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Figures and figure supplements

Dopamine drives *Drosophila sechellia* adaptation to its toxic host

Sofía Lavista-Llanos, et al.

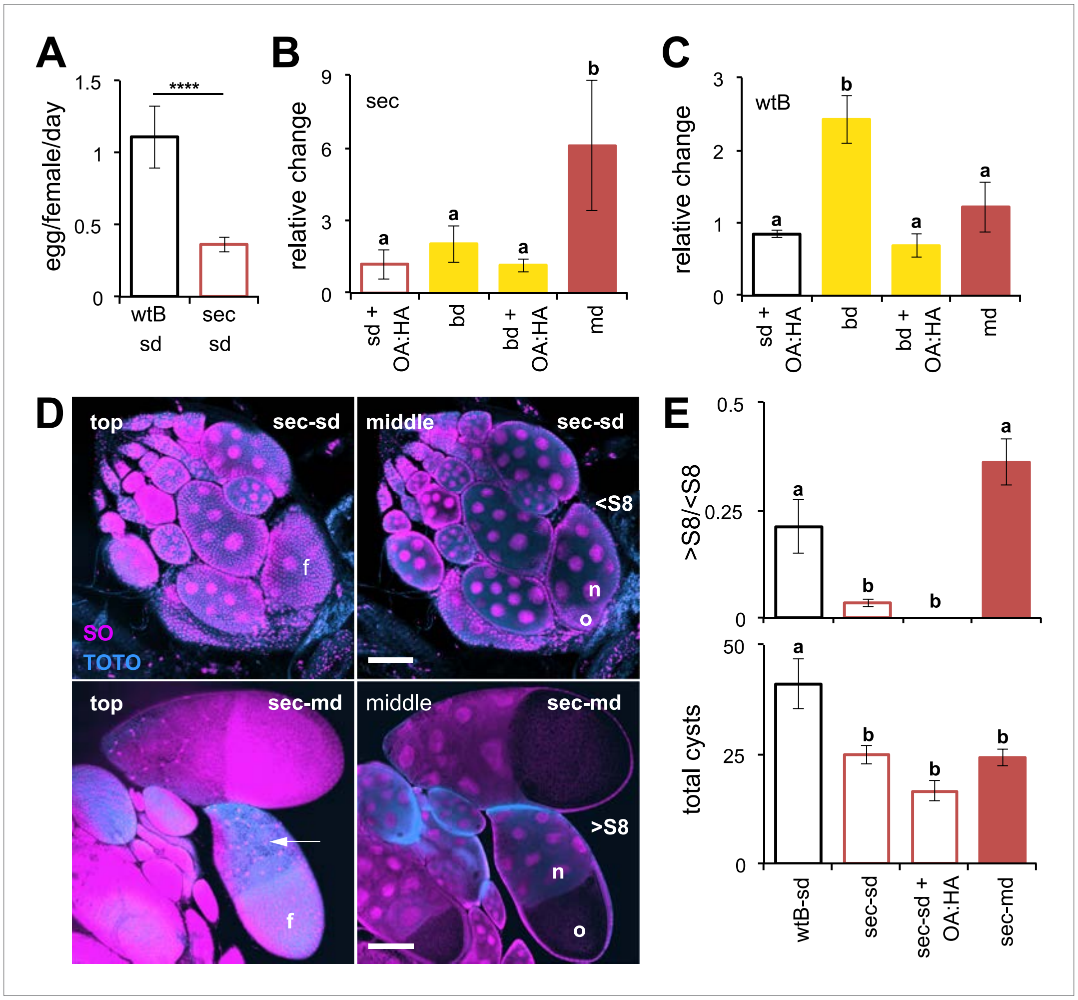

Figure 1. Morinda increases egg production in *D. sechellia*. (**A**–**C**) Egg production (egg/female/day) (*N* > 20) (**A**) and its relative change (*N* > 5) (**B** and **C**) in *D. sechellia* (14021–0248.25, sec) and *D. melanogaster* wild-type Berlin (wtB) fed a standard diet (sd), or morinda diet (md), or banana diet (bd), or diets supplemented with morinda carboxylic acids (+OA:HA). (**D**) Confocal images showing the surface (top) or the interior (middle) of ovarioles stained with nucleic acid specific dyes (sytox orange (SO) and TOTO) of *D. sechellia* (14021–0248.25) fed a standard diet (sec-sd) or a morinda diet (sec-md). The follicle cells (f) surrounding the oocyte (o) or stretched over the nurse cells (n) (arrow) are indicated for early (<S8) or vitellogenic cysts (>S8). Scale bar 100 μm. (**E**) Rate of vitellogenesis (>S8/<S8, top graph) and number of cysts (total cysts, lower graph) (*N* > 8) in *D. sechellia* (14021–0248.25, sec) and *D. melanogaster* wild-type Berlin (wtB) fed a standard diet (sd), or a morinda diet (md), or a diet supplemented with morinda carboxylic acids (+OA:HA). Different letters denote significant differences (p < 0.01) using ANOVA followed by Tukey's test (**B**–**E**); ****p < 0.00001 using Student's *t* test to compare species (**A**). Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.003

