The effect of co- and superinfection on the adaptive dynamics of vesicular stomatitis virus

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Abstract

In many infectious diseases, hosts are often simultaneously infected with several genotypes of the same pathogen. Much theoretical work has been done on modelling multiple infection dynamics, but empirical evidences are relatively scarce. Previous studies have demonstrated that coinfection allows faster adaptation than single infection in RNA viruses. Here, we use experimental populations of the vesicular stomatitis Indiana virus derived from an infectious cDNA, to show that superinfection dynamics promotes faster adaptation than single infection. In addition, we have analysed two different periodicities of multiple infection, daily and separated 5 days in time. Daily multiple infections allow higher fitness increases than multiple infections taking place every 5 days. We propose that the effect of superinfection on fitness is mainly influenced by the time elapsed between the first and the second infection, since shorter time intervals offer more opportunities to competition between resident and invading populations.

Keywords: Coinfection; Superinfection; Experimental evolution; Vesicular stomatitis Indiana virus; RNA viruses

1. Introduction

In natural systems, parasite infections are usually a mixture of different genotypes (Read and Taylor, 2001). Several theoretical works have modelled the evolution of infectious diseases under multiple infection dynamics (Nowak and May, 1994; May and Nowak, 1995; Mosquera and Adler, 1998; Saldaña et al., 2003). Experimental studies employing several parasitic models have shown that the outcome of infection dynamics depends on initial conditions, such as the relative frequency at inoculation or, for superinfection, the temporal spacing and order of inoculation (Berchieri and Barrow, 1990; Hart and Cloyd, 1990; Taylor et al., 1997; Lipsitch et al., 2000). It is well-known that the transmission rates of individual strains can be reduced by intra-host competition, especially when they are introduced into an already infected host (Read and Taylor, 2000). In this sense, some experimental studies have shown that avirulent lines are capable of overgrowing or excluding virulent genotypes when the avirulent line is administered first or at high frequency in the inoculum (Berchieri and Barrow, 1990; Sernicola et al., 1999; Read and Taylor, 2000).

RNA virus populations are extremely heterogeneous due to their high mutation rates, resulting in the emergence of infections with multiple coexisting viral strains (reviewed in Domingo and Holland, 1997). Viral density is initially low with respect to the density of available hosts and thus, single infections prevail (each host is infected by one strain at a time), whereas later on, multiple infections can occur. Coinfections take place when different viral strains infect the same multicellular host simultaneously, whereas superinfection occurs when an infected host undergoes a secondary infection. The same scheme applies at the inter-host level or for cells at the intra-host level.

Multiple infections are common among viral infections and are important for adaptive dynamics. For instance, Wang et al. (2000) reported mutualistic interaction between coinfecting strains of human immunodeficiency virus type 1 (HIV-1) subtype B. Multiple infections can also involve different viral species, as is the case of hepatitis C virus, which can coexist...
with other viruses, as HIV-1 (Laskus et al., 2000), or hepatitis B virus (Dimitrakopoulos et al., 2000). Sometimes the presence of one virus appears not to modify the virulence of the other (Gobbi et al., 2000), whereas in other cases it seems to enhance (Lee et al., 2002) or repress (Schuttler et al., 2002) the virulence of the other.

Since multiple infections promote competition between the different strains, viral populations reach higher fitness than in single infections, as shown in several experimental evolution studies (Clarke et al., 1994; Miralles et al., 2001; Cuevas et al., 2003). Using hematopoietic necrosis (IHNV) and pancreatic necrosis (IPNV) viruses, Alonso et al. (1999) showed that coinfections resulted in a systematic displacement of the necrosis (IPNV) viruses, whereas when cells were first infected with IHNV and later superinfected with IPNV, both viruses coexisted without loss of productivity. A related experimental approach was carried out by Miralles et al. (2001) using vesicular stomatitis virus (VSV). In this work, experimental populations were evolved under single infections, as well as coinfections and superinfections with a time delay of 6 h between the first and the second infection. Populations evolved under coinfection regimes showed higher fitness than populations evolved under single infections. In contrast, superinfection did not produce any significant effect on fitness compared to single infection. However, the question arises whether the time delay might not be short enough to permit efficient propagation of the invading viral strains, thus failing to establish an effective competition between strains. This latter possibility is supported by a study carried out with the basidiomycete fungus Mycrobotrium violaceum (Hood, 2003), which concluded that generally, the genotype inoculated first had a highly significant advantage, although multiple genotypes were detected in a minority of cases.

Here, we were interested in assessing whether superinfection dynamics had a significant effect on the evolution of RNA viral populations. To do so, we reduced the time delay with respect to Miralles et al. (2001) work from 6 to 3 h; thus increasing the probability that invading viruses could effectively compete with the resident population. In our experiment, large populations sizes were employed to start each infection passage and hence, competition events among viral strains might tend to fix the fittest ones (Miralles et al., 1999). RNA virus populations show very fast rates of evolution in constant environments (Novella et al., 1995). Moreover, it is important to remark that negative-sense RNA viruses show rates of homologous recombination very much lower than those of mutation and hence, can be considered as effectively asexual (Chare et al., 2003). Therefore, we can discard recombination between resident and invading populations in our experimental setting. Finally, we extended previous work by studying two periodicities of infection: daily multiple infections, as done by Miralles et al. (2001), and a 5-day periodicity. The results confirmed that populations undergoing coinfection showed higher fitness than populations undergoing single infection or superinfection and, more importantly, showed that superinfection promotes higher fitness than single infections. Finally, we also detected a significant effect of the periodicity of infection.

