Supporting Information

Savage and Zamudio 10.1073/pnas.1106893108

SI Methods

Egg Mass Collection and Husbandry. We collected *L. yavapaiensis* egg masses from five populations in Arizona in March 2008 (Fig. S1). We detached partial clutches and shipped them overnight to Cornell University. Frogs were housed in a 12 h/12 h light/dark regimen at 30% humidity (±3%) and 21 °C (±1 °C) to mimic winter field conditions. Upon hatching, we equally divided larvae from each clutch into eight replicate 16 × 30 × 9 cm plastic cages filled with dechlorinated laboratory water. We fed larvae 90% spirulina algae and 10% fish flakes ad libitum, replacing water with fresh dechlorinated laboratory water three times per week. Variable survival resulted in unequal numbers of frogs per replicate, ranging from one to five per cage (Table S3). We transferred metamorphosed frogs to 16 × 30 × 9 cm plastic cages containing plastic perches and kept moist with a film of dechlorinated laboratory water. Frogs were fed 3-wk-old pathogen-free crickets ad libitum. Only metamorphs reaching the stage of complete tail resorption (i.e., past Gosner stage 46) were used in experimental infections.

***Bd* and Chytridiomycosis Field Surveys.** We surveyed populations for *Bd* during winter (January and February) of 2007 through 2010 (1). We categorized each dead individual testing positive for *Bd* as a chytridiomycosis mortality event. Additionally, we collected frogs with signs of chytridiomycosis (i.e., skin redness, lethargy, and loss of righting ability) for overnight observation, and categorized these as *Bd* mortalities if death occurred within 24 h and the individuals were *Bd*-positive. We estimated *Bd* infection and mortality prevalence with 95% Clopper–Pearson binomial confidence intervals (2).

Analysis of Risk Factors. We calculated relative risk (RR) using Eq. (**1**):

$$\mathrm{RR} = (p^{+} \times c^{-}) \,/\, (p^{-} \times c^{+}), \qquad [1]$$

where p^{+} is the frequency of frogs with allele x that died, p^{-} is the frequency of frogs with allele x that did not die, c^{-} is the frequency of frogs without allele x that died, and c^{+} is the frequency of frogs without allele x that did not die. Significance of each RR value was assessed by Fisher exact test and sequential Bonferroni correction (3).

1. Savage AE, Sredl MJ, Zamudio KR (2011) Disease dynamics vary spatially and temporally in a North American amphibian. *Biol Conserv* 144:1910–1915.
2. Clopper C, Pearson S (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 26:404–413.
3. Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, Frost SDW (2006) Automated phylogenetic detection of recombination using a genetic algorithm. *Mol Biol Evol* 23: 1891–1901.

Fig. S1. (*A*) Egg mass collection localities in Arizona for the five *L. yavapaiensis* populations used in experimental *Bd* infections. (*B*) Photo of AC shows typical *L. yavapaiensis* habitat. (*C*) Image of a *Bd*-infected frog found dead with signs of chytridiomycosis at CIC. (*D*) Image of a healthy frog from CIC.

