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ORIGINAL ARTICLE

Why is HIV not vector-borne?

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Abstract

Many pathogens of humans are blood borne, including HIV, Malaria, Hepatitis B and C, West Nile virus, Dengue, and other viral hemorrhagic fevers. Although several of these pathogens are transmitted by blood-feeding arthropods, HIV is not. A number of properties of HIV and its life cycle have been identified as proximate explanations for the absence of arthropod transmission, but little consideration has been given to why HIV has not evolved this form of transmission. We consider the empirical evidence for arthropod transmission, and suggest that mechanical transmission has not evolved in HIV because such strains would induce a faster onset of AIDS during infection, which would thereby limit their ability to spread. On the other hand, it is not as clear why biological transmission has not occurred. Available data suggests that a lack of appropriate genetic variation in HIV is one explanation, but it is also possible that a conflict between natural selection occurring within and between infected individuals has prevented its evolution instead. We discuss the potential significance of these ideas, and argue that taking such an evolutionary perspective broadens our understanding of infectious diseases and the potential consequences of public health interventions.

Introduction

Approximately 40 million people are infected with the human immunodeficiency virus (HIV) worldwide (UNAIDS 2006). Infections by this lentivirus from the family *Retroviridae* are characterized by a long asymptomatic period after host immune defenses control the initial infection. During this subsequent chronic phase of infection, HIV slowly diminishes the host's immune functioning by targeting CD4+ T lymphocytes for infection. Within these cells the virus replicates by hijacking the intracellular molecular machinery to transcribe viral RNA, and eventually the productively infected cells die (Fauci 1988). Over time, the density of virions in the blood stream increases and the immune system functioning becomes progressively compromised, leaving HIV-infected individuals increasingly susceptible to opportunistic infections.

Being blood-borne, HIV is transmitted via contact with the blood of an infected individual: through transfusions, needle-sharing, sexual contact or from mother to child during childbirth or breast-feeding. Initially there were concerns that HIV might be vector-borne (e.g., transmitted via mosquitoes) but it has since become widely accepted that such transmission does not occur at any significant level (Bockarie and Paru 1996). This current belief stems both from epidemiological data and experimental studies that directly examine the potential for HIV transmission via arthropods (Lawrence 1987; Lifson 1988; Bockarie and Paru 1996).

Why is HIV not vector-borne (throughout this article we use the term 'vector' synonymously with 'arthropod')? The majority of medical scientists, when asked this question, will offer the following explanations (Bockarie and Paru 1996): (i) HIV concentrations in the blood are too low during human infection to permit vector transmission; (ii) HIV is unable to survive long enough outside of humans (or primates) for vector transmission; (iii) HIV is not able to replicate within arthropod vectors. Each of these explanations has empirical support (Lifson 1988; Bockarie and Paru 1996) and thus all three are good explanations for the lack of vector transmission in HIV. If most arthropods pick up very little HIV when feeding on humans, and if the level of HIV in (or on) these

vectors quickly decays, then no significant vector transmission is expected to occur.

The above findings provide a satisfying proximate explanation for the lack of vector transmission in HIV. *Given the current characteristics of HIV*, these three features of the virus make it unlikely that vector transmission will occur. In this article, we approach this question from an evolutionary perspective. Therefore, rather than taking the characteristics of HIV as given, we are interested in understanding why HIV has evolved these particular features and not others instead. For example: (i) why has HIV not evolved a replication strategy that results in viral concentrations high enough to allow vector transmission? (ii) why has HIV not evolved to be more durable and thus to survive longer outside of humans? (iii) why has HIV not evolved the ability to replicate in arthropod vectors?

Asking such questions might strike many readers as strange since much of evolutionary biology proceeds by asking why certain features of organisms are as we observe them rather than by asking why some features are absent. This is only natural, since it is difficult to decide which, among the infinite number of 'missing features', should be the focus of study. It is useful to view such questions as falling on a continuum, from questions about the lack of traits that are virtually non-existent in all taxa (e.g., why do dogs not have wheeled appendages) to questions about the lack of traits that are common in some populations or species but not others (e.g., why do German Shepherds not have curly hair?). Our contention is that, by asking such questions about the absence of some traits, we can gain deeper insight into biology. To quote philosopher Arthur Eddington (1927), ''…the contemplation in natural science of a wider domain than the actual leads to a far better understanding of the actual.''.

As in all science, however, not all questions are interesting, and this serves as our primary guide for focusing on some missing features and not others. In particular, we focus on the lack of vector transmission in HIV, because of the profound epidemiological significance of its absence. An arthropod-transmissible form of HIV would clearly exacerbate the already devastating impact of the disease, opening up routes of transmission to groups previously at low-risk (e.g., children). Therefore, it is worth asking why, from an evolutionary standpoint, this has not occurred (Weiss 2001). More to the point, if there *are* conditions under which HIV could have evolved vector transmission we would do well to understand these, not only from the standpoint of scientific curiosity, but also to prevent such an outcome in the future.

In this article, we address the question of why HIV lacks vector transmission, both through a consideration of available empirical data and through the construction of mathematical models. Although our results are necessarily speculative, we believe that they shed some light on the evolutionary biology of HIV, and on the evolutionary biology of blood-borne pathogens more generally.

Why is HIV not vector-borne?

