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RESEARCH ARTICLE

High Heritability Is Compatible with the Broad Distribution of Set Point Viral Load in HIV Carriers

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Abstract

Set point viral load in HIV patients ranges over several orders of magnitude and is a key determinant of disease progression in HIV. A number of recent studies have reported high heritability of set point viral load implying that viral genetic factors contribute substantially to the overall variation in viral load. The high heritability is surprising given the diversity of host factors associated with controlling viral infection. Here we develop an analytical model that describes the temporal changes of the distribution of set point viral load as a function of heritability. This model shows that high heritability is the most parsimonious explanation for the observed variance of set point viral load. Our results thus not only reinforce the credibility of previous estimates of heritability but also shed new light onto mechanisms of viral pathogenesis.

Author Summary

Following an initial peak in viremia, the viral load in HIV infected patients settles down to a set point which remains more or less stable during chronic HIV infection. This set point viral load is one of the key factors determining the rate of disease progression. The extent to which it is determined by the virus versus host genetics is thus central to developing a better understanding of disease progression. Here we develop an analytical model that describes the changes of the distribution of set point viral load in the HIV carrier population over a full cycle of transmission. Applying this model to patient data we find that the most parsimonious explanation for the observed large variation of set point viral load across HIV patients is that set point viral load is highly heritable from donors to recipients. This implies that set point viral load is to a considerable extent under the genetic control of the virus.

Introduction

The time course of viral load in HIV infected patients follows a characteristic pattern. During primary infection the viral load rapidly grows to very high levels. The peak viremia is attained within the first few weeks of infection. Thereafter the viral load declines rapidly over a period of several months and eventually settles down at a much lower level referred to as the viral set point. Set point viral load (spVL) is a central characteristic of the course of the disease. Firstly, the virus load measurements do fluctuate in patients, the time average of the viral load remains remarkably close to the spVL in most of patients over the time scale of several years [1, 2]. Secondly, higher spVL is associated with faster disease progression [3].

The stability of spVL within patients is in strong contrast to the enormous variation in spVL observed between patients. While variation in spVL between patients ranges over 3–4 orders of magnitude [3–6], the time trend over longitudinal viral load measurements typically changes by less then 0.1 log per year [1, 2]. Given that spVL is a key predictor of disease progression, there is considerable interest in identifying the host and viral genetic factors underlying the variation in spVL.

A well known example for the influence of naturally occurring variation in human genetic factors on viral load is the Δ32 deletion in the *CCR5* gene [7]. Moreover polymorphisms in HLA-B and C alleles have been associated with variance in virus load and genome-wide association studies (GWAS) showed that about 20% of the variance in log spVL can be attributed to specific single nucleotide polymorphisms [8–11]. 20% is likely a lower bound for the overall contribution of host genetic factors, because GWAS generally suffer from the problem that they can only identify common genetic variants with strong effects and do not account for epistatic effects between host genes [12].

Natural variation in the virus can also affect spVL. For example the transmission of a *nef*-deficient virus through a contaminated blood sample resulted in a low viral load in the recipients [13]. Moreover, several studies have reported a correlation between predicted replicative capacity and viral load [14–16]. As this prediction is based only on the viral genotype a patient carries, this implies that naturally occurring variation in viruses does affect viral load. A number of recent studies attempted to estimate the contribution of the viral genotype to the variation in spVL by quantifying the statistical association of viral load between donors and recipients either directly in donor-recipient pairs or through phylogenetic analysis [17–22]; for reviews see Müller et al. [23] and Fraser et al. [24]. A meta-analysis of previously published donor-recipient studies correcting for various co-factors such as age and sex yielded a heritability of 33% with a 95% confidence interval of 20–46% [24]. The two studies that inferred heritability based on phylogenetic methods provided the most extreme estimates with 5.7% reported by Hodcroft et al. [22] and 59% reported by Alizon et al. [21]. While the phylogenetic approaches have an advantage over the donor-recipient based approaches in that they can use much larger patient populations, it is currently unclear to what extent the underlying assumptions of the phylogenetic approaches of no selection and high frequency of sampling affect the robustness of these results.

The discrepant estimates call for a better quantitative understanding of the underlying factors determining heritability of log spVL in HIV. To this end we develop here a quantitative model that describes the change of the distribution of log spVL in a patient population in relation to heritability over a full transmission cycle. The model extends the approach of Shirreff et al. [25] and is similar in spirit to the integral projection models in ecology that are used to describe the temporal changes of distributions of a continuous phenotypic trait in populations [26–28]. In contrast to many applications in ecology, the application to distributions of log spVL has the advantage that all relevant processes and populations for which data are available,

are numerically well approximated by a Gaussian function. This fact enables us to obtain complete analytical understanding of how spVL changes through time based on a model parametrized by available data.

Results

We consider the change of the spVL distribution over one full reproduction cycle on the epidemiological level, i.e. from the current to the next generation of patients. We divide the patient population into "carriers" (HIV infected individuals prior to selection for transmission), "donors" (individuals that have been selected for transmission) and "recipients" (individuals that have just been infected by donors). Furthermore, we divide the reproduction cycle into three steps: (i) selection of donors from the carriers with replacement according to their transmission potential, (ii) transmission from donors to recipients, and (iii) intrahost evolution of the virus from the start of infection to the next transmission. Finally, we explicitly distinguish between factors contributing to set point viral load with regard to being transmissible (i.e. viral genetic factors) versus being non-transmissible (i.e. host genetic factors, environmental factors, or any interaction between host, virus, and the environment). A schematic overview over the effects of these steps on the distribution of log spVL is shown in Fig. 1.

In the Supplementary Materials we show how the change of the spVL distribution can be computed for any distribution over a full transmission cycle. If all populations and processes are well approximated by Gaussian functions, then an approximation to the resulting log spVL distributions can be computed analytically (see Methods and Supplementary Materials). Assuming that the population is in equilibrium we obtain for the mean, $\tilde{M}_C$, variance, $\tilde{V}_C$, and heritability, h^2, the following expressions:

$$\tilde{M}_C = \mu_o + \mu_i\left(1 + \frac{v_e + v_o}{\tilde{V}_C - v_e}\right), \tag{1}$$

$$\tilde{V}_C = \frac{v_t + v_i}{2}\left(1 + \sqrt{1 + 4\frac{v_e + v_o}{v_t + v_i}}\right) + v_e, \tag{2}$$

$$h^2 = 1 - \frac{v_e}{\tilde{V}_C}. \tag{3}$$

Here the parameters μ_o and v_o characterize the transmission potential [6], i.e. the overall probability of a patient to transmit the infection as a function of log spVL (Fig. 1(o)–(i)). This transmission potential is given by the product of the rate of transmission per contact and the disease duration. As the former increases and the latter decrease with increasing spVL, the transmission potential has a maximum at intermediate levels of spVL [6]. The parameter v_e gives the variance of the contribution of host/environmental effects on log spVL. The parameter v_t describes the variance due to the bottleneck at transmission from donor to recipient, as a founder strain is selected randomly from the diverse population in the donor (Fig. 1(i)–(ii)). The parameters μ_i and v_i describe the mean and variance of the contribution of intrahost evolution to log spVL (Fig. 1(ii)–(iii)).

Our model assumes that the bottleneck at transmission is neutral with regard to selection on set point viral load. Note, that the assumption is without loss of generality. This is important because there is evidence for selection at transmission [29], although it is unclear whether selection acts on spVL. Any selective effect at transmission, however, can be subsumed into the parameter μ_i. Hence, the effect of selection is effectively incorporated in our model.

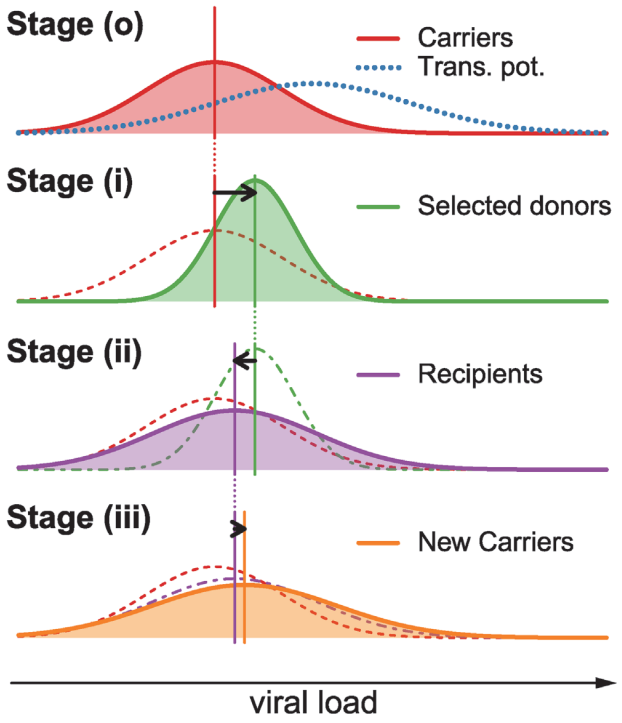

Fig 1. Graphical example of the change in the distribution of log spVL in the population over one reproduction cycle. During one full reproduction cycle, the distribution goes through the following steps: (o) The log spVL distribution within a population follow a Gaussian function with mean M_C and variance V_C (red curve). The transmission potential (blue dotted line) selects a subset of this population as donors (see Equation 5). (i) The transmission potential selects donors from the carrier population in (o) with mean M_D that lies between the mean of the carriers, M_C, and the mean of the transmission potential, μ_o. The resulting variance in log spVL in the selected donors is smaller than in the carrier population (see Equation 7). (ii) The selected donors transmit to new hosts, thus randomizing the host/environmental contributions and lowering the population mean log spVL and increasing the population variance. The variance is further increased by a transmission bottleneck and sampling effect on the level of the individual donors.(iii) Within-host evolution of log spVL may further increase or decrease the population mean, while always increasing or not affecting the variance. This completes a full reproduction cycle. In equilibrium, the individual changes in mean and variance in stages (i), (ii) and (iii) is such that the overall change in mean and variance from stage (o) to (iii) is zero.

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The parameter for the mean contribution by the host/environment, μ_e, does not appear in equations 1 or 2. This is because the equations refer to the phenotypic value of spVL, i.e. the sum of the genetic contributions of the virus and the contributions from the host/environment. Any large environmental/host effect on the mean can always be compensated by correspondingly strong genetic effect of the virus on the mean but with opposite sign.