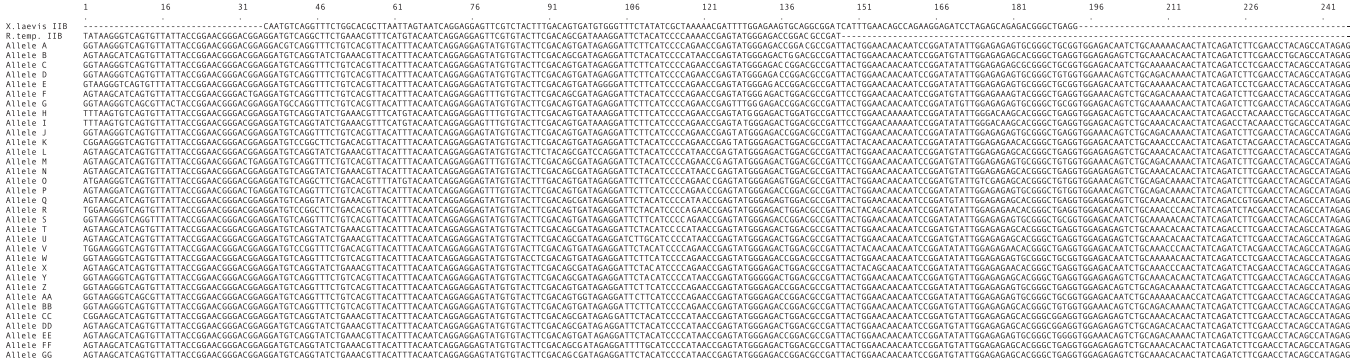

Fig. S2. Alignment of 33 MHC class IIB alleles recovered from 99 *Bd*-infected *L. yavapaiensis* individuals. Alleles were aligned to *Silurana* (*Xenopus*) *laevis* and *Lithobates* (*Rana*) *temporaria* partial MHC class IIB sequences from GenBank (accession nos. EF210752.1 and FJ876299.1, respectively).

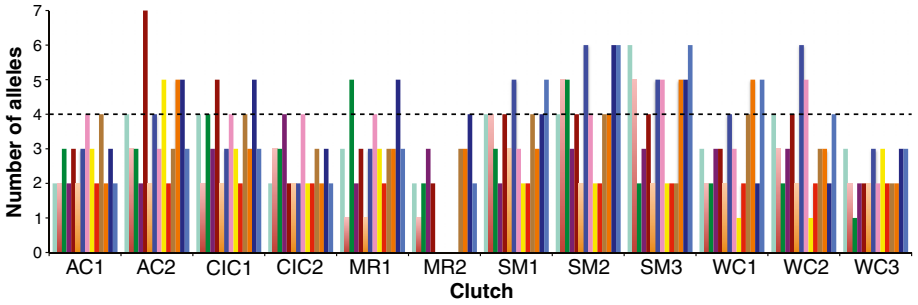

Fig. S3. Number of microsatellite alleles recovered for each *Bd*-infected *L. yavapaiensis* clutch. Colors represent each of the 14 genotyped microsatellite loci. The dotted line shows the threshold above which multiple paternity was inferred.

Table S1. Laboratory-reared, *Bd*-infected *L. yavapaiensis* individuals, housing conditions, change in mass over the course of the experiment, maximum *Bd* infection intensity (in genome equivalents), and MHC class IIB PBR genotypes

