) sources of genetic variability, and (*c*) forces affecting natural selection on transmission at within- and between-host levels.

A critical aspect of evolutionary ecology of infectious disease is the transmission rate, β, which can influence processes such as epidemic spread, postepidemic fade-outs, and low-level persistence (Anderson & May 1991). The complexity of the infection process within hosts means that transmission rates usually differ between hosts (Lloyd-Smith et al. 2005, Woolhouse et al. 1997) and between types of pathogens, from viruses to bacteria to eukaryotic Protozoa. Generality may be established by "unpacking" β into various components, especially at the within-host and between-host levels, and asking how these components provide opportunities for selection on transmission (Bull 1994, Day & Proulx 2004, Frank 1996, Real & Biek 2007). Opportunities for selection on between-host transmission depend on the ability to survive the passage among host individuals, and may be limited for pathogens that do not replicate outside the primary host, either in vectors or in environmental reservoirs. Within hosts, however, infectivity, replication, pathogenicity, and dissemination from various host cells or tissues (tissue tropism) can differ between pathogens (see sidebar, Unpacking Pathogen Transmission) and will influence transmission by altering infection

UNPACKING PATHOGEN TRANSMISSION

Fitness of pathogens depends critically on transmission: the ability to translocate to new (uninfected) hosts, especially when conditions within infected hosts become unsuitable for further persistence (Frank & Schmid-Hempel 2008, Lipsitch & Moxon 1997). Dividing the process of transmission into components helps us understand how natural selection on pathogen tansmission occurs. First, infectivity describes a pathogen's ability to invade hosts after contacts with infected hosts, arthropod vectors, or environmental reservoirs. Infectivity is often measured as the dose (e.g., number of bacterial cells or viral particles) required to successfully invade host cells or tissues and is sometimes referred to as aggressiveness or colonization of hosts (Thomas & Elkinton 2004). Infectivity and within-host dynamics also depend critically on tissue tropism, within-host multiplication, and pathogenicity. These same processes influence dissemination, the ability of a pathogen to successfully leave an infected host to be translocated to another host. Translocation, movement of a pathogen from an infected host to other hosts, may then follow multiple paths, including direct contact between hosts, movement by arthropod vectors, or by contact with an environmental reservoir. How transmission and within-host dynamics relate to virulence can be quite variable between types of pathogens, depending on details of host cell recognition, host immune responses, pathogen evasion of host defenses, and pathogen multiplication.

Table 1 Modes of transmission between hosts

Horizontal	Examples
Direct	Contact between infected and susceptible individuals (e.g., sexually transmitted diseases, predation or cannibalism, respiratory droplets, shared needles among intravenous drug users, or by poor medical or agricultural practices)
Vector-borne	*Mechanical*: without amplification of the pathogen in the vector (typhus in house flies, myxoma virus and Rift Valley fever virus in mosquitoes) *Biological*: the vector is a site of reproduction or part of the life cycle of the pathogen (plague in flea guts, *Plasmodium* in mosquitoes, *Borrelia* in ticks, *Trypanosoma* and typhus in flies' guts and mosquitoes, many viruses in mosquitoes)
Environmental reservoirs	No growth or amplification of pathogen [spores of bacteria (anthrax) or fungi, viral capsules]. Continued replication of pathogen or parasite (*E. coli*, cholera in water and in biofilms on copepod exoskeletons)
Vertical	
Maternal to egg or seed, placental in mammals and some other vertebrates, transovariol in insects and other arthropods	HIV in humans, *Wolbachia* in arthropods and nematodes (increased transmission by manipulating host reproduction), plant viruses in insect vectors

intensities, incubation times from infection to dissemination, and infectious periods (Bull 1994, Day 2001, Levin & Antia 2001, Lipsitch & Moxon 1997). For example, persistent infections by human immunodeficiency virus (HIV) attacking helper T cells or by malaria parasites invading red blood cells vary in intensity during phases of infection (pathogen replication) and have extensive infectious periods. Acute infections, such as those caused by the measles virus invading epithelium and then spreading to other cells, or the plague bacteria (*Yersinia pestis*) attacking macrophages, have shorter infectious periods before they are either cleared by the immune system or the host dies. Selection within hosts greatly influences transmission as pathogens become adapted to invading specific tissues that facilitate dissemination from hosts and translocation between hosts.

This is not to say that between-host transmission is less variable and has fewer opportunities for selection, but evolutionary responses at the between-host level may be less predictable because of greater environmental variation. Alternative modes of between-host transmission are intensively studied in environmental contexts (**Table 1**), and changes in transmission pathways often require extensive evolutionary change. But this reminds us to distinguish between pathogen ecology and disease, the damage caused in hosts as a consequence of infection (see sidebar, Virulence, Pathogenicity, and Disease). Much disease results from spillovers to incidental or other dead-end hosts, with high virulence but few possibilities for transmission back to definitive hosts. For example, many diseases affecting humans have wildlife origins, such as severe acute respiratory syndrome (SARS) caused by coronavirus originating in bats (Wang et al. 2006) and renal failure and respiratory syndromes caused by hantaviruses from rodents (Zeier et al. 2005). These viruses are pathogenic in humans, but the majority of transmission is stopped by failure to replicate to high enough numbers to disseminate from hosts (and by public health interventions). Pathogen fitness from spillovers is usually zero, but nonetheless, spillovers offer large opportunities for selection. Evolution of new transmission pathways from spillovers forms a basis for expanding pathogen host ranges and disease emergence (Cleaveland et al. 2007, Kuiken et al. 2006, Webby et al. 2004, Wolfe et al. 2007, Woolhouse et al. 2001).

Intensity: the number (concentration) of a pathogen infecting an individual; intensity varies among host tissues; only some tissues or cells result in dissemination (see tissue tropism)

VIRULENCE, PATHOGENICITY, AND DISEASE

Finding a definition of virulence that is useful in all conexts and pleases everyone is virtually impossible (Bull 1994; Casadevall & Pirofski 2001; Day 2001, 2002; Lipsitch & Moxon 1997; Thomas & Elkinton 2004), as virulence is the outcome of interactions between pathogens and hosts. In some contexts, virulence describes pathogen multiplication and subsequent damage to hosts, measured as morbidity (lower host vigor), reduced reproduction, or death. In this usage, virulence relates to the rate of multiplication within a host, includes scavenging for nutrients, toxin production and evading host clearance, and may closely track within-host pathogen fitness. In many evolutionary models, virulence refers to disease, measured as host damage or mortality, and thus may not relate directly to pathogen fitness. On the other hand, pathogenicity is the ability for pathogens to cause disease in hosts, and is often used synonymously with virulence. Pathogenesis within hosts depends on pathogen exploitation of cells and tissues, toxicity produced by the pathogens, and/or immune responses of hosts. In many definitions, both pathogenicity and virulence include infectivity, the ability of a pathogen to invade a host (Thomas & Elkinton 2004). In this review of pathogen transmission, I find it useful to consider infectivity separately from virulence (focused on host damage) and pathogenicity (focused on pathogens' abilities to cause disease). Finally, many infections are asymptomatic within their hosts (pathogens have low pathogenicity and are avirulent), illustrating that infection and disease are also not completely synonymous.