The above results are applicable if, (i) if the population is approximately in equilibrium, and (ii) all populations and processes are numerically well approximated by Gaussian functions.

Assumption (i) has been discussed in detail previously [6, 25, 30, 31]. In essence, this assumption is supported by three observations. Firstly, the mean of the spVL distribution coincides with the optimum of the transmission potential (see Fig. 2 and Fraser et al. [6]). Secondly, the rate of change of spVL has decreased over the last 25 years [31]. Thirdly, the rate of evolution is sufficiently rapid such that a spVL that is optimal for transmission could have evolved over the course of the epidemic [25]. These findings suggest that the distribution of set point

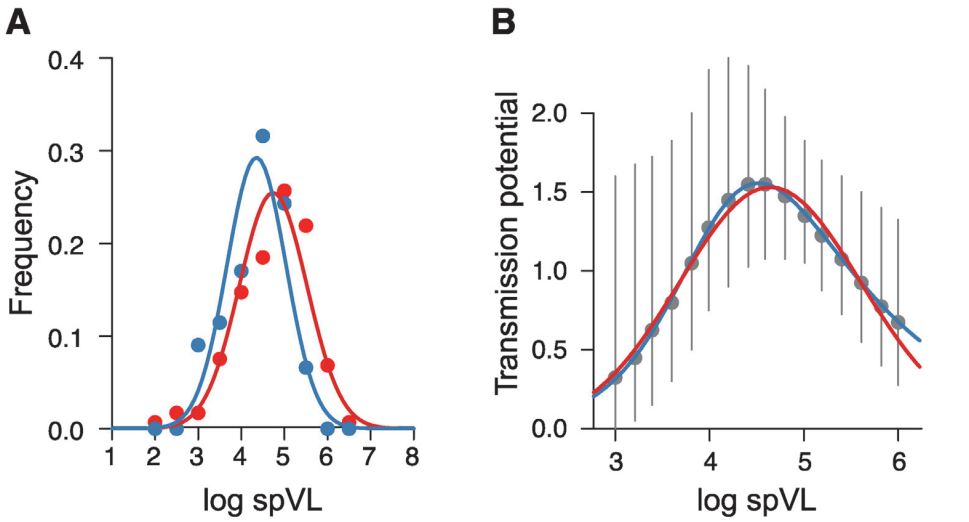

Fig 2. Viral load distributions and transmission potential estimated from patient cohorts as extracted from the corresponding graphs in Fraser et al. [6]. (**A**) The viral load distributions in the a Zambian and a Dutch cohort (see Fraser et al. [6]). The lines correspond to the best fits of a Gaussian to the distribution of log spVL. The null hypothesis that log spVL is normally distributed cannot be rejected based on a test that tests whether the residuals between model and fit themselves are normally distributed. The estimated mean and standard deviation are 4.74 and 0.61 for the Zambian data (red) and 4.35 and 0.47 for the Amsterdam data (blue). (**B**) The transmission probability according to the functions for transmissibility and duration of disease as a function of viral load as provided in Fraser et al. [6]. The grey circles and lines represent the mean and 95% confidence interval of the transmission potential as estimated from the Zambian and Amsterdam cohorts by Fraser et al. [6]. The blue line represents the corresponding theoretically derived transmission potential as provided by Fraser et al. [6]. The red line corresponds to the best fit of a log Gaussian to the estimated transmission potential. The parameters of the fitted log Gaussian are $\mu_o = 4.64 \pm 0.021$ and $v_o = 0.96 \pm 0.025$ (estimate ± standard deviation).

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viral load is indeed approximately in equilibrium, which in turn makes it is plausible to assume that the environmental and genetic factors determining set point viral load are also in equilibrium.

Regarding assumption (ii), we note that a Gaussian function describes a distribution or process by a main effect (mean) and some variational noise (variance). Thus in absence of any better knowledge, a Gaussian distribution is a natural starting point to describe any process and simply represents a second order approximation to an unknown distribution. We can assess the validity of describing the distributions of spVL in carriers and the transmission potential graphically using available data. Inspection of Fig. 2A and Figure S1 in Supplementary S1 Text shows that the viral load amongst carriers is indeed numerically well approximated by a Gaussian with mean log spVL, $M_C \approx 4.5$, and variance in log spVL, $V_C \approx 0.5$. Also the fit of a Gaussian to the transmission potential (see Fig. 2B) is a very good approximation (mean $\mu_o \approx 4.6$ and variance $v_o \approx 1.0$), even though the transmission potential as estimated by Fraser et al. [6] is slightly right-skewed.

There are no data to inform the shape of the processes of transmission and intrahost evolution. Using a description that has a mean effect with some variation around this mean is natural. Nonetheless, we test the effect of numerical deviations from a Gaussian with the following simulations. Firstly, we use the exact right-skewed transmission potential as given by Fraser et al. [6]. The analytical approximations for the distribution of the population in equilibrium remain excellent when the substantial deviations of the transmission potential from a Gaussian

are incorporated (see Figure S2). Secondly, we study the robustness towards deviations from Gaussian functions in the processes describing intrahost evolution and the transmission bottleneck. Even when both processes are strongly skewed, the analytical approximations for mean and variance are excellent (typically less than 2% deviation, see Figure S3 in Supplementary S1 Text).

To assess what heritability values are compatible with the observed mean and variance of log SPVL in the carrier population we take a simple approach that is in essence Approximate Bayesian Computing with rejection sampling. To this end we define plausible prior distributions for the parameters of the model. Sampling randomly from the priors we determine the resulting means and variances of log spVL in carriers and reject sets of parameters that lead to means and variances outside a defined permissible range. The set of accepted parameters gives the posterior distribution.

For the range of permissible mean log spVL we assume $4 < \tilde{M}_C < 5$, which is compatible but somewhat larger than the observed range in the studies reported by Fraser et al. [6] and Geskus et al. [5] (see Fig. 2A and Supplementary Materials, Section E). For the permissible range of variances of log spVL we assume that $0.3 < \tilde{V}_C < 0.8$, which again is compatible but somewhat larger than the values reported by Fraser et al. [6] and Geskus et al. [5] (see Supplementary Materials, Section E).

We use uniform priors for all parameters. The parameters μ_o and v_o, which describe mean and variance of the transmission potential, have thus far only been estimated only by a single peer reviewed study (Fraser et al. [6] and Fig. 2B; see also [32]). To account for uncertainty in the estimates of these parameters we use $4 < \mu_o < 5$ and $0.5 < v_o < 1.5$. Estimates for remaining parameters cannot be easily derived from the existing literature. To account for uncertainty in these parameters we assume $0 < v_e < 1$; $-1 < \mu_i < 1$; $0 < v_i < 0.3$ and $0 < v_t < 0.3$.

Fig. 3 shows the posterior parameter distribution from the rejection sampling. Different colors in the scatter plots indicate different levels of mean heritability at given parameter combinations. The contour lines show the density of posterior distribution. The key result shown in

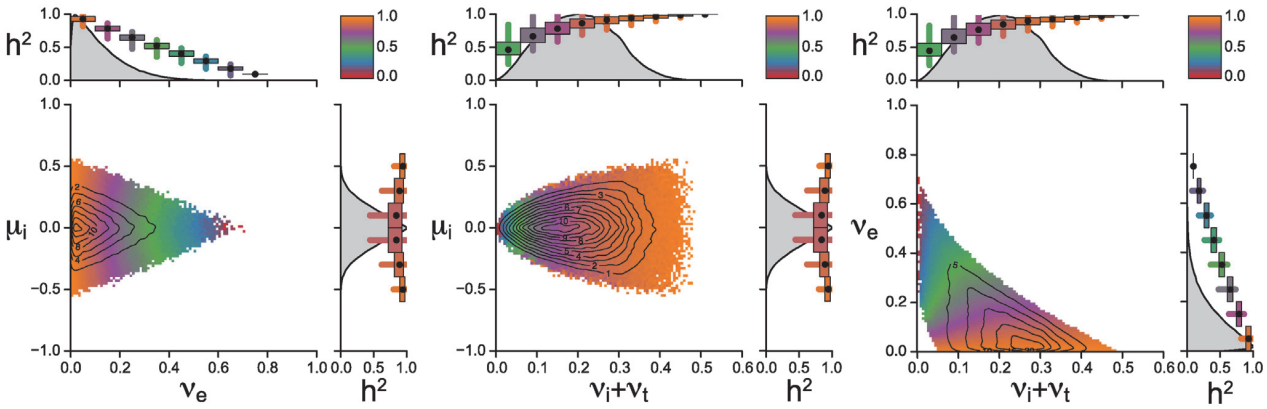

Fig 3. Posterior distribution of parameters from the rejection sampler. We report pairwise scatterplots of the parameters μ_i, v_e and the compound parameter $v_i + v_t$, since these two parameters only appear as a sum in all equations for the mean, variance and heritability. 10^7 random sets of parameter values are sampled randomly from the uniform priors described by the ranges on the *x* and *y* axes. Around 1.9% of the randomly generated parameter combinations yield values for mean and variance of log spVL that are compatible with the acceptance criterion $4 < \tilde{M}_C < 5$, $0.3 < \tilde{V}_C < 0.8$. The contour lines show the two-dimensional kernel density estimate of the posterior sample. The colours reflect the mean heritability of binned parameter combinations and are stacked such that points with lower heritability lie on top of points with higher heritability. The small plots to the top and right of the scatterplots show the posterior density estimate along a single parameter dimension, as well as the mean (black dot), and 50% (boxes) and 95% quantiles (lines) of heritabilities along those parameter dimensions. Most of the probability mass occurs at low values of μ_i and v_e.

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the figure is that the majority of accepted parameter values result in high values of heritability (purple to orange color at contour lines of highest posterior densities). While low values of heritability are also compatible with the observed mean and variance of log spVL, they occur rarely in the posterior sample and are at the edges of the prior distributions (red to blue areas). The center of mass of the posterior sample is in areas with high heritability, higher in fact then what would seem compatible with current estimates of heritability and host genetic factors. There are two factors not included in this analysis: measurement error of spVL and prior knowledge of the host contribution to spVL. Increasing the measurement accuracy of spVL would increase heritability estimates based on both donor-recipient pairs and phylogenetic inference. Incorporating prior knowledge of the host genetic contribution would set an upper bound on the estimates of heritability in our analysis. Thus accounting for these two factors bring the center of mass of the heritability distribution closer to the measured values of heritability.

