Supplementary Information for:

Low potential for evolutionary rescue from climate change in a tropical fish

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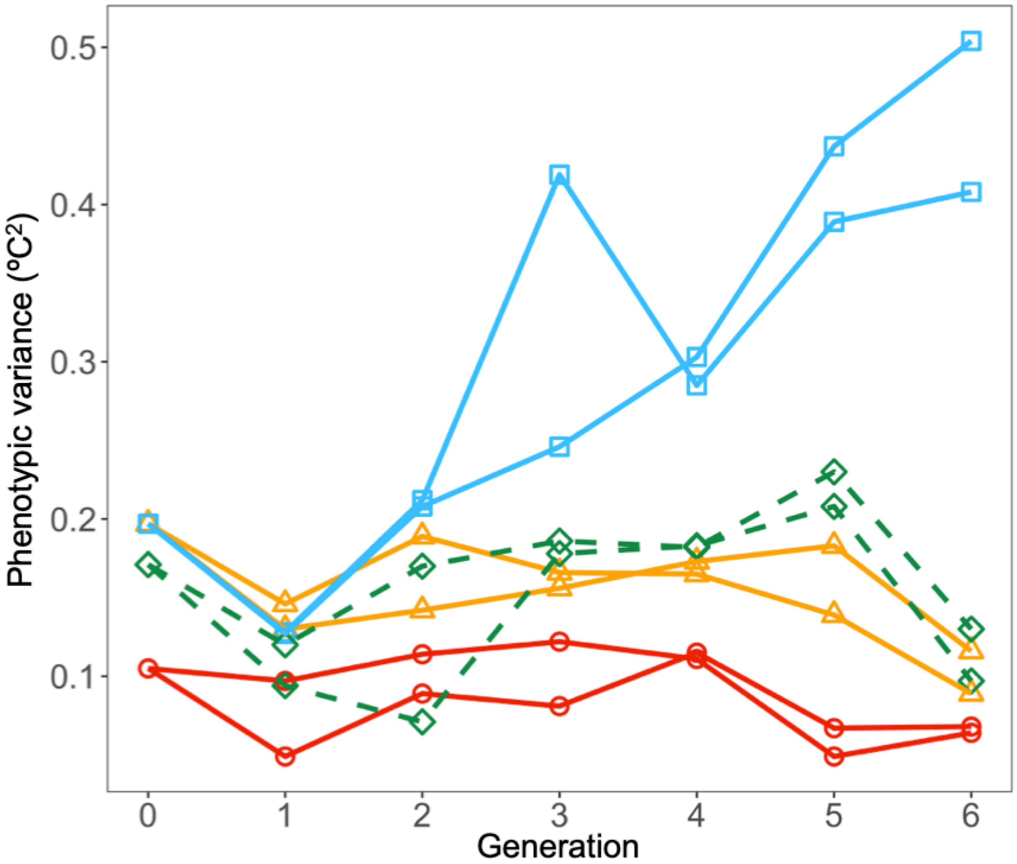
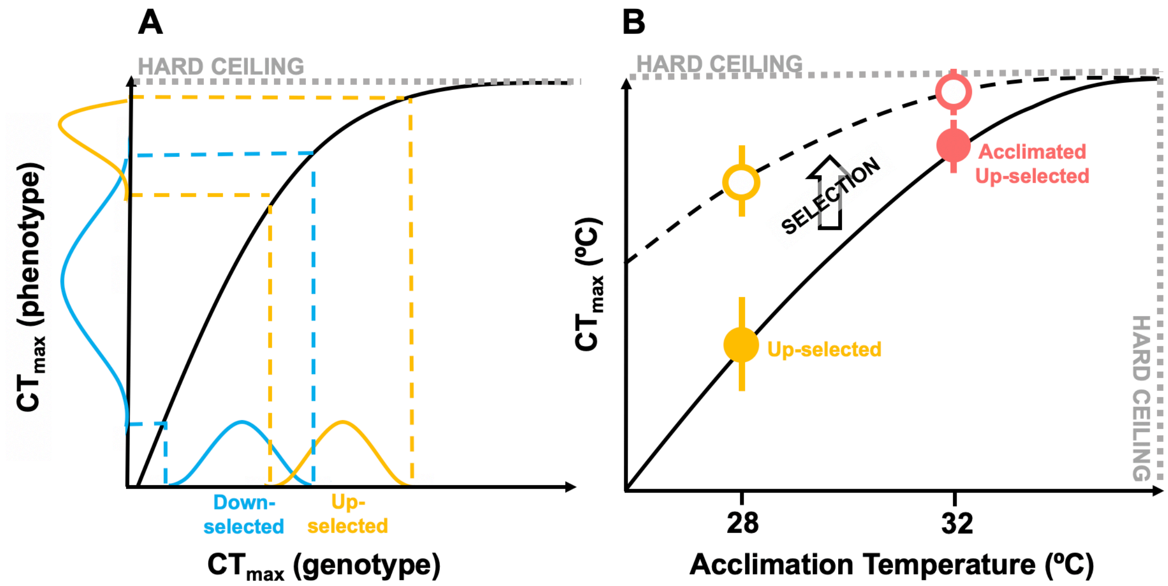
www.pnas.org/cgi/doi/10.1073/pnas.2011419117



