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

Sarah Cobey[1,*], Mercedes Pascual[1,2] and Ulf Dieckmann[3]

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

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 trade-off: a phenotype suitable for infecting one set of hosts poorly infects another. Introducing and analysing a simple evo-epidemiological model, here we study how such a trade-off 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 trade-off, 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: pathways of phenotypic evolution may lead through regions of low fitness or, especially if mutations interact epistatically, the genotypes required along these pathways may be unlikely or even impossible to appear. 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 trade-off. 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 *et al.* (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 multi-host 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 trade-offs and to adopt generalist strategies when trade-offs 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 trade-off in host specialization are expected to influence

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

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 favoured for a broad range of both weak and strong trade-offs, (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 trade-off 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. Kida *et al.* 1994; Ito *et al.* 1999). Thus, the chemistry of receptor binding creates a trade-off 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 analyse how the host range of influenza changes with trade-off 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. MATERIAL AND 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 $p = P(\alpha2,6) = 1$. In our model, the virus's probability of infecting via an α2,3-receptor, $P(\alpha2,3)$, is related to $P(\alpha2,6)$ through a trade-off with strength s (Egas *et al.* 2004),

$$P(\alpha2,3)^{1/s} + P(\alpha2,6)^{1/s} = 1. \tag{3.1}$$

This trade-off 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 $0.5 < P(\alpha2,6) - P(\alpha2,3) < 0.8$ and a high degree of specialization if $P(\alpha2,6) - P(\alpha2,3) \geq 0.8$. 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, $|P(\alpha2,6) - P(\alpha2,3)| \leq 0.5$ (figure 1b).

Epidemiological dynamics follow the susceptible–infected–recovered–susceptible 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 owing 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 γ_i, the rate of infection by λ_i and the rate of recovery by ν_i. Below, we explicitly show the equations for each state of the intermediate host,

$$\frac{dS_m}{dt} = \gamma_m R_m - \lambda_m S_m, \tag{3.2a}$$

$$\frac{dI_m}{dt} = \lambda_m S_m - \nu_m I_m \tag{3.2b}$$

and $$\frac{dR_m}{dt} = \nu_m I_m - \gamma_m R_m. \tag{3.2c}$$

The force of infection in the intermediate host, λ_m, equals the sum of the *per capita* rates of acquiring infections from
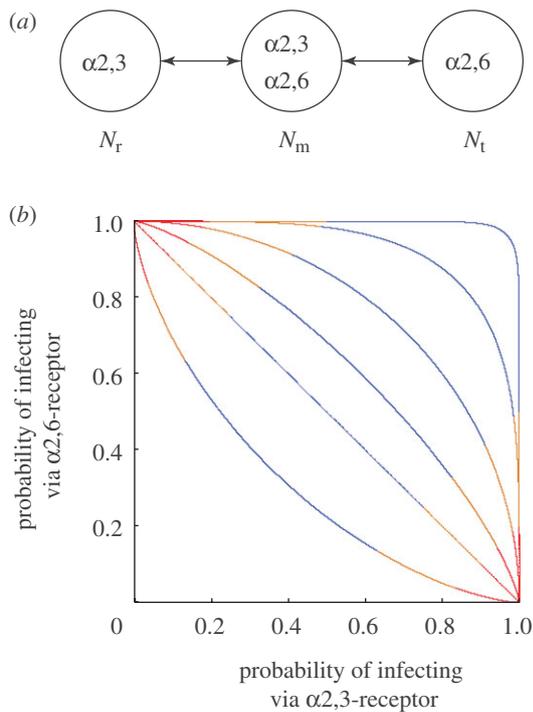
Figure 1. (*a*) Transmission structure of host community, highlighting receptor conformations in three host populations: reservoir hosts (waterfowl; r), intermediate hosts (pigs and chickens; m) and target hosts (humans; t). Population sizes in each class are denoted by N_i, with $i =$ r, m, t. (*b*) Trade-off for receptor preference. The strength of the trade-off is given by s, with $s < 1$ characterizing a weak trade-off and $s > 1$ a strong trade-off. Moving away from the origin, the curves correspond to $s = 1.5, 1, 0.75, 0.5, 0.25$ and 0.05. Colours indicate the degree of specialization on the nearby receptor: red (high specialization), orange (low specialization) and blue (negligible specialization: generalists).

