The Advantage of Sex in the RNA Virus $\phi 6$

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Manuscript received January 22, 1997
Accepted for publication July 2, 1997

ABSTRACT

When laboratory populations of the RNA bacteriophage $\phi 6$ are subjected to intensified genetic drift, they experience a decline in fitness. These experiments demonstrate that the average effect of mutations is deleterious, and they are used to suggest that Muller's ratchet can operate in these viruses. However, the operation of Muller's ratchet does not alone guarantee an advantage of sex. When $\phi 6$ populations were subjected to a series of bottlenecks of one individual and then crossed, the measured advantage of sex was not significant. To determine whether a small sample size, as opposed to allelism or another explanation, can account for the negative result, we repeated the $\phi 6$ experiments by crossing a larger set of populations. We found that bottlenecked populations of $\phi 6$ could recover fitness through mutations. However, hybrids produced by crossing the populations recovered an additional amount over the contribution of mutations. This additional amount, which represents an advantage of sex to $\phi 6$, was determined to be significantly greater than zero. These results provide indirect support for an advantage of sex through Muller's ratchet. However, we also use our experimental design and results to propose an alternative to Muller's ratchet as a model for the evolution of sex.

ORGANISMS with small population size are subjected to intensified genetic drift and therefore experience an increased rate of genetic fixation and extinction. If the average mutation is deleterious, this effect of drift is to decrease the fitness of the population. A distinction can be made in monitoring the decline of fitness; either the fixation of deleterious alleles or the extinction of genomes with no mutations can be followed. The latter is of particular evolutionary interest because it is the basis of Muller's ratchet, the hypothesis that sex (genetic exchange) evolved in response to genetic drift in a population with a high deleterious mutation rate (MULLER 1964; FELSENSTEIN 1988).

Muller's ratchet pertains to the fact that whenever drift causes the extinction of genomes that are free of mutations, the loss is effectively irreversible in an asexual population. A back mutation can recreate a mutation-free genome, but the backward rate is expected to be much smaller than the forward rate (HAIGH 1978; MAYNARD SMITH 1978). A forward mutation can occur at any site of the genome, but a backward mutation has to be at the site that restores the original mutation-free sequence. The advantage of sex is that it makes up for the low backward rate by using recombination or chromosome reassortment to recreate (from mutated genomes) genomes with no or fewer mutations. Muller's ratchet also requires that compensatory mutations (ones that correct a deleterious mutation without restoring the original sequence) are rare. If compensatory mutations are sufficiently common, Muller's ratchet can be stopped without the recreation of mutation-free genomes (WAGNER and GABRIEL 1990). It is expected that compensatory mutations are less common than deleterious mutations because it is easier to destroy function than to correct or improve function by a random mutation.

RNA viruses offer an unique opportunity to study Muller's ratchet (CHAO 1994). These viruses have extraordinarily high rates of mutation. Whereas the mutation rate of DNA is 10^{-9} to 10^{-10} errors per nucleotide replication, the rate for RNA is 10^{-3} to 10^{-5}. Furthermore, many RNA viruses can reproduce sexually. Whenever two or more parent viruses co-infect the same host cell, hybrid progeny can be produced by genetic exchange between the parent genomes. In some RNA viruses, the exchange is through recombination. In others, recombination is lacking and exchange is achieved through genome segmentation. A genome is segmented into several RNA molecules and hybrid progeny are reassortants containing segments descending from the various co-infecting parents. However, some RNA viruses lack recombination and are not segmented. These viruses are effectively asexual even when many viruses co-infect the same host cell.

Laboratory studies have shown that some of the requirements for Muller's ratchet may be met in RNA viruses. If viral populations are subjected to intensified drift by being forced through a succession of bottlenecks of one virus, they accumulate deleterious mutations and experience an overall reduction in fitness (CHAO 1990; DUARTE *et al.* 1992). Thus, deleterious mutations are clearly more common than both back and compensatory mutations. However, the operation of Muller's ratchet does not necessarily provide an advantage for sexual reproduc-

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tion. When CHAO *et al.* (1992) crossed a series of bottlenecked populations of the RNA bacteriophage $\phi 6$ and measured the fitness of the resulting hybrids, they found that the advantage of sex to a few hybrids was significantly greater than zero, but the overall advantage to all hybrids was not. Their failure to obtain a significant overall advantage may have been simply due to a small sample size, but there are alternative explanations (SEE DISCUSSION and CHAO *et al.* 1992). One possibility is that a region of the $\phi 6$ genome may be a mutational hotspot. As a result, mutations are more likely to be allelic, and sex is not advantageous because it is less able to recreate genomes with no or fewer mutations.

