

Figure S1: The percentage of activated sperm that have spikes is not significantly increased at 27°C. *In vitro* sperm activation using two different activators was measured for males raised at 20°C or 27°C. Activated sperm were scored as having spikes or pseudopods.

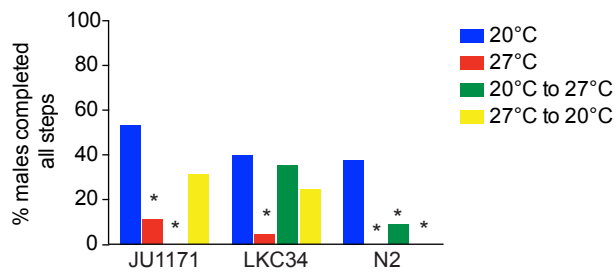


Figure S2: Males raised 27°C continuously fail to complete mating more often than males raised at 20°C. As each step in the mating assay can result in the loss of a male from the mating process, we calculated the percentage of males that completed all steps successfully. LKC34 was the most temperature insensitive in this assay only showing a decreased percentage of males completing mating when males were raised continuously at 27°C. N2 was the most temperature sensitive showing decreased percentage of males completing mating with any exposure to elevated temperature ($p < 0.05$, Fisher's exact test).

Table S1: Percentage of activated sperm

	% round spermatids (n)	% sperm w/ pseudopods (n)	% sperm w/ spikes(n)	Total Sperm
JU1171				
SB 20°C	86.9 (932)	12.5 (134)	0.6 (7)	1073
SB 27°C	85.9 (201)	13.7 (32)	0.4 (1)	234
Pronase 20°C	20.5 (102)	77.5 (386)	2.0 (10)	498
Pronase 27°C	29.4 (89)	68.6 (208)	2.0 (6)	303
Zinc 20°C	20.0 (219)	62.8 (688)	17.2 (188)	1095
Zinc 27°C	40.8 (361)	50.9 (450)	8.3 (73)	884
LKC34				
SB 20°C	84.4 (562)	13.5 (90)	2.1 (14)	666
SB 27°C	81.9 (730)	17.3 (154)	0.8 (7)	891
Pronase 20°C	14.6 (64)	84.9 (372)	0.5 (2)	438
Pronase 27°C	13.7 (106)	85.6 (661)	0.7 (5)	772
Zinc 20°C	26.7 (336)	69.2 (871)	4.1 (51)	1258
Zinc 27°C	44.3 (166)	48.0 (180)	7.7 (29)	375
N2				
SB 20°C	82.2 (619)	17.1 (129)	0.7 (5)	753
SB 27°C	85.2 (621)	13.4 (98)	1.4 (39)	728
Pronase 20°C	23.3 (107)	75.2 (346)	1.5 (7)	460
Pronase 27°C	26.9 (319)	69.8 (828)	3.9 (39)	1186
Zinc 20°C	22.3 (236)	76.3 (806)	1.4 (15)	1057
Zinc 27°C	46.4 (510)	47.27 (520)	6.36 (70)	1100

Table S2: Percentage of males that completed mating versus transferred sperm

Strain and assay	% males in each temperature treatment			
	20°C (n)	27°C (n)	upshift (n)	downshift (n)
JU1171				
Completed Mating**	53.3 (24)	11.1 (5)	0.0 (0)	31.1 (14)
Inseminated	62.0 (31)	5.0 (2)	0.0 (0)	0.0* (0)
LKC34				
Completed Mating**	40.0 (24)	4.4 (2)	35.6 (16)	24.4 (11)
Inseminated	38.0 (19)	0.0 (0)	13.33* (4)	0.0* (0)
N2				
Completed Mating**	37.8 (17)	0.0 (0)	8.9 (4)	0.0 (0)
Inseminated***	46.0 (23)	5.0 (2)	3.33 (1)	0.0 (0)

* $p < 0.05$ Fisher's exact test between the number of male that completed mating and transferred sperm in a given strain at a given temperature

** Total number of males scored in mating experiments was 45 except for LKC34 at 20°C which was 60 males.

*** Total number of males scored in transfer experiments was 50.