There are two broad reasons why a trait of interest (in this case vector-transmission) might not evolve. First, the necessary genetic variation for the trait might arise only very rarely (if at all). For instance, the evolution of RNA viruses, such as HIV, could be strongly constrained by the size of their genome (Holmes 2003). Second, the necessary form and strength of natural selection might not be present for the trait to evolve, at least over the timescale under consideration. Thus, from an evolutionary standpoint, HIV is not vector-borne because either the necessary genetic variation for such transmission has never arisen, or the necessary selective factors that would make such variants increase in frequency over the relevant timescale have not occurred. Our evolutionary explanation for why HIV is not vector-borne will be sought within these two possibilities.

Before beginning to consider these explanations, we must be more precise about our definition of vector-transmission. There are two different processes that might result in vector-transmission, and that are often lumped under this single heading. The first is simple mechanical transmission of a pathogen by arthropods. This occurs when the arthropod acts solely as a means of physical transport of viral particles between hosts (e.g., having viral particles in and around mouthparts). The second is biological transmission. This occurs when the virus replicates within the arthropod vector during the time period between feeding events.

Genetic variation

How likely is it that HIV has not evolved vector transmission because of a lack of appropriate genetic variation? The most direct way to assess this possibility is to determine if genetic variants capable of vector transmission are currently present in the HIV population. Unfortunately, performing such an assay on all genotypes within the population would be next to impossible. Furthermore, if variants capable of vector transmission are selectively disadvantageous, then their frequency in the population might be extremely low. Nevertheless, there are some studies available that take this approach as far as is possible.

A second approach to addressing this issue is to examine the closely related viruses of HIV. If some of HIVs close relatives have evolved vector-transmission, then the

premise that genetic variation for this route of transmission in HIV does not exist would be significantly weakened. Below we review both types of evidence, for both mechanical and biological transmission.

Mechanical transmission: A number of studies working directly with HIV have assayed its ability to be transmitted mechanically by arthropods. Such transmission is often difficult to assess under natural conditions, and therefore most studies have employed artificial experimental setups, using a variety of arthropods including mosquitoes, stable flies, and Tabanids (e.g., horse flies and deer flies). Although the findings are somewhat mixed, mechanical transmission appears possible in principle. For example, HIV was found to be viable for up to 10 days in African soft ticks, and perhaps even up to 14 days (Humphrey-Smith et al. 1993; see also Humphrey-Smith and Chastel 1988). Likewise, Webb et al. 1989 found that HIV can remain infectious for up to 8 days in the gut of *Cimex hemipterus*. Most research on this topic has stressed the need for large bloodmeal size, however, because HIV tends not to have a very high viral titre during infection in humans (Lifson 1988; Webb et al. 1989; Foil and Issel 1991; Bockarie and Paru 1996). This has led to a focus on arthropods, like ticks, whose bloodmeal sizes are often 70 times larger than that of mosquitoes (Humphrey-Smith et al. 1993). It has also been suggested that squashing mosquitoes while they are feeding, and subsequently scratching the area (which might result in lacerations) could increase the likelihood of transmission as well (Siemens 1987).

Other studies of closely related viruses suggest that, in principle, there is no obvious barrier to mechanical transmission of HIV by arthropods. In fact, it has also been suggested that mechanical vector transmission might be the route through which HIV was initially transmitted to humans (Eigen et al. 2002). At least three other retroviruses can be transmitted mechanically, including Bovine leukemia virus, Friend murine leukemia virus, and equine infectious anemia virus (Foil and Issel 1991; Humphrey-Smith et al. 1993). The latter (equine infectious anemia virus) is believed to be a close relative of HIV (McClure et al. 1988), and epidemiological evidence suggests that vector transmission might play a significant role in its transmission (Foil and Issel 1991). Interestingly, however, all three of these retroviruses tend to reach viral titres in their hosts that are much higher than those typical of HIV (Foil and Issel 1991).

It has also been suggested that some Hepatitis viruses can be transmitted mechanically by arthropods (Jupp et al. 1983), although this has been controversial (Kuno 2004). Even if this does occur, however, the viral levels of HIV in humans are thought to be about 10 to 100 times lower than that of some hepatitis viruses (Foil and Issel 1991), again implicating HIVs low titre during infection of humans as the primary reason that mechanical transmission does not occur in this virus.

Biological transmission: Despite evidence that mechanical transmission of HIV by arthropods can occur, there is no evidence that biological transmission is possible. For example, although Humphrey-Smith et al. (1993) found that HIV can remain viable in ticks for up to 2 weeks, they failed to find any evidence that HIV can replicate in these vectors. Evidence has been reported of HIV-related nucleic acids being found in Tsetse flies from central Africa (Becker et al. 1986), but this has been controversial, and epidemiological evidence is not indicative of vector transmission (Noireau et al. 1987). Furthermore, experiments using cell cultures from arthropods have demonstrated that HIV is not capable of replicating in these cells (Srinivasan et al. 1987). In fact, no evidence exists to date that any retrovirus is capable of biological transmission by arthropods (Webb et al. 1989; Foil and Issel 1991; Kuno 2004; Kuno and Chang 2005). It remains unclear exactly why such replication is not possible, but functional constraints on receptor use in arthropods versus mammals provides one proximate explanation (van den Heuvel et al. 1999). Alternatively, it remains possible that appropriate genetic variation can arise, but that selection simply does not favor the spread of biological transmission in HIV or other retroviruses.

Selection

The above empirical findings suggest that, in principle, HIV is capable of being transmitted mechanically. In practice, however, the typical HIV viral titre in the bloodstream of humans is too low for significant vector-borne transmission to occur (Lifson 1988; Webb et al. 1989; Foil and Issel 1991; Bockarie and Paru 1996). There is evidence of genetic variation for differences in viremia in HIV-infected patients (Kanki et al. 1999) and this therefore suggests that mechanical transmission should be evolutionarily feasible if it were selectively advantageous. Thus, we are forced to ask: why have genetic variants of HIV that induce higher viremia, and thus that transmit mechanically via arthropods, not increased in frequency?