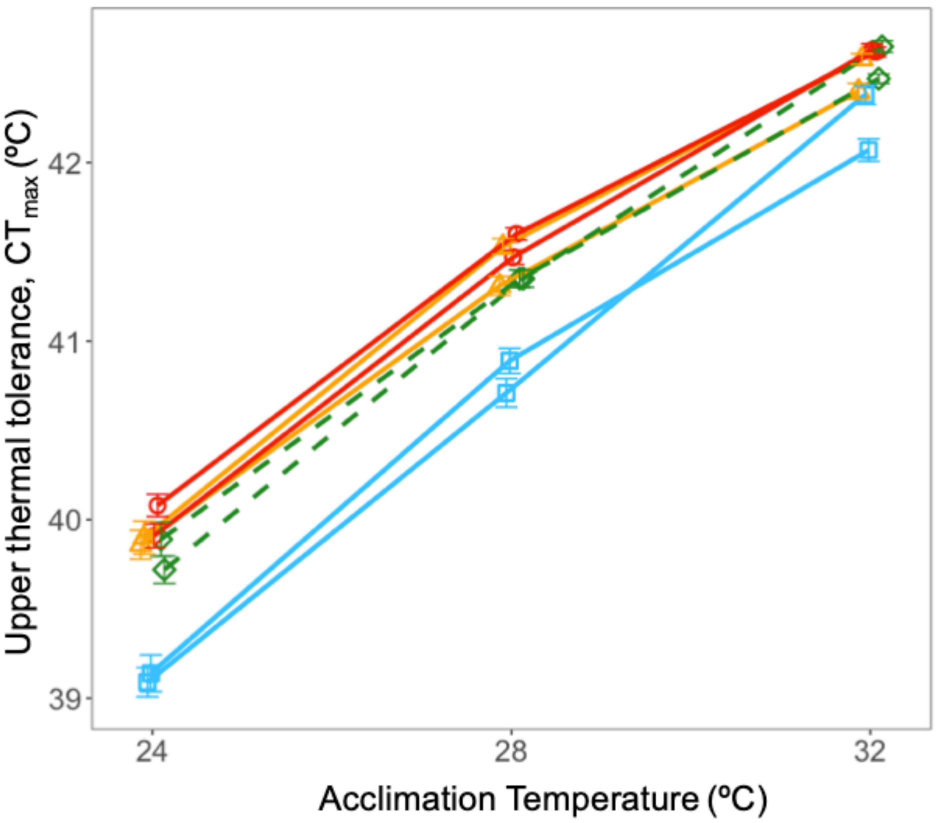
Supplementary Figure 1: Phenotypic variance (°C^2) in each selected line at each generation. Up-selected (orange triangles), Down-selected (blue squares), Acclimated Up-selected (red circles) and Control (green diamonds).