The figure also highlights that generally wider priors would not change the posterior distribution because parameter values at the upper end of priors are never accepted. The change of the mean capacity of the virus to induce spVL through intrahost evolution, μ_i, is restricted to values smaller than 0.6 and decreases with increasing variance generated by the host/environment effects, v_e. Increasing v_e corresponds to decreasing heritability (see eq. 3) and thus high levels of μ_i require high levels of heritability. The center of mass of the posterior sample suggests that the most parsimonious explanation of the observed mean and variance of log spVL implies both small intrahost evolution and high heritability.

One criticism leveled against the transmission potential as quantified in [6] is that it does not appropriately reflect transmissions occuring during the acute or the AIDS phase. In the Supplementary Material, Section F.3, we show that our quantitative results are robust towards using a corrected transmission potential.

Discussion

The above analysis shows that the most parsimonious explanation of the observed distribution of spVL in HIV carrier populations requires high heritability of spVL. Although low heritability values are also compatible with the observed distribution of spVL in HIV carrier populations, parameter combinations resulting in these low values have a small probability and occur at the edge of the realistic parameter range. The skepticism with which the estimated heritability values have been met in the field suggests that the general expectation is that heritability of spVL should be low. In contrast, our analysis shows that high heritability values are not only compatible with, but are also the more parsimonious explanation of the observed distribution in spVL in HIV carrier populations.

Low heritability only occurs if the processes of intrahost evolution and the transmission bottleneck have a weak effect on spVL, i.e. if the parameters μ_i, v_i and v_t are small. An intuition can be obtained by noting that in equilibrium the variance generating and variance eliminating processes balance out. The transmission potential only exerts weak selection on log spVL and therefore only marginally reduces variance. The decrease of variance by selection for transmission has to be compensated by an increase in variance by intrahost evolution and the transmission bottleneck. For too low heritability, the genetic variance generated by intrahost evolution and transmission bottlenecks would overwhelm the reduction of variance due to selection by the transmission potential. While there are to our knowledge no data that allow to estimate the variance generated at transmission, v_t, the posterior distributions of μ_i and v_i are broadly compatible with the observed changes of virus load within patients [1, 2, 33].

Taken together our analysis suggests that the most parsimonious explanation of the distribution of log spVL is high h^2 but low v_t, μ_i and v_i. Hence, heritability is high while the processes

of intrahost evolution and transmission bottleneck have a small effect on the capacity of the virus to modulate log spVL. High heritability implies a substantial genetic control of the log spVL by the virus. The observation that at the same time the contribution of intrahost evolution to spVL is small raises an interesting question: How can a strongly heritable trait show little intrahost evolution? Given the otherwise ample evidence for rapid intrahost evolution of HIV such as escape from drugs or the immune response, the absences of intrahost evolution of spVL is surprising. Generally a trait is expected to respond to selection, if (i) the trait is heritable, (ii) there is phenotypic variation of the trait in a population, and (iii) the trait is linked to fitness. That spVL is heritable has been reported previously [23, 24] and our analysis reinforces the credibility of these findings. That there is phenotypic variation in the control of spVL by the virus is plausible given the large genetic variation of the virus population within an individual. What remains is whether it is conceivable that the capacity of a viral genotype to induce spVL is only weakly linked to fitness. One hypothesis that could reconcile high heritability with little intrahost evolution is that variation in viral load between patients is in part due to virus-induced activation of target cells. Difference in activation rate of target cells has previously been argued to account for a substantial part of the variation in viral load [4]. Furthermore, if target cell activation is at least partially under the control of the virus, then this control may indeed be weakly linked to intrahost fitness. If the target cell activation is systemic (i.e. not locally confined to the inducing virus) then increased target cell activation increases the pool of susceptible cells, but the benefit of increased target cell activation is not confined to the producer virus. As a result selection for virus induced activation rate is expected to be neutral or nearly neutral [34]. Indeed, an explicit model of the evolution of log spVL for a virus induced control of target cell activation can reconcile high heritability with absence of intrahost evolution [35].

Our modeling approach is based on describing how the distribution of a continuous phenotypic trait, here log spVL, changes in a population over a full cycle of reproduction. This approach is closely related to the method of integral projection models, which has been developed and widely applied in ecology and population biology [26–28, 36, 37]. The approach can in principle describe how arbitrary distributions change over time as a function of processes such as selection and reproduction. Here we are able to obtain a full analytical description of the temporal change of the spVL distribution, because all relevant distributions and processes can be well approximated by Gaussian functions. We also show that our analytical results remain robust even for substantial deviations numerical deviations from Gaussian functions (see Supplementary Materials, Section F). Moreover, the model can be parametrized on the basis of available data. There are ample data for mean and variance of spVL and also most of the parameters can be confined to plausible ranges based on the literature.

Our study clearly supports that high heritability is compatible with the observed distribution of log spVL in HIV carriers. High heritability of spVL does not preclude that also the host genotype has a considerable effect on virus load. However, it does lead to the expectation that over the course of infection the capacity to induce higher spVL should increase considerably unless this capacity is only weakly linked to intrahost fitness. This sheds new light onto the mechanisms controlling viral load. There should be identifiable genetic variation in the virus population that is associated with viral load, and moreover, the loci associated with control of viral load should be weakly linked to intrahost fitness. Genome-wide association studies mapping viral genetic polymorphisms to variance in log spVL seem a natural approach to test this prediction. A recent study by Bartha et al. [38] was unable to identify any statistical associations, but was powered only to detect individual non-synonymous mutations with an effect size of $>4\%$ on heritability. Larger studies will thus be required to identify whether and which viral polymorphisms are associated with set point viral load.

Methods

In the following sections we derive an analytical model that describes the change of mean and variance of spVL in the population of HIV carriers as a function of the heritability of spVL. We account for the virus and host effects by subdividing the phenotype (i.e. log spVL) into genetic and environmental/host components. Generally we denote the changes in mean and variance in the carrier, donor, and recipient populations with the subscripts *C*, *D* and *R*, respectively. We use greek letters for the parameters of the model and latin letters for the variables. When referring to the phenotype (i.e. log spVL) we use upper-case letters and when referring to the genotype we use lower-case letters.

Distribution of log spVL in carrier population

The spVL in a patient is generally determined by viral genetic factors, host genetic factors, the environment and interactions between these factors. Since only the virus is transmitted from donors to recipients, we subsume all non-transmissible effects such as the host genetic factors, environmental effects and all interactions between host, virus and the environment generically under "environmental effects", *e*. The transmissible effects due to the viral genotype are the "genotypic effects", *g*. The "phenotype" spVL is then given by *g*+*e*.

We assume that the distribution of log spVL in the carrier population is given by a normal distribution $\mathcal{N}(M_C, V_C)$, where M_C and V_C are the mean and variance, respectively. The transmission potential, defined as the overall probability of transmission of an HIV carrier integrated over the entire course of the disease, is assumed to be a function of log spVL which can be well approximated by a normal distribution $\mathcal{N}(\mu_o, v_o)$ (see Fig. 2). Here μ_o is the log spVL at which the transmission potential is maximal and v_o characterizes how strongly the transmission potential selects for transmission at μ_o.

We assume that *g* and *e* are independent and normally distributed in the carrier population with $\mathcal{N}(m_C, v_C)$ and $\mathcal{N}(\mu_e, v_e)$, respectively. Here m_C and v_C are the variables that describe the mean and variance of the distribution of viral genotypes in the carrier population. Note that here the independence of *g* and *e* refers to the quantitative contribution of virus and host to spVL. Importantly, this independence does not imply an absence of virus genotype by host genotype interactions, such as an interaction between a particular viral epitope and a host HLA molecule. Genotype by genotype interactions are non-transmissible and thus subsumed in *e*. The parameters μ_e and v_e describe mean and variance of the distribution of environmental effects, which comprise host effects, interactions and any non-transmissible effect. The distribution of phenotype log spVL in the carrier population is then given by a normal distribution with mean and variance,

$$M_C = m_C + \mu_e, \qquad \text{and} \qquad V_C = v_C + v_e. \tag{4}$$

Selection of donors

Selection for transmission acts on log spVL, i. e. on the sum of the genotypic and environmental effects, and is given by the transmission potential. Specifically, the probability of transmission for a given log spVL, ϕ, is given by (see Fig. 2B),

$$S(\phi) = \frac{1}{\sqrt{2\pi v_o}} e^{-\frac{(\phi-\mu_o)^2}{2v_o}}. \tag{5}$$

Applying the above transmission potential to the carrier population, we find that the genotype and phenotype in the donor population are again normally distributed (see Supplementary Materials, Equations B6 and B7). The donor genotype has mean and variance,

$$m_D = \frac{m_C(v_e + v_o) + (\mu_o - \mu_e)v_C}{v_C + v_e + v_o}, \qquad \text{and} \qquad v_D = \frac{v_C(v_e + v_o)}{v_e + v_o + v_C}. \tag{6}$$

The donor phenotype has mean and variance (see Supplementary Materials, Equations B8 and B9),

$$M_D = \frac{M_C v_o + \mu_o V_C}{v_o + V_C}, \qquad \text{and} \qquad V_D = \frac{V_C v_o}{v_o + V_C}. \tag{7}$$

Note, that the mean and variance of the environmental effects (i.e. the host effect) is not given by the differences between the phenotypic and genotypic values, because environment and genotype in the donors are correlated. This is because selection for transmission acts on the sum of environmental and genotypic effects. In other words selection for transmission selects a subset of viral genotypes and host genotypes, and host and viral genotypes are correlated, because selection operates on their combined effect.

Transmission to recipients

When the virus is transmitted from the donor to the recipient population, the virus is "harvested" from a non-random distribution of environmental effects (and thus also from a non-random set of hosts). The harvested virus is then redistributed over a random set of new hosts/environments in the recipient population. Thus all environmental effects in the donor population are erased at transmission and the environmental contribution in the recipients is redrawn from $\mathcal{N}(\mu_e, v_e)$. To account for the fact that the virus population experiences a strong bottleneck from recipient to donor, we assume that the viral genotype is not transmitted exactly from donor to recipient but instead is assumed to be randomly drawn out of a distribution of genotypes in the donor patient. Assuming that this distribution is normal with mean m_D and variance v_t we obtain that both genotype and
phenotype in the donor population are normally distributed. The recipient genotype has mean and variance,

$$m_R = m_D, \qquad \text{and} \qquad v_R = v_D + v_t. \tag{8}$$

The recipient phenotype has mean and variance,

$$M_R = m_R + \mu_e^0, \qquad \text{and} \qquad V_R = v_R + v_e^0, \tag{9}$$

where μ_e^0 and v_e^0 are the mean and variance of the host/environmental effects prior to infection. The environmental effects are redrawn randomly, because they are not inherited from one transmission to the next. Note, that we assume here that the bottleneck at transmission is neutral. This assumption does not imply that there is no selection at the transmission stage, but rather that the bottleneck is neutral with regard to the spVL that the transmitted strains will eventually cause. Any selection at and after transmission on the viral genotypic contribution to log spVL is subsumed in the next step, intrahost evolution.

