Supporting Information

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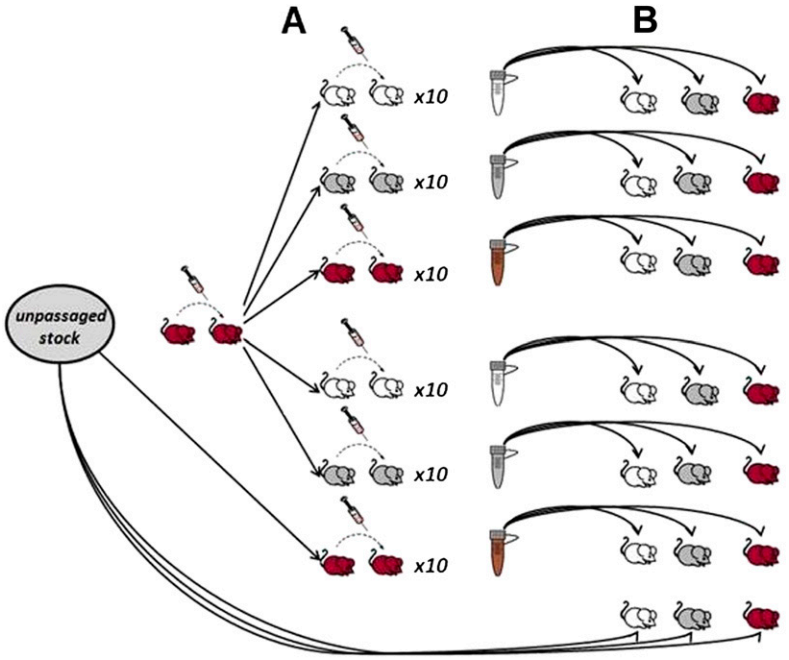

Fig. S1. Experimental design of serial passages and tests. (*A*) Virus was grown in tissue culture to create an unpassaged stock. Five of six passage lines began from a stock virus that had been passaged in BALB/c^{dd} animals for two rounds, and one was started directly from our unpassaged stock (see *Materials and Methods*). Virus was serially passaged for 10 rounds through genetically identical individuals from each of three *MHC* genotypes (brown mice-BALB/c^{dd}; gray mice-BALB/c^{bb}; white mice-BALB/c^{kk}). Two independent passage lines were conducted in each of the three *MHC* genotypes to produce six postpassage stocks (color coded tubes). (*B*) During the test phase, groups of animals (12 individuals per group) from each *MHC* genotype were infected with unpassaged stock to obtain baseline fitness and virulence measures. Six groups of animals (6–15 individuals per group) from each *MHC* genotype were also infected with one of the six postpassage stocks and pathogen fitness and virulence measures were compared.

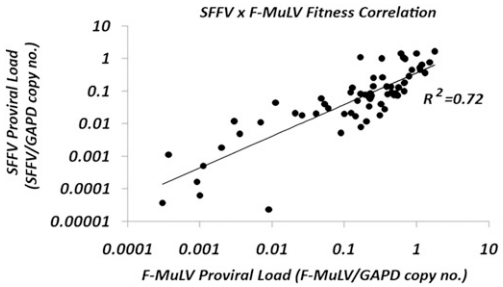

Fig. S2. Correlation between Friend murine leukemia virus (F-MuLV) and spleen focus forming virus (SFFV) titers. We tested the prediction that F-MuLV and SFFV proviral loads would be highly correlated within the same individual. An ANOVA was performed, and demonstrated a highly significant and positive association between measures of F-MuLV and SFFV proviral loads (R^2 = 0.72, $F_{1,64}$ = 169.96, $P < 0.0001$).

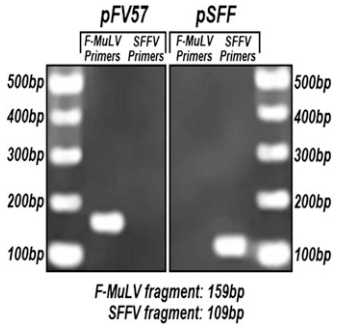

Fig. S3. F-MuLV and SFFV primer sets are virus-specific. Plasmids containing either the entire F-MuLV (pFV57) or SFFV genome (pSFF) were used as template DNA in PCR reactions to confirm that F-MuLV and SFFV primer sets used in this study, specifically amplified from the respective target genomes.

