Effect of population patchiness and migration rates on the adaptation and divergence of vesicular stomatitis virus quasispecies populations

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The effect of migration among different isolated virus quasispecies populations on their adaptation and diversity was analysed through experimental evolution. An in vitro cell system was employed to simulate migration of vesicular stomatitis virus between isolated homogeneous host cell populations. The results clearly demonstrated a positive correlation between the migration rate and the magnitude of the mean fitness reached by the virus quasispecies populations. The results also showed, although less clearly, that fitness differences among quasispecies decreased with the magnitude of migration. These results are in close agreement with predictions of standard population genetics theory. These results can be explained in terms of the spread of beneficial mutations, originating in a single isolated quasispecies, through the entire system formed by the different quasispecies populations contained in different host cell populations.

Introduction

Evolution happens whenever allelic frequencies in populations move away from the pre-existing, Hardy–Weinberg, equilibrium. The main forces that break down this equilibrium and cause populations to evolve are natural selection, mutation, migration and random genetic drift (Hartl & Clark, 1988). In order to understand the evolutionary dynamics of RNA viruses, it is important to study the relative role that each of these factors plays in virus diversity and evolution. Recently, a large effort has been devoted to understanding some of them. For instance, the combined effect of random drift and the accumulation of deleterious mutations (Muller’s ratchet) was shown to be an important factor that decreases virus fitness and that might drive virus populations close to extinction (Chao, 1990; Duarte et al., 1992, 1993; Clarke et al., 1993; Novella et al., 1995b, 1996; Elena et al., 1996; Escarmís et al., 1996). Also, the effect of natural selection acting on newly arisen beneficial mutations has been determined to be a key factor in understanding the great adaptability of RNA virus quasispecies (Clarke et al., 1993, 1994; Elena et al., 1996, 1998; Novella et al., 1995a, 1999). From the opposite standpoint, virus populations (and, in general, microbial populations) have recently been employed as models to test classical predictions of evolutionary theory. However, no attention has yet been paid to the study of the role of migration among virus populations in the evolution of virus quasispecies.

The effect of population patchiness (i.e. compartmentalization of a large population in small, local populations) on the spread, fitness and variability of virus populations has not been thoroughly studied from an experimental point of view. A few studies have focussed on analysing the patterns of spatial variation in natural populations. For example, Rodríguez-Cerezo et al. (1991) sampled tobacco mild green mosaic tobamovirus from host populations that were either geographically separated or from small, contiguous areas. They found a rather homogeneous, genetically stable virus population, despite the geographical distances, reflecting strong migration and subsequent virus spread and homogenization. Similar results were found by Rodríguez-Cerezo et al. (1989) with pepper mild mottle tobamovirus. Nichol (1987, 1988) studied epizootic epidemics of the New Jersey serotype of vesicular stomatitis virus (VSV); his data were later analysed by Moya & García-Arenal (1995), who found that the level of differentiation among populations did not increase with time, reflecting limited isolation among populations and indicating that the virus population could be seen as panmictic because of easy dispersal. In contrast, Guerri et al. (1991) found a limited pattern of migration in citrus tristeza closterovirus, because the

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virus population was highly variable at the point where it was initially introduced, but this variability rapidly decreased away from this initial infection point. A similar pattern of limited migration was observed among geographically distant isolates of parainfluenza virus type 1 (Dave et al., 1997). Hepatitis G virus shows a more complex pattern of geographical distribution (Viazov et al., 1997). Because isolates from Africa and southern Asia clustered together in a phylogenetic tree, and isolates from Europe, America and central Asia join in a different cluster, no sign of migration of variants among these two clusters was apparent [see part VI of Gibbs et al. (1995) for an extensive list of related studies].