In this review, rather than attempting the huge task of covering the full spectrum of pathogens from viruses to bacteria, fungi, and pathogenic macroparasites, I unpack β for plague, the infamous disease caused by the gram negative bacterium *Y. pestis*. Two features make plague an excellent model for studying pathogen evolutionary ecology. First, *Y. pestis* naturally occurs in wild rodents (see sidebar, What Is a Plague Focus?), but also spills over to many other mammals, is highly virulent to most mammals, and is transmitted both by fleas and direct contact with infected individuals. Plague was responsible for some of the most sensational pandemics in human history when *Y. pestis* emerged from wild rodents in Asia into the eastern Mediterranean in 550 AD, the Black Death of Europe beginning in 1347, and the modern worldwide pandemic originating in China in the late 1800s (Achtman et al. 2004, Gage & Kosoy 2005, Perry & Fetherston 1997,

WHAT IS A PLAGUE FOCUS?

Ecological and epidemiological studies of plague use the term "focus" to describe persistence of *Y. pestis* in wild (sylvatic) populations of rodents and their fleas, with occasional spillover to humans (Anisimov et al. 2004; Duplantier et al. 2005; Gage & Kosoy 2005; Tong et al. 2005a,b). Geographically, numerous plague foci have been identified based on the predominant rodent host in each region: For instance, gerbils (*Rhombomys opimus*) in the desert focus in Kazakhstan, marmots (*Marmota himalayana*) in the Qinghai-Gansu-Tibetan grasslands, black rats (*Rattus rattus*) in Madagascar, and prairie dogs (*Cynomys ludovicianus*) in the western Great Plains in the United States. Phenotypic and genotypic differences between foci are apparent at many levels, from bacterial physiology and pathogenicity to genetic differences based on simple sequence repeats, IS100 and SNP genotypes, plasmid variation, and genomic changes such as insertions, inversions, and gene rearrangements. Plague foci also differ in whether *Y. pestis* remains enzootic while continually cycling at low levels, or are characterized by sporadic epizootics. Boundaries between foci are often poorly defined, and genotyping of plague isolates shows that foci identified by physiological traits may be epidemiologically linked (e.g., Lowell et al. 2007). The presumption in Asia is that *Y. pestis* is specifically adapted to hosts in each geographic focus, although this has not been experimentally determined.

Pollitzer 1954). Second, within-host dynamics of plague are well known, including molecular, cellular, and genomic aspects of infectivity, mechanisms of pathogenesis, and factors that promote dissemination in both mammals and fleas (Carniel & Hinnebusch 2004; Perry & Fetherston 1997, 2007). In a similar vein, intraspecific variation in transmission pathways is also well-documented in influenza A viruses (Webby et al. 2004).

Finally, I briefly discuss the widespread hypothesis that long-term stability and persistence of parasite-host interactions depend on trade-offs between transmission and virulence: Pathogens that quickly overwhelm their hosts are expected to have lower transmission and persistence (Bull 1994, Day 2001, Dybdahl & Storfer 2003, Ebert & Bull 2003, Ewald 1994, Frank 1996, Galvani 2003, MacKinnon & Read 2004). Instead, I examine an alternative hypothesis that virulence may be a secondary consequence of within-host dynamics during infections, especially when damage to hosts is unrelated to rates of within-host replication in tissues that can lead to dissemination (de Roode et al. 2005, Graham et al. 2005, Levin & Antia 2001, Levin et al. 1999, Meyers et al. 2003). The strength of transmission-virulence trade-offs will thus depend on details of within-host dynamics along the path from infection to dissemination (Ganusov & Antia 2003) and trade-offs may disappear altogether when populations and contact rates between individuals are spatially structured (Cross et al. 2007, Gandon et al. 2002).

R_0: the number of infections arising from an infected individual in a susceptible population; like reproductive rates of free-living organisms, summarizes multiplication of infections

FITNESS AND THE REPRODUCTIVE RATIO R_0

As in all evolutionary models, we need a measure of fitness. For pathogens (and parasites), no fitness measure exists that is completely divorced from hosts: Most pathogens have no fitness without hosts. The basic reproduction ratio, R_0, the number of secondary infections arising from an initial infection in a population of susceptible hosts (Heesterbeek 2002, Roberts 2007), is perhaps the best candidate as a relative fitness measure, especially over the short term. As with any fitness measure, precaution should be taken to ensure that time scales of R_0 and selection episodes match (Fenton et al. 2002). Over longer time periods, relative fitness of pathogen strains will be influenced by host-pathogen coevolution and frequency-dependent selection, and simple maximization of R_0 may not lead to higher pathogen fitness (Dieckmann 2002). Like transmission, R_0 has several components but is especially sensitive to transmission rate, the infectious period of hosts (the inverse of host mortality rates caused by disease), and the number of susceptible hosts (Begon et al. 2002, Frank 1996, Lipsitch & Moxon 1997, McCallum et al. 2001). Even for the same value of R_0, changing transmission rates and infectious periods can change the speed and duration of epidemics. For example, a pathogen might have a high R_0, but immunizing infections may rapidly exhaust the local pool of susceptibles, leading to local extinctions of the pathogen and zero local fitness (Grenfell 2001). The measles virus during the prevaccine era in England provides a good example: high R_0 but still driven to local extinction (Bjørnstad et al. 2002).

Additionally, R_0 can be influenced by host heterogeneity, with greater heterogeneity in transmission (i.e., a few hosts responsible for most new infections) generally increasing R_0 (Dwyer et al. 2002, Lloyd-Smith et al. 2005, Roberts 2007, Woolhouse et al. 1997). However, heterogeneity in transmission can also lower the probability of pathogens invading new populations because of the increased chance that only poorly transmitting individuals are introduced (Lloyd-Smith et al. 2005).

Calculation of R_0 for vector borne pathogens is complicated by an additional transmission term, the entomological infection rate (Smith et al. 2005, Webb et al. 2006), which exacerbates transmission heterogeneity. Regardless, with some caution R_0 can be used as a fitness measure to predict the outcome of competition between strains of pathogens within hosts and the evolution of virulence (Ebert & Bull 2003, Gupta 2005, Lipsitch & Nowak 1995).

