frontiers

Supplementary Material

Characterization of reproductive diapause in male *Drosophila melanogaster*

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Supplementary figures

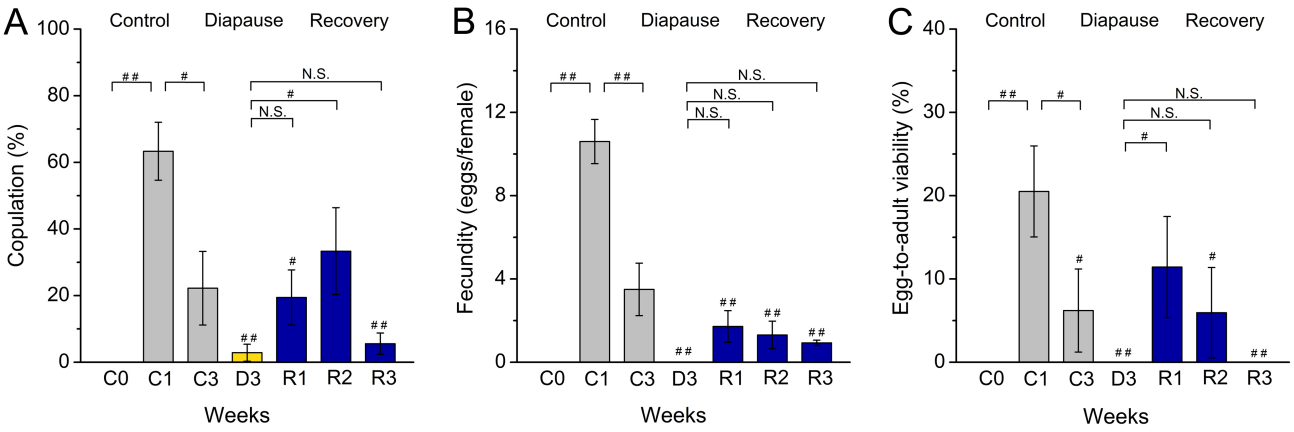

Fig. S1. Diapause conditions affect copulation, fecundity and egg to adult viability. All data are shown for crossing between identical partners (pairing virgin female - unmated male of the same condition). Newly eclosed flies (C0) of either sexe do not show any copulation behaviour and there is no oviposition or progeny. **A.** Three week diapausing flies (both, females and males) (D3) display strong reduction in copulation in comparison to the 1-week control flies, but copulation increases after recovery from diapause (R1) to the level of 3-week control (C3) flies. **B.** Fecundity after copulation of identical partners is negligible for diapausing females (D3) (crossed with diapausing males). Post-diapausing, recovered (R1-R3) females after crossing with recovered males (R1-R3) display lower fecundity than control flies (C1-C3). **C** Although female flies that recovered from diapause for 1-3 weeks (R1-R3) and then crossed with recovered male partners (R1-R3) lay fewer eggs than the controls (C1), the egg-to-adult viability of eggs produced by R1 females is similar to those of the control C1 flies. Data are shown as mean ± S.E.M , and average 6 independent replicates with 5-7 fly couples in each replicate, n = 30-42 fly couples (male and female). Data significantly different from the control C1 flies or between groups is indicated with $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (Kruskal–Wallis test followed by pairwise comparisons using Wilcoxon rank sum test). N.S. Not significant. See Fig 3 for the same parameters after crossing of C0-R3 flies with a control (C1) partner.

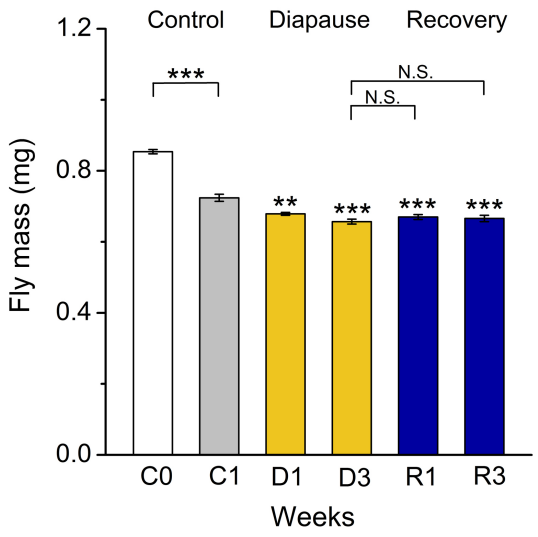

Fig. S2. The body mass of diapausing flies decreases. Fly wet mass (mg) of unmated males of *D. melanogaster,* including 3-6 h old flies (C0), and those kept in normal conditions for 1 or 3 weeks (C1 and C3), or in diapause conditions for 3 weeks (D3) and thereafter recovery for 1-3 weeks (R1-R3). Data significantly different from the controls (C1) are indicated with *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (ANOVA followed with Tukey test). Data represent 6 replicates with 6-15 flies in each replicate, n = 36-90 flies.

Supplementary table

Table S1. Primers for qPCR (all displayed 5'–3')

Primer	Sequence	Reference
rp49 F *rp49* R	ATC GGT TAC GGA TCG AAC AA GAC AAT CTC CTT GCG CTT CT	Kubrak et al., 2014
Akh F *Akh* R	AGA CCT CCA ACG AAA TGC TG GTG CTT GCA GTC CAG AAA GAG	Hentze et al., 2015
pepck F *pepck R*	TCA ATG GCG AAT CCT GCT AC TCC TTC ACG TCC ACC TTA TCC	Hentze et al., 2015
bmm F *bmm R*	GGT CCC TTC AGT CCC TCC TT GCT TGT GAG CAT CGT CTG GT	Hentze et al., 2015
NLaz F *NLaz R*	GGT GAA TGC GGC CAT CAA TC AAT GGC TGC GTC GGG TAA AA	This study
TotA F *TotA R*	AAT TCT TCA ACT GCT CTT ATG TGC TTT GGA GTC ATC GTC CTG GG	This study
soti	TCA GCG AGG CGA GTA CTT TG TCC GAG GTC CAA GAG AGG TT	This study
twe	GCA AAT AGA TCA GGA TGG CGA G CTC CTG CAC GGC TGA TTT TC	This study
Drs	GCA CTC TGA TTC AAA ACC GA GGT CTC GTT GTC CCA GAC	This study

Unless otherwise stated primers were designed using free online software Primer Blast, BLAST database from NIH, Bethesda, Maryland, USA (http:blast.ncbi.nlm.gov/Blast/cgi). For the rest of the primers the correspon-ding references are shown.

References to primers

Hentze, J.L., Carlsson, M.A., Kondo, S., Nässel, D.R., Rewitz, K.F. (2015) The neuropeptide allostatin A regulates metabolism and feeding decisions in *Drosophila*. Sci Rep 5,11680.

Kubrak, O.I., Kucerova, L., Theopold, U., and Nässel, D.R. (2014). The Sleeping Beauty: How Reproductive Diapause Affects Hormone Signaling, Metabolism, Immune Response and Somatic Maintenance in Drosophila melanogaster. PLoS ONE 9, e113051.