

Supplementary Information

Male fertility thermal limits predict vulnerability to climate warming

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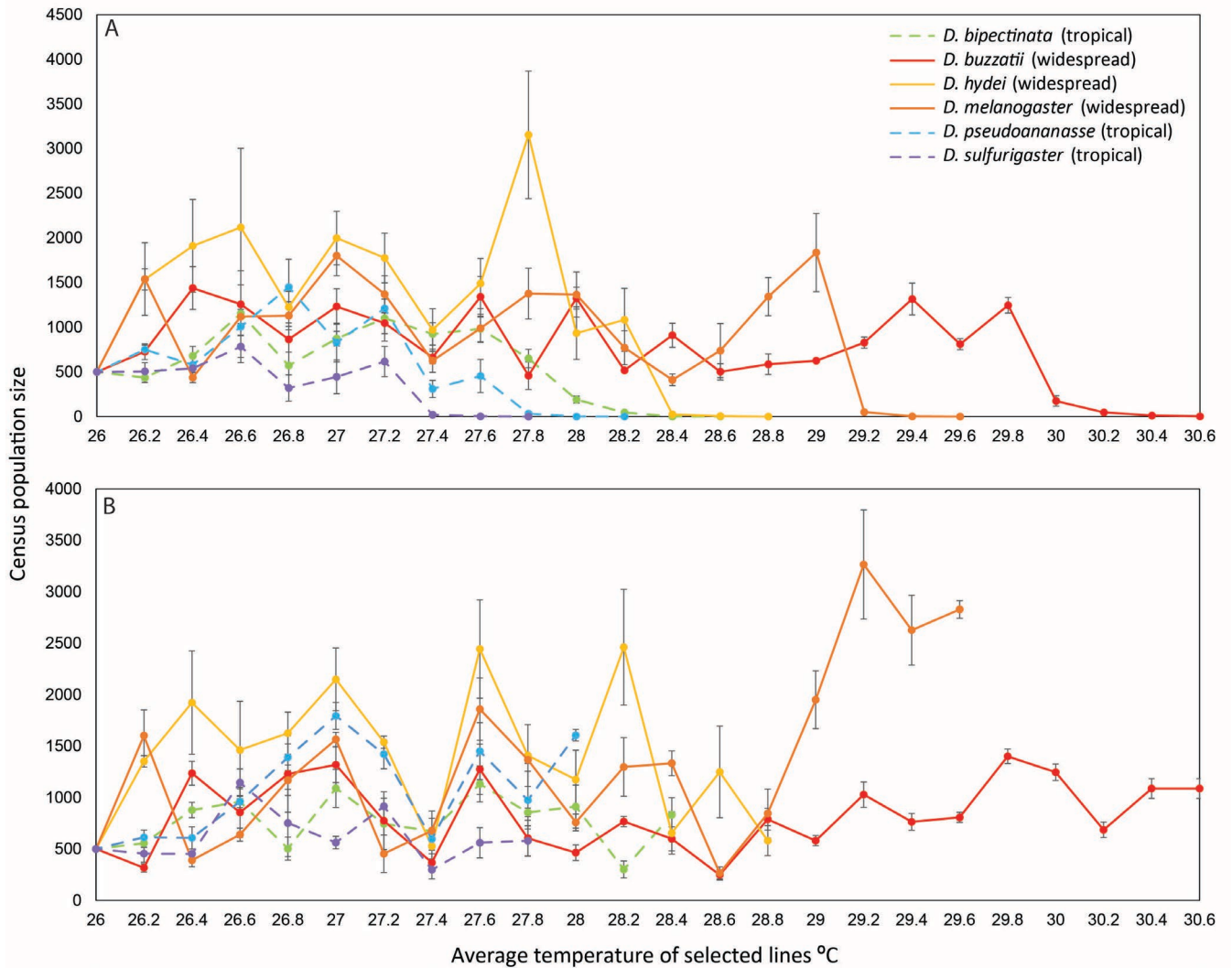


Figure S1. Changes in population size in selected (A) and control lines (B) in tropical (dashed lines) and widespread (solid lines) *Drosophila* species. Four replicate selection lines of *D. bipectinata* (tropical), *D. buzzatii* (widespread cactophilic), *D. hydei* (widespread), *D. melanogaster* (widespread), *D. pseudoananassae* (tropical) and *D. sulfurigaster* (tropical) were initiated under a fluctuating temperature regime averaging 26 °C (\pm 3 °C) and exposed to a 0.2 °C increase in average temperature every two weeks (approximately one generation). Census population size (\pm SEM) was estimated at the end of each two-week warming period by assessing the census population size of each replicate line. The source data is provided in the Source data file.