It is equally useful to "unpack" R_0 into components that relate to fitness and opportunities for selection: contacts, probability of transmission per contact, and infectious periods (Real & Biek 2007). Estimating R_0 usually requires the kind of detailed population data available for some human epidemics, such as measles, influenza, SARS, and malaria (Bjørnstad et al. 2002, Lipsitch et al. 2003, Mills et al. 2004, Smith et al. 2005), but robust methods can still be applied to wildlife populations when less detailed data are available (Ferrari et al. 2005, Real & Biek 2007). For pathogens that persist beyond initial outbreaks, R_0 can still be estimated by measuring seroprevalence among individuals of susceptible and infected classes; for instance, brucellosis in adult and juvenile bison in Yellowstone National Park (Dobson & Meagher 1996).

Seroprevalence: the proportion of animals (vertebrates) in a population that carry antibodies (termed seropostive), indicating current or past infection

GENETIC VARIABILITY OF PATHOGENS

A pathogen's abilities to adapt to novel transmission pathways, to maintain transmission in the face of ecological change, and to circumvent host immune responses depend on stores of genetic variation. Genetic variability in pathogens is potentially limitless and arises in bacteria by horizontal gene transfer (HGT) between species, transposition of insertion elements within genomes, reassortment and homologous recombination following HGT, and mutation (Grenfell et al. 2004, Moran & Plague 2004, Ochman & Moran 2001, Thomson & Parkhill 2004, Wren 2003). HGT is less common in viruses, but a poor replication repair mechanism results in high mutation rates at the nucleotide level. Segmented viruses reassort when several genotypes coinfect the same host cells, leading to potentially dramatic changes in both transmission and pathogenicity (e.g., Baigent & McCauley 2003, Baranowski et al. 2001, Boots et al. 2003, Ferguson et al. 2003b, Webby et al. 2004). The best-known example is for influenza A, where transmission depends on viral hemagglutinin (HA) surface proteins preferentially binding to sialic acid receptors on host cell surfaces, and mutations in HA determine both infectivity and host range of influenza variants. Given the importance of reassortment and HGT in microbes, and sexual reproduction in eukaryotic pathogens, standing genetic variation within pathogen populations will depend critically on the frequency of coinfection of hosts by multiple pathogen genotypes.

Here I describe genetic variation in plague, in light of lessons learned from comparisons between *Yersinia* species, including *Y. pestis*, and other bacteria. First, multiple genetic analyses suggest that *Y. pestis* evolved only 1500 to 20,000 years ago in central Asia from its closest relative, *Y. pseudotuberculosis* (Achtman et al. 2004, Wren 2003), an enteric pathogen of vertebrates transmitted by the oral-fecal route that chronically infects macrophages in lymph nodes near the gut. The plague bacterium *Y. pestis* shifted to flea-borne transmission, primarily of rodents, while still attacking macrophages in lymph nodes, resulting in swelling (also called buboes). This transition included escape to the bloodstream and widespread septicemia leading to host death (Carniel 2003, Wren 2003). Despite these major changes, all *Yersiniae* share a 4.6 Mb main chromosome with approximately 98% DNA sequence similarity between *Y. pestis* and *Y. pseudotuberculosis*, and slightly more divergence from *Y. enterocolitica*, another enteric pathogen (Skurnik et al. 2000). Most differences are synonymous mutations, pointing to strong purifying selection that limits genetic variability at the nucleic acid level.

The plague genome is like most bacteria in having signatures of HGT between species, plasmid acquisition and loss, and abundance of mobile genetic (insertion) elements. The ease of genetic transformation in *Y. pestis* has been experimentally demonstrated by coinfection of flea guts with *Y. pestis* and antibiotic resistant *E. coli*, and transfer of antibiotic resistant genes to *Y. pestis* (Hinnebusch et al. 2002a). Similarly, homologous recombination between serotypes of the bacterium *Vibrio cholerae* has been induced within biofilms on the exoskeletons of marine copepods that serve as environmental reservoirs for human infections (Blokesch & Schoolnik 2007). A primary

Table 2 The most important factors promoting pathogenicity and transmission of *Y. pestis* (after Brubaker 2003, Hinnebusch 2005, Perry & Fetherston 1997)

Within-host infection and/or pathogenicity	Location in genome	Type	Function
pgm (pigmentation locus)	Chromosomal	HPI and *hms* loci, many genes	Yersiniabactin iron uptake systems necessary for vertebrate host invasion
F1 (Fraction 1, outer capsule)	pFra plasmid (also called pMT)	Major gene *caf1*, several modifiers	Impedes phagocytosis by macrophages, by hindering binding of macrophages
pH 6 antigen	Chromosomal	Single gene	Part of adhesive rod-like pili, expressed between pH 5 and 6.7 in phagolysosomes of macrophages, prevents phagocytosis
Yops (*Yersinia* outer proteins)	pCD1 plasmid (also called pCad, pPYV in other *Yersiniae*)	Locus with many genes	Attack of macrophages, type III secretion system, cytotoxicity, immune suppression
LcrV	pCD1 plasmid	Major gene, modifiers	Immunosuppression, up-regulation of interleukin-10 to prevent inflammation response
pla (plasminogen activator)	pPla plasmid (also called pPCP1, pPST)	Single gene	Protease breaks down fibrin, promotes escape from site of injection by fleas, possible IL-8 inhibition
Between-host transmission			
pgm	Chromosomal	*hmsHFSRT*, *M* (heme storage loci)	Protein matrix for biofilm formation in flea foregut and proventriculus, degraded above 37°C
Ymt (*Yersinia* murine toxin)	pFra plasmid	Single gene	Phospholipase D, survival in flea gut

example of HGT in plague is the chromosomal high pathogenicity island (HPI), which carries genes coding proteins necessary for invasion of vertebrate hosts (**Table 2**). HPI has high sequence similarity to pathogenicity islands in *Salmonella*, *E. coli*, *Klebsiella*, and *Enterobacter* (Thomson & Parkhill 2004). Other genes, such as the toxic complexes (e.g., *Ymt*, **Table 2**), are homologs of insect pathogens. The *Yersiniae* also share plasmids necessary for pathogenicity and transmission (**Table 2**). The *Yersinia* outer proteins (*Yops*), encoded on the 75 kb plasmid pCD1, include a hypodermic needle-like type III secretion system for intracellular injection of cytotoxic and immunosuppressive proteins. The *Yops* have sequence similarity and synteny to pathogenicity islands from *Salmonella*. Two other plasmids, pFra (110 kb) and pPla (9.5 kb) are unique to *Y. pestis* and encode genes for invading macrophages and for biofilm formation and persistence in flea guts (Hinnebusch 2005).