Supplementary Figure 2: The presence of a hard ceiling for CT$_{max}$ generates A) a non-linear mapping of the genotypic values onto the phenotypic values in CT$_{max}$ and B) a decrease in plasticity after selection for increased upper thermal tolerance.

In panel A the graphical model suggests how the presence of a hard-upper limit in CT$_{max}$ (grey striped line) affects the mapping of individual differences in CT$_{max}$ at the genotypic level into phenotypic differences. According to this model, the presence of the hard-upper limit in CT$_{max}$ introduces non-linearity in the mapping between the two levels that affects the distribution of the phenotypic values. We illustrate this effect for the Down-selected lines (blue) and the Up-selected lines (orange) which show similar variation at the genotypic level. This scenario assumes that CT$_{max}$ is affected by a large number of loci with alleles of small effects (The infinitesimal model; (1–3)), and that genetic variance is not affected by selection (4). Due to the non-linearity of the genotype-phenotype map, variation at the phenotypic level differs between the two lines; the closer mean CT$_{max}$ is to the upper limit, the narrower and more skewed the phenotypic distribution of CT$_{max}$ is. This difference in the distribution of CT$_{max}$ is what we observed at the F$_6$ between the Up- and Down-selected lines (Fig. 4).

Panel B represents the effects of selection on the reaction norm of CT$_{max}$ in the presence of the hard ceiling. The two curves represent the reaction norm of CT$_{max}$ with acclimation temperature. The acclimation effect on CT$_{max}$ (i.e. plasticity in CT$_{max}$) is represented by the steepness of the slope between the two acclimation temperatures. The lower curve (solid line filled dots) represents the reaction norm of CT$_{max}$ before selection (see (5) for an empirical assessment of this reaction norm). After selection to increase CT$_{max}$ (dash line, open dots), there is an upwards shift in the Up-selected lines acclimated to 28°C (orange open point in panel B). However due to the presence of the hard ceiling the Acclimated Up-selected line have less potential to increase in CT$_{max}$. This generates a decrease in plasticity (shallower slope).



Supplementary Figure 3: Upper thermal tolerance (CT_{max}) measured in all selected lines at the last generation (F_6) after acclimation at 24, 28 and 32°C. Mean ± SE of each replicate line (*n* = 60 individuals): Up-selected (orange triangles), Down-selected (blue squares), Acclimated Up-selected (red circles) and Control (green diamonds). Estimates from lme model: contrast with Control at 24 °C: Up-selected 0.08 ± 0.08, t = 0.96, p = 0.36; Down-selected -0.69 ± 0.08, t = -8.70, p < 0.001; Acclimated Up 0.19 ± 0.08, t = 2.375, p = 0.04. At 28 °C: Up-selected 0.07 ± 0.09, t = 0.83, p = 0.42; Down-selected -0.55 ± 0.09, t = -6.42, p < 0.001; Acclimated Up 0.19 ± 0.09, t = 2.22, p = 0.04. At 32 °C: Up-selected -0.07 ± 0.09, t = -0.83, p = 0.42; Down-selected -0.33 ± 0.08, t = -3.87, p = 0.002; Acclimated Up +0.07 ± 0.09 °C, t = 0.77, p = 0.46.

Supplementary Table 1: Summary table of the mean trial duration, CT_{max}, standard deviation, selection differential and selection response at each generation for each replicate. The selection response in the Up and Down lines is relative to the Control lines.

