Supplemental Material

Temperature-dependence of spontaneous mutation rates

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Mutation rate estimates

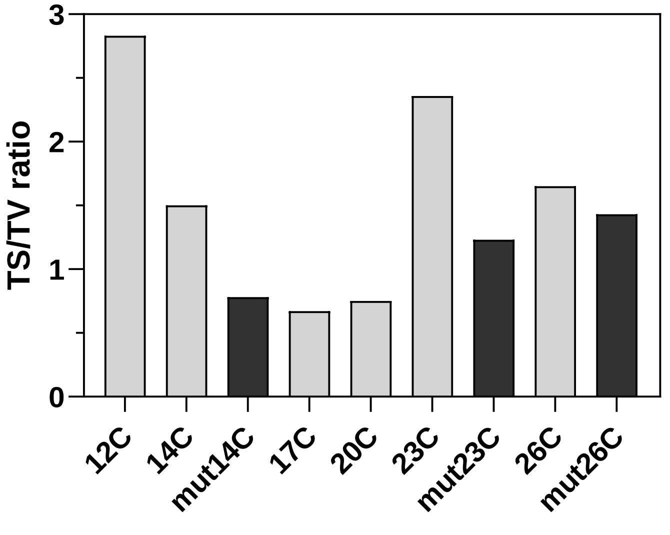
Supplemental Table S1: Outline of number of callable sites and mutation rates previously estimated for this species at 20°C on the basis of an older version of the draft genome (Oppold and Pfenninger 2017) in comparison to the rates reported at the same temperature in this study.

	Oppold & Pfenninger 2017	Present study (20°C)
n° callable sites	1.23E08	1.27E08
µ total	4.15E-09 (3.13E-09 to 0.54E-08)	4.20E-09 (3.12E-09 to 5.45E-09)
µSNM	2.12E-09 (1.39E-09 to 3.03E-09)	3.36E-09 (2.43E-09 to 4.47E-09)
µSNI	2.13E-09 (na)	8.55E-10 (2.65E-10 to 1.39E-09)

Mutator lines

Supplemental Table S2: Details of the identified mutator lines with number of detected de novo mutations, resulting total mutation rates (µtotal) and the fold increase when compared to the mean mutation rates of the remaining MAL at the same temperature.

temp	MA line ID	mutations	µtotal	fold increase to mean rate of temp
14	B1 (mutator)	49	9.32E-08	20
23	D3 (mutator)	42258	1.29E-04	9769
23	D4 (mutator)	34319	9.71E-05	7358
23	D8 (mutator)	29304	7.73E-05	5856
26	E2-11 (mutator)	18	1.04E-07	4

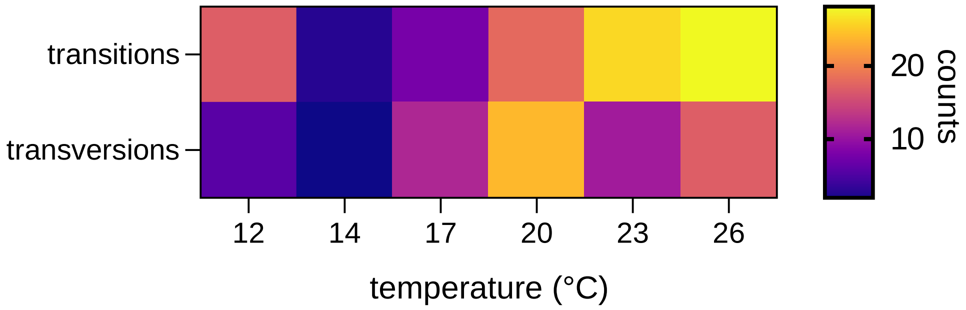


Supplemental Figure S1: Ratios between transition (TS) and transversions (TV) based on summed up numbers per temperature. Ratios of mutator lines (‘mut’) highlighted in dark.

Temperature-dependence of mutation rates

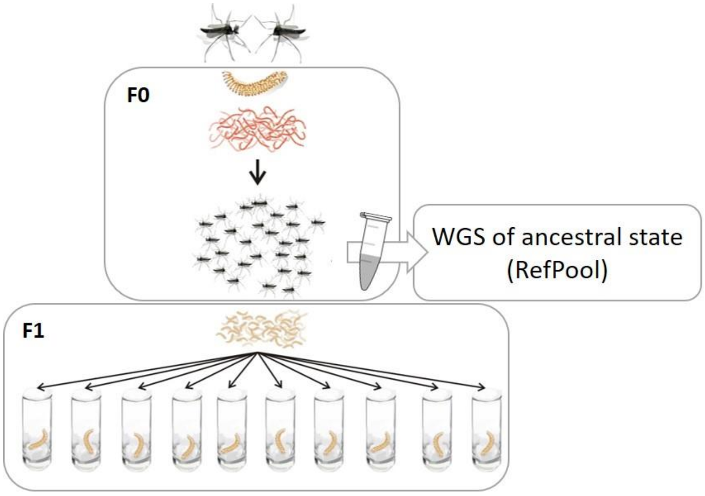
Supplemental Table S3: Test statistics of linear regression vs second order polynomial regression model to explain the observed relation between mutation rates and temperature. Akaike information criterion (AIC) to compare model fit.

	μ_{total}	μ_{SNM}	μ_{SNI}
linear	$r^2 = 0.28$ AIC = 8	$r^2 = 0.30$ AIC =8	$r^2 = 0.002$ AIC = 8
2nd order polynomial	$r^2 = 0.98$ AIC =6	$r^2 = 0.99$ AIC =6	$r^2 = 0.73$ AIC =6



Supplemental Figure S2: Heatmap of detected transitions versus transversions per experimental temperature. Due to the low number of SNMs per MAL it is not possible to use replicate ratios for statistical testing of the transition-transversion ratios.

Supplemental Methods



Supplementary Figure S3: Detailed overview of the onset of the mutation accumulation experiment using a single egg clutch. To represent the ancestral parents the offspring was whole genome sequenced (WGS) as pooled sample (RefPool).