Consistent with the hypothesis that plague originated in central Asia, the greatest genetic variation is found in central Asian plague foci (see sidebar, What Is a Plague Focus?). Variation in plasmid size and content naturally occurs, and a recently discovered 5.9 kb plasmid is emerging in China, but its role in pathogenesis or transmission is unknown (Anisimov et al. 2004). Plasmid variation correlates with phenotypic characteristics, including pathogenicity, but some of this variation is an artifact of spontaneous plasmid loss during laboratory passage (Anisimov et al. 2004, Lowell et al. 2007). Five recently published complete genomes of *Y. pestis* echo the lack of nucleotide variation described above, but also revealed more than 200 pseudogenes, representing

as much as 5% of the genomes (Wren 2003, Chain et al. 2004, Thomson & Parkhill 2004, Tong et al. 2005b). Many pseudogenes in *Y. pestis* have functioning homologs in *Y. pseudotuberculosis* and *Y. enterocolitica* that code for pathogenesis and general physiology for the enteric lifestyle. Genetic analyses of plague foci in China have revealed unique pseudogenes in different foci (Tong et al. 2005b) and variation in plague virulence factors yersiniabactin and heme storage systems (Tong et al. 2005a,b). A newly identified isolate from the Tibetan plateau in China is avirulent in humans (Song et al. 2004), but adaptive significance for plague transmission and persistence in wild rodent populations remains to be determined.

Plague is also highly variable in that its genome contains an abundance of simple sequence repeats (e.g., AT, CAAA: Klevytska et al. 2001) and insertion sequences from transposable elements (e.g., IS100: Motin et al. 2002). Both occur within reading frames of genes and potentially influence pathogen fitness, but their greatest utility has been in genotyping systems for delineation of geographical isolates of plague, including long-recognized biovars (*Antiqua* from Africa, *Medievalis* in central Asia, *Orientalis* from eastern Asia) and newly recognized types (*pestoides* in central Asia, *Microtus* in China) that differ in characteristics such as glycerol fermentation and nitrate reduction (Achtman et al. 2004, Anisimov et al. 2004, Lowell et al. 2007, Zhou et al. 2004). Simple sequence repeats have been especially useful for identifying plague transmission and spillovers on small spatial scales (Girard et al. 2004, Lowell et al. 2005).

Finally, epistatic gene interactions likely play a major role in genetic differences in pathogenicity and transmission between *Y. pestis* isolates (Chain et al. 2004). For example, *Y. pestis* lacks O-antigens of the outer lipopolysaccharides, which are virulence factors in *Y. pseudotuberculosis*. This loss is adaptive because O-antigens interfere with the activity of the plasminogen activator *pla* (**Table 2**), a critical factor for transport of bacteria within hosts (Kukkonen et al. 2004). These kinds of gene interactions could limit or generally promote evolution of new transmission pathways.

WITHIN-HOST DYNAMICS

The within-host level presents the greater challenge for understanding pathogen transmission, as the diversity of mechanisms used by pathogens to invade and subdue hosts is staggering. Discovery of each new pathogen serves to highlight the underlying biotic diversity remaining to be uncovered. For example, the recent emergence of SARS from the wild game markets of Guangdong province in southern China includes genetic intermediates between coronaviruses in bats, civet cats in wild game markets, and humans: It appears that SARS jumped to humans after evolution in civet cats, not directly from bats to humans (Wang et al. 2006). Pathogen diversity, however, points to the great opportunities for selection within hosts, and that pathogenesis and within-host dynamics represent adaptive radiations in the most classical sense.

Cell and tissue invasion and/or persistence within hosts is mediated by different genetic and biochemical mechanisms in different types of pathogens: For example, viruses recognize specific surface cell receptors for binding and entering host cells; bacteria have adhesion mechanisms for cell and tissue invasion; and Protozoa such as the *Plasmodium* sp. that cause malaria express variable surface proteins to prevent vertebrate immune systems from consistently recognizing them during persistent infections. The within-host equation has two sides: rates of pathogen replication versus rates of clearance by innate and adaptive immune responses of hosts. The constant exposure of potential hosts to microbes is usually thwarted by outer coverings of organisms: skin, cuticle, and mucus membranes (Levin & Antia 2001). Unless pathogens can persist closer to the outside, a premium exists for pathogens to make contact with cells and tissues with access to deeper tissues and host resources. A good example is *Staphylococcus aureus*, which invades epithelia at the anterior nares of humans and contacts lymphoid tissues of tonsils and adenoids. The related *S. epidermidis*

invades squamous cell epithelia on skin. A big difference between them is that *S. aureus* circumvents both innate and immune responses of hosts, whereas *S. epidermidis* lacks those genes but has a pH-altering arginine system to increase persistence in skin (Massey et al. 2006). Similar contrasts can be made between *Y. pestis* and its two relatives, *Y. pseudotuberculosis* and *Y. enterocolitica*: *Y. pestis* acquired plasmids encoding genes for flea-borne transmission, deeper invasion of the host, and acute infection resulting in septicemia and death (**Table 2**), while losing function in genes for persistence with low pathogenicity in mesenteric lymph nodes (Chain et al. 2004, Wren 2003).

Tracing within-host pathways of infection, replication, and dissemination provides the framework for understanding the opportunities for selection at this level. As before, I focus on within-host dynamics of *Y. pestis* and point to factors that influence both pathogenicity and transmission (**Table 2**).

Getting In: Infectivity

The plague bacterium does not use specific cell surface receptors for infection (see sidebar, CCR5-Δ32, HIV Resistance, and Plague: Putting the Hype in Hypovirulence) but instead has four requirements for invading and replicating within mammalian hosts: gaining iron, avoiding phagocytosis, attacking macrophages, and spreading from the primary site of infection to lymph nodes, liver, and spleen, ultimately to the blood stream and septicemic infection for uptake and transmission by fleas (Sebbane et al. 2005). Plague infections present different symptoms—bubonic (swollen lymph nodes), primary septicemia (fulminating in the blood stream), or primary pneumonic in lung tissues—and each tends to arise from different transmission pathways. Bubonic plague arises mainly from bites of infected fleas, primary septicemia may arise from infected fleas but also from ingestion of infected animals by predation or cannibalism, whereas primary pneumonia is from inhalation of respiratory droplets or other fluids from infected individuals. Further, each route has a different dose-response: Flea-borne transmission requires 10^4 or more bacteria, whereas primary septicemia and pneumonic infections from direct contacts with infected hosts can

