Supplementary Information

Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton.

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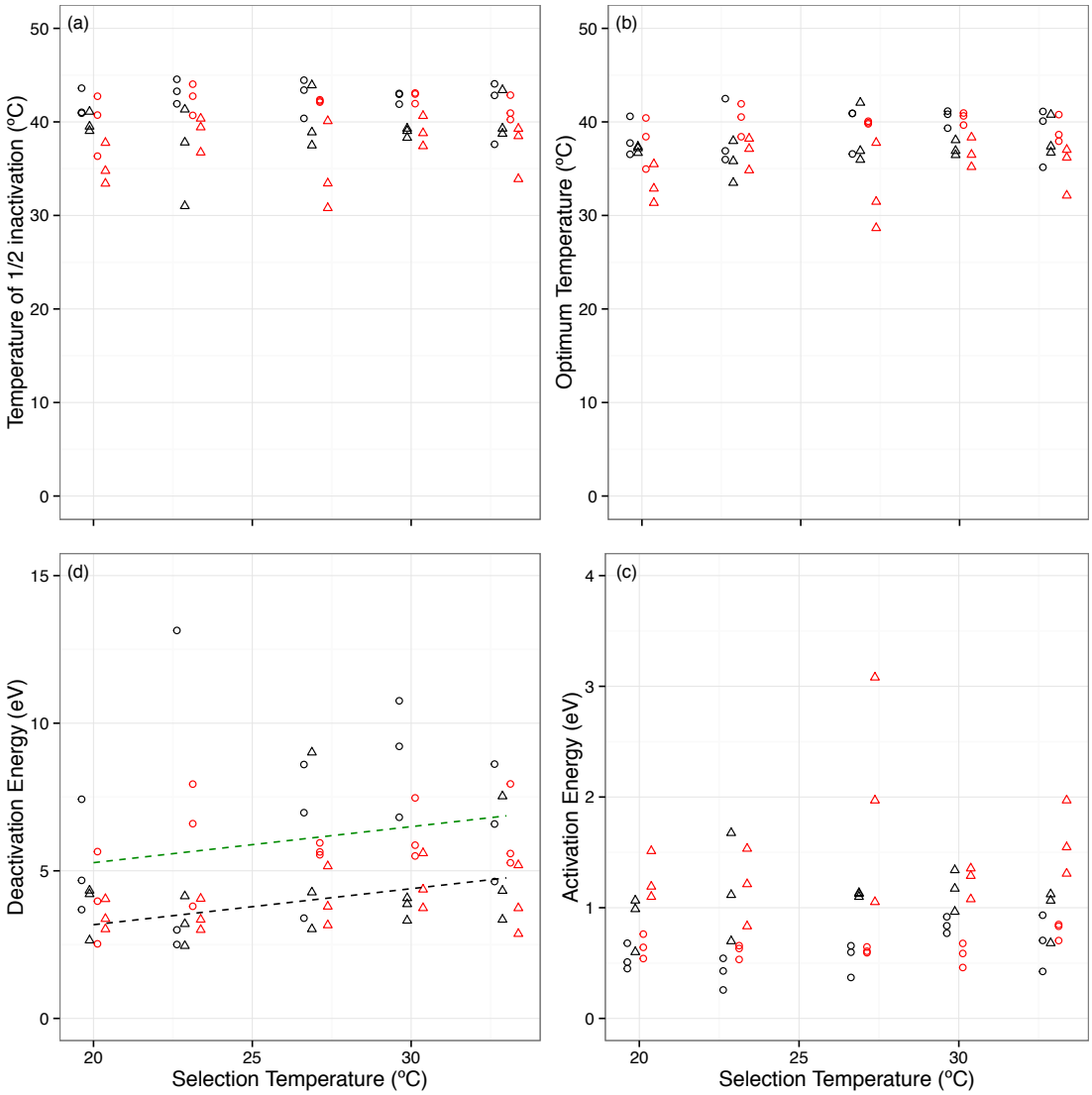

Figure S1. Effects of selection temperature on metabolic traits. In (a-d) circles show the metabolic traits of photosynthesis and triangles those of respiration after exposure to different selection temperatures for ~10 (black) and ~100 generations (red). In (c) fitted broken lines show the significant linear relationship between the deactivation energy and selection temperature for photosynthesis (green) and respiration (black) (see Table S4).

