Interim Report IR-10-027

Ecological factors driving the long-term evolution of influenza’s host range
Sarah Cobey (scobey@hsph.harvard.edu)
Mercedes Pascual (pascual@umich.edu)
Ulf Dieckmann (dieckmann@iiasa.ac.at)

Approved by
Detlof Von Winterfeldt
Director
July 2011

Interim Reports on work of the International Institute for Applied Systems Analysis receive only limited review. Views or opinions expressed herein do not necessarily represent those of the Institute, its National Member Organizations, or other organizations supporting the work.
Ecological factors driving the long-term evolution
of influenza’s host range

Sarah Cobey¹*, Mercedes Pascual¹,², and Ulf Dieckmann³

¹Department of Ecology and Evolutionary Biology, 830 North University Avenue,
University of Michigan, Ann Arbor, Michigan 48109, USA
²Howard Hughes Medical Institute, USA
³Evolution and Ecology Program, International Institute for Applied Systems Analysis
(IIDSA), Schlossplatz 1, A-2361 Laxenburg, Austria

*Author and present address for correspondence: Center for Communicable Disease
Dynamics, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA
02115, USA (scobey@hsph.harvard.edu)
Abstract

The evolution of a pathogen’s host range is shaped by the ecology of its hosts and by the physiological traits that determine host specificity. For many pathogen traits there is a tradeoff: a phenotype suitable for infecting one set of hosts poorly infects another. Introducing and analyzing a simple evo-epidemiological model, here we study how such a tradeoff is expected to affect evolution of the host ranges of influenza viruses. We examine a quantitative trait underlying host specificity, given by an influenza virus’s degree of adaptation to certain conformations of sialic acid receptors, and investigate how this receptor preference evolves in a minimal network of host species, including humans, that differ in life history and receptor physiology. Using adaptive dynamics theory, we establish thresholds in interspecific transmission rates and host population sizes that govern the emergence and persistence of human-adapted viruses. These ecological thresholds turn out to be largely independent of the strength of the evolutionary tradeoff, underscoring the importance of ecological conditions in determining a disease’s host range.

Keywords: influenza; host range; adaptive dynamics; emerging infectious diseases
1. INTRODUCTION

Several challenges complicate the task of predicting evolution. One is the presence of evolutionary constraints: It may not be possible to optimize two phenotypic traits simultaneously, because a high value in one trait rules out high values in the other.

Another problem concerns attainability: Evolutionary pathways may lead through regions of low fitness or, if mutations interact epistatically, may be difficult to map to phenotypes in the first place. Yet another class of problems arises from the environment or ecology in which evolution occurs: The fitness of a trait may be frequency-dependent, being influenced by the phenotypes of other individuals. Fitness can also be affected by population size, spatial interactions, and extrinsic factors, and these relationships can be nonlinear and dynamic.

Predicting evolution of host ranges in pathogens requires confronting several of these problems at once. Many pathogens show adaptations to specific host or tissue types and are unable to infect other hosts or tissues without undergoing extensive adaptation (Baranowski et al. 2001; Webby et al. 2004). Such adaptation often comes at the expense of the ability to infect an original host type, and thus presents an evolutionary constraint in the form of a tradeoff. Pathogens tend to undergo extreme changes in population size during the same period in which rapid evolution occurs. Host immunity and host demography furthermore often impose frequency-dependent selection.

Given this complexity, it is not surprising that there is little general theory for the evolution of host ranges in pathogens. This is unfortunate, considering the ubiquity of zoonoses: Most pathogens of humans infect at least one other species (Woolhouse &
Gowtage-Sequeria (2005). Existing models address host range indirectly. For example, Parker (2003) used optimization principles to show how parasitic helminths may expand their host range through trophic transmission to acquire complex life cycles. Gandon (2004) developed predictions for the evolution of virulence and transmission in a multihost environment. Some insights might also be gained by interpreting host range as a resource-choice problem for pathogens. In Levins’s (1962) classic approach, consumers are predicted to specialize under strong tradeoffs and to adopt generalist strategies when tradeoffs are weak. His model, like Parker’s, assumes that the optimal strategy will prevail. When selection is frequency-dependent, however, optimization principles are likely to give qualitatively incorrect predictions (Dieckmann et al. 2002; Egas et al. 2004; Koelle et al. 2005).

Our goal in this study is to develop basic predictions for the evolution of influenza’s host range. Host range here refers to the specificity and diversity of pathogens in the host community. We choose influenza because of its importance to the health of animal populations and its interesting constraints and ecology. At the same time, the methods of analysis presented here are general and might be of interest also with regard to many other pathogens. Our analysis focuses on how host ecology and a tradeoff in host specialization are expected to influence evolutionary outcomes in the long run. We do not consider the mechanistic details of evolutionary attainability here, since the genotype-to-phenotype maps relevant to influenza’s host range are poorly known (Baigent & McCauley 2003). Like Levins’s approach, ours ignores environmental variation, such as seasonality, and assumes that viral population dynamics roughly equilibrate between successful invasions of pathogen strategies. These simplifications allow us to obtain
general results about the structure of host ranges in a heterogeneous host environment, when adaptation is restricted by a single evolutionary constraint. We find that (i) specialists are favored for a broad range of both weak and strong tradeoffs, (ii) the scope for specialist coexistence sensitively depends on interspecific transmission rates and host population sizes, whereas (iii) these dependencies are only weakly affected by tradeoff strength.

2. BACKGROUND

The host range of many viruses is constrained by cell recognition (Baranowski et al. 2001). Influenza viruses all bind to cell-surface oligosaccharides with a terminal sialic acid. Sialic acids fall into one of two general types of conformations: the Neu5Acα(2,3)-Gal linkage or the Neu5Acα(2,6)-Gal linkage. The intestinal and/or respiratory epithelia of waterfowl, horses, and dogs contain mainly cells with α2,3-linked sialic acids, whereas the upper respiratory epithelia of cats and humans are dominated by α2,6-linked sialic acid receptors (Baigent & McCauley 2003). Pigs, the alleged “mixing vessels” of influenza viruses (Webster et al. 1992), contain both types of receptors in their respiratory tracts (Scholtissek et al. 1998). Chickens also possess both types of receptors (Gambaryan et al. 2002). Experiments have shown that most viruses cannot replicate in host tissue of dissimilar receptor type, and viruses preferring one receptor type can often sustain some replication in any host possessing that type, even if they are adapted to another species (e.g., Ito et al. 1999; Kida et al. 1994). Thus, the chemistry of receptor binding creates a tradeoff between the ability of influenza viruses to invade cells of one type or the other.
The distribution of α2,3- and α2,6-linked receptors in the host community presents an interesting evolutionary challenge: In a population of diverse potential hosts, under what circumstances will viruses evolve new receptor preferences? The emergence of avian influenza subtype H5N1 in humans has been ascribed to high interspecific mixing in backyard farms, large population sizes in the expanding commercial poultry industry, and the presence of intermediate hosts (pigs or chickens) that serve as ecological and evolutionary bridges between waterfowl and humans (Bulaga et al. 2003; Liu et al. 2003; Webster 2004; Webster & Hulse 2004). How easily could α2,6-adapted mutant viruses invade in these different environments, and would they be able to coexist in the long run with α2,3-adapted resident viruses?