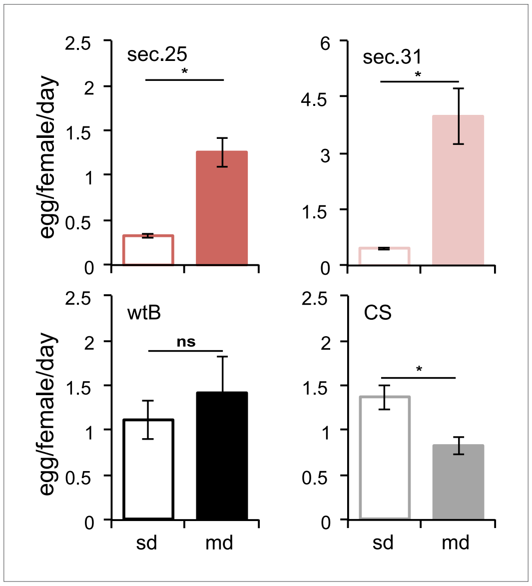

Figure 1—figure supplement 1. Morinda stimulates egg production in *D. sechellia*. Egg production (egg/female/day) in *D. sechellia* 14021–0248.25 (sec.25, *N* = 3), *D. sechellia* 14021–0248.31 (sec.31, *N* = 3), *D. melanogaster* wild-type Berlin (wtB, *N* = 3) and *D. melanogaster* Canton-S (CS, *N* = 3), fed a standard diet (sd) or morinda diet (md). ns = non-significant, *p < 0.05 using Student's *t* test to compare diets in each group. Error bars represent s.e.m.
DOI: 10.7554/eLife.03785.004

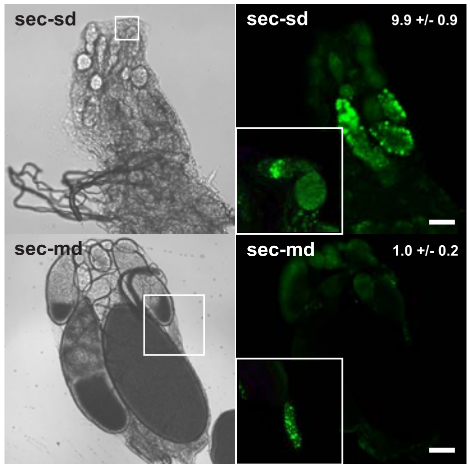

Figure 1—figure supplement 2. Apoptosis in *D. sechellia* ovaries. Confocal fluorescent images (right) and light transmission images (left) of *D. sechellia* (14021–0248.25, sec) ovaries of flies fed a standard diet (sd) (top) or morinda diet (md) (bottom), stained with the vital die acridine orange to label apoptosis (***Arama and Steller, 2006***). The number of apoptotic cysts per ovary (average ± s.e.m.) is indicated for a standard diet (N = 11) and morinda diet (N = 10). Insets show in detail apoptosis occurring at the germanium (top) and an example of the occasional apoptotic cysts in ovaries of flies fed morinda (bottom). Scale bar 20 μm.
DOI: 10.7554/eLife.03785.005