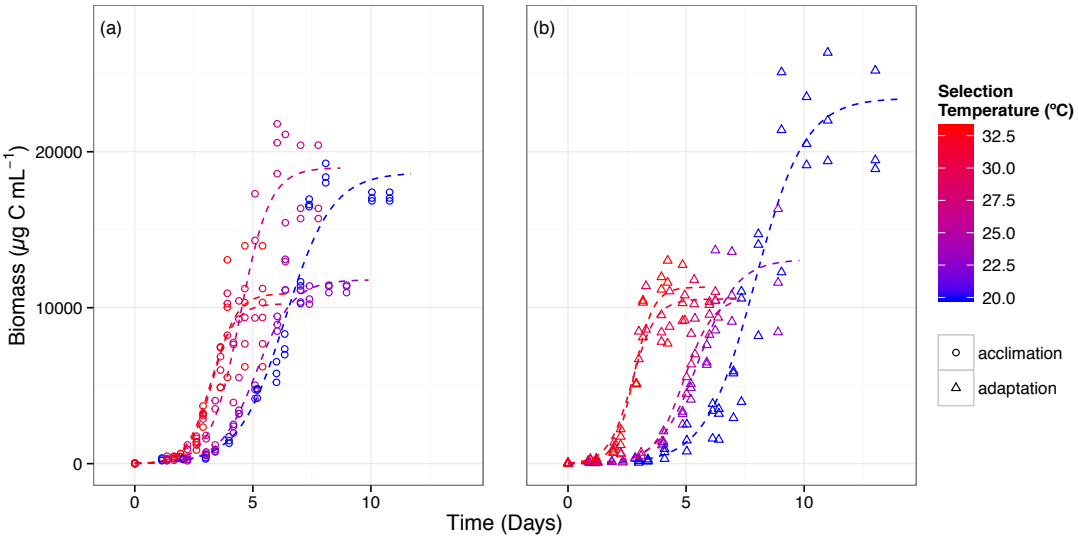

Figure S2. Effects of temperature on population dynamics. Sigmoid growth curves were measured for populations following short-term (10 generations; a) and long-term warming (100 generations; b) at 20ºC (blue), through to 33ºC (red). Analyses reveal that at 33ºC, growth rates (*r*) are higher after long-term warming. Fitted lines are based on mean parameters at each growth temperature of non-linear least squares regression using a sigmoid growth curve equation *(n = 3)* (see Methods Eq. 5).

