

A Linear Relationship between Fitness and the Logarithm of the Critical Bottleneck Size in Vesicular Stomatitis Virus Populations[∇]§

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We explored the relationship between fitness change and population size during transmission in vesicular stomatitis populations of very high fitness. The results show a linear correlation between the logarithm of the critical bottleneck size (population size at which there are no significant fitness changes after 20 passages) and the initial fitness of the population. In addition, limits to fitness increases during large-population passages of very-high-fitness strains were abolished by increasing the population size during transmission, indicating that beneficial variation is still available in these populations.

Viral evolution is the result of selection and random drift operating on the genetic variation that arises during replication. Random drift dominates during bottleneck transmissions because sampling effects lead to the loss of beneficial mutations or the fixation of deleterious mutations. Bottlenecks are common during natural infections, particularly among respiratory viruses. In an earlier report, we found a correlation between the fitness of vesicular stomatitis virus (VSV) strains and the critical bottleneck size (CBS) in low-fitness populations subjected to repeated severe bottlenecks (transmission sizes of 2 to 30 PFU) (8). We defined the CBS as the number of virus particles used for transmission that resulted in no net fitness changes at the end of 20 passages. Later, we and others reported that limits to fitness gains (and fitness losses) during large-population passages were consistent with a linear relationship between initial fitness and the logarithm of the CBS (9, 10). Miralles et al. (6) argued against this relationship and proposed that limits to further fitness gains reflected the exhaustion of beneficial variation. Their main criticism was that a linear relationship observed at small bottleneck sizes could not be extrapolated to bottleneck sizes that were several orders of magnitude larger. To resolve this question, we tested whether the relationship between the logarithm of the CBS and fitness was still linear for strains with high fitness.

We generated several VSV populations with fitness levels between 5.5 and 15 in BHK-21 cells (Table 1) through 35 to 50 large-population passages (2×10^5 PFU/passage unless otherwise indicated) of monoclonal antibody-resistant mutant (MARM) U, a strain that has neutral fitness compared to the wild type (wt) and differs from the wt only in a genetic marker that provides resistance to monoclonal antibody II (5). Each strain was obtained from an independent replica of MARM U

passages except strain Marilyn, which is the progeny of Bonnie after 20 additional passages at 2×10^4 PFU/passage, and Victoria, which is the progeny of Marilyn after 20 additional passages at 4×10^6 PFU/passage. All passages were done at a constant multiplicity of infection of 0.1 PFU/cell by using flasks of the appropriate size for each population size; for all passages, the number of cells infected equals the number of PFU used for infection. For each population, we carried out six replicas of 20 passages at the expected CBS, and for some of them, we also carried out passages at transmission sizes below or above the expected CBS (Table 1). We determined the fitness of the progeny for each replica in competitions between the test MARM virus and reference wt as previously described (5). For each competition, we mixed the test mutant and wt (2×10^5 PFU), and we infected a monolayer of BHK-21 cells at a multiplicity of infection of 0.1 and incubated the flask for 20 to 24 h at 37°C. We then determined the MARM/wt ratios before (R0) and after (R1) competition by using triplicate plaque assays in the presence and absence of monoclonal antibody II, and we calculated fitness by dividing R1 by R0. Each fitness value was calculated as the average of three to six independent determinations.

Our results showed that the correlation between the logarithm of the CBS and fitness was linear within the range of initial fitness from previous work and for the strains reported here (Fig. 1) (for a complete data set, see Table S1 in the supplementary material). Furthermore, as predicted, passages at population sizes below the CBS consistently led to fitness losses, with two exceptions: the fitness loss in strain Marilyn was not significant after correction for multiple testing ($P = 0.09$), and strain Bob gained fitness at and below the predicted CBS. These results confirmed our suggestion that sampling effects influence virus evolution at large-population transmissions if viral fitness is sufficiently high. Furthermore, Marilyn had significant fitness gains at a transmission size 20-fold larger than the CBS. Thus, beneficial variation was still present even for strains such as the high-fitness strain.