Intrahost evolution

After transmission the virus population in the recipient may change in a directed fashion according to intrahost evolution. Assuming that the overall change of the viral genotype due to

intrahost evolution can be approximated by a normal distribution we find that the distribution of genotypes and phenotypes in the next generation of carriers, C' is again normal. The distribution of the genotypes has a mean and variance

$$m_{C'} = m_R + \mu_i, \qquad \text{and} \qquad v_{C'} = v_R + v_i. \tag{10}$$

The parameter μ_i thus describes any genetic change in the virus that affects log spVL across all patients in the same way. The parameter v_i describes genetic changes that affect log spVL in a manner that is specific to the patient, i.e. it describes the effect of changes of log spVL due to genetic interactions between virus and host. As the environmental effects comprise the immune response by the host, the mean and variance in environmental effects may change in coevolution with the virus through μ_e^i and v_e^i, respectively. Thus we obtain for mean and variance of the distribution of phenotypes,

$$M_{C'} = m_{C'} + \mu_e^0 + \mu_e^i, \qquad \text{and} \qquad V_{C'} = v_{C'} + v_e^0 + v_e^i. \tag{11}$$

Note, that any selection for spVL at the transmission bottleneck can now be interpreted as a genotypic change that occurs during intrahost evolution. Thus the overall model is appropriate both for non-selective and selective bottlenecks.

Heritability

Heritability, h^2, is defined as fraction of genotypic variance relative to phenotypic variance in the carrier population [39]. Thus we have,

$$h^2 = \frac{v_C}{V_C} = 1 - \frac{v_e}{V_C}. \tag{12}$$

Heritability can be estimated in a parent-offspring regression [39], where h^2 is equal to the regression slope b. Donor-recipient pairs can be seen as parent-offspring pairs, where care must be taken since the donors are not randomly selected from the carrier population but are selected according to the transmission potential. Since, however, we are measuring the heritability of spVL and donors are selected based on spVL, the regression of recipients on selected donors is equal to heritability of spVL in carriers [24, 39].

Mean and variance of log spVL at equilibrium

We now have a complete analytical description how mean and variance of log spVL change from the current to the next generation of carriers. The fact that the log spVL that maximizes the transmission potential and the mean of the distribution of log spVL in the carrier populations (see Fig. 2 and Fraser et al. [6]) are both around 4.5, we can assume that the process is roughly at equilibrium. In equilibrium we have that the mean and variance of the distribution of phenotypes does not change, i.e. $M_{C'} = M_C$ and $V_{C'} = V_C$. This will be fulfilled if the genetic and environmental contributions are also at equilibrium, implying in particular that $\mu_e = \mu_e^0 + \mu_e^i$ and $v_e = v_e^0 + v_e^i$ (see Supplementary Materials section C.1). Using Equation 12 we can express the equilibrium mean and variance of log spVL as a function of v_e, the variance of the

contribution of the host/environment to log spVL (see Supplementary Materials, Equations C9 and C10),

$$\tilde{M}_C = \mu_o + \mu_i\left(1 + \frac{v_e + v_o}{\tilde{V}_C - v_e}\right), \tag{13}$$

$$\tilde{V}_C = \frac{v_t + v_i}{2}\left(1 + \sqrt{1 + 4\frac{v_e + v_o}{v_t + v_i}}\right) + v_e. \tag{14}$$

or as a function the heritability h^2 (see Supplementary Materials, Equations C12 and C13),

$$\tilde{M}_C = \mu_o + \mu_i\left(1 + \frac{(1-h^2)\tilde{V}_C + v_o}{h^2\tilde{V}_C}\right), \tag{15}$$

$$\tilde{V}_C = \frac{v_t + v_i}{2(h^2)^2}\left(1 + \sqrt{1 + \frac{4(h^2)^2 v_o}{v_t + v_i}}\right). \tag{16}$$

Supporting Information

S1 Text. Supplementary Methods and Figures.
(PDF)

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Author Contributions

Conceived and designed the experiments: SB GEL CF. Performed the experiments: SB GEL CF. Analyzed the data: SB GEL CF. Contributed reagents/materials/analysis tools: SB GEL CF. Wrote the paper: SB GEL CF.

References

1. O'Brien TR, Rosenberg PS, Yellin F, Goedert JJ (1998) Longitudinal HIV-1 RNA levels in a cohort of homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol 18: 155–61. doi: 10.1097/00042560-199806010-00007 PMID: 9637580
2. Sabin CA, Devereux H, Phillips AN, Hill A, Janossy G, et al. (2000) Course of viral load throughout HIV-1 infection. J Acquir Immune Defic Syndr 23: 172–7. doi: 10.1097/00042560-200002010-00009 PMID: 10737432
3. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, et al. (1996) Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272: 1167–70. doi: 10.1126/science.272.5265.1167 PMID: 8638160
4. Bonhoeffer S, Funk GA, Günthard HF, Fischer M, Müller V (2003) Glancing behind virus load variation in HIV-1 infection. Trends Microbiol 11: 499–504. doi: 10.1016/j.tim.2003.09.002 PMID: 14607066
5. Geskus RB, Prins M, Hubert JB, Miedema F, Berkhout B, et al. (2007) The HIV RNA setpoint theory revisited. Retrovirology 4: 65. doi: 10.1186/1742-4690-4-65 PMID: 17888148
6. Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP (2007) Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. Proc Natl Acad Sci U S A 104: 17441–6. doi: 10.1073/pnas.0708559104 PMID: 17954909

7. Meyer L, Magierowska M, Hubert JB, Rouzioux C, Deveau C, et al. (1997) Early protective effect of CCR-5 delta 32 heterozygosity on HIV-1 disease progression: relationship with viral load. The SEROCO Study Group. AIDS 11: F73–8. doi: 10.1097/00002030-199711000-00001 PMID: 9302436

8. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, et al. (2007) A whole-genome association study of major determinants for host control of HIV-1. Science 317: 944–7. doi: 10.1126/science.1143767 PMID: 17641165

9. Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, et al. (2009) Common genetic variation and the control of HIV-1 in humans. PLoS Genet 5: e1000791. doi: 10.1371/journal.pgen.1000791 PMID: 20041166

10. Dalmasso C, Carpentier W, Meyer L, Rouzioux C, Goujard C, et al. (2008) Distinct genetic loci control plasma HIV-RNA and cellular HIV-DNA levels in HIV-1 infection: the ANRS Genome Wide Association 01 study. PLoS One 3: e3907. doi: 10.1371/journal.pone.0003907 PMID: 19107206

11. Limou S, Le Clerc S, Coulonges C, Carpentier W, Dina C, et al. (2009) Genomewide association study of an AIDS-nonprogression cohort emphasizes the role played by HLA genes (ANRS Genomewide Association Study 02). J Infect Dis 199: 419–26. doi: 10.1086/596067 PMID: 19115949

12. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, et al. (2009) Finding the missing heritability of complex diseases. Nature 461: 747–53. doi: 10.1038/nature08494 PMID: 19812666

13. Learmont JC, Geczy AF, Mills J, Ashton LJ, Raynes-Greenow CH, et al. (1999) Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1. A report from the Sydney Blood Bank Cohort. N Engl J Med 340: 1715–22. doi: 10.1056/NEJM199906033402203 PMID: 10352163

14. Kouyos RD, von Wyl V, Hinkley T, Petropoulos CJ, Haddad M, et al. (2011) Assessing predicted HIV-1 replicative capacity in a clinical setting. PLoS Pathog 7: e1002321. doi: 10.1371/journal.ppat.1002321 PMID: 22072960

15. Quiñones-Mateu ME, Ball SC, Marozsan AJ, Torre VS, Albright JL, et al. (2000) A dual infection/competition assay shows a correlation between ex vivo human immunodeficiency virus type 1 fitness and disease progression. J Virol 74: 9222–33. doi: 10.1128/JVI.74.19.9222-9233.2000 PMID: 10982369

16. Barbour JD, Hecht FM, Wrin T, Segal MR, Ramstead CA, et al. (2004) Higher CD4+ T cell counts associated with low viral pol replication capacity among treatment-naive adults in early HIV-1 infection. J Infect Dis 190: 251–6. doi: 10.1086/422036 PMID: 15216458

17. Tang J, Tang S, Lobashevsky E, Zulu I, Aldrovandi G, et al. (2004) HLA allele sharing and HIV type 1 viremia in seroconverting Zambians with known transmitting partners. AIDS Res Hum Retroviruses 20: 19–25. doi: 10.1089/088922204322749468 PMID: 15000695

18. Hecht FM, Hartogensis W, Bragg L, Bacchetti P, Atchison R, et al. (2010) HIV RNA level in early infection is predicted by viral load in the transmission source. AIDS 24: 941–5. doi: 10.1097/QAD.0b013e328337b12e PMID: 20168202

19. Hollingsworth TD, Laeyendecker O, Shirreff G, Donnelly CA, Serwadda D, et al. (2010) HIV-1 transmitting couples have similar viral load set-points in Rakai, Uganda. PLoS Pathog 6: e1000876. doi: 10.1371/journal.ppat.1000876 PMID: 20463808

20. van der Kuyl AC, Jurriaans S, Pollakis G, Bakker M, Cornelissen M (2010) HIV RNA levels in transmission sources only weakly predict plasma viral load in recipients. AIDS 24: 1607–8. doi: 10.1097/QAD.0b013e32833b318f PMID: 20539098

21. Alizon S, von Wyl V, Stadler T, Kouyos RD, Yerly S, et al. (2010) Phylogenetic approach reveals that virus genotype largely determines HIV set-point viral load. PLoS Pathog 6: e1001123. doi: 10.1371/journal.ppat.1001123 PMID: 20941398

22. Hodcroft E, Hadfield JD, Fearnhill E, Phillips A, Dunn D, et al. (2014) The contribution of viral genotype to plasma viral set-point in HIV infection. PLoS Pathog 10: e1004112. doi: 10.1371/journal.ppat.1004112 PMID: 24789308

