

Table S1. Logistic regression models used to fit cold mortality curves for each genotype at each temperature. Shown are the analysis of deviance results and LT_{50} estimates with standard error calculated using binomial (Figs. 1,2) and quasibinomial variance functions.

Genotype	Factors	Temperature (°C)	Df.	Deviance	Residual Df.	Residual Deviance	$LT_{50} \pm SE$ (binomial)	$P(> \chi)^1$	F	$Pr(>F)^2$	SE (quasibinomial)
Berlin-K	Null Time	-4	1	228.29	29	244.736	74.13±1.17 (min)	<2.2e-16 ***	444.68	<2.2e-16 ***	±0.84 (min)
Canton-S					28	16.446	98.22±1.55 (min)				
Hikone-A-S	Null Time	-4	1	314.33	34	374.86	98.22±1.55 (min)	<2.2e-16 ***	177.82	7.672e-15 ***	±2.06 (min)
Oregon-R-C					33	60.52	109.64±1.56 (min)				
Berlin-K	Null Time	-4	1	269.67	31	301.525	109.64±1.56 (min)	<2.2e-16 ***	282.08	<2.2e-16 ***	±1.52 (min)
Canton-S					30	31.852	104.63±2.51 (min)				
Hikone-A-S	Null Time	-4	1	175.82	41	370.46	104.63±2.51 (min)	<2.2e-16 ***	36.194	4.486e-07 ***	±5.55 (min)
Oregon-R-C					40	194.64	129.04±2.28 (min)				
Berlin-K	Null Time	-4	1	244.67	34	358.47	129.04±2.28 (min)	<2.2e-16 ***	54.069	1.92e-08 ***	±4.86 (min)
Canton-S					33	113.80	11.15±0.26 (h)				
Hikone-A-S	Null Time	-2	1	233.62	37	272.040	11.15±0.26 (h)	<2.2e-16 ***	221.30	<2.2e-16 ***	±0.27 (h)
Oregon-R-C					36	38.418	4.76±0.12 (h)				
Berlin-K	Null Time	-2	1	356.75	36	397.76	4.76±0.12 (h)	<2.2e-16 ***	48.682	4.088e-08 ***	±0.27 (h)
Canton-S					35	41.00	12.20±0.39 (h)				
Hikone-A-S	Null Time	-2	1	149.15	37	266.89	12.20±0.39 (h)	<2.2e-16 ***	54.084	1.132e-08 ***	±0.65 (h)
Oregon-R-C					36	117.74	10.59±0.22 (h)				
Berlin-K	Null Time	-2	1	249.9	37	344.65	10.59±0.22 (h)	<2.2e-16 ***	104.89	3.271e-12 ***	±0.35 (h)
Canton-S					36	94.76	12.17±0.22 (h)				
Hikone-A-S	Null Time	-2	1	303.5	37	378.9	12.17±0.22 (h)	<2.2e-16 ***	131.72	1.384e-13 ***	±0.33 (h)
Oregon-R-C					36	75.4	14.86±0.62 (h)				
Berlin-K	Null Time	0	1	136.53	30	207.691	14.86±0.62 (h)	<2.2e-16 ***	46.855	1.615e-07 ***	±0.57 (h)
Canton-S					29	71.162	11.38±0.32 (h)				
Hikone-A-S	Null Time	0	1	220.39	35	279.910	11.38±0.32 (h)	<2.2e-16 ***	77.892	2.583e-10 ***	±0.55 (h)
Oregon-R-C					34	59.522	26.52±0.88 (h)				
Berlin-K	Null Time	0	1	129.49	26	206.284	26.52±0.88 (h)	<2.2e-16 ***	22.644	6.966e-05 ***	±2.09 (h)
Canton-S					25	76.794	18.06±0.60 (h)				
Hikone-A-S	Null Time	0	1	78.539	32	154.712	18.06±0.60 (h)	<2.2e-16 ***	21.748	5.623e-05 ***	±1.13 (h)
Oregon-R-C					31	76.173					