In this work, we defined the CBS based on the (lack of) fitness change after 20 passages. We have previously shown that the fitness change during the first 20 passages was domi-

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TABLE 1. TPS used for the different VSV strains included in this study

TPS ^a	Relative size for strain ^g							
	MARM N (0.38 ± 0.01)	MARM U (1.05 ± 0.08)	MARM X (3.05 ± 0.04)	MARM U35 (5.5 ± 0.3)	Marco (6.8 ± 0.8)	Bonnie (8.0 ± 1)	Marilyn (10.7 ± 0.8) ^f	Victoria (15.4 ± 1.4)
Above CBS	5 ^b	100 ^d					4 × 10 ⁶	
At CBS	2 ^b	4 ^d or 5 ^{b,e}	30 ^b	3 × 10 ^{2d}	5 × 10 ³	1 × 10 ⁴	2 × 10 ⁵	
Below CBS		1 ^c	5 ^b	30 ^d		1 × 10 ³	2 × 10 ⁴	2 × 10 ⁵

^a Number of PFU during transmission relative to the predicted CBS.

^b Data from reference 8.

^c Data from reference 7.

^d Data from reference 4.

^e The same population size used for MARM U was tested in another strain, MARM C (0.91 ± 0.03).

^f The same population sizes used for Marilyn were tested in another strain, Bob (10.7 ± 1.5).

^g The values in parentheses below each strain indicate the average fitness of the population before passing.

nated by the sampling of preexisting variation (4). Therefore, the fitness increases we observed were conceivably due to beneficial mutations carried over from the previous passages. However, it is also possible that these mutations were generated de novo during replication and sampled when the population size at transmission was increased sufficiently. Finally, more-complex interactions among the larger number of variants during replication at higher transmission population sizes (TPS) may contribute to the overall fitness increases.

We do not have a solid explanation for why strain Bob behaves differently from the rest of the strains. One possibility

is that Bob is located on a different fitness peak than the other strains (3), and its beneficial mutation rate is increased. Bonnie, Marilyn, and Victoria are a continuation of the same replica of passages, and it is reasonable to propose that they are all in different sites of the same fitness peak. In contrast, of all the high-fitness populations, only Marco and Bob were generated independently. Nevertheless, the predictions of the model were generally good for all the strains in the data set, suggesting that displacement into different fitness peaks is probably infrequent.

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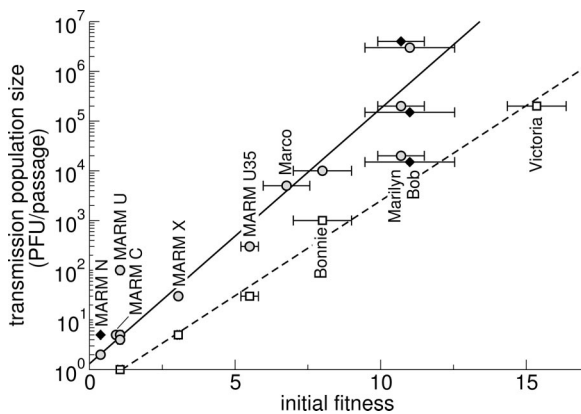


FIG. 1. TPS versus initial fitness of the VSV strain. Symbols represent the fitness change after 20 passages: open squares, significant fitness loss; gray circles, no significant fitness change; filled diamonds, significant fitness gain. Data for Bob are moved slightly to the lower right because otherwise they overlap with data for Marilyn. TPS is the CBS for data represented as gray circles, because those correspond to regimens in which there was not a fitness change. We set our significance level to an α value of 0.05 and corrected for multiple testings using the false-discovery-rate correction (1, 2). The solid line is the best linear fit of initial fitness versus log transmission size for gray circles, and the dashed line represents the same for open squares.