Compartmentalization must not only be understood at the macroscopic population level. It is also present within a single infected individual; different virus variants can be isolated from different tissues, reflecting the adaptation of quasispecies to local cellular conditions. From their original organ, viruses can diffuse to different organs already infected (or not) with another local variant by means of the blood or any other organic fluid. Once the variants come in contact, a competition for resources begins and the better-adapted always displaces the less fit. Empirical examples of this within-individual compartmentalization are not rare. Different distributions of human immunodeficiency virus type 1 (HIV-1) variants in individual patients were observed between blood and semen (Delwart et al., 1998; Zhu et al., 1996), blood and cervical secretions (Poss et al., 1995, 1998), blood and lungs (Ilescu et al., 1994), and among blood, lymph nodes, spleen, bone marrow, liver, testis, lung, brain and cerebrospinal fluid (van’t Wout et al., 1998; Wong et al., 1997), but the isolates from the same compartment always showed a greater identity among sequences. Similar results have been reported for simian immunodeficiency virus (Reinhart et al., 1998) and hepatitis C virus (Maggi et al., 1997; Navas et al., 1998).

The basic migration model

The model we have employed in order to make predictions about the effect of virus migration among isolated virus patches is an extension of the simple one-dimensional stepping-stone model proposed by Kimura & Weiss (1964), with two modifications: (i) our patches, instead of being linearly connected, were distributed in a circle (as in Fig. 2), eliminating the odd behaviour of populations at the edge, and (ii) we have allowed selection to act on any beneficial mutation that arises in any of the isolated quasispecies populations. The model starts by assuming that all isolated patches are initially infected by polymorphic quasispecies populations of equal composition and that, at a given moment, a beneficial mutation arises in a single isolated population and reaches a significant frequency within this population prior to migration. Hence, migration takes place only in a clockwise direction with a rate \( m \), and a sample of this beneficial mutation migrates to the next patch. After sufficient continuous migration, this beneficial mutation will spread throughout the entire set of patches (hereafter, metapopulation). Let us assume that, in the \( i \)-th isolated quasispecies population, a beneficial mutation appears and reaches a frequency of \( p_{i,t} \). At this moment, migration takes place, and a fraction \( m p_{i,t} \) of individuals carrying the beneficial mutation moves from the population and a fraction \( m p_{i-1,t} \) of individuals from the \( i-1 \)-th population, which also carries the beneficial mutation, is incorporated (obviously, at the beginning of the process, this fraction will be zero.) Similarly, a fraction \( m(1-p_{i,t}) \) of genotypes not carrying the beneficial mutation also moves away from the \( i \)-th population and a fraction \( m(1-p_{i-1,t}) \) is incorporated from the \( i-1 \)-th population. Once each individual population is founded by this mixture of genotypes, selection takes place by means of differential growth rates for each genotype. If the fitness of the genotype carrying the beneficial mutation is \( 1+s \), where \( s \) is a coefficient representing the selective advantage of the beneficial mutation, and the fitness of the wild-type genotype is 1, the frequency of the beneficial genotype, after migration and selection, can be obtained by means of the expression:

\[
p_{i,t+1} = (1+s)[(1-m)p_{i,t} + mp_{i-1,t}]/\bar{W}_{i,t}
\]

where \( \bar{W}_{i,t} = 1 + s(1-m)p_{i,t} + tsp_{i-1,t} \) represents the mean fitness of the virus quasispecies population resident in the \( i \)-th isolated patch at moment \( t \). The average fitness across patches can be computed at any moment as the arithmetic mean of the \( \bar{W}_{i,t} \) values. In a similar way, we can compute the variance (or any other measure of differences) for fitness among patches. As an illustration, Fig. 1 shows the trajectories for both mean fitness and standard deviation of fitness across patches for the three different migration rates employed below and for a number of selection coefficients. Several conclusions can be drawn from Fig. 1. (i) The mean fitness across patches increases with the intensity of migration as a consequence of the spread of (any possible) beneficial mutation through the entire metapopulation, reaching a maximum, stable value. (ii) The variance for fitness among patches decreases as the migration rate increases as a consequence of the mixing of genotypes. Without migration, a stable polymorphism can be attained. (iii) For a given migration rate, the maximum level of average fitness depends upon the magnitude of the selective advantage. (iv) For low migration rates and high selection coefficients, transient polymorphism can appear (middle-right panel), although in the long run, differences among patches tend to be eliminated, and even the most variable case is far less polymorphic than that obtained without migration.

