Cost of Host Radiation in an RNA Virus

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ABSTRACT

Although host radiation allows a parasite to expand its ecological niche, traits governing the infection of multiple host types can decrease fitness in the original or alternate host environments. Reasons for this reduction in fitness include slower replication due to added genetic material or modifications, fitness trade-offs across host environments, and weaker selection resulting from simultaneous adaptation to multiple habitats. We examined the consequences of host radiation using vesicular stomatitis virus (VSV) and mammalian host cells in tissue culture. Replicate populations of VSV were allowed to evolve for 100 generations on the original host (BHK cells), on either of two novel hosts (HeLa and MDCK cells), or in environments where the availability of novel hosts fluctuated in a predictable or random way. As expected, each experimental population showed a substantial fitness gain in its own environment, but those evolved on new hosts (constant or fluctuating) suffered reduced competitiveness on the original host. However, whereas evolution on one novel host negatively correlated with performance on the unselected novel host, adaptation in fluctuating environments led to fitness improvements in both novel habitats.

HOST radiation allows a parasite to expand its ecological niche by adapting to one or more novel hosts. Niche expansion can reduce competition (Futuyma and Moreno 1988; Rainey and Travisano 1998), allowing access to a greater diversity of resources (hosts) when competing to produce progeny. But the evolution of traits that produce generalist lineages that can infect several hosts may be costly to an individual parasite for several reasons:

1. Whereas rapid replication is generally advantageous (because more progeny are produced per infection or parallel infections are faster established), the ability to infect multiple hosts may involve added genetic material or modifications. Therefore, a slower-replicating generalist could be competitively disadvantaged on the original host (for examples, see Ebert 1998).
2. Theories of ecological specialization generally assume that adaptations to different habitats are antagonistic; alleles beneficial in one habitat impair performance in others and this drives species to specialize (Levins 1968). Hence, traits advantageous on the novel host may trade off with competitive ability on the original or alternate hosts (Gould 1979; Olmsted *et al.* 1984; Fry 1990).
3. Because simultaneous adaptation to different habitats exposes only a fraction of a generalist lineage to any given habitat, its response to selection in one habitat is weaker than that of a specialist lineage confined to the habitat.

The specialist lineage is thus predicted to evolve faster than the generalist lineage (Whitlock 1996). For the same reason, generalists are more prone to fitness reduction due to accumulation of mutations that are deleterious in some habitats but neutral (or nearly neutral) in others (Kawecki 1994, 1998). This cost would occur even when performance in different habitats is affected by different nonoverlapping loci (absence of trade-offs).

Viruses radiate by expanding their host range, which is the cellular environments where a virus produces progeny. Host range may be described in terms of host genotype, host species, target cell (tissues) within a host, or even the ability to overcome different antivirals or nonspecific immune responses. Even closely related viruses can have very different host ranges. For example, influenza A infects many species of birds and mammals (including whales, pigs, and humans), whereas influenza B is generally restricted to humans (Kingsbury 1991). Both A and B generate variability through segment reassortment when multiple viruses coinfect the same cell, but A is highly variable in its forms of surface glycoproteins, which presumably allows it to switch easily between hosts and to trigger worldwide pandemics (Webster *et al.* 1995).

Previous studies indicate that evolved generalist viruses are competitively inferior on their original host (*e.g.*, Chao *et al.* 1977; Novella *et al.* 1995a; Crill

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et al. 2000). Hence, viral attenuation (weakening) via passage on a novel host is the typical strategy employed to develop live vaccines, which can elicit an immune response without inducing disease (Bull 1994; Ebert 1998). Less explored is whether adaptation of viruses to one novel host allows them to better compete on an alternate novel host (*e.g.*, Weaver *et al.* 1999), that is, whether the fitness improvement is solely beneficial on the selected host or, alternatively, permits increased performance on an unselected novel host as well. Also intriguing is whether simultaneous adaptation to multiple novel hosts poses a greater challenge to viruses than adaptation to each novel host alone (Weaver *et al.* 1999).