Here we present the results of a study expanding the analysis of CHAO *et al.* (1992). To determine whether the previous negative results were due to small sample size or an alternative explanation, we reestimated the overall advantage of sex to $\phi 6$ by examining a larger number of crosses. A significantly greater than zero advantage is revealed by the larger sample size. These results provide indirect support for an advantage of sex by Muller's ratchet, but also suggest an alternative model for the evolution of sex in these viruses.

The RNA bacteriophage $\phi 6$: A single clone of $\phi 6$ was first isolated from field samples of bean straw and characterized by VIDAVER *et al.* (1973). $\phi 6$, or any phage remotely resembling it, has never been reisolated from nature, despite attempts by several investigators (K. A. VIDAVER, personal communication; L. CHAO and L. MINDICH, unpublished results). It is a virulent phage that has as its host the bacterium *Pseudomonas phaseolicola*, which is the phytopathogen responsible for blight of beans. Cells infected with $\phi 6$ yield a burst size between 200 and 400 progeny phages after a latent period of ~100 min. $\phi 6$ has the characteristic high mutation rate of RNA viruses (DOMINGO and HOLLAND 1994), and temperature-sensitive mutations appear spontaneously at a frequency of 0.5% (L. MINDICH, personal communication). The genome of $\phi 6$ is 13,379 nucleotides in size and it is segmented into three RNA molecules, each of which constitutes 22, 30 and 48% of the genome (SEMANCIK *et al.* 1973; MCGRAW *et al.* 1986; GOTTLIEB *et al.* 1988; MINDICH *et al.* 1988). Segment reassortment is the mechanism of genetic exchange because recombination between segments is rare or nonexistent in the laboratory (MINDICH *et al.* 1976).

MATERIALS AND METHODS

Culture conditions and media: All phage and bacteria were grown, plated, crossed, incubated and diluted at 25° in LC medium (MINDICH *et al.* 1976). Agar concentrations in plates were 1.5 and 0.7% for bottom and top LC agar, respectively. Volume of top agar was 3 ml and that of lawns was 200 μl. Lawns were made from overnight bacterial cultures.

Phage and bacterial stocks: $\phi 6$ strains are from CHAO *et al.* (1992). *P. phaseolicola* was purchased from American Type Culture Collection (ATCC no. 21781). *P. pseudocaligenes* ERA (MINDICH *et al.* 1976) was obtained from L. MINDICH (Department of Microbiology, Public Health Institute of the City of New York). Bacteria were stored in 4:6 glycerol/LC (v/v) at −20°. Phage lysates were prepared by plating 10^4 plaque-purified phage (unless otherwise indicated) with top agar and a *P. phaseolicola* lawn. After 24 hr the plaques in the top agar were resuspended in 4 ml of LC broth and centrifuged at 3000 rpm for 10 min. The phage lysate was the supernatant, filtered (0.22 mm, Durapore, Millipore) to remove bacteria. Lysates were stored at 4° for up to 1 month. For long-term storage of phage, plaques were cut from a *P. phaseolicola* lawn, resuspended in 4:6 glycerol/LC and stored at −20°.

Crosses: In a hybrid cross of two $\phi 6$ strains, an adsorption mixture was prepared by combining the strains (each at a final concentration of 1.25×10^9/ml) and exponentially growing *P. phaseolicola* (at a final concentration of 2×10^8/ml). In a selfed cross the mixture contained the same concentration of *P. phaseolicola*, but only one strain was added at a final concentration of 2.5×10^9/ml. After a 40-min incubation with no shaking, each mixture was placed on ice to stop bacterial growth. Then, 2.5×10^3 phage of each mixture were plated with top agar and a *P. phaseolicola* lawn and incubated for 18 hr. The resulting plaques were harvested and filtered to produce the hybrid and selfed populations.

Mass selection: To start a mass selection, ~2.5×10^3 phage from the hybrid and selfed populations were plated with top agar and a *P. phaseolicola* lawn. After an 18-hr growth cycle, the resulting plaques were harvested and a filtered lysate was prepared. Then, 2.5×10^3 phage from the lysates were plated and incubated for a second 18-hr growth cycle. After six growth cycles, filtered lysates were prepared and used as the mass-selected selfed and hybrid populations. Within each cycle the inoculum of 2.5×10^3 phage increased to a population of ~$3–4 \times 10^{11}$.