Individual	Pop	Replicate	Clutch	Survived, d	Survived?	No./ cage	0 DPI mass, g	Death/ 35DPI mass, g	Change in mass	PBR hetero- zygosity	Max. inf. intensity	PBR Allele 1	PBR Allele 2
CIC 1.1.A1 TUFI-1	CIC	1	1	7	No	2	1.36	0.91	−0.45	Hom	515.7	A	A
CIC 1.1.A1 TUFI-2	CIC	1	1	3	No	2	1.21	1.41	0.2	Hom	515.7	A	A
CIC 1.1.A2 TUFI-1	CIC	2	1	16	No	3	1.12	0.97	−0.15	Hom	625.6	A	A
CIC 1.1.A2 TUFI-2	CIC	2	1	20	No	3	0.81	1.2	0.39	Het	625.6	A	D
CIC 1.1.A2 TUFI-3	CIC	2	1	14	No	3	1.38	1.15	−0.23	Hom	625.6	A	A
CIC 1.1.B1 TUFI-1	CIC	3	1	2	No	3	1.96	1.9	−0.06	Hom	NA	A	A
CIC 1.1.B1 TUFI-3	CIC	3	1	4	No	3	1.88	1.98	0.1	Hom	NA	A	A
CIC 1.1.B2 TUFI-1	CIC	4	1	5	No	1	1.45	1.65	0.2	Het	NA	A	D
CIC 1.2.A1 TUFI-1	CIC	5	1	6	No	1	1.11	1.14	0.03	Hom	1777.7	A	A
CIC 1.2.A2 TUFI-1	CIC	6	1	15	No	4	1.07	1.28	0.21	Hom	948.9	A	A
CIC 1.2.A2 TUFI-2	CIC	6	1	21	No	4	1.38	1.6	0.22	Hom	784.89	A	A
CIC 1.2.A2 TUFI-3	CIC	6	1	21	No	4	1.51	1.52	0.01	Hom	784.89	A	A
CIC 1.2.A2 TUFI-4	CIC	6	1	29	No	4	1.8	1.62	−0.18	Hom	784.89	A	A
CIC 1.2.B1 TUFI-1	CIC	7	1	13	No	5	2.82	3.2	0.38	Hom	144.58	A	A
CIC 1.2.B1 TUFI-2	CIC	7	1	13	No	5	1.51	1.32	−0.19	Hom	144.58	A	A
CIC 1.2.B1 TUFI-3	CIC	7	1	7	No	5	0.98	0.97	−0.01	Hom	144.58	A	A
CIC 1.2.B1 TUFI-4	CIC	7	1	7	No	5	1.1	1.29	0.19	Hom	144.58	A	A
CIC 1.2.B1 TUFI-5	CIC	7	1	14	No	5	1.24	1.43	0.19	Hom	144.58	A	A
CIC 1.2.B2 TUFI-1	CIC	8	1	8	No	2	2.37	2.15	−0.22	Hom	227.4	A	A
CIC 1.2.B2 TUFI-2	CIC	8	1	6	No	2	1.85	1.5	−0.35	Hom	227.4	A	A
CIC 2.1.A1 TUFI-1	CIC	9	1	20	No	1	1.17	1.22	0.05	Hom	1669.24	A	A
CIC 2.1.A2 TUFI-1	CIC	10	2	16	No	4	1.16	1.04	−0.12	Het	981.96	A	C
CIC 2.1.A2 TUFI-2	CIC	10	2	23	No	4	1.46	1.32	−0.14	Hom	981.96	A	A
CIC 2.1.A2 TUFI-3	CIC	10	2	3	No	4	1.27	1.49	0.22	Het	981.96	A	G
CIC 2.1.A2 TUFI-4	CIC	10	2	3	No	4	1.14	1.22	0.08	Hom	981.96	A	A
CIC 2.1.B1 TUFI-1	CIC	11	2	29	No	1	1.48	1.11	−0.37	Hom	441.87	A	A
CIC 2.1.B2 TUFI-1	CIC	12	2	22	No	1	0.97	1.13	0.16	Hom	860.38	A	A
CIC 2.2.A1 TUFI-1	CIC	13	2	12	No	3	1.1	1.4	0.3	Het	301.87	A	I
CIC 2.2.A1 TUFI-2	CIC	13	2	13	No	3	1.01	1.05	0.04	Hom	301.87	A	A
CIC 2.2.A1 TUFI-3	CIC	13	2	13	No	3	1.33	0.95	−0.38	Hom	301.87	A	A