This study examines questions about the costs associated with host radiation in a model RNA virus, vesicular stomatitis virus (VSV). When replicate virus populations are allowed to evolve on a novel host, is there systematic evidence this weakens their ability to compete on the original host? Are viruses evolved on one novel host advantaged (or disadvantaged) when competing on an unselected novel host? Are viruses that adapt in fluctuating host environments disadvantaged relative to viruses that evolve on only a single host?

MATERIALS AND METHODS

Viruses and host cells: The VSV (family *Rhabdoviridae*) genome is a single-stranded RNA molecule of negative polarity and ~11 kb, organized into five genes and a small 3′ noncoding region (Wagner 1991). VSV has been widely used as a model in RNA virus evolution (Elena *et al.* 2000; Moya *et al.* 2000) and provides a good system to explore the evolution of host radiation. Its characteristics include short generation times and extremely high rates of spontaneous mutation ($\sim 10^{-3}$–10^{-5} substitutions per nucleotide and round of replication; Drake and Holland 1999). More important, VSV infects a wide range of hosts in nature (including insects and mammals), but this characteristic has not been fully explored (Wagner 1991). Cell attachment of VSV is known to be pH-dependent (Fredericksson and Whitt 1998) and may involve common phospholipids of the cell membrane (*e.g.*, phosphatidylserine), but mammalian cells can vary considerably in degree of susceptibility to VSV infection (Wagner 1991). Viruses in this study were originally derived from the Mudd-Summer strain of the VSV Indiana serotype (hereafter wild type, or wt). MARM *C* (Holland *et al.* 1991) is a mouse I_1-monoclonal antibody (I_1-mAb; VandePol *et al.* 1986) resistant mutant, containing an $Asp^{259} \rightarrow Ala$ substitution in the surface glycoprotein (G); this amino acid substitution permits replication of MARM *C* under I_1-mAb levels that completely neutralize wt.

Three mammalian hosts were used in this study. Baby hamster kidney (BHK) cells, typically used to propagate VSV in our laboratory, served as the original host, whereas Madin-Darby canine kidney (MDCK) cells and human epithelial carcinoma (HeLa) cells served as novel hosts. BHK cells are derived from fibroblasts, cells that live in the spaces between other cells and secrete the proteins of the extracellular matrix (Adams 1990). The novel hosts are derived from epithelia; MDCK originated from epithelial cells covering the bounding cavities of canine kidney tubules, and HeLa cells are derived from tumor tissues of the human cervix (Adams 1990). The BHK cells are maintained in our laboratory and the HeLa and MDCK cells were obtained from the European Collection of Cell Cultures (ECACC).

Media and culture conditions: Cell monolayers were grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) containing either 5% heat-inactivated newborn bovine calf serum and 0.06% protease peptone 3 (BHK) or 10% heat-inactivated fetal calf serum (HeLa and MDCK). Cells were grown to a density of $\sim 10^5$ cells/cm^2 in 25-cm^2 plastic flasks for infections, or in 100-cm^2 dishes for routine maintenance. Cells were incubated at 37°, 95% relative humidity, and 5% CO_2 atmosphere.

Cell monolayers were infected at a multiplicity of infection of 0.01 viruses per cell to avoid the appearance of defective-interfering particles characteristic of high-multiplicity infections with VSV (Horodyski *et al.* 1983). Viral particles were enumerated by plaque assays using confluent cell monolayers under DMEM solidified with 0.7% agarose. Differential quantitation of genetically marked MARM clones and total virus was done by parallel platings in the presence and absence of I_1-mAb in the agarose overlay, respectively.