Line	Generation	Mean trial duration (mins)	Mean CT_{max}	SD	Selection Differential	Selection Response
Up1	0	44.2	41.27	0.44	-	-
	1	45.5	41.65	0.36	0.44	0.23
	2	44.6	41.39	0.38	0.33	0.09
	3	45.0	41.49	0.39	0.37	0.11
	4	45.5	41.64	0.42	0.39	0.01
	5	45.3	41.59	0.43	0.38	0.35
	6	45.8	41.75	0.34	0.4	0.08
Up2	0	44.2	41.27	0.44	-	-
	1	44.9	41.48	0.38	0.44	0.06
	2	44.4	41.33	0.43	0.36	0.02
	3	44.7	41.40	0.41	0.43	0.02
	4	45.7	41.72	0.41	0.4	0.08
	5	45.4	41.63	0.37	0.38	0.39
	6	46.4	41.92	0.30	0.35	0.25
Down1	0	44.2	41.27	0.44	-	-
	1	44.3	41.28	0.36	-0.50	-0.13
	2	43.1	40.94	0.46	-0.41	-0.37
	3	42.8	40.83	0.5	-0.53	-0.56
	4	44.2	41.27	0.55	-0.56	-0.37
	5	42.1	40.63	0.66	-0.63	-0.61
	6	43.1	40.94	0.71	-0.75	-0.73
Down2	0	44.2	41.27	0.44	-	-
	1	44.7	41.40	0.36	-0.5	-0.02
	2	43.5	41.05	0.46	-0.40	-0.25
	3	42.3	40.69	0.65	-0.53	-0.69
	4	44.0	41.21	0.53	-0.69	-0.42
	5	42.4	40.71	0.62	-0.59	-0.53
	6	42.8	40.85	0.64	-0.75	-0.82
Acclimated Up1	0	35.3	42.58	0.32	-	-
	1	35.6	42.69	0.31	-	-

	2	34.9	42.48	0.34	-	-
	3	35.4	42.61	0.35	-	-
	4	35.8	42.75	0.33	-	-
	5	35.6	42.69	0.22	-	-
	6	36.1	42.84	0.25	-	-
Acclimated Up2	0	35.3	42.58	0.32	-	-
	1	35.8	42.73	0.22	-	-
	2	35.6	42.68	0.30	-	-
	3	36.0	42.79	0.28	-	-
	4	35.7	42.70	0.34	-	-
	5	35.8	42.75	0.26	-	-
	6	35.9	42.77	0.26	-	-

Realised heritability and SE for each replicate line

We estimated realized heritability h^2 as the slope of the regression of the cumulated response to selection on the cumulated selection differential across generation (OLS model). We used a linear mixed effect model where the response variable was the per generation line average cumulated response to selection and the predictor variables were the per generation line average cumulated selection differential, generation and treatment. Replicate was a random factor. This means that our sample size per treatment was $n = 12$ (i.e. 6 generations × 2 replicates). Alternatively, realized heritability can be estimated by the ratio between the cumulated response to selection (Rc) and the cumulated selection differential (Sc), or OLS regression or Generalized Least Square models (GLS) on individual data. GLS models can include information about heteroscedasticity and autocorrelation in the residuals across generation due to drift and other factors. Thus, if the three methods (Rc/Sc, OLS or GLS) provide unbiased estimates of the realized heritability h^2, they provide different estimates of the standard error of h^2 (5 page 599). When performed on every individual in each line at each generation, the OLS method underestimates the standard error in h^2 (6), while standard errors provided by the ratio Rc/Sc (see equation below) and GLS models are more realistic and relatively similar, although standard errors provided by GLS models tend to be smaller when h^2 is small and the number of generations of selection limited. However, compared with these estimates based on individual data, our method based on line means is expected to overestimate the standard error in h^2. To assess this overestimation, we used the equation provided by Walsh and Lynch (eq. 18.18 page 600 in 5) to estimate the standard error in h^2 in the Rc/Sc method. This equation shows that the variance of the estimated realized heritability $\hat{b}_t$ is:

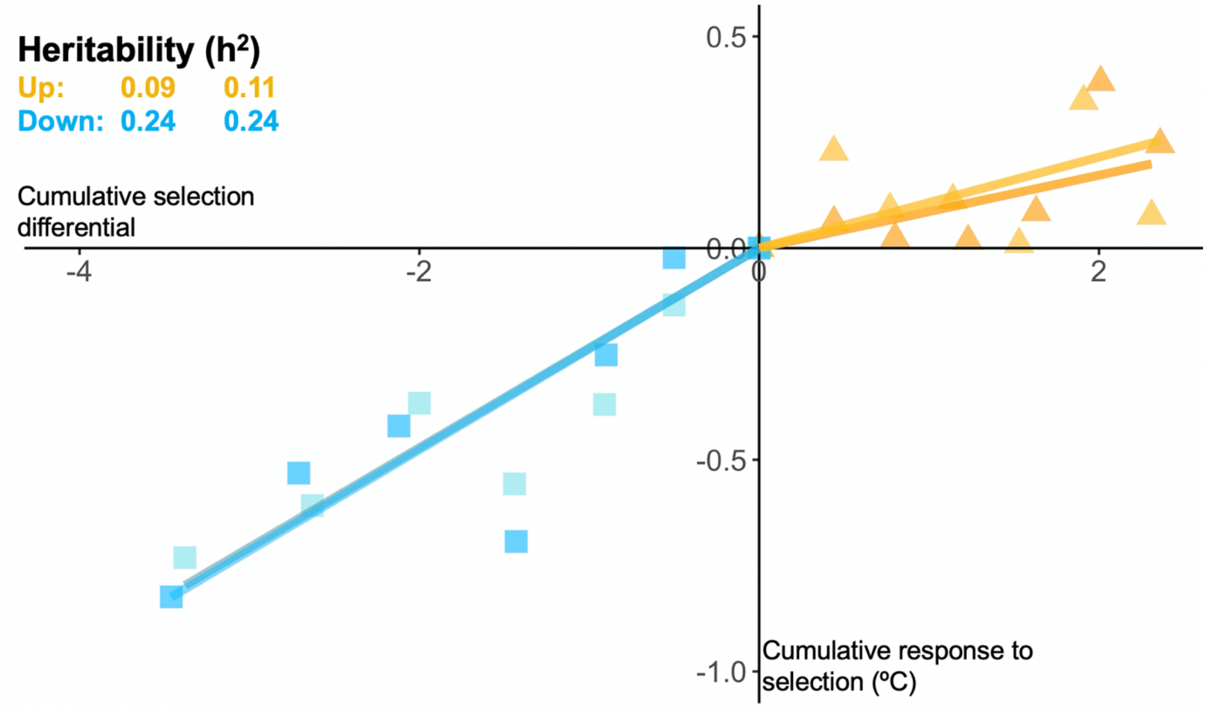
$$Var(\hat{b}_t) = \frac{Var[R_c(T)]}{S_c^2(T)} \approx \frac{(T/N)\hat{h}_r^2\sigma_z^2 + \sigma_z^2/M_T}{S_c^2(T)}$$

where $\hat{h}_r^2$ is the ratio of the cumulated response over the cumulated selection differential, T is the number of generations (6 in our case), N is the number of individuals selected at each generation (150 in our case), σ_z^2 is the phenotypic variance (we used the variance at the starting generation because it is the one estimated with the strongest accuracy), $S_c^2(T)$ is the square of the cumulated selection differential, and M_T is the size of the population (450 in our case). The SE of the realized heritability will be the square root of $Var(\hat{b}_t)$.

The cumulative selection differentials and cumulative responses to selection used for these calculations along with the resulting SE are shown in the table below and heritability for each replicate line is shown in the figure below. Considering the large sample size in each line used in this experiment, standard errors in realized heritability calculated using the Rc/Sc method are smaller than the standard errors obtained with the OLS model performed on line means. We decided to present the latter, more conservative estimates, in the main document.