Here we analyze how the host range of influenza changes with tradeoff strength in a simple evo-epidemiological model in which influenza viruses can adapt their receptor preference. We first assume that host species are epidemiologically equivalent except for their receptor types. Subsequently, we adopt more realistic assumptions and explore how the evolutionary dynamics of influenza viruses are modulated by two major components of influenza’s ecology, interspecific transmission rates and the relative abundances of different host species.

3. METHODS

(a) Epidemiological dynamics

We consider a community with three host populations. One population, with abundance $N_r$, represents the waterfowl reservoir and has only α2,3-receptors. Another population, with abundance $N_t$, represents the “target” population (e.g., cats or humans) and has only
α2,6-receptors. The third population, with abundance $N_m$, represents intermediate hosts such as pigs and chickens that possess both receptor types. We assume there are contacts between the reservoir and intermediate hosts ($N_r$ and $N_m$) and between the intermediate and target hosts ($N_m$ and $N_t$), but not between the reservoir and the target hosts (figure 1a).

Whether a contact between infected and susceptible host individuals results in transmission of the influenza virus depends on the host’s receptor type and the virus’s receptor preference $p$. We define $p$ as the virus’s probability of infecting via an α2,6-receptor; a perfect α2,6-specialist thus has $p = P(\alpha 2,6) = 1$. In our model, the virus’s probability of infecting via an α2,3-receptor, $P(\alpha 2,3)$, is related to $P(\alpha 2,6)$ through a tradeoff with strength $s$ (Egas et al. 2004),

$$P(\alpha 2,3)^{1/s} + P(\alpha 2,6)^{1/s} = 1.$$ (1)

This tradeoff can be tuned to be weak ($s < 1$) or strong ($s > 1$). For later reference, we introduce three broad categories of viral phenotypes: α2,6-specialists, α2,3-specialists, and generalists. We consider an α2,6-specialist to have a low degree of specialization if $0.5 < P(\alpha 2,6) - P(\alpha 2,3) < 0.8$ and a high degree of specialization if $P(\alpha 2,6) - P(\alpha 2,3) \geq 0.8$. The criteria for α2,3-specialization are analogous. A virus is considered adapted to a receptor if it is specialized to that receptor. Generalist preferences comprise the remaining cases, $|P(\alpha 2,6) - P(\alpha 2,3)| \leq 0.5$ (figure 1b).

Epidemiological dynamics follow the susceptible-infected-recovered-susceptible (SIRS) model. The transition of a host from recovered to susceptible indirectly captures
two kinds of processes, the replenishment of susceptible hosts via births and deaths and
the loss of immunity due to antigenic evolution by the pathogen. Our model represents
these dynamics by six ordinary differential equations. The equations follow the rates
dS/dt and dI/dt at which the abundances of susceptible and infected hosts change in each
of the three host populations. Since we assume constant population sizes, the rates dR/dt
at which the number of recovered hosts changes in each of the three host populations
follow from those equations. For each host in population i = r (“reservoir”), m
(“intermediate”), t (“target”), the rate of susceptible replenishment is given by γᵢ, the rate
of infection by λᵢ, and the rate of recovery by νᵢ. Below we explicitly show the equations
for each state of the intermediate host,

\[
\frac{dS}{dt} = \gamma_m R_m - \lambda_m S_m \quad , \quad (2a)
\]
\[
\frac{dI}{dt} = \lambda_m S_m - \nu_m I_m \quad , \quad (2b)
\]
\[
\frac{dR}{dt} = \nu_m I_m - \gamma_m R_m \quad . \quad (2c)
\]

The force of infection in the intermediate host, λₘ, equals the sum of the per
capita rates of acquiring infections from contacts with infected members of all host
populations, \( \lambda_m = \lambda_{mr} + \lambda_{mm} + \lambda_{mt} \). We initially assume that transmission rates are
frequency-dependent (Keeling & Rohani 2007). This leads to the following form of the
transmission term, illustrated here for the rate of new infections in the intermediate host
caused by contact with reservoir hosts,
\[ \lambda_{mr}S_m = \max[P(\alpha2,3),P(\alpha2,6)]\beta_{mr}\left(\frac{c_{mr}S_m}{N_r + c_{mr}N_m}\right)I_r, \]  

(3)

where \( \beta_{ij} \) is the baseline rate at which an infected individual in host population \( j \) transmits infection to a susceptible individual in host population \( i \). The transmission rate \( \beta_{ij} \) takes into account physical and behavioral differences between the host populations that affect the likelihood of infection given a contact. The effective transmission rate between two different populations is further modified by the appropriate receptor probability [in equation (3), \( \max[P(\alpha2,3),P(\alpha2,6)] \)], and the fraction of contacted hosts that are susceptible [in equation (3), \( \frac{c_{mr}S_m}{N_r + c_{mr}N_m} \)]. To specify this susceptible fraction, we introduce \( c_{mr} \), the ratio of the probabilities per unit time of inter-population (between intermediate and reservoir hosts) and intra-population (among reservoir hosts) contact.

The denominator, \( N_r + c_{mr}N_m \), is thus proportional to the expected total number of hosts contacted by an infected reservoir host during a given time period, and the numerator, \( c_{mr}S_m \), is proportional to the expected number of susceptible intermediate hosts contacted by an infected reservoir host during the same time period.

For simplicity, we initially assume \( c_{ij} = c_{ji} = c \), before relaxing this assumption later. Under this assumption, \( c \) controls the degree of mixing between host populations.

For \( c = 0 \), all contacts occur within the separate host populations. In this situation, if \( S_r/N_r \approx 1 \), the effective transmission rate equals the baseline rate \( \beta_{in} \) and no contacts are potentially wasted on hosts in other populations. The case \( c = 1 \) implies free mixing between reservoir and intermediate hosts and between intermediate and target hosts. As \( c \)
approaches infinity, the effective transmission rate between host populations $i$ and $j$
eq 1), and the effective transmission rate within host populations drops to zero. A more restrictive interpretation of our parameterization is that $c_{ij}$ represents the fraction of population $j$ in the range of population $i$, implying $c \in [0,1]$

$c_{ij}$ can also be interpreted as the integrated product of the spatial frequency distributions for hosts $i$ and $j$. We further assume that the between-population transmission rates $\beta_{ij}$ equal the average of the two corresponding within-population transmission rates,

$$\beta_{ij} = \beta_{ji} = \frac{\beta_{ii} + \beta_{jj}}{2}.$$ (4)

Extending these conventions to infections arising from contacts with infected hosts from all three host populations, we obtain,

$$\lambda_m = \max[P(\alpha 2, 3), P(\alpha 2, 6)] \left( \frac{\beta_{ir}c_{ir}I_r}{N_r + c_{ir}N_m} + \frac{\beta_{mr}I_m}{c_{mr}N_r + N_m + c_{mr}N_t} + \frac{\beta_{mr}c_{mr}I_t}{c_{mr}N_m + N_t} \right).$$ (5)

Equations for the other host populations are analogous (equations S1 and S2). As equation (5) illustrates, in our model infection of the intermediate host occurs via the receptor type to which the infecting virus is better adapted. By modeling all mortality implicitly in the rate of susceptible replenishment, our model assumes that infections are acute and do not kill hosts, and that natural mortality acts only on recovered hosts.
(b) Evolutionary dynamics

To model the evolution of host range, we test the ability of a mutant virus with receptor preference $p_1$ to invade a community of hosts infected with a resident virus of receptor preference $p_2$. To constrain the problem, we assume that in each host population, the resident virus has reached its endemic equilibrium, and that the ability of the mutant to invade the resident is given by its instantaneous growth rate when rare in the environment determined by the resident. This growth rate, also known as the mutant’s invasion fitness in the resident’s environment (Metz et al. 1992), is given by the dominant eigenvalue of the Jacobian of the rare mutant’s epidemiological dynamics (electronic supplementary material). The endemic equilibrium and the dominant eigenvalue are calculated numerically, since both are determined by polynomial equations of orders in excess of four.