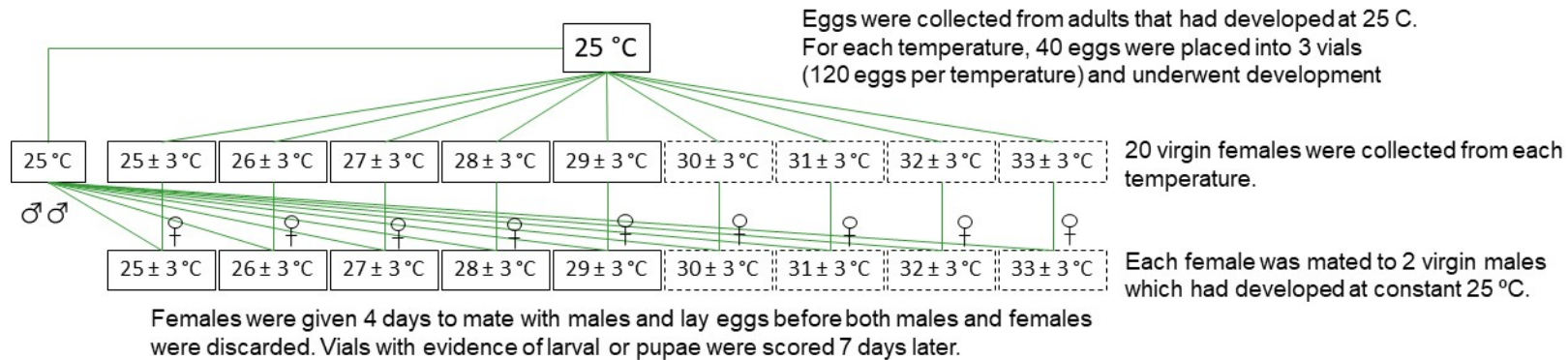
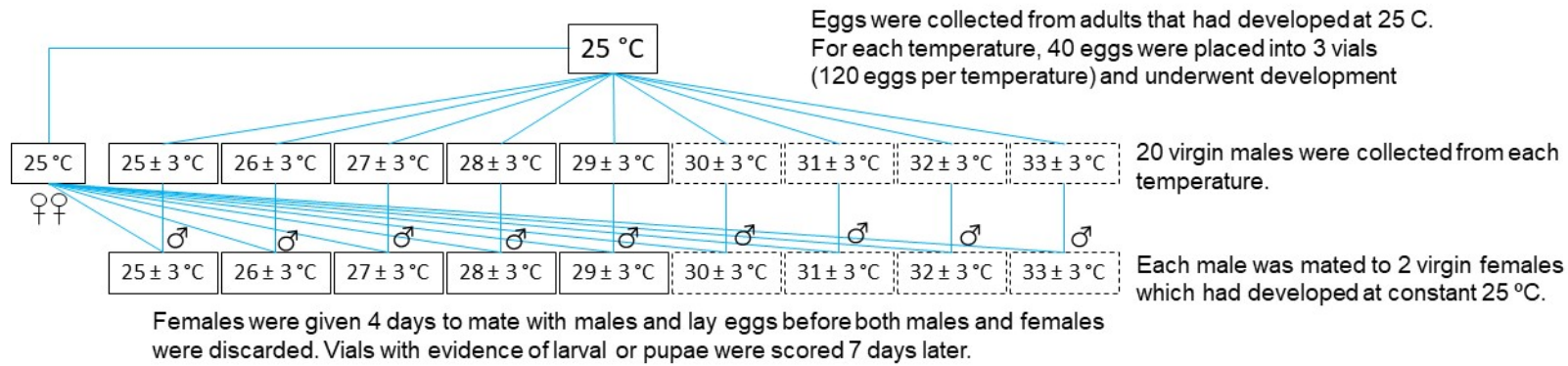
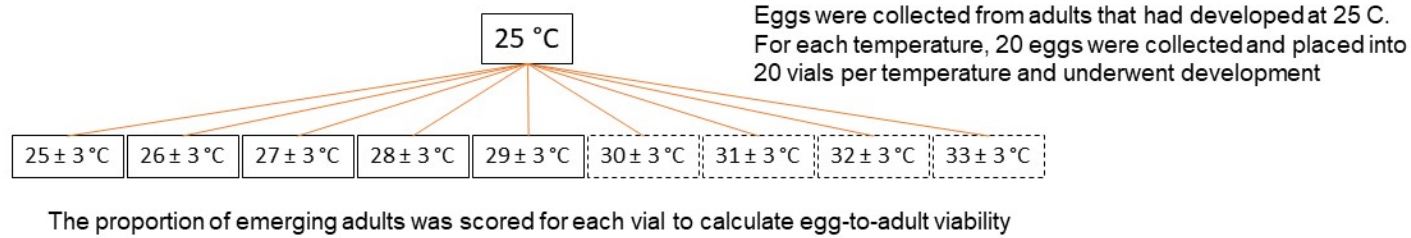