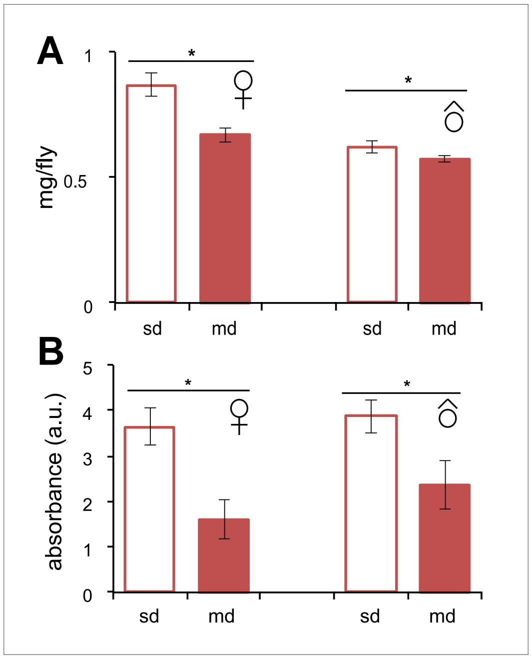

Figure 1—figure supplement 3. Feeding behavior in *D. sechellia*. (**A**) Individual weight (mg/fly) (*N* > 20) of *D. sechellia* (14021–0248.25) mated females and males grown on standard diet (sd) or morinda diet (md). (**B**) Food intake (*N* = 3) measured as light absorbance (a.u., arbitrary units) of ingested sulforhodamine B added to a standard diet (sd) or morinda diet (md). *p < 0.01 using Student's *t* test to compare diets in each group. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.006