By determining the growth rate of every possible mutant phenotype against every possible resident phenotype, we obtain pairwise invasibility plots (PIPs). PIPs show which phenotypes are uninvasible once attained and which phenotypes can be attained through the succession of small and advantageous mutational steps. The former phenotypes are called evolutionarily stable, the latter convergence stable. Our assumptions and approach are an application of the theory of adaptive dynamics (Dieckmann & Law 1996; Geritz et al. 1998; Metz et al. 1996).
4. RESULTS

(a) Effects of tradeoff strength in a neutral host ecology

We first examine how host range evolves when the host populations are epidemiologically equivalent in every respect but their receptors: hosts share the same population sizes and rates of contact, recovery, and susceptible replenishment, but their receptors vary. For simplicity, we assume $c = 1$, implying free mixing between reservoir and intermediate hosts and between intermediate and target hosts.

For very weak tradeoffs ($s \leq 0.5$ in figure 2a), a complicated dynamic emerges. The PIPs show two strategies that are both evolutionarily and convergence stable, but only locally. Which strategies are realized depends on the phenotype of the initial resident and on the mutational step size. For $s = 0.5$, starting from a perfect $\alpha_{2,3}$-specialist (i.e., from a resident with $p = 0$), mutants that are slightly better adapted to the target host than the residents can invade up to $p \approx 0.23$ (where $P(\alpha_{2,3}) \approx 0.97$). If mutations are always small, this resident, which shows a low degree of $\alpha_{2,3}$-specialization, will persist indefinitely. However, there is evidence that in some subtypes of influenza viruses, single mutations can effect large changes in receptor binding. If mutations are large, mutants with sufficiently high $p$ can still invade when tradeoffs are very weak. At $s = 0.5$, invasions by mutants with very high $p$ leads to a resident strategy at $p \approx 0.97$ (where $P(\alpha_{2,3}) \approx 0.23$, corresponding to low $\alpha_{2,6}$-specialization). This other attractor is also locally evolutionarily and convergence stable.

As the tradeoff strengthens, the two local attractors disappear, and only the repellor previously separating them remains. The two perfect specialists (at $p = 0$ and $p = 1$) thus become evolutionary end points. If mutational step sizes are small, only one
perfect specialist will arise from a given starting condition. For example, if $s = 0.75$, a
resident starting at $p = 0.5$ can be progressively invaded by mutants with smaller $p$ until
arriving at perfect $\alpha_{2,3}$-specialization. As before, which specialist appears depends on
the phenotype of the initial resident. Figure 2 also shows that if mutational step sizes are
large, a mutant better adapted to $\alpha_{2,6}$-receptors (i.e., with $P(\alpha_{2,6})$ above $\approx 0.7$) can
invade a perfect $\alpha_{2,3}$-specialist and evolve increasing $\alpha_{2,6}$-specialization, and vice versa.

Assuming that large mutations can occur and that multiple specialists are able to
arise, will they coexist? Reflecting the plots about their main diagonal reveals areas of
mutual invasibility, or protected dimorphic coexistence: both the mutant and the resident
have positive invasion fitness in the environment of the other type. Evaluating the
selection gradient in the regions of coexistence shows whether this coexistence is
transient or evolutionarily stable. When the tradeoff is very weak ($s = 0.05, 0.25$, and
$0.5$), we see the basins of attraction for the equilibria described previously (figure 2b). In
addition, we find a third attractor within the region of coexistence that is also locally
evolutionarily stable. This kind of attractor is sometimes referred to as a singular
coalition (Geritz et al. 1998). At $s = 0.5$, this attractor occurs where one resident is highly
$\alpha_{2,6}$-specialized and the other is highly $\alpha_{2,3}$-specialized. For stronger tradeoffs ($s = 0.75$
and above), this attractor is absent, and perfect specialists can coexist as evolutionary end
points.

In summary, if large mutations are possible, a neutral ecology almost always
gives rise to pairs of specialists that are able to coexist in the long run; generalists only
appear when the tradeoff is extremely weak ($s = 0.05$). These results appear robust for
reasonable variations in ecological parameters (figures S1 and S2). Our analysis up to this

point reveals additional features of the evolution of host range in this system. First, PIPs are not anti-symmetric, that is, they are not invariant under reflection about the main diagonal and the subsequent exchange of signs. This demonstrates that selection for receptor preference is frequency-dependent (Meszéna et al. 2001). Second, evolutionary branching, the endogenous generation of two different phenotypes from a single phenotype through frequency-dependent disruptive selection (Metz et al. 1992; Geritz et al. 1998), cannot occur in this system for a wide range of plausible ecological parameters (electronic supplementary material). Third, once tradeoff strength increases to the point that perfect specialists are evolutionary end points, further increases in tradeoff strength have virtually no effect on the invasion potential of strong α2,6-specialists.

(b) Effects of host ecology

We now explore how a range of relevant ecological features affect our results. First, we allow hosts to vary in their rates of contact, recovery, and loss of infectiousness. Second, we investigate a modified version of our model that might better capture the dynamics of fecal-oral and aerosol transmission between and within the reservoir and intermediate hosts. Third, we examine the effects of two possible long-term intervention strategies, changing the sizes of intermediate and target hosts and the degree of mixing between different host populations.

(i) Differences in host demography and epidemiology

Natural host populations differ not only in their receptors but also in their demographic and epidemiologic rates. We therefore investigate two main features of host populations,
the rate $\gamma$ at which susceptible hosts are replenished and the pathogen’s basic reproduction ratio $R_0$ in each host population.

The rate $\gamma$ in equations (2a) and (2c) approximates the net effects of birth, death, immigration, emigration, and loss of immunity. We choose relatively high values of $\gamma$ (1/3 and 1/6 months$^{-1}$, respectively) for reservoir and intermediate hosts, implying that a recovered individual will, on average, be replaced every three or six months by a susceptible host. In the intermediate hosts, such replacement mainly occurs through culling or sale. In the reservoir hosts, it occurs mainly through loss of immunity and migration. We initially assume that $\gamma$ is approximately fourfold smaller (1/2 y$^{-1}$) in the target hosts. This choice reflects influenza’s relatively fast antigenic evolution in humans, the longer lifespan of the target population, and a high rate of immigration and emigration events.