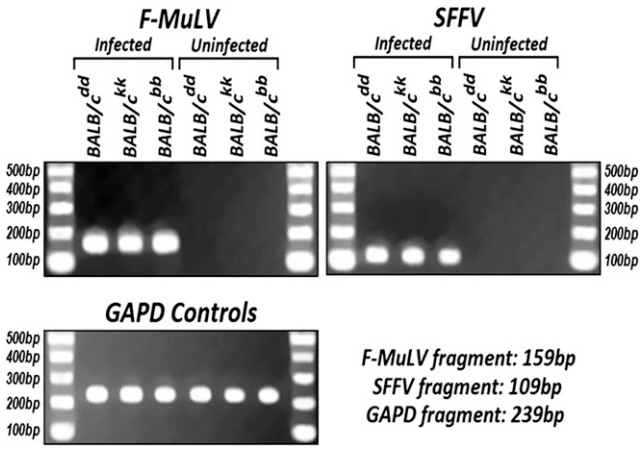

Fig. S4. Viral primers do not amplify endogenous retroviral sequences. Primers specific to both the F-MuLV and SFFV viruses were designed and tested on DNA isolated from uninfected and infected animals from each host genotype to ensure that primer sets were specific to infectious virus and not germ-line–encoded endogenous retroviral sequences. GAPDH (*GAPD*) amplifications for each individual are provided as internal controls for the presence of quality template DNA in each PCR (see quantitative PCR methods for cycling conditions).

Table S1. Passaged viruses are more fit than unpassaged virus

Virus genotype	Mean fitness ± SD	*P* value (unpassaged vs. postpassage virus)
Proviral load measures	(F-MuLV/GAPDH copy no.)	
Unpassaged (infecting BALB/c^{dd}) (n = 11)	0.01 ± 0.01	—
Unpassaged (infecting BALB/c^{kk}) (n = 10)	0.01 ± 0.01	—
Unpassaged (infecting BALB/c^{bb}) (n = 12)	0.004 ± 0.01	—
BALB/c^{dd} (Replicate 1 virus) (n = 9)	0.35 ± 0.24	($F_{1,18}$ = 40.52, P < 0.0001)
BALB/c^{dd} (Replicate 2 virus) (n = 7)	0.33 ± 0.19	($F_{1,16}$ = 35.36, P < 0.0001)
Between replicate comparison		($F_{1,14}$ = 0.04, P = 0.85)
BALB/c^{kk} (Replicate 1 virus) (n = 14)	1.05 ± 0.52	($F_{1,22}$ = 143.81, P < 0.0001)
BALB/c^{kk} (Replicate 2 virus) (n = 9)	0.67 ± 0.48	($F_{1,17}$ = 97.64, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 1.93, P = 0.18)
BALB/c^{bb} (Replicate 1 virus) (n = 13)	0.53 ± 0.44	($F_{1,23}$ = 147.73, P < 0.0001)
BALB/c^{bb} (Replicate 2 virus) (n = 10)	0.31 ± 0.16	($F_{1,20}$ = 122.16, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 0.96, P = 0.34)
Infectious virus particles	(FFU/spleen)	
Unpassaged (infecting BALB/c^{dd}) (n = 12)	115.33 ± 52.76	—
Unpassaged (infecting BALB/c^{kk}) (n = 12)	149 ± 105.42	—
Unpassaged (infecting BALB/c^{bb}) (n = 12)	152.8 ± 61.24	—
BALB/c^{dd} (replicate 1 virus) (n = 9)	15,969.87 ± 7384.44	($F_{1,19}$ = 230.76, P < 0.0001)
BALB/c^{dd} (replicate 2 virus) (n = 7)	14,953.48 ± 3752.78	($F_{1,17}$ = 187.56, P < 0.0001)
Between replicate comparison		($F_{1,14}$ = 0.013, P = 0.91)
BALB/c^{kk} (replicate 1 virus) (n = 13)	4,048.16 ± 3134.84	($F_{1,23}$ = 293.16, P < 0.0001)
BALB/c^{kk} (replicate 2 virus) (n = 9)	21,514.16 ± 10696.89	($F_{1,19}$ = 82.60, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 58.66, P < 0.0001)
BALB/c^{bb} (replicate 1 virus) (n = 13)	21,556.68 ± 16833.63	($F_{1,23}$ = 183.18, P < 0.0001)
BALB/c^{bb} (replicate 2 virus) (n = 10)	12,550.07 ± 4041.63	($F_{1,20}$ = 177.30, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 1.25, P = 0.28)