CCR5-Δ32, HIV RESISTANCE, AND PLAGUE: PUTTING THE HYPE IN HYPOVIRULENCE

Details of pathogen invasion of host cells and tissues are key to understanding their evolutionary ecology. For instance, some individuals in northern Europe infected with HIV-1 do not develop AIDS because they are homozygous for a mutation in a gene coding for the cytokine receptor CCR5 on the surface of CD4+ T-cells (Dean et al. 1996). HIV-1 binds to CCR5 to infect CD4+ T-cells, eventually depleting the T-cell population, leading to collapse in immune defense and AIDS. Mutant receptors (CCR5-Δ32) are nonfunctional and prevent HIV-1 binding. The frequency of CCR5-Δ32 is 0.05 to 0.15 in northern European populations, but is absent from others (Dean et al. 2002). It is unlikely that HIV-1 selected for increased CCR5-Δ32 within the single generation since its emergence. Alternatively, it has been suggested that the high frequency of CCR5-Δ32 resulted from selection for resistance to plague since the Black Death in medieval times (Galvani & Slatkin 2003). But other evidence does not neatly fit the plague hypotheses: In mice, *Y. pestis* infection is the same in $CCR5^-$ and $CCR5^+$ strains (Mecsas et al. 2004), several other nonfunctional CCR5 alleles are also in high frequency in human populations (Blanpain et al. 2000), and the CCR5-Δ32 allele has been recovered from European skeletons from the Bronze Age (2900 BP) long before plague invaded (Hummel et al. 2005). Although bacterial disease is an unlikely cause, nonfunctional CCR5 receptors are frequent and selection via disease resistance to viruses that bind cell surface receptors (small pox?) is plausible as an explanation for their high frequency (Blanpain et al. 2000, Galvani & Slatkin 2003, Novembre et al. 2005, Sabeti et al. 2005).

begin from fewer than 10 bacteria. The differences in infectivity can be explained in part by lack of expression of mammalian virulence factors below 26°C, temperatures typical of flea vectors. The switch to a mammalian host expression profile rapidly begins when the temperature increases to 37°C (Perry & Fetherston 1997, Straley & Perry 1995). Direct contact with infected mammalian hosts permits transmission of fully virulent bacteria.

Gaining iron. As with many bacteria, iron is essential but is not readily available within mammalian hosts. Soon after invasion, *Yersiniae* synthesize an efficient iron uptake system [the siderophore, details go beyond the scope of this review (Perry & Fetherston 1997)]. The siderophore is based on the protein yersiniabactin, which has broad homology among the *Enterobacteriaceae*. Several systems in *Y. pestis* provide iron uptake after initial infection and are important in maintaining infection, for instance, within buboes (Perry & Fetherston 1997, Sebbane et al. 2006b).

Avoiding phagocytosis. Among the first cells encountered by an invading pathogen are macrophages, especially monocytes, with polymorphonuclear leukocytes (PMN) being summoned later (Sebbane et al. 2005). Bacteria attacked by PMNs are mainly destroyed early in infection, but those engulfed by monocytes begin to express virulence factors that make them resistant to further attack (Brubaker 2003, Perry & Fetherston 1997). The mechanisms for avoiding phagocytosis are the *F1* protein capsule, the outer cover that prevents macrophage adhesion, and the Ph 6 antigen, a rod-like pilus system. A second function for Ph 6 is to adhere to host cells so that the needle-like *Yops* can stab them. Without these factors, flea-transmitted infections could not proceed, but *F1* is not necessary for transmission by direct host-host contact: Strains lacking the *F1* capsule can be fully virulent if injected subcutaneously or directly transmitted via the lungs (Perry & Fetherston 1997).

Attacking macrophages. Part of plague's mystique is the apparent speedy onset of symptoms: Hosts die 24–48 h after they sicken, but 5–7 days after infection. At the molecular level, *Y. pestis* manipulates the innate immune response early during infection while transitioning from intracellular escape of macrophages to extracellular growth and destroying macrophages. *Yops*, the type III secretion system, works in conjunction with *LcrV* to: (*a*) inhibit phagocytosis, (*b*) lower production of cytokines TNF-α and IFN-γ that recruit PMN and natural killer cells, (*c*) induce production of the anti-inflammatory cytokine interleukin-10, and (*d*) eventually cause macrophage self-destruction (Brubaker 2003; Sebbane et al. 2006b). Suppressing symptoms during bacterial replication delays onset of many disease symptoms until after *Y. pestis* is bacteremic in the blood stream.

Moving from the site of primary infection. Transition of *Y. pestis* to an invasive pathogen that avoids blood clotting and tissue repair mechanisms at the point of infection is facilitated by the plasminogen activator, *pla*. The role of *pla* is to make *Y. pestis* just sticky enough, balancing adhesion of bacterial cells for invasion while allowing the bacteria to escape to deeper tissues. *Pla* cleaves fibrin and makes plasmin to break down cell junctions and basement membranes, and possibly inhibits production of interleukin-8 and the recruitment of PMNs (Perry & Fetherston 1997). Both bubonic plague from flea transmission and pneumonic plague after invasion of mucous membranes in lungs depend on *pla* (Lathem et al. 2007, Sebbane et al. 2006a).

Adaptive immunity. Adaptive immunity dramatically changes transmission dynamics, potentially halting subsequent infections because the humeral response clears pathogens before they

proliferate. This is, of course, the basis of vaccination programs, with lifelong immunity as a goal. The ability of pathogens to evade adaptive immunity is a broad topic that generates more questions than answers, although a pair of broad axes—innate versus specific immunity and constituitive versus induced expression—provides a useful framework (Schmid-Hempel & Ebert 2003). Within-host dynamics of *Y. pestis* depend on evasion of both constituitive and induced innate responses of mammalian hosts. Specific acquired immune response to plague infections seems straightforward: Survivors are usually seropositive for *F1* and *LcrV* antibodies, and a wide range of vaccines incorporating these two antigens are in development. Some rodents are resistant to plague infection (Gage & Kosoy 2005), but *Y. pestis* is highly pathogenic in most mammals and mechanisms of resistance to *Y. pestis* remain to be determined in any species.

Generally, at least five aspects of specific acquired immunity alter the ability of hosts to clear infections and create within-host selection on subsequent transmission: (*a*) life long immunity, as in measles, where subsequent outbreaks depend on a supply of newborn susceptibles (Bjørnstad et al. 2002); (*b*) chronic infections maintained via mechanisms of within-host antigenic variation, either via mutation (HIV) or recombination/reassortment (e.g., *Plasmodium*, the bacteria *Neisseria*); (*c*) transient immunity, with several serotypes circulating within populations, such as cholera (Koelle et al. 2006); (*d*) cross-immunity, so that previous exposure to pathogens with similar epitopes provides some immunity [responses may be positive or may cause disease via cytokine overreactions, such as dengue virus serotypes (Graham et al. 2005)]; and (*e*) antigenic drift between subsequent infections, either by mutation and/or reassortment between concomitantly infecting viral types, such as influenza A [new strains reinvade previously exposed populations (Ferguson et al. 2003b)].