Experimental populations: A single clone of MARM *C* was used to found four replicate populations in each of five treatments: BHK (B), HeLa (H), MDCK (M), correlated-fluctuating HeLa-MDCK (CF), and random-fluctuating HeLa-MDCK (RF). The CF treatment featured alternating passages on HeLa and MDCK cells, whereas a random number generator decided fluctuation in the RF treatment. At the start of the experiment, each population was allowed to infect an overnight monolayer of the particular host. After 45 min incubation to allow virus adsorption, excess virus was removed and each mixture was incubated for an additional 47 hr. The propagation cycle was repeated using a diluted sample of the resultant viral progeny and a newly grown host monolayer. A total of 25 cycles were conducted for each population. The 48-hr transfer cycle ensured that the slower-growing non-BHK populations attained stationary densities. By passage five, we observed that all populations reached stationary density at 24 hr; but, for consistency, the 48-hr cycle was maintained throughout the experiment. Each cycle represents approximately four generations of viral evolution (Miralles *et al.* 2000). Therefore, each experiment proceeded for ~100 generations. Following daily propagation, a sample from each population was stored in a −80° freezer for further study.

Competition assays and fitness: We used the fitness assay developed by Holland *et al.* (1991). A MARM clone (or population) was mixed 1:1 with wt virus and the mixture was used to infect a cell monolayer as described above. Progeny were collected after 24 hr (BHK) or 48 hr (MDCK or HeLa), diluted 10^4-fold, and used to initiate the next competition transfer by infection of a fresh monolayer. At least two competition passages were carried out for each fitness estimate. The ratio of competitors was determined by plating, which yielded the proportion of MARM (p_t) to wt ($1 - p_t$) at passage number t. The antilogarithm of the slope of the regression

$$\ln \frac{p_t}{1 - p_t} = \ln \frac{p_0}{1 - p_0} + t \ln W$$

is taken as an estimate of the fitness of the MARM competitor relative to wt (Elena *et al.* 1998).

RESULTS

Preliminary measurements: Replicated ($n = 6$) assays on BHK showed that the mean fitness of MARM *C* relative to wt did not differ significantly from 1.0 (1.051 ±

0.055 SEM; $t_5 = 0.3480$, $P = 0.7420$), confirming that MARM *C* is a neutral variant on the original host. For greater accuracy, we divided this estimate by 1.051 to normalize the mean fitness of the MARM *C* ancestor to 1.0; identical scaling was used whenever wt served as the common competitor to gauge fitness changes on BHK.

Use of wt as the common competitor on HeLa and MDCK was problematic because wt was outcompeted by viruses evolved on these hosts: its presence was undetectable after day 1 of our multiday competition assays (see MATERIALS AND METHODS). To circumvent this problem, we propagated wt for eight passages on HeLa and MDCK independently and then isolated a single clone designated wt_H and wt_M, respectively. Replicated ($n = 3$) assays yielded mean fitnesses of MARM *C* relative to wt_H of 0.357 ± 0.032 on HeLa and of MARM *C* relative to wt_M of 0.414 ± 0.027 on MDCK. [These two values are not significantly different ($t_4 = 0.2347$, $P = 0.8260$), suggesting that the fitness of MARM *C* is similar in both novel environments prior to evolution.] We similarly adjusted measurements involving wt_H and wt_M as above.

Adaptation in simple environments: Viruses evolved in simple environments (BHK, MDCK, or HeLa) are expected to increase in fitness relative to the ancestor (HOLLAND *et al.* 1991; CLARKE *et al.* 1993; NOVELLA *et al.* 1995b; MIRALLES *et al.* 1999, 2000). To test this prediction, we competed three replicates of each population against a common competitor (wt_M or wt_H) at generation 100 and compared each dataset to that obtained for the MARM *C* ancestor in identical assays. [To compute significance levels for these comparisons, we employed the sequential Bonferroni criterion (RICE 1989)].

Eleven of 12 populations showed significant increases in fitness and the lone exception, population B4, was marginally not significant ($P = 0.0531$). By treating each B population as a single observation, the grand mean fitness exceeded 1.0 ($W = 1.934 \pm 0.127$; $t_3 = 6.3533$, one-tail $P = 0.0039$), and no fitness heterogeneity was detected among the populations (one-way ANOVA: $F_{3,20} = 0.6264$, $P = 0.6063$). Greater overall increases were observed in the H and M populations (Table 1), indicating that VSV is not optimally adapted to BHK and that less margin for improvement exists when the virus evolves further on its original host. More importantly, a nested ANOVA (population within host environment) showed no significant effect of novel host environments on fitness ($F_{1,6} = 1.6063$, $P = 0.2520$), although heterogeneity was detected among replicate populations ($F_{6,16} = 3.4891$, $P = 0.0212$). Thus, we conclude that populations in the H and M groups evolved similarly on their respective novel hosts.