Paired-growth experiments: For a paired-growth experiment (CHAO 1990), a $\phi 6$ strain and a genetically marked reference $\phi 6$ were mixed at a 1:1 ratio and plated with top agar and a *P. phaseolicola* lawn. After 18 hr, a filtered lysate was prepared from the resulting plaques. An aliquot from the lysate was then plated to start a second growth cycle. A total of three cycles was completed. The ratio of phage:reference during paired growth was monitored by marking the reference $\phi 6$ with a spontaneous host range mutation that allowed growth on an alternate host *P. pseudocaligenes* (MINDICH *et al.* 1976). The marked reference $\phi 6$ made clear plaques on mixed lawns of *P. phaseolicola* and *P. pseudocaligenes* (200:1 ratio), whereas the unmarked phage made turbid plaques. The number of plaques per paired-growth plate and mixed lawn plate was kept between 200 and 600. A maximum of 600 was chosen to minimize overlap between plaques on the paired-growth plates. Genetic exchange between overlapping plaques would have confounded the fitness estimates. Within a paired-growth plate, the phage population increased from the initial inoculum of 200–600 phage to ~$3–4 \times 10^{11}$.

Fitness estimates: Fitness (W) of a phage was defined as the relative change of the ratio of phage:reference $\phi 6$ per growth cycle, or $W^t = R_t/R_o$, where R_t and R_o are the ratios, respectively, at the start and after t paired-growth cycles. W was estimated by fitting $\log(W)$ by the least squares method (SOKAL and ROHLF 1995) to paired-growth data, and its units are per growth cycle.

Statistical analyses: Fitness data were log-transformed before analysis. All tests were one-tailed because fitness is expected to increase through mass selection.

EXPERIMENTAL DESIGN

CHAO *et al.* (1992) examined the advantage of sex by crossing nine $\phi 6$ strains that had been subjected to

40 bottlenecks of one individual virus (CHAO 1990). However, they did not perform all the possible crosses. Instead, three strains were chosen and crossed with all others for a total of 21 crosses. To increase sample size, we crossed for this study all possible pairs of the same nine strains for a total of 36 crosses. Crosses previously performed by CHAO *et al.* (1992) were repeated to eliminate block effects (SOKAL and ROHLF 1995).

Sex is advantageous if the cross of two strains yields a hybrid population that contains a hybrid (or ressortant) whose fitness is higher than either of the two parents. However, isolating such a hybrid in a viral cross is difficult because reassortants are produced at a low frequency of ~5–20% (MINDICH *et al.* 1976). Parental genotypes are more common because they are produced by cells infected with only one parent phage and by those infected by both parents. To identify the presence of possible rare hybrids of higher fitness, we followed the approach of CHAO *et al.* (1992) and enriched for such genotypes by subjecting the hybrid population to mass selection. This was achieved by propagating the population through several growth cycles with a larger bottleneck of 2.5×10^3 phage. With a larger bottleneck, selection operates with minimal drift, and hybrid populations containing higher fitness reassortants should evolve a higher fitness. To control for the possibility that a hybrid population improves because of back and compensatory mutations (see above), we also "selfed" the parent strains and subjected them to mass selection. Sex is then advantageous if a hybrid population after mass selection has a higher fitness than both of the two selfed parental populations after mass selection.

Hybrid and selfed populations after mass selection, hereafter simply hybrid and selfed phage, were measured for fitness by paired-growth experiments (CHAO *et al.* 1992). Fitness determined by paired-growth experiments is relative to a reference $\phi 6$ (CHAO 1990), which is a genetically marked derivative of original $\phi 6$ clone that gave rise to the nine strains used in the present study. The reference $\phi 6$ represents, therefore, a $\phi 6$ before being subjected to successive bottlenecks (and genetic drift) and before it has accumulated deleterious mutations and acquired a lower fitness. However, because the genetic marker is slightly deleterious, the fitness of the reference $\phi 6$ is 5.5% lower than that of the original unmarked $\phi 6$ (CHAO 1990). All fitness values presented here were divided by 1.055 to adjust for the effect of the marker, and $W = 1$ indicates that a phage has the same fitness as the original $\phi 6$. The accuracy of the 5.5% value does not affect our later estimates of the advantage of sex and mutations because those estimates are relative, and any error is canceled. Except for the purpose making our results comparable to our previous publications (CHAO 1990; CHAO *et al.* 1992), an adjustment is otherwise inconsequential for this study.