Figure S2 Initial trait assessment experimental design

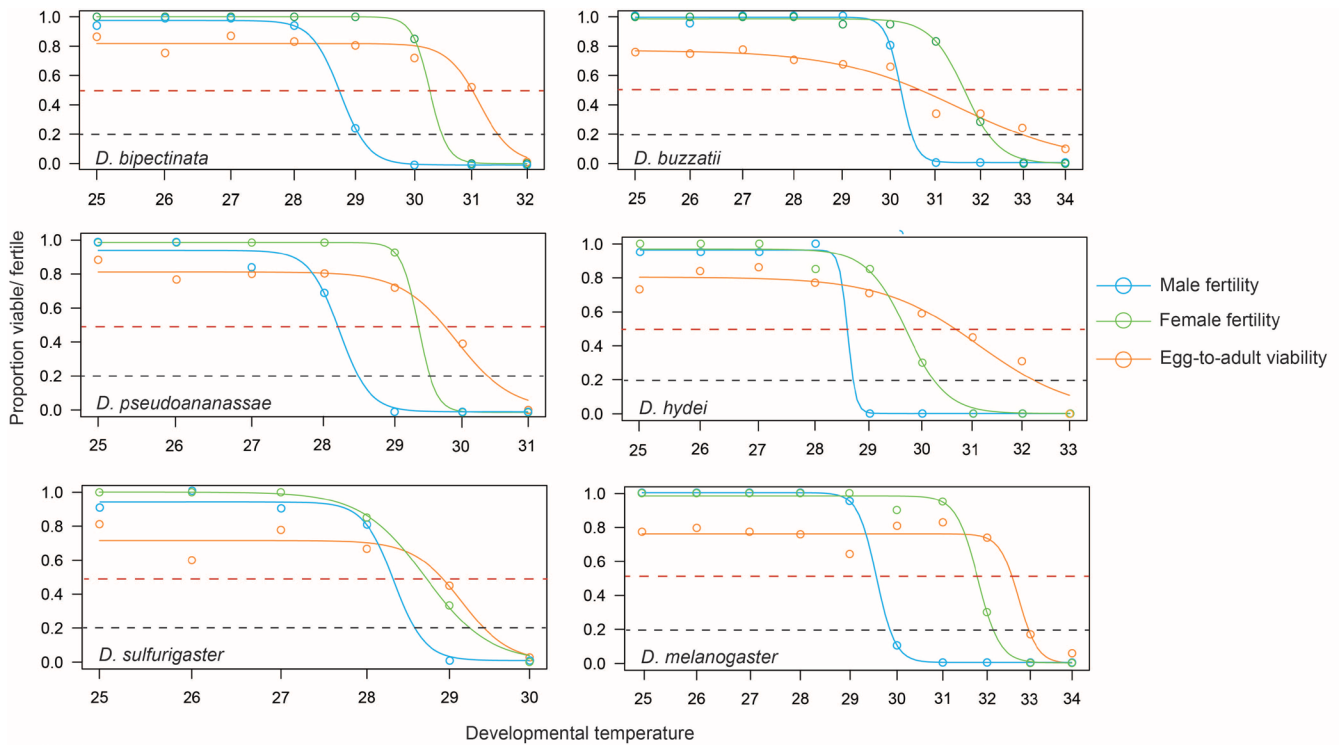


Figure S3. The relationship between mean developmental temperature (°C) and mean male fertility (blue), female fertility (green) and egg-to-adult viability (orange) for *D. bipectinata* (tropical), *D. buzzatii* (widespread cactophilic), *D. pseudoananassae* (tropical), *D. hydei* (widespread), *D. sulfurigaster* (tropical) and *D. melanogaster* (widespread). The solid lines represent the fitted three-parameter log-logistic dose-response model. The dashed lines indicate where fertility/viability is 50% (red) and 20% (black). Note different scales on the x- axis. Source data are provided as a Source Data file.