This work analyses the effect of population subdivision and subsequent migration of complex mixtures of virus among different patches on the adaptability and diversity of the RNA virus VSV. In contrast with the indirect studies mentioned above, which used field data, employing an in vitro system, we have measured directly the effect of different migration rates on both overall virus fitness and variability in fitness. Does gene flow have any appreciable effect in the evolution of virus
Population subdivision in an RNA virus

![Graphs showing trajectories for mean fitness across populations and standard deviation for fitness.](image)

**Fig. 1.** Trajectories for mean fitness across populations (left column) and standard deviation for fitness (right column). Plots were obtained by using equation (1) and the following combination of parameters: (a) no migration, \( m = 0 \); (b) low migration, \( m = 0.05 \); (c) high migration, \( m = 0.5 \). For each migration rate, five different values for the coefficient of selection, \( s \), were also employed. In all cases, the initial frequency for the beneficial allele in the first population has been set to \( p_0 = 0.1 \).

Is the variability introduced by migration meaningful compared with the enormous amount of variability due to the high mutation rates of RNA viruses? These are the questions we want to address in this study.

**Methods**

- **VSV clones.** The two clones employed in the present study were derived from a clone of the Mudd–Summer strain of the VSV Indiana serotype, and have been propagated for many years under laboratory conditions.

One clone, MARM C, had an Asp\(^{359} \rightarrow \text{Ala} \) substitution in the G surface protein. This substitution allows the mutant to replicate under MAb 1\( \_1 \) concentrations that neutralize the wild-type clone (Vandepol et al., 1986). All the migration experiments were done with MARM C. The other virus clone employed was a MAb 1\( \_1 \)-sensitive, surrogate wild-type. This wild-type was employed as a common competitor during the competition experiments. To avoid any further genetic change in the wild-type clone, a large volume with a high titre (~ 10\(^{10} \) p.f.u./ml) was produced and kept at \(-80^\circ\text{C}\) in 1 ml aliquots.

The fitness of MARM C relative to the wild-type has been determined many times under conditions identical or nearly identical to those...
were tested experimentally: completely isolated quasispecies populations, and high genetic flow between quasispecies populations (among others: Holland et al., 1991; Duarte et al., 1992, 1993; Clarke et al., 1993; Miralles et al., 1997), as well as 15 times during the course of the present experiment, always giving a relative value of one (mean ± SEM of 1.000 ± 0.036; Student’s *t* = 0.0005, *P* = 0.9996). Thus, the mutation conferring the MARM phenotype can be considered to be selectively neutral.

**Cell lines and culture conditions.** Baby hamster kidney cells (BHK-K) were grown as monolayers under Dulbecco’s modified Eagle’s minimum essential medium (DMEM) containing 5% newborn calf serum and 0.06% proteose peptone 3. Cells were grown in 25 cm² plastic flasks for infections or in 100 cm² plates for routine maintenance.

The I₁ MAb employed in the competition experiments was produced and characterized by Lefrancois & Lyles (1982) and Vandepol et al. (1986). Professor John J. Holland kindly provided the hybridoma cells that produce this antibody. We propagated hybridoma cells in DMEM containing 20% bovine calf serum, 2 µg/ml thymidine, 0.1 µg/ml glycine and 14 µg/ml hypoxanthine in large flasks to produce many litres of high titre neutralizing MAb, which was stored at −20 °C until use. All cell lines were maintained in incubators at 37 °C and with a 5% CO₂ atmosphere.

**Experimental design.** Fig. 2 shows a schematic diagram of the experimental treatments. Basically, three different migration rates (*m*) were tested experimentally: completely isolated quasispecies populations (*m* = 0), low genetic flow between quasispecies populations (*m* = 0.05) and high genetic flow between quasispecies populations (*m* = 0.5). These three values were chosen to cover the widest possible range of parameters. Each flask simulates an isolated patch (either a host population or an organ within a body) containing about 7.5 × 10⁶ host cells. After 1 day of infection and completion of the cytopathic effect, the number of virus particles was about 5 × 10⁶ per ml.