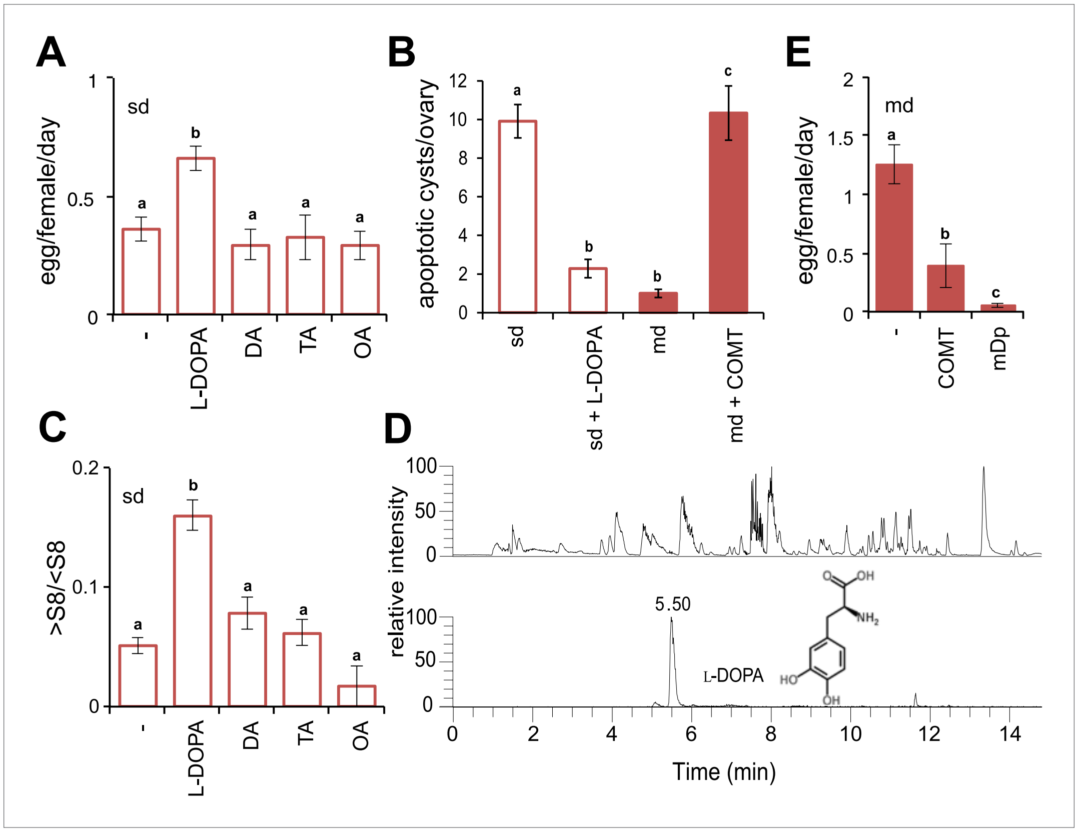

Figure 2. Morinda L-DOPA is required to stimulate egg production. (**A**) Egg production (egg/female/day) (*N* > 6) (**B**) quantification of apoptosis (apoptotic cysts/ovary) (*N* > 6) and (**C**) rate of vitellogenesis (>S8/<S8) (*N* > 12) in *D. sechellia* (14021–0248.25) flies fed a non-supplemented (–) standard diet (sd) or a standard diet supplemented with L-3,4-dihydroxyphenylalanine (1 mg/ml, L-DOPA); dopamine (1 mg/ml, DA); tyramine (2 mg/ml, TA) or octopamine (2 mg/ml, OA); or a non- pretreated morinda diet (md) or a morinda diet pre-treated with catechol-O-methyltransferase (2.5 U per gram of fruit, md + COMT). Different letters denote significant differences ($p < 0.01$) using ANOVA followed by Tukey's test. Error bars represent s.e.m. (**D**) The total ion chromatogram (top trace) shows all compounds present in morinda extract, and the extracted ion chromatogram (lower trace) corresponds to the exact mass of sum formula of L-3,4-dihydroxyphenylalanine (L-DOPA) present in the fruit; as analysed by UHPLC-MS. (**E**) Egg production (eggs/female/day) (*N* > 3) in *D. sechellia* (14021–0248.25) flies fed a morinda diet (md) non-pre-treated (–), pre-treated with catechol-O-methyltransferase (2.5 U per gram of fruit, COMT) or α-methyl-DOPA (0.4 mM, mDp). Different letters denote significant differences ($p < 0.01$) using ANOVA followed by Tukey's test. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.007