23. Müller V, Fraser C, Herbeck JT (2011) A strong case for viral genetic factors in HIV virulence. Viruses 3: 204–16. doi: 10.3390/v3030204 PMID: 21994727

24. Fraser C, Lythgoe K, Leventhal GE, Shirreff G, Hollingsworth TD, et al. (2014) Virulence and pathogenesis of HIV-1 infection: an evolutionary perspective. Science 343: 1243727. doi: 10.1126/science.1243727 PMID: 24653038

25. Shirreff G, Pellis L, Laeyendecker O, Fraser C (2011) Transmission selects for HIV-1 strains of intermediate virulence: a modelling approach. PLoS Comput Biol 7: e1002185. doi: 10.1371/journal.pcbi.1002185 PMID: 22022243

26. Easterling MR, Ellner SP, Dixon PM (2000) Size-specific sensitivity: Applying a new structured population model. Ecology 81: 694–708. doi: 10.1890/0012-9658(2000)081%5B0694:SSSAAN%5D2.0.CO;2

27. Ellner SP, Rees M (2006) Integral Projection Models for Species with Complex Demography. Am Nat 167: 410–428. doi: 10.1086/499438 PMID: 16673349

28. Coulson T (2012) Integral projections models, their construction and use in posing hypotheses in ecology. Oikos 121: 1337–1350. doi: 10.1111/j.1600-0706.2012.00035.x

29. Carlson JM, Schaefer M, Monaco DC, Batorsky R, Claiborne DT, et al. (2014) HIV transmission. Selection bias at the heterosexual HIV-1 transmission bottleneck. Science 345: 1254031. doi: 10.1126/science.1254031 PMID: 25013080

30. Lythgoe KA, Pellis L, Fraser C (2013) Is HIV short-sighted? Insights from a multistrain nested model. Evolution 67: 2769–82. doi: 10.1111/evo.12166 PMID: 24094332

31. Herbeck JT, Müller V, Maust BS, Ledergerber B, Torti C, et al. (2012) Is the virulence of HIV changing? A meta-analysis of trends in prognostic markers of HIV disease progression and transmission. AIDS 26: 193–205. doi: 10.1097/QAD.0b013e32834db418 PMID: 22089381

32. Williams BG (2011) Determinants of sexual transmission of HV: implications for control. arXiv:11084715.

33. Shankarappa R, Margolick JB, Gange SJ, Rodrigo AG, Upchurch D, et al. (1999) Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. J Virol 73: 10489–502. PMID: 10559367

34. Bartha I, Simon P, Müller V (2008) Has HIV evolved to induce immune pathogenesis? Trends Immunol 29: 322–8. doi: 10.1016/j.it.2008.04.005 PMID: 18524680

35. Hool A, Leventhal GE, Bonhoeffer S (2013) Virus-induced target cell activation reconciles set-point viral load heritability and within-host evolution. Epidemics 5: 174–80. doi: 10.1016/j.epidem.2013.09.002 PMID: 24267873

36. Childs DZ, Rees M, Rose KE, Grubb PJ, Ellner SP (2004) Evolution of size-dependent flowering in a variable environment: construction and analysis of a stochastic integral projection model. Proc Biol Sci 271: 425–34. doi: 10.1098/rspb.2003.2597 PMID: 15101702

37. Coulson T, MacNulty DR, Stahler DR, vonHoldt B, Wayne RK, et al. (2011) Modeling effects of environmental change on wolf population dynamics, trait evolution, and life history. Science 334: 1275–8. doi: 10.1126/science.1209441 PMID: 22144626

38. Bartha I, Carlson JM, Brumme CJ, McLaren PJ, Brumme ZL, et al. (2013) A genome-to-genome analysis of associations between human genetic variation, HIV-1 sequence diversity, and viral control. Elife 2: e01123. doi: 10.7554/eLife.01123 PMID: 24171102

39. Falconer DS (1981) Introduction to quantitative genetics. Longman (New York), 2nd edition.

High heritability is compatible with the broad distribution of set point viral load in HIV carriers: Supplementary Text S1

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Contents

A Distribution of genotype and phenotype in the different populations along a full replication cycle

In this section we will derive expressions for the distributions of genotypes g, environment e and phenotype ϕ in the populations of carriers C, donors D, recipients R and new carriers E. The phenotype ϕ refers here to the log set point virus load (log spVL). The genotype g refers to the virus and the environment e refers to all non-transmissible contribution to log spVL, i.e. the contributions from the host genotype, from the interactions between host and viral genotypes and from the environment. Generally, $p_{x,Y}$ will denote the distribution of $x \in \{g, e, \phi\}$ in the population $Y \in \{C, D, R, E\}$. The phenotype $\phi(g, e)$ is a function of the genotype g and the environment e. The simplest assumption is that g and e contribute additively,

$$\phi(g, e) = g + e. \tag{A1}$$

A.1 Carrier population

Let the joint distribution of genotypes and environments in the carrier population be $p_{ge,C}(g, e)$. Assuming that genotypes and environments are independently distributed we have,

$$p_{ge,C}(g, e) = p_{g,C}(g)p_{e,C}(e). \tag{A2}$$

The distribution of the phenotype ϕ in the carrier population is,

$$\begin{aligned} p_{\phi,C}(\phi) &= \iint p_{ge,C}(g, e|\phi)p_{g,C}(g)p_{e,C}(e)\, dgde && \text{(A3)}\\ &= \iint \delta(\phi - (g + e))p_{g,C}(g)p_{e,C}(e)\, dgde && \text{(A4)}\\ &= \int p_{g,C}(g)p_{e,C}(\phi - g)\, dg && \text{(A5)}\\ &= [p_{g,C} * p_{e,C}](\phi). && \text{(A6)} \end{aligned}$$

Here, δ is the Dirac-delta function and the asterisk denotes the convolution of the distributions $p_{g,C}$ and $p_{e,C}$.

A.2 Donor population

Donors are selected from the current distribution of carriers according to their fitness $S(\phi)$ which depends on their phenotype $\phi = g + e$. The joint distribution of g and e in selected donors is,

$$p_{ge,D}(g, e) = \frac{1}{Z_s} p_{ge,C}(g, e)S(g + e) = \frac{1}{Z_s} p_{g,C}(g)p_{e,C}(e)S(g + e), \tag{A7}$$

where Z_s is a normalization constant,

$$\begin{aligned} Z_s &= \iint p_{ge,C}(g, e)S(g + e)dgde = \iint p_{g,C}(g)p_{e,C}(e)S(g + e)dedg && \text{(A8)}\\ &= \iint p_{g,C}(g)p_{e,C}(\phi - g)S(\phi)d\phi dg && \text{(A9)}\\ &= \int p_{\phi,C}(\phi)S(\phi)d\phi. && \text{(A10)} \end{aligned}$$

We can then write the joint distribution of genotypes g and phenotypes ϕ in the selected donors,

$$p_{g\phi,D}(g,\phi) = \int p_{ge,D}(g,e)\delta(\phi-(g+e))de \tag{A11}$$

$$= \frac{1}{Z_s}\int p_{g,C}(g)p_{e,C}(e)S(g+e)\delta(\phi-(g+e))de \tag{A12}$$

$$= \frac{1}{Z_s}p_{g,C}(g)p_{e,C}(\phi-g)S(\phi). \tag{A13}$$

The distribution of genotypes irrespective of the phenotype then is $p_{g,D}(g,\phi)$ marginalized over ϕ,

$$p_{g,D}(g) = \int p_{g,D}(g,\phi)d\phi = \frac{1}{Z_s}\int p_{g,C}(g)p_{e,C}(\phi-g)S(\phi)d\phi. \tag{A14}$$

Similarly, the distribution of the phenotype ϕ in the selected donors is,

$$p_{\phi,D}(\phi) = \int p_{g,D}(g,\phi)dg = \frac{1}{Z_s}\int p_{g,C}(g)p_{e,C}(\phi-g)S(\phi)dg = \frac{1}{Z_s}[p_{g,C}*p_{e,C}](\phi)S(\phi). \tag{A15}$$

A.3 Recipient population

The distribution of genotypes in the recipient population is shaped by the transmission function $\mathcal{T}(g_R,g_D)$, which determines the genotype g_R of a recipient given that the genotype of the donor was g_D. So the distribution of g in the recipients is $\mathcal{T}$ integrated over all genotypes in the donor population,

$$p_{g,R}(g_R) = \int \mathcal{T}(g_R,g_D)p_{g,D}(g_D)\,dg_D \tag{A16}$$

$$= \frac{1}{Z_s}\iint \mathcal{T}(g_R,g_D)p_{g,C}(g_D)p_{e,C}(\phi-g_D)S(\phi)\,d\phi\,dg_D. \tag{A17}$$

We can write the distribution of phenotype in the recipient population as,

$$p_{\phi,R}(\phi_R) = \int p_{g,R}(g_R)p_{e,R}(\phi_R-g_R)dg_R \tag{A18}$$

$$= \iint \mathcal{T}(g_R,g_D)p_{g,D}(g_D)p_{e,R}(\phi_R-g_R)dg_Ddg_R \tag{A19}$$

$$= \int p_{g,D}(g_D)dg_D\int \mathcal{T}(g_R,g_D)p_{e,R}(\phi_R-g_R)dg_R \tag{A20}$$

$$= \int p_{g,D}(g_D)[\mathcal{T}*p_{e,R}](\phi_R,g_D)dg_D. \tag{A21}$$

Inserting equation (A14),

$$p_{\phi,R}(\phi_R) = \frac{1}{Z_s}\iint [\mathcal{T}*p_{e,R}](\phi,g)\,p_{g,C}(g)p_{e,C}(\phi'-g)S(\phi')\,d\phi'\,dg. \tag{A22}$$

A.4 Evolved population (after intrahost evolution)

Let $\mathcal{E}_g(g_E, g_R)$ be the function that evolves the genotype within the host. The distribution of genotypes in the evolved recipients is then,

$$p_{g,E}(g_E) = \int \mathcal{E}_g(g_E, g_R) p_{g,R}(g_R) dg_R. \tag{A23}$$

Inserting equation (A17),

$$p_{g,E}(g_E) = \frac{1}{Z_s} \iiint \mathcal{E}_g(g_E, g_R) \mathcal{T}(g_R, g_D) p_{g,C}(g_D) p_{e,C}(\phi - g_D) S(\phi)\, d\phi\, dg_D\, dg_R. \tag{A24}$$