On the other hand, there is no evidence to suggest that biological transmission can occur, even in principle. This might be because the relevant genetic variation for such transmission has not appeared (or perhaps is not possible). Alternatively, perhaps such variation does occasionally arise, but that such strains are acted against by natural selection. In this section we use some mathematical calculations to elucidate potential reasons why selection might not favor increased viremia and thus

mechanical vector transmission in HIV. We then consider the same question for biological transmission.

Mechanical transmission: To understand the form and strength of selection shaping the evolution of HIV viremia it is helpful to quantify the important properties of HIV epidemiology in terms of a mathematical model. At present HIV infection is increasing in prevalence in the human population (UNAIDS 2006). The simplest description of this is exponential growth in the number of infected individuals;

$$\frac{dy}{dt} = ry \tag{1}$$

where $y(t)$ is the number of HIV-positive individuals at time t, and r is the per capita growth rate. Although the rate of increase of HIV appears to be slowing in recent years, exponential growth nevertheless provides a useful benchmark for its initial spread in humans.

The per capita growth rate will depend on various properties of HIV, including its mode of transmission. To derive an expression for the per capita growth rate of HIV-positive individuals in terms of underlying epidemiological parameters, we first need to specify a model for the epidemiological dynamics. This model will allow for both sexual transmission of HIV as well as insect transmission, and therefore it will also track the number of insects carrying HIV. Using $w(t)$ for the number of insects carrying HIV at time t, and $y(a,t)$ as the number of HIV-positive people who were infected a years ago, we specify the dynamics as

$$\frac{dw(t)}{dt} = vab_1 \int_0^\infty y(s,t)ds - \mu w(t) \tag{2a}$$

$$\frac{\partial y(a,t)}{\partial t} = -\frac{\partial y(a,t)}{\partial a} - \delta(a)y(a,t) \tag{2b}$$

with boundary condition $y(0,t) = xab_2w(t) + x\beta \int_0^\infty y(s,t)ds$. In equations (2), v is the population size of insects free of HIV, a is the insect-biting rate, b_1 is the probability of an insect picking up HIV, given it feeds on an infected human, and μ is the per capita loss rate of infected insects (which subsumes both insect mortality and the decay of HIV stores in or on the insect). Note that, although many insects display a characteristic time lag between feeding events, for simplicity we have ignored this. Also note that, for simplicity, equations (2) implicitly assume that the likelihood of an insect picking up HIV from an infected human is constant across all infection ages (i.e., it does not depend on a). The parameter b_2 is the probability that an insect-carrying HIV infects a human when feeding, and x is the number of HIV-negative people. We assume that the number of susceptible insects and people are both constant over the timescale of interest because HIV infection is still increasing in prevalence in the human population (UNAIDS 2006). The parameter β is the transmission rate through sexual contact of HIV, and is assumed to be independent of infection age. Our assumption is justified on the basis that we are concerned with average viremia throughout the entire infection, and viral loads during the acute infection phase are strongly correlated with viral loads during the subsequent chronic phase (Kelley et al. 2007). Results in Appendix 1 also show that the main conclusions are not altered by relaxing this assumption. Lastly, $\delta(a)$ is a function describing the mortality rate of HIV-positive people as a function of infection age. In particular, we will suppose that $\delta(a)$ has the form

$$\delta(z) = \begin{cases} \delta_0 & z<\tau \\ \infty & z \geq \tau \end{cases} \tag{3}$$

where τ is the time during the infection at which AIDS develops.

This model is analyzed in Appendix 1 to show that the asymptotic per capita rate of increase, r, is defined implicitly, as a function of various epidemiological parameters, by the equation:

$$r + \delta_0 = x\left(\frac{va^2b_1b_2}{\mu} + \beta\right)\left(1 - e^{-(r+\delta_0)\tau}\right). \tag{4}$$

Some of the parameters in equation (4) will depend on the level of viremia in humans, and therefore viremia will affect the per capita rate of spread of HIV. In particular, the amount of HIV picked up by an arthropod during a feeding, b_1, is expected to increase with viremia. Similarly, evidence suggests that sexual transmission rate, β, also increases with viremia (Operskalski et al. 1997; Tovanabutra et al. 2002; Wawer et al. 2005). Lastly, evidence also shows that high levels of viremia lead to a more rapid development of AIDS, and thus a lower value of τ in untreated patients (Mellors et al. 1997; Levy 1998; Raffanti et al. 2004).

With these specifications, our question about the evolution of mechanical transmission of HIV can now be cast in population-genetic terms. Suppose the predominant strain of HIV is one that gives rise to a viremia too low for vector transmission (i.e., $b_1 \approx 0$). Also suppose that a mutant strain arises that produces a viremia high enough for mechanical vector transmission. Assuming that multiple infections do not occur (an assumption that we relax below), the rate of change in frequency, p, of this mutant strain is (Day and Gandon 2007)

$$\frac{dp}{dt} = p(1-p)(r_B - r_A) \tag{5}$$

where r_A is the per capita growth rate of the original strain and r_B is the per capita growth rate of the mutant

