

Life history adaptation under climate warming magnifies the agricultural footprint of a cosmopolitan insect pest

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Predictions of how thermal adaptation via increased resource acquisition affects the agricultural impact of insect pests based on protein biophysics.

Biological rates of ectotherms show an empirically well-described relationship with temperature that closely mirrors the thermodynamic performance of enzymes (Hochachka and Somero 2002; Angilletta 2009), because biological rates are governed at the biochemical level by the enzymatic reaction rate, k_{cat} (Evans and Polanyi 1935; Eyring 1935):

$$k_{cat} \propto A_B e^{-E_a/RT} \quad (\text{Eq. S1})$$

where E_a is the activation energy required for the enzymatic reaction to occur, R is the universal gas constant ($0.002 \text{ kcal mol}^{-1}$), T is temperature in Kelvin and A_B is a species and rate specific constant for enzyme catalysis (Clarke 2004; Dell et al. 2011). Equation 1 thus describes an increase in reaction rate with temperature (**Fig. 1A**, main text). The observed decline in biological rate at temperatures exceeding the organism's thermal optimum is attributed to a reduction in the proportion of functional enzyme due to reversible inactivation via protein unfolding (Bloom et al. 2005; DePristo et al. 2005; Bershtein et al. 2017; Echave and Wilke 2017; Agozzino and Dill 2018) (**Fig. 1A**). The proportion of folded protein ready to do work in a biological process follows a Boltzmann probability as a function of the Gibbs free energy of folding, ΔG , which is thus a measure of protein stability (DePristo et al. 2005):

$$P_{\text{fold}} = \prod_{i=1}^n \frac{1}{1 + A_{D,i} e^{\Delta G_i(T)/RT}} \quad (\text{Eq. S2})$$

Where constant A_D and stability, ΔG , is given for each of a set of n proteins that act in sequence and contribute multiplicatively to a biological rate (Chen and Shakhnovich 2010; Echave and Wilke 2017). At a benign temperature of 25°C , most natural proteins occur in functional (properly folded) state and the mean value of the Gibbs energy is negative (mean $\Delta G \sim -7 \text{ kcal mol}^{-1}$; (DePristo et al. 2005; Chen and Shakhnovich 2009). However, ΔG is comprised of an enthalpy term (ΔH) and a temperature-dependent entropy term (ΔS) (Eyring and Polanyi 2013) that reduces to: $\Delta G(T) = \Delta H - T\Delta S$ over the ecologically relevant temperature range of most organisms (Chen and Shakhnovich 2010). Based on values from the literature (Chen and Shakhnovich 2010; Dill et al. 2011), $\Delta S \sim -0.25$ to $-0.50 \text{ kcal/mol K}^{-1}$ for a protein of typical length. From equation S2 it is therefore clear that warm temperatures increase entropy making ΔG less negative, leading to a rapid non-linear reduction in the fraction of functional protein (**Fig. 1A**).

The reaction rate kinetics of equation S1 have been combined with the protein folding stability of equation S2 to describe temperature-dependent biological rates and even fitness in simple scenarios (Bershtein et al. 2017; Echave and Wilke 2017). We here place these biophysical predictions into a life history framework incorporating acquisition and allocation trade-offs (Jong and Noordwijk 1992) to predict the temperature dependence of population growth rate:

$$r(T) \propto (1 - c)^M * M^b * k_{cat} * P_{\text{fold}} \quad (\text{Eq. S3})$$

where M is accumulated energy reserves, c is the survival cost of acquiring energy, and b is the allometric exponent for how growth and reproduction scale with M .

While there is variation in biological rates across organisms and for different traits (Clarke 2004; Angilletta 2009; Huey and Kingsolver 2011), inherent enzyme properties governing the temperature-dependence of protein stability and reaction rate have been shown to evolve slowly (Dill et al. 2011). Instead, thermal adaptation often proceeds via costly compensatory responses involving upregulation of molecular chaperones such as heat-shock proteins that aid protein folding at hot temperature (Feder et al. 2000), and enzyme catalysts that allow reactions to take place at cold temperature despite thermodynamic constraints (Hochachka and Somero 2002). Here we illustrate how such thermal compensation via increased energy acquisition can affect growth rates of ectotherm pests facing changing climates (see **Fig. 1C**, main text). Assume that the energy reserves, M , of an organism can be allocated with fraction p to produce molecules that increase enzyme reaction rate, and with fraction q to molecules that aid protein folding stability, leaving $M(1-p-q)$ reserves for growth and reproduction. Inserting these expressions into equation S3 gives:

$$r'(T) = (1 - c)^M * [M(1 - p - q)]^b * pMA_B e^{-\frac{Ea}{RT}} * \prod_1^i \frac{1}{1 + \frac{A_D}{qM} e^{\Delta G_i(T)/RT}} \quad (\text{Eq. S4})$$

We can find the optimal strategy of energy acquisition (increasing M at cost c) and allocation (of M to p and q) at different temperatures and for different costs of growth by numerically solving for the combination of M , p and q that maximize r' .

Eq. S4 is well-supported in its qualitative sense. Eqs. S1 and S2 have received strong empirical support from in vitro measures of enzymes (Dill et al. 2011; Echave and Wilke 2017; Agozzino and Dill 2018) and recapitulates the asymmetry of empirically estimated thermal reaction norms of quantitative traits (Angilletta 2009). Thermal compensation via molecular chaperones is also well-understood (Hochachka and Somero 2002) and has been shown to trade-off with growth and reproduction (Feder et al. 2000). However, the quantitative solution to the multidimensional trade-off described by eq. S4 depends on the mechanistic basis of thermal sensitivity down to its finer details and exactly how energy reserves invested in thermal compensation affect folding stability and reaction rate, relationships that are not empirically well-defined nor completely understood (e.g. Eqs. S1 and S2 describe phenomenological rather than mechanistic relationships). Thus, our aim is only to provide qualitative predictions. We do this by using eq. S4 to illustrate how thermal niches can evolve via compensatory energy acquisition and allocation under a relatively high ($c = 0.9$) or low ($c = 0.8$) cost of energy acquisition, when an organism is adapting to a cold (23°C) or hot (35°C) temperature (**Fig. 1C**, main text). For other parameters we first assumed values that correspond to those commonly observed in the literature: $\Delta G_{T=298K} = -7 \text{ kcal mol}^{-1}$; $\Delta S \sim -0.25 \text{ kcal mol}^{-1} \text{ K}^{-1}$; $n = 100$ proteins; $b = 0.75$, $Ea = 20 \text{ kcal mol}^{-1}$ ($\sim 0.7\text{eV}$). A_B , and A_D were set to 1 for simplicity.