BETWEEN-HOST DYNAMICS

Discovering transmission routes parallels the study of ecological dispersal: Where do pathogens and infected hosts move, how do they get there, and how often does transmission occur by these routes? Transmission between hosts is the product of rates of replication and dissemination from hosts, translocation between hosts, and infectivity of hosts upon contact (Lipsitch & Moxon 1997), and falls into two broad categories: horizontal and vertical (Burnet & White 1972, Ewald 1994; see **Table 1**). Horizontal transmission can occur via direct contact between infected and susceptible individuals, translocation by arthropod vectors such as mosquitoes, ticks, and fleas, or contact with an infectious environmental reservoir. At the population level, each aspect of horizontal transmission has different consequences for contact rates between hosts, and whether contacts are influenced most by the density of a population or by the frequency of infected individuals. Transmission via direct contact or by vectors will be most influenced by the frequency of infected individuals (vectors) in a population, whereas transmission from a reservoir will be influenced by overall population density (Begon et al. 2002, McCallum et al. 2001, Webb et al. 2006).

The ability of pathogens to replicate outside their primary hosts will greatly increase opportunities for transmission. Some pathogens have resting stages for environmental persistence, for example, the spores of the anthrax bacterium. In other cases, the resting states can be transported though the environment, such as oocysts of *Toxoplasma gondii* shed by domestic cats into streams and translocated to marine waters where they infect sea otters on the Pacific coast of the United States. (Conrad et al. 2005). Pathogens that amplify within an environmental reservoir have even greater potential for transmission [e.g., cholera on marine copepod exoskeletons, but see a recent consideration of additional human-human transmission (Pascual et al. 2006)].

Vertical transmission occurs between generations within a single host lineage or species, with pathogens transmitted directly from parents to offspring via reproductive cells or tissues. Thus,

studies comparing evolution of virulence under horizontal and vertical transmission are common, and virulence is expected to be lower with vertical transmission because of the potential for reduced fitness of pathogens that damage parents (Ewald 1994, Frank 1996). For example, laboratory experiments with barley stripe mosaic virus (Stewart et al. 2005) and microsporidian parasites of *Daphnia* (Vizoso & Ebert 2005) demonstrated lower virulence when transmission was vertical, and that virulence was influenced by the frequency of vertical transmission. Models suggest lowered virulence because vertical transmission evolves by increased host survival and because between-host pathogen bottlenecks result in loss of high virulence genes and lower within-host competition between pathogen strains (Bergstrom et al. 1999). Why relatively few vertically transmitted pathogens or symbionts exist is perhaps a mystery: Once the ability to infect reproductive tissues has been reached, lowered virulence would seem to quickly follow.

A classic example of the power of vertical transmission to influence pathogen/host dynamics is seen in the diverse proteobacteria *Wolbachia*, intracellular parasites of a wide array of insects and nematodes (Werren 1997, Stevens et al. 2001). *Wolbachia* increase vertical transmission by manipulating the reproductive biology of their hosts via parthenogenesis, sex ratio manipulation, and cytoplasmic incompatibility between host species (Stevens et al. 2001, Stouthamer et al. 1999, Werren 1997). *Wolbachia* are often pathogenic to their hosts, especially when horizontally transmitted, but the power of vertical transmission to influence host-pathogen dynamics is underscored by the diverse effects *Wolbachia* have on their hosts (Werren 1997) and by the discovery of other bacterial symbionts of insects with similar effects on reproduction (Zchori-Fein et al. 2004).

Vector-borne transmission is hypothesized to select for greater virulence, especially if high pathogen replication and high parasitemia (viremia or bacteremia) in circulatory systems is needed to infect vectors and to complete the life cycle (Ewald 1994). Pathogens may be transmitted mechanically, without pathogen replication, on the mouthparts of blood-feeding or plant-feeding arthropods. Transmission should be increased, however, by replication of the pathogen in the vector, called biological transmission. Pathogens such as mosquito-borne viruses or malaria are more easily transmitted when the pathogens amplify to high viremia or parasitemia in vectors. An interesting twist of biological transmission is that the microbe may be pathogenic to the vector and thus generate a cost. For example, increased transmission of malaria, *Plasmodium chabaudi*, from mice to the mosquito *Anopheles stephensi* reduces survival via higher parasite burdens in the mosquitoes (Ferguson et al. 2003a). Contrasts between mechanical and biological transmission of plague by fleas are considered below.

Multiple Transmission Routes of *Y. pestis*

The multiple routes of transmission by *Y. pestis* illustrate opportunities for selection at the between-host level, and have profound implications for the severity of disease, speed of epidemics, and persistence within plague foci (see sidebar, What Is a Plague Focus?). A worldwide feature of plague is that outbreaks in wild rodent populations are sporadic, and that plague is often undetectable in times between large-scale epizootics and high mortality (Anisimov et al. 2004, Gage & Kosoy 2005, Stapp et al. 2004). Transmission by *Y. pestis* is flea-borne,by respiratory droplets, and/or by ingestion of infected animals by either predation or cannibalism (Gage & Kosoy 2005, Perry & Fetherston 1997, Webb et al. 2006, Wilder et al. 2008). The plague bacterium lacks adaptations for saprophytic life and does not form spores for environmental persistence, so reservoirs such as those seen for anthrax, cholera, or *E. coli* are considered unlikely. Vertical transmission in mammals or fleas, via pathogen-filled blood in the feces, has not been generally demonstrated (Gage & Kosoy 2005).

Direct transmission by respiratory droplets rapidly fulminates to pneumonic plague and host death, most likely because directly transmitted bacteria already express mammalian virulence factors at 37°C (Lathem et al. 2007, Motin et al. 2004, Perry & Fetherston 1997). The lethal dose for *Y. pestis* expressing the high temperature profile is fewer than 10 bacilli and temperature dependent gene expression influences pathogenicity in mice (Perry & Fetherston 1997), along with higher expression of *Yops*, *LcrV*, and *F1* (Motin et al. 2004). However, direct transmission requires close contact, and rapid onset and death of hosts makes it unlikely that this route leads to widespread epidemics (Webb et al. 2006). The last confirmed outbreak of primary pneumonic plague in humans in the United States was in 1925 in Los Angeles. Large-scale outbreaks of pneumonic plague occurred during winter in Manchuria in 1910–1911 and 1920–1921 among miners living in cramped quarters (Pollitzer 1954).

The dominant model for flea-borne plague transmission is based on the Oriental rat flea (*Xenopsylla cheopis*), peridomestic black rats (*Rattus rattus*), and spillover to humans (Pollitzer 1954). Efficient infection of fleas requires blood meals from bacteremic mammalian hosts with more than 10^8 bacteria per milliliter of blood, whereas transmission back to a mammalian host requires several thousand bacteria being returned by biting fleas (Engelthaler et al. 2000, Lorange et al. 2005). The bites of blocked fleas, those with a mass of biofilm and bacteria filling the spiny proventriculus in the midgut, are exceptionally proficient—fleas reflux bacteria into bite wounds and feed voraciously because feeding is never to repletion. Bacteria continue to replicate within fleas (Engelthaler et al. 2000), and persistence of *Y. pestis* within fleas requires at least two specific adaptations (**Table 2**): a biofilm, whose secretion depends on the *Y. pestis HmsHFRS* operon, and protection from degradation by *Ymt*, a phospholipase D (Hinnebusch et al. 1996, 2002b; Jarrett et al. 2004). Biofilms also form in guts of the nematode *C. elegans* after experimental infection of *Y. pestis* (Darby et al. 2002, Joshua et al. 2003), and in the closely related *Y. pseudotuberculosis*. Serotypes differ in their ability to form biofilms, suggesting that preadaptation for biofilms facilitated the colonization of flea guts (Erickson et al. 2006). The roles of biofilms in transmission in blocked fleas is clear, but the possibility remains that biofilms in *Y. pestis* also function in allowing long-term persistent infections in unblocked fleas.