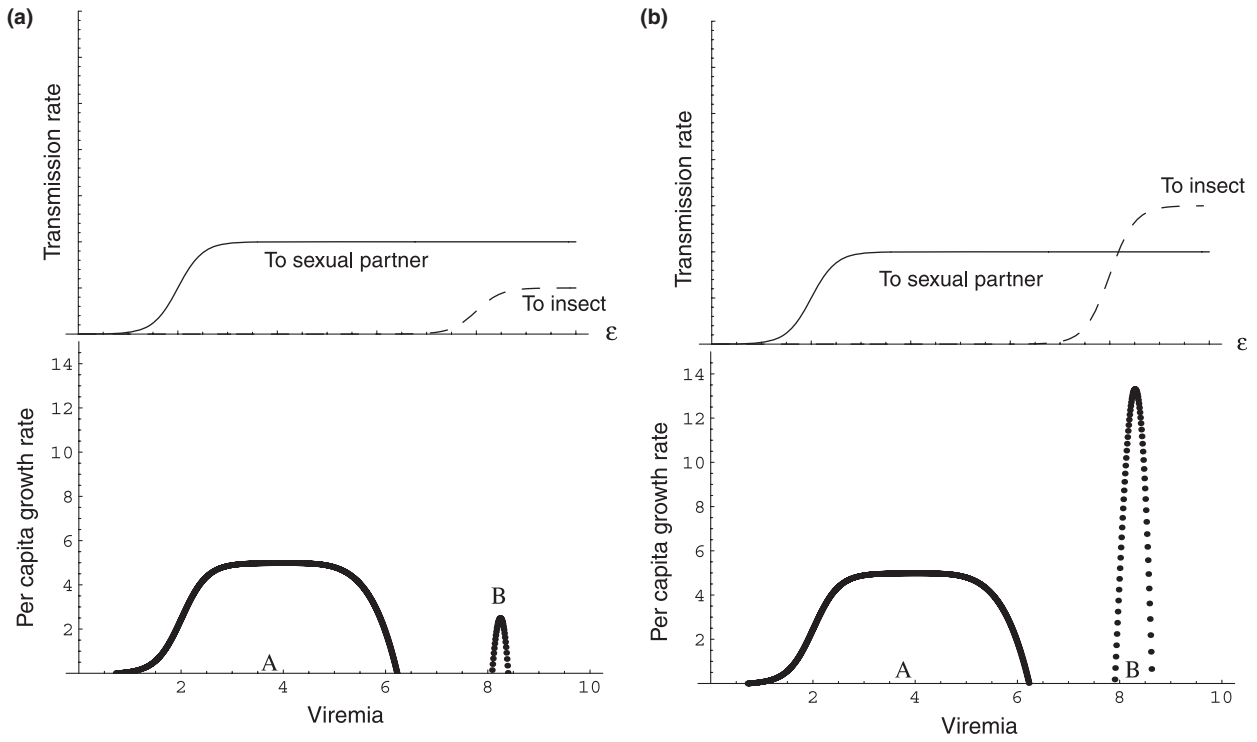

Figure 1. Sexual transmission rate (solid line) and vector transmission rate (dashed line) as a function of viremia, ϵ. The resulting per capita growth rate based on equation (4) is also plotted (growth rate is negative where is falls below the horizontal axis, meaning that such strains can never increase in number). Letters 'A' and 'B' denote the sexually transmitted and vector transmitted genotypes considered in model (5) of the text. Parameter values: $\delta_0 = 1/65$, $a = 1$, $v = 100$, $x = 5$, $\mu = 15$. Panel (a) – vector transmission is selectively disadvantageous. $b_1(\varepsilon) = \frac{1}{4}(1 + \tanh(2(\varepsilon - 8)))$, $\beta(\varepsilon) = \frac{1}{2}(1 + \tanh(2(\varepsilon - 2)))$, $b_2 = 0.945$. Figure is drawn with the growth rate of 'B' less than that of 'A' but still positive for illustrative purposes only. Negative values of r_B also readily occur with only slight changes in parameters values. Panel (b) – vector transmission is selectively advantageous. $b_1(\varepsilon) = \frac{3}{4}(1 + \tanh(2(\varepsilon - 8)))$, $\beta(\varepsilon) = \frac{1}{2}(1 + \tanh(2(\varepsilon - 2)))$, $b_2 = 1.1$.

strain. Specifically, r_A and r_B are implicitly defined, from equation (4), as

$$r_A + \delta_0 \approx x\beta\left(1 - e^{-(r_A+\delta_0)\tau_A}\right), \qquad (6a)$$

and

$$r_B + \delta_0 \approx x\left(\frac{va^2 b_1 b_2}{\mu} + \beta\right)\left(1 - e^{-(r_B+\delta_0)\tau_B}\right), \qquad (6b)$$

respectively. Equations (6) make the implicit assumption that the increased viremia of the mutant strain does not significantly increase its sexual transmission rate. More precisely, sexual transmission must be a concave function of viremia with an optimal viral load that is lower than for vector transmission for the following argument to hold. Empirical evidence in support of this functional form for HIV exists (Quinn et al. 2000; Fraser et al. 2007).

Two qualitatively distinct evolutionary outcomes are predicted depending upon parameter values (Fig. 1). First, a comparison of equations (6a) and (6b) reveals that r_B will be smaller than r_A (i.e., the mutant strain will decrease in frequency) and HIV will be predominately sexually transmitted, when the following conditions hold: vector mortality, or the HIV decay rate, is high (i.e., large μ), vector population size is small (i.e., small v), vector biting rate is small (i.e., small a), or the HIV transfer rates to and from vectors, b_1 and b_2, are small. Indeed, r_B is not only less than r_A in such situations (as in Fig. 1), but often negative as well, meaning that vector transmissible strains will not only decrease in frequency but in absolute numbers. Therefore, some or all of the above conditions must hold if this analysis is to explain why HIV has not evolved mechanical vector transmission. Conversely, if the opposite conditions hold, then the mutant will increase in frequency, and both sexual and mechanical vector transmission will play a significant role in the disease's epidemiology (Fig. 1).