Supplementary Table 2: Realized heritability estimates for each replicate. Realised heritability is calculated using two methods, the ratio of the cumulative response to selection and the cumulative selection differential (Rc/Sc) and using ordinary least square (OLS) regression of the cumulative response to selection on the cumulative selection differential using the mean of each line at each generation. The standard error (SE) for each replicate is calculated as the SE of the realized heritability estimated as the ratio between the cumulated response and the cumulated differential using the equation outlined in the methods section and taken from Walsh and Lynch (6).

Line	Cumulative selection differential (Sc)	Cumulative response to selection (Rc)	Realised heritability (Rc/Sc)	Realised heritability (OLS)	SE (Rc/Sc)	SE (OLS)
Up1	2.31	0.08	0.03	0.09	0.011	0.035
Up2	2.36	0.25	0.10	0.11	0.015	0.025
Down1	-3.38	-0.73	0.22	0.24	0.014	0.024
Down2	-3.46	-0.82	0.24	0.24	0.014	0.031



Supplementary Figure 4: Realised heritability (h^2) of upper thermal tolerance (CT_{max}) in wild-caught zebrafish in each replicate line. The realised heritability was estimated for each line (2 lines per treatment) as the slope of the regression of the cumulative selection differential on the cumulative response to selection using a linear model passing through the origin. Slopes are presented for the two Down-selected lines (blue), two Up-selected lines (orange). Two shades are used for each of the replicate lines within a treatment and data points represent the mean of each replicate ($n \approx 450$) over six generations of selection.

Supplementary Table 3: Overview for the number of spawners and larvae at each generation. Estimated: Number of males (M) and females (F) in each selection line at each generation. Box success rate: Percentage of spawning boxes that were set up that were successful (i.e. contained eggs). Number of M and F spawned: Maximum and minimum number of males and females that contributed eggs to the next generation. Larvae: Estimated #: estimated number of larvae when they were moved into the large aquaria at 7-10 days post fertilisation. Actual #: number of larvae that survived until the start of the selection episode (6 weeks old). Mortality %: total larvae mortality from estimated until actual counts.

			Estimated				Number of M and F spawned							Larvae		
Generation (parents)	**Treatment**	**Replicate line**	**F**	**M**	**Unknown sex**	**Box success rate %**	**Total min**	**Total max**	**Min F**	**Max F**	**Min M**	**Max M**	**Generation (larvae)**	**Estimated #**	**Actual #**	**Mortality %**
5	Down	1	61	86	17	26	36	108	17	51	19	57	6	1212	780	36
5	Down	2	46	87	19	31	39	113	16	46	23	67	6	909	439	52
5	Up	1	80	72	1	88	42	126	21	63	21	63	6	1320	541	59
5	Up	2	87	68	4	71	58	152	28	84	27	68	6	1325	530	60
5	Control	1	79	74	0	86	36	106	18	53	18	53	6	906	387	57
5	Control	2	66	107	2	75	53	158	20	60	33	98	6	1340	671	50
5	Acclimated Up	1	73	90	2	69	66	163	36	73	30	90	6	1487	558	62
5	Acclimated Up	2	84	71	0	38	55	140	26	75	29	65	6	1045	517	51
4	Down	1	60	80	3	31	43	127	18	54	25	73	5	1115	590	47
4	Down	2	53	59	5	37	37	105	19	53	18	52	5	1115	485	57
4	Up	1	72	76	3	87	48	140	23	69	25	73	5	1280	690	46
4	Up	2	81	73	2	64	52	154	27	81	25	73	5	1485	645	57
4	Control	1	57	91	2	44	44	130	19	57	25	73	5	905	360	60
4	Control	2	60	89	1	35	35	105	17	51	18	54	5	625	305	51
4	Acclimated Up	1	71	85	3	57	49	144	20	59	29	85	5	1775	625	65
4	Acclimated Up	2	57	77	2	37	45	133	19	56	26	77	5	1415	550	61
3	Down	1	51	51	48	32	34	82	15	44	19	56	4	792	440	44
3	Down	2	39	-	-	22	17	50	9	26	8	24	4	516	307	41
3	Up	1	53	53	44	66	48	142	21	63	27	80	4	1395	920	34
3	Up	2	63	63	24	54	46	134	21	61	25	73	4	1225	825	33
3	Control	1	51	51	48	50	38	110	17	50	21	61	4	520	212	59