Cost of adaptation in simple environments: We hypothesized that adaptation to a novel host would decrease competitive ability on the original host. To test this idea, we competed each H and M population at generation 100 against wt on BHK, with replication ($n = 3$, HeLa-evolved; $n = 6$, MDCK-evolved). Adaptation to HeLa detracted from competitive ability on BHK, and each population was found to be significantly less fit than the ancestor (Table 1). Pooling these estimates, the mean fitness of HeLa-adapted viruses was <1.0 ($W = 0.288 \pm 0.043$; $t_3 = 7.4837$, one-tail $P = 0.0025$), with no significant difference among populations (one-way ANOVA: $F_{3,8} = 0.7020$, $P = 0.5769$).

In contrast, data for the M group were more variable; these populations competed very well on BHK (Table 1), and populations M1 and M3 still have a fitness significantly greater than MARM *C*.

However, a more subtle cost of host radiation is evident for the M group (Figure 1): the better these viruses performed on MDCK, the worse they fared on the original host. This negative trend was highly significant (Pearson's correlation with $r = -0.9968$, 2 d.f., one-tail $P = 0.0016$); in addition, a nested ANOVA (population within host environment) confirmed that the evolved host environment strongly affects fitness on BHK ($F_{1,6} = 8.6946$, $P = 0.0257$). Hence, fitness of HeLa-evolved viruses agrees with the trade-off hypothesis, whereas a subtle (but detectable) cost existed for the MDCK-evolved viruses.

Fitness in unselected hosts: To test whether adaptation to one novel host is associated with performance on the unselected novel host, we competed each M population against wt_H on HeLa and each H population against wt_M on MDCK, with replication ($n = 3$). Results (Table 1 and Figure 2) showed that all eight evolved populations performed worse than the ancestor on the unselected host. Each of the four H populations showed significantly lower fitness on MDCK than that of the MARM *C* ancestor. In contrast, only one of the M populations (M1) had a significantly lower fitness on HeLa. However, treating each M population as one observation, the mean fitness was significantly <1.0 ($W = 0.472 \pm 0.056$; $t_3 = 9.4564$, one-tail $P = 0.0013$), with no significant difference among populations (one-way ANOVA: $F_{3,8} = 0.1996$, $P = 0.8938$). A two-way ANOVA (Table 2) confirmed that the selective environment affects subsequent performance on the unselected host. In particular, the highly significant interaction between treatment and competition environments prompted our conclusion that adaptation to one novel host negatively correlated with fitness on the alternate novel host.

Adaptation in fluctuating environments: To test whether adaptation in fluctuating environments is more costly when viruses grow on the original host, we competed each CF and RF population against wt on BHK cells with replication ($n = 3$). Results (Table 1) showed that CF and RF populations were less fit than their ancestor on BHK, and that the fitness disadvantage was equal in magnitude to that observed for viruses adapted to HeLa alone. A Tukey's HSD test (SOKAL and ROHLF 1995) confirmed homogeneity among the CF, RF, and H populations ($P = 0.9853$), whereas the M populations