On the first day, five flasks for each of the three migration rates were infected with equal samples of the original MARM C clone. These five isolated populations were ordered in a circle (metapopulation) and migration always occurred in a clockwise direction, from the *i*-th flask to the *i* + 1-th flask, at the rates indicated. The next day, and until the end of the experiment, a sample from each flask was taken and diluted 10⁻¹-fold in fresh DMEM. In order to infect the next day’s flask, we always added 200 µl diluted virus (i.e. 10³ viruses) to the fresh BHK-K confluent monolayer. In order to avoid the appearance of defective interfering particles, the m.o.i. under this regime of batch transfers was kept as low as ~0.013 virus per cell.

As shown in Fig. 2, the difference between the three migration regimes is based on whether the sample comes from the same *i*-th flask or from the migration of virus from the *i* − 1-th flask into the resident virus quasispecies population in the *i*-th flask. For the non-migration experiments (*m* = 0), the 200 µl sample for the *i*-th flask came from the previous day’s *i*-th flask. For the low genetic flow regime (*m* = 0.05), 190 µl came from the day before’s *i*-th flask and 10 µl immigrated from the *i* − 1-th flask. Finally, for the high migration rate regime (*m* = 0.5), 100 µl came from the *i*-th flask and another 100 µl immigrated from the *i* − 1-th flask.

After addition of the inoculum, flasks sat for 45 min inside the incubator to allow virus adsorption. After this time, inoculum was removed and fresh DMEM supplemented with 2% newborn calf serum was added.

Each evolution line was maintained for a total of 25 daily transfers, the equivalent of approximately 100 generations of RNA(+) mediated replication.

The basic design shown in Fig. 2 was replicated independently four times in order to have enough statistical power to discern any trends in the results. Hence, at the end of the experiment, we had: 4 replicated
experimental blocks $\times$ 3 migration rates $\times$ 5 flasks = 60 samples in which the fitness relative to wild-type was measured in triplicate assays.

**Relative fitness assays.** At the end of each evolution experiment, the resulting evolved MARM C populations were assayed for relative fitness in triplicate (Holland et al., 1991). Each derived MARM C quasispecies population was mixed, in three independent test tubes, with a known amount of wild-type virus and the initial ratio for each replicate mixture. $R_p$ was determined by plaque assays with and without MAB $I_1$ in the agarose overlay medium. Each competition mixture was serially transferred for a sufficient number of passages to obtain good estimates in the agarose overlay medium. Each competition mixture was serially transferred for a sufficient number of passages to obtain good estimates of relative fitness, as follows. At each transfer, the resulting virus mixture was diluted by a factor of 10$^4$ and used to initiate the next competition passage by infection of a fresh cell monolayer. The ratio of MARM C to wild-type was determined by plating with and without MAB $I_1$ in the overlay agarose medium at different transfers. These determinations gave the proportion of MARM C to wild-type at transfer $t$, $R_p$. Fitness was defined as $W = R_p/R_w$ (Chao, 1990) and obtained by fitting lnW to the time series data by the least-squares method (Sokal & Rohlf, 1995).

**Statistical analysis.** The linear model used to fit the data is a two-level nested analysis of variance (ANOVA) (model II) in which the variable ‘isolated patch’ is nested within ‘migration rate’, which is itself nested within the ‘experimental block’. In other words, a given fitness value ($W_i$) will be defined as the $l$-th fitness determination ($l = 1, 2, 3$) done for the $k$-th patch ($k = 1, 2, 3, 4, 5$) when the migration rate was the $j$-th ($j = 1, 2, 3$) and for the $i$-th replicated block ($i = 1, 2, 3, 4$). Formally, this is equivalent to the expression:

$$W_{ijkl} = \mu + B_i + M_j + D_{ik} + \varepsilon_{ijkl}$$

where $\mu$ is the overall mean fitness and $B$, $M$ and $D$ are, respectively, a block effect, a migration rate within blocks effect and a patch within migration rates within blocks effect. Finally, $\varepsilon_{ijkl}$ is the error term. Usually, it is assumed that $B$, $M$, $D$ and $\varepsilon_{ijkl}$ are normally distributed with mean zero and positive variances (Sokal & Rohlf, 1995). Because the model described in the Introduction makes directional predictions, a one-tailed test was employed when convenient. All statistical analyses described were done with the SPSS 8.0.1S for Windows package (Norusis, 1992).

