

**Supplemental material for**

**Chronic exposure to warm temperature  
causes low sperm abundance and quality in  
*Drosophila melanogaster***

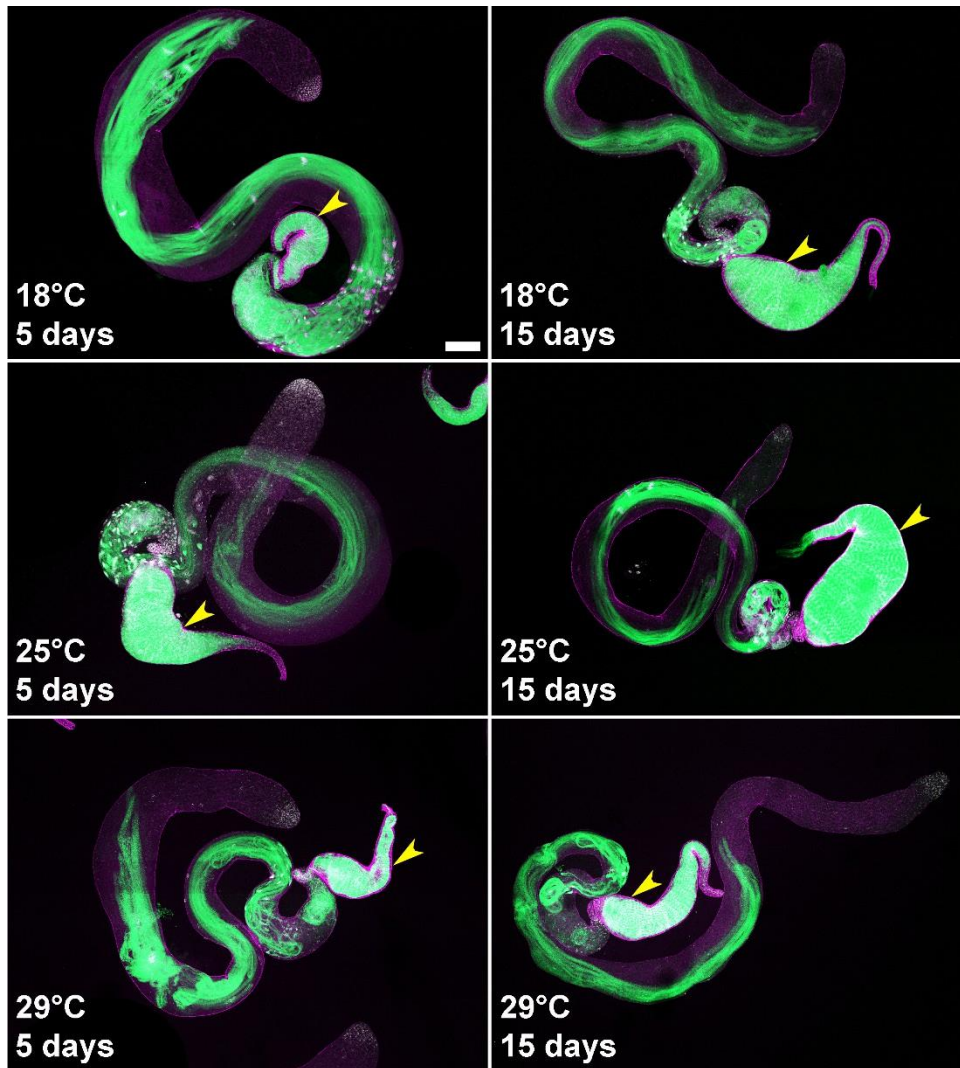
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SUPPLEMENTAL FIGURE



**Figure S1. Whole testes from males maintained at different temperatures.**

Representative whole testes images from *w\**; *ProtB-GFP*; *dj-GFP* males incubated at 18°C, 25°C, or 29°C for five or 15 days used for analysis shown in Fig. 3C,D.

Phalloidin (magenta), actin; GFP (green), Protamin B (labels germ cell nuclei starting in late canoe stage) and Don Juan (labels sperm tails); DAPI (white), nuclei.

## SUPPLEMENTAL TABLES

**Table S1. Numbers of spermatheca pairs analyzed for sperm motility**

Temperature	5 days*	10 days*	15 days*	20 days*
18°C	10	16	14	22
25°C	7	17	25	12
29°C	9	17	13	18

\*100% of sperm-containing spermathecae had motile sperm.

**Table S2. Numbers of eggs in different categories from 12-hour collection**

**testing effects of temperature on sperm quality**

Temperature	Dead*	Stage 1*	Stage 2+*	Unfertilized*	Total
25°C	45 (20) <sup>#</sup>	9 (4)	164 (73)	08 (3.5)	226
29°C	184 (66)	46 (16)	0 (0)	50 (18)	280

\*These categories are explained in the text and Fig. 6C, and these same data are graphed in Fig. 6D.

<sup>#</sup>Percentages are shown in parentheses.

## SUPPLEMENTAL MOVIE LEGENDS

**Movie S1. Representative spermatheca pair containing sperm from *w\**; *ProtB-GFP*; *dj-GFP* males maintained at 18°C for 20 days.** Sperm show normal motility in both spermathecae (arrows) and in seminal receptacle (asterisk). GFP (green), Protamin B (sperm nuclei) and Don Juan (sperm tails).

**Movie S2. Representative spermatheca pair containing sperm from *w\**; *ProtB-GFP*; *dj-GFP* males maintained at 25°C for 20 days.** Sperm show normal

motility in both spermathecae (arrows) and in seminal receptacle (asterisk). GFP (green), Protamin B (sperm nuclei) and Don Juan (sperm tails).

**Movie S3. Representative spermatheca pair with sperm from *w\**; *ProtB-GFP*; *dj-GFP* males maintained at 29°C for 20 days.** Sperm show normal motility in both spermathecae (arrows) and in seminal receptacle (asterisk). The spermatheca on the left has fewer than 10 spermatozoa. GFP (green), Protamin B (sperm nuclei) and Don Juan (sperm tails).