Due to the evolution of the virus genetics, the host-virus interactions can change. This would result in a change in the distribution of e in the evolved population. Let $\mathcal{E}_e(e_E, e_R)$ be the function that evolves the interactions within the host. The distribution of environmental factors in the evolved recipients is then,

$$p_{e,E}(e_E) = \int \mathcal{E}_e(e_E, e_R) p_{e,R}(e_R) de_R. \tag{A25}$$

We can write the distributions of phenotypes as,

$$\begin{aligned} p_{\phi,E}(\phi_E) &= \int p_{g,E}(g_E) p_{e,E}(\phi_E - g_E)\, dg_E && \text{(A26)} \\ &= \frac{1}{Z_s} \int \cdots \int \mathcal{E}_g(g_E, g_R) \mathcal{T}(g_R, g_D) p_{g,C}(g_D) p_{e,C}(\phi - g_D) S(\phi) \\ &\quad \times \mathcal{E}_e(\phi_E - g_E, e_R) p_{e,R}(e_R)\, de_R\, d\phi\, dg_D\, dg_R\, dg_E && \text{(A27)} \\ &= \frac{1}{Z_s} \iiint \mathcal{T}(g_R, g_D) p_{g,C}(g_D) p_{e,C}(\phi - g_D) S(\phi)\, d\phi\, dg_D\, dg_R \\ &\quad \times \int \left[\mathcal{E}_g * \mathcal{E}_e\right](\phi_E; g_R, e_R) p_{e,R}(e_R)\, de_R. && \text{(A28)} \end{aligned}$$

B Analytical solution assuming normal distributions

While the above expressions hold for any distribution, the integral cannot be solved in the general case. If we assume normal distributions for all the different processes, we are able to derive closed-form expressions.

B.1 Carriers

We assume that the distributions $p_{g,C}$ and $p_{e,C}$ are normally distributed,

$$p_{g,C} = \frac{1}{\sqrt{2\pi v_C}} \exp\left\{-\frac{(m_C - g)^2}{2v_C}\right\}, \tag{B1}$$

$$p_{e,C} = \frac{1}{\sqrt{2\pi \nu_e}} \exp\left\{-\frac{(\mu_e - e)^2}{2\nu_e}\right\}. \tag{B2}$$

Here, (m_C, v_C) and (μ_e, ν_e) are the means and variances of the genotype and environmental distributions respectively.

Since the convolution of two Gaussian distributions with means μ_1 and μ_2 and variances σ_1^2 and σ_2^2 is also a Gaussian with mean $\mu_{12} = \mu_1 + \mu_2$ and variance $\sigma_{12}^2 = \sigma_1^2 + \sigma_2^2$, the distribution of phenotypes in the carrier population $p_{\phi,C}$ is also normal with mean,

$$M_C = m_C + \mu_e, \tag{B3}$$

and variance,

$$V_C = v_C + \nu_e. \tag{B4}$$

B.2 Selected donors

Additionally, the product of two Gaussians is also a Gaussian (not necessarily normalized) with mean,

$$\mu_p = \frac{\mu_1\sigma_2^2 + \mu_2\sigma_1^2}{\sigma_1^2 + \sigma_2^2},$$

and variance,

$$\sigma_p^2 = \frac{\sigma_1^2\sigma_2^2}{\sigma_1^2 + \sigma_2^2}.$$

Thus $p_e \equiv p_{e,C}$ is symmetric around the mean μ_e such that,

$$p_e(\phi - g) = p_e((g + 2\mu_e) - \phi),$$

and equation (A14) becomes,

$$p_{g,D}(g) = \frac{1}{Z_s} p_{g,C}(g)[p_e * S](g + 2\mu_e). \tag{B5}$$

The convolution of p_e and S has mean $\mu_e + \mu_o$ and variance $\nu_e + \nu_o$. If we write,

$$A(g) = [p_e * S](g + 2\mu_e),$$

then A is a Gaussian with variance $\nu_e + \nu_o$ and mean $\mu_e + \mu_o - 2\mu_e = \mu_o - \mu_e$. From the product formula above, $p_{g,D} \sim \mathcal{N}(m_D, v_D)$,

$$m_D = \frac{m_C(\nu_e + \nu_o) + (\mu_o - \mu_e)v_C}{v_C + \nu_e + \nu_o}, \tag{B6}$$

$$v_D = \frac{v_C(\nu_e + \nu_o)}{v_C + \nu_e + \nu_o}. \tag{B7}$$

The distribution of phenotypes in the donor population follows from equation (A15) directly. So, $p_{\phi,D} \sim \mathcal{N}(M_D, V_D)$,

$$M_D = \frac{M_C\nu_o + \mu_o V_C}{\nu_o + V_C}, \tag{B8}$$

$$V_D = \frac{V_C\nu_o}{V_C + \nu_o}. \tag{B9}$$

B.3 Recipients

The transmission function $\mathcal{T}$ determines the viral genotype of the recipient, given that the genotype of the donor was g_R. We assume that $\mathcal{T}$ is normally distributed around g_R with variance ν_t. Thus equation (A17) becomes

$$p_{g,R}(g_R) = \int p_t(g_R - g_D) p_{g,D}(g_D)\, dg_D,$$

where p_t is a Gaussian with zero mean and variance ν_t. This integral is again a convolution, such that $p_{g,R} \sim \mathcal{N}(m_R, v_R)$ with,

$$m_R = m_D, \tag{B10}$$
$$v_R = v_D + v_t. \tag{B11}$$

Equivalently for the phenotype distribution in the recipients, from equation (A21),

$$p_{\phi,R}(\phi_R) = \int p_{t+e}(\phi_R - g_D) p_{g,D}(g_D)\, dg_D,$$

where p_{t+e} is a Gaussian with mean μ_e^0 and variance $v_t + \nu_e^0$. Thus the convolution is again Gaussian and $p_{\phi,R} \sim \mathcal{N}(M_R, V_R)$,

$$M_R = m_D + \mu_e^0, \tag{B12}$$
$$V_R = v_D + v_t + \nu_e^0. \tag{B13}$$

B.4 New carriers

The same as for transmission, we assume that the evolver functions for the viral and environmental contribution is $\mathcal{E}_g \sim \mathcal{N}(g_R + \mu_i, \nu_i^g)$ and $\mathcal{E}_e \sim \mathcal{N}(e_R + \mu_e^i, \nu_e^i)$, respectively. The evolved population of new carriers has a genotype distribution given by equation (A23).

$$p_{g,E}(g_E) = \int p_{Eg}((g_E - \mu_i) - g_R) p_{g,R}(g_R) dg_R,$$

where p_E has mean zero and variance ν_i^g, such that $p_{g,E} \sim \mathcal{N}(m_{C'}, v_{C'})$,

$$m_{C'} = m_R + \mu_i, \tag{B14}$$
$$v_{C'} = v_R + \nu_i^g. \tag{B15}$$

The distribution of phenotypes in the evolved population as a function of the distribution in the recipient population is,

$$p_{\phi,E}(\phi_E) = \iiint p_{g,R}(g_R) \mathcal{E}_g(g_E, g_R) p_{e,R}(e_R) \mathcal{E}_e(\phi_E - g_E, e_R)\, dg_R\, dg_E\, de_R.$$

Let $p_{Ee}(x)$ be a normal distribution with mean zero and variance ν_e^i,

$$p_{\phi,E}(\phi_E) = \iint p_{g,R}(g_R) \mathcal{E}_g(g_E, g_R) \int p_{e,R}(e_R) p_{Ee}((\phi_E - g_E - \mu_e^i) - e_R)\, dg_R\, dg_E\, de_R \tag{B16}$$
$$= \iint p_{g,R}(g_R) \mathcal{E}_g(g_E, g_R) f_1(\phi_E - g_E)\, dg_R\, dg_E, \tag{B17}$$

with f_1 a normal distribution with mean $\mu_e^0 + \mu_e^i$ and variance $\nu_e^0 + \nu_e^i$. Integrating the convolutions further,

$$p_{\phi,E}(\phi_E) = \int f_1(\phi_E - g_E)\, dg_E \int p_{g,R}(g_R) p_{Eg}(g_E - g_R - \mu_i)\, dg_R \tag{B18}$$

$$= \int f_1(\phi_E - g_E) f_2(g_E)\, dg_E, \tag{B19}$$

where f_2 is a normal distribution with mean $m_R + \mu_i$ and variance $v_R + \nu_i^g$.
The distribution of the phenotype follows from the convolution of f_1 and f_2, such that $p_{\phi,E} \sim \mathcal{N}(M_{C'}, V_{C'})$,

$$M_{C'} = m_R + \mu_i + \mu_e^0 + \mu_e^i, \tag{B20}$$

$$V_{C'} = v_R + \nu_i^g + \nu_e^0 + \nu_e^i \tag{B21}$$

C Equilibrium solutions for mean and variance of spVL

Concerning log spVL under the assumption of normal distributions, we have the following expressions for the distribution of log spVL in the current carriers and the carriers in the following generation,

$$\phi_C \sim \mathcal{N}\left(m_C + \mu_e, v_C + \nu_e\right), \tag{C1}$$

$$\phi_{C'} \sim \mathcal{N}\left(\frac{m_C(\nu_e + \nu_o) + (\mu_o - \mu_e)v_C}{v_C + \nu_e + \nu_o} + \mu_i + \mu_e^0 + \mu_e^i, \frac{v_C(\nu_e + \nu_o)}{v_C + \nu_e + \nu_o} + \nu_t + \nu_i^g + \nu_e^0 + \nu_e^i\right). \tag{C2}$$

The system is said to be in equilibrium when the distribution in phenotype not longer changes from one generation to the next, thus,

$$m_C + \mu_e = \frac{m_C(\nu_e + \nu_o) + (\mu_o - \mu_e)v_C}{v_C + \nu_e + \nu_o} + \mu_i + \mu_e^0 + \mu_e^i, \tag{C3}$$

$$v_C + \nu_e = \frac{v_C(\nu_e + \nu_o)}{v_C + \nu_e + \nu_o} + \nu_t + \nu_i^g + \nu_e^0 + \nu_e^i. \tag{C4}$$