**Results**

An SPSS data file containing the raw fitness values is available by anonymous ftp from the following address: ftp://serbio.uv.es/pub/incomming/elenal/entre demes.sav

Prior to further analysis, the assumptions of the ANOVA were tested. Neither the normality of data nor the homogeneity of variances among migration rates was fulfilled. However, a natural logarithm transformation of the data was enough to eliminate these problems. As the conclusions were not influenced by this transformation, we opted to report the analysis based on the non-transformed data in order to keep the meaning of the values presented clearer.

The first observation that arises from our results is that a significant increase in fitness was attained for all the evolved lines. This observation is in agreement with many previous reports where large populations of VSV allowed to evolve under batch culture conditions readily show an increase in fitness (Clarke et al., 1993; Elena et al., 1996, 1998; Novella et al., 1995a, 1999). In this case, the mean overall fitness after the evolutionary process, pooling together all the estimates, was $1.8565 \pm 0.0540$, which is significantly larger than the value reported above for the ancestral MARM C clone ($t_{195} = 13.2018$, 1-tail $P < 0.0001$).

**Among-patches migration rate has an effect on the overall fitness**

Table 1 shows the two-level nested ANOVA for fitness (Sokal & Rohlf, 1995). Three observations are important from this table. Firstly, it is possible to detect a slightly significant level of heterogeneity between the results obtained in each one of the four replicates ($P = 0.0343$). Despite the parallelism observed in the results from each line, it is logical to expect some degree of heterogeneity between blocks due to the random nature of the mutations that appeared independently in each replicate.

Secondly, a strongly significant effect of migration rate was detected ($P < 0.0001$). The final fitness reached under different migration regimes is clearly dependent on whether or not migration took place during the evolution of each quasispecies population. Migration, of course, allows any possible beneficial mutation that arose within an individual quasispecies population to spread throughout the metapopulation.

Thirdly, there is no significant overall effect among different patches within a metapopulation ($P = 0.4223$). Nonetheless, this is the expected result taking into consideration that one of

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate</td>
<td>34.0414</td>
<td>3</td>
<td>11.3471</td>
<td>4.7703</td>
<td>0.0343</td>
</tr>
<tr>
<td>Migration rate within replicate</td>
<td>19.0297</td>
<td>8</td>
<td>2.3787</td>
<td>9.4769</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Patch within migration rate</td>
<td>12.0496</td>
<td>48</td>
<td>0.2510</td>
<td>1.0398</td>
<td>0.4223</td>
</tr>
<tr>
<td>Error</td>
<td>28.9651</td>
<td>120</td>
<td>0.2414</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The fitness value obtained in the non-migration regime was 100 obtained for the fitness observed under each adaptation of the virus to the batch culture conditions, we can express the fitness gain due only to migration took place as the fitness gain due only to migration. By conducting an independent analysis for each different migration rate, we can gain more insight into this question (see below).

Virus fitness increases with the intensity of migration

In order to gain more insight into the effect of migration on the fitness of virus metapopulations, we computed the mean fitness for each of the three migration rates and each of the four replicated blocks (3 rates × 4 replicates = 12 mean values). Taking the mean fitness obtained for the case where no migration took place as the fitness gain due only to the adaptation of the virus to the batch culture conditions, we can express the fitness observed under each m > 0 as a percentage reflecting the excess in fitness gain relative to the fitness obtained for m = 0:

$$100 \times \frac{[\bar{W}(m)] - W(0)]}{[\bar{W}(0)]} \quad (2)$$

Fig. 3(a) shows the mean (± SEM) of the transformed data. The fitness value obtained in the non-migration regime was $1.5510 \pm 0.2275$; the value obtained for the low-migration regime was $1.8389 \pm 0.1597$, whereas it was $2.1721 \pm 0.3952$ for the high-migration regime. Hence, on average, the increase in fitness due to migration was about 18.6% when migration occurs at low frequency ($m = 0.05$), whereas this percentage increased to 40.1% for high migration rates ($m = 0.5$). A Spearman’s correlation test (Sokal & Rohlf, 1995) showed a significant correlation between the magnitude of the fitness gain and the magnitude of the migration rate ($r_s = 0.8126$; 10 degrees of freedom, 1-tail $P = 0.0007$): as the migration rate increases, so does the mean fitness reached by the metapopulation. The reason for this correlation, as stated above, is that a high migration rate allows a beneficial mutation to spread more easily from the isolated quasispecies population where it appeared to the entire metapopulation.