CIC 2.2.A2 TUFI-1	CIC	14	2	5	No	2	1.46	1.99	0.53	Hom	358.9	A	A
CIC 2.2.A2 TUFI-2	CIC	14	2	6	No	2	1.6	2.01	0.41	Hom	358.9	A	A
CIC 2.2.B1 TUFI-1	CIC	15	2	20	No	1	1.67	1.51	−0.16	Hom	948.9	A	A
CIC 2.2.B2 TUFI-1	CIC	16	2	11	No	1	1.95	2.1	0.15	Hom	146.9	A	A
AC 1.1.A1 TUFI-1	AC	17	3	8	No	3	1.04	0.7	−0.34	Hom	766.3	E	E
AC 1.1.A1 TUFI-2	AC	17	3	15	No	3	1.08	1.04	−0.04	Hom	766.3	A	A
AC 1.1.A1 TUFI-3	AC	17	3	16	No	3	1.16	1.47	0.31	Hom	766.3	A	A
AC 1.1.A2 TUFI-1	AC	18	3	35	Yes	3	3.08	2.34	-0.74	Het	625.5	A	Q
AC 1.1.A2 TUFI-2	AC	18	3	35	Yes	3	2.88	2.47	-0.41	Het	625.5	A	B
AC 1.1.A2 TUFI-3	AC	18	3	35	Yes	3	2.4	2.09	-0.31	Het	625.5	A	B
AC 1.1.B1 TUFI-1	AC	19	3	35	Yes	1	1.96	1.59	-0.37	Het	214.3	A	B
AC 1.2.A2 TUFI-1	AC	20	3	35	Yes	1	1.43	0.89	-0.54	Het	114.1	A	X
AC 1.2.B2 TUFI-1	AC	21	3	23	No	1	1.41	1.2	−0.21	Hom	931.4	E	E
AC 2.1.A1 TUFI-1	AC	22	4	1	No	1	0.98	0.8	−0.18	Hom	NA	A	A
AC 2.2.A1 TUFI-1	AC	23	4	3	No	1	1.31	1.04	−0.27	Hom	NA	C	C
AC 2.2.A2 TUFI-1	AC	24	4	35	Yes	2	0.97	0.93	-0.04	Het	328.8	A	Y
AC 2.2.A2 TUFI-2	AC	24	4	35	Yes	2	1.26	1.27	0.01	Het	328.8	A	W
AC 2.2.B1 TUFI-1	AC	25	4	26	No	4	1.49	1.41	−0.08	Het	1161.3	C	B
AC 2.2.B1 TUFI-2	AC	25	4	26	No	4	1.05	1.3	0.25	Hom	1161.3	A	A
AC 2.2.B1 TUFI-3	AC	25	4	24	No	4	1.19	1.33	0.14	Hom	1161.3	A	A
AC 2.2.B1 TUFI-4	AC	25	4	24	No	4	1.2	1.31	0.11	Hom	1161.3	C	C
MR 1.2.A2 TUFI-1	MR	26	5	22	No	1	0.77	1.07	0.3	Het	1291.91	AA	A
MR 1.2.B2 TUFI-1	MR	27	5	28	No	3	1.3	1.51	0.21	Het	811.98	H	A
MR 1.2.B2 TUFI-2	MR	27	5	28	No	3	1.54	1.63	0.09	Het	811.98	C	S
MR 2.2.B2 TUFI-3	MR	27	5	29	No	3	0.94	1.28	0.34	Hom	811.98	C	GG
MR 2.1.B2 TUFI-1	MR	28	5	9	No	1	1.24	1.32	0.08	Het	137.6	A	FF
WC 2.2.A1 TUFI-1	WC	29	6	16	No	3	1.67	2.89	1.22	Het	1338.32	D	O
WC 2.2.A1 TUFI-2	WC	29	6	15	No	3	1.14	1.31	0.17	Het	1338.32	O	BB
WC 2.2.A1 TUFI-3	WC	29	6	11	No	3	0.87	0.6	−0.27	Het	1338.32	A	K
WC 2.2.A2 TUFI-1	WC	30	6	3	No	3	0.99	1.43	0.44	Het	855.11	O	EE
WC 2.2.A2 TUFI-2	WC	30	6	2	No	3	1.17	1.08	−0.09	Het	855.11	A	J
WC 2.2.A2 TUFI-3	WC	30	6	14	No	3	0.99	1.17	0.18	Het	855.11	P	A
WC 2.2.B1 TUFI-1	WC	31	6	11	No	2	0.95	1.01	0.06	Het	266.1	O	F