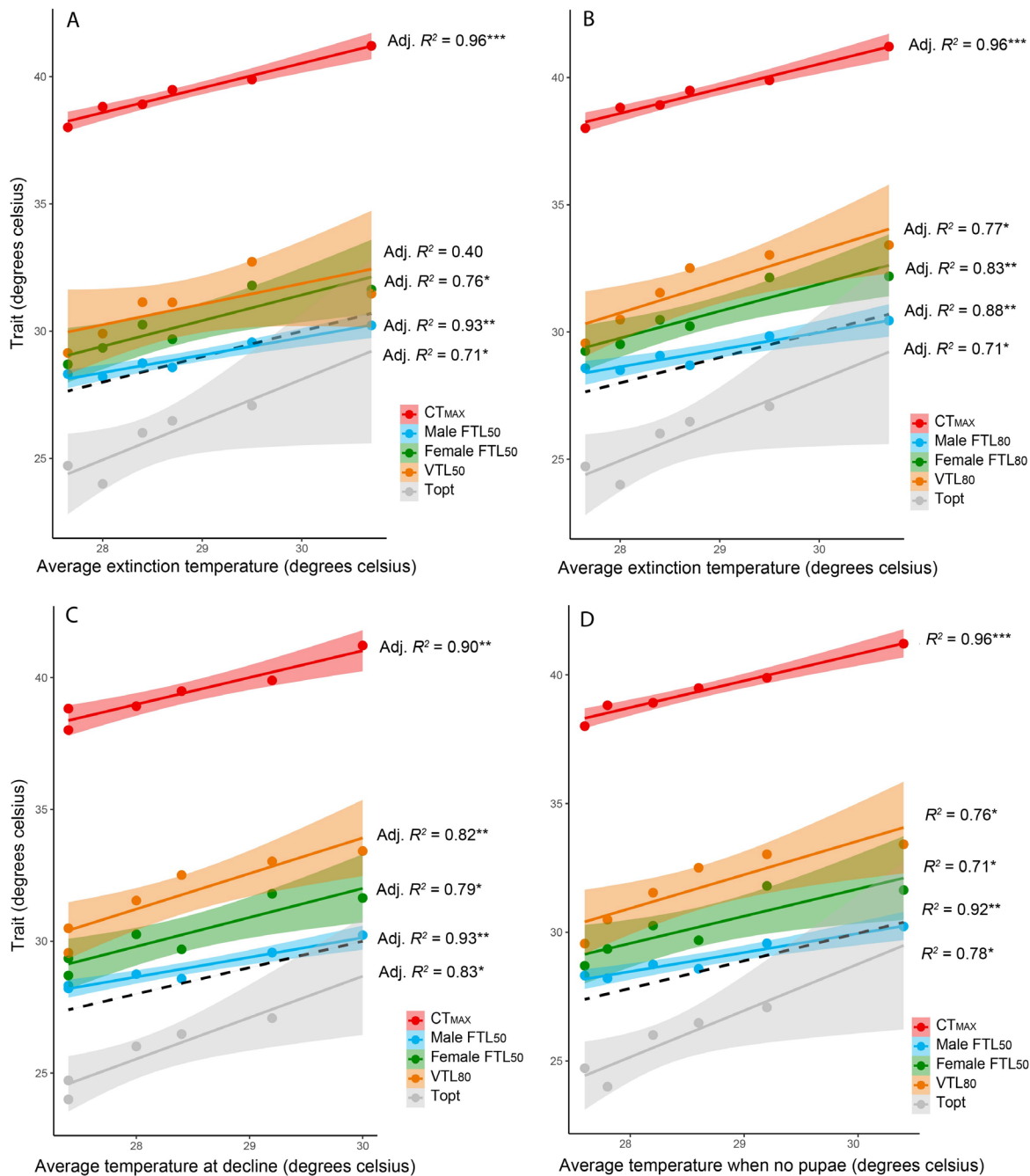


Figure S4. Associations between different estimates of thermal tolerance and average extinction temperature (A, B), average temperature at decline (C) and average temperature when there are no longer pupae (D) across 6 *Drosophila* species. Each point represents mean thermal tolerance for each species, the solid lines represent the fitted linear models and the shaded areas are the 95% confidence interval of these models. The dashed black line represents the direct relationship (slope = 1) between tolerance and average extinction (A/B), decline (C), no pupae temperature (D). The source data is provided in the Source data file.

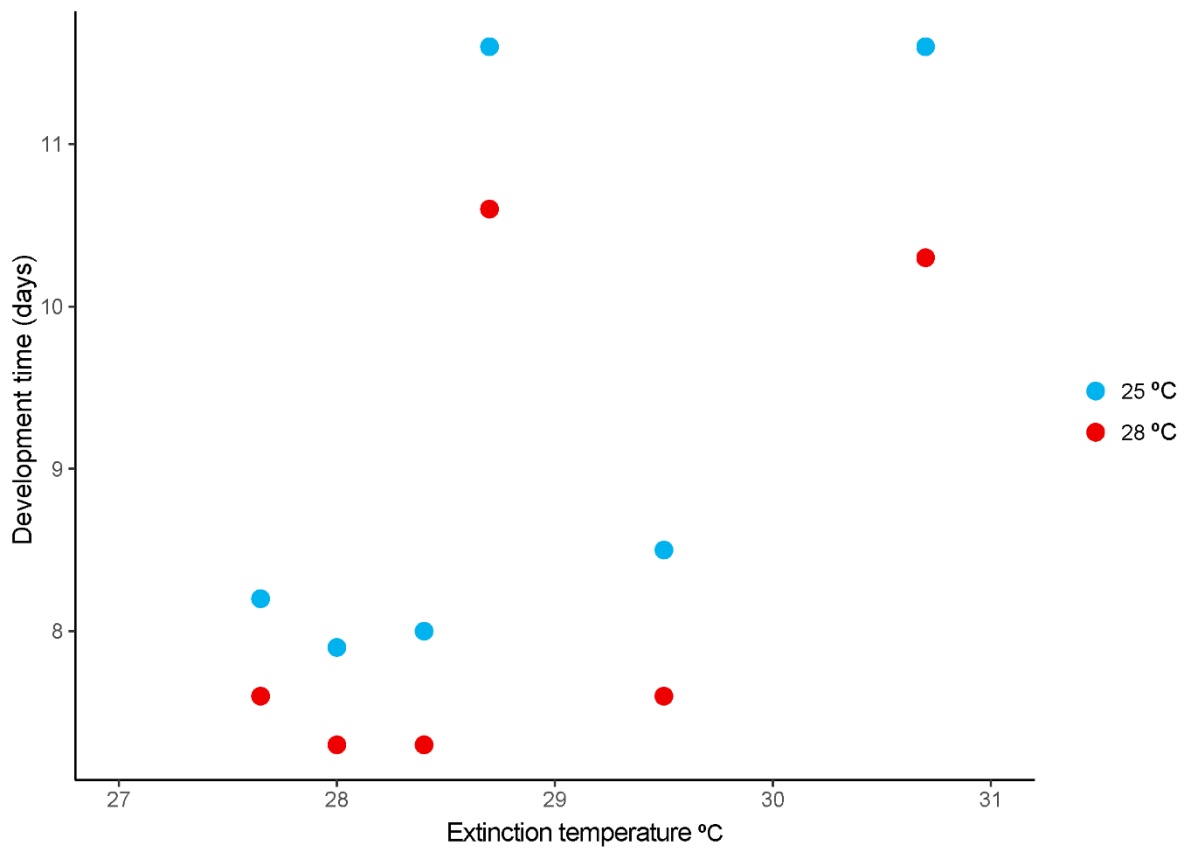


Figure S5. The relationship between average egg-to-adult development time at fluctuating 25 °C (blue) and 28 °C (red) and average extinction temperature. Each point represents the mean development time for each single species. See Table S2 for temperature regimes. The source data is provided in the Source data file.