3	Control	2	60	60	30	59	44	117	18	54	26	78	4	1135	655	42
3	Acclimated Up	1	57	69	24	67	48	138	16	48	32	90	4	1897	895	53
3	Acclimated Up	2	41	57	52	48	33	93	12	34	21	60	4	1260	610	52
2	Down	1	39	-	-	32	34	101	11	33	26	68	3	1135	775	32
2	Down	2	56	-	-	33	38	109	14	41	24	68	3	1078	640	41
2	Up	1	57	-	-	51	43	129	18	54	25	75	3	1235	535	57
2	Up	2	57	-	-	55	46	134	21	61	25	71	3	1530	935	39
2	Control	1	66	-	-	47	42	119	18	52	24	69	3	1517	700	54
2	Control	2	62	-	-	52	46	130	20	58	26	74	3	990	593	40
2	Acclimated Up	1	41	-	-	70	48	139	17	50	31	89	3	1875	945	50
2	Acclimated Up	2	41	-	-	58	52	149	21	59	31	90	3	1455	880	40
2	Down	1	51	84	15	-	45	135	17	51	28	84	3	1325	940	29
1	Down	2	45	85	20	-	31	95	12	36	19	57	2	845	540	36
1	Up	1	60	80	10	-	35	105	15	45	20	60	2	830	600	28
1	Up	2	63	79	8	-	37	111	14	42	23	69	2	1055	610	42
1	Control	1	54	79	17	-	35	105	16	48	19	57	2	870	600	31
1	Control	2	42	83	25	-	35	105	14	42	21	63	2	1200	650	46
1	Acclimated Up	1	42	72	6	-	33	99	13	39	20	60	2	1644	870	47
1	Acclimated Up	2	33	76	11	-	34	102	13	39	21	63	2	1345	870	35
0	Down	1	36	109	5	-	32	96	10	30	22	66	1	1090	953	13
0	Down	2	30	112	8	-	31	93	9	27	22	66	1	950	610	36
0	Up	1	57	89	4	-	33	99	13	39	20	60	1	825	770	7
0	Up	2	45	85	20	-	38	114	15	45	23	69	1	963	900	7
0	Control	1	54	81	15	-	31	93	14	42	17	51	1	700	635	9
0	Control	2	33	106	11	-	30	90	11	33	19	57	1	550	550	0
0	Acclimated Up	1	63	79	8	-	40	120	18	54	22	66	1	1330	1060	20
0	Acclimated Up	2	54	90	6	-	40	120	17	51	23	69	1	1690	1500	11

References

1. M. Kimura, A stochastic model concerning the maintenance of genetic variability in quantitative characters. *Proc. Natl. Acad. Sci. U. S. A.* **54**, 731–736 (1965).

2. R. Lande, The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genet. Res.* **26**, 221–235 (1975).

3. M. G. Bulmer, The Effect of Selection on Genetic Variability. *Am. Nat.* **105**, 201–211 (1971).

4. N. H. Barton, M. Turelli, Adaptive landscapes, genetic distance and the evolution of quantitative characters. *Genet. Res.* **49**, 157–173 (1987).

5. R. Morgan, *et al.*, Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conserv. Physiol.* **7** (2019).

6. B. Walsh, M. Lynch, *Evolution and Selection of Quantitative Traits* (Oxford University Press, 2018).