

## Size of Genetic Bottlenecks Leading to Virus Fitness Loss Is Determined by Mean Initial Population Fitness

ISABEL S. NOVELLA,<sup>1</sup> SANTIAGO F. ELENA,<sup>2</sup> ANDRES MOYA,<sup>2</sup> ESTEBAN DOMINGO,<sup>3</sup>  
AND JOHN J. HOLLAND<sup>1\*</sup>

*Department of Biology and Institute for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0116,<sup>1</sup> and Departament de Genetica and Servei de Bioinformatica, Facultat de Biologia, Universitat de Valencia, 46100 Burjassot, Valencia,<sup>2</sup> and Centro de Biologia Molecular “Severo Ochoa” (Consejo Superior de Investigaciones Cientificas, Universidad Autonoma de Madrid), Cantoblanco, 28049 Madrid,<sup>3</sup> Spain*

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**Genetic bottlenecks are important events in the genetic diversification of organisms and colonization of new ecological niches. Repeated bottlenecking of RNA viruses often leads to fitness losses due to the operation of Muller’s ratchet. Herein we use vesicular stomatitis virus to determine the transmission population size which leads to fitness decreases of virus populations. Remarkably, the effective size of a genetic bottleneck associated with fitness loss is greater when the fitness of the parental population increases. For example, for starting virus populations with low fitness, population transfers of five-clone-to-five-clone passages resulted in a fitness increase. However, when a parental population with high fitness was transferred, 30-clone-to-30-clone passages were required simply to maintain fitness values.**

Genetic bottlenecks are responsible for founder effects. The founder effect is a bias in the genetic differences between populations which stem from the original founders of the population. Founder effects can be very important in the evolution of species (12, 21, 23, 26, 35). Consequences of the genetic drift which accompanies bottleneck transmission include temporal loss of polymorphism (1, 3, 25, 27, 30) and fixation of alleles independently of their selective value (24, 28). When followed by geographical isolation, bottlenecks might be an important factor in speciation (14, 21, 29, 34). Genetic bottlenecks accelerate the gradual accumulation of deleterious mutations in the absence of recombination, an effect known as Muller’s ratchet (11, 18, 20, 22, 23). When mutation rates are high, sampling of a small subpopulation frequently leads to loss of the more fit and more common genomes (master sequences). If most mutations are deleterious (10), repetitive, progressive loss of more fit genomes should often result in fitness declines (Muller’s ratchet). Muller’s ratchet has been shown to cause significant fitness loss in unicellular organisms (2) and RNA viruses (4, 9). Fitness is a complex parameter, difficult to measure for sexual organisms. However, RNA viruses, by virtue of their high mutation rates (8, 31) and short genome doubling times, represent excellent objects to quantitate fitness variation. For viruses replicating in a defined environment, fitness can be equated to overall replicative ability to produce infectious progeny, and growth competition experiments reproducibly measure relative fitness and fitness variation (4, 17). Fitness losses due to bottleneck events can be counterbalanced by subsequent transmission of large populations. This has been suggested by several theoretical studies (13, 15, 19, 22, 32) and verified experimentally using vesicular stomatitis virus (VSV) (6, 17). Here we study the transmission size (number of infectious particles sampled from a virus population) which, upon repeated transfers, avoids loss of viral fitness. What is likely most important in observing a deleterious effect of genetic bottle-

necks on virus fitness is the probability that the transmission population has lower fitness than the parental population from which the transmission sample is taken. As is discussed below, this probability will depend on a number of factors in addition