These considerations provide the conditions required for the spread of a mutant with mechanical vector transmission, but they do not tell us the time frame over which such spread occurs. For example, it would be useful to know by how much arthropods must increase

the overall transmission rate of HIV if such strains are to have increased significantly in frequency during the period of time in which HIV has been evolving in humans. The calculations in Appendix 2 demonstrate that the factor by which vectors must increase the overall transmission rate of HIV, in order for the relative frequency of the mutant, $p/(1-p)$, to increase by a factor of K over a period of T years, is given by

$$\frac{1-e^{-(r_A+\delta_0)\tau_A}}{1-e^{-(\ln K+(r_A+\delta_0)T)\tau_B/T}}\left(1+\frac{\ln K}{(r_A+\delta_0)T}\right). \qquad (7)$$

All parameters in equation (7) can be estimated except K, T, and τ_B (Appendix 2), yielding Fig. 2.

First, note that changes in K (the amount of increase that must occur for evolutionary change to be deemed 'significant') have very little effect on predictions (Fig. 2). Therefore, we can view any of these curves as general requirements for significant evolution of vector transmission to have occurred during the past 30 to 50 years. Also, as expected, the curves are decreasing, reflecting the fact that a smaller benefit of vector transmission is required to generate significant evolution if evolution has longer to act. More interestingly we can see that, if the increased viremia that allows for vector transmission also results in the development of AIDS after only 5 years as opposed to 8 years, then arthropods would need to cause a doubling of HIV transmission rate for appreciable evolution to have occurred. On the other hand, if increased viremia results in the development of AIDS after only 1 year, then arthropods would need to increase HIV transmission rate by 5- or 6-fold for appreciable evolution to have occurred. Unfortunately, there are currently no estimates available of these parameters, but these calculations nevertheless suggest that significant mechanical transmission could have evolved within the last 30–50 years under biologically plausible conditions. Therefore, the lack of vector transmission in HIV cannot immediately be attributed to an insufficient evolutionary history of HIV in humans.

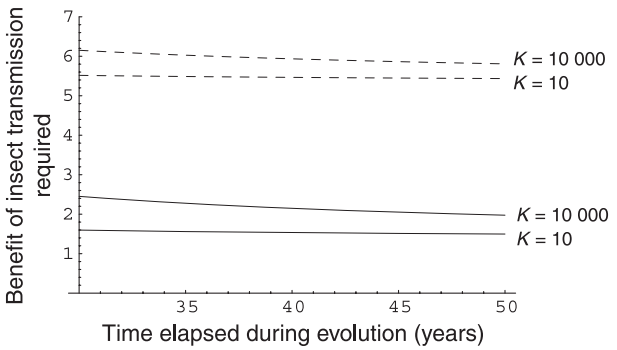

Figure 2. The factor by which vectors must increase the overall transmission rate of HIV in order for a vector transmissible virus to increase in relative frequency by a factor of K, as a function of the amount of time over which evolution occurs (between 30 and 50 years for HIV in humans). Solid lines assume that the increased viremia caused by the vector-transmissible virus decreases the time until the development of AIDS from $\tau_A = 8$ to $\tau_B = 5$ years. Dashed lines assume a reduction from $\tau_A = 8$ to $\tau_B = 1$ year. The parameter K has very little effect over several orders of magnitude, meaning that the benefit of vector transmission required for it to evolve over 30–50 years is determined largely by the value of τ_B.

Biological transmission: Although there is no evidence suggesting that the required genetic variation for biological transmission is possible in HIV (or any other retrovirus; Foil and Issel 1991; Kuno 2004; Kuno and Chang 2005; Webb et al. 1989), it is nevertheless instructive to consider whether there might also be reasons associated with the nature and strength of selection for why such transmission has not evolved.

Equation (5) can again be used in this context, with equation (4) again defining the per capita growth rate for different strains of HIV. Biological transmission need not require an increased viremia in humans, however, because the pathogen would replicate to transmissible levels once in the arthropod vector. As a result, strains that are capable of biological vector transmission need not result in the more rapid development of AIDS. Without some associated cost, however, biological vector transmission would clearly enhance the growth rate of HIV and thus would readily evolve. Thus, if an absence of such transmission is to be explained in terms of selection (as opposed to an explanation based on a lack of genetic variation) then there must be some associated cost.

There are at least two biologically plausible mechanisms through which such a cost might arise. First, effective biological vector transmission might require evolutionary changes that reduce HIVs capacity for sexual transmission. In this case, the cost stems from an evolutionary trade-off between these two transmission routes. The sexually transmitted form would have a growth rate defined by

$$r_A+\delta_0 = x\beta\left(1-e^{-(r_A+\delta_0)\tau}\right) \qquad (8a)$$

whereas the vector-transmitted form would have a growth rate defined by

$$r_B+\delta_0 = x\frac{va^2b_1b_2}{\mu}\left(1-e^{-(r_B+\delta_0)\tau}\right). \qquad (8b)$$

The parameter values in equations (8) *could* readily be such that the growth rate of the vector-transmitted strain was less than that of the sexually transmitted strain. There is, however, no *a priori* reason why this would be expected rather than the reverse. Therefore, it does not provide a very compelling answer as to why biological

transmission has not evolved in HIV, particularly given that it is absent in all other retroviruses as well.