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,

$$\lambda_{mr} S_m = \max[P(\alpha 2, 3), P(\alpha 2, 6)]\beta_{mr}\left(\frac{c_{mr} S_m}{N_r + c_{mr} N_m}\right) I_r,$$
$$(3.3)$$

where β_{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 β_{ij} takes into account physical and behavioural 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 (3.3), $\max[P(\alpha 2, 3), P(\alpha 2, 6)]$), and the fraction of contacted hosts that are susceptible (in equation (3.3), $(c_{mr} S_m/(N_r + c_{mr} N_m))$). To specify this susceptible fraction, we introduce c_{mr}, the ratio of the probabilities per unit time of interpopulation (between intermediate and reservoir hosts) and intrapopulation (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 $S_i/N_i \approx 1$, the effective transmission rate equals the baseline rate β_{ii}, 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 equals β_{ij} (again assuming $S_i/N_i \approx 1$), and the effective transmission rate within host populations drops to zero. A more restrictive interpretation of our parametrization 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 β_{ij} equal the average of the two corresponding within-population transmission rates,

$$\beta_{ij} = \beta_{ji} = \frac{\beta_{ii} + \beta_{jj}}{2}. \qquad (3.4)$$

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

$$\lambda_m = \max[P(\alpha 2, 3), P(\alpha 2, 6)]$$
$$\times \left(\frac{\beta_{mr} c_{mr} I_r}{N_r + c_{mr} N_m} + \frac{\beta_{mm} I_m}{c_{mr} N_r + N_m + c_{mt} N_t} + \frac{\beta_{mt} c_{mt} I_t}{c_{mt} N_m + N_t}\right). \qquad (3.5)$$

Equations for the other host populations are analogous (electronic supplementary material, equations (S1) and (S2)). As equation (3.5) illustrates, in our model, infection of the intermediate host occurs via the receptor type to which the infecting virus is better adapted. By modelling 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 (see the 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; Metz *et al.* 1996; Geritz *et al.* 1998).

4. RESULTS

(a) *Effects of trade-off 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 trade-offs ($s \leq 0.5$ 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 trade-offs 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 trade-off 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 endpoints. 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 ≈ 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 trade-off 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 trade-offs ($s = 0.75$ and above), this attractor is absent, and perfect specialists can coexist as evolutionary endpoints.

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 trade-off is extremely weak ($s = 0.05$). These results appear robust for reasonable variations in ecological parameters (electronic supplementary material, 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 (see the electronic supplementary material). Third, once trade-off strength increases to the point that perfect specialists are evolutionary endpoints, further increases in trade-off strength have virtually no effect on the invasion potential of strong $\alpha 2,6$-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 faecal–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 γ at which susceptible hosts are replenished and the pathogen's basic reproduction ratio R_0 in each host population.