RAL-208	0									
Null Time	1	228.50	33	343.50	16.59±0.29 (h)	<2.2e-16 ***	63.125	4.554e-09 ***	±0.56 (h)	
Berlin-K	2									
Null Time	1	208.65	32	277.372	16.04±0.44 (h)	<2.2e-16 ***	108.19	1.241e-11 ***	±0.62 (h)	
Canton-S	2									
Null Time	1	92.496	34	157.095	30.01±0.72 (h)	<2.2e-16 ***	50.261	4.068e-08 ***	±0.98 (h)	
Hikone-A-S	2									
Null Time	1	77.289	27	115.797	36.14±0.80 (h)	<2.2e-16 ***	54.647	7.529e-08 ***	±0.95 (h)	
Oregon-R-C	2									
Null Time	1	170.85	34	217.546	19.75±0.67 (h)	<2.2e-16 ***	124.28	9.942e-13 ***	±0.79 (h)	
RAL-208	2									
Null Time	1	113	36	196.483	33.66±0.80 (h)	<2.2e-16 ***	42.688	1.541e-07 ***	±1.31 (h)	
Berlin-K	4									
Null Time	1	104.93	23	140.491	28.63±2.52 (h)	<2.2e-16 ***	66.612	4.229e-08 ***	±3.16 (h)	
Canton-S	4									
Null Time	1	147.81	23	208.055	40.00±2.14 (h)	<2.2e-16 ***	50.2	4.167e-07 ***	±3.67 (h)	
Hikone-A-S	4									
Null Time	1	143.74	23	201.738	46.96±2.24 (h)	<2.2e-16 ***	37.817	3.443e-06 ***	±4.37 (h)	
Oregon-R-C	4									
Null Time	1	107.35	23	132.166	18.29±1.61 (h)	<2.2e-16 ***	12.482	0.00187 **	±4.74 (h)	
RAL-208	4									
Null Time	1	173.86	23	207.15	54.41±2.01 (h)	<2.2e-16 ***	104.33	8.195e-10 ***	±2.60 (h)	
Berlin-K	6									
Null Time	1	175.46	38	285.67	4.41±0.12 (d)	<2.2e-16 ***	56.293	6.125e-09 ***	±0.21 (d)	
Canton-S	6									
Null Time	1	139.59	45	360.93	3.92±0.12 (d)	<2.2e-16 ***	30.037	1.944e-06 ***	±0.26 (d)	
Hikone-A-S	6									
Null Time	1	64.546	42	409.90	4.46±0.18 (d)	9.43e-16 ***	8.0909	0.006911 **	±0.52 (d)	
Oregon-R-C	6									
Null Time	1	127.48	47	239.22	2.89±0.11 (d)	<2.2e-16 ***	57.197	1.313e-09 ***	±0.17 (d)	
RAL-208	6									
Null Time	1	216.65	45	348.85	4.26±0.12 (d)	<2.2e-16 ***	62.006	6.116e-10 ***	±0.22 (d)	

¹ Test using the binomial variance function

² Test using the quasibinomial variance function

Table S2. Analysis of deviance showing the effects of time, genotype, and the time-by-genotype interaction on mortality at each temperature tested using both binomial and quasibinomial variance functions.

	Temperature (°C)	Df.	Deviance	Residual Df.	Residual Deviance	Binomial	Quasibinomial	
						$P(> \chi)$	F	$Pr(>F)$
Null	-4			173	1693.18			
Time		1	927.68	172	765.50	<2.2e-16 ***	341.8791	<2.2e-16 ***
Genotype		4	268.17	168	497.33	<2.2e-16 ***	24.7072	5.092e-16 ***
Time x Genotype		4	80.07	164	417.26	<2.2e-16 ***	7.3775	1.730e-05 ***
Null	-2			188	1752.30			
Time		1	748.23	187	1004.07	<2.2e-16 ***	238.105	<2.2e-16 ***
Genotype		4	499.52	183	504.55	<2.2e-16 ***	39.740	<2.2e-16 ***
Time x Genotype		4	137.23	179	367.32	<2.2e-16 ***	10.917	6.071e-08 ***
Null	0			160	1235.72			
Time		1	417.38	159	818.34	<2.2e-16 ***	114.2882	<2.2e-16 ***
Genotype		4	339.83	155	478.51	<2.2e-16 ***	23.2636	5.389e-15 ***
Time x Genotype		4	79.86	151	398.64	<2.2e-16 ***	5.4672	0.0003876 ***
Null	2			167	1092.30			
Time		1	297.42	166	794.88	<2.2e-16 ***	159.134	<2.2e-16 ***
Genotype		4	467.90	162	326.98	<2.2e-16 ***	62.588	<2.2e-16 ***
Time x Genotype		4	24.98	158	302.01	5.084e-05 ***	3.341	0.01173 *
Null	4			119	986.81			
Time		1	582.10	118	404.71	<2.2e-16 ***	156.5885	<2.2e-16 ***
Genotype		4	180.45	114	224.26	<2.2e-16 ***	12.1359	3.31e-08 ***
Time x Genotype		4	12.34	110	211.92	0.01501 *	0.8297	0.509
Null	6			221	1673.78			
Time		1	632.73	220	1041.05	<2.2e-16 ***	148.5252	<2.2e-16 ***
Genotype		4	118.35	216	922.69	<2.2e-16 ***	6.9455	2.846e-05 ***
Time x Genotype		4	1.86	212	920.83	0.7612	0.1092	0.9792

Table S3. Analysis of deviance showing the effects of time, temperature, genotype, and their interactions on mortality tested using the quasibinomial variance function. Results using the binomial variance function are presented in Table 1.