The second way in which a cost of biological transmission might arise is through a conflict between natural selection acting on transmission of HIV between hosts, and natural selection acting on the virus' replicative capacity within a host. It is well documented in HIV (and other retroviruses) that extensive genetic variation arises within an infected host via mutation. If vector-transmissible strains suffer a cost in terms of their replicative ability within humans, then this within-host natural selection acting against vector transmission might be enough to prevent its spread.

Modeling the evolutionary consequences of this in HIV is difficult because each infected human will harbor a suite of genetic variants, some of which will be better at exploiting the human host. This will cause evolutionary change in the genetic composition of HIV within the infected individuals. At the same time, this suite of strains is also being transmitted to new hosts via sexual contact and potentially vector transmission. The assumption of the above hypothesis is then that the strains that are better able to transmit to new hosts via vector transmission are not the ones best able to compete for resources within a host.

The simplest way to abstract these processes into a tractable model that still retains the fundamental processes at work is to make an assumption of superinfection. Specifically, we suppose that humans almost always harbor only a single strain, but occasionally new strains arise by mutation. When such a mutation occurs, the mutant then either takes over the host or dies out instantaneously, resulting in a single strain infection once again (Levin and Pimentel 1981; Nowak and May 1994). In keeping with our earlier notation we will use B to denote the vector-transmissible form, and A to denote the form best able to compete within a host and thus to transmit sexually.

Letting μ be the rate at which new mutations arise within an infected host, turning either an A pathogen into a B pathogen or vice versa, and using $\sigma_{i \to j}$ to denote the probability that an i mutant so produced will take over a host originally infected with type j, where i and j are either A or B, model (5) can be extended to yield (Day and Proulx 2004; Day and Gandon 2006)

$$\frac{\mathrm{d}p}{\mathrm{d}t} = p(1-p)(r_\mathrm{B} - r_\mathrm{A}) + \mu(1-p)\sigma_{\mathrm{A}\to\mathrm{B}} - \mu p \sigma_{\mathrm{B}\to\mathrm{A}} \quad (9)$$

with r_A and r_B again given by equations (6). The hypothesis under consideration supposes that within-host competition always favors the sexually transmitted form, and thus we take $\sigma_{\mathrm{A}\to\mathrm{B}} = 0$ in equation (9). In this case, there are then two possible evolutionary outcomes. First, if $r_\mathrm{B} - r_\mathrm{A} > \mu\sigma_{\mathrm{B}\to\mathrm{A}}$, then the frequency of vector transmission will ultimately evolve to the equilibrium value $\hat{p} = 1 - (\mu\sigma_{\mathrm{B}\to\mathrm{A}}/(r_\mathrm{B} - r_\mathrm{A}))$.On the other hand, if $r_\mathrm{B} - r_\mathrm{A} < \mu\sigma_{\mathrm{B}\to\mathrm{A}}$, then vector transmission will never evolve. In other words, if the significance of within-host evolution is large relative to the benefit of vector transmission, then vector transmission will never evolve. This will be true whenever the mutation rate of the virus is high (i.e., large μ) and when the selective advantage of sexual transmission in terms of within-host competition is large. The first of these is certainly true of most retroviruses, although the second requirement is less well documented. Nevertheless, this might provide a selective explanation for why no retrovirus appears to have evolved biological vector transmission.

Discussion

HIV transmission via arthropods was a serious concern upon the discovery of this virus. Experiments and epidemiological data have unequivocally demonstrated, however, that such vector transmission does not occur at any significant level, and various aspects of HIV biology have been implicated as proximate reasons (Bockarie and Paru 1996). These reasons do not offer an explanation for why vector transmission has not evolved, however, and as Weiss (2001) points out, we ought to seriously consider whether such evolution might occur in the future (for a summary, see Table 1).

Existing data suggest that the lack of mechanical vector transmission in HIV is not due to genetic constraints. While ecological constraints, such as number of vectors and biting rates, may limit vector transmission in certain areas, these constraints would likely not explain why HIV has not evolved this form of transmission in areas where vector-borne diseases (e.g. malaria) are endemic. Rather, there must presumably be a reason why such transmission is selectively disadvantageous in HIV. The calculations presented above offer one possibility. Effective mechanical vector transmission can be brought about only through the evolution of higher levels of viremia, and this also results in a more rapid onset of AIDS. This reduces the duration over which such strains can be transmitted from an infected human, more than is made up for by the occurrence of vector transmission. It also remains possible that insufficient time has elapsed for the evolution of vector transmission to occur, but our calculations suggest that this is not a very compelling possibility.

On the other hand, existing data is largely consistent with the hypothesis that biological vector transmission has not evolved in HIV because of genetic constraints. At the same time, it is not possible to rule out a selective explanation instead. In particular, if there is a genetic

Table 1. Main conclusions of the study

		Evidence of capability of HIV for vector transmission	Evidence of vector transmission in related viruses	Evidence of genetic constraint	Why a lack of vector transmission?
Mechanism of vector transmission	Mechanical; vector acts solely as a means of physical transport of viral particles	HIV remains viable for considerable time in ticks (Humphrey-Smith and Chastel 1988; Humphrey-Smith et al. 1993) and *C. hemipterus* (Webb et al. 1989).	In Bovine leukemia virus, Friend murine leukemia virus, equine infectious anemia virus (Foil and Issel 1991; Humphrey-Smith et al. 1993).	Data not consistent with a genetic constraint.	Selectively disadvantageous since it requires higher levels of viremia, resulting in faster onset of AIDS.
	Biological; virus replicates within the vector	Little evidence of replication within potential vectors (Srinivasan et al. 1987).	No evidence (Foil and Issel 1991; Kuno 2004; Kuno and Chang 2005; Webb et al. 1989)	Data is consistent with a genetic constraint.	Genetic trade-off between replication in human host and insect vector.

trade-off between efficient replication in humans and replication in arthropod vectors, then a conflict between selection favoring effective replication within humans, and selection favoring arthropod transmission between humans can readily prevent biological transmission from evolving. This is particularly likely when the mutation rate of the virus is high, and thus might provide an explanation for the lack of biological vector transmission in all retroviruses.