The rate γ in equations (3.2a) and (3.2c) approximates the net effects of birth, death, immigration, emigration and loss of immunity. We choose relatively high values of γ (1/3 and 1/6 month^{-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 γ is approximately fourfold smaller (1/2 yr^{-1}) in the target hosts. This choice reflects influenza's relatively fast antigenic evolution in humans, the longer lifespan of the
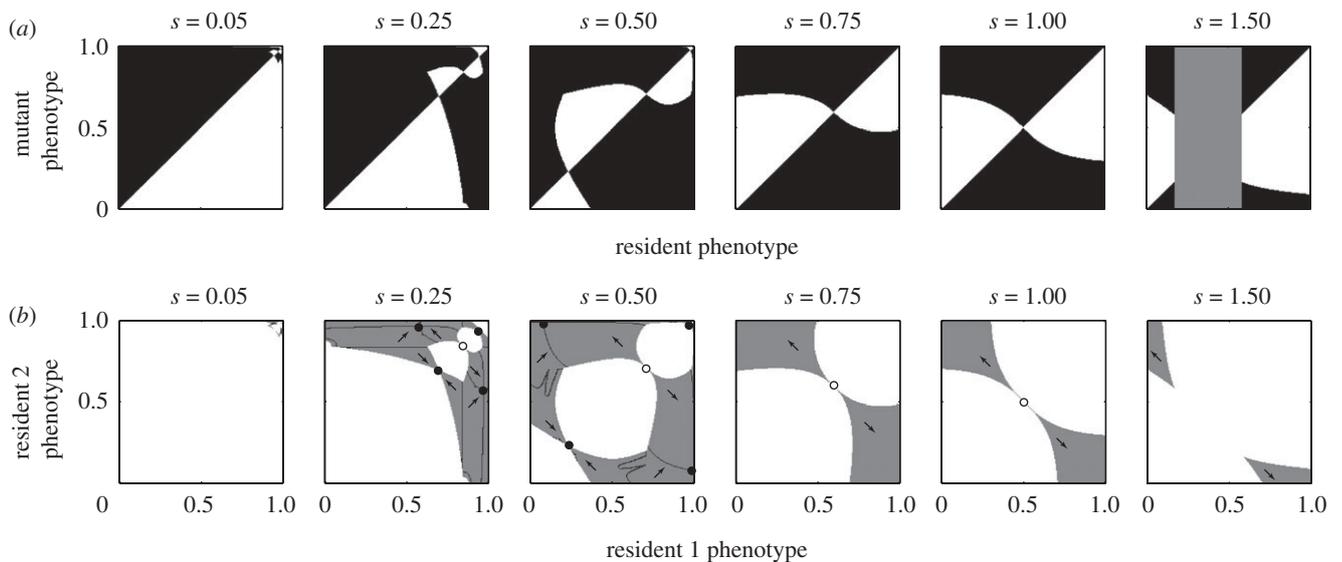
Figure 2. Evolutionary outcomes in a neutral host ecology. (*a*) PIPs for different trade-off strengths *s* for $N_t = N_m = N_r$, $c = 1$, $\beta_{rr} = \beta_{mm} = \beta_{tt} = 1/3$ day^{-1}, $\nu_r = \nu_m = \nu_t = 1/6$ day^{-1} and $\gamma_r = \gamma_m = \gamma_t = 1/180$ day^{-1}. Black (white) areas indicate where the mutant has a positive (negative) growth rate in the endemic environment determined by the resident. Grey areas indicate regions in which the resident phenotype is not viable. (*b*) Trait evolution plots for the PIPs in (*a*). Grey 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).

target population and a high rate of immigration and emigration events.

Better estimates are available for the epidemiological rates of transmission and recovery in influenza's different host populations (electronic supplementary material, 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 $R_{0,m\to m} = \beta_{mm}/\nu_m$. Our parameters yield R_0 values that are highest for reservoir hosts ($R_{0,r\to 4} = 4$ for a perfect $\alpha 2,3$-specialist), lowest for target hosts ($R_{0,t\to t} = 1.5$ for a perfect $\alpha 2,6$-specialist) and intermediate for intermediate hosts ($R_{0,m\to m} = 1.75$ for either perfect specialist). These choices of R_0 and γ 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 trade-off is not especially weak (*s* larger than ≈ 0.25), perfect $\alpha 2,3$-specialists tend to dominate: they are the evolutionary endpoint from the majority of starting conditions, assuming small mutational step sizes (electronic supplementary material, 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 γ of susceptible replenishment (electronic supplementary material, figures S3 and S4).

(ii) *Density-dependent transmission*

In wild waterfowl, influenza viruses appear to be transmitted predominantly by the faecal–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 (see the 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). By 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 (3.4), the force of infection for the intermediate host is then

$$\lambda_m = \max[P(\alpha 2, 3,), P(\alpha 2, 6)]$$
$$\times \left(\beta_{mr} c_1 I_r + \beta_{mm} I_m + \frac{\beta_{mt} c_2 I_t}{c_2 N_m + N_t} \right). \qquad (4.1)$$

The shift from frequency-dependent to density-dependent transmission requires a change in the value and dimensions of β_{ij} for $i, j \in \{m, r\}$. We choose β_{ij} so that

