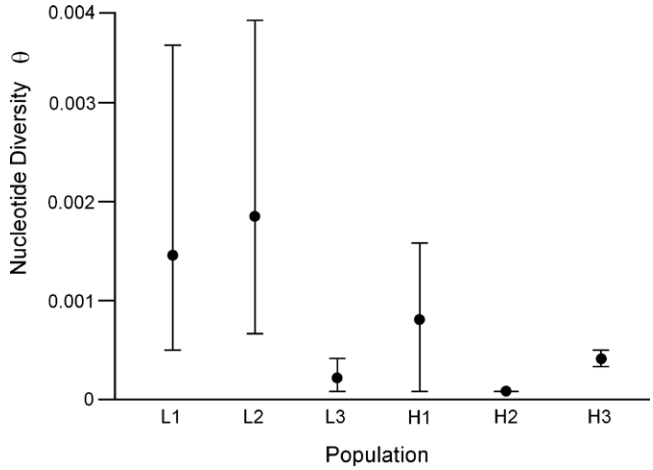

Figure 3. Population average nucleotide diversity (θ). θ was computed using a maximum likelihood approach. Each value of θ is the average of 5 estimates, enabling the computation of a 95% confidence interval.

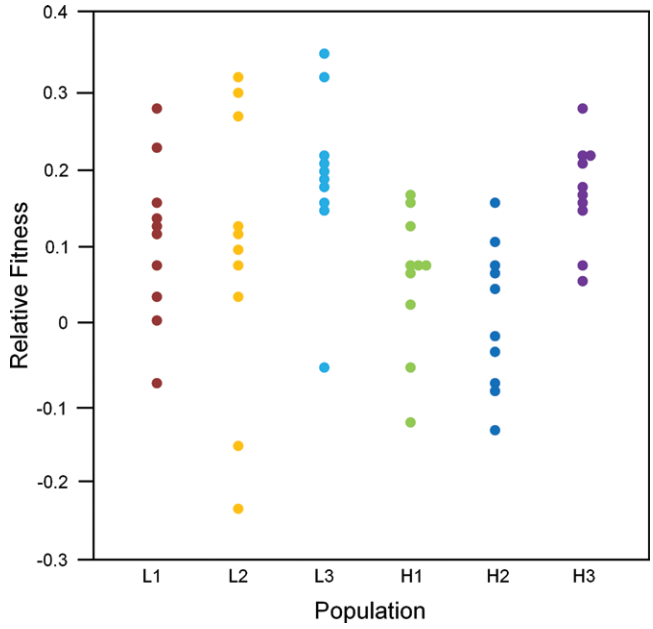

Figure 4. Relative fitness estimates for clones obtained from asexually and sexually evolved populations. Ten clones were isolated from each of 3 low MOI (L1, L2, L3) and high MOI (H1, H2, H3) phage φ6 populations. Each point is the mean of 3 relative fitness estimates for each clone. Global mean standard error < 0.013. Some points offset for clarity.

counterparts following 300 generations of experimental evolution. Although both treatment groups showed similar diversity in protein-coding regions, the asexually evolved populations were more diverse in noncoding regions (7 mutations vs. none, see Figure 2). These results appear to contradict theory that predicts greater genetic diversity in coinfecting virus populations relative to clonal ones (Frank 2001) and suggest that the reassortment (sexual exchange) of genomic segments may not generate increased genetic variation that would foster faster adaptation in these RNA viruses (Chao 1988; Chao 1992). Even if our conclusions are in error, and there are no significant differences in the genetic diversities of the 2 treatments, this result still is at odds with the theoretical expectation that viral populations experiencing high levels of coinfection should show significantly greater standing genetic variation than those evolved under clonal conditions.

Following 300 generations of viral evolution, the mean level of nucleotide diversity in our experimental populations was almost an order of magnitude lower than those reported among RNA virus populations infecting humans (Elena, Codoner, and Sanjuan 2003) and within experimentally

evolved populations of RNA phage Qβ ($\theta = 0.0017, 0.0018$; these estimates derived from our analysis of Bacher et al. 2003. One explanation is that the mutation rate of phage φ6 (estimated to be 2.7×10^{-6} reversions per generation; Chao et al. 2002; Burch et al. 2007; Ferris et al. 2007) is generally lower than that of most RNA viruses (Drake 1993; Drake and Holland 1999). Although the sexually evolved populations were especially lacking in genetic diversity, this dearth of mutations did not appear to be due to a lower relative mutation rate. We previously observed that the sexually evolved viruses had a greater tendency to generate spontaneous host-range mutants when challenged to grow on a novel host species (Montville et al. 2005), suggesting they are not mutation limited. However, this difference in ability to generate host-range mutants was most likely due to differing genetic architectures between the asexually and sexually evolved strains (e.g., presence of preexisting mutations in the sexually evolved strains, which were required for a 2-step mutation process to infect the new host). Thus, the available evidence does not indicate that differences in mutation rate can explain our current results.

Clonal Interference

One possible explanation is that clonal interference caused genetic diversity to be higher in the asexually evolved populations, on average, compared with the sexually evolved populations. Recombination (breaking and joining of homologous sequences) is rare or nonexistent in phage φ6, but reassortment of segments readily occurs. Populations of the virus evolved under low MOI were not allowed to experience frequent reassortment, thus preventing these lineages from combining multiple beneficial mutations that arose in different genetic backgrounds. A consequence was that mutants of equal (or very similar) fitnesses might have been maintained together in the population for longer times due to inefficiency of selection. The relatively greater number of inferred haplotypes in the asexually evolved populations was consistent with the hypothesis that clonal interference maintained neutral genetic variation in these populations but not in the sexually evolved lineages. This hypothesis is further supported by our observations that clone fitness was not highly divergent among viruses within each asexually evolved population and that much of the diversity was found in noncoding regions. However, we note that other processes, such as purifying selection, can produce the same results.