TABLE 1

Mean fitness of evolved populations on three hosts

Strain	BHK	HeLa	MDCK
H1	0.281 ± 0.173 *	2.136 ± 0.438 *	0.402 ± 0.086 *
H2	0.210 ± 0.060 *	2.510 ± 0.257 *	0.305 ± 0.040 *
H3	0.252 ± 0.039 *	5.097 ± 1.008 *	0.013 ± 0.003 *
H4	0.408 ± 0.080 *	3.132 ± 0.658 *	0.154 ± 0.023 *
M1	3.510 ± 0.798	0.354 ± 0.151 *	3.532 ± 0.115 *
M2	1.449 ± 0.346	0.488 ± 0.260	5.131 ± 0.976 *
M3	3.835 ± 1.069	0.618 ± 0.341	3.229 ± 0.604 *
M4	1.528 ± 0.251	0.428 ± 0.208	5.271 ± 0.506 *
CF1	0.097 ± 0.021 *	3.301 ± 0.224 *	5.179 ± 2.339
CF2	0.121 ± 0.016 *	4.074 ± 0.399 *	6.671 ± 0.133 *
CF3	0.261 ± 0.102 *	3.038 ± 0.585 *	3.963 ± 1.044 *
CF4	0.178 ± 0.048 *	3.955 ± 0.556 *	3.752 ± 0.850 *
RF1	0.506 ± 0.078 *	3.905 ± 0.271 *	8.618 ± 2.979 *
RF2	0.301 ± 0.286 *	4.164 ± 0.820 *	3.028 ± 0.214 *
RF3	0.196 ± 0.027 *	4.174 ± 0.865 *	5.424 ± 2.015 *
RF4	0.371 ± 0.077 *	4.883 ± 0.691 *	4.614 ± 0.687 *

Adjusted by dividing by mean fitness of the MARM *C* ancestor relative to wt (BHK), wt_H (HeLa), or wt_M (MDCK). * indicates fitness significantly different from the ancestor (one-tail *t*-tests; sequential Bonferroni correction).

were clearly different ($P < 0.0001$). Figure 1 shows the comparison between the fitness on the original host and that attained in multiple novel habitats, where overall fitness across fluctuating environments was computed as the geometric mean of fitness values in the HeLa and MDCK environments (Gillespie 1991).

To determine whether evolution in fluctuating host environments limits viral adaptation, we competed each derived CF and RF population against wt_H on HeLa and against wt_M on MDCK, with replication ($n = 3$). Results (Table 1 and Figure 2) showed that each CF and RF population improved significantly on HeLa cells. More importantly, these populations competed as well as viruses evolved on HeLa alone (Tukey's HSD: CF, RF, and H group, $P = 0.0765$; M group, $P < 0.0001$). Fitness gains on MDCK were more variable; one of eight populations (CF1) did not improve significantly on MDCK (Table 1). However, its mean fitness is second highest among CF populations and the lack of statistical significance is likely due to measurement error. Treating each CF population as a single replicate, the grand mean is significantly >1.0 ($W = 4.891 \pm 0.671$; $t_3 = 5.7950$, one-tail $P = 0.0051$), with no significant difference among

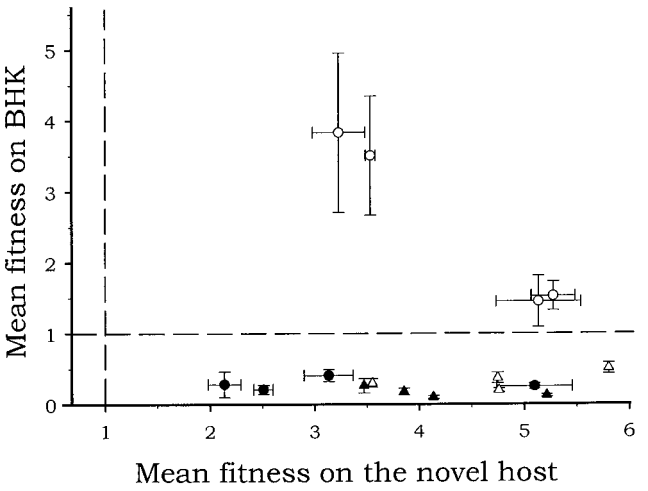

Figure 1.—Genetic correlation between fitness on the original BHK host and that in novel host environment(s). Each point represents the mean (±SEM) of replicate measurements. Values for populations evolved in fluctuating environments (CF, RF) are geometric means across novel cell types. (●) H populations; (○) M populations; (▲) CF populations; (△) RF populations.