Table S1. Collection information and details on the number of iso-female lines used to establish mass-bred populations for each species, the number of generations populations were maintained in the laboratory before the trait assessment or warming evolution experiment was commenced and the average development time from egg to adult at fluctuating 25 and 28 °C (see Table S2).

Species (species subgroup)	Distribution (tropical/ widespread)	Collection location (latitude, longitude)	Number of lines	Generations after collection (initial assessment/ warming experiment)	Development time at 25° C / 28° C (days)
<i>D. bipectinata</i> (ananassae)	tropical	18.21 S, 145.78 E	20	10 / 11	8.0 / 7.3
<i>D. birchii</i> (montium)	tropical	18.21 S, 145.78 E	20	15 / NA	NA
<i>D. bunnanda</i> (montium)	tropical	18.21 S, 145.78 E	10	15 / NA	NA
<i>D. buzzatii</i> (mulleri)	widespread	37.65 S, 144.77 E	15	7 / 8	11.6 / 10.3
<i>D. hydei</i> (hydei)	widespread	37.80 S, 145.00 E	20	5 / 6	11.6 / 10.6
<i>D. melanogaster</i> (melanogaster)	widespread	37.82 S, 145.02 E	20	13 / 14	8.5 / 7.6
<i>D. pseudoananassae</i> (ananassae)	tropical	18.21 S, 145.78 E	20	9 / 10	7.9 / 7.3
<i>D. serrata</i> (montium)	widespread	18.21 S, 145.78 E	15	15 / NA	NA
<i>D. simulans</i> (melanogaster)	widespread	37.82 S, 145.02 E	20	14 / NA	NA
<i>D. sulfurigaster</i> (nasuta)	tropical	18.21 S, 145.78 E	20	10 / 11	8.2 / 7.6

Table S2 Temperature regime of cabinets. Relative humidity was kept constant at 90%.

Lights	Time	Control Regime	Selection + 1.2 °C	Selection + 2.2 °C	Selection + 3.4 °C	Selection + 4.2 °C
0	0:00	24 °C	25.2 °C	26.2 °C	27.4 °C	28.2 °C
0	3:00	23.5 °C	24.7 °C	25.7 °C	26.9 °C	27.7 °C
1	6:00	24.5 °C	25.7 °C	26.7 °C	27.9 °C	28.7 °C
1	7:00	26 °C	27.2 °C	28.2 °C	29.4 °C	30.2 °C
1	8:00	27 °C	28.2 °C	29.2 °C	30.4 °C	31.2 °C
1	9:00	28 °C	29.2 °C	30.2 °C	31.4 °C	32.2 °C
1	11:00	29 °C	30.2 °C	31.2 °C	32.4 °C	33.2 °C
1	15:00	28 °C	29.2 °C	30.2 °C	31.4 °C	32.2 °C
1	17:00	27 °C	28.2 °C	29.2 °C	30.4 °C	31.2 °C
0	18:00	26 °C	27.2 °C	28.2 °C	29.4 °C	30.2 °C
0	20:00	25 °C	26.2 °C	27.2 °C	28.4 °C	29.2 °C
0	23:00	24 °C	25.2 °C	26.2 °C	27.4 °C	28.2 °C
	Average	26 °C	27.2 °C	28.2 °C	29.4 °C	30.2 °C
	Maximum	29 °C	30.2 °C	31.2 °C	32.4 °C	33.2 °C

Table S3 Temperatures when species subjected to experimental warming began to decline, became sterile (no pupae) and went extinct, averaged across four replicate lines.

Species	Average temperature at decline	Average temperature when sterile	Average temperature at extinction	Minimum temperature at extinction	Maximum temperature at extinction
<i>D. bipectinata</i> (T)	28.0 °C	28.15 °C	28.4 °C	25.9 °C	31.4 °C
<i>D. buzzatii</i> (W)	30.0 °C	30.25 °C	30.7 °C	28.2 °C	33.7 °C
<i>D. hydei</i> (W)	28.4 °C	28.60 °C	28.7 °C	26.2 °C	31.7 °C
<i>D. melanogaster</i> (W)	29.2 °C	29.20 °C	29.5 °C	27.0 °C	32.5 °C
<i>D. pseudoananassae</i> (T)	27.4 °C	27.80 °C	28.0 °C	25.5 °C	31.0 °C
<i>D. sulfurigaster</i> (T)	27.4 °C	27.60 °C	27.65 °C	25.15 °C	30.65 °C

T = tropical species, W = widespread species

Table S4 Correlations (pairwise, two-sided Pearson) between traits across six species (Five species for comparisons with *Topt*. Significant correlations ($P < 0.05$, uncorrected) are highlighted in bold. Source data are provided as a Source Data file.

