



**Figure S4** *fem* transcripts in male embryos treated with *Am-tra2* dsRNA-2. The male *fem* mRNAs of individuals 48 hours after egg-laying were studied using semiquantitative RT-PCR. Early embryos were injected with 33 pg of *Am-tra2* dsRNA-2 (lanes 1-7), 67 pg of *Am-tra2* dsRNA-2 (lanes 8-19) or ddH<sub>2</sub>O (lanes 20-22). The untreated male and female controls (labeled as n.i.) are shown in lanes 23-26 and 27-28, respectively. NC denotes our control PCR in which no cDNA was added (lane 29). Fragments were resolved by agarose gel electrophoresis and stained with ethidium bromide. The corresponding fragment of the male *fem* transcripts has a size of 458 bp. We used amplification of the cDNAs of the gene *elongation factor 1α* (*ef-1α*) as a relative control to semiquantify *csd* transcripts across embryonic samples.