Better estimates are available for the epidemiologic rates of transmission and recovery in influenza’s different host populations (table S1). A standard measure of a pathogen’s fitness in a population is its basic reproduction ratio $R_0$, which measures the expected total number of secondary infections caused by a primary infection in an otherwise fully susceptible host population. For a perfect specialist in a population of intermediate hosts with $c = 1$, the total number of secondary cases in its own population is $R_{0,m\rightarrow m} = \beta_{mm}/\nu_m$. Our parameters yield $R_0$ values that are highest for reservoir hosts ($R_{0,t\rightarrow r} = 4$ for a perfect $\alpha_2,3$-specialist), lowest for target hosts ($R_{0,t\rightarrow t} = 1.5$ for a perfect $\alpha_2,6$-specialist), and intermediate for intermediate hosts ($R_{0,m\rightarrow m} = 1.75$ for either perfect
specialist). These choices of $R_0$ and $\gamma$ allow the highest disease prevalence to be reached in reservoir hosts and the highest levels of immunity in target hosts.

Changing the demography and epidemiology of the different host populations predictably breaks the symmetry in evolutionary outcomes. In general, if mixing is complete ($c = 1$) and the tradeoff is not especially weak ($s$ larger than $\approx 0.25$), perfect $\alpha_{2,3}$-specialists tend to dominate: they are the evolutionary end point from the majority of starting conditions, assuming small mutational step sizes (figures S3-S5). Even if large mutations are possible, $\alpha_{2,6}$-specialists often cannot invade perfect $\alpha_{2,3}$-specialists, or such invasion is feasible only for perfect or nearly perfect $\alpha_{2,6}$-specialists. This restriction on $\alpha_{2,6}$-specialist invasion is much more sensitive to differences in $R_0$ among host populations than to the rates $\gamma$ of susceptible replenishment (figures S3, S4).

(ii) Density-dependent transmission

In wild waterfowl, influenza viruses appear to be transmitted predominantly by the fecal-oral route via contamination of shared water sources. Water is presumably also the route by which they infect domesticated animals, including pigs and chickens. Pigs and chickens generally crowd at high densities and permit aerosol transmission (electronic supplementary material). To test the robustness of our conclusions, we now assume that transmission rates under waterborne and aerosol transmission in reservoir and intermediate hosts scale more closely with the abundances than with the frequencies of infected hosts, resulting in density-dependent transmission (Keeling & Rohani 2007). In contrast, aerosol transmission involving the target hosts is better represented by
frequency-dependent transmission, as transmission rates between target and intermediate hosts quickly saturate with respect to population size.

A modified version of our model thus assumes density-dependent transmission within and between reservoir and intermediate hosts, and frequency-dependent transmission within target hosts and between target and intermediate hosts. We also distinguish the amount of mixing between reservoir and intermediate hosts \( (c_1) \) from that between intermediate and target hosts \( (c_2) \). Analogous to equation (2d), the force of infection for the intermediate host is then

\[
\lambda_m = \max[P(\alpha 2, 3), P(\alpha 2, 6)] \left( \beta_{mr} c_1 I_t + \beta_{mm} I_m + \frac{\beta_{mr} c_2 I_t}{c_2 N_m + N_t} \right).
\]

The shift from frequency-dependent to density-dependent transmission requires a change in the value and dimensions of \( \beta_{ij} \) for \( i,j \in \{m,r\} \). We choose \( \beta_{ij} \) so that the initial growth rates in each host are identical to the frequency-dependent case with \( N_t = N_m = 100 \) individuals. We assume that transmission is limited by the abundance of viruses in, and contact opportunities of, infecting hosts, and thus let the transmission rates equal those of the infecting host population: \( \beta_{tm} = \beta_{mn} \) and \( \beta_{mr} = \beta_{tr} \). For simplicity, we also assume that the transmission rate between intermediate and target hosts equals that within the target population: \( \beta_n = \beta_m = \beta_{nt} \). A complete description of this model version is provided by equations (S3) to (S5) (electronic supplementary material). We now explore the consequences of this varied form of transmission in the context of possible intervention strategies.
(iii) *Sizes of intermediate and target host populations*

The abundances of the intermediate and target hosts have nonlinear effects on the ability of $\alpha_{2,6}$-specialists to invade perfect $\alpha_{2,3}$-specialists. In general, increasing the size of the intermediate host population diminishes the ability of $\alpha_{2,6}$-specialists to invade when perfect $\alpha_{2,3}$-specialists are endemic. In contrast, increasing the size of the target host population improves the ability of $\alpha_{2,6}$-adapted viruses to invade. These patterns hold for our frequency-dependent and density-dependent models, and also for neutral and non-neutral host ecologies (figures S6-S9).

There are notable quantitative differences in the evolutionary outcomes resulting from the two different transmission modes. Unsurprisingly, frequency-dependent transmission attenuates the effects of increasing abundances. In otherwise neutral host ecologies, even when the population of intermediate hosts is twice as large as the population of target hosts, invasion by $\alpha_{2,6}$-adapted viruses with a low degree of specialization is still possible when perfect $\alpha_{2,3}$-specialists are resident (figure S6a).

Similarly, invasion by $\alpha_{2,6}$-specialists is still possible when the population of target hosts is roughly a fifth as large as those of the other hosts (figure S8). In an otherwise neutral host ecology, density-dependent transmission between reservoir and intermediate hosts also permits $\alpha_{2,6}$-invasion when intermediate host abundance is quite high (figure S6b). In contrast, differences in $R_0$ and $\gamma$ among host populations greatly restrict the population sizes allowing $\alpha_{2,6}$-invasion (figures S7 and S9). For intermediate tradeoff strengths (e.g., $s = 0.75$ and $s = 1$), $\alpha_{2,6}$-specialists cannot invade and coexist if the size of target host population is lower than those of the other host populations, or if the size of
intermediate host population exceeds those of the other host populations. Remarkably,

the sizes of target and intermediate host populations that form the threshold for the

invasion of α2,6-specialists do not change substantially as tradeoff strength varies from s

= 0.25 to s = 1.5.

(iv) Contacts among host populations

It is interesting to ask whether an intervention that reduces c₁ (the degree of mixing

between reservoir and intermediate hosts) has a greater effect on host-range evolution

than one that reduces c₂ (the degree of mixing between the intermediate and target hosts).

We find that the ability of α2,6-specialists to invade and coexist with α2,3-specialists

increases as transmission rates among host populations decline. This result holds when

parameters c₁ and c₂ are considered under density-dependent transmission in either

neutral or non-neutral host ecologies (figures S11 and S12). It also holds under

frequency-dependent transmission when c₁ and c₂ are varied together (figure S10).

Nonetheless, a neutral host ecology permits invasion of viruses with a low degree of

α2,6-specialization even when contacts between hosts from different populations are

roughly as likely as those between hosts in the same population. Under more realistic

host ecologies, opportunities are much more restricted (figures S11b and S12b). For all

but the weakest tradeoffs, an increase in c₁ will quickly limit the invasion potential of

α2,6-adapted viruses. A greater increase in c₂ is necessary to cause the same effect.
5. DISCUSSION

We have shown how the evolution of host range, predicated on a single tradeoff, can be shaped by frequency-dependent selection, tradeoff strength, transmission mode, and host ecology. As expected, very weak tradeoffs favor generalist strategies. Unexpectedly, however, weak tradeoffs can promote the evolution and coexistence of viral phenotypes specialized on alternative receptor types, assuming large mutations are possible. In that case, both host ecology and tradeoff strength nonlinearly affect the ability of $\alpha_{2,6}$-adapted mutants to invade when $\alpha_{2,3}$-specialists are resident. The invasion of $\alpha_{2,6}$-adapted viruses is facilitated by low inter-population transmission rates, low abundances of intermediate hosts, and high abundances of target hosts (figure 3). Interestingly, these conditions are relatively insensitive to tradeoff strength. Except at extremely weak tradeoffs, epidemiological coexistence implies evolutionary coexistence; if perfect specialists cannot coexist evolutionarily, extremely well-adapted specialists can.