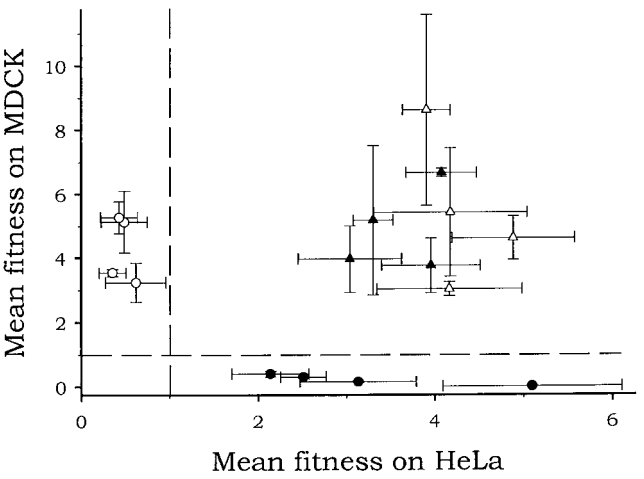

Figure 2.—Genetic correlation between fitness on the two novel hosts (HeLa and MDCK) for the H (●), M (○), CF (▲), and RF (△) populations. Each point represents the mean (±SEM) of replicate measurements.

TABLE 2

Analysis of variance for fitness of H and M populations in the unselected novel host

Source of variation	SS	d.f.	MS	*F*	*P*
Treatment environment	5.2715	1	5.2715	4.8988	0.0321
Competition environment	2.0069	1	2.0069	1.8650	0.1790
Interaction	139.5019	1	139.5019	129.6382	<0.0001
Error	47.3478	44	1.0761		
Total	194.1281	47			

populations (one-way ANOVA: $F_{3,8} = 0.9895$, $P = 0.4453$). Similarly, CF and RF populations competed as well on MDCK as viruses in the M group (Tukey's HSD test: $P = 0.6981$), but H populations were distinctly different ($P < 0.0001$). We concluded that simultaneous evolution on HeLa and MDCK did not constrain viral adaptation to novel hosts.

DISCUSSION

Does the radiation of parasites into novel host environments affect their ability to compete on the original host? Do environments that fluctuate in the availability of novel hosts limit adaptation? We examined the consequences of host radiation using VSV and mammalian host cells as a model system and these studies provide three pertinent results.

First, viruses that evolve on a novel host experience substantial improvements in fitness, but show reduced competitive ability in the original host. For viruses evolved on HeLa cells the fitness cost matched predictions; fitness of these populations on the original host was reduced below that of their ancestor. Evidently, traits that promote viral growth in cancerous epithelial cells (HeLa) oppose infection in fibroblast cells of connective tissue (BHK). For viruses evolved on MDCK cells the cost was less straightforward. These viruses retained strong competitive ability on the original host, but this negatively correlated with their performance on the evolved host. That is, the more fit evolved viruses were on MDCK, the worse they competed on BHK.

Second, adaptation of VSV to one novel host does not correlate with improved performance on an unselected novel host. When viruses radiate into novel host environments, the possibility exists that generally beneficial traits will fix in the population. For example, more rapid processing of RNA polymerase, increased RNA polymerase affinity for the substrate, or an increased encapsidation efficiency will assist in replication in all hosts. In contrast, other traits, such as changes affecting membrane receptors, cellular cytoskeleton protein components, ribosomes, or Golgi membranes might only allow adaptation to a specific host. Our results suggest that cell-specific mutations tend to spread in viral populations. HeLa-adapted viruses became less fit on MDCK, and MDCK-adapted strains became worse competitors on HeLa. Whereas these results support the general notion that fitness trade-offs across habitats drive species to specialize (Levins 1968), this result does not demonstrate a cost of host radiation, but rather it involves the specificity of viral adaptation to a particular host niche.