RESULTS

The fitness of hybrid and selfed phage are summarized in Table 1. For comparison, the initial fitness of the nine strains, after a succession of 40 bottlenecks of one individual, but before selfing and mass selection, are included. These initial values are from CHAO *et al.* (1992). Table 1 was analyzed for two effects. Did selfing and mass selection improve fitness of the selfed phage? Did crossing and mass selection improve fitness of the hybrid phage?

Mean improvement of selfed phage: Any fitness improvement by a phage after selfing and mass selection has to be due to back and compensatory mutations. Such a mutational improvement was defined as $I_M = \log(W_2/W_1)$, where W_1 is the initial fitness of the strain and W_2 is the mean fitness of selfed phage of the same strain. For example, from Table 1, I_M for 41D was $\log(W_2) - \log(W_1) = (-0.187) - (-0.280) = 0.093$.

I_M was estimated for each strain, and the mean ± SEM averaged over the nine strains was $\bar{I}_M = 0.082 \pm 0.0302$, which was significantly greater than zero by a one-tailed paired *t*-test (SOKAL and ROHLF 1995; Table 2).

Improvement of hybrid phage: If a more fit reassortant is produced by a cross, the fitness of the resulting hybrid phage should be greater than that of the two selfed phage that were derived from the two parent strains that were crossed to create the hybrid. Thus, improvement for each hybrid phage was defined as $I_S = \log(W_3/W_4)$, where W_3 is the fitness of the hybrid phage, and W_4 is the higher selfed mean fitness of the two parent phage. For example (see Figure 1 for a graphical interpretation), $\log(W)$ for selfed 41D and selfed 37J phage were, respectively, −0.187 and −0.204 (Table 1). Thus, for the cross 41D × 37J, $\log(W_3) = -0.066$, $\log(W_4) = -0.187$, and $I_S = 0.120$. I_S was subscripted to denote that any nonzero value is due to genetic variation generated by sex (reassortment) and over what is achieved by mutations alone in the selfed phage populations.

I_S was estimated for each of the 36 crosses, and the average for all crosses was $\bar{I}_S = 0.038$ (Table 3). However, the statistical significance of $\bar{I}_S$ could not be determined from the standard error of the sample because many of the I_S values in Table 3 were estimated by using the same W_4 value. For example, $\log(W_4) = -0.110$ for all of the eight I_S values involving 41G. The multiple use of W_4 to calculate more than one I_S creates nonindependence because W_4 is not estimated without error. There were multiple estimates of W_4, but only a total of five (Table 1), and some crosses (those involving 41G, 41F, and 41E) required using the same W_4 more than five times.

We determined therefore the statistical significance of $\bar{I}_S$ by randomization methods (MANLY 1994). The null hypothesis was $\bar{I}_S = 0$, or that for any given cross

TABLE 1

Fitness of selfed and hybrid phage

	Log (W)									
			Hybridized with							
Strain	Initial	Selfed	41D	41E	37C	37I	37J	41F	41G	41I
37A	−0.122 ± 0.0215	−0.119 ± 0.0221	0.025	−0.001	0.012	0.028	0.010	−0.084	−0.031	−0.189
41D	−0.280 ± 0.0114	−0.187 ± 0.0450		−0.073	−0.294	−0.254	−0.066	−0.028	−0.017	−0.025
41E	−0.184 ± 0.0206	−0.117 ± 0.0059			−0.046	0.027	−0.060	−0.152	−0.042	−0.084
37C	−0.546 ± 0.0326	−0.242 ± 0.0139				−0.325	−0.210	−0.180	−0.072	−0.085
37I	−0.332 ± 0.0353	−0.296 ± 0.0366					−0.129	−0.170	−0.072	−0.112
37J	−0.331 ± 0.0054	−0.204 ± 0.0669						−0.081	−0.030	−0.068
41F	−0.142 ± 0.0266	−0.110 ± 0.0272							−0.076	−0.180
41G	−0.122 ± 0.0217	−0.062 ± 0.0054								−0.087
41I	−0.224 ± 0.0213	−0.207 ± 0.0257								

Names of the strains are from CHAO *et al.* (1992). All fitness values are presented as $\log_{10}(W)$. Initial and selfed values are mean ± SEM, n = 3 and 5, respectively. Hybrid values are single measurements.