Among-patches variability decreases with the intensity of migration

Following Sokal & Rohlf (1995), we obtained the among-patches variance for fitness. Once we got these values, we followed the same logic as in the previous paragraph, re-scaling the variances by that estimated for $m = 0$:

$$100 \times \frac{[\bar{W}(m)] - W(0)]}{[\bar{W}(0)]} \quad (3)$$

Similarly to the previous case, the meaning of this relative among-patches variance is straightforward: the reduction in the differences among quasispecies populations induced by migration relative to the maximum possible differences (i.e. no migration). Fig. 3(b) shows the mean (± SEM) of these data. Despite the fact that none of these among-patches variances was significant ($P > 0.05$ in all cases), a Spearman’s test revealed a significant negative correlation between migration rate and the among-patches variance in fitness ($r_s = -0.5718$; 10 degrees of freedom, 1-tail $P = 0.0260$). As predicted by the model, the larger the migration rate, the smaller the differences among quasispecies populations.

Discussion

This is the first report that analyses directly the effect on fitness of migration among isolated virus populations. The first important conclusion from the present work is the close agreement between predictions well known from standard population genetic theory and our experimental results. We have detected significant effects of migration on both the magnitude of fitness attained by the entire metapopulation and the degree of differentiation between quasispecies populations contained within different patches. The larger the rate of migration, the higher the mean fitness value reached. In contrast, the larger the migration rate, the lower the variance for fitness obtained among quasispecies populations resident in different patches. These two observations are a consequence of the same phenomenon, the spread of genotypes carrying beneficial mutations throughout the entire metapopulation. When a beneficial mutation appears in an isolated quasispecies
population, it reaches fixation within that patch with a speed proportional to the advantage it provides to the molecules bearing it. Once the mutation is fixed, the mean fitness of this population has increased from 1 to the final value of 1 + s. However, the viruses in the remaining patches do not benefit from the presence of this mutation and their fitness remains around 1 (this is true unless some different beneficial mutation increases their fitness to 1 + s', where s ≠ s'). Hence, the average fitness of the entire metapopulation will be much smaller than 1 + s. However, if migration takes place, the genotypes carrying the beneficial mutation will spread to the neighbouring patches, making their fitnesses increase as well. Furthermore, the mean fitness across patches will also increase to a value near 1 + s.

It is important to point out here that we have been able to observe these correlations when data from many different experiments were pooled together. It is not appropriate to do the same analysis based on data from single experiments. The reason for this is that different mutations, with different fitness effects and arising at different moments in each experiment, would or would not generate significant correlations. For example, if a mutation with an extremely beneficial effect on fitness arises early in the experiment when m = 0.05, it will spread through the metapopulation by the end of the experiment despite the low migration rate, hence, pushing up mean fitness across patches. In contrast, imagine that for the same experimental block, the beneficial mutation that arises in the m = 0.5 regime has a small effect (or, alternatively, appears late in the experiment); it will not be spread to the entire metapopulation by the end of the experiment and, hence, the mean fitness across patches will be smaller than the value obtained for m = 0.05. In the long run, however, for a given distribution of mutation effects and times of appearance, the larger the migration rate, the faster it will spread across patches and, consequently, the higher the average fitness at the end of a fixed number of generations.