Trait		CTmax	Male FTL ₅₀	Female FTL ₅₀	VTL ₈₀
Male FTL ₅₀	Correlation	0.917			
	<i>P</i>	0.01			
	df	4			
Female FTL ₅₀	Correlation	0.857	0.923		
	<i>P</i>	0.03	0.01		
	df	4	4		
VTL ₈₀	Correlation	0.915	0.835	0.887	
	<i>P</i>	0.01	0.04	0.02	
	df	4	4	4	
<i>Topt</i>	Correlation	0.79	0.85	0.79	0.90
	<i>P</i>	0.11	0.07	0.11	0.04
	df	3	3	3	3

Table S5. Results from a paired *t*-test (two tailed) examining mean differences between average temperature at extinction, maximum temperature at extinction, average temperature when the population size of selected lines was significantly lower than control lines (decline temperature) and average temperature when pupae were no longer present in selected lines (sterile temperature) and thermal tolerance traits CTmax, VTL₈₀, male FTL₅₀, female FTL₅₀ and T_{opt} across the six species exposed to experimental warming. Source data are provided as a Source Data file.

Trait 1	Trait 2	Mean difference	<i>t</i>	df	<i>P</i>
Average extinction temperature	CTmax	-10.56	-132.05	5	<0.001
	VTL ₈₀	-2.93	-10.25	5	<0.001
	Male FTL ₅₀	-0.12	-0.74	5	0.494
	Female FTL ₅₀	-1.42	-6.28	5	0.002
	T _{opt}	2.79	8.61	4	0.001
Maximum extinction temperature	CTmax	-7.56	-94.55	5	<0.001
Average decline temperature	CTmax	-10.99	-86.50	5	<0.001
	VTL ₈₀	-3.36	-12.04	5	<0.001
	Male FTL ₅₀	-0.54	-4.14	5	0.008
	Female FTL ₅₀	-1.84	-8.52	5	<0.001
	T _{opt}	2.42	8.69	4	<0.001
Average sterile temperature (no pupae)	CTmax	-10.76	-126.82	5	<0.001
	VTL ₈₀	-3.13	-10.38	5	<0.001
	Male FTL ₅₀	-0.31	-2.23	5	0.076
	Female FTL ₅₀	-1.61	-6.46	5	0.001
	T _{opt}	2.62	8.01	4	0.001

Table S6. Results from generalized linear mixed models (binomial distribution, logit link function) assessing the effect of treatment (control or selection) and temperature (control or selected (warmer) developmental temperatures) on male sterility on species that had undergone selection for experimental warming tolerance. Replicate lines are nested within treatment as a random effect. Source data are provided as a Source Data file.

Warming	Species	Predictor	Estimate ± SE	df	χ^2	<i>P</i>	Semi-partial R^2 (CI)
1.2 °C	<i>D. bipectinata</i>	Intercept	0.868 ± 0.208	1	17.364	<0.001	
		temperature	0.413 ± 0.203	1	4.135	0.042	0.032 (0.00-0.12)
		treatment	0.009 ± 0.204	1	0.002	0.966	<0.001 (0.00-0.04)
		temp*treat	-0.083 ± 0.208	1	0.170	0.680	0.001 (0.00-0.05)
		Rep (intercept)	Variance ± SD = 0.008 ± 0.088				
	<i>D. buzzatii</i>	Intercept	1.947 ± 0.316	1	37.997	<0.001	
		temperature	-0.143 ± 0.240	1	0.355	0.552	0.002 (0.00-0.04)
		treatment	-0.177 ± 0.261	1	0.372	0.542	0.003 (0.00-0.05)
		temp*treat	-0.014 ± 0.240	1	0.004	0.953	<0.001 (0.00-0.03)
		Rep (intercept)	Variance ± SD = 0.203 ± 0.451				
	<i>D. hydei</i>	Intercept	1.881 ± 0.243	1	60.038	<0.001	
		temperature	-0.320 ± 0.243	1	1.743	0.187	0.011 (0.00-0.07)
		treatment	0.041 ± 0.243	1	0.028	0.867	<0.001 (0.00-0.03)
		temp*treat	-0.215 ± 0.243	1	0.783	0.376	0.005 (0.00-0.05)
		Rep (intercept)	Variance ± SD = <0.001 ± <0.001				
	<i>D. melanogaster</i>	Intercept	3.290 ± 0.458	1	51.611	<0.001	
		temperature	-0.374 ± 0.458	1	0.665	0.415	0.004 (0.00-0.05)
		treatment	0.374 ± 0.458	1	0.665	0.415	0.004 (0.00-0.05)
		temp*treat	0.374 ± 0.458	1	0.665	0.415	0.004 (0.00-0.05)
		Rep (intercept)	Variance ± SD = <0.001 ± <0.001				
<i>D. pseudoananassae</i>	Intercept	-0.171 ± 0.424	1	0.163	0.687		
	temperature	0.666 ± 0.193	1	11.898	<0.001	0.054 (0.01-0.14)	
	treatment	0.177 ± 0.425	1	0.174	0.676	0.004 (0.00-0.05)	
	temp*treat	0.201 ± 0.191	1	1.101	0.294	0.006 (0.00-0.05)	
	Rep (intercept)	Variance ± SD = 1.155 ± 1.075					
2.2 °C	<i>D. buzzatii</i>	Intercept	2.584 ± 0.411	1	39.628	<0.001	
		temperature	0.376 ± 0.342	1	1.214	0.271	0.008 (0.00-0.06)
		treatment	0.368 ± 0.363	1	1.024	0.312	0.010 (0.00-0.06)
		temp*treat	0.361 ± 0.341	1	1.118	0.290	0.007 (0.00-0.06)
		Rep (intercept)	Variance ± SD = 0.098 ± 0.313				
	<i>D. melanogaster</i>	Intercept	3.208 ± 0.534	1	36.097	<0.001	
		temperature	0.004 ± 0.419	1	<0.001	0.993	<0.001 (0.00-0.03)
		treatment	0.576 ± 0.467	1	1.520	0.218	0.011 (0.00-0.07)
		temp*treat	-0.018 ± 0.419	1	0.002	0.965	<0.001 (0.00-0.03)
		Rep (intercept)	Variance ± SD = 0.316 ± 0.562				
3.4 °C	<i>D. buzzatii</i>	Intercept	2.784 ± 0.419	1	44.101	<0.001	
		temperature	0.156 ± 0.419	1	0.138	0.710	0.001 (0.00-0.05)
		treatment	-0.082 ± 0.419	1	0.038	0.845	<0.001 (0.00-0.04)
		temp*treat	0.509 ± 0.419	1	1.476	0.224	0.012 (0.00-0.08)
		Rep (intercept)	Variance ± SD = <0.001 ± <0.001				
4.2 °C	<i>D. buzzatii</i>	Intercept	0.457 ± 0.368	1	1.532	0.216	
		temperature	1.752 ± 0.383	1	20.934	<0.001	0.259 (0.141-0.39)
		treatment	-0.427 ± 0.367	1	1.355	0.244	0.022 (0.000-0.10)
		temp*treat	-0.588 ± 0.350	1	2.828	0.093	0.046 (0.002-0.14)
		Rep (intercept)	Variance ± SD = 0.181 ± 0.425				