After finding the optimal strategy in the four scenarios, resulting thermal reaction norms for population growth rate were calculated and are depicted in **Fig. 1C** of the main text, along with the optimal energy acquisition strategy in each scenario. Cold-adapted populations outcompete hot-adapted population at cold temperature, but growth rates and the predicted

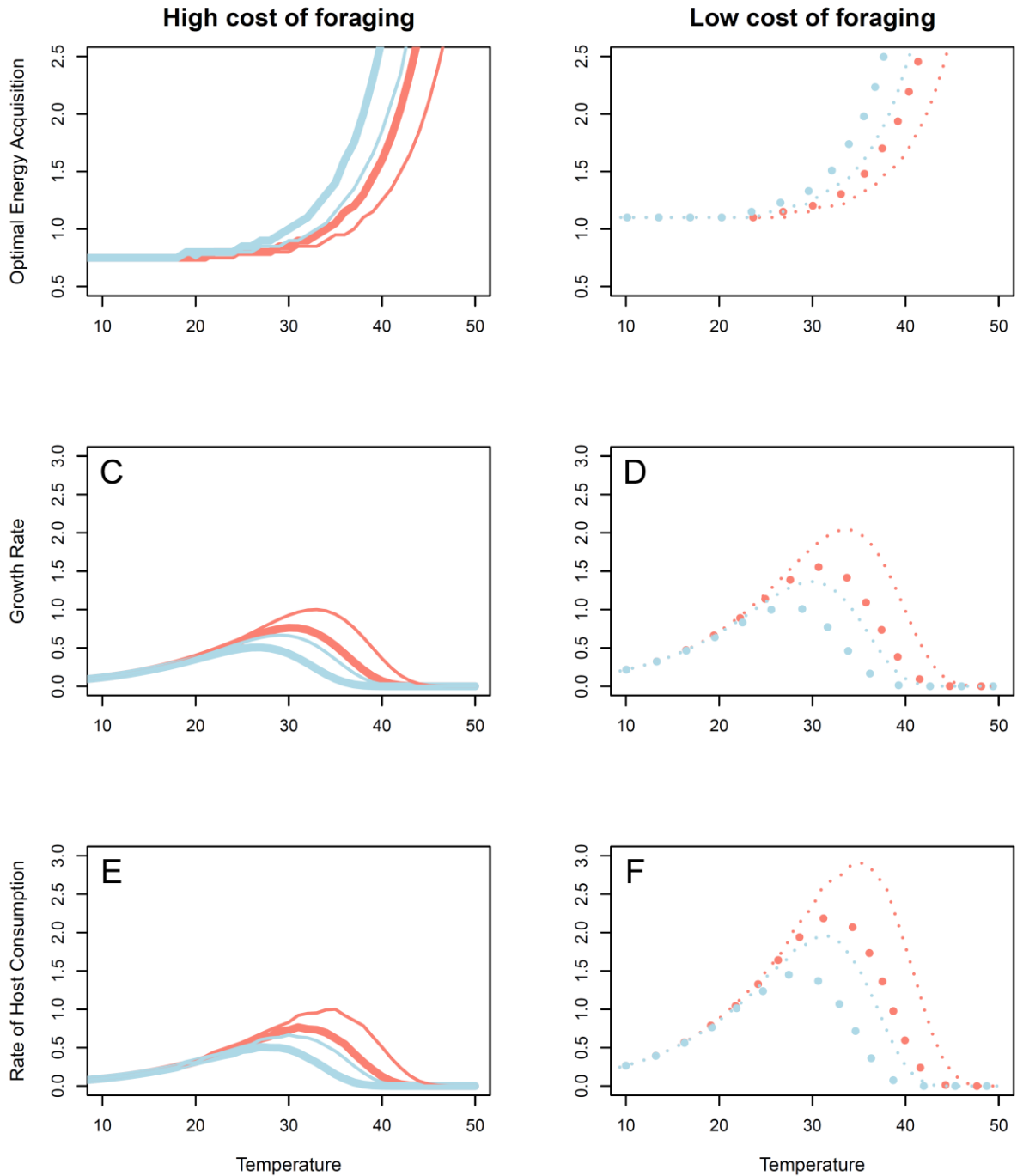
impact on agriculture are greater at warm temperatures and for the hot-adapted population due to reduced thermodynamic constraints on reaction rates (Gillooly et al. 2002; Brown et al. 2004). Lower costs of feeding favour increased energy acquisition and allocation to maintenance, which improves thermal performance and increases thermal niche breadth (**Fig. 1C**, main text). Increased feeding effort is particularly beneficial at hot temperature because there are increased demands on maintaining protein folding at these temperatures (**Fig. 1A**, main text). Thermal adaptation via increased energy acquisition mediates an even greater impact on the rate of host consumption (given by the product of r' and M – the latter assumed to be proportional to per capita host consumption) (**Fig. 1D**, main text, **Fig. C-F**).

To further illustrate how warming temperatures affect energy acquisition and the predicted impact on agriculture, we calculated the optimal life-history strategy (M' , p' and q') at each temperature under different assumptions of the thermal sensitivity of protein folding (Supplementary Note 1 Figure, below). We then calculated the associated population growth rate and rate of host consumption for each temperature and scenario. This confirmed that, if increased energy acquisition and allocation can compensate effects of temperature on molecular failure rates, greater thermal sensitivity favours increased foraging effort (**Fig. A, B**). Increased thermal sensitivity implies low fitness at high temperature per definition, so population growth rates remain low in this scenario (**Fig. C, D**) as thermal compensation via increased growth effort also comes at a cost (c). However, if costs of feeding are low, increased energy acquisition is more favourable, which increases population growth rate, and even more so, agricultural impact (**Fig. E, F**). In fact, the strategy to increase M at stressful temperature causes populations experiencing temperatures slightly hotter than those that maximize population growth rate to have the greatest agricultural impact (compare **Fig. C** vs. **E** & **Fig. D** vs. **F**).

We note that protein folding can also be compromised by acute cold stress (Feder et al. 2000; Hochachka and Somero 2002). However, such cold temperatures are typically not experienced during the active seasons of tropical and sub-tropical insects (Deutsch et al. 2008; Johansson et al. 2020), as the case for *C. maculatus* (Baur et al. 2023), and were therefore not modelled. Likewise, stressful temperatures affect organisms in a multitude of ways. Thus, our particular example using protein folding is merely meant to illustrate a general principle and was chosen based on the simple fact that the modelled relationships are empirically well-supported in qualitative sense (see above). Other physiological phenomena that scale with temperature, such as metabolic expenditure (Gillooly et al. 2001) and the production of harmful reactive oxygen species (Dowling and Simmons 2009), are likely to simultaneously contribute to increasing molecular failure rates at stressful temperature.

Finally, similar to previous models (Bershtein et al. 2017; Echave and Wilke 2017), we here assumed that growth and reproduction of complex organisms are proportional to the rate at which they generate functional enzyme, which is clearly simplistic considering the ecological complexity affecting the link between rates of biochemical reactions and population growth rate. Yet, this simplicity allows forming general predictions and is supported qualitatively by the fact that a range of different biological rates scale predictably with temperature (Brown et al. 2004; Angilletta 2009). R code used to produce predictions is available as Supplementary Code 1.