Table S1. Cont.

Individual	Pop	Replicate	Clutch	Survived, d	Survived?	No./ cage	0 DPI mass, g	Death/ 35DPI mass, g	Change in mass	PBR hetero- zygosity	Max. inf. intensity	PBR Allele 1	PBR Allele 2
WC 2.2.B1 TUFI-2	WC	31	6	6	No	2	1.2	1.02	−0.18	Het	266.1	O	N
WC 1.1.A1 TUFI-1	WC	32	6	2	No	1	1.28	1.01	−0.27	Hom	NA	F	F
WC 1.1.A2 TUFI-1	WC	33	6	7	No	4	1.5	1.03	−0.47	Hom	526.4	F	F
WC 1.1.A2 TUFI-2	WC	33	6	6	No	4	1.08	1.05	−0.03	Het	526.4	Z	F
WC 1.1.A2 TUFI-3	WC	33	6	7	No	4	1.04	1.12	0.08	Hom	526.4	A	A
WC 1.1.A2 TUFI-4	WC	33	6	5	No	4	0.82	0.73	−0.09	Hom	526.4	A	A
WC 1.1.B2 TUFI-1	WC	34	6	9	No	1	1.07	0.51	−0.56	Het	400.1	A	CC
WC 3.1.B TUFI-1	WC	35	7	5	No	1	1.51	1.13	−0.38	Hom	NA	A	A
WC 3.1.C TUFI-1	WC	36	7	4	No	2	2.21	1.91	−0.3	Hom	NA	A	A
WC 3.1.C TUFI-2	WC	36	7	5	No	2	1.63	1.94	0.31	Hom	NA	A	A
SM 1.1.A1 TUFI-1	SM	37	8	7	No	3	0.8	0.9	0.1	Hom	133.6	A	A
SM 1.1.A1 TUFI-2	SM	37	8	7	No	3	0.81	0.96	0.15	Hom	133.6	A	A
SM 1.1.A1 TUFI-3	SM	37	8	2	No	3	0.82	0.72	−0.1	Hom	133.6	L	L
SM 1.1.A2 TUFI-1	SM	38	8	35	Yes	1	1.18	1.49	0.31	Het	264.7	A	Q
SM 1.1.B1 TUFI-1	SM	39	8	7	No	1	0.94	0.97	0.03	Hom	611.7	A	A
SM 1.2.B1 TUFI-1	SM	40	8	1	No	2	1.2	1.2	0	Hom	332.30	A	A
SM 1.2.B1 TUFI-2	SM	41	8	17	No	2	1.24	1.04	−0.2	Hom	332.30	A	A
SM 1.2.B2 TUFI-1	SM	42	8	1	No	2	1.27	1.33	0.06	Het	1302.09	A	U
SM 1.2.B2 TUFI-2	SM	42	8	22	No	2	1.36	1.17	−0.19	Hom	1302.09	A	A
SM 1.2.A1 TUFI-1	SM	43	9	10	No	1	2	2.1	0.1	Hom	549.22	A	A
SM 2.1.A1 TUFI-1	SM	44	9	35	Yes	1	1.41	0.93	-0.48	Hom	125.6	B	B
SM 2.1.A2 TUFI-1	SM	45	9	10	No	2	1.22	1.01	−0.21	Hom	886.98	B	B
SM 2.1.A2 TUFI-2	SM	45	9	19	No	2	1.28	1.31	0.03	Hom	886.98	B	B
SM 2.1.B1 TUFI-1	SM	46	9	6	No	2	0.96	1.02	0.06	Hom	359.6	B	B
SM 2.1.B1 TUFI-2	SM	46	9	7	No	2	1.42	0.58	−0.84	Hom	359.6	R	R
SM 2.1.B2 TUFI-1	SM	47	9	7	No	1	0.95	0.65	−0.3	Het	371.3	B	E
SM 2.2.B2 TUFI-1	SM	48	9	9	No	1	2.65	1.45	−1.2	Het	422.3	B	DD
SM 2.2.B1 TUFI-1	SM	49	9	35	Yes	1	0.96	0.92	−0.04	Het	321.8	B	Q
SM 2.3.B2 TUFI-1	SM	50	8	35	Yes	1	1.09	0.9	−0.19	Het	593.6	B	N
SM 3.1.A1 TUFI-1	SM	51	10	35	Yes	1	1.15	0.82	−0.33	Het	408.4	A	B
SM 3.1.A2 TUFI-1	SM	52	10	35	Yes	1	1.56	1.57	0.01	Het	523.2	A	T
SM 3.1.B1 TUFI-1	SM	53	10	35	Yes	1	1.26	1.36	0.1	Het	149.4	A	Q
SM 3.2.A1 TUFI-1	SM	54	10	7	No	1	0.74	0.82	0.08	Hom	288.4	A	A
SM 3.2.B2 TUFI-1	SM	55	10	5	No	1	0.8	0.8	0	Hom	NA	C	C
SM 3.2.B1 TUFI-1	SM	56	10	17	No	1	1.29	1.1	−0.19	Hom	1411.24	C	C
SM 3.2.A2 TUFI-1	SM	57	10	14	No	1	0.99	1.01	0.02	Het	754.32	A	V