Tradeoff strength varies among influenza viruses. Viable intermediate phenotypes with dual receptor functionality have been reported for some subtypes but not for others. Matrosovich et al. (2001) identified a lineage of H9N2 from wild aquatic birds and poultry that retained relatively high binding affinity for both avian $\alpha_{2,3}$- and porcine $\alpha_{2,6}$-receptors. Likewise, some avian-adapted H2N2 viruses from 1957 show a weak tradeoff in binding to $\alpha_{2,3}$- and $\alpha_{2,6}$-receptors, which might have allowed them to gain a foothold in the human or pig population and then undergo further adaptations to $\alpha_{2,6}$-receptor types (Liu et al. 2009). In contrast, strains of H1N1 and H3N2 from humans and pigs often show only weak affinity for $\alpha_{2,3}$-sialosides, and exhibit a complete change in receptor preference resulting from only a few amino-acid substitutions (Matrosovich et
al. 2000). Our model predicts that weak tradeoffs should allow invasion of less well
adapted types (e.g., H2N2), and also that subtypes with higher tradeoff strengths would
more readily give rise to the long-term coexistence of specialists. The second pattern
echoes the observation that the subtypes often found circulating in pigs and humans
(H1N1 and H3N2) show affinity either to \( \alpha_{2,3} \) or \( \alpha_{2,6} \)-receptors, but not to both
simultaneously.

Our results lend strong support to the idea that certain host ecologies facilitate
expansions of a disease’s host range. We find that, fortunately, coexistence of specialists
is much more difficult in influenza’s natural ecology than in a neutral one. Low inter-
population transmission rates, small intermediate host populations, and large target host
populations all increase the fraction of hosts that are susceptible to \( \alpha_{2,6} \)-mutants by
limiting exposure to \( \alpha_{2,3} \)-viruses in the intermediate host. Low transmission rates
between the intermediate and target hosts (low \( c_2 \)) reduce the fraction of target hosts’
contacts with intermediate hosts, some fraction of which resist infection due to previous
exposure to \( \alpha_{2,3} \)-adapted viruses. This reduction thus opposes a potential “dilution
effect” of wasting contacts on incompetent (here, immune) hosts (Schmidt & Ostfeld
2001). While the effect of increasing the population of target hosts is unsurprising, a less
intuitive result is that large populations of intermediate hosts, by supporting increased
exchange of \( \alpha_{2,3} \)-adapted viruses with the reservoir, reduce the fraction of hosts
potentially susceptible to \( \alpha_{2,6} \)-adapted viruses. Of course, large populations of
intermediate hosts in nature could pose an increased risk for the emergence of \( \alpha_{2,6} \)-adapted viruses if host abundance correlates positively with the pathogen’s genetic
diversity. This result nonetheless underscores the major roles of immunity in the
intermediate host population and of the rates of contact between target and intermediate hosts.

Investigations of the system’s nonequilibrium dynamics could be useful. Influenza outbreaks are seasonal in most animals, and transmission rates are likely to be seasonal. If the amplitude of epidemic oscillations is sufficiently high, equilibria of viral evolution can be different from those predicted here (White et al. 2006). Adaptation is also fundamentally probabilistic. Although we established a threshold for invasion based on positive growth of a mutant when rare, negative growth rates in nature may stochastically generate chains of mutations and transmission that are long enough to allow significant adaptation and ultimately positive growth (Andre & Day 2005; Antia et al. 2003). In other words, it may be possible for α2,6-adapted viruses to gain a foothold outside the areas of positive growth in the analyses presented here.

Increasing detail on receptor specificity in different viruses will help address questions of evolutionary attainability. The tradeoff between α2,3- and α2,6-preference provides a rough approximation of patterns in relative binding ability (Gambaryan et al. 2005). Receptor binding ability is only one small, though critical, determinant of a disease’s host range (Baigent & McCauley 2003). It might be feasible to model additional adaptations indirectly as a change in tradeoff strength, which we might expect to diminish over time as compensatory mutations arise at the receptor-binding site and in other genes.

This work shows that the evolution of host range may be as sensitive to ecological considerations as it is to the physiological details of adaptation. The long-term diversity of influenza viruses, for all realistic tradeoffs, is highly sensitive to transmission rates and population sizes. Naturally or artificially acquired immunity in intermediate hosts and the
dilution of contacts among competent hosts are key to reducing the long-term ability of

440 $\alpha_{2,6}$-adapted viruses to persist.
Acknowledgements

We thank two anonymous reviewers, as well as Andrew Dobson, Casey Schneider-Mizell, Katia Koelle, and Åke Brännström for useful comments. S.C. was funded by the U.S. National Committee for IIASA, the Rackham Graduate Student Research Grant, and a NSF Graduate Research Fellowship. This work was begun while S.C. participated in the Young Scientists Summer Program at IIASA. M.P. received support from the James S. McDonnell Foundation through a Centennial Fellowship. M.P. is an investigator of the Howard Hughes Medical Institute. U.D. gratefully acknowledges support by the European Commission, the European Science Foundation, the Austrian Science Fund, the Austrian Ministry of Science and Research, and the Vienna Science and Technology Fund.
References


Figure captions

Figure 1. (a) Transmission structure of host community, highlighting receptor conformations in three host populations: reservoir hosts (waterfowl; r), intermediate hosts (pigs and chickens; m), and target hosts (humans; t). Population sizes in each class are denoted by \( N_i \) with \( i = r, m, t \). (b) Tradeoff for receptor preference. The strength of the tradeoff is given by \( s \), with \( s < 1 \) characterizing a weak tradeoff and \( s > 1 \) a strong tradeoff. Moving away from the origin, the curves correspond to \( s = 1.5, 1, 0.75, 0.5, 0.25, \) and \( 0.05 \). Colors indicate the degree of specialization on the nearby receptor: red (high specialization), orange (low specialization), and blue (negligible specialization: generalists).