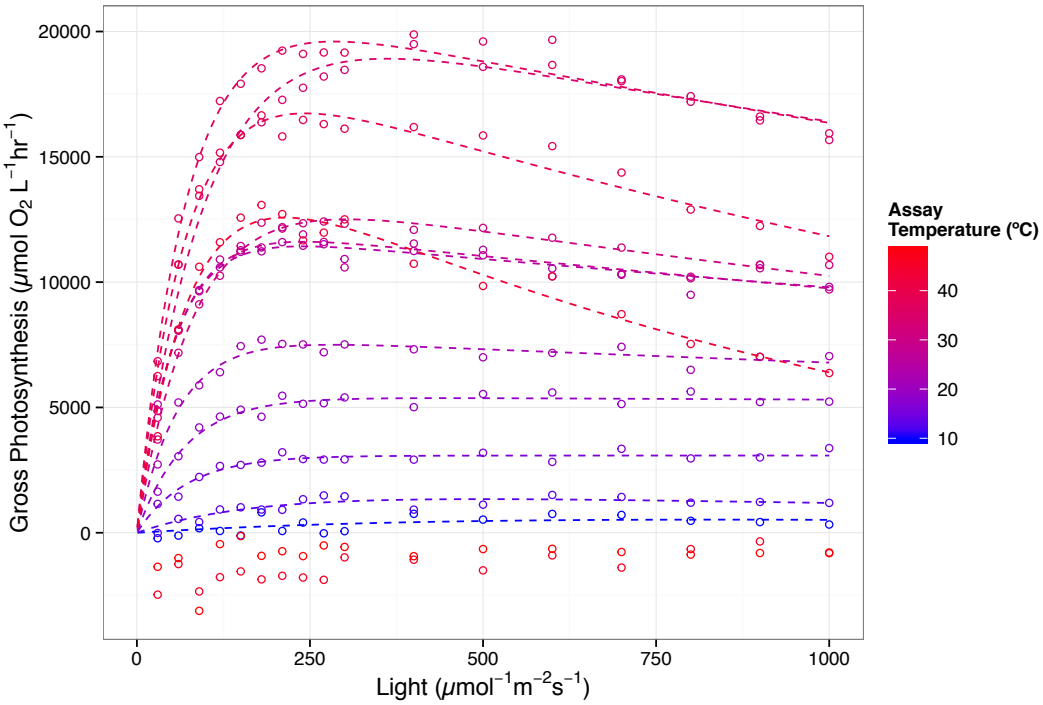

Figure S3. Photosynthesis irradiance curves used to characterise the acute temperature response of photosynthesis. Rates of gross photosynthesis (*P*) were measured at various light intensities across the full range of acute temperatures (10 – 49°C), characterising the metabolic thermal niche of *Chlorella vulgaris*. Here data are presented for one replicate at the long-term ancestral temperature regime (20ºC). Lines represent the best fit to the photoinhibition model using non-linear least squares regression (see Methods Eq. 6).

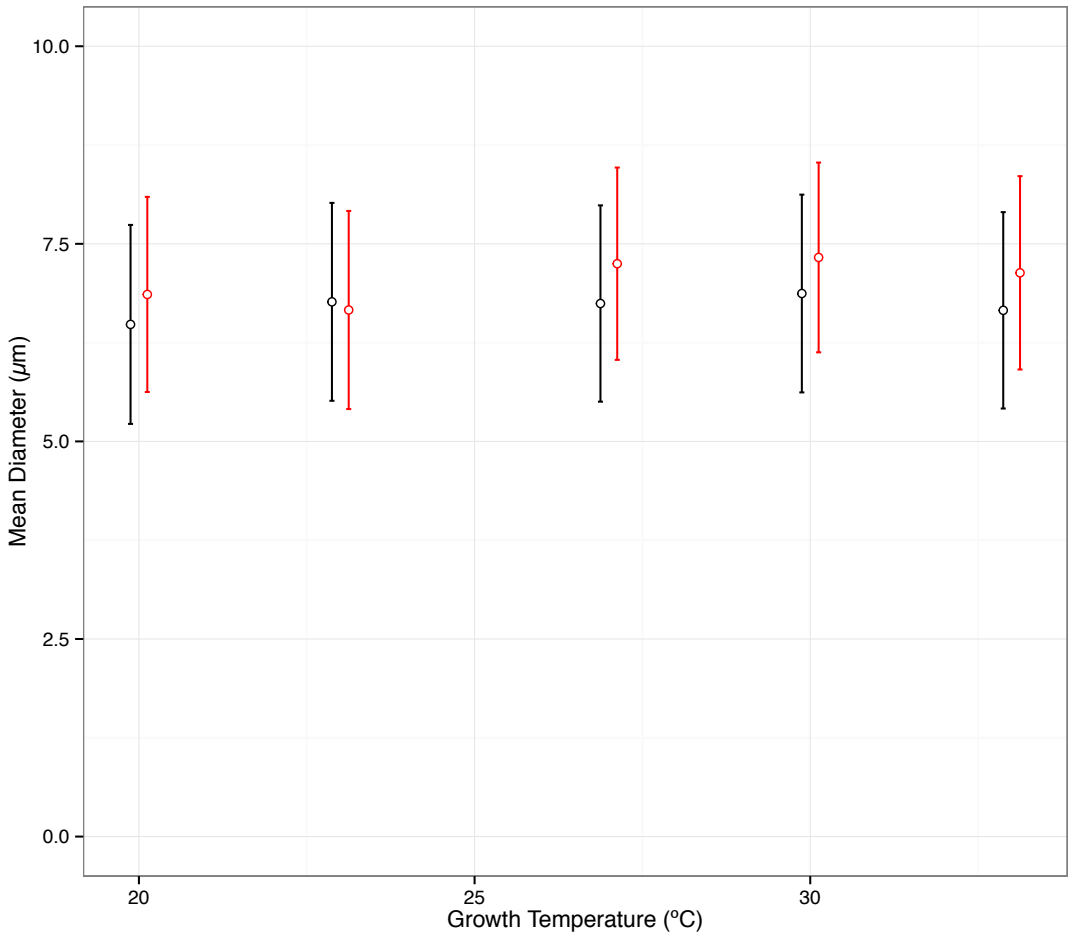

Figure S4. Effects of selection temperature on cell size. Equivalent spherical diameter was measured for each replicate after short-term (black circles) and long-term (red circles) exposure to each selection temperature. Mean cell diameter was estimated for each replicate population as the anti-log of the average $\log_{10}$ cell diameter. Body size did not vary between selection temperatures or between long-term versus short-term warming. The error bars represent 1 standard deviation from the mean.