Optimal energy acquisition and its agricultural impact



In **A)** and **B)**, the optimal strategy of energy acquisition (M') at each temperature in a scenario of high ($c = 0.9$; left panels) or low ($c = 0.8$; right panels) costs of foraging. 100 (thin lines) or 300 (thick lines) multiplicatively acting proteins were assumed to contribute to growth and/or reproduction. These proteins were either relatively stable ($\Delta G_{T=298K} = -7$ kcal mol⁻¹; salmon) or less stable ($\Delta G_{T=298K} = -6$ kcal mol⁻¹; light blue). More numerous, and less stable, proteins contribute to increased thermal sensitivity and benefit increases in M' . In **C)** and **D)**, the resulting population growth rate, and in **E)** and **F)** the rate of host consumption, are shown at each temperature for each scenario. Note that these results represent optimal solutions to the multidimensional life-history trade-offs and could either stem from genetic adaptation or adaptive plasticity. Results in panels C-F are scaled by the maximum of the scenario depicted by thin and full lines in salmon colour ($\Delta G_{T=298K} = -7$ kcal mol⁻¹; $n = 100$, $c = 0.9$).

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Supplementary Data 1: Gene annotations for all heat stress and reproduction genes (see separate file). P-values are based on hypergeometric tests for over-representation and corrected for multiple testing using the Benjamini-Hochberg method (false discovery rates).

Supplementary Data 2: Gene ontology for all heat stress and reproduction genes (see separate file). P-values are based on hypergeometric tests for over-representation and corrected for multiple testing using the Benjamini-Hochberg method (false discovery rates).

Supplementary Table 1: Gene expression analyses

Supplementary Table 1a: Nested ANOVA of allocation response based on the 134 antagonistic genes (corresponds to Figure 3D of main text. The model was fit using the "aov" function and was specified as:

```
Allocation ~ Evolution.regime*temperature*Origin + Error(Population)
```

Error: Population

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regime	2	1320027	660013	0.122	0.8867
Temperature	2	140331406	70165703	12.972	0.0030 **
Origin	2	311216068	155608034	28.768	0.0002 ***
Regime:Origin	4	238677450	59669362	11.031	0.0024 **
Residuals	8	43272241	5409030		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temperature	2	4.3e+09	2.2e+09	262.982	1.63e-13 ***
Regime:Temperature	4	1.7e+07	4.2e+06	0.514	0.7264
Temperature:Origin	4	9.5e+07	2.4e+07	2.867	0.0553 .
Regime:Temperature:Origin	8	1.1e+08	1.4e+07	1.738	0.1610
Residuals	17	1.4e+08	8.3e+06		

Supplementary Table 1b: Nested ANOVA of allocation response based on all the 21 overlapping genes upregulated in response to reproduction. The model was fit using the "aov" function and was specified as:

Allocation ~ Evolution.regime*temperature*Origin + Error(Population)

Error: Population

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regime	2	251496	125748	11.964	0.00394 **
Temperature	2	23331	11666	1.110	0.37548
Origin	2	56967	28483	2.710	0.12629
Regime:Origin	4	115818	28954	2.755	0.10385
Residuals	8	84084	10510		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temperature	2	1117957	558978	72.545	4.74e-09 ***
Regime:Temperature	4	33218	8304	1.078	0.3983
Temperature:Origin	4	73571	18393	2.387	0.0918 .
Regime:Temperature:Origin	8	38745	4843	0.629	0.7433
Residuals	17	130990	7705		

Supplementary Table 1c: Nested ANOVA of allocation response based on all the 113 overlapping genes upregulated in response to heat shock. The model was fit using the "aov" function and was specified as:

Allocation ~ Evolution.regime*temperature*Origin + Error(Population)

Error: Population

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regime	2	2697138	1348569	0.251	0.783913
Temperature	2	137047700	68523850	12.755	0.003248 **
Origin	2	305102729	152551364	28.396	0.000232 ***
Regime:Origin	4	235845545	58961386	10.975	0.002475 **
Residuals	8	42979006	5372376		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temperature	2	4.21e+09	2.1e+09	258.02	1.91e-13 ***
Regime:Temperature	4	1.63e+07	4.08e+06	0.500	0.736
Temperature:Origin	4	9.14e+07	2.28e+07	2.800	0.059 .
Regime:Temperature:Origin	8	1.15e+08	1.44e+07	1.761	0.155
Residuals	17	1.39e+08	8.16e+06		

Supplementary Table 1d: Nested ANOVA of allocation to reproduction based on all the 1269 differentially expressed genes in response to mating. The model was fit using the "aov" function and was specified as:

```
Allocation ~ Evolution.regime*Temperature*Origin + Error(Population)
```

Error: Population

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regime	2	1.4e+10	6.8e+09	3.82	0.0685 .
Temperature	2	9.1e+09	4.5e+09	2.52	0.142
Origin	2	1.4e+10	6.8e+09	3.76	0.070 .
Regime:Origin	4	6.2e+09	1.5e+09	0.86	0.527
Residuals	8	1.4e+10	1.8e+09		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temperature	2	7.9e+10	3.6e+10	23.11	1.4e-05 ***
Regime:Temperature	4	4.8e+09	1.2e+09	0.69	0.61
Temperature:Origin	4	4.2e+09	1.1e+09	0.62	0.65
Regime:Temperature:Origin	8	7.8e+09	9.7e+08	0.57	0.79
Residuals	17	2.9e+10	1.7e+09		

Supplementary Table 1e: Nested ANOVA of allocation to stress response based on all the 765 differentially expressed genes in response to heat shock. The model was fit using the "aov" function and was specified as:

```
Allocation ~ Evolution.regime*temperature*Origin + Error(Population)
```