Figure 2. Evolutionary outcomes in a neutral host ecology. (a) Pairwise invasibility plots for different tradeoff strengths \( s \) for \( N_t = N_m = N_r, c = 1, \beta_{rr} = \beta_{mm} = \beta_{tt} = 1/3 \text{ days}^{-1}, \nu_t = \nu_m = \nu_i = 1/6 \text{ days}^{-1}, \) and \( \gamma_t = \gamma_m = \gamma_i = 1/180 \text{ days}^{-1} \). Black (white) areas indicate where the mutant has a positive (negative) growth rate in the endemic environment determined by the resident. Gray areas indicate regions in which the resident phenotype is not viable. (b) Trait evolution plots for the pairwise invasibility plots in (a). Gray areas indicate phenotype pairs that are mutually invasible and that therefore can coexist and coevolve. Black lines are evolutionary isoclines at which the selection pressure on one phenotype vanishes. Circles correspond to evolutionary attractors if filled and to evolutionary repellors if open. Arrows show the directions, at the quadrant level, of positive selection pressures (for better readability, such arrows are shown here only for the largest bounded regions).
**Figure 3.** Conditions that permit the coexistence of perfect specialists, assuming frequency-dependent transmission, realistic ecological parameters of host populations (table S1), and a linear tradeoff \( s = 1 \). Parameter combinations that permit specialist coexistence are in gray. Coexistence is evolutionarily stable for higher tradeoffs \( s = 0.75 \) and above), but not for weaker tradeoffs; however, even at weaker tradeoffs, extremely well adapted viruses are able to coexist (see text, fig. 2). (a) Effects of the relative population size \( N_m/N_r = N_m/N_t \) of intermediate hosts and of the degree \( c_1 \) of mixing between reservoir and intermediate hosts. (b) Effects of the relative population size \( N_t/N_r = N_t/N_m \) of target hosts and of the degree \( c_2 \) of mixing between intermediate and target hosts.

**Short title for page headings:** Evolution of influenza’s host range
Figure 1

(a)

(b)
Figure 2

(a)

(b)
Figure 3

(a) degree of mixing between intermediate and target hosts

relative abundance of intermediate hosts

degree of mixing between reservoir and intermediate hosts

relative abundance of target hosts

degree of mixing between intermediate and target hosts
Electronic Supplementary Material

for

Ecological factors driving the long-term evolution
of influenza’s host range

Sarah Cobey, Mercedes Pascual, and Ulf Dieckmann

Contents

I. Equations for intermediate and target hosts for model with frequency-dependent transmission

II. The Jacobian of the model with frequency-dependent transmission

III. Default parameters (table S1)

IV. Equations for model with density-dependent transmission

V. Figures
   a. Captions
   b. Effects of tradeoff strength in a neutral ecology
      i. Figure S1 ($R_0 = 1.5$)
      ii. Figure S2 ($R_0 = 4$)
   c. Effects of host ecology
      i. Differences in host demography and epidemiology
         1. Figure S3 ($c = 1$, $R_0$ constant, varying $\gamma$)
         2. Figure S4 ($c = 1$, varying $R_0$, constant $\gamma$)
         3. Figure S5 ($c = 1$, varying both $R_0$ and $\gamma$)
      ii. Intermediate and target host population sizes
         1. Figure S6 (varying $N_m$ in a neutral ecology)
         2. Figure S7 (varying $N_m$ in a non-neutral ecology)
         3. Figure S8 (varying $N_l$ in a neutral ecology)
         4. Figure S9 (varying $N_l$ in a non-neutral ecology)
      iii. Relative rates of interspecific transmission
         1. Figure S10: Varying $c_1$ (reservoir-intermediate host) and $c_2$
            (target-intermediate host) identically when all transmission
            is frequency-dependent
         2. Figure S11: Varying $c_1$ only with density dependence
         3. Figure S12: Varying $c_2$ only with density dependence

VI. References
I. Equations for intermediate and target hosts for model with frequency-dependent transmission

SIRS equations for the population of intermediate hosts are given in the main text (eqs. 2a-c). The corresponding SIRS equations for the population of reservoir hosts are

\[
\frac{dS_i}{dt} = \gamma_i R_i - P(\alpha 2, 3) \left( \frac{\beta_{il} I_t}{N_t + cN_m} + \frac{\beta_{im} c I_m}{cN_t + N_m + cN_t} \right) S_i ,
\]  

(S1a)

\[
\frac{dI_i}{dt} = P(\alpha 2, 3) \left( \frac{\beta_{il} I_t}{N_t + cN_m} + \frac{\beta_{im} c I_m}{cN_t + N_m + cN_t} \right) S_i - \nu_i I_i ,
\]  

(S1b)

\[
\frac{dR_i}{dt} = \nu_i I_i - \gamma_i R_i .
\]  

(S1c)

Analogously, SIRS equations for the population of target hosts are

\[
\frac{dS_t}{dt} = \gamma_t R_t - P(\alpha 2, 6) \left( \frac{\beta_{it} I_i}{N_t + cN_m} + \frac{\beta_{im} c I_m}{cN_t + N_m + cN_t} \right) S_t ,
\]  

(S2a)

\[
\frac{dI_t}{dt} = P(\alpha 2, 6) \left( \frac{\beta_{it} I_i}{N_t + cN_m} + \frac{\beta_{im} c I_m}{cN_t + N_m + cN_t} \right) S_t - \nu_i I_t ,
\]  

(S2b)

\[
\frac{dR_t}{dt} = \nu_i I_t - \gamma_t R_t .
\]  

(S2c)
II. The Jacobian of the model with frequency-dependent transmission

The Jacobian matrix of a rare mutant’s epidemiological dynamics is given by

\[
J = \begin{bmatrix}
P_1(\alpha,3)\left(\frac{\beta_{i}S_i}{N_i + cN_m}\right) - v_i & P_1(\alpha,3)\left(\frac{\beta_{m}S_m}{cN_i + N_m + cN_i}\right) & 0 \\
\max\{P_1(\alpha,2,3), P_1(\alpha,2,6)\}\left(\frac{\beta_{i}S_i}{N_i + cN_m}\right) & \max\{P_1(\alpha,2,3), P_1(\alpha,2,6)\}\left(\frac{\beta_{m}S_m}{cN_i + N_m + cN_i}\right) - v_m & \max\{P_1(\alpha,2,3), P_1(\alpha,2,6)\}\left(\frac{\beta_{m}S_m}{cN_m + N_i}\right) \\
0 & P_1(\alpha,2,6)\left(\frac{\beta_{i}S_i}{cN_i + N_m + cN_i}\right) & P_1(\alpha,2,6)\left(\frac{\beta_{m}S_m}{cN_m + N_i}\right) - v_i
\end{bmatrix}
\]

\(P_1\) refers to the phenotype of the rare mutant virus. The elements \(J_{ij}\) are the instantaneous per capita rates of mutant infections spreading from infected hosts in population \(j\) to susceptible hosts in population \(i\). Host abundances at the endemic equilibrium of the resident virus are denoted by an asterisk.

III. Default parameters

We choose parameters in keeping with general observations on the relative growth rates of different influenza subtypes in different hosts (Webster et al. 1992) (Table S1):

- The rates of loss of immunity, \(\gamma_i\), are qualitative estimates based on several observations. Rates are highest in waterfowl, since they appear to have little long-term immunity to influenza. The intermediate hosts, as domesticated animals, also have relatively high turnover. Turnover rates in the target population are low due to longer host lifespans and long-lasting immunity (whose loss of immunity is here a proxy for antigenic evolution). However, we assume they are offset by relatively high host mobility (migration).
The assumption of frequent, regular contact (suitable for transmission) between intermediate hosts and target hosts such as humans, in both rural and more industrial settings, is supported by serological surveys of pigs (Brown et al. 1995; Olsen et al. 2000; Yu et al. 2007) and by observations on asymptomatic pig-farm workers (Campitelli et al. 1997; Halvorson et al. 1983; Karunakaran et al. 1983; Myers et al. 2006; Olsen et al. 2002; Sivanandan et al. 1991) and poultry workers (Koopmans et al. 2004).