Biological transmission by fleas contrasts sharply with transmission by mechanical means, for instance on mouthparts, where a flea may be expected to return only a few hundred or a thousand bacteria to the next host. The short persistence time of *Y. pestis* under these conditions would lower opportunities for transmission (Webb et al. 2006, Wilder et al. 2008). But blockage cuts off the fleas' life spans by starvation, creating a trade-off between virulence and transmission in *X. cheopis*. The 4–7 day period necessary for blockage lowers the epidemic potential of plague transmitted by fleas (Webb et al. 2006), and blockage is uncommon in many of the hundreds of fleas that can also transmit *Y. pestis* (Gage & Kosoy 2005). Many flea species have been found to transmit while partially blocked, and the recent demonstration that infected fleas can efficiently transmit without blockage during the first 24–96 h after infection (Wilder et al. 2008) suggests that the trade-off between efficient transmission and blockage in fleas is not general. A mechanism that allows *Y. pestis* to remain infectious in fleas remains to be determined, with likely importance of biofilms. A potential limit on persistence in fleas is that the biofilm-forming *hmHFRS* operon has temperature-dependent expression, and at high temperatures, biofilm proteins degrade and fleas clear infections (Gage & Kosoy 2005, Perry & Fetherston 1997).

Influence on Host Behavior

Pathogen manipulation of host or vector behavior provides an opportunity for selection on transmission, and numerous examples of host behavior being altered adaptively to affect pathogen

transmission can be found (Moore 2002). As a precaution, adaptive manipulation should be distinguished from simple morbidity of infected hosts: Many infected animals change behavior simply because they are sick. For instance, there is no evidence that flea behavior has been adaptively manipulated to favor plague transmission, unless the increased feeding rate of blocked fleas may be counted as a pathogen adaptation. Alternatively, the rapid feeding could be a desperate final act. This leads to coevolutionary conflicts between *Y. pestis* and fleas: Fleas have higher clearance rates at higher temperature, so rather than feeding voraciously and favoring plague transmission, the fleas' best strategy would be to seek to cure their infections on the warmest spot in their environment or on mammalian hosts.

It has been suggested that plague could influence the social structure of their hosts, and that Asian rodents such as ground squirrels and gerbils have lower sociality than their North American relatives that only experienced plague during the past century (Biggins & Kosoy 2001). Among North American prairie dogs (*Cynomys* spp.), population dynamics of highly social black-tailed prairie dogs (*C. ludovicianus*) have changed to a classical metapopulation, with local extinctions during plague outbreaks followed by recolonization within 2–4 years (Antolin et al. 2006). The less-social white-tailed prairie dog (*C. leucurus*) has simply declined in overall abundance, without such dramatic population fluctuations (Antolin et al. 2002). Social contacts influence rates of spread of pathogens (Cross et al. 2007, Keeling & Grenfell 2000, Naug & Smith 2007, Webb et al. 2006), but whether differences in social structure provide an opportunity for selection for *Y. pestis* to alter its transmission or persistence by changing the social behavior of its hosts remains to be seen.

Climate and Transmission Cycles

Seasonality in transmission cycles is ubiquitous (Altizer et al. 2006, Harvell et al. 2002) with the possibility that pathogens evolve seasonally adapted types, as seen in cholera in Bangladesh (Koelle et al. 2006). Unfortunately, most associations between disease and seasonal or long-term climate cycles are based on correlations (Pascual & Dobson 2005) without elucidating mechanisms that could be unpacked into within- and between-host opportunities for selection. Cholera transmission is via contaminated water, and outbreaks in Bangladesh are driven by high rainfall and flooding when the El Niño Southern Oscillation (ENSO) is active. Cholera serotypes (i.e., surface antigens and immune responses to each type) change in frequency over time as well, so transmission dynamics are determined by both between-host and within-host selection. Which mode of selection mode predominates is unknown (Koelle et al. 2006).

In the relatively arid western United States, human plague cases in New Mexico and Arizona (Enscore et al. 2002) and die-offs of black-tailed prairie dogs on the Great Plains (Stapp et al. 2004) are more frequent when ENSO sparks above average precipitation: Springs are warmer and summers are cooler. Similarly, temperature and rainfall variation triggers plague in gerbils in Kazakhstan (Stenseth et al. 2006) and human plague cases in Vietnam (Cavanaugh & Marshall 1972). These climate links suggest that plague transmission changes in response to an inverted trophic cascade (Stapp 2007): Rodent populations in enzootic plague reservoirs increase (milder weather equals more food/higher rodent survival equals higher flea growth/survival and temperature-related pathogen survival), thus increasing transmission and subsequent outbreaks (Enscore et al. 2002, Collinge et al. 2005). This indirect connection has been implicated in hantavirus outbreaks in the southwestern United States (Yates et al. 2002), but evidence for the longer-term trophic cascade linking climate to plague epidemiology is generally lacking (Davis et al. 2005). A more likely mechanism is that high temperature and lower humidity directly reduce survival of fleas or bacteria. High temperature increases clearance of *Y. pestis* infections from fleas and lowers transmission (Cavanaugh 1971, Gage & Kosoy 2005, Kartman & Prince 1956).

Adaptation to large-scale climate variability in *Y. pestis* could be constrained by the trade-off between microclimates in the flea (low but variable temperature) and mammal (high temperature) parts of the transmission cycle. Operons that control overall temperature dependent expression are not linked with the transmission and virulence genes (Motin et al. 2004), thus pleiotropic temperature regulation could limit responses to selection.

TRANSMISSION BETWEEN POPULATIONS

The details of transmission between populations are poorly understood. Pathogens can be detected after they invade, their spread can be measured and speed and waves of epidemics in continuous host populations can be modeled. Some spectacular large scale patterns have been traced, such as the spread of West Nile virus, influenza A virus, *Borrelia*, *Salmonella*, and *Campylobacter* along migratory flyways of birds (Reed et al. 2003). The spread of rabies also provides excellent examples including riverine barriers to spread in raccoon populations in the northeastern United States (Smith et al. 2002). However, with the exception of pathogens that affect humans (e.g., SARS, West Nile virus), agriculture (e.g., hoof and mouth virus), and conspicuous forest trees (e.g., Dutch elm disease and chestnut blight in North America), where index cases can be identified by epidemiological trace back, routes of transmission in natural populations remain elusive.