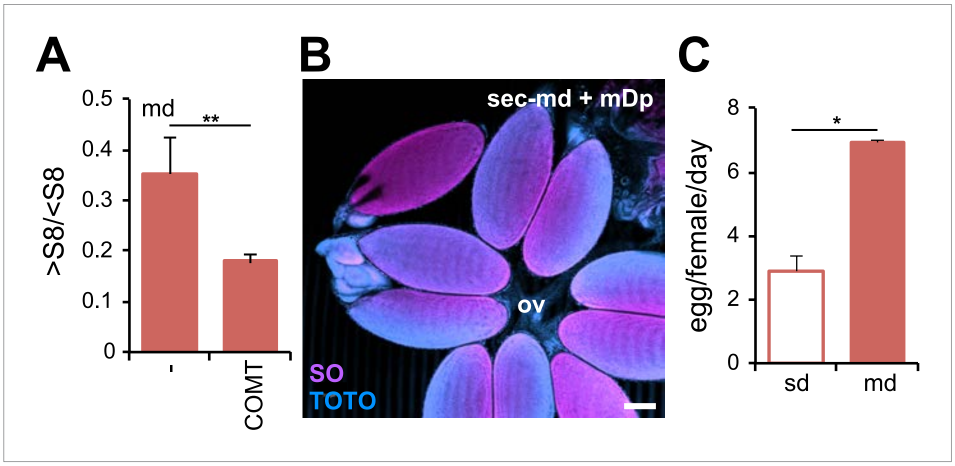

Figure 2—figure supplement 1. *D. sechellia* female fertility is modulated by morinda. (**A**) Rate of vitellogenesis (>S8/<S8) ($N > 6$) in *D. sechellia* (14021–0248.25) flies fed a morinda diet (md) non-pre-treated (−) or pre-treated with catechol-O-methyltransferase (2.5 U per gram of fruit, COMT). **$p < 0.002$ using Student's *t* test. Error bars represent s.e.m. (**B**) Confocal image shows *D. sechellia* (14021–0248.25, sec) egg retention in the ovary of a fly fed a morinda diet (md) supplemented with α-methyl-DOPA (0.4 mM, mDp). Dissected ovaries were stained with nucleic-acid-specific dyes (sytox orange (SO) and TOTO). The oviduct (ov) is indicated. Scale bar, 100 μm. (**C**) Bar graph shows ovipositon (egg/female/day) ($N = 3$) stimulated in *D. sechellia* (14021–0248.25) flies offered fresh morinda (md) as oviposition substrate compared to flies offered a standard oviposition substrate (sd). *$p < 0.02$ using Student's *t* test. Error bars represent s.e.m.
DOI: 10.7554/eLife.03785.008

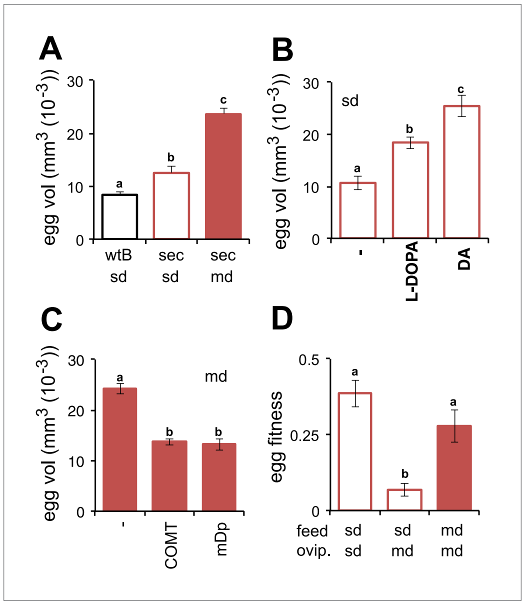

Figure 3. Morinda enhances early fitness. (**A**–**C**) Volume (mm^3(10^{-3})) of *D. melanogaster* wild-type Berlin (wtB) and *D. sechellia* (14021–0248.25, sec) eggs produced by flies fed a standard diet (sd) or morinda diet (md) ($N > 15$) (**A**); *D. sechellia* (14021–0248.25) flies fed a non-supplemented (−) standard diet (sd), or supplemented with L-3,4-dihydroxyphenylalanine (1 mg/ml, L-DOPA) or dopamine (1 mg/ml, DA) ($N > 23$) (**B**); *D. sechellia* (14021–0248.25) flies fed a non-pre-treated (−) morinda diet (md), or pre-treated with catechol-O-methyltransferase (2.5 U per gram of fruit, COMT) or α-methyl-DOPA (0.4 mM, mDp) ($N > 10$) (**C**). (**D**) Egg hatching rate ($N > 5$) in *D. sechellia* (14021–0248.25) fed (feed) a standard diet (sd) or morinda diet (md), ovipositing (ovip.) in either media. Different letters denote significant differences ($p < 0.01$) using ANOVA followed by Tukey's test. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.009