Table S1 provides means, SDs, sample sizes (n), and results of one-way ANOVAs comparing unpassaged and postpassage virus fitness within each of the three host genotypes. Based on proviral load measures, the mean fitness of unpassaged virus across the three *MHC* genotypes is 0.009 F-MuLV/GAPDH copies and the mean fitness across the six postpassage stocks is 0.54 F-MuLV/GAPDH copies (a 54-fold difference). Biologically, this can be interpreted to mean that at the end of 12 d of infection with unpassaged virus, ~1% of host genomes from spleen cells contain an integrated F-MuLV provirus, but infection with postpassage viruses results in over 50% of host genomes containing an integrated F-MuLV provirus (assuming a 1:1 host cell:provirus ratio). Infectious virus particle measures show a similar pattern. The mean fitness of unpassaged virus across the three *MHC* genotypes is 139.04 focus forming units (FFU)/spleen and the mean fitness of postpassage stocks is 15098.74 FFU/spleen. This finding represents a 109-fold increase in the number of infectious virus particles found in the spleens of animals infected with postpassage virus stocks. Therefore, pathogen adaptation during serial passage has produced a high fitness pathogen phenotype.

Table S2. Passaged viruses are more virulent than unpassaged virus

Virus genotype	Mean virulence ± SD [spleen size (g)]	*P* value (unpassaged vs. postpassage virus)
Unpassaged (infecting BALB/c^{dd}) (n = 11)	0.17 ± 0.02	—
Unpassaged (infecting BALB/c^{kk}) (n = 10)	0.16 ± 0.02	—
Unpassaged (infecting BALB/c^{bb}) (n = 12)	0.18 ± 0.02	—
BALB/c^{dd} (replicate 1 virus) (n = 9)	3.02 ± 0.08	($F_{1,18}$ = 729.83, P < 0.0001)
BALB/c^{dd} (replicate 2 virus) (n = 7)	2.73 ± 0.17	($F_{1,16}$ = 376.47, P < 0.0001)
Between replicate comparison		($F_{1,14}$ = 1.55, P = 0.23)
BALB/c^{kk} (replicate 1 virus) (n = 14)	2.90 ± 0.21	($F_{1,22}$ = 304.47, P < 0.0001)
BALB/c^{kk} (replicate 2 virus) (n = 9)	3.32 ± 0.10	($F_{1,17}$ = 492.98, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 1.92, P = 0.18)
BALB/c^{bb} (replicate 1 virus) (n = 13)	2.81 ± 0.13	($F_{1,23}$ = 709.82, P < 0.0001)
BALB/c^{bb} (replicate 2 virus) (n = 10)	3.46 ± 0.14	($F_{1,20}$ = 747.03, P < 0.0001)
Between replicate comparison		($F_{1,21}$ = 5.47, P = 0.03)

Table S2 provides means, SDs, sample sizes (n), and results of one-way ANOVAs comparing virulence between unpassaged and postpassage virus stocks within each of the three host genotypes. Virulence measures associated with infection by unpassaged vs. postpassage viruses for the same groups listed in the table were compared using one-way ANOVAs. Mean spleen size was 0.17 g for animals infected with unpassaged virus and 3.04 g for animals infected with postpassage viruses. Therefore, the observed increases in pathogen fitness in our postpassage stocks after serial passage resulted in an ~18-fold increase in virulence (splenomegaly) associated with infection.

Table S3. Linear model comparisons

	Proviral loads		Infectious particles		Splenomegaly	
Model	AIC score	*P* value	AIC score	*P* value	AIC score	*P* value
Host genotype virus genotype **genotype*genotype**	527.38	<0.0001	486.85	<0.0001	410.62	<0.0001
Virus genotype **genotype*genotype**	565.18	0.0003	540.24	<0.0001	437.98	0.0006
Genotype*genotype	562.09	0.0002	549.80	<0.0001	443.38	0.0013