Table S1. Results of the linear mixed effects model analysis for growth trajectories (see Methods). Random effects on the slope and intercept were determined at the level of replicates nested within selection temperatures. The results of the model selection procedure on the fixed effect terms are given and the most parsimonious models are highlighted in bold. Analyses reveal that growth rates changed significantly through time and that growth trajectories were significantly different between selection temperatures.

Model	**d.f.**	**AIC**	**logLik**	***L*-ratio**	***p***
random effects structure					
random = ~1 | id)					
corrected structure = varPower() & corARMA(q = 1)					
fixed effects structure					
1. **growth rate ~ 1 + time * selection temperature**	**14**	**-140**	**84**		
2. growth rate ~ 1 + time + selection temperature	10	-123	71.3	25.4	**< 0.001**
3. growth rate ~ 1 + time	6	-53	32.9	77.0	**< 0.001**

Table S2. Trajectory of exponential growth rate at the various selection temperatures. Parameters are estimated from a mixed effects model (see Methods adn Table S1) and treatment contrasts were made using Tukey's least significant difference tests. Contrasts significant at the 0.05 level for the slope of the growth trajectory are 20ºC vs 27ºC, 20ºC vs 30ºC, 20ºC vs 33ºC, 23ºC vs 27ºC, 23ºC vs 30ºC, 23ºC vs 33ºC, 27ºC vs 33ºC and 30ºC vs 33ºC. Significant contrasts for the intercept at 0.05 significance are 20ºC vs 27ºC, 20ºC vs 30ºC, 20ºC vs 33ºC, 23ºC vs 27ºC, 23ºC vs 30ºC and 23ºC vs 33ºC.

Growth Temperature	Slope (95% CI)	Intercept (95% CI)
20ºC	0.000152 (-0.000260 – 0.000566)	0.87 (0.85 – 0.89)
23ºC	0.00122 (-0.000288 – 0.00274)	1.14 (1.08 – 1.19)
27ºC	0.00452 (0.00239 – 0.00665)	1.42 (1.34 – 1.49)
30ºC	0.00556 (0.00329 – 0.00783)	1.58 (1.49 – 1.66)
33ºC	0.0130 (0. 00929 – 0.0168)	1.48 (1.36 – 1.60)

Table S3. Mean and standard errors for the metabolic traits governing the thermal response curves for *Chlorella vulgaris* following fitting to a modified Sharpe-Schoolfield equation using non-linear least squares regression.