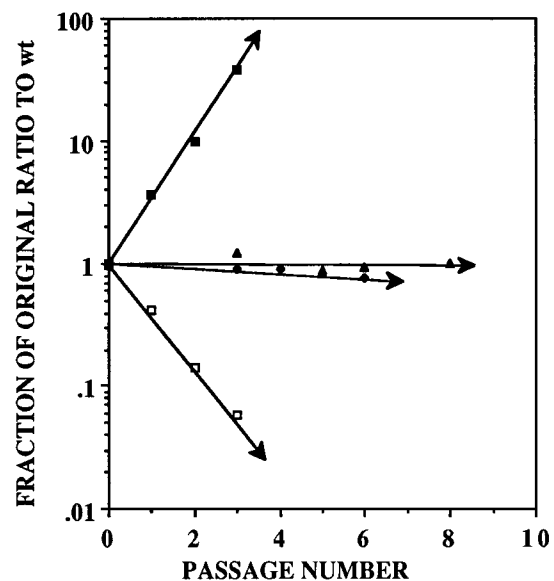


FIG. 1. Fitness vector diagrams showing the relative fitness of parental MARM populations. The vectors represent changes in the original ratios of MARM to the wild type (wt) during competition passage of virus mixtures. Methods for cell culture, virus infections, and MARM-wild type competitions are described elsewhere (6, 7, 9, 10, 17). The neutral MARM U clone (black triangles) was obtained by plaque selection under I1-containing agar layers and has an Asp-259→Ala substitution in the surface G glycoprotein. The MARM C clone (black diamonds) was obtained after 20 plaque-to-plaque transfers of MARM U and one amplification passage. The MARM X clone (black squares) has an Asp-257→Val substitution in the G glycoprotein, was obtained after 61 consecutive diluted passages in BHK cells, and shows a high-fitness phenotype. The MARM N clone (open squares), a low-fitness virus, was obtained after plaque-to-plaque transfers of MARM U and a single amplification passage (6, 7, 9, 10, 17).

\* Corresponding author. Mailing address: Department of Biology and Institute for Molecular Genetics, 9500 Gilman Dr., University of California, San Diego, La Jolla, CA 92093-0116. Phone: (619) 534-2520. Fax: (619) 534-7108.

TABLE 1. Virus fitness values after passage of parental MARM clonal populations<sup>a</sup>

MARM clone	Passage dynamics	Replicate	Fitness	ST	df	<i>P</i>	
X	Control		3.05 ± 0.03				
		5-to-5					
		A	1.25 ± 0.02	382.794	1, 128	<0.001	
		B	1.68 ± 0.03	256.664	1, 112	<0.001	
		C	2.10 ± 0.03	86.568	1, 112	<0.001	
		D	1.24 ± 0.03	137.739	1, 144	<0.001	
		E	1.72 ± 0.03	235.702	1, 96	<0.001	
		F	2.5 ± 0.2	2.863	1, 96	0.094	
		Mean	1.7 ± 0.2	5.7250	5	0.001	
		30-to-30	A	3.9 ± 0.3	2.80	1, 79	0.098
			B	2.5 ± 0.6	6.16	1, 70	0.015
			C	4.3 ± 0.7	7.26	1, 79	0.009
			D	2.1 ± 0.3	4.29	1, 70	0.042
			E	2.6 ± 0.3	0.99	1, 79	0.322
	F		2.6 ± 0.2	0.00	1, 79	0.974	
	Mean	3.0 ± 0.4	0.1613	5	0.439		
U	Control		1.0 ± 0.2				
		5-to-5					
		A	0.978 ± 0.006	0.085	1, 208	0.771	
		B	0.992 ± 0.008	0.009	1, 192	0.925	
		C	0.85 ± 0.02	2.491	1, 176	0.116	
		D	2.03 ± 0.06	31.220	1, 96	<0.001	
		E	1.26 ± 0.03	3.42	1, 96	0.068	
		F	1.67 ± 0.06	11.615	1, 144	0.001	
	Mean	1.3 ± 0.2	0.8134	5	0.226		
C	Control		0.91 ± 0.03				
		5-to-5					
		A	2.0 ± 0.1	291.921	1, 96	<0.001	
		B	1.08 ± 0.01	11.684	1, 176	0.001	
		C	1.086 ± 0.008	24.522	1, 176	<0.001	
		D	0.95 ± 0.01	158.882	1, 177	<0.001	
		E	0.979 ± 0.009	3.270	1, 176	0.072	
		F	0.91 ± 0.06	0.020	1, 192	0.888	
	Mean	1.2 ± 0.2	1.2921	5	0.126		
N	Control		0.38 ± 0.01				
		5-to-5					
		A	0.48 ± 0.02	12.689	1, 59	0.001	
		B	0.47 ± 0.01	12.736	1, 59	0.001	
		C	0.75 ± 0.02	85.52	1, 77	<0.001	
		D	0.47 ± 0.02	9.394	1, 59	0.003	
		E	0.63 ± 0.09	5.216	1, 59	0.026	
		F	0.52 ± 0.03	16.044	1, 59	<0.001	
		Mean	0.55 ± 0.05	3.0476	5	0.014	
		2-to-2	A	0.35 ± 0.02	0.02	1, 69	0.878
			B	0.39 ± 0.03	0.01	1, 69	0.923
			C	0.51 ± 0.03	2.91	1, 69	0.093
			D	0.46 ± 0.05	45.48	1, 63	<0.001
			E	0.32 ± 0.02	3.13	1, 69	0.081
	F		0.34 ± 0.03	4.11	1, 69	<0.05	
	Mean	0.38 ± 0.01	0.2714	5	0.399		