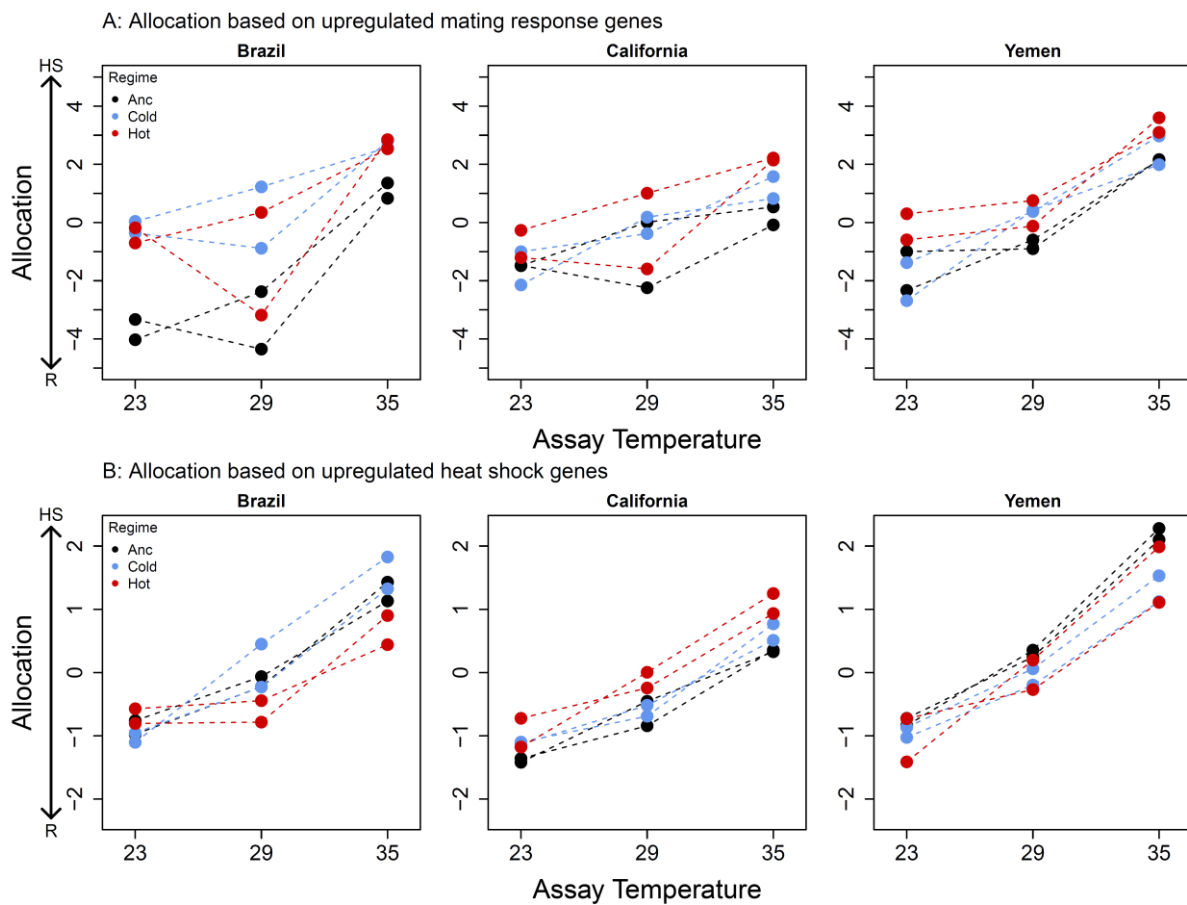
Error: Population

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regime	2	5563026	2781513	1.31	0.32080
Temperature	2	24185478	12092739	5.72	0.02872 *
Origin	2	93953143	46976571	22.21	0.00054 ***
Regime:Origin	4	50331286	12582822	5.95	0.01601 *
Residuals	8	16921967	2115246		

Error: Within

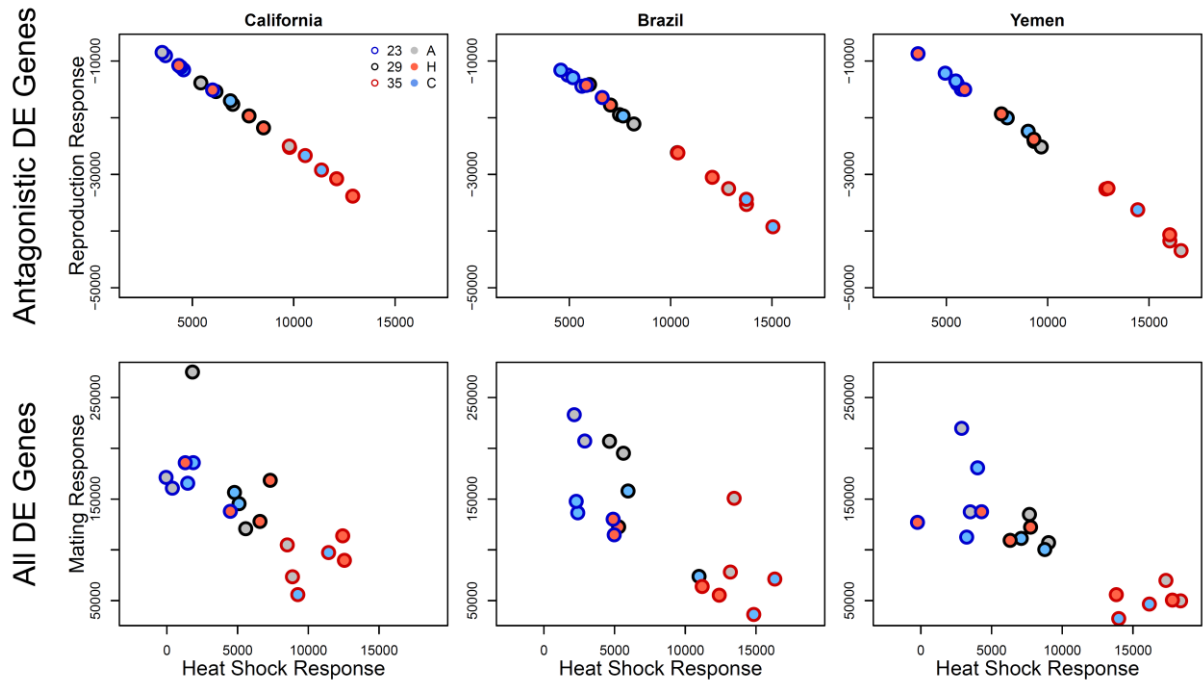
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temperature	2	1.08e+09	5.42e+08	196.21	1.8e-12 ***
Regime:Temperature	4	8.28e+06	2.07e+06	0.75	0.572
Temperature:Origin	4	3.24e+07	8.10e+06	2.94	0.052 .
Regime:Temperature:Origin	8	3.66e+07	4.58e+06	1.66	0.181
Residuals	17	4.69e+07	2.76e+06		

Supplementary Figure 1: Allocation trade-off between reproduction and heat stress response in experimental evolution lines based on overlapping genes.



A: allocation based on expression of the 21 genes upregulated in response to mating in hot-adapted (red), cold-adapted (blue) and ancestors (black), reared at 23, 29, or 35°C. B: the same patterns for 113 genes upregulated in response to heat shock. Allocation towards stress response increased with assay temperature for both sets of genes. There was not general effect of evolution regime across the three backgrounds when analyzing the 113 genes upregulated in response to heat shock, while ancestors showed significantly higher expression of the 21 genes upregulated in response to mating. Each line's gene expression was based on transcriptomic data from a single RNA library consisting of 10 pooled female abdomens.

Supplementary Figure 2: Allocation trade-off between reproduction and heat stress response in experimental evolution lines based on all significant genes.



Top panels: expression responses based on the 134 antagonistic genes in hot-adapted (red fill), cold-adapted (blue fill) and ancestors (grey fill), reared at 23 (blue border), 29 (black border) or 35°C (red border). Bottom panels: the same patterns for analyses based on all genes differentially expressed in response to heat stress (765 genes) and mating (1269 genes). There was not general effect of evolution regime across the three backgrounds in either analysis, but a strong effect of rearing temperature, with allocation towards stress response and away from reproduction at 35°C. Each line's gene expression was based on transcriptomic data from a single RNA library consisting of 10 pooled female abdomens.