Our analysis might also be extended to include other forms of transmission, for instance needle transmission (see Bruneau et al. 1997 for the efficiency of needle exchange programs). Several evolutionary consequences of this are possible depending both on the level of viremia required for such transmission to occur and the resulting transmission rate. For instance, if needle transmission can be achieved with a lower viremia than sexual transmission, and if this leads to a sufficiently high transmission rate, less virulent strains could be favored. Conversely, if needle transmission requires a high viremia and leads to a sufficiently high transmission rate, more virulent strains would be favored. The only situation in which enhanced needle use could lead to the evolution of vector-borne transmission would be if effective needle transmission requires a viremia close to that of vector-borne transmission, while leading to a much higher transmission rate than vector-borne transmission. This way, strains with high viremia could be maintained in the population through needle transmission, and vector-borne transmission would then occur largely as a byproduct.

Our conclusions in this article are necessarily speculative, but such speculation is a necessary part of the initial stages of any research. One of our aims is to stimulate future research into the evolutionary biology of HIV transmission. From the results presented here, a number of different directions might be taken to ground these evolutionary ideas more firmly in empirical data. One possibility would be to examine more closely mechanical vector transmission in immunodeficiency viruses of other species. For example, more data on the epidemiological patterns of SIV and its potential for alternative routes of transmission would be enormously useful. Since SIV is believed to be at the evolutionary ancestor of HIV, it would be very interesting to know if the longer evolutionary history it has had with its host has resulted in different transmission patterns. To the best of our knowledge, there are no empirical studies testing the potential for vector transmission of SIV. Another fruitful approach might be to conduct artificial selection experiments with HIV in arthropod tissue culture. Experiments have demonstrated that HIV cannot currently replicate significantly in arthropod cells, but no study to our knowledge has attempted to select for the evolution of HIV replication in such cells. One could even imagine doing such experiments with both mammalian and arthropod cell cultures to determine of the evolutionary trade-off postulated here actually occurs.

Ultimately, it will require innovative experiments and empirical studies to push the boundaries of our knowledge of HIV, and the use of evolutionary biology as a powerful tool for designing sensible intervention strategies. These kinds of studies are beginning to appear for other aspects of HIV biology (e.g., see Müller et al. 2006 for an interesting evolutionary analysis of HIV virulence) but more work on transmission biology would be useful. For example, if further empirical research validated the hypothesis presented here, that mechanical vector transmission has not evolved because of its associated mortal-

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ity costs, this would then have important implications for how we attempt to stop the spread of HIV. Strategies such as condom use, while beneficial for reducing the extent of sexual transmission, could thereby enhance the relative benefit of vector transmission, *potentially* resulting in the evolution of this new route of transmission. The use of antibiotics against bacterial pathogens has clearly brought home the fact that pathogens can readily evolve the means to circumvent our control measures, and there is no reason to expect things to be any different for other control measures. The use of antiviral medication, on the other hand, not only reduces sexual transmission but also the level of viremia, and therefore would presumably not move the selective balance more towards vector transmission. It is only by asking these kinds of questions, however, that we will have a chance at preventing adverse future outcomes.

Finally, the question of biological vector transmission addressed here is really a special case of the more general question of the evolution of a pathogen's host range. Why do some pathogens have a relatively broad taxonomic host range while others are much more conservative? This continues to be an interesting and important question in the evolutionary ecology of parasites (Poulin 2007) and there are some theoretical results predicting when we might expect different outcomes (Gandon 2004). From the standpoint of human diseases this is also clearly an important question since emerging diseases, such as pandemic influenza, are precisely instances in which a pathogen evolves a different host range. A better understanding of the evolutionary biology of parasite host ranges is an important goal for future research.

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Appendix 1 – Derivation of per capita growth rate

Model (2) of the main text predicts an eventual exponential increase in the number of HIV-positive people (assuming overall transmission rates are high enough). We can calculate the rate of increase, r, in the following way. First, because the population dynamics of the vector occur on a shorter timescale than that for HIV infection, we can treat the variable w as though it is always maintained in quasi-equilibrium. Setting equation (2) equal to zero, the quasi-equilibrium value is given by

$$w(t) \approx \frac{vab_1}{\mu} \int_0^\infty y(s,t)\mathrm{d}s. \qquad \text{(A1-1)}$$

Thus, model (2) can be simplified to the single equation

$$\frac{\partial y(a,t)}{\partial t} = -\frac{\partial y(a,t)}{\partial a} - \delta(a)y(a,t) \qquad \text{(A1-2)}$$

with boundary condition $y(0,t) = x\left(\frac{va^2b_1b_2}{\mu} + \beta\right) \int_0^\infty y(s,t)\mathrm{d}s$. Equation (A1-2) can then be solved by separation of variables. In particular, we postulate a solution of the form $y(a, t) = A(a)T(t)$. Substituting this into (A1-2) then yields

$$\begin{aligned} AT' &= -A'T - \delta AT \\ T'/T &= -A'/A - \delta \end{aligned}.$$