<sup>a</sup> All MARM clonal populations employed here have been described previously (6, 7, 9, 10, 17). Control values refer to fitness of parental MARM populations before passage. Fitness was estimated from the slope of the regression of the logarithm of the ratio between MARM virus and the wild type with passage number. Statistical tests were carried out as previously described (6, 9, 10). ST is the statistical test applied to compare slopes, which was the Snedecor test for comparing the slopes of replicates with those of the corresponding controls (MARM populations prior to passages) and Student's *t* test for comparing mean values. df, degrees of freedom of the ST value; *P*, probability that a replicate and the control have an equal slope. Passage dynamics refers to the number of clones pooled and passed during each of 20 consecutive *n*-plaque-to-*n*-plaque transfers (see text).

to transmission size, and parental population fitness is shown here to be one of them.

## MATERIALS AND METHODS

**Cells and viruses.** BHK<sub>21</sub> cells and VSV Indiana serotype (Mudd-Summers strain) were employed for all experiments. Methods for cell culture and viruses have been described elsewhere (10, 17). We have used, as parental populations, different genetically marked, antibody-resistant mutant (MARM) clones of VSV. The genetic marker allowed us to determine the changes in the ratio of mutant

to wild-type virus during direct competition experiments, a parameter which reflects the fitness of the MARM relative to that of the wild type (17). Parental populations (Fig. 1) included a high-fitness clone (MARM X;  $W = 3.05 \pm 0.03$ ), a low-fitness clone (MARM N;  $W = 0.38 \pm 0.01$ ), and two neutral clones (MARM U [ $W = 1.0 \pm 0.2$ ] and MARM C [ $W = 0.91 \pm 0.03$ ]).

**Virus passages.** Six replicate aliquots of each viral population were each subjected to 20 transfers. All the MARM clones were subjected to five-clone-to-five-clone passages. In addition, MARM N was subjected to 2-clone-to-2-clone passages, and MARM X was subjected to 30-clone-to-30-clone passages. Transfers consisted of randomly picking either 2, 5, or 30 well-isolated plaques of MARM virus clones, mixing them together, and then using appropriate dilutions

of this mixture to seed new BHK<sub>21</sub> monolayers for formation of the next series of well-isolated plaques. The following day, the same number of plaques (progeny of single virus particles) was picked and mixed. It should be stressed that although the progeny virus in each plaque (clone) contains approximately 10<sup>6</sup> infectious particles, only a single infectious particle initiates each isolated plaque, and the yield from the requisite number of plaques is pooled and diluted prior to each of 20 consecutive transfers to form new plaques. Therefore, only 2, 5, or 30 infectious particles are sampled from each plating at each passage, i.e., the plated effective population transfer size is exactly 2, 5, or 30 viral particles. In this situation, population size and population structure are correlated. The larger the sampled population size, the greater the number of variants that will be included, i.e., the more genetically complex it will be. A greater number of sampled variants should increase the probability of sampling a higher-fitness genome and thereby avoiding Muller's ratchet operation. In all transfer experiments, relative fitness was initially determined for the parental MARM clones and again after the 20 serial passages were completed. During the relative fitness competition passages employed here, the two competing virus populations (MARM and the wild type) can increase their fitness because of the Red Queen effect (7). However, this involves "constant running to stay in the same (relative fitness) place" until rather rare stochastic fitness leaps lead to competitive exclusion (extinction) of one or the other population (7).