2. Materials and methods

2.1. VSV populations

Viral strains employed in this study were obtained from a full-length infectious VSV cDNA clone, a chimeric genome which consisted of partial sequences from different strains (Whelan et al., 1995). Specifically, we employed two genotypes provided by Sanjuán et al. (2004a): a wild-type genotype (Whelan et al., 1995) and a mutant genotype surrogate MARM RSV. This mutant presents substitution A-3853 → C in the positive strand (Asp-259 → Ala in the G surface protein), which confers the ability to grow in the presence of monoclonal antibody 11 [mAb-resistant mutant (MARM) phenotype] at a concentration that inhibits wild-type growth (Vandepol et al., 1986). Evolution experiments were carried out by employing MARM RSV genotype, whereas the wild-type genotype was used as a common competitor in the competition assays. The MARM RSV has previously shown to be selectively neutral relative to the wild-type (Sanjuán et al., 2004a, 2004b).

To ensure that most of the variability was generated during the experimental passages and was not already present at the beginning of the experiment, the ancestral MARM RSV obtained from transfection of the cDNA clone was amplified in a 96-well plate with ~10^4 BHK-21 cells, thus producing less than a viral generation, and then aliquoted and kept at −80 °C.

2.2. Cell lines and culture conditions

Baby hamster kidney cells (BHK-21) were grown as monolayers in Dulbecco modified Eagle’s minimum essential medium (DMEM) containing 10% heat-inactivated foetal calf serum. Cells were grown in 24-well plates (containing 0.5 mL of medium) for infections, and in 100 cm² petri plates (containing 15 mL of medium) for routine maintenance. Cell cultures were maintained in incubators at 37 °C and with 5% CO₂ atmosphere.

2.3. Experimental evolution

Each 24-well plate mimicked a metapopulation and each single well containing ~2 × 10⁵ cells represented a single host. For each treatment, the host population size (i.e. the number of wells) was 23. Each well was labelled with identification (f), and transfer (n) numbers. The 24th well was seeded with cells but not infected, acting as a control for the absence of cross contamination between wells.

The experiment was initiated by infecting the first set of 24-well plates with ~2 × 10⁴ MARM RSV plaque forming units (pfu) per well (multiplicity of infection 0.1). This starting viral population was not previously adapted to the laboratory environment. We thus incubated for 48 h post-infection before sampling viruses to allow completion of the cytopathic effect, which resulted in a final titre of ~1.0 × 10⁵ pfu/mL. The well denoted (f, n) was sampled and diluted properly to infect well (f, n + 1) with ~2.0 × 10⁴ MARM RSV pfu, and so on. For the
case of no migration (single infection), the well ($f, n + 1$) was infected with viruses from well ($f, n$). For coinfection, well ($f, n + 1$) was infected with viruses from wells ($f, n$) (resident virus) and ($g \neq f, n$) (immigrant virus), $g$ being randomly chosen without replacement from the 23 wells of a given plate (i.e. metapopulation). Analogously, for superinfection, well ($w, n + 1$) was infected with viruses from well ($w, n$) (resident virus) and after a 3 h incubation, viruses from well ($g \neq w, n$) were added.

In all, five different transfer regimes were carried out by using ten 24-well plates (two replicates per treatment). The first treatment corresponded to single infection, with no migration between wells. There were two different coinfection treatments; one followed a daily periodicity of coinfection ($p1$), whereas the other followed a 5-day periodicity ($p1/5$). Finally, there were two different superinfection treatments, corresponding to each of the two periodicities. The migration frequency (proportion of immigrant to resident population) was 0.05 for both coinfection and superinfection treatments. The five transfer procedures were maintained for a total of 25 passages, the equivalent of $\sim 100$ viral generations (Miralles et al., 2000).

2.4. Relative fitness assays

After the completion of the evolution experiment, competition assays were carried out as previously described (Holland et al., 1991). Briefly, three replicates of each evolving MARM RSV population were each mixed with a known amount of wild-type, and used to infect a cell monolayer. Analogous to the evolution experiment, infection was allowed to proceed for 48 h. Then, the resulting population was suitably diluted and used to initiate the next competition passage by infecting a fresh monolayer with $\sim 2.0 \times 10^6$ pfu/well. The ratio of MARM RSV to wild-type was determined by plaque assays with and without monoclonal antibody I1 in agarose overlay medium. These determinations gave the proportion of MARM RSV to wild-type at transfer $t$, $R_t$. Two competition passages were carried out for each fitness assay. The fitness of MARM RSV relative to wild-type was estimated as $W = 1 + (b/\ln D)$, where $b$ is the regression coefficient of $\ln R_t$ against days of transfer and $D$ is the dilution factor (Levin et al., 2000).