We note that the likelihood of clonal interference should increase with elevated population size (Miralles et al. 2000). Although in the original experimental evolution study an attempt was made to create equivalent effective population sizes (i.e., ~500 individuals) across the high and low MOI treatments (Turner and Chao 1998), it was later shown that a limit to coinfection in phage φ6 probably caused the effective population size at high MOI to be between 200 and 300 individuals (Turner et al. 1999). These effective population sizes were thus similar, but the difference might have enhanced the opportunity for clonal interference to operate under low MOI conditions.

The high MOI populations, by contrast, could have experienced reassortment that combined multiple beneficial mutations (i.e., mutated segments) into the same background, allowing more efficient fixation and reduced overall population genetic diversity (Fisher 1930; Muller 1932). Such a scenario would be consistent with the Fisher–Muller hypothesis, which states that recombination will increase the rate of adaptation. However, we found no evidence that the sexually evolved populations showed higher average fitness than their asexually evolved counterparts; rather the within-population fitness diversity was roughly equal between the 2 treatments. This result implied that the sexually evolved populations did not experience greater fitness gains and did not have lower genetic variance in fitness than the asexually evolved populations.

Mutational Robustness

Our results are also consistent with the reported difference in evolved genetic robustness (phenotypic constancy in the face of mutational change) among the φ6 populations. We previously found that when mutation accumulation was used to randomly introduce mutations into the virus populations, the sexually evolved lineages were less robust than the asexually evolved lineages (Montville et al. 2005). By definition, robust populations should show greater diversity than brittle ones because robustness essentially creates greater neutrality among variants within a population. Therefore, the greater number of inferred haplotypes in the asexually evolved populations is expected based on earlier robustness results with these evolved populations. One possibility for future study would be to use next-generation sequencing to explore rare diversity in the evolved populations and to identify whether the purported effects of clonal interference and/or differing evolved robustness caused the asexually evolved populations to retain variation that might be beneficial in new environments, possibly enhancing their evolvability.

A test of the advantage of sex using this same study system showed somewhat different results; low MOI asexual and high MOI sexual populations of φ6 were compared for their abilities to purge mutations of known deleterious effect (Froissart et al. 2004). The results indicated that standing genetic diversity existed for longer in the sexually evolved populations, evidenced by deleterious mutations purged significantly faster in absence of coinfection (asexual conditions). The explanation was frequent coinfection resulted in complementation, which buffered the effects of the deleterious mutations and caused selection to be inefficient at purging them. However, there was 1 key difference between that previous study and the present one. In the study by Froissart et al. (2004), only the fate of deleterious mutations was examined, whereas in this present study, all mutations in the sequenced regions, including beneficial and neutral mutations, were considered. It may be that discrepancy in results between the 2 studies can be explained by clonal interference. Competing beneficial mutations of similar effect may be maintained longer in asexual populations, increasing population genetic diversity.

Virus Segmentation Not for Sex

Taken together, the results show that sexually evolved populations of phage φ6 do not maintain higher levels of genetic diversity (this study), do not adapt faster to a fixed environment (Turner and Chao 1998), and do not purge deleterious mutations faster via reassortment (Froissart et al. 2004). Additionally, reassortment among φ6 phages facilitates complementation, and deleterious alleles, cheating genes, and defective segments are maintained longer than they are in asexually evolved populations (Turner and Chao 1998; Turner and Chao 1999; Froissart et al. 2004). Although evidence shows that reassortment allows φ6 populations to recover from the effects of accumulated mutations due to Muller's ratchet (Chao et al. 1992), these combined results indicate that sex is not generally beneficial in evolving populations of the virus and that clonal interference is apparently a stronger force than complementation for maintenance of standing genetic diversity.

The evolutionary ecology of RNA virus segmentation has been perhaps studied more intensely in phage φ6 than in any other segmented virus. In the broadest sense, it appears that genetic exchange in φ6 may be an occasionally useful side effect of a segmented genetic architecture, which evolved for another purpose (Pressing and Reanney 1984; Nee 1989). A likely possibility is that segmentation evolved to promote greater packaging efficiency of RNA and better control of gene expression in the virus (Onodera et al. 1998; Qiao et al. 2000). This possibility echoes the important difference between the evolutionary origins of sex versus its maintenance, a difference that is often discussed in the evolution of sex literature. Although segmentation in RNA viruses might have evolved for a different purpose, reassortment may be occasionally useful and evolutionarily important in these viruses even if it occurs rarely.

Funding

US National Science Foundation (DBI-0310205, DBI-0535910, and DEB-0804039 to J.J.D.; DBI-0630707 to S.D.; DEB-0743616 to S.V.E.; and DEB-0452163 to P.E.T.). J.J.D. was also supported by the Professional Staff Congress of CUNY.

Acknowledgments

We thank A. Jethra, H. McCreery, R. Montville, S. Remold, and L. Chao for laboratory assistance and members of the Turner Laboratory helpful discussion. We also acknowledge the Noble Foundation Workshop on Viral Evolution for fostering excellent discussions within the virus evolution community.

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Received October 19, 2012; First decision April 1, 2013; Accepted June 4, 2013

Corresponding Editor: John Logsdon