Het, heterozygous; Hom, homozygous.

Table S2. Terminal branch evolution among 33 PBR sequences from five *L. yavapaiensis* populations experimentally infected with *Bd*

Terminal branch of ML phylogeny	Codon site under positive selection	Normalized dN − dS	*P* value	Amino acid change
Single likelihood ancestral counting				
Leading to allele EE	3	18.1	0.09	Gly → His
Fixed-effects likelihood				
Leading to allele EE	3	1.1	0.03	Gly → His
Leading to allele EE	18	0.93	0.03	Ser → Lys
Leading to allele Q	46	0.74	0.04	Leu → Val
Random-effects likelihood				
Leading to allele EE	3	3.21	0.99	Gly → His
Leading to allele EE	18	3.32	0.99	Ser → Lys

ML phylogeny of the 82-codon PBR alignment was used as the input tree for selection on particular codons within branches of the phylogeny. We used three different codon-based methods, all implemented with the HyPhy statistical software (1). The most conservative method, single likelihood ancestral counting, infers ancestral codon state and then calculates normalized expected and observed nonsynonymous (dN) and synonymous (dS) substitutions at each site (1). The fixed-effects likelihood method directly estimates rates of nonsynonymous and synonymous substitutions at each site, whereas the random-effects likelihood approach fits a distribution of substitution rates across sites and then infers the rate at which each site evolves (1). When the normalized difference between nonsynonymous and synonymous substitutions (dN − dS) equals 0, the site is neutrally evolving; when dN − dS > 0, the site is positively selected; and when dN − dS < 0, the site is negatively selected.

1. Pond SL, Frost SDW, Muse SV (2005) HyPhy: Hypothesis testing using phylogenies. *Bioinformatics* 21:676–679.

Table S3. Survival from hatching through start of experimental infections for laboratory-reared *L. yavapaiensis*

Clutch	Larvae hatched (%)	Larvae reaching Gosner stage 45 (%)	Frogs surviving metamorphosis (beyond Gosner stage 46) (%)
CIC1	143 (100)	118 (83)	32 (27)
CIC2	134 (100)	123 (92)	24 (20)
AC1	188 (100)	91 (48)	14 (15)
AC2	205 (100)	119 (58)	14 (12)
MR1	195 (100)	11 (6)	5 (57)
MR2	118 (100)	2 (2)	2 (2)
MR3	0	—	—
WC1	96 (20)	54 (56)	7 (13)
WC2	255 (100)	167 (65)	16 (10)
WC3	117 (100)	41 (35)	5 (12)
SM1	247 (100)	159 (64)	17 (11)
SM2	155 (100)	98 (63)	13 (13)
SM3	253 (100)	115 (45)	9 (8)
Total	1,810	1,044 (58)	158 (15)
Mean	144 (85)	87.5 (55)	13.2 (16)