	Df.	Deviance	Residual Df.	Residual Deviance	<i>F</i>	<i>Pr(>F)</i>
Null			1035	8975.8		
Time	1	1040.65	1034	7935.2	320.7772	<2.2e-16 ***
Temperature	5	2060.02	1029	5875.2	126.9991	<2.2e-16 ***
Genotype	4	459.95	1025	5415.2	35.4449	<2.2e-16 ***
Time x Temperature	5	1271.95	1020	4143.3	78.4150	<2.2e-16 ***
Time x Genotype	4	312.71	1016	3830.6	24.0983	<2.2e-16 ***
Temperature x Genotype	20	919.13	996	2911.4	14.1660	<2.2e-16 ***
Time x Temperature x Genotype	20	293.45	976	2618.0	4.5228	2.192e-10 ***

Table S4. Logistic regression models used to fit curves for mortality at -4°C with altered humidity. Shown are the analysis of deviance results and LT₅₀ estimates with standard error calculated using binomial and quasibinomial variance functions.

Genotype	Treatment	Factors	Df.	Deviance	Residual Df.	Residual Deviance	LT ₅₀ ±SE (min) (binomial)	$P(> \chi)^1$	F	$Pr(>F)^2$	SE (quasibinomial)
Berlin-K	Dry	Null			29	229.60	50.94±2.61				
		Time	1	178.03	28	51.57		< 2.2e-16 ***	107.21	4.44e-11 ***	±3.37
	Control	Null			29	197.219	47.89±3.06				
		Time	1	145.00	28	52.222		< 2.2e-16 ***	80.29	1.025e-09 ***	±4.11
	Humid	Null			29	198.823	52.90±2.93				
		Time	1	161.9	28	36.923		< 2.2e-16 ***	134.09	3.439e-12 ***	±3.22
Canton-S	Dry	Null			29	249.914	114.15±3.27				
		Time	1	158.62	28	91.297		< 2.2e-16 ***	46.669	2.016e-07 ***	±6.02
	Control	Null			29	261.87	108.76±3.19				
		Time	1	169.38	28	92.49		< 2.2e-16 ***	44.565	3.032e-07 ***	±6.22
	Humid	Null			29	261.39	122.00±3.24				
		Time	1	152.16	28	109.22		< 2.2e-16 ***	41.358	5.785e-07 ***	±6.21
Hikone-A-S	Dry	Null			29	220.21	123.98±4.14				
		Time	1	109.13	28	111.08		< 2.2e-16 ***	17.209	0.0002819 ***	±10.41
	Control	Null			29	315.66	111.40±2.84				
		Time	1	197.75	28	117.90		< 2.2e-16 ***	13.262	0.001088 **	±10.96
	Humid	Null			29	237.195	121.99±2.99				
		Time	1	167.89	28	69.302		< 2.2e-16 ***	24.403	3.269e-05 ***	±7.85
Oregon-R-C	Dry	Null			23	198.397	177.35±4.50				
		Time	1	176.59	22	21.808		< 2.2e-16 ***	213.67	8.253e-13 ***	±4.09
	Control	Null			23	275.116	147.02±3.70				
		Time	1	239.93	22	35.187		< 2.2e-16 ***	148.83	2.895e-11 ***	±4.70
	Humid	Null			23	253.085	153.07±4.37				
		Time	1	203.86	22	49.223		< 2.2e-16 ***	96.867	1.611e-09 ***	±6.33
RAL-208	Dry	Null			29	234.54	115.27±3.75				
		Time	1	127.90	28	106.64		< 2.2e-16 ***	38.12	1.147e-06 ***	±6.88
	Control	Null			29	248.47	111.32±3.69				
		Time	1	132.42	28	116.05		< 2.2e-16 ***	35.594	2.005e-06 ***	±7.13
	Humid	Null			29	242.50	125.34±4.74				
		Time	1	90.538	28	151.96		< 2.2e-16 ***	20.514	0.0001003 ***	±9.96

¹ Test using the binomial variance function

² Test using the quasibinomial variance function

Figure S1. Cold survival data for A) -4°C , B) -2°C , C) 0°C , D) 2°C , E) 4°C , and F) 6°C . At least five independent pools of 10 males were sampled at five time points for each temperature. Plotted is the proportion dead for each independent pool of males and the fitted line using logistic regression that was used to infer LT_{50} . Some data points are overlapping.