Supplementary Table 2: univariate models of thermal plasticity

THERMAL PLASTICITY: in Ancestral founders

Supplementary Table 2a ### Lifetime Reproductive Success

offspring ~ temp * origin + experiment * temp + (1 | date)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
temp	33.5466	2	7.029	0.0002538	***
origin	9.5749	2	98.301	0.0001588	***
experiment	18.0062	2	7.672	0.0012656	**
temp:origin	2.5896	4	73.275	0.0435995	*
temp:experiment	14.2318	4	8.365	0.0008606	***

Supplementary Table 2b ### Development Time

dev ~ origin * temp

	Sum Sq	Df	F value	Pr(>F)	
origin	0.24	2	11.8464	0.003013	**
temp	1131.34	2	56919.7122	< 2.2e-16	***
origin:temp	0.06	4	1.5213	0.275500	
Residuals	0.09	9			

Supplementary Table 2c ### Metabolic Rate

log(co2) ~ origin * temp + log(aw.mean) + (1 | Date) + (1 | Tube)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
origin	2.0253	2	102.021	0.1372207	
temp	107.8867	2	3.456	0.0007681	***
log(aw.mean)	96.3264	1	93.599	4.745e-16	***
origin:temp	1.7845	4	93.323	0.1385070	

Supplementary Table 2d ### Adult Weight

aw.before ~ origin * temp2 + (1 | Date)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
origin	3.8808	2	103.965	0.0236895	*
temp	28.1112	2	2.744	0.0148681	*
origin:temp	6.2839	4	103.983	0.0001437	***

Supplementary Table 2e ### Early Fecundity

```
eggs ~ origin * temp2 + (1 | Tube) + (1 | Date)
```

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
origin	4.7965	2	90.591	0.010471	*
temp	8.2937	2	2.949	0.061544	.
origin:temp	4.1946	4	98.993	0.003522	**

Supplementary Table 2f *### Water loss ###*

```
log(wvp.corr) ~ origin * temp2 + (1 | Tube)
```

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
origin	1.3167	2	96.187	0.2728	
temp	262.1866	2	78.984	<2e-16	***
origin:temp	1.1731	4	87.864	0.3282	

Supplementary Table 2g *### Weigh Loss ###*

```
log(aw.loss) ~ origin * temp2 + log(aw.mean) + (1 | Tube) + (1 | Date)
```

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
origin	2.5414	2	91.487	0.08431	.
temp	8.3343	2	3.792	0.04093	*
log(aw.mean)	0.0008	1	100.813	0.97774	
origin:temp	2.0975	4	98.218	0.08687	.

Supplementary Table 3: univariate models of thermal adaptation

ADAPTATION: Evolution Regime Differences

Supplementary Table 3a ### Lifetime Reproductive Success

offspring ~ temp*origin*regime+experiment * temp + (1|pop)+(1| pop:temp)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
temp	22.9443	1	6.81	0.0021500	**
origin	0.7091	2	5.99	0.5291548	
regime	48.9568	1	6.23	0.0003605	***
experiment	8.7884	2	1023.46	0.0001643	***
temp:origin	2.0129	2	6.02	0.2141043	
temp:regime	1.2883	1	6.37	0.2972912	
origin:regime	0.6842	2	6.00	0.5399057	
temp:experiment	19.6974	2	1024.06	4.036e-09	***
temp:origin:regime	0.4912	2	6.02	0.6344217	

Supplementary Table 3b ### Development Time

devtime~temp*origin*regime + (1|pop) + (1|pop:temp)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
temp	8926.9130	1	6	9.472e-11	***
origin	8.4124	2	6	0.01817	*
regime	10.5487	1	6	0.01751	*
temp:origin	5.0516	2	6	0.05173	.
temp:regime	108.4421	1	6	4.595e-05	***
origin:regime	0.2305	2	6	0.80084	
temp:origin:regime	2.0679	2	6	0.20743	

Supplementary Table 3c ### Metabolic Rate

log(co2)~temp*origin*regime+log(aw)+ (1|pop)+(1|pop:temp)+(1|date)+(1|tube)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
temp	623.9250	1	12.55	4.435e-12	***
origin	1.0236	2	6.36	0.41175	
regime	1.8975	1	13.67	0.19050	
log(aw)	185.8547	1	328.30	2.2e-16	***
temp:origin	2.1438	2	6.50	0.19270	
temp:regime	1.1742	1	12.82	0.29850	
origin:regime	2.1009	2	6.54	0.19735	
temp:origin:regime	4.8510	2	7.16	0.04659	

Supplementary Table 3d ### Adult Weight

```
aw ~ temp2 * origin * regime + (1 | pop) + (1 | pop:temp)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
```

	F	Df	Df.res	Pr(>F)	
temp	4.5182	1	5.6231	0.080762	.
origin	13.2373	2	5.9595	0.006420	**
regime	70.9266	1	5.9626	0.000158	***
temp:origin	7.5042	2	5.8614	0.024192	*
temp:regime	11.5186	1	5.9896	0.014644	*
origin:regime	2.4900	2	6.0374	0.162713	
temp:origin:regime	3.7048	2	6.1139	0.088317	.

Supplementary Table 3e ### Early Fecundity

```
eggs~temp*origin*regime+egg.counter+(1|pop)+(1|pop:temp)+(1|date)+(1|tube)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
```

	F	Df	Df.res	Pr(>F)	
temp	78.7184	1	17.11	8.229e-08	***
origin	16.9281	2	6.07	0.003293	**
regime	11.5846	1	17.08	0.003362	**
egg.counter	35.4965	1	343.36	6.331e-09	***
temp:origin	0.5990	2	6.22	0.578234	
temp:regime	8.2913	1	17.14	0.010344	*
origin:regime	2.6100	2	7.32	0.139386	
temp:origin:regime	0.5344	2	7.37	0.607089	

Supplementary Table 3f ### Water loss

```
log(wvp)~temp*origin*regime+log(aw)+(1|pop)+(1|pop:temp)+(1|date)+(1|tube)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
```