W_3 was equivalent to a random deviate of W_4. Because each W_4 was based on five independent estimates (see Table 1), we could use randomization to generate the distribution of the chance deviation for each cross and then determine average chance deviation for the 36 crosses. $\bar{I}_S$ was therefore significantly greater than zero if the average chance deviation was greater than our estimate of $\bar{I}_S$ = 0.038 <5% of the times. A 5% cutoff on one side of the distribution corresponds to a one-tailed test.

To obtain the distribution of the average chance deviation, we conducted a total of 10,000 randomization trials. A single trial consisted of deriving 36 random deviates, one for each of the 36 crosses. A random deviate was obtained by first randomly choosing (with replacement) one of the five independent estimates of the $\log(W_4)$ that pertained to a given cross. The difference between the chosen independent estimate and the mean value of the $\log(W_4)$ for the cross was the random deviate, and the resulting 36 random deviates were averaged to yield an average chance deviation.

TABLE 2

Fitness improvement of selfed phage

Strain	I_M
37A	0.003
41D	0.093
41E	0.067
37C	0.301
37I	0.036
37J	0.127
41F	0.032
41G	0.060
41I	0.017
	$\bar{I}_M$ = 0.082*

$\bar{I}_M$, mean I_M value averaged over all nine strains.

* Significantly greater than zero ($P < 0.01$; d.f. = 8; one-tailed).

Thus, each trial replicates the data structure used to calculate $\bar{I}_S$. The average chance deviation for each trial was then compared to $\bar{I}_S$ = 0.038.

Our randomization determined that the average chance deviation was $>\bar{I}_S$ in only two of 10,000 trials. Thus, $\bar{I}_S$ = 0.038 was significantly greater than zero ($P < 0.0002$; one- tailed).

DISCUSSION

We began these studies to determine whether an advantage of sex through Muller's ratchet could be met in RNA viruses (CHAO 1988, 1992; see Introduction). In our first study (CHAO 1990), we tested whether the average mutation is deleterious. The expectation is that

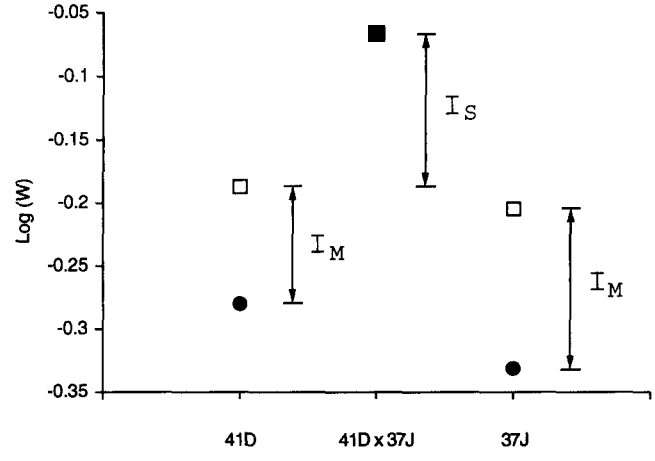

FIGURE 1.—Relationship between fitness (W) of selfed and hybrid phage and I_S and I_M. Fitness values are presented as $\log_{10}(W)$ on the ordinate axis and are taken from Table 1. Because this figure is only for the purpose of illustration, fitness values are presented for only one cross (41D × 37J). Initial fitness of phage (●); fitness of selfed (□) and hybrid (■) phage after mass selection. I_M for a strain is the difference between its (□) and (●). Because (□; 41D) is greater than (□; 37J), I_S is the difference between (■) and (□; 41D).