the initial growth rates in each host are identical to the frequency-dependent case with $N_r = N_m = 100$ 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: $\beta_{rm} = \beta_{mm}$ and $\beta_{mr} = \beta_{rr}$. For simplicity, we also assume that the transmission rate between intermediate and target hosts equals that within the target population: $\beta_{tt} = \beta_{tm} = \beta_{mt}$. A complete description of this model version is provided by the electronic supplementary material, equations (S3)–(S5). 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. By 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 (electronic supplementary material, 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 (electronic supplementary material, figure S6*a*). 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 (electronic supplementary material, 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 (electronic supplementary material, figure S6*b*). By contrast, differences in R_0 and γ among host populations greatly restrict the population sizes, allowing $\alpha 2,6$-invasion (electronic supplementary material, figures S7 and S9). For intermediate trade-off strengths (e.g. $s = 0.75$ and $s = 1$), $\alpha 2,6$-specialists cannot invade and coexist if the size of the target host population is lower than those of the other host populations, or if the size of the 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 $\alpha 2,6$-specialists do not change substantially as trade-off 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 c_1 (the degree of mixing between reservoir and intermediate hosts) has a greater effect on host-range evolution than one that reduces c_2 (the degree of mixing between the intermediate and target hosts). We find that the ability of $\alpha 2,6$-specialists to invade and coexist with $\alpha 2,3$-specialists increases as transmission rates among host populations decline. This result holds when parameters c_1 and c_2 are considered under density-dependent transmission in either neutral or non-neutral host ecologies (electronic supplementary material, figures S11 and S12). It also holds under frequency-dependent transmission when c_1 and c_2 are varied together (electronic supplementary material, figure S10). Nonetheless, a neutral host ecology permits invasion of viruses with a low degree of $\alpha 2,6$-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 (electronic supplementary material, figures S11*b* and S12*b*). For all but the weakest trade-offs, an increase in c_1 will quickly limit the invasion potential of $\alpha 2,6$-adapted viruses. A greater increase in c_2 is necessary to cause the same effect.

5. DISCUSSION

We have shown how the evolution of host range, predicated on a single trade-off, can be shaped by frequency-dependent selection, trade-off strength, transmission mode and host ecology. As expected, very weak trade-offs favour generalist strategies. Unexpectedly, however, weak trade-offs 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 trade-off 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 interpopulation transmission rates, low abundances of intermediate hosts and high abundances of target hosts (figure 3). Interestingly, these conditions are relatively insensitive to trade-off strength. Except at extremely weak trade-offs, epidemiological coexistence implies evolutionary coexistence; if perfect specialists cannot coexist evolutionarily, extremely well-adapted specialists can.

Trade-off 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 a 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 trade-off 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). By 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 trade-offs should allow invasion of less well-adapted types (e.g. H2N2), and also that subtypes with higher trade-off 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
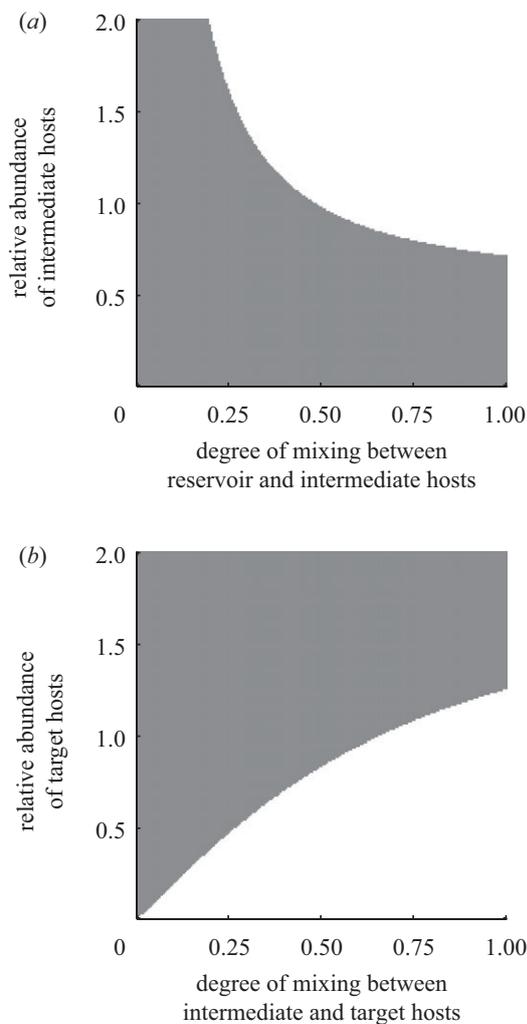
Figure 3. Conditions that permit the coexistence of perfect specialists, assuming frequency-dependent transmission, realistic ecological parameters of host populations (electronic supplementary material, table S1) and a linear trade-off ($s = 1$). Parameter combinations that permit specialist coexistence are in grey. Coexistence is evolutionarily stable for higher trade-offs ($s = 0.75$ and above), but not for weaker trade-offs; however, even at weaker trade-offs, extremely well-adapted viruses are able to coexist (see text, figure 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.

humans (H1N1 and H3N2) show affinity to either $\alpha 2,3$- 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 interpopulation 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 owing 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 non-equilibrium 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 (Antia *et al.* 2003; Andre & Day 2005). In other words, it may be possible for $\alpha 2,6$-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 trade-off between $\alpha 2,3$- and $\alpha 2,6$-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 trade-off 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 trade-offs, 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 $\alpha 2,6$-adapted viruses to persist.