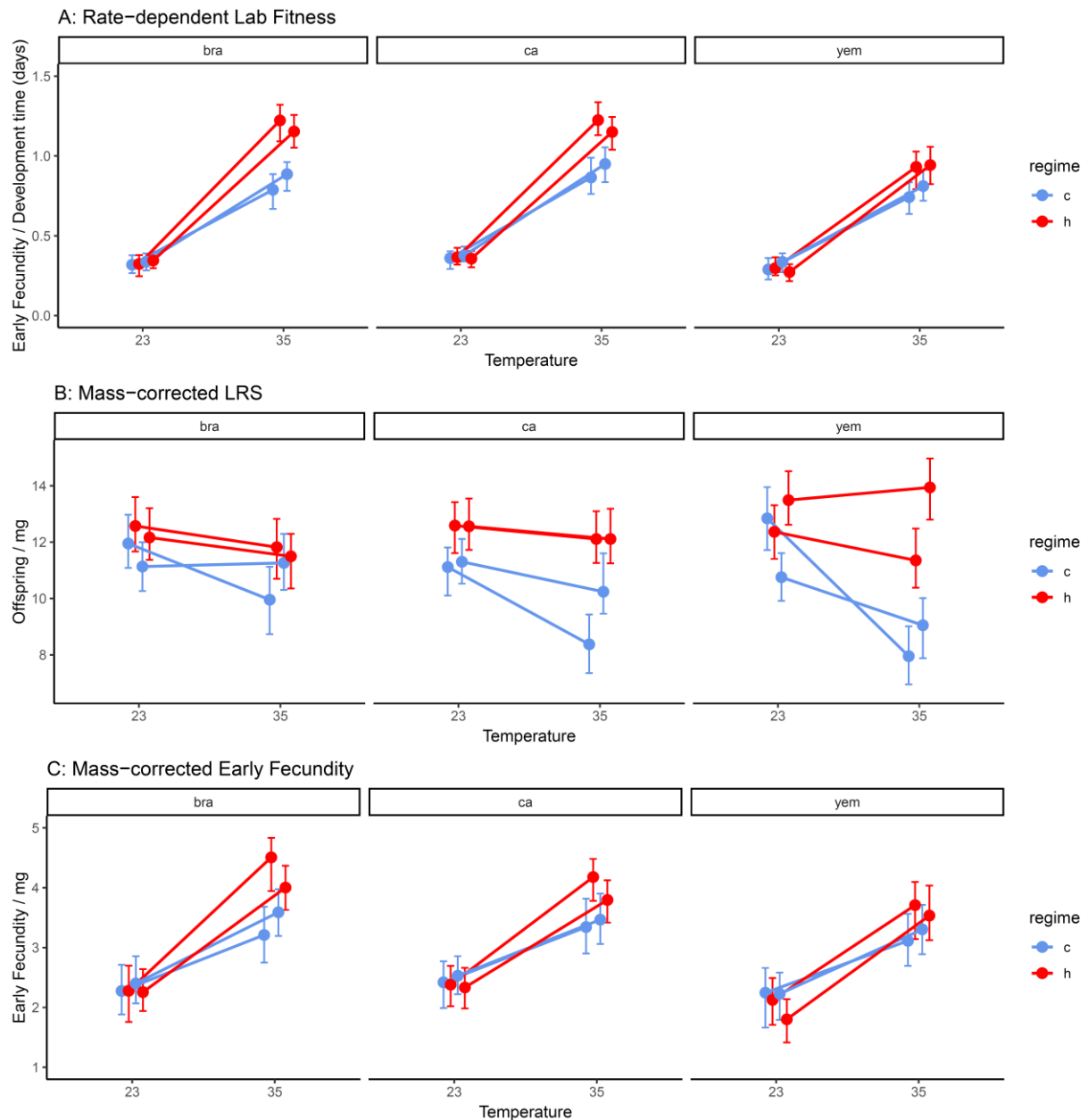
	F	Df	Df.res	Pr(>F)	
temp	941.0332	1	6.947	1.123e-08	***
origin	1.5900	2	5.752	0.28203	
regime	10.1036	1	8.310	0.01240	*
log(aw)	0.9647	1	289.272	0.32684	
temp:origin	0.2615	2	5.592	0.77884	
temp:regime	9.3107	1	7.250	0.01777	*
origin:regime	0.3662	2	5.751	0.70845	
temp:origin:regime	0.8626	2	5.827	0.46975	

Supplementary Table 3g ### Weight Loss

```
log(weight.loss+1e-04)~temp*origin*regime+log(aw.mean)+(1|pop)+(1|pop:temp)+(1|date)+(1|tube)
Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
```

	F	Df	Df.res	Pr(>F)	
temp	72.6328	1	18.35	8.47e-08	***
origin	10.3677	2	6.31	0.01012	*
regime	0.9370	1	19.04	0.34519	
log(aw.mean)	5.5838	1	359.36	0.01866	*
temp:origin	2.3715	2	6.26	0.17110	
temp:regime	7.3232	1	17.93	0.01450	*
origin:regime	0.3279	2	6.74	0.73130	
temp:origin:regime	1.1515	2	6.79	0.37102	

Supplementary Figure 3: Alternative estimates of laboratory fitness in experimental evolution lines



In **A**) as a proxy for fitness of each line at the conditions and two thermal conditions used in experimental evolution, we calculated a rate-dependent measure as early fecundity per female divided by development time (which corresponds roughly to generation time; mean time from start of egg laying until the emergence of reproductively mature adults) from Dataset 2. As eggs laid early should have an advantage due to being first to utilize the host resource, the calculation should give a reasonable estimate of laboratory fitness during experimental evolution. In **B**) LRS is shown corrected for mean body mass of females and in **C**) early fecundity corrected for mass is shown. All three measures show that hot-adapted lines outcompete cold-adapted lines at 35°C. Differences are less clear at 23°C, even after correcting for the larger size of females from hot-adapted lines. Whiskers represent 95% CIs based on parametric bootstrap.

Supplementary Table 4: Summary of models of host consumption in experimental evolution lines

Analyses of host consumption in hot and cold regime

Supplementary Table 4a ### Total host biomass consumed:

loss.ctrl1.fem ~ regime*origin*temp + (1|pop) + (1|temp:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
regime	22.8112	1	5.9715	0.003115	**
origin	0.5881	2	5.9551	0.584685	
temp	0.5359	2	11.7887	0.598759	
regime:origin	1.3612	2	5.9962	0.325530	
regime:temp	3.2465	2	11.8820	0.075016	.
origin:temp	0.8559	4	11.8547	0.517510	
regime:origin:temp	0.4674	4	11.9324	0.758747	

Supplementary Table 4b ### Consumed host biomass per beetle emerging:

cons.beetle ~ regime*origin*temp + (1|pop) + (1|temp2:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
regime	16.3913	1	5.9410	0.006877	**
origin	0.6981	2	5.9144	0.534344	
temp	1.9469	2	11.5953	0.186625	
regime:origin	1.4082	2	5.9818	0.315383	
regime:temp	0.5502	2	11.7669	0.590976	
origin:temp	0.6558	4	11.7140	0.634330	
regime:origin:temp	0.6467	4	11.8705	0.639931	

Supplementary Table 4c ### Consumed host biomass per egg laid:

cons.egg ~ regime*origin*temp + (1|pop) + (1|temp:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
regime	15.2788	1	5.9118	0.008137	**
origin	0.0864	2	5.8858	0.918403	
temp	34.6239	2	11.4288	1.412e-05	***
regime:origin	0.4961	2	5.9513	0.632026	
regime:temp	0.0631	2	11.6719	0.939182	
origin:temp	0.9797	4	11.5938	0.455681	
regime:origin:temp	3.3172	4	11.8327	0.048229	*

Supplementary Table 4d ### Consumed host biomass per beetle biomass:

cons.biomass ~ regime*origin*temp + (1|pop) + (1|temp:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)	
regime	0.5985	1	5.7284	0.469870	
origin	1.3658	2	5.6298	0.328431	
temp	10.5747	2	10.7792	0.002873	**
regime:origin	0.6121	2	5.7766	0.573975	
regime:temp	2.6759	2	11.0591	0.112765	
origin:temp	0.4299	4	10.8771	0.784332	
regime:origin:temp	1.0298	4	11.1742	0.433849	

Analyses of beetle traits in hot and cold regime

Supplementary Table 4e ### Minimum development time of emerging beetles:

NB.DAYS.DVLP ~ regime*origin*temp + (1|pop) + (1|temp:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
regime	0.9963	1	6	0.35675
origin	1.1980	2	6	0.36494
temp	754.7321	1	6	1.538e-07 ***
regime:origin	3.5293	2	6	0.09700 .
regime:temp	1.4842	1	6	0.26885
origin:temp2	1.3395	2	6	0.33040
regime:origin:temp	3.9220	2	6	0.08141 .