Thus we can ask, where do opportunities for selection for between-population transmission arise? A recent stochastic model by Antia et al. (2003) suggests that even small increases in R_0 below the epidemic level can lead to some transmission chains establishing in naïve populations. This suggests that the greatest opportunities for selection occur within hosts or vectors, with a premium on increased pathogen dissemination from hosts (or vectors) that successfully disperse while infected. It is here that perhaps the clearest trade-offs between virulence and transmission exist: Pathogens that damage hosts (or vectors) limit their transmission between populations.

Transmission can be studied by population genetic analyses to infer gene flow of pathogens from spatial patterns of molecular genetic variation, from within host variation to large-scale patterns (Grenfell et al. 2004). Worldwide differentiation of the obligate human gut bacterium, *Helicobacter pylori*, follows long-term historical patterns of human migration and suggests that between-population transmission has been rare (Falush et al. 2003). Distributions of genotypes of rabies virus in Canada suggests several emergences of the virus from arctic foxes into other foxes farther south (Real et al. 2005). Population genetic studies of plague on small scales suggest that outbreaks are localized, with single *Y. pestis* clones being transmitted during each epizootic (Girard et al. 2004, Lowell et al. 2005). Comparison of patterns of molecular genetic variation within hosts to patterns between hosts, and scaling up to the level of the host population, provides a framework inferring how within-host selection relates to transmission (Grenfell et al. 2004). In particular, it provides a mechanism for examining how opportunities for selection may be limited by population bottlenecks during the infection process, genetic variation generated within hosts and immune responses within hosts, relative to the standing variation in the pathogen population as a whole.

Small World Effects in a Metapopulation Setting

More interesting dynamics, and opportunities for selection, arise when populations are subdivided so that mixing between local areas is reduced. For infectious diseases, where local fade-outs may be expected, dynamics quickly resemble those seen in classical metapopulations, with local extinctions and recolonizations of subpopulations (Hess 1996, Keeling et al. 2004). In general, the issue in disease metapopulations is pathogen persistence: Transmission rates within and between

subpopulations must balance with infectious periods and the supply of susceptible hosts within subpopulations. Pathogen-host metapopulations have been considered at three levels. The first is the within-host infrapopulation. In terms of responses to selection, this level should follow the same within-host dynamics of infection, replication, and dissemination discussed above. Evolutionary processes will ensue within these infrapopulations if they include multiple infections and a sexually reproductive stage, or for viruses and bacteria if infections persist within hosts long enough for mutations or novel recombination to arise (Grenfell et al. 2004).

The second level is where hosts are subdivided and where pathogens become locally extinct. Prevaccination measles in Great Britain provides one of the best-studied cases, where the measles virus goes extinct within small towns after all susceptible individuals are infected and develop immunity (Keeling et al. 2004). In this case, selection for persistence in small populations is weak because measles are able to persist in the larger populations in cities. Local extinction implies that rates of colonization of local host populations, between-population transmission, has to be greater than the local extinction rate, with dynamics much like free-living organisms in classical metapopulations. Models suggest that selection will act strongly on latent and infectious periods, with a premium on maintaining infections to span between-population dispersal by infected hosts or arthropod vectors (Cross et al. 2007).

The third level is where both host and pathogen become locally extinct, so that both the pathogen and host undergo metapopulation dynamics. This is the situation for the epidemiology of plague as described for prairie dogs in North America (Antolin et al. 2006, Snäll et al. 2008) and gerbils in Kazakhstan (Davis et al. 2007). Theory suggests that pathogens can persist in metapopulations if pathogens have other hosts that may act as local reservoirs (Gog et al. 2002). For plague foci, whether regional persistence of this highly virulent pathogen depends on other hosts acting as reservoirs, or whether the spatial arrangement of host patches creates the spatial and temporal heterogeneity that would allow persistence remains to be seen (Antolin et al. 2006, Gage & Kosoy 2005). The two alternatives would have dramatically different effects on transmission rates: In the case of reservoirs, transmission could remain low but steady, but in the second case, persistence would depend on recolonization of host patches occurring at a greater rate than the sum of between-population transmission and local (epizootic) R_0.

TRANSMISSION AND VIRULENCE CAN BE SEPARATE OBJECTS OF SELECTION

Finally, much research focuses on the evolution of virulence in pathogens, with a focus on potential evolutionary conflicts between high virulence and transmission rates, and that highly virulent pathogens will ultimately reduce R_0 and pathogen fitness. In its simplest form, the trade-offs imply that virulent pathogens will reduce the chance of transmission because of damage to hosts (Ewald 1994). But the universality of the trade-off has been questioned on both theoretical and empirical grounds (Dybdahl & Storfer 2003, Ebert & Bull 2003, Frank 1996, Ganusov 2003, Ganusov & Antia 2003, MacKinnon & Read 2004). Two examples illustrate the problem. First, trade-offs will be strongest where host damage (virulence) has the greatest effect in slowing transmission (Ganusov & Antia 2003). For instance, the bacterium *Neisseria meningitidis* is genetically variable and shows greater variation in transmissibility than virulence (Taha et al. 2002). *Neisseria* most commonly causes low-virulence throat and nose infections in humans, but occasionally also infects other tissues such as the spinal cord where they cause severe encephalitis. However, transmission is only from the nasopharyngeal infections, and depends on capsule switching and immune escape of new types, which arise by transformation and recombination between serotypes in hosts with

multiple infections. No trade-off between virulence and transmission occurs because virulence in the spinal cord is coincidental and unrelated to transmission (cf. Ganusov 2003).

Second, pathogens with high virulence and transmission can persist in subdivided populations, as long as between-population transmission is high enough to invade new populations before the infected local population becomes extinct (Boots et al. 2003). In particular, higher virulence is expected for pathogens infecting subdivided populations with the possibility of long-distance transmission (perhaps by vectors) because the pathogen escapes local populations that have evolved defenses. This may explain the high virulence of plague, as discussed above, and suggests that pathogens transported long distance by modern human travel may remain virulent, as seen in rabbit hemorrhagic disease transported to Asia from Europe (Boots et al. 2003).

In the end, the evolutionary ecology of pathogen transmission is best understood via opportunities for selection unpacked along pathways from within-host replication and dissemination to long-distance movement of pathogens between populations. Evaluating which level of selection provides the greatest opportunity will help predict how transmission routes may evolve. But the prediction will also depend on understanding the impact on pathogen fitness and whether sufficient genetic variation exists for evolutionary response. Details of pathogenesis within hosts tell us whether transmission will incur costly trade-offs, or whether transmission and pathogenesis are unlinked.

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