Motivation for density-dependent transmission between the reservoir and intermediate host populations comes from Brown et al. (2000), Ly et al. (2007), and Alexander (2000).
Table S1. Default parameter values used in non-neutral models. Note that “individuals” in the denominator of $\beta_{rr}$ and $\beta_{mm}$ in the model with density-dependent transmission is a pseudo-unit.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_r$</td>
<td>Rate of recovery in reservoir hosts</td>
<td>1/(12 days)</td>
<td>Hulse-Post et al. (2005)</td>
</tr>
<tr>
<td>$\nu_{ma}$</td>
<td>Rate of recovery in intermediate hosts</td>
<td>1/(7 days)</td>
<td>Hinshaw et al. (1981), Brown (2000), Van der Goot et al. (2003)</td>
</tr>
<tr>
<td>$\nu_t$</td>
<td>Rate of recovery in target hosts</td>
<td>1/(6 days)</td>
<td>Leekha et al. (2007); Carrat et al. (2008)</td>
</tr>
<tr>
<td>$\gamma_r$</td>
<td>Rate of susceptible replenishment in reservoir hosts</td>
<td>1/(90 days)</td>
<td>Kida et al. (1980); Hulse-Post et al. (2005)</td>
</tr>
<tr>
<td>$\gamma_{ma}$</td>
<td>Rate of susceptible replenishment in intermediate hosts</td>
<td>1/(180 days)</td>
<td></td>
</tr>
<tr>
<td>$\gamma_t$</td>
<td>Rate of susceptible replenishment in target hosts</td>
<td>1/(730 days)</td>
<td></td>
</tr>
<tr>
<td>$c (c_1, c_2)$</td>
<td>Expected probability that a member of one host population can contact another host population (for reservoir and intermediate hosts, for intermediate and target hosts)</td>
<td>1.0 (except where explicitly varied)</td>
<td></td>
</tr>
<tr>
<td>$\beta_{rt}$</td>
<td>Transmission rate among target hosts</td>
<td>1/(4 days)</td>
<td>Saenz et al. (2006)</td>
</tr>
</tbody>
</table>

Model with frequency-dependent transmission

| $\beta_{rr}$ | Transmission rate among reservoir hosts | 1/(3 days) | |
| $\beta_{mm}$ | Transmission rate among intermediate hosts | 1/(4 days) | Saenz et al. (2006) |

Model with density-dependent transmission

| $\beta_{rr}$ | Transmission rate among reservoir hosts | 1/(300 days · individuals) | |
| $\beta_{mm}$ | Transmission rate among intermediate hosts | 1/(400 days · individuals) | Saenz et al. (2006) |
IV. Equations for model with density-dependent transmission

All parameters and variables are as defined in the main text.

**Reservoir, r**

\[
\frac{dS_r}{dt} = \gamma_r R_r - P(\alpha_{2,3}) S_r \left( \beta_r I_r + \beta_m c_1 I_m \right) \quad (S3a)
\]

\[
\frac{dI_r}{dt} = P(\alpha_{2,3}) S_r \left( \beta_r I_r + \beta_m c_1 I_m \right) - \nu_r I_r \quad (S3b)
\]

\[
\frac{dR_r}{dt} = \nu_r I_r - \gamma_r R_r \quad (S3c)
\]

**Intermediate host, m**

\[
\frac{dS_m}{dt} = \gamma_m R_m - \max[P(\alpha_{2,3}),P(\alpha_{2,6})] S_m \left( \beta_{m} c_1 I_r + \beta_{m} c_2 I_m + \frac{\beta_m c_2 I_m}{c_2 N_m + N_t} \right) \quad (S4a)
\]

\[
\frac{dI_m}{dt} = \max[P(\alpha_{2,3}),P(\alpha_{2,6})] S_m \left( \beta_{m} c_1 I_r + \beta_{m} c_2 I_m + \frac{\beta_m c_2 I_m}{c_2 N_m + N_t} \right) - \nu_m I_m \quad (S4b)
\]

\[
\frac{dR_m}{dt} = \nu_m I_m - \gamma_m R_m \quad (S4c)
\]

**Target host, t**

\[
\frac{dS_t}{dt} = \gamma_t R_t - P(\alpha_{2,6}) S_t \left( \frac{\beta_{m} c_2 I_m}{c_2 N_t + N_m} + \frac{\beta_n I_t}{N_t + c_2 N_m} \right) \quad (S5a)
\]

\[
\frac{dI_t}{dt} = P(\alpha_{2,6}) S_t \left( \frac{\beta_{m} c_2 I_m}{c_2 N_t + N_m} + \frac{\beta_n I_t}{N_t + c_2 N_m} \right) - \nu_t I_t \quad (S5b)
\]

\[
\frac{dR_t}{dt} = \nu_t I_t - \gamma_t R_t \quad (S5c)
\]
V. Figures

**Figure S1.** Pairwise invasibility (a) and trait evolution (b) plots for hosts that are identical except for their receptor preferences. Parameters are identical to figure 2, except \( v_r = v_m = v_t = 1/4.5 \text{ days}^{-1} \) (and thus intraspecific \( R_0 \) for the appropriate specialist in each species is 1.5). Gray areas in (a) indicate regions where the resident is inviable, whereas in (b) they denote regions of coexistence. In the trait evolution plots, black lines are isoclines and black circles correspond to evolutionary attractors if filled and repellors if open. Arrows show the direction at the quadrant level of selection pressure. For clarity, they are sometimes shown extending outside the plot, though phenotypes are bounded by the axes.

**Figure S2.** Pairwise invasibility (a) and trait evolution (b) plots for hosts that are identical except for their receptor preferences. Parameters are identical to figure 2, except \( v_r = v_m = v_t = 1/12 \text{ days}^{-1} \) (and thus \( R_0 \) for the appropriate specialist in each species is 4). Gray areas in (b) denote regions of coexistence. In the trait evolution plots, black lines are isoclines and black circles correspond to evolutionary attractors if filled and repellors if open. Arrows show the direction at the quadrant level of selection pressure. For clarity, they are sometimes shown extending outside the plot, though phenotypes are bounded by the axes.
Figure S3. Pairwise invasibility (a, c) and trait evolution (b, d) plots for host populations differing in their rates of susceptible replenishment $\gamma$ but not $R_0$. In all plots, $\gamma_r = 1/90$ days$^{-1}$ and $\gamma_m = 1/180$ days$^{-1}$. The intraspecific $R_0$ for all hosts is 2 ($\beta_r = \beta_mm = \beta_t = 1/3$ days$^{-1}$, $v_r = v_m = v_t = 1/6$ days$^{-1}$). Hosts have equal population sizes, populations mix freely ($c = 1$), and transmission rates are frequency-dependent. In (a) and (b), $\gamma = 1/730$ days$^{-1}$. In (c) and (d), $\gamma = 1/7300$ days$^{-1}$. Gray areas in (a) indicate regions where the resident is inviable, whereas in (b) they denote regions of coexistence. In the trait evolution plots, black lines are evolutionary isoclines and black circles correspond to evolutionary attractors if filled and repellors if open. Arrows show the direction at the quadrant level of selection pressure. For clarity, they are sometimes shown extending outside the plot, though phenotypes are bounded by the axes.