From equation (C4) we readily find the equilibrium solution for v_C,

$$\tilde{v}_C = \frac{\nu_t + \nu_i + (\nu_e^0 + \nu_e^i - \nu_e)}{2}\left(1 \pm \sqrt{1 + 4\frac{\nu_e + \nu_o}{\nu_t + \nu_i + (\nu_e^0 + \nu_e^i - \nu_e)}}\right). \tag{C5}$$

The equilibrium solution of m_C as a function of v_C is then,

$$\tilde{m}_C = (\mu_o - \mu_e) + \left(\mu_i + \mu_e^0 + \mu_e^i - \mu_e\right)\left(1 + \frac{\nu_e + \nu_o}{\tilde{v}_C}\right). \tag{C6}$$

If we assume that at equilibrium, the distributions of environmental factors no longer change from one generation of carriers to the next, then,

$$\mu_e' \equiv \mu_e^0 + \mu_e^i = \mu_e, \tag{C7}$$

$$\nu_e' \equiv \nu_e^0 + \nu_e^i = \nu_e, \tag{C8}$$

where the prime signifies the values of mean and variance of environmental factors in the new generation of carriers. Thus the equilibrium solutions for the phenotype distribution are,

$$\tilde{M}_C = \tilde{m}_C + \mu_e = \mu_o + \mu_i \left(1 + \frac{\nu_e + \nu_o}{\tilde{V}_C - \nu_e}\right), \tag{C9}$$

$$\tilde{V}_C = \tilde{v}_C + \nu_e = \frac{\nu_t + \nu_i^g}{2}\left(1 \pm \sqrt{1 + 4\frac{\nu_e + \nu_o}{\nu_t + \nu_i^g}}\right) + \nu_e. \tag{C10}$$

We can express the equilibrium solutions in terms of the heritability h^2, where

$$\nu_e = (1 - h^2)\tilde{V}_C. \tag{C11}$$

65 Inserting into equation (C10),

$$\tilde{V}_C = \frac{\nu_t + \nu_i}{2}\left(1 + \sqrt{1 + 4\frac{(1-h^2)\tilde{V}_C + \nu_o}{\nu_t + \nu_i}}\right) + (1 - h^2)\tilde{V}_C.$$

By rearranging the terms we get,

$$\tilde{V}_C h^2 \frac{2}{\nu_t + \nu_i} - 1 = \sqrt{1 + 4\frac{(1-h^2)\tilde{V}_C + \nu_o}{\nu_t + \nu_i}}.$$

Squaring both sides yield the quadratic equation,

$$\tilde{V}_C^2 - \frac{\nu_t + \nu_i}{(h^2)^2}\tilde{V}_C - \frac{(\nu_t + \nu_i)\nu_o}{(h^2)^2} = 0.$$

that has the solutions,

$$\tilde{V}_C = \frac{\nu_t + \nu_i}{2(h^2)^2}\left(1 \pm \sqrt{1 + \frac{4(h^2)^2\nu_o}{\nu_t + \nu_i}}\right).$$

Keeping only the non-negative solution and inserting equation (C11) in the expression for $\tilde{M}_C$,

$$\tilde{M}_C = \mu_o + \mu_i\left(1 + \frac{(1-h^2)\tilde{V}_C + \nu_o}{\tilde{V}_C - (1-h^2)\tilde{V}_C}\right) = \mu_o + \mu_i\left(1 + \frac{(1-h^2)\tilde{V}_C + \nu_o}{h^2\tilde{V}_C}\right), \tag{C12}$$

$$\tilde{V}_C = \frac{\nu_t + \nu_i}{2(h^2)^2}\left(1 + \sqrt{1 + \frac{4(h^2)^2\nu_o}{\nu_t + \nu_i}}\right). \tag{C13}$$

C.1 Equilibrium of environmental factors

In the main text we argue that there is good evidence that the phenotypic distribution of spVL is approximately in equilibrium, and thus $M_{C'} = M_C$ and $V_{C'} = V_C$. In the above derivation, we assume that this also implies an equilibrium of the environmental factors,

$$\mu_e' \equiv \mu_e^0 + \mu_e^i = \mu_e,$$
$$\nu_e' \equiv \nu_e^0 + \nu_e^i = \nu_e.$$

It is straightforward to see that if both the distributions for g and e are in equilibrium, then the distribution for ϕ is also in equilibrium. There are, however, certain special cases that can be considered where an equilibrium of ϕ does not imply an equilibrium of g and e. Firstly, the distribution of ϕ might converge faster to an equilibrium value than the distributions of g and e. This would imply that the contributions of the virus and the environment to the variance in spVL might still be changing over time. Consequently, heritability may also still be changing over time. Secondly, the contributions of g and e may be diverging in opposite directions, such that the change in the distribution of g cancels out the change in the distribution of e on the population level. This scenario, however, is unlikely as it requires the viral and host/environmental factors that influence spVL to increase or decrease indefinitely. Thirdly, the change in g and e on the population level is described by a stable limit cycle, such that the distribution in spVL in the population is constant through time, $\phi(t) = g(t) + e(t) \equiv \tilde{\phi}$. While stable limit cycles can appear in theoretical models, they are rarely observed in real complex biological systems, due to the delicate balance required between the variables. Furthermore, this balance has to be maintained on a population level, which would require some sort of synchrony between the evolutionary changes happening in each individual host. We therefore argue that it is most conceivable that the equilibrium of spVL in the population also implies an equilibrium of the distribution of viral and environmental effects.

D Connection to integral projection models

Our description of the distributions of log spVL change over generations has strong parallels to *integral projection models* used in ecology to describe how the composition of population with continuous traits changes over discrete time (2–4). In this formalism, the number of individuals with trait y in generation $t + 1$ is given by (2),

$$n(y, t + 1) = \int_{\Omega} k(y, x)n(x, t)dx. \tag{D1}$$

Here, $k(y, x)$ is called the kernel and defines the number of offspring with trait y produced by an offspring of trait x in generation t.

Heritability can be viewed as the regression of offspring on parents, i.e. new carriers on old carriers. As we assume the distribution of log spVL in carriers to be normal, the conditional distribution of log spVL in new carriers given an log spVL current carriers is,

$$p(\phi_{C'}|\phi_C = \varphi) \sim \mathcal{N}\left(M_C + \sqrt{\frac{V_C}{V_{C'}}}\rho(\varphi - M_{C'}), (1 - \rho^2)V_C\right), \tag{D2}$$

where $\rho = \sqrt{V_{C'}/V_C}h^2$ is the correlation coefficient between carriers in subsequent generations. Thus the projection kernel $k(\phi_{C'}, \phi_C) = p(\phi_{C'}|\phi_C)$.

E Viral load in Geskus et al. (1)

We extracted the viral load measurements from the pdf file of Geskus et al. (1) to provide a further estimate of mean and variance of viral load. This study is also based on the Amsterdam cohort, but the patient population is not identical to the one used in Fraser et al. (5). Excluding

measurements that were under the detection limit we estimate a mean of 4.22 logs with a variance of 0.59. The fitted line in figure S1 shows that the distribution is well approximated by a normal distribution, although a statistical test reveals a significant deviation from normality. Note that the viral loads reported in Geskus et al. (1) are not spVLs, but include also repeated measurements from individual patients. As a consequence the sample variance is likely an overestimate of the real variance of spVL.

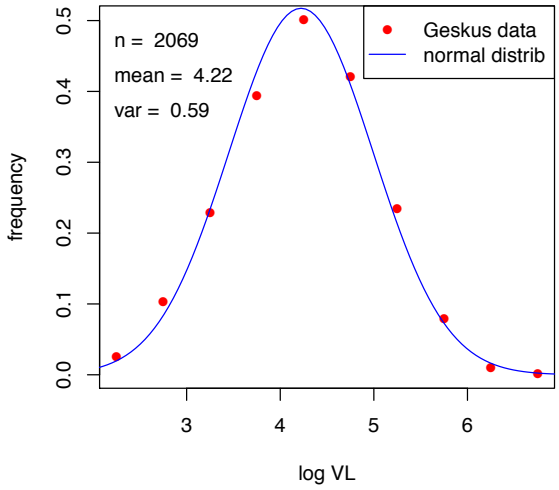


Figure S1. Distribution of spVL in donors and recipients in Geskus et al. (1). The plot is confined to viral load measured between years 1 and 5 after serovonversion.

F Deviations from normality

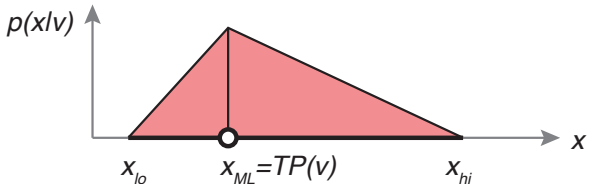
F.1 Exact transmission potential

In this section we assess to what degree the normal approximation to the transmission potential (TP) results in a distribution of spVL in HIV carriers that is different from using the TP as reported in Fraser et al. (5). We also account for uncertainty in the transmission potential by accounting for the confidence intervals in the reported TP. To this end we simulate 20 reproduction cycles (i.e. selection for donors, transmission and intrahost evolution) in a population of $N = 10^5$ individuals. At each reproduction cycle the number the donors of the N recipients are selected in the following manner:

(a) The maximum likelihood estimate for the number of infections caused by an individual with spVL v, as well as the upper and lower bounds of the confidence are determined by linear interpolation of the TP from (5).

(b) We then construct a triangular distribution for the probability of x secondary infections at spVL v between the lower x_{lo} and upper x_{hi} bounds of the confidence interval, such that

the probability of x secondary infections fulfils $p(x_{\text{lo}}|v) = p(x_{\text{hi}}|v) = 0$ and $\text{argmax}_x p(x|v) = x_{\text{ML}} = \text{TP}(v)$. The value of $p(x_{\text{ML}})$ is such that $\int_{x_{\text{lo}}}^{x_{\text{hi}}} p(x)dx = 1$.



(c) The number of secondary infections x_i at the current reproduction cycle for each individual i is then sampled from the constructed distribution for each corresponding spVL v_i.

(d) Donors for all new recipients are picked randomly from the donor population with probability proportional to x_i.

The simulated distribution of spVL in carriers after 20 cycles is shown in Figure S2. The normal approximation is in very good agreement with the simulated distribution.