Supplementary Table 4f ### Mean body mass of emerging beetles:

beetle.size ~ regime*origin*temp + (1|pop) + (1|temp:pop)

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
regime	21.8705	1	5.8145	0.0036987 **
origin	2.6164	2	5.7760	0.1552477
temp	131.3228	1	4.7673	0.0001191 ***
regime:origin	0.5676	2	5.8428	0.5952821
regime:temp	7.8662	1	5.0982	0.0369797 *
origin:temp	12.4183	2	4.7663	0.0128767 *
regime:origin:temp	1.1448	2	5.1029	0.3884123

Supplementary Table 4g ### Model explaining host consumption from life history traits:

#fecundity ="eggs" #Mean adult mass ="beetle.size" #juvenile survival = "survival"

loss.ctrl ~ scale(beetle.size)+scale(survival)+scale(eggs)+temp

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.97480	0.01037	94.027	< 2e-16 ***
scale(beetle.size)	0.07982	0.01121	7.117	2.08e-10 ***
scale(survival)	0.10423	0.01117	9.331	4.45e-15 ***
scale(eggs)	0.41430	0.01309	31.641	< 2e-16 ***

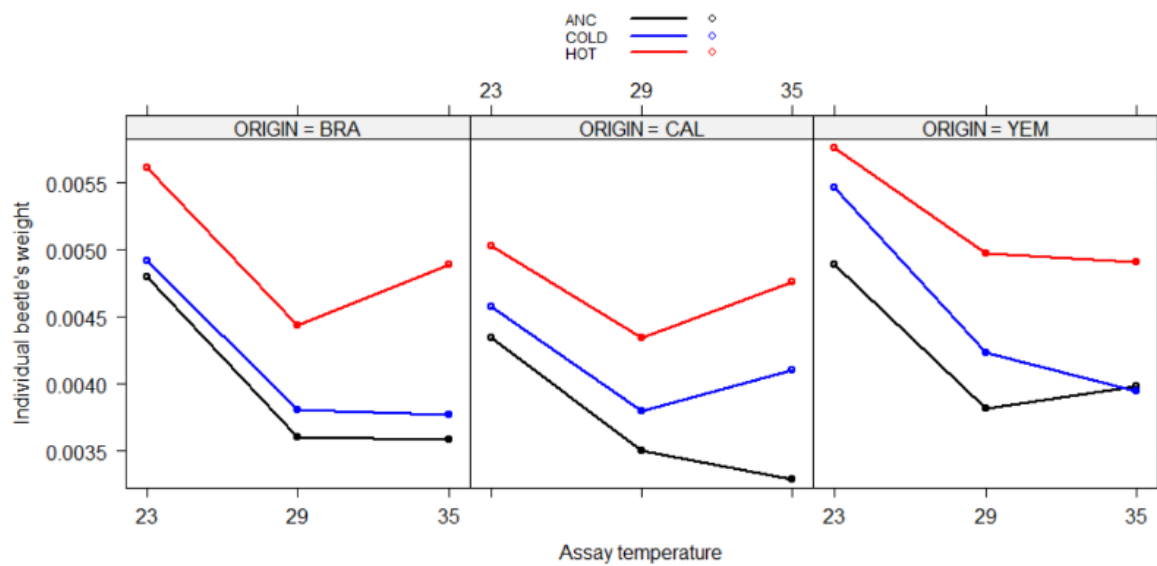
Residual standard error: 0.1031 on 95 degrees of freedom

(10 observations deleted due to missingness)

Multiple R-squared: 0.9273, Adjusted R-squared: 0.925

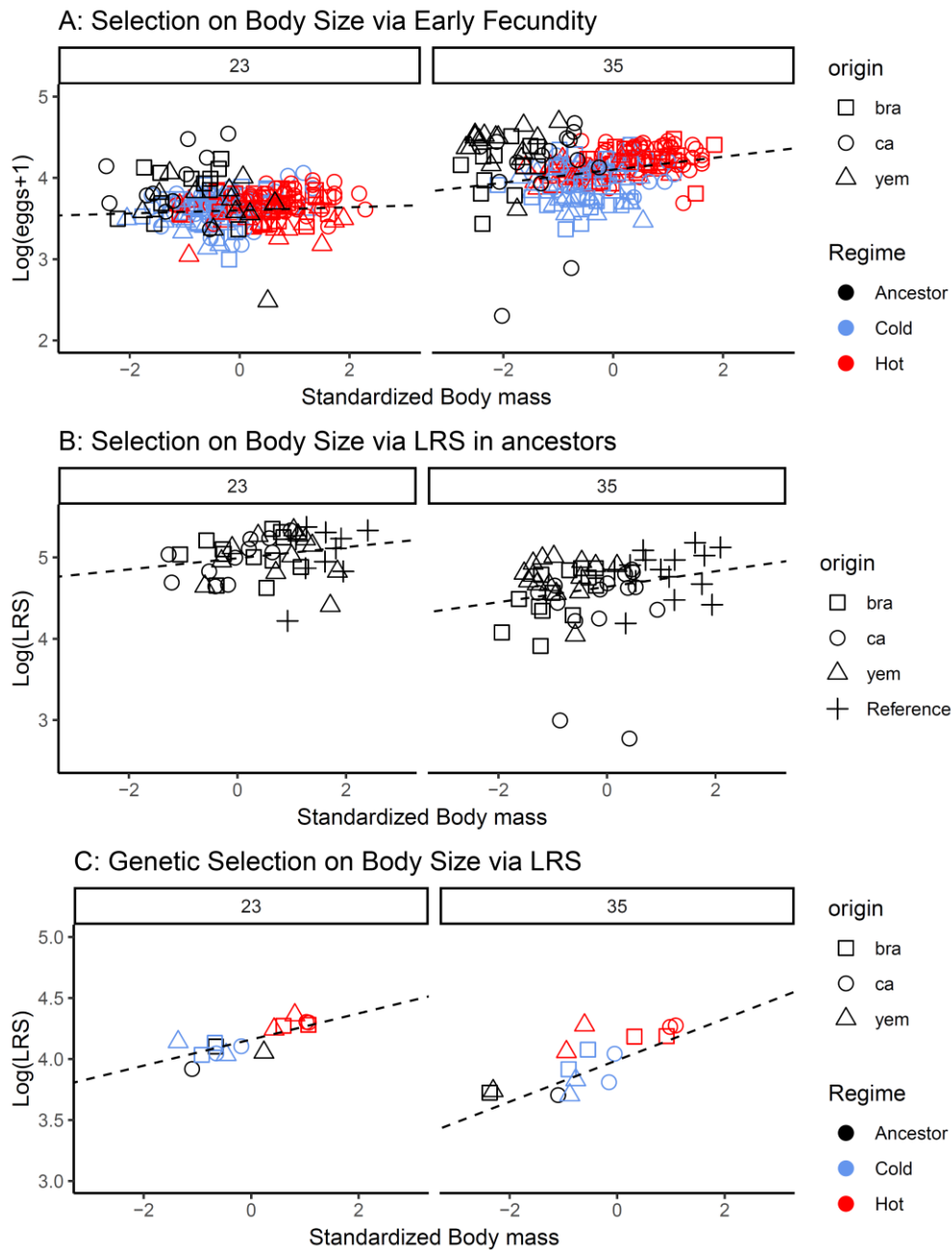
F-statistic: 403.7 on 3 and 95 DF, p-value: < 2.2e-16

Supplementary Figure 4: Mean body mass of beetles emerging from the assays of host consumption.