REFERENCES

Andre, J. B. & Day, T. 2005 The effect of disease life history on the evolutionary emergence of novel pathogens. *Proc. R. Soc. B* **272**, 1949–1956. (doi:10.1098/rspb.2005.3170)

Antia, R., Regoes, R. R., Koella, J. C. & Bergstrom, C. T. 2003 The role of evolution in the emergence of infectious diseases. *Nature* **426**, 658–661. (doi:10.1038/nature02104)

Baigent, S. J. & McCauley, J. W. 2003 Influenza type A in humans, mammals and birds: determinants of virus virulence, host-range and interspecies transmission. *BioEssays* **25**, 657–671. (doi:10.1002/bies.10303)

Baranowski, E., Ruiz-Jarabo, C. M. & Domingo, E. 2001 Evolution of cell recognition by viruses. *Science* **292**, 1102–1105. (doi:10.1126/science.1058613)

Bulaga, L. L., Garber, L., Senne, D. A., Myers, T. J., Good, R., Wainwright, S., Trock, S. & Suarez, D. L. 2003 Epidemiologic and surveillance studies on avian influenza in live-bird markets in New York and New Jersey, 2001. *Avian Dis.* **47**, 996–1001. (doi:10.1637/0005-2086-47.s3.996)

Dieckmann, U. & Law, R. 1996 The dynamical theory of coevolution: a derivation from stochastic ecological processes. *J. Math. Biol.* **34**, 579–612. (doi:10.1007/BF02409751)

Dieckmann, U., Metz, J. A. J., Sabelis, M. W. & Sigmund, K. 2002 *Adaptive dynamics of infectious diseases. In pursuit of virulence management.* Cambridge Studies in Adaptive Dynamics. Cambridge, UK: Cambridge University Press.

Egas, M., Dieckmann, U. & Sabelis, M. W. 2004 Evolution restricts the coexistence of specialists and generalists: the role of trade-off structure. *Am. Nat.* **163**, 518–531.

Gambaryan, A. S., Yamnikova, S. S., Lvov, D. K., Robertson, J. S., Webster, R. G. & Matrosovich, M. N. 2002 Differences in receptor specificity between the influenza viruses of duck, chicken, and human. *Mol. Biol.* **36**, 429–435. (doi:10.1023/A:1016023700050)

Gambaryan, A., Yamnikova, S., Lvov, D., Tuzikov, A., Chinarev, A., Pazynina, G., Webster, R., Matrosovich, M. & Bovin, N. 2005 Receptor specificity of influenza viruses from birds and mammals: new data on involvement of the inner fragments of the carbohydrate chain. *Virology* **334**, 276–283. (doi:10.1016/j.virol.2005.02.003)

Gandon, S. 2004 Evolution of multihost parasites. *Evolution* **58**, 455–469.

Geritz, S. A. H., Kisdi, É., Meszéna, G. & Metz, J. A. J. 1998 Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* **12**, 35–57. (doi:10.1023/A:1006554906681)

Ito, T., Kawaoka, Y., Nomura, A. & Otsuki, K. 1999 Receptor specificity of influenza A viruses from sea mammals correlates with lung sialyloligosaccharides in these animals. *J. Vet. Med. Sci.* **61**, 955–958. (doi:10.1292/jvms.61.955)

Keeling, M. J. & Rohani, P. 2007 *Modeling infectious diseases in humans and animals.* Princeton, NJ: Princeton University Press.