Given that exponential growth at rate r occurs, we have $T'/T = r$, and therefore $A(a)$ satisfies the equation $-(r + \delta) = A'/A$. This has the solution

$$A(a) = A_0 \exp\left[-\int_0^a (r + \delta(s))\mathrm{d}s\right]. \qquad \text{(A1-3)}$$

Substituting solution (A1-3) into the boundary condition for equation (A1-2) we then obtain

$$1 = x\left(\frac{va^2 b_1 b_2}{\mu} + \beta\right)\int_0^\infty \mathrm{e}^{-\int_0^s (r+\delta(z))\mathrm{d}z}\mathrm{d}s. \qquad \text{(A1-4)}$$

Finally, making use of (3) from the main text, equation (A1-4) simplifies as

$$1 = x\left(\frac{va^2 b_1 b_2}{\mu} + \beta\right)\int_0^\tau \mathrm{e}^{-(r+\delta_0)s}\mathrm{d}s$$

or

$$r + \delta_0 = x\left(\frac{va^2 b_1 b_2}{\mu} + \beta\right)\left(1 - \mathrm{e}^{-(r+\delta_0)\tau}\right). \qquad \text{(A1-5)}$$

Equation (A1-5) implicitly defines the growth rate, r, as a function of the epidemiological parameters.

If we want to take into account the fact that, depending on the age of infection, hosts might be more or less infectious we get

$$1 = x\left(\frac{va^2 b_2}{\mu}\int_0^\tau b_1(s)\mathrm{e}^{-(r+\delta_0)s}\mathrm{d}s + \int_0^\tau \beta(s)\mathrm{e}^{-(r+\delta_0)s}\mathrm{d}s\right) \qquad \text{(A1-6)}$$

In this case solving the integration requires a numerical approximation. Also, we need to make an extra assumption to link age of infection and infectious state (both for vector-borne and sexual transmission).

If we take a decreasing function of the shape $b_1\mathrm{e}^{-ka}$ and $\beta\,\mathrm{e}^{-ka}$, where k is a positive constant indicating the speed of decrease in transmission, we can write:

$$1 = x\left(\frac{va^2 b_2}{\mu} b_1 \int_0^\tau \mathrm{e}^{-(r+\delta_0+k)s}\mathrm{d}s + \beta\int_0^\tau \mathrm{e}^{-(r+\delta_0+k)s}\mathrm{d}s\right) \qquad \text{(A1-7)}$$

We thus end-up with almost the same expression as before:

$$r + \delta_0 + k = x\left(\frac{va^2 b_1 b_2}{\mu} + \beta\right)\left(1 - \mathrm{e}^{-(r+\delta_0)\tau}\right) \qquad \text{(A1-8)}$$

To summarize, if we assume the decrease in transmission can be approximated by an exponential function, the same analysis holds by simply modifying the value of, δ_0.

Appendix 2 – Analysis of the time frame of mutant spread

First, equations (6) can be combined to obtain

$$\left(\frac{va^2 b_1 b_2}{\beta\mu} + 1\right) = \frac{r_\mathrm{B} + \delta_0}{1 - \mathrm{e}^{-(r_\mathrm{B}+\delta_0)\tau_\mathrm{B}}} \Big/ \frac{r_\mathrm{A} + \delta_0}{1 - \mathrm{e}^{-(r_\mathrm{A}+\delta_0)\tau_\mathrm{A}}} \qquad \text{(A2-1)}$$

where the quantity on the left-hand side of (A2-1) is the factor by which arthropods must increase overall HIV transmission to yield a given value of the per capita growth rate, r_B. Furthermore, from equation (5) it can be shown that the relative frequency of the mutant allele, $h = p/(1 - p)$ changes exponentially over time, according to

$$\frac{\mathrm{d}h}{\mathrm{d}t} = (r_\mathrm{B} - r_\mathrm{A})h. \qquad \text{(A2-2)}$$

Therefore, the value of $(r_\mathrm{B} - r_\mathrm{A})$ required for h to increase by a factor of K in T time units is $(\ln K)/T$. As a result, we can express the required value of r_B as a function of K, T, and r_A as $r_\mathrm{B} = (\ln K)/T + r_\mathrm{A}$. Substituting this into the right-hand side of equation (A2-1) yields

$$\frac{1 - \mathrm{e}^{-(r_\mathrm{A}+\delta_0)\tau_\mathrm{A}}}{1 - \mathrm{e}^{-(\ln K + (r_\mathrm{A}+\delta_0)T)\tau_\mathrm{B}/T}}\left(1 + \frac{\ln K}{(r_\mathrm{A} + \delta_0)T}\right). \qquad (A2-3)$$

Lastly, we can estimate r_A from existing data. The number of HIV-infected people in the world has increased from approximately 10 million to approximately 35 million in the 11 years between 1991 and 2002 (UNAIDS 2006). Assuming an exponential increase, this yields a value of $r_A \approx 0.114$. Further, using a life expectancy of 65 years, we can estimate $\delta_0 = 1/65$, and also $\tau_A \approx 8$ years as the rough time elapsed between HIV infection and the development of AIDS. This leaves the parameters K, T, and τ_B when plotting Fig. 2. The parameter K is seen to have very little effect over several orders of magnitude (Fig. 2). Therefore, the benefit of vector transmission (in terms of transmission rate) required for it to evolve within 30–50 years is determined largely by the value of τ_B, which is the time elapsing before the onset of AIDS for the vector-transmissible strain.