Third, simultaneous adaptation of viruses to two novel hosts did not limit their ability to compete on each host separately. In contrast, viruses evolved in fluctuating habitats performed as well as those evolved in simple novel environments. This was true whether environmental fluctuations in host availability occurred in a random or correlated (predictable) fashion. Furthermore, previous experiments in eastern equine encephalitis virus (Weaver *et al.* 1999) and VSV (Novella *et al.* 1999) also show that alternating host cycles do not limit adaptation. Taken together, these findings contradict the idea that weaker response to selection, or an increased mutational load, reduces the fitness of generalists below that of specialists (Kawecki 1994, 1998; Whitlock 1996).

We observed that fluctuating environments constrained the ability of viruses to compete on their original host. Whereas MDCK-evolved viruses maintained strong competitive ability on the ancestral host, viruses evolved in fluctuating MDCK-HeLa environments did not receive this benefit. Rather, fitness on the original host was reduced to that of viruses evolved on HeLa alone, demonstrating that one of the two novel habitats determined competitive performance. For this reason, genetic changes involving adaptation to MDCK must differ from those conferring an advantage in fluctuating environments.

Selection for host expansion in VSV: VSV infects mammals and insects and can be transmitted by arthropod vectors (Wagner 1991). Thus, exposure to novel and/or fluctuating host environments is likely to be important in VSV's evolution. This suggests that generalist variants of VSV capable of infecting more than one host type are selectively favored at least for transitions from old host to new hosts. Assuming that beneficial effects of host radiation in VSV compensate for any associated costs, then an adaptive mechanism should facilitate VSV's ability to jump between hosts. Perhaps host switches are simplified because VSV is an RNA virus that inherently mutates at very high rates (Drake and

Holland 1999); this may explain why the vast majority of arboviruses that alternate between hosts have RNA genomes. In addition, one of the major limitations regarding host range is the existence of appropriate receptors, and niche expansion may be easier for VSV because it seems to exploit a receptor (phosphatidylserine) common to most animal cells (Wagner 1991).

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LITERATURE CITED

Adams, R. L. P., 1990 *Cell Culture for Biochemists*. Elsevier, Amsterdam.

Bull, J. J., 1994 Virulence. Evolution **48:** 1423–1437.

Chao, L., B. R. Levin and F. M. Stewart, 1977 A complex community in a simple habitat: an experimental study with bacteria and phage. Ecology **58:** 369–378.

Clarke, D. K., E. A. Duarte, A. Moya, S. F. Elena, E. Domingo *et al.*, 1993 Genetic bottlenecks and population passages cause profound fitness differences in RNA viruses. J. Virol. **67:** 222–228.

Crill, W. D., H. A. Wichman and J. J. Bull, 2000 Evolutionary reversals during viral adaptation to alternating hosts. Genetics **154:** 27–37.

Drake, J. W., and J. J. Holland, 1999 Mutation rates among RNA viruses. Proc. Natl. Acad. Sci. USA **96:** 13910–13913.

Ebert, D., 1998 Experimental evolution of parasites. Science **282:** 1432–1435.

Elena, S. F., M. Dávila, I. S. Novella, J. J. Holland, E. Domingo *et al.*, 1998 Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet. Evolution **52:** 309–314.

Elena, S. F., R. Miralles, J. M. Cuevas, P. E. Turner and A. Moya, 2000 The two faces of mutation: extinction and adaptation in RNA viruses. IUBMB Life **49:** 1–5.

Frederickson, B. L., and M. A. Whitt, 1998 Attenuation of recombinant vesicular stomatitis viruses encoding mutant glycoproteins demonstrates a critical role for maintaining a high pH threshold for membrane fusion in viral fitness. Virology **240:** 349–358.

Fry, J. D., 1990 Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. Am. Nat. **136:** 569–580.

Futuyma, D. J., and G. Moreno, 1988 The evolution of ecological specialization. Annu. Rev. Ecol. Syst. **19:** 207–233.

Gillespie, J. H., 1991 *The Causes of Molecular Evolution*. Oxford University Press, New York.

Gould, F., 1979 Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae*. Evolution **33:** 791–802.