Putting these results into the biological ‘ballpark’

The choice of migration rate values in our experiment might be considered arbitrary and, perhaps, far from those expected to be important in the real world. It would be desirable to test many more migration rates, but the size of the experiment would then make it impractical. However, in order to put our conclusions in a context of real parameters, we can carry out the following thought-experiment. Imagine an individual infected by a variant of any virus. This individual moves into a population where the resident variant is different. Assuming that the virus load per infected individual is a constant, then the rate of migration is \( m = \frac{1}{N} \), where \( N \) is the host population size. Clearly, for populations of moderate size, the migration rate is well below our lowest value of \( m = 0.05 \). The problem is still the lack of information about a true value for the migration rate in natural populations. In a pioneering study done by Grassly et al. (1999) with HIV-1, the migration rate has been indirectly inferred from sequence data to be as high as \( m = 0.0039 \) per day (averaging estimates from subtypes A and B and \( env \) and \( gag \) genes). Putting this value into equation (1), and using a selective advantage for the immigrant of \( s = 0.05 \), we get an average fitness, after 100 generations, of 1.0270, with an among-patches standard deviation of 0.0206. Thus, compared with the case of no migration, the gain in fitness due to migration is 1.08% (equation 2), and the reduction in variability among patches is 7.84% (equation 3). To take a more extreme example, for a host population with \( N = 10^6 \) (i.e. \( m = 10^{-6} \)) and the same value for \( s \) as above, the average fitness is 1.01003 (0.003%), with a standard deviation of 0.0223 (0.45%).

A completely different picture can be drawn for migration among tissues within an infected individual. In this case, it is logical to believe that migration rates must be much higher. However, in contrast to the growing experimental evidence for quasispecies heterogeneity among tissues and organs, the proper analysis to infer gene flow is still under way. Nevertheless, qualitative conclusions about the magnitude of within-patient migration can be drawn. Delwart et al. (1998) analysed HIV-1 clones isolated from peripheral blood mononuclear cells (PBMC) and non-spermatozoal semen mononuclear cells (NSMC) from five patients. In all five patients analysed, they found that isolates from both cell types clustered together significantly, indicating a continuous incorporation of variants from one tissue into the other. Furthermore, some significant clusters were formed exclusively by isolates from NSMC, others by a majority of isolates from NSMC, but also containing a few PBMC isolates. This last observation could be a reflection of the role played by blood cells in the spread of variants through the entire body. Similar results were obtained by van’t Wout et al. (1998), also with isolates of HIV-1 from different organs. They also found significant clusters containing isolates from different tissues. Poss et al. (1998) made an excellent and detailed study of the degree of differentiation between HIV-1 isolated from PBMC and cervical secretions. They found a significant phenetic structure for sequences from these two cellular origins. In addition, for all the patients analysed, they found that variants isolated from mucus at any time-point were different from samples taken at the preceding time-points, indicating a continuous input of variants from a different reservoir. Comparable results have also been reported by Ball et al. (1994), Hughes et al. (1997) and Wong et al. (1997). All of these examples clearly suggest that the within-patients migration rate among organs should be quite large and within the range of values employed in our study.

Migration and degree of virulence

Without migration, the virus quasispecies populations resident in a given patch will develop an intermediate level of virulence that will be the result of a balance between the virus’
necessity for fast replication and the fact that it is damaging its food supply by killing host cells (Levin & Pimentel, 1981; Bremermann & Pickering, 1983; Frank, 1992; Antia et al., 1994). Migration implies that single host populations will be invaded by new, different virus genotypes. Competition for the limited number of host cells will occur between the immigrant and the resident virus quasispecies populations. Under this situation, virulence levels increase and the virus strain with the higher basic reproductive rate will overtake the less-virulent strains (Bremermann & Thieme, 1989; Frank, 1992; Nowak & May, 1994; Mosquera & Adler, 1998). These two predictions of the ecological theory for evolution of host–parasite systems are also confirmed by our results: a higher level of virulence was obtained when migration took place, mixing genotypes, whereas a lower level was attained without migration.

This effect of pathogen migration can also be employed to explain why, in human infections, person-to-person transmission favours less-virulent variants than, for instance, waterborne or airborne transmission (Ewald, 1994). Person-to-person transmission will be equivalent to a low migration rate, since an extremely harmful pathogen will affect its host’s ability to move and hence the possible number of contacts and the spread of the infection. In contrast, water or airborne transmission will be equivalent to a high migration rate, since they do not depend on the effect of the parasite on the motility of the host and will occur at a host-independent rate.

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