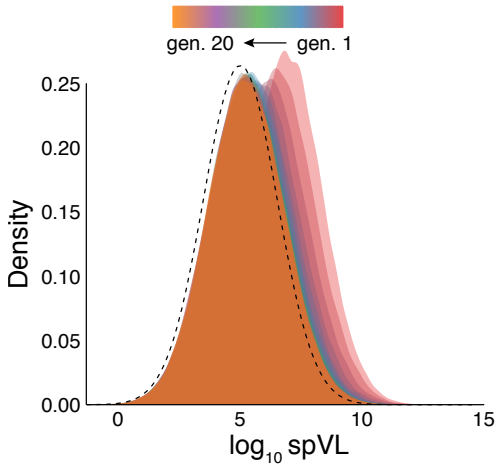


Figure S2. Simulated distribution of spVL in HIV carriers after 20 reproduction cycles when using the exact transmission potential together with the reported confidence intervals. Other parameters are $\mu_o = 4.5$, $\nu_o = 1$, $\mu_e = 3$, $\nu_e = 1$, $\mu_i = 0.2$, $\nu_i = 0.3$, $\nu_t = 0.2$. The starting population is assumed normally distributed with mean $m_g = 4$ and $v_g = 0.4$. The dashed line shows the equilibrium under the normal approximation to the transmission potential.

F.2 Skewness in intrahost evolution and transmission bottleneck

To test the effect of deviations from normality of the processes of intrahost evolution and the transmission bottleneck we sampled from a skew-normal instead of a normal distribution for

both processes. The skew-normal distribution is characterized by a location, a shape and a scale parameter that together define mean, variance and skewness of the distribution. If the shape parameter is zero, the distribution has no skewness and reduces to normal distribution. To sample from the skew-normal distribution we used the `rsnorm` function of the VGAM package in R (6). Figure S3 shows the effect of skewness in processes of intrahost evolution and the transmission bottleneck mean, variance and skewness of the spVL distribution in the carrier population by varying skewness in both processes from -0.9 to 0.9. The key result is that the analytical results for mean and variance of the spvL distribution remain excellent approximations even for strong skewness in the processes of intrahost evolution and the transmission bottleneck.

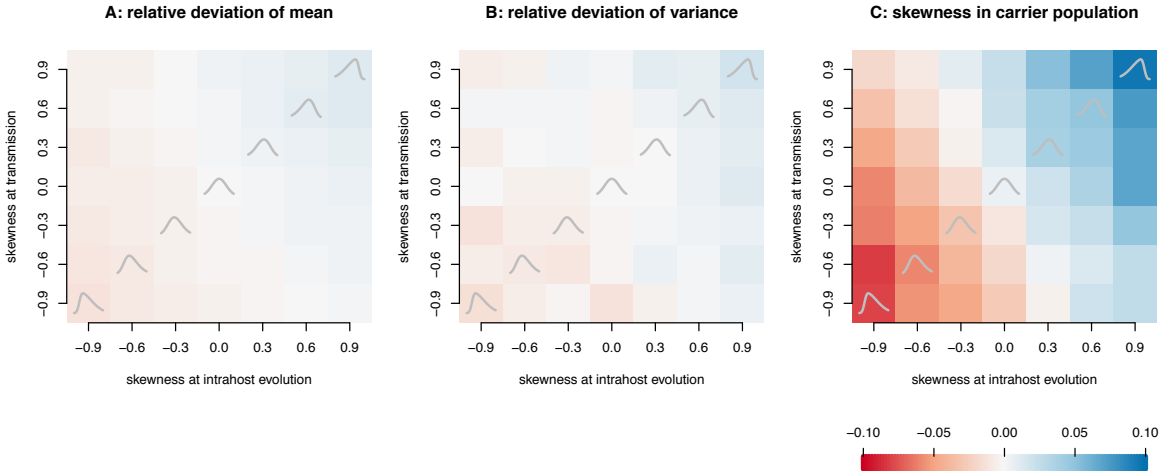


Figure S3. The effect of skewness in the processes of intrahost evolution and the transmission bottleneck on mean, variance and skewness of simulated distributions of spVL in carriers. Panel A shows the relative deviation of the computed mean from the analytical mean (eq. C12), i.e. the difference of computed and analytical mean divided by the analytical mean. Panel B shows the corresponding relative deviation from the analytical variance (eq. C13). Panel C shows the skewness of the distribution of spVL in the carrier population. The grey lines show distributions with the corresponding level of skewness. The color legend applies to all panels. Generally the relative deviation of mean and variance remains below a few percent even for large skewness in the processes of intrahost evolution and the transmission bottleneck. Also the absolute level of skewness in the simulated distributions (panel C) remains below 0.1. Taken together this indicates that even strongly skew processes lead to small effects on the resulting distribution of spVL in HIV carriers. Parameters of the simulation are $\mu_o = 4.5, \nu_o = 1, \mu_e = 3, \nu_e = 1, \mu_i = 0.2, \nu_i = 0.3, \nu_t = 0.2$. The population size used in the simulation is 200000.

F.3 Influence of the acute and AIDS phase on the transmission potential

One concern regarding the transmission potential from Fraser et al. (5) is that it neglects transmission from the acute and the AIDS phase of the infection. This is addressed in more detail in the supplementary material of Fraser et al. (5). As described therein the required correction de-

pends on the assumed model of sexual mixing and partner exchange rate. One way to account for the contribution of these phases is to add a constant term to the transmission potential. This term was estimated in Fraser et al. (5) to be 0.67 (0.32-1.23 95% c. i.) for primary infection and 0.50 (0.31-0.96 95% c. i.) for pre-AIDS/AIDS. A reasonable range for this constant, c, is thus $[0, 2]$.

We performed simulations to compare the equilibrium mean and variance for a transmission potential with a constant c to the analytical expression obtained assuming $c = 0$ (see figure S4). The simulations show that both mean and variance increase with increasing c. Adding a constant to the transmission potential results in overall weaker selection for viral load. This leads to a general increase in variance. The mean increases because the transmission potential is weaker in opposing the force of intrahost evolution towards higher spVL.

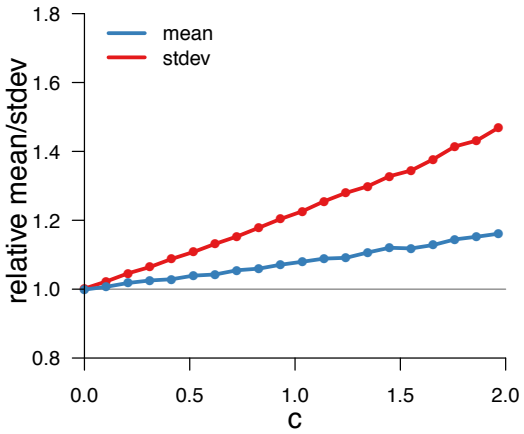


Figure S4. The effect of adding a contribution of the acute and AIDS phases to the overall transmission potential. We show the relative increase of mean and standard deviation compared to the analytical solution (eqs. C12 and C13) as a function of the constant c that is added to the transmission potential. This constant c spans a realistic range of contributions from the acute and AIDS phase as described in Fraser et al. (5). Parameters of the simulation are $\mu_o = 4.5, \nu_o = 1, \mu_e = 3, \nu_e = 1, \mu_i = 0.2, \nu_i = 0.3, \nu_t = 0.2$.

Furthermore, we tested the effect a corrected transmission potential by repeating the rejection sampling procedure using $c = 1.2$ (see figure S5). Using a corrected transmission potential generally narrows down the acceptable parameter ranges (because of the effect of increasing variance and mean shown in figure S4). The areas of highest posterior probability remain in regions of high heritability. Thus, in summary, modifying the transmission potential to account for the contributions of the acute and AIDS phase does not change the two key conclusions, namely that high heritability is the most parsimonious explanation for the observed mean and variance of spVL and that the forces of intrahost evolution must be weak.

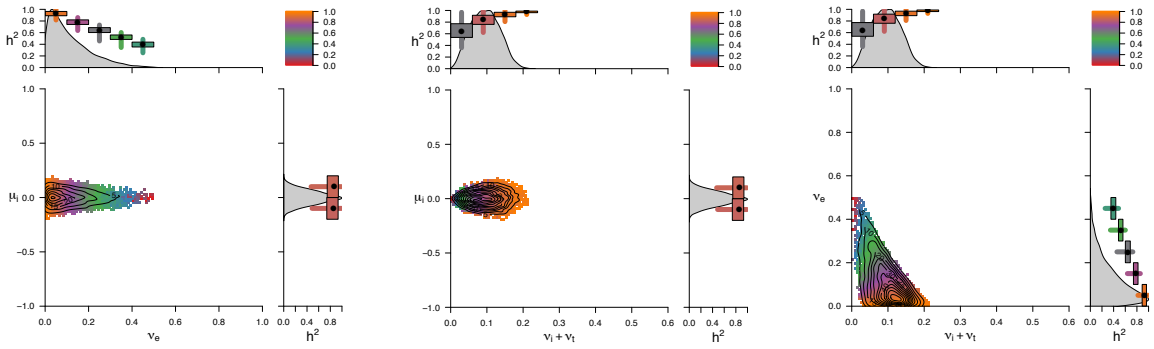


Figure S5. Posterior distribution of parameters from the rejection sampler assuming a transmission potential plus a constant $c = 1.2$. The figure is analogous to figure 3 in the main text. Since no analytical solutions are available for the modified transmission potential we performed simulations to measure the approximate equilibrium mean and variance. Because of the higher computational demands we sampled $40'000$ random sets of parameter values from these restricted priors: $0 < \nu_e < 0.6; 0 < \mu_i < 0.3; 0 < \nu_i, \nu_t < 0.15$. For comparison, however, we plot the accepted parameters over the same range as in figure 3 in the main text.

References

1. Geskus RB, Prins M, Hubert JB, Miedema F, Berkhout B, et al. (2007) The HIV RNA setpoint theory revisited. Retrovirology 4: 65. doi:10.1186/1742-4690-4-65.

2. Easterling MR, Ellner SP, Dixon PM (2000) Size-specific sensitivity: Applying a new structured population model. Ecology 81: 694–708.

3. Ellner SP, Rees M (2006) Integral Projection Models for Species with Complex Demography. Am Nat 167: 410–428.

4. Coulson T, MacNulty DR, Stahler DR, vonHoldt B, Wayne RK, et al. (2011) Modeling effects of environmental change on wolf population dynamics, trait evolution, and life history. Science 334: 1275–8. doi:10.1126/science.1209441.

5. Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP (2007) Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. Proc Natl Acad Sci U S A 104: 17441–6. doi:10.1073/pnas.0708559104.

6. Yee TW (2013) VGAM: Vector Generalized Linear and Additive Models. R package version 0.9-2.