Holland, J. J., J. C. de la Torre, D. K. Clarke and E. Duarte, 1991 Quantitation of relative fitness and great adaptability of clonal populations of RNA viruses. J. Virol. **65:** 2960–2967.

Horodyski, F. M., S. T. Nichol, K. R. Spindler and J. J. Holland, 1983 Properties of DI particle resistant mutants of vesicular stomatitis virus isolated from persistent infections and from undiluted passages. Cell **33:** 801–810.

Kawecki, T. J., 1994 Accumulation of deleterious mutations and the evolutionary cost of being a generalist. Am. Nat. **144:** 833–838.

Kawecki, T. J., 1998 Red queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. Am. Nat. **152:** 635–651.

Kingsbury, D. W., 1991 Orthomyxoviridae and their replication, pp. 527–541 in *Fundamental Virology*, Ed. 2, edited by B. Fields and D. Knipe. Raven Press, New York.

Levins, R., 1968 *Evolution in Changing Environments*. Princeton University Press, Princeton, NJ.

Miralles, R., P. J. Gerrish, A. Moya and S. F. Elena, 1999 Clonal interference and the evolution of RNA viruses. Science **285:** 1745–1747.

Miralles, R., A. Moya and S. F. Elena, 2000 Diminishing returns of population size in the rate of RNA virus adaptation. J. Virol. **74:** 3566–3571.

Moya, A., S. F. Elena, A. Bracho, R. Miralles and E. Barrio, 2000 The evolution of RNA viruses: a population genetics view. Proc. Natl. Acad. Sci. USA **97:** 6967–6973.

Novella, I. S., D. K. Clarke, J. Quer, E. A. Duarte, C. H. Lee *et al.*, 1995a Extreme fitness differences in mammalian and insect hosts after continuous replication of vesicular stomatitis virus in sandfly cells. J. Virol. **69:** 6805–6809.

Novella, I. S., E. A. Duarte, S. F. Elena, A. Moya, E. Domingo *et al.*, 1995b Exponential increases of RNA virus fitness during large population transmission. Proc. Natl. Acad. Sci. USA **92:** 5841–5844.

Novella, I. S., C. L. Hershey, C. Escarmis, E. Domingo and J. J. Holland, 1999 Lack of evolutionary stasis during alternating replication of an arbovirus in insect and mammalian cells. J. Mol. Biol. **287:** 459–465.

Olmsted, R. A., R. S. Baric, B. A. Sawyer and R. E. Johnston, 1984 Sindbis virus mutants selected for rapid growth in cell culture display attenuated virulence in animals. Science **225:** 424–427.

Rainey, P. B., and M. Travisano, 1998 Adaptive radiation in a heterogeneous environment. Nature **394:** 69–72.

Rice, W. R., 1989 Analyzing tables of statistical tests. Evolution **43:** 223–225.

Sokal, R. R., and F. J. Rohlf, 1995 *Biometry*, Ed. 3. Freeman, San Francisco.

VandePol, S. B., L. Lefrancois and J. J. Holland, 1986 Sequence of the major antibody binding epitopes of the Indiana serotype of vesicular stomatitis virus. Virology **148:** 312–325.

Wagner, R. R., 1991 *Rhabdoviridae* and their replication, pp. 489–503 in *Fundamental Virology*, Ed. 2, edited by B. Fields and D. Knipe. Raven Press, New York.

Weaver, S. C., A. C. Brault, W. Kang and J. J. Holland, 1999 Genetic and fitness changes accompanying adaptation of an arbovirus to vertebrate and invertebrate cells. J. Virol. **73:** 4316–4326.

Webster, R. G., W. J. Bean and G. T. Gorman, 1995 Evolution of influenza viruses: rapid evolution and stasis, pp. 531–543 in *Molecular Basis of Viral Evolution*, edited by A. Gibbs, C. H. Calisher and F. García-Arenal. Cambridge University Press, Cambridge, UK.

Whitlock, M. C., 1996 The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. Am. Nat. **148:** S65–S77.

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