Shown a means for each regime on each genetic background. Note that the ancestors (black) are included for illustrative purposes but were not included in the statistical analyses of cold (blue) and hot (red) regimes, presented in the anova tables

Supplementary Figure 5: Selection on female body mass at different temperatures



A) Regression of logarithmized early fecundity on variance standardized body mass for each female trio from dataset 1 & 2. **B)** Regression of logarithmized lifetime reproductive success (number of adult offspring produced) and variance standardized body mass for female trios from the experiment on ancestral populations that measured both traits on the same individuals. **C)** Regression of logarithmized mean lifetime reproductive success on mean (variance standardized) body mass for each line used in the experiment. Selection gradients on size are given by the regression slopes. These slopes were estimated across all data. In panel A, this was done after first removing effects of evolution regime (cold/hot/ancestor). In each case the selection gradient is steeper at 35°C compared to 23°C.

Supplementary Table 5: Selection on adult body mass

Supplementary Table 5a

Selection on body mass (aw) via early fecundity in evolution lines ###
Corresponds to panel A of Figure S6

`log(eggs + 1) ~ temp*scale(aw)+selection*temp`

	Estimate	Std. Error	t value	Pr(> t)
(Intercept - ancestor)	3.82066	0.04461	85.636	< 2e-16 ***
temp35	0.47389	0.06821	6.948	1.29e-11 ***
scale(aw)	0.02080	0.02303	0.903	0.36694
regime.c	-0.20117	0.04993	-4.029	6.56e-05 ***
regime.h	-0.18576	0.05943	-3.126	0.00189 **
temp35:scale(aw)	0.05512	0.03186	1.730	0.08432 .
temp35:regime.c	-0.13462	0.07497	-1.796	0.07322 .
temp35:regime.h	0.04767	0.08700	0.548	0.58399

Residual standard error: 0.2475 on 456 degrees of freedom
Multiple R-squared: 0.496, Adjusted R-squared: 0.4882
F-statistic: 64.1 on 7 and 456 DF, p-value: < 2.2e-16

Anova Table (Type II tests)

	Sum Sq	Df	F value	Pr(>F)
temp	21.7727	1	355.4800	< 2.2e-16 ***
scale(aw)	0.5945	1	9.7067	0.001952 **
regime	3.6228	2	29.5747	8.38e-13 ***
temp:scale(aw)	0.1833	1	2.9927	0.084316 .
temp:regime	0.7136	2	5.8252	0.003176 **
Residuals	27.9294	456		

Supplementary Table 5b

#at 23C:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	3.82377	0.04125	92.707	< 2e-16 ***
scale(aw)	0.01923	0.01912	1.006	0.315681
reimge.c	-0.20117	0.04484	-4.486	1.15e-05 ***
regime.h	-0.18576	0.05337	-3.480	0.000601 ***

Residual standard error: 0.2223 on 226 degrees of freedom
Multiple R-squared: 0.08268, Adjusted R-squared: 0.0705
F-statistic: 6.79 on 3 and 226 DF, p-value: 0.0002109

Supplementary Table 5c

#at 35C:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.28339	0.05393	79.420	< 2e-16 ***
scale(aw)	0.07973	0.02523	3.160	0.00179 **
regime.c	-0.33578	0.06101	-5.504	9.9e-08 ***
regime.h	-0.13808	0.06932	-1.992	0.04755 *

Residual standard error: 0.27 on 230 degrees of freedom
Multiple R-squared: 0.1872, Adjusted R-squared: 0.1765
F-statistic: 17.65 on 3 and 230 DF, p-value: 2.396e-10

Supplementary Table 5d

Selection on body mass (aw) via LRS in ancestors ###
Corresponds to panel B of Figure S6

`log(total.fecundity+1) ~ temp *scale(aw.before)`

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	4.98807	0.06024	82.809	< 2e-16	***
temp35	-0.34478	0.07855	-4.389	2.85e-05	***
scale(aw.before)	0.06846	0.06275	1.091	0.278	
temp35:scale(aw.before)	0.02664	0.07984	0.334	0.739	

Residual standard error: 0.3645 on 99 degrees of freedom
Multiple R-squared: 0.2666, Adjusted R-squared: 0.2443
F-statistic: 11.99 on 3 and 99 DF, p-value: 9.177e-07

Anova Table (Type II tests)

Response: `log(total.fecundity + 1)`

	Sum Sq	Df	F value	Pr(>F)	
temp	2.5541	1	19.2210	2.905e-05	***
scale(aw.before)	0.6367	1	4.7919	0.03094	*
temp:scale(aw.before)	0.0148	1	0.1114	0.73928	
Residuals	13.1551	99			

Supplementary Table 5e

#at 23C:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	5.01642	0.03976	126.179	<2e-16	***
scale(aw.before)	0.05996	0.04021	1.491	0.143	

Residual standard error: 0.2667 on 43 degrees of freedom
Multiple R-squared: 0.04917, Adjusted R-squared: 0.02706
F-statistic: 2.224 on 1 and 43 DF, p-value: 0.1432

Supplementary Table 5f

#at 35C:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	4.61273	0.05575	82.733	<2e-16	***
scale(aw.before)	0.09304	0.05624	1.654	0.104	

Residual standard error: 0.4246 on 56 degrees of freedom
Multiple R-squared: 0.04659, Adjusted R-squared: 0.02956
F-statistic: 2.736 on 1 and 56 DF, p-value: 0.1037

Supplementary Table 5g

Genetic selection on body mass (aw) via LRS ###
Corresponds to panel C of Figure S6

log(LRS) ~ temp*scale(aw)

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.12760	0.03152	130.946	< 2e-16 ***
temp35	-0.10609	0.04432	-2.394	0.02418 *
scale(aw)	0.12201	0.03590	3.398	0.00219 **
temp235:scale(aw)	0.03371	0.04608	0.731	0.47107

Residual standard error: 0.118 on 26 degrees of freedom
Multiple R-squared: 0.6828, Adjusted R-squared: 0.6462
F-statistic: 18.65 on 3 and 26 DF, p-value: 1.158e-06

Anova Table (Type II tests)

Response: log(LRS)

	Sum Sq	Df	F value	Pr(>F)
temp	0.07752	1	5.570	0.02605 *
scale(aw)	0.55746	1	40.057	1.059e-06 ***
temp:scale(aw)	0.00744	1	0.535	0.47107
Residuals	0.36183	26		

Supplementary Table 5h

#At 23C:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.15518	0.01961	211.93	< 2e-16 ***
scale(aw)	0.10715	0.02029	5.28	0.000149 ***

Residual standard error: 0.07593 on 13 degrees of freedom
Multiple R-squared: 0.682, Adjusted R-squared: 0.6575
F-statistic: 27.88 on 1 and 13 DF, p-value: 0.000149

Supplementary Table 5i

#At 35C:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	3.98632	0.03836	103.93	< 2e-16 ***
scale(aw)	0.16991	0.03970	4.28	0.000896 ***

Residual standard error: 0.1485 on 13 degrees of freedom
Multiple R-squared: 0.5849, Adjusted R-squared: 0.5529
F-statistic: 18.32 on 1 and 13 DF, p-value: 0.0008964