Kida, H., Ito, T., Yasuda, J., Shimizu, Y., Itakura, C., Shortridge, K. F., Kawaoka, Y. & Webster, R. G. 1994 Potential for transmission of avian influenza-viruses to pigs. *J. Gen. Virol.* **75**, 2183–2188. (doi:10.1099/0022-1317-75-9-2183)

Koelle, K., Pascual, M. & Yunus, M. 2005 Pathogen adaptation to seasonal forcing and climate change. *Proc. R. Soc. B* **272**, 971–977. (doi:10.1098/rspb.2004.3043)

Levins, R. 1962 Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. *Am. Nat.* **96**, 361–373. (doi:10.1086/282245)

Liu, M. *et al.* 2003 The influenza virus gene pool in a poultry market in South Central China. *Virology* **305**, 267–275. (doi:10.1006/viro.2002.1762)

Liu, J. F., Stevens, D. J., Haire, L. F., Walker, P. A., Coombs, P. J., Russell, R. J., Gamblin, S. J. & Skehel, J. J. 2009 Structures of receptor complexes formed by hemagglutinins from the Asian Influenza pandemic of 1957. *Proc. Natl Acad. Sci. USA* **106**, 17 175–17 180. (doi:10.1073/pnas.0906849106)

Matrosovich, M., Tuzikov, A., Bovin, N., Gambaryan, A., Klimov, A., Castrucci, M. R., Donatelli, I. & Kawaoka, Y. 2000 Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J. Virol.* **74**, 8502–8512. (doi:10.1128/JVI.74.18.8502-8512.2000)

Matrosovich, M. N., Krauss, S. & Webster, R. G. 2001 H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. *Virology* **281**, 156–162. (doi:10.1006/viro.2000.0799)

Meszéna, G., Kisdi, É., Dieckmann, U., Geritz, S. A. H. & Metz, J. A. J. 2001 Evolutionary optimization models and matrix games in the unified perspective of adaptive dynamics. *Selection* **2**, 193–210. (doi:10.1556/Select.2.2001.1-2.14)

Metz, J. A. J., Nisbet, R. M. & Geritz, S. A. H. 1992 How should we define fitness for general ecological scenarios? *Trends Ecol. Evol.* **7**, 198–202. (doi:10.1016/0169-5347(92)90073-K)

Metz, J. A. J., Geritz, S. A. H., Meszéna, G., Jacobs, F. J. A. & Van Heerwaarden, J. S. 1996 Adaptive dynamics: a geometrical study of the consequences of nearly faithful reproduction. In *Stochastic and spatial structures of dynamical systems* (eds S. J. Van Strien & S. M. Verduyn Lunel), pp. 183–231. Amsterdam, The Netherlands: North Holland.

Parker, G. A., Chubb, J. C., Ball, M. A. & Roberts, G. N. 2003 Evolution of complex life cycles in helminth parasites. *Nature* **425**, 480–484. (doi:10.1038/nature02012)

Schmidt, K. A. & Ostfeld, R. S. 2001 Biodiversity and the dilution effect in disease ecology. *Ecology* **82**, 609–619. (doi:10.1890/0012-9658(2001)082[0609:BATDEI]2.0.CO;2)

Scholtissek, C., Hinshaw, V. S. & Olsen, C. W. 1998 Influenza in pigs and their role as the intermediate host. In *Textbook of influenza* (eds K. G. Nicholson, R. G. Webster & A. J. Hay), pp. 137–145. Malden, MA: Blackwell Science.

Webby, R., Hoffmann, E. & Webster, R. 2004 Molecular constraints to interspecies transmission of viral pathogens. *Nat. Med.* **10**, S77–S81. (doi:10.1038/nm1151)

Webster, R. G. 2004 Wet markets: a continuing source of severe acute respiratory syndrome and influenza? *Lancet* **363**, 234–236. (doi:10.1016/S0140-6736(03)15329-9)

Webster, R. G. & Hulse, D. J. 2004 Microbial adaptation and change: avian influenza. *Rev. Sci. Tech. l'Office Int. Epizooties* **23**, 453–465. (doi:10.1080/08910600500533012)

Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. & Kawaoka, Y. 1992 Evolution and ecology of influenza-A viruses. *Microbiol. Rev.* **56**, 152–179. (doi:10.1111/j.1751-0813.1992.tb09940.x)

White, A., Greenman, J. V., Benton, T. G. & Boots, M. 2006 Evolutionary behaviour in ecological systems with trade-offs and non-equilibrium population dynamics. *Evol. Ecol. Res.* **8**, 387–398.

Woolhouse, M. E. J. & Gowtage-Sequeria, S. 2005 Host range and emerging and reemerging pathogens. *Emerg. Infect. Dis.* **11**, 1842–1847.