## RESULTS

The results of each series of 20 consecutive transfers of small population samples are shown in Table 1. Following 20 consecutive two-clone-to-two-clone passages, the low-fitness ( $W = 0.38$ ) parental MARM N clone showed slight changes or no significant changes in fitness. However, when the virus transmission size was raised to five virus particles at each transfer, fitness gains were observed in all series. After five-clone-to-five-clone passages of neutral MARMs C ( $W = 0.91$ ) and U ( $W = 1.0$ ), results were similar to those obtained for MARM N after two-clone-to-two-clone passages (i.e., either no change or slight gains in fitness). However, similar results were observed with the high-fitness MARM X clone ( $W = 3.1$ ) only when the number of viral particles passed each time was 30. Smaller virus transmission populations (i.e., five clones) underwent significant decreases in fitness.

This work demonstrates that the bottleneck size and genetic diversity required to suppress Muller's ratchet is strongly dependent on the mean fitness of the initial population. This result can be explained by the probability of sampling variants (infectious particles) of lower, equal, or greater fitness than the average fitness exhibited by the original population. Variants of equal or greater fitness should be sampled more frequently from populations with low fitness (such as MARM N), because highly fit populations (such as MARM X) have a lower probability of undergoing further mutational improvement in their replicative efficiency. The subclones derived from a clonal MARM X population have, on average, lower fitness than the parental clone mean fitness (10).

## DISCUSSION

The results reported here suggest that the number of infectious units of a virus transmitted to a susceptible host might often be an important modulator of disease even beyond the increased risk associated with larger infectious doses of any microbial pathogen. At the height of infection in any individual host, the complex viral population (quasispecies) may often attain high mean fitness as the result of massive replication, mutation, and rapid selection of more-fit variants during competition within the population. Under these circumstances, a high-fitness parental population will include a higher proportion of lower-fitness subclones (10), and a bottleneck transmission to a new host is more likely to include only progeny of lower fitness than the parental (donor) ensemble. The higher the fitness that has been reached by the donor quasispecies, the more likely it will be that the viral population will undergo

genetic bottlenecks during low-dosage transmissions (i.e., a greater number of viral particles will be needed to avoid Muller's ratchet effect). As mentioned earlier, a number of factors can influence the probability of virus fitness declines during transmission of small virus samples. These include the number of transferred infectious particles and the structure of the parental population, as demonstrated here. Other important factors not examined in this study could include inflammatory and immune responses, host cell heterogeneity, temperature variations, and route of virus entry, etc.

Finally, early after bottleneck transmission, the replicating viral population will probably have suboptimal fitness, and very early transmission of just a few particles to a new host might often restore virus fitness by including favorable mutations. This might happen because deleterious mutations in genomes which already have low fitness are likely to produce a mutational meltdown (severe debilitation and lethality), so surviving progeny would mainly include variants which are either as fit or more fit than the parental ensemble. Thus, transmission size may variably modulate virus replicative efficiency and resulting disease processes. Intrahost transmission events are probably at least as important for evolution of virus fitness as are inter-host transmissions, but the former might often be difficult to investigate. The dynamics of human immunodeficiency virus type 1 turnover in infected individuals is an example (5, 16, 33). With human immunodeficiency virus production of at least 10<sup>8</sup> to 10<sup>9</sup> virions per day and as many or more CD4 T cells being depleted each day in an infected individual, it is not at all obvious whether cell-to-cell bottleneck transmissions might be more or less common than (nonbottleneck) transmissions of larger virus populations. This probably varies in different lymphoid tissues, in different infected individuals, and at different stages of infection, but it could have profound importance for viral evolution and host response.

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