We used two criteria to identify which of the above three linear models was most appropriate to summarize patterns of pathogen adaptation and virulence. First, we compared AIC (Akaike's Information Criteria) scores from each of the three models, which are reported in the above table along with *P* values associated with the effect of interest in each analysis (boldface). The scores and *P* values above are based on model analysis of pooled data between our two replicate experiments. The lowest AIC score identifies the best model. Of our three models, the model incorporating the main effects of host genotype, virus genotype, and the interaction effect between these two variables (denoted "*MHC* familiarity" above) resulted in the lowest AIC scores. This result is because the main effects of host genotype and virus genotype are significant in almost every comparison (see Table S4). The second criterion we used to identify the best model was how robust a given model was when replicate experiments were analyzed independently. The only model of our three to conserve all significant effects when data were analyzed independently was the same model identified by the lowest AIC score. Therefore, we reported *P* values associated with this model in the article. More detailed summary statistics including parameter estimates, effect sizes, and independent analyses for each replicate experiment are available upon request.

Table S4. Pathogen adaptation is host genotype-specific

Effect	Pooled data	Replicate experiment 1	Replicate experiment 2
Proviral load			
Host genotype	($F_{2,167}$ = 20.20, d.f.=2, P < 00001)	($F_{2,88}$ = 11.99, P < 0.0001)	($F_{2,79}$ = 11.64, P < 0.0001)
Virus genotype	($F_{5,167}$ = 1.73, P = 0.13)	($F_{2,88}$ = 0.21, P = 0.81)	($F_{2,79}$ = 6.96, P = 0.0017)
Genotype*genotype	($F_{10,167}$ = 4.57, P < 0.0001)	($F_{4,88}$ = 5.22, P = 0.0009)	($F_{4,79}$ = 4.38, P = 0.0032)
Infectious particles			
Host genotype	($F_{2,170}$ = 34.82, P < 0.0001)	($F_{2,90}$ = 11.24, P < 0.0001)	($F_{2,80}$ = 27.67, P < 0.0001)
Virus genotype	($F_{5,170}$ = 5.52, P < 0.0001)	($F_{2,90}$ = 3.72, P = 0.03)	($F_{2,80}$ = 0.20, P = 0.82)
Genotype*genotype	($F_{10,170}$ = 6.35, P < 0.0001)	($F_{4,90}$ = 8.09, P < 0.0001)	($F_{4,80}$ = 4.47, P = 0.003)
Splenomegaly			
Host genotype	($F_{2,167}$ = 15.56, P < 0.0001)	($F_{2,88}$ = 8.51, P = 0.0004)	($F_{2,79}$ = 9.64, P = 0.0002)
Virus genotype	($F_{5,167}$ = 4.58, P = 0.0006)	($F_{2,88}$ = 8.02, P = 0.0007)	($F_{2,79}$ = 5.50, P = 0.006)
Genotype*genotype	($F_{10,167}$ = 4.01, P < 0.0001)	($F_{4,88}$ = 4.11, P = 0.005)	($F_{4,79}$ = 5.87, P = 0.0004)

A standard least-squares multiple regression model was used to test for host genotype*virus genotype interaction effects. By incorporating the main effects of host genotype and virus genotype into a standard least-squares model we are able to remove variation in our data that is explained by differences among host genotypes in their average suceptibility to infection with adapted viruses, as well as differences in average pathogen fitness among our postpassage stocks. Two replicate experiments were conducted in this study. For each experiment a single virus line was derived from each of the three host genotypes. In total, six virus lines were created (three host genotypes × two replicate virus lines per host background). For "pooled data" estimates reported above, a generalized linear model was constructed that modeled replicate virus lines as independent samples (n = 6 total virus lines) for a total of 18 host-genotype by pathogen-genotype combinations. In addition, each replicate experiment was analyzed independently (n = 3 total virus lines) for a total of nine host-genotype by pathogen-genotype combinations. *F*-statistics, degrees of freedom, and *P* values for all tests are reported above. All *P* values reported for interaction effects are significant, and indicate that passaged pathogens are significantly more fit and virulent when infecting hosts carrying a familiar versus an unfamiliar *MHC* genotype (see Fig. 4). Moreover, the significance of these effects is conserved when replicate experiments are analyzed independently. Thus, pathogen fitness tradeoffs between host *MHC* genotypes that arise during serial passage provide a selective advantage to hosts carrying unfamiliar (i.e., rare) *MHC* genotypes through increased resistance to infectious disease.