Figure S4. Pairwise invasibility (a) and trait evolution (b) plots for host populations differing in their $R_0$ but not their rate of susceptible replenishment. Here, intraspecific $R_0$ is 4 in the reservoir ($\beta_r = 1/3$ days$^{-1}$, $v_r = 1/12$ days$^{-1}$), 1.75 in the intermediate host ($\beta_mm = 1/4$ days$^{-1}$, $v_m = 1/7$ days$^{-1}$), and 1.5 in the target host ($\beta_t = 1/4$ days$^{-1}$, $v_t = 1/6$ days$^{-1}$), as in table S1. Hosts have identical population sizes and rates of susceptible replenishment ($\gamma_r = \gamma_m = \gamma_t = 1/180$ days$^{-1}$), populations mix freely ($c = 1$), and transmission rates are frequency-dependent. Gray areas in (a) indicate regions where the resident is inviable, whereas in (b) they denote regions of coexistence. In the trait evolution plots, black lines are evolutionary isoclines and black circles correspond to evolutionary attractors if filled and repellors if open. Arrows show the direction at the quadrant level of selection pressure.
**Figure S5.** Pairwise invasibility \((a, c)\) and trait evolution \((b, d)\) plots allowing both intraspecific \(R_0\) and rates of susceptible replenishment to vary among hosts. Parameters are the same as those used for figure S4, except where noted, and rates of susceptible replenishment are the same ones used for figure S3 and listed in table S1. For \((c)\) and \((d)\), intraspecific \(R_0\) in the reservoir \((R_0 = 2; \nu_i = 1/6 \text{ days}^{-1})\) is lower than in \((a)\) and \((b)\), though in both cases it is still higher than in the intermediate \((R_0 = 1.75)\) and target hosts \((R_0 = 1.5)\). Gray areas in \((a)\) indicate regions where the resident is inviable, whereas in \((b)\) they denote regions of coexistence. In the trait evolution plots, black lines are evolutionary isoclines and black circles correspond to evolutionary attractors if filled and repellors if open. Arrows show the direction at the quadrant level of selection pressure.

**Figure S6.** Coexistence plots showing the effects of changing intermediate host abundance in a neutral ecology, assuming \((a)\) frequency-dependent and \((b)\) density-dependent transmission. Ecological parameters are the same as those used in figure 2 (for all hosts, \(R_0 = 2\) and \(\gamma = 1/180 \text{ days}^{-1}\)). Pairwise invasibility and trait evolution plots corresponding to where \(N_m = N_i = N_f\) with frequency-dependent transmission are shown in figure 2. Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S7.** Coexistence plots showing the effects of changing intermediate host abundance in a non-neutral ecology, assuming \((a)\) frequency-dependent and \((b)\) density-dependent transmission. Ecological parameters are the same as those used in table S1.
Pairwise invasibility and trait evolution plots corresponding to where $N_m = N_t = N_r$ with frequency-dependent transmission are shown in figure S5(a, b). Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S8.** Coexistence plots showing the effects of changing target host abundance in a neutral ecology, assuming frequency-dependent transmission. Ecological parameters are the same as those used in figure 2 (for all hosts, $R_0 = 2$ and $\gamma = 1/180$ days$^{-1}$). Pairwise invasibility and trait evolution plots corresponding to where $N_t = N_m = N_r$ with frequency-dependent transmission are shown in figure 2. Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S9.** Effects of changing target host abundance in a non-neutral ecology, assuming frequency-dependent transmission. Ecological parameters are the same as those used in table S1. Pairwise invasibility and trait evolution plots corresponding to where $N_t = N_m = N_r$ with frequency-dependent transmission are shown in figure S5(a,b). Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S10.** Coexistence plots showing effects of changing the degree of mixing between populations ($c = c_1 = c_2$) when all transmission rates are frequency-dependent. Coexistence plots are shown for (a) neutral and (b) non-neutral ecologies. Pairwise invasibility and trait evolution plots corresponding to the case where $c = 1$ are shown in figure 2 and figure S5(a, b), respectively, assuming frequency-dependent transmission.
Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S11.** Coexistence plots showing effects of changing only the scaling on rates of interspecific transmission between the reservoir and intermediate host populations ($c_1$) in (a) neutral and (b) non-neutral ecologies, assuming density-dependent transmission between the reservoir and intermediate host populations. Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.

**Figure S12.** Coexistence plots showing effects of changing only the degree of mixing between the intermediate and target populations ($c_2$) in (a) neutral and (b) non-neutral ecologies, assuming density-dependent transmission between the reservoir and intermediate host populations. Plus signs indicate areas of coexistence, which correspond to the gray regions of trait evolution plots.
Figure S1

(a) $s = 0.05$ $s = 0.25$ $s = 0.5$

(b) $s = 0.05$ $s = 0.25$ $s = 0.5$
Figure S2

(a) $s = 0.05$

(b) $s = 0.25$

(c) $s = 0.5$

(d) $s = 0.75$

(e) $s = 1$

(f) $s = 1.5$

(mutant phenotype vs. resident phenotype)

(resident 2 phenotype vs. resident 1 phenotype)
Figure S3

(a) $s = 0.05$  $s = 0.25$  $s = 0.5$

(b) $s = 0.05$  $s = 0.25$  $s = 0.5$
Figure S3 (continued)
Figure S4

(a) $s = 0.05$ $s = 0.25$ $s = 0.5$

(b) $s = 0.05$ $s = 0.25$ $s = 0.5$
Figure S5

(a) $s = 0.05$  

(b) $s = 0.05$  

resident 2 phenotype  

resident 1 phenotype  

resident phenotype  

mutant phenotype  

$s = 0.25$  

$s = 0.5$  

$s = 0.75$  

$s = 1$  

$s = 1.5$
Figure S6

(a) Region of inviability

(b) Relative intermediate host abundance

(s = 0.05, s = 0.25, s = 0.5, s = 0.75, s = 1, s = 1.5)
Figure S7

(a) $\delta = 0.05$  \hspace{1cm} $\delta = 0.25$  \hspace{1cm} $\delta = 0.5$

(b) $\delta = 0.05$  \hspace{1cm} $\delta = 0.25$  \hspace{1cm} $\delta = 0.5$

region of inviability

relative intermediate host abundance

resident 1 phenotype

resident 2 phenotype
Figure S8
Figure S9
Figure S10

(a) $s = 0.05$  

(b) $s = 0.05$
Figure S11

(a) $s = 0.05$ | $s = 0.25$ | $s = 0.5$

(b) $s = 0.05$ | $s = 0.25$ | $s = 0.5$

Legend:
- 0.0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0
- degree of mixing

responder 2 phenotype vs. responder 1 phenotype
Figure S12
VI. References


at increased risk of infection with zoonotic influenza virus? Clinical Infectious Diseases 42, 14-20.


Van der Goot, J. A., de Jong, M. C. M., Koch, G. & van Boven, M. 2003 Comparison of the transmission characteristics of low and high pathogenicity avian influenza A virus (H5N2). Epidemiology and Infection 131, 1003-1013.