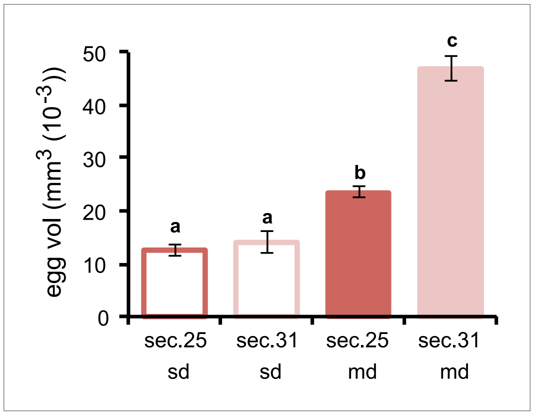

Figure 3—figure supplement 1. Volume of *D. sechellia* eggs is modulated by morinda diet. Volume (mm^3 (10^{-3})) of *D. sechellia* 14021–0248.25 (sec.25) and *D. sechellia* 14021–0248.31 (sec.31) eggs produced by flies fed a standard diet (sd) or morinda diet (md) ($N > 7$). Different letters denote significant differences ($p < 0.01$) using ANOVA followed by Tukey's test. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.010

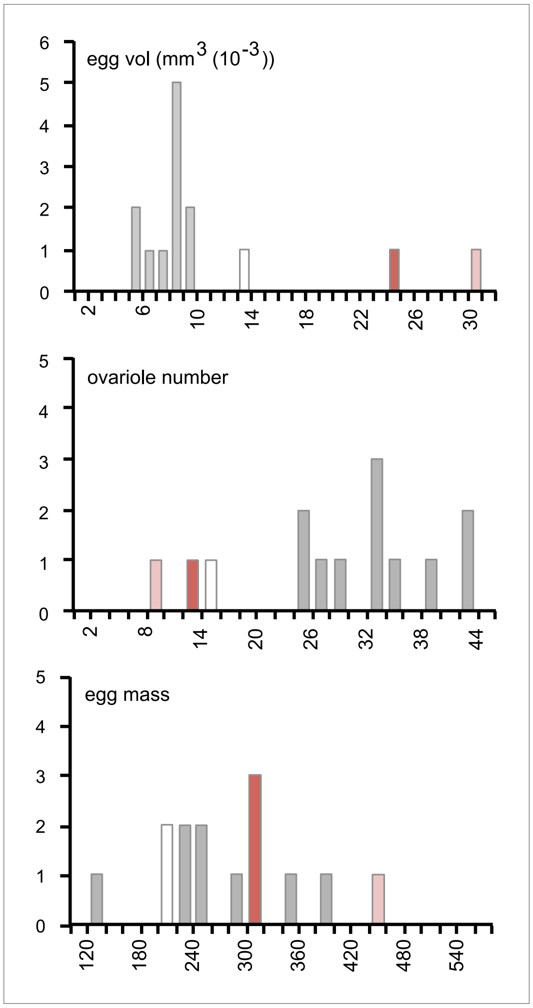

Figure 3—figure supplement 2. Female resource investment on fertility is conserved in *D. sechellia.* Histograms of egg volume (mm^3 (10^{-3})) (top), ovariole number (middle), and egg mass production (egg mass, calculated as egg volume × ovariole number) (bottom) in *D. sechellia* 14021–0248.25 (red bar) and *D. sechellia* 14021–0248.31 (light red bar) fed a morinda diet, compared to 12 Drosophila siblings (*D. ananassae* (14024–0371.13), *D. erecta* (14021–0224.01), *D. melanogaster* (wild type Berlin and 14021–0231.36), *D. mojavensis* (15081–1352.22), *D. persimilis* (14011–0111.49), *D. pseudoobscura* (14011–0121.94), *D. sechellia* (14021–0248.25, white bar), *D. simulans* (14021–0251.195), *D. virilis* (15010–1051.87), *D. willistoni* (14030–0811.24), *D. yakuba* (14021–0261.01), (data from ***Markow et al., 2009***), (grey bar)) fed a standard diet.
DOI: 10.7554/eLife.03785.011