Significant *p*-values ($\alpha = 0.05$) for fixed effects are in bold.

Table S7. Results from linear mixed models (gaussian distribution, identity link function) assessing the effect of treatment (control or selection) and temperature (control or selected (warmer) developmental temperatures) on male fertility on species that had undergone selection for experimental warming tolerance. Replicate lines are nested within treatment as a random effect. Source data are provided as a Source Data file.

Warming	Species	Predictor	Estimate ± SE	df	χ^2	<i>P</i>	Semi-partial R^2 (CI)
1.2 °C	<i>D. bipectinata</i>	Intercept	8.054 ± 1.365	1	34.806	<0.001	
		temperature	1.174 ± 0.668	1	3.091	0.104	0.020 (0.00-0.09)
		treatment	0.159 ± 1.365	1	0.014	0.907	<0.001 (0.00-0.04)
		temp*treat	0.240 ± 0.668	1	0.129	0.720	0.001 (0.00-0.04)
		Rep (intercept)	Variance ± SD = 9.729 ± 3.119				
	<i>D. buzzatii</i>	Intercept	18.368 ± 2.427	1	52.277	<0.001	
		temperature	0.103 ± 1.227	1	0.007	0.933	<0.001 (0.00-0.03)
		treatment	-2.013 ± 2.427	1	0.688	0.407	0.015 (0.00-0.08)
		temp*treat	-2.808 ± 1.227	1	5.233	0.022	0.029 (0.001-0.1)
		Rep (intercept)	Variance ± SD = 35.06 ± 5.92				
	<i>D. hydei</i>	Intercept	25.013 ± 1.385	1	326.10	<0.001	
		temperature	-2.238 ± 1.151	1	3.784	0.052	0.023 (0.00-0.09)
		treatment	0.326 ± 1.385	1	0.055	0.814	<0.001 (0.00-0.03)
		temp*treat	-1.276 ± 1.151	1	1.230	0.268	0.008 (0.00-0.06)
		Rep (intercept)	Variance ± SD = 4.76 ± 2.18				
	<i>D. melanogaster</i>	Intercept	28.271 ± 1.258	1	505.24	<0.001	
		temperature	-5.420 ± 1.266	1	18.341	<0.001	0.119 (0.04-0.22)
		treatment	1.445 ± 1.356	1	1.135	0.287	0.010 (0.00-0.06)
		temp*treat	2.558 ± 1.266	1	4.084	0.043	0.030 (0.001-0.10)
		Rep (intercept)	Variance ± SD = 1.90 ± 1.38				
<i>D. pseudoananassae</i>	Intercept	4.587 ± 0.995	1	21.239	<0.001		
	temperature	2.177 ± 0.477	1	20.881	<0.001	0.103 (0.03-0.20)	
	treatment	1.749 ± 0.100	1	3.089	0.079	0.069 (0.01-0.16)	
	temp*treat	1.465 ± 0.477	1	9.451	0.002	0.049 (0.01-0.13)	
	Rep (intercept)	Variance ± SD = 6.11 ± 2.47					
2.2 °C	<i>D. buzzatii</i>	Intercept	48.743 ± 3.610	1	182.29	<0.001	
		temperature	4.980 ± 2.763	1	3.249	0.071	0.020 (0.00-0.08)
		treatment	1.500 ± 3.610	1	0.173	0.678	0.002 (0.00-0.04)
		temp*treat	-0.177 ± 2.763	1	0.004	0.949	<0.001 (0.00-0.03)
		Rep (intercept)	Variance ± SD = 43.21 ± 6.57				
	<i>D. melanogaster</i>	Intercept	43.679 ± 1.837	1	565.65	<0.001	
		temperature	1.616 ± 1.566	1	1.064	0.302	0.007 (0.00-0.06)
		treatment	0.141 ± 1.835	1	0.006	0.939	<0.001 (0.00-0.03)
		temp*treat	-0.115 ± 1.566	1	0.005	0.942	<0.001 (0.00-0.03)
		Rep (intercept)	Variance ± SD = 7.33 ± 2.71				
3.4 °C	<i>D. buzzatii</i>	Intercept	29.052 ± 1.928	1	227.13	<0.001	
		temperature	1.336 ± 1.664	1	0.645	0.422	0.005 (0.00-0.06)
		treatment	0.882 ± 1.923	1	0.209	0.647	0.002 (0.00-0.05)
		temp*treat	1.031 ± 1.664	1	0.384	0.536	0.003 (0.00-0.05)
		Rep (intercept)	Variance ± SD = 6.496 ± 2.549				
4.2 °C	<i>D. buzzatii</i>	Intercept	29.409 ± 2.344	1	157.35	<0.001	
		temperature	25.979 ± 2.344	1	122.79	<0.001	0.512 (0.40-0.62)
		treatment	-2.858 ± 2.344	1	1.486	0.223	0.012 (0.00-0.09)
		temp*treat	-3.254 ± 2.344	1	1.926	0.165	0.016 (0.00-0.08)
		Rep (intercept)	Variance ± SD = <0.001 ± 0.001				