TABLE 3

Improvement of hybrid phage

	I_S when hybridized with							
Strain	41D	41E	37C	37I	37J	41F	41G	41I
37A	0.144	0.116	0.131	0.147	0.129	0.026	0.031	−0.070
41D		0.044	−0.108	−0.068	0.120	0.082	0.045	0.161
41E			0.071	0.144	0.057	−0.042	0.020	0.033
37C				−0.083	−0.006	−0.070	−0.010	0.122
37I					0.075	−0.060	−0.010	0.095
37J						0.029	0.032	0.136
41F							−0.014	−0.070
41G								−0.025

Mean I_S value averaged over all 36 crosses is $\bar{I}_S = 0.038$, which was significantly greater than zero ($P < 0.0002$; one-tailed). See text for details of statistical analysis.

for Muller's ratchet to operate the rate of back and compensatory mutations must be smaller than that of deleterious mutations. A smaller rate would be manifested by an average deleterious effect of mutations. Thus, we imposed intensified genetic drift on populations of the RNA bacteriophage $\phi 6$ and monitored the effect of accumulating mutations. Our result showed that the average mutational effect was indeed deleterious because the populations experienced an overall decline in fitness. This result may be part of a more general phenomenon because a similar outcome has been reported for another RNA virus (DUARTE *et al.* 1992).

However, whether these $\phi 6$ experiments actually demonstrate Muller's ratchet can be questioned because the intensified genetic drift was promoted by imposing a bottleneck of a single phage. Such a small bottleneck causes fixation, which negates an advantage of sex because there is no longer genetic variation in the population for recreating genomes with no or fewer mutations. However, aside from fixation, the difference between Muller's ratchet *sensu stricto* and our experiments is small. Both require drift and that the average effect of mutations is deleterious. Thus, when we initiated our experiments, we were not concerned that fixation occurred. Our eventual goal may have been to demonstrate an advantage of sex through Muller's ratchet, but we wanted first to maximize our chances of determining whether the accumulation of mutations through genetic drift leads to a fitness decline. That was best achieved by using a bottleneck of one individual. If we succeeded, it seemed likely that we would also succeed in demonstrating Muller's ratchet by increasing the size of the bottleneck. The latter would be a future study.

After completing our mutation accumulation experiments with $\phi 6$, we tested whether the deleterious mutations that had accumulated in our $\phi 6$ strains were randomly distributed. As indicated above, if mutations are not random, the operation of Muller's ratchet, even if we were eventually able to demonstrate it, does not by itself translate into an advantage of sex. If most mutations in $\phi 6$ occur mainly in one segment, reassortment cannot recreate a mutation-free genome. Because there is no or very little intrasegment recombination in $\phi 6$ (see above), mutations in the same segment are essentially allelic. Given that $\phi 6$ has only three segments, the probability of allelism is expected to be high. The distribution of mutations across the various segments could be determined by mapping, but such an approach, although possible and currently being undertaken, is laborious and would have limited our sample size. Thus, we opted in our initial studies (CHAO *et al.* 1992) to use hybridization and mass selection for determining whether the distribution of deleterious mutations in $\phi 6$ favors sexual reproduction.

Hybridization and mass selection (see EXPERIMENTAL DESIGN and RESULTS for details) estimates the quantities I_S and I_M, which represent fitness recovery (improvement) due to sex and mutations, respectively, by $\phi 6$ strains that have accumulated deleterious mutations. Recovery is achieved by expanding population size and allowing natural selection to operate unhindered by genetic drift, and sex by crossing the strains before population expansion. If two strains are crossed and they have accumulated deleterious mutations that are not allelic, the I_S value for the cross is expected to be greater than zero.

When we (CHAO *et al.* 1992) first measured the mean values $\bar{I}_M$ and $\bar{I}_S$ for a set of $\phi 6$ strains, we found that both quantities were greater than zero, but only $\bar{I}_M$ was significantly so. In the present study, we reestimated $\bar{I}_M$ and $\bar{I}_S$ with a larger data set and show that both quantities are significantly greater than zero, and our new estimates are $\bar{I}_M = 0.082$ and $\bar{I}_S = 0.038$. Thus, the deleterious mutations in our strains are not likely to be allelic.

We interpret these combined results to provide indirect support for an advantage of sex by Muller's ratchet, recognizing that direct evidence requires that we use a larger bottleneck in propagating our viral populations.

However, as these studies were completed, it became apparent that our experimental design actually provided an alternative model to Muller's ratchet. This model, which we first proposed in CHAO *et al.* (1992), overcame the problem of fixation by supposing that viral populations are subdivided. If each subpopulation were fixed for a different deleterious mutation, mutation-free genomes could be recreated by genetic exchange between the subpopulations.