Selection Temperature	Exposure	Flux	$b(T_c)$ (μmol O_2 μg C^{-1} h^{-1} @ 25ºC)	E_a (eV)	E_h (eV)	T_h (ºC)	T_{opt} (ºC)
20	long-term	P	1.66 (0.239)	0.648 (0.0637)	4.05 (0.903)	39.9 (1.89)	37.9 (1.59)
20	short-term	P	1.59 (0.33)	0.546 (0.0688)	5.26 (1.12)	41.9 (0.877)	38.3 (1.2)
23	long-term	P	0.758 (0.0174)	0.607 (0.0381)	6.11 (1.22)	42.5 (0.972)	40.3 (1.02)
23	short-term	P	1.41 (0.117)	0.409 (0.083)	6.21 (3.47)	43.3 (0.756)	38.5 (2.03)
27	long-term	P	0.93 (0.0222)	0.615 (0.0158)	5.71 (0.122)	42.2 (0.0732)	39.9 (0.0825
27	short-term	P	1.69 (0.0717)	0.541 (0.0874)	6.32 (1.54)	42.7 (1.22)	39.5 (1.45)
30	long-term	P	0.765 (0.0616)	0.575 (0.0631)	6.28 (0.604)	42.7 (0.362)	40.4 (0.389)
30	short-term	P	1.15 (0.249)	0.841 (0.043)	8.93 (1.15)	42.6 (0.368)	40.4 (0.56)
33	long-term	P	0.801 (0.0549)	0.795 (0.0461)	6.27 (0.843)	41.4 (0.782)	39.1 (0.855)
33	short-term	P	1.44 (0.466)	0.687 (0.147)	6.61 (1.15)	41.5 (1.98)	38.8 (1.84)
20	long-term	R	0.492 (0.0551)	1.27 (0.125)	3.48 (0.3)	35.3 (1.29)	33.2 (1.21)
20	short-term	R	0.45 (0.064)	0.884 (0.144)	3.73 (0.544)	39.9 (0.622)	37.1 (0.2)
23	long-term	R	0.188 (0.026)	1.19 (0.203)	3.47 (0.31)	38.8 (1.09)	36.7 (0.997)
23	short-term	R	0.379 (0.0212)	1.16 (0.283)	3.27 (0.487)	36.7 (3.03)	35.8 (1.29)
27	long-term	R	0.178 (0.0298)	2.03 (0.587)	4.03 (0.59)	34.8 (2.77)	32.6 (2.69)
27	short-term	R	0.387 (0.0153)	1.12 (0.0105)	5.44 (1.82)	40.1 (1.95)	38.3 (1.9)
30	long-term	R	0.17 (0.0197)	1.24 (0.0839)	4.57 (0.546)	39 (0.935)	36.7 (0.916)
30	short-term	R	0.445 (0.105)	1.16 (0.109)	3.75 (0.228)	38.9 (0.292)	37.1 (0.475)
33	long-term	R	0.16 (0.013)	1.61 (0.194)	3.93 (0.679)	37.2 (1.68)	35.1 (1.51)
33	short-term	R	0.449 (0.17)	0.955 (0.139)	5.07 (1.26)	40.5 (1.47)	38.3 (1.26)

Table S4. Results of an Analysis of Covariance for each metabolic trait with interactions between selection temperature, exposure (short- or long-term warming) and metabolic flux (*P* or *R*). Parameters included in the most parsimonious model are highlighted in bold.

Parameter	Effect	d.f.	F value	P value
$b(T_c)$	selection temperature	1,52	13.7	**< 0.001**
	metabolic flux	1,52	274.58	**< 0.001**
	exposure	1,52	40.9	**< 0.001**
	selection temperature*metabolic flux	1,52	0.198	0.65
	selection temperature*exposure	1,52	6.93	**< 0.05**
	metabolic flux*exposure	1,52	2.38	0.13
	selection temperature*metabolic flux*exposure	1,52	1.36	0.25
E_a	selection temperature	1,52	2.44	0.11
	metabolic flux	1,52	56.8	**< 0.001**
	exposure	1,52	7.29	**< 0.01**
	selection temperature*metabolic flux	1,52	0.0004	0.98
	selection temperature*exposure	1,52	0.024	0.88
	metabolic flux*exposure	1,52	4.8	**< 0.05**
	selection temperature*metabolic flux*exposure	1,52	0.91	0.34
E_h	selection temperature	1,52	4.71	**< 0.05**
	metabolic flux	1,52	17.5	**< 0.001**
	exposure	1,52	1.78	0.19
	selection temperature*metabolic flux	1,52	0.42	0.52
	selection temperature*exposure	1,52	0.09	0.76
	metabolic flux*exposure	1,52	0.39	0.53
	selection temperature*metabolic flux*exposure	1,52	0.002	0.96
T_h	selection temperature	1,52	0.15	0.34
	metabolic flux	1,52	36.4	**< 0.001**
	exposure	1,52	4.73	**< 0.05**
	selection temperature*metabolic flux	1,52	0.335	0.57
	selection temperature*exposure	1,52	0.211	0.65
	metabolic flux*exposure	1,52	1.38	0.25
	selection temperature*metabolic flux*exposure	1,52	0.23	0.64
T_{opt}	selection temperature	1,52	2.23	0.133
	metabolic flux	1,52	28.8	**< 0.001**
	exposure	1,52	2.75	0.10
	selection temperature*metabolic flux	1,52	0.04	0.85
	selection temperature*exposure	1,52	0.02	0.88
	metabolic flux*exposure	1,52	5.81	**< 0.05**
	selection temperature*metabolic flux*exposure	1,52	0.0001	0.99