Significant *p*-values ($\alpha = 0.05$) for fixed effects are in bold.

Table S8. Results from linear mixed models (gaussian distribution, identity link function) assessing the effect of treatment (control or selection) and temperature (control or selected (warmer) developmental temperatures) on CTmax on species that had undergone selection for experimental warming tolerance. Replicate lines are nested within treatment as a random effect. Source data are provided as a Source Data file.

Warming	Species	Predictor	Estimate ± SE	df	χ^2	<i>P</i>	Semi-partial R^2 (CI)
2.2 °C	<i>D. buzzatii</i>	Intercept	41.560 ± 0.034	1	1.4e6	<0.001	
		temperature	-0.052 ± 0.034	1	2.339	0.126	0.007 (0.00-0.04)
		treatment	-0.014 ± 0.034	1	0.164	0.685	0.001 (0.00-0.02)
		temp*treat	-0.013 ± 0.034	1	0.138	0.710	<0.001 (0.00-0.02)
		Rep (intercept)	Variance ± SD = <0.001 ± <0.001				
	<i>D. melanogaster</i>	Intercept	41.054 ± 0.012	1	1.2e7	<0.001	
		temperature	-0.103 ± 0.012	1	60.94	<0.001	0.173 (0.11-0.25)
		treatment	0.005 ± 0.012	1	0.167	0.683	0.001 (0.00-0.02)
		temp*treat	-0.007 ± 0.012	1	0.311	0.577	0.001 (0.00-0.02)
		Rep (intercept)	Variance ± SD = <0.001 ± <0.001				
3.4 °C	<i>D. buzzatii</i>	Intercept	41.570 ± 0.018	1	5.3e6	<0.001	
		temperature	-0.081 ± 0.018	1	19.92	<0.001	0.078 (0.03-0.15)
		treatment	0.003 ± 0.018	1	0.021	0.884	<0.001 (0.00-0.02)
		temp*treat	0.005 ± 0.018	1	0.083	0.774	<0.001 (0.00-0.02)
		Rep (intercept)	Variance ± SD = <0.001 ± 0.001				
4.2 °C	<i>D. buzzatii</i>	Intercept	41.359 ± 0.021	1	4.0e6	<0.001	
		treatment	-0.003 ± 0.021	1	0.015	0.903	<0.001 (0.00-0.04)
		Rep (intercept)	Variance ± SD = 0.002 ± 0.041				

Significant *p*-values ($\alpha = 0.05$) for fixed effects are in bold.

Table S9 Estimates of mean CTmax, male FTL₅₀, female FTL₅₀ and VTL₈₀ for the 10 species where these traits were assessed.

Species	CTmax (±SEM)	Male FTL ₅₀ (±SEM)	Female FTL ₅₀ (±SEM)	VTL ₈₀ (±SEM)
<i>D. bipectinata</i>	38.92 ± 0.04	28.75 ± 0.09	30.26 ± 0.10	31.54 ± 0.25
<i>D. birchii</i>	38.11 ± 0.04	25.80 ± 0.22	27.99 ± 0.06	NA
<i>D. bunnanda</i>	38.42 ± 0.05	26.69 ± 0.16	28.08 ± 0.11	NA
<i>D. buzzatii</i>	41.21 ± 0.05	30.23 ± 0.11	31.64 ± 0.09	33.42 ± 0.37
<i>D. hydei</i>	39.49 ± 0.05	28.58 ± 0.34	29.69 ± 0.10	32.51 ± 0.26
<i>D. melanogaster</i>	39.89 ± 0.03	29.57 ± 0.07	31.8 ± 0.08	33.03 ± 0.09
<i>D. pseudoananassae</i>	38.82 ± 0.06	28.21 ± 0.09	29.35 ± 0.11	30.49 ± 0.15
<i>D. serrata</i>	38.61 ± 0.04	27.24 ± 0.08	28.47 ± 0.21	NA
<i>D. simulans</i>	38.93 ± 0.04	29.32 ± 0.13	NA	NA
<i>D. sulfurigaster</i>	38.01 ± 0.05	28.32 ± 0.12	28.7 ± 0.10	29.56 ± 0.15