Part of the appeal of population subdivision model is that our experiments provide estimates of some of the parameters necessary for evaluating the model. In this respect, it is fortunate that we chose to measure $\bar{I}_M$ and $\bar{I}_S$ and to determine by hybrization and mass selection whether the deleterious mutations in our ϕ6 strains were allelic. Had we mapped the mutations, the question of allelism may have been settled, but we would not know for certain whether an advantage is generated. For example, nonallelism should result in an advantage, but could mutations mask the effect? $\bar{I}_S$ and $\bar{I}_M$ allows us to make the assessment. In light of our new and better estimate of $\bar{I}_S$, we end by presenting a summary of the model.

There are initially segmented (sexual) and nonsegmented (asexual) ϕ6. Their population is fragmented into isolated subpopulations, possibly because of population structure in the population of the host bacterium. It is assumed that the isolation is sufficiently complete to prevent selection among subpopulations. Furthermore, the subpopulations are small, possibly because most individuals fail to reproduce and the infection of most host populations are initiated by a single phage. Thus, genetic drift is strong and each subpopulation is effectively a clonal lineage that is continuously bottlenecked. Because the subpopulations are isolated from each other, genetic drift operates equally on both segmented and nonsegmented phage. As the subpopulations accumulate deleterious mutations through drift, their fitness is reduced.

Periodically, the host bacteria form larger and longer-lasting patches. During the existence of these patches, phage from many subpopulations colonize the same patch. The phage achieve large populations and improve their fitness through mass selection. Because nonsegmented phage are asexual, they rely entirely on mutations for improving fitness. $\bar{I}_M$ provides an estimate of magnitude of the improvement. With our present estimate of $\bar{I}_M = 0.082$, fitness is expected to improve by a factor of $10^{0.082} = 1.21$. On the other hand, segmented phage are sexual, and they can count on both mutations and sex. Thus, they improve first by an amount $\bar{I}_M$ and then, with our present estimate of $\bar{I}_S = 0.038$, by a factor of $10^{0.038} = 1.09$. The outcome is that segmented phage increase in frequency relative to nonsegmented phage after many episodes of genetic drift and mass selection. During the drift phase, segmented and nonsegmented phage lose equally. During mass selection, they race to recover their fitness loss, and segmented phage have the advantage.

Our demonstration that $\bar{I}_S$ is greater than zero is critical for the model. In addition to sample size and allelism (see above), a third reason why $\bar{I}_S$ may not be greater than zero is that the contribution of compensatory mutations and sex during mass selection could have been equal. In other words, the fitness of selfed and hybrid phage are the same and, due the to way we calculate $\bar{I}_S$ and $\bar{I}_M$ (see RESULTS), $\bar{I}_S = 0$. In such an event, although sex truly helps the recovery of the hybrid phage, it is not evolutionarily favored because segmented and nonsegmented phage recover equally (albeit by different mechanisms) during mass selection in the larger patches. Given the high mutation rate of RNA viruses, the role of compensatory mutations during mass selection cannot be minimal. Our estimate of $\bar{I}_M$ being more than was twice as large as $\bar{I}_S$ attests to that. However, more importantly, our estimate of $\bar{I}_S > 0$ shows that there is a significant contribution of sex, despite the large effect of mutations.

The importance of small populations and genetic drift in viruses may be questioned because viral populations can rapidly achieve extraordinarily high numbers. However, population size without additional information on the history of the population can be misleading. In our genetic drift and mutation accumulation experiments (CHAO 1990), the ϕ6 lineages were expanded to a population size of 8×10^9 after each bottleneck of one phage, but the expansion was not sufficient to stop genetic drift. Recent evidence suggests that viral populations in nature may be frequently subjected to small transmission bottlenecks, possibly of one virus, during the initial entry into a host (DUARTE *et al.* 1994). For example, when the envelope region of human immunodeficiency virus was monitored within single human hosts over the time course of infection, genetic variation was almost nonexistent initially, but then increased greatly during the later stages of infection (YAMAGUCHI and GOJOBORI 1997). The increased variation was not due to reinfection because the later isolates were phylogenetic descendants of the original virus. If the natural history of most viruses is a succession of transmission bottlenecks, Muller's ratchet or a similar process may be actively operating in wild viral populations.

We thank S. O. NG, L. E. WONG and C. D. MATTHEWS for laboratory assistance; L. MINDICH and H. R. REVEL for advice, phage and bacteria; E. RUSSEK-COHEN for statistical assistance; and A. CLARK for comments on manuscript. This work was supported in part by a National Institute of General Medical Sciences grant to L. CHAO.

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Communicating editor: A. G. CLARK