3. Results

Normality and homocedasticity were confirmed for each dataset (data not shown). As expected, a significant increase in average relative fitness was observed in all infections dynamics with respect to the ancestral population (Fig. 1).

Initially, we assessed whether periodicity had an effect on the experimental evolution dynamics. We detected a significant effect of periodicity (Table 1) as well as a significant interaction between periodicity and treatment (coinfection or superinfection), suggesting that differences between treatments depended on periodicity. Therefore, subsequent data analyses were carried out separately for each periodicity.

3.1. Periodicity 1/5

We detected a significant effect of the mode of multiple infection (Fig. 1, nested ANOVA, $F = 128.738$, $p = 0.001$), whereas there was no significant effect of the experimental replicate ($F = 1.656$, $p = 0.180$) or the viral isolate (i.e. well) ($F = 0.830$, $p = 0.887$). Specifically, viral populations undergoing coinfection dynamics showed marginally higher fitness than populations undergoing superinfection ($F = 17.038$, $p = 0.054$) with no replicate ($F = 0.867$, $p = 0.424$) or isolate effects ($F = 0.786$, $p = 0.898$). More interestingly, superinfection populations showed higher fitness than single infection populations ($F = 128.825$, $p = 0.008$), although there was some replicate effect ($F = 3.283$, $p = 0.042$), but no isolate effect ($F = 1.009$, $p = 0.473$).

3.2. Periodicity 1

Again, there was a significant effect of the mode of multiple infection (nested ANOVA, $F = 89.625$, $p = 0.002$), but not of treatment ($F = 0.054$). Isolate ($F = 0.424$) and replicate ($F = 0.887$) effects were not significant, although isolate ($F = 0.424$) and replicate ($F = 0.887$) effects were not significant. Isolate ($F = 0.424$) and replicate ($F = 0.887$) effects were not significant.

Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>d.f.</th>
<th>$F$</th>
<th>$p$</th>
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</thead>
<tbody>
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<td>Treatment</td>
<td>0.938</td>
<td>1</td>
<td>63.903</td>
<td>0.001</td>
</tr>
<tr>
<td>Periodicity</td>
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<td>1</td>
<td>47.847</td>
<td>0.002</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.546</td>
<td>1</td>
<td>37.190</td>
<td>0.004</td>
</tr>
<tr>
<td>Replicate</td>
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<td>4</td>
<td>11.251</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isolate</td>
<td>0.230</td>
<td>176</td>
<td>0.892</td>
<td>0.804</td>
</tr>
<tr>
<td>Error</td>
<td>0.538</td>
<td>368</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The first two factors were factorial (thus having an interaction term) and fixed. The factor “replicate” (random) was nested within the interaction term. The factor “isolate” (random) was nested within “replicate”. Type III sums of squares (SS), degrees of freedom (d.f.), $F$-statistics and null-hypothesis probabilities ($p$) are shown.
the isolate ($F = 1.208$, $p = 0.098$). Moreover, a significant effect of the experimental replicate was detected ($F = 24.298$, $p < 0.001$). Coinfection dynamics promoted higher fitness increases than superinfection dynamics ($F = 52.411$, $p = 0.019$) with replicate ($F = 33.715$, $p < 0.001$) but no isolate effects ($F = 1.259$, $p = 0.098$). Superinfection dynamics showed higher fitness than single infections ($F = 46.219$, $p = 0.021$) with again, replicate ($F = 12.065$, $p < 0.001$) but no isolate effect ($F = 1.141$, $p = 0.228$).

In sum, coinfection promoted higher fitness increases than superinfection, which in turn promoted higher fitness increases than single infections (Fig. 1). Finally, notice that periodicity remarkably affected the magnitude of fitness improvement (Fig. 2). There was a positive correlation between periodicity and fitness for both types of infection, the correlation being zero, coinfection would take place, yielding the maximum fitness (Saldan˜a et al., 2003). Although some studies have shown that superinfection dynamics can involve a benefit for viral populations, coinfection generally represents the best option for maximizing viral competition for host resources. Whereas coinfection enables the effective contribution of the whole genetic pool to viral adaptation, cellular and viral factors impede this contribution in superinfection dynamics.

Finally, our model system does not allow testing the effect of immune system, which is especially important for superinfection dynamics. Immune-driven selection could be considered as the major factor encountered by viruses infecting vertebrates. We can though speculate that, since immune system response triggers a secondary response off, it would mainly restrict infection by invading viral populations and therefore, differences between superinfection and coinfection dynamics should be even more stressed in real infections than in our experimental system.

Acknowledgements

We are grateful to Dr. Santiago F. Elena for comments, discussions and critical reading of the manuscript. We thank R. Martínez for technical assistance. This study was financially supported by the Spanish Ministerio de Ciencia y Tecnología.
(grant BFU2005-00503) and the Wellcome Trust (grant 071979) to A.M. J.M.C. and R.S were funded by the Wellcome Trust and C.S.I.C., respectively. F.Y.C. enjoyed a predoctoral fellowship from the Universitat de València.

References