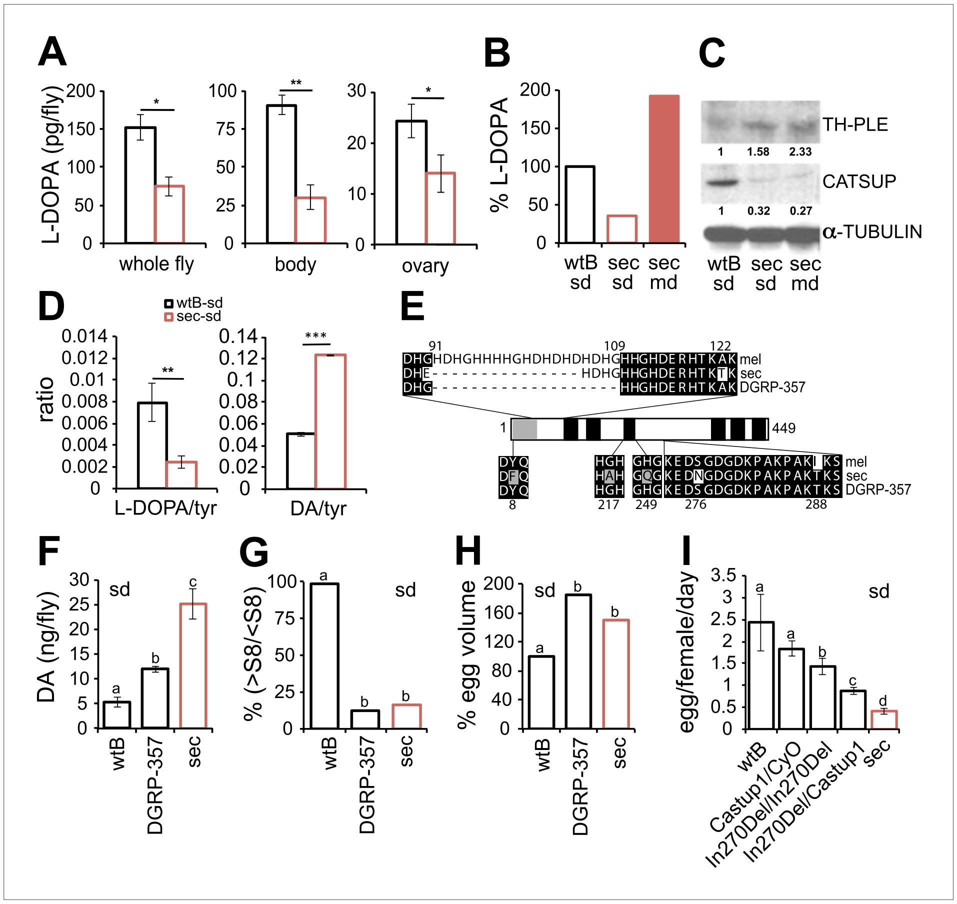

Figure 4. Dopamine metabolism is impaired in *D. sechellia*. (**A**) L-3,4-dihydroxyphenylalanine quantification (L-DOPA pg/fly) ($N = 3$) in whole fly, bodies and ovaries of female *D. melanogaster* wild-type Berlin (wtB) and *D. sechellia* (14021–0248.25, sec) fed a standard diet (sd). *$p < 0.05$ and **$p < 0.002$ using Student's *t* test. (**B**) Relative L-3,4-dihydroxyphenylalanine (% pg L-DOPA per mg body, % L-DOPA) ($N = 3$) in female *D. melanogaster* wild-type Berlin (wtB) and *D. sechellia* (14