Figure S1A

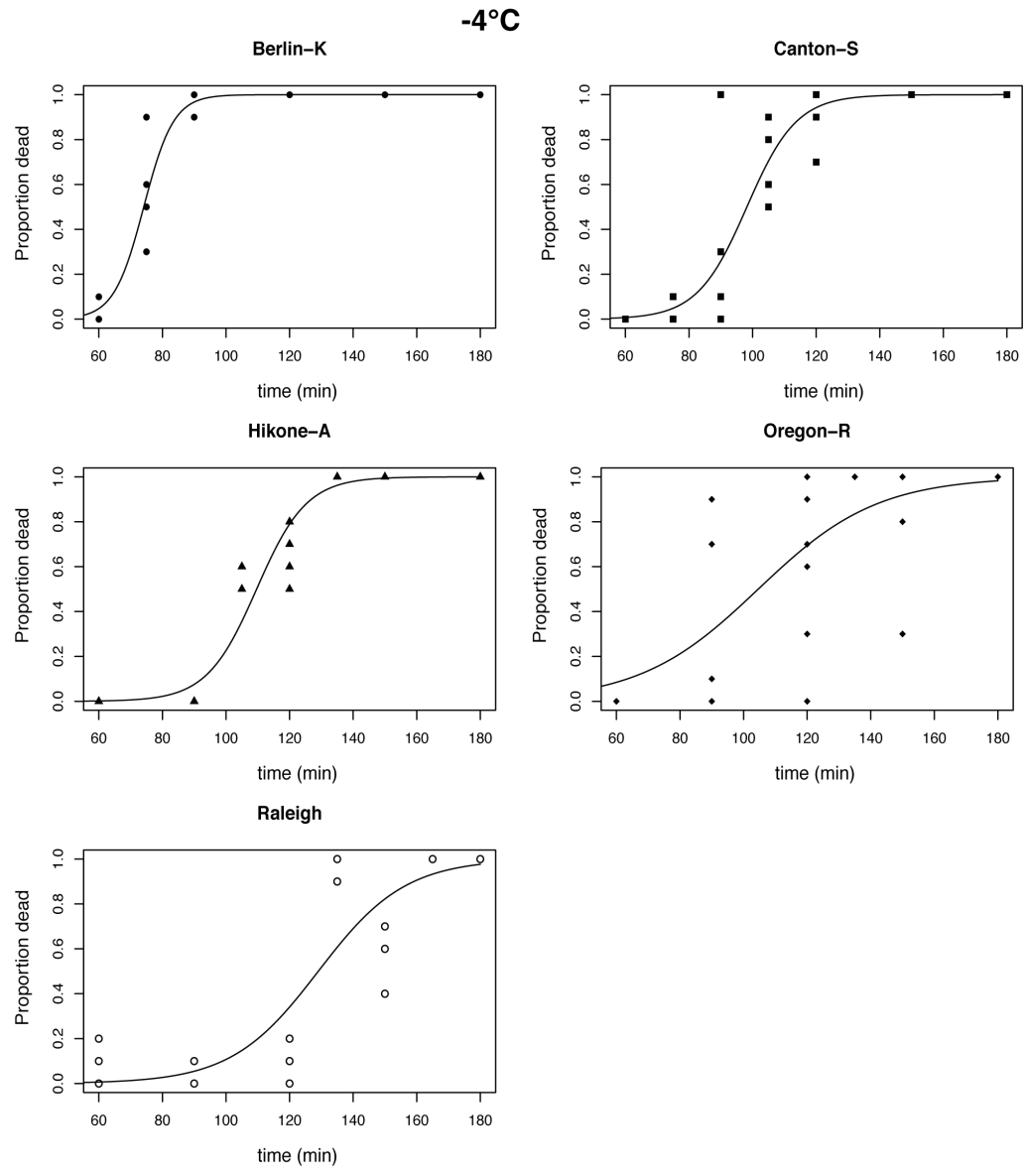


Figure S1B

-2°C

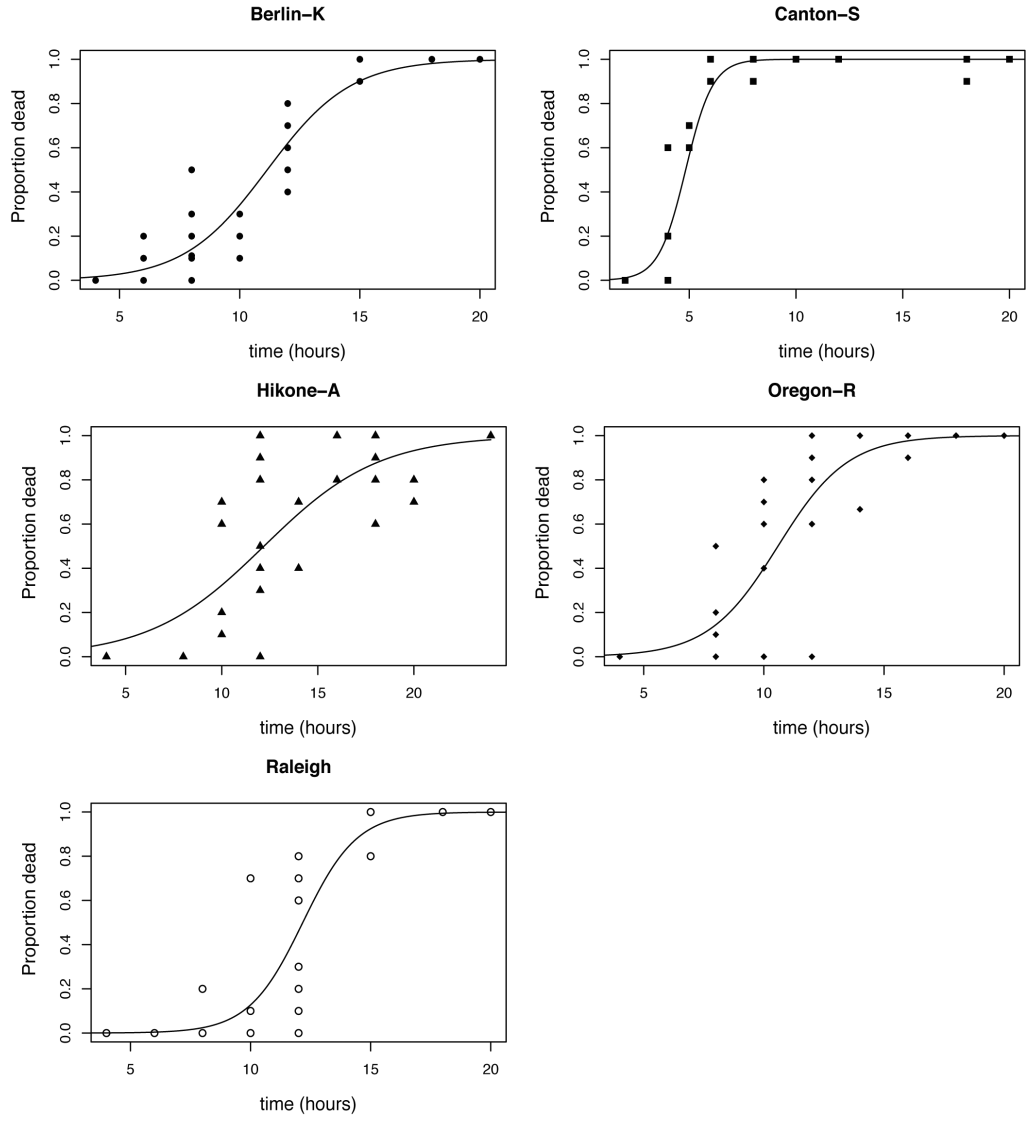


Figure S1C

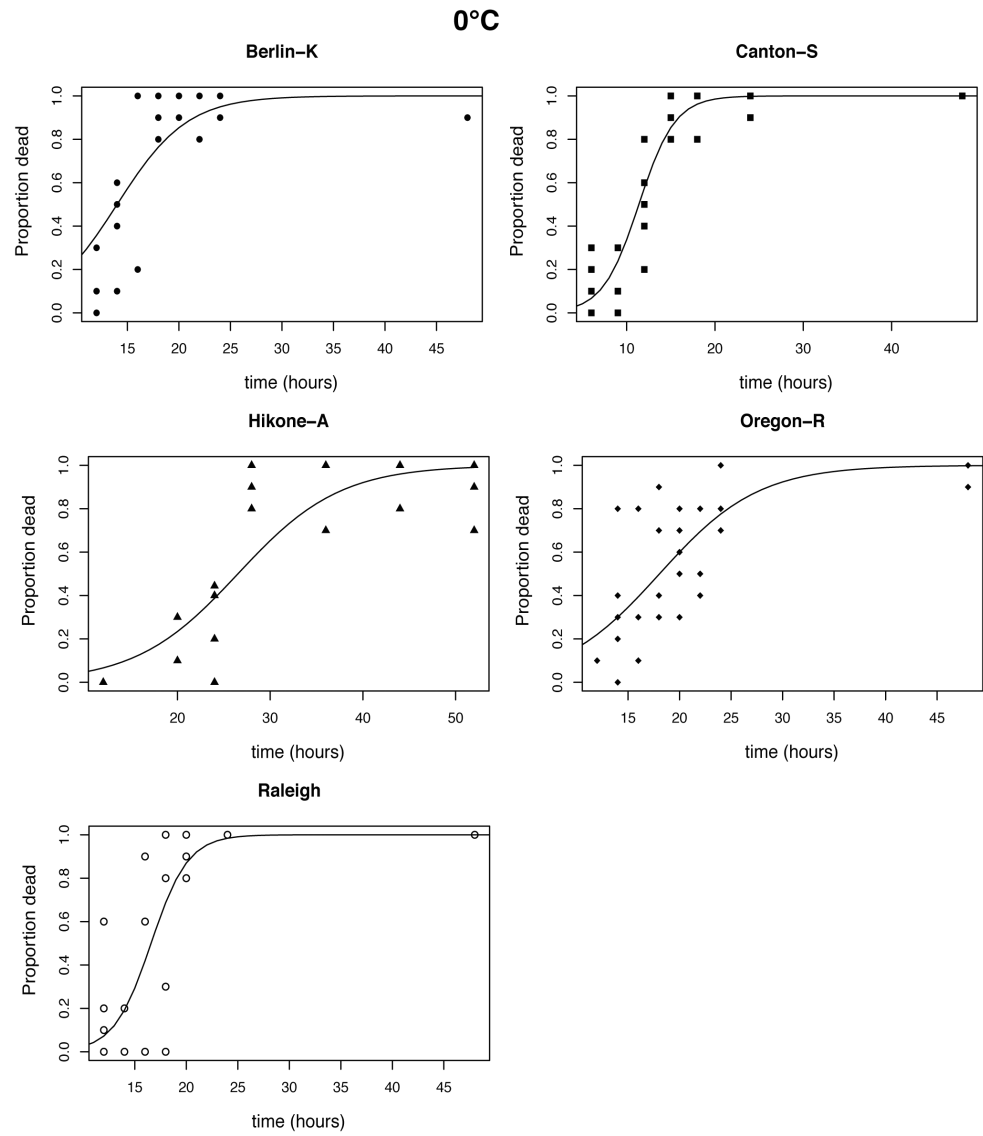


Figure S1D

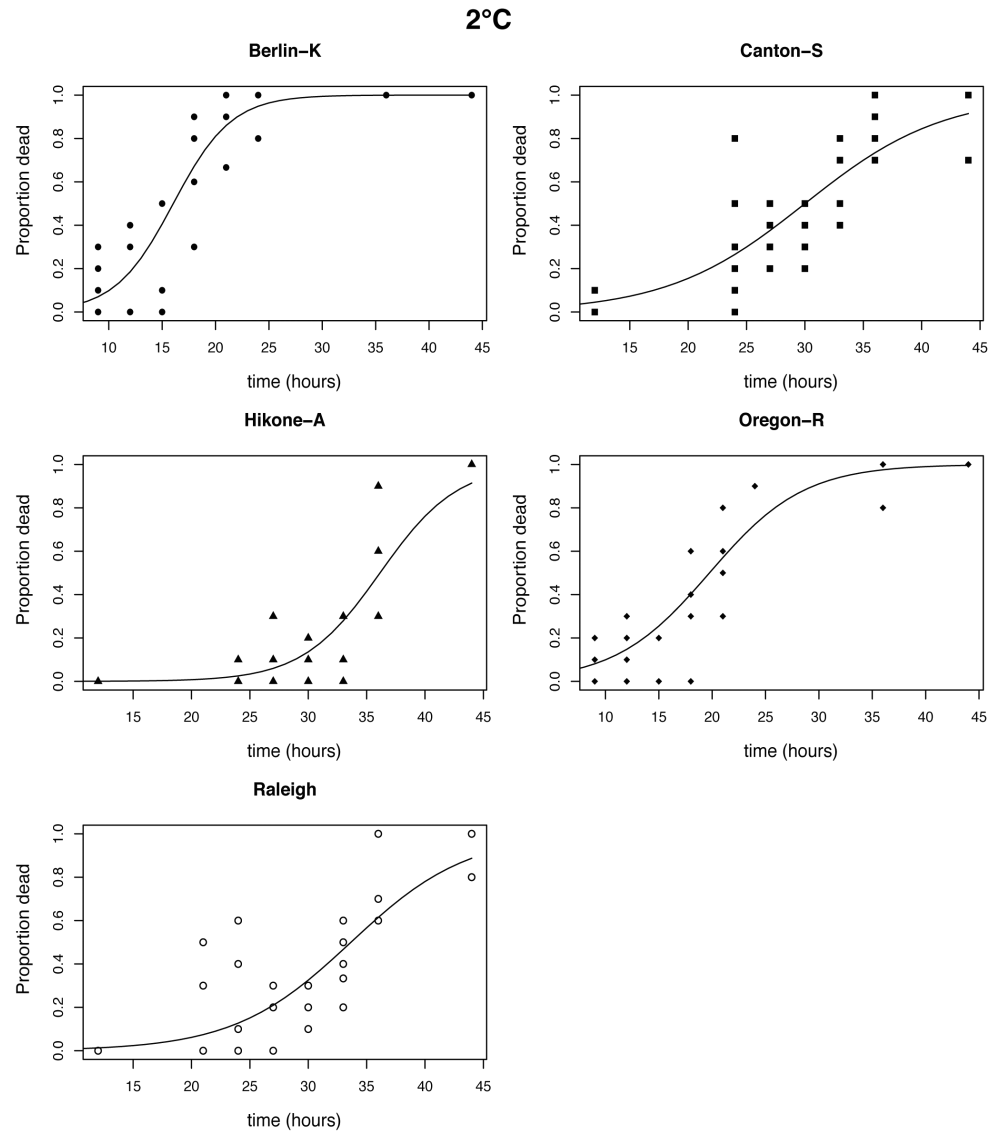


Figure S1E

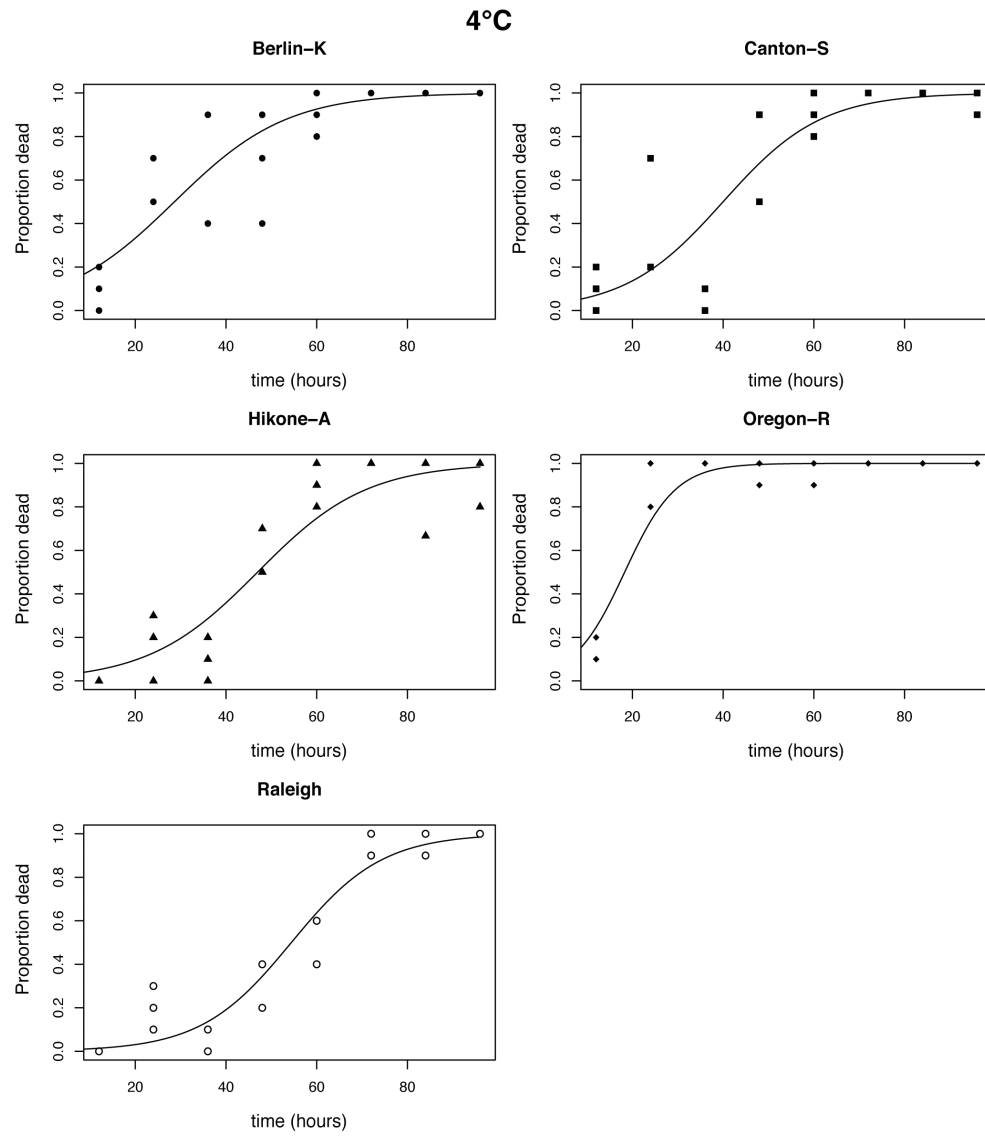


Figure S1F

6°C

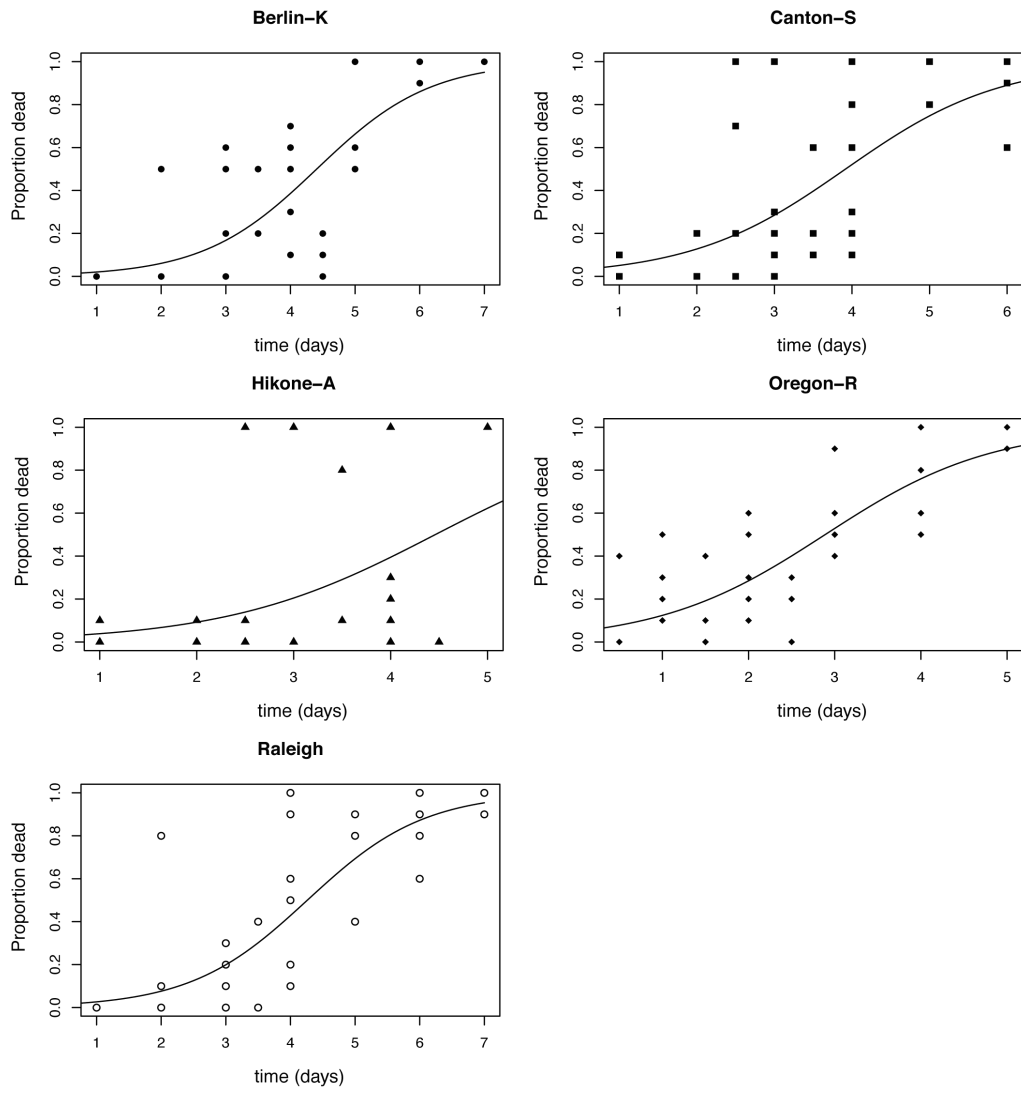


Figure S2. There is very little change in mass during freezing at -80°C followed by our weighing protocol. Flies were briefly anesthetized and weighed while alive (Live). After freezing for 24 hours at -80°C , flies were weighed again using the protocol described in the methods (Dead). There were consistent, but very small (1.5-3% of wet mass) decreases in mass for the majority of individual flies that were similar across genotypes, resulting in a significant effect of freezing when using a paired t-test ($P_{t\text{-test}} < 0.001$). The figure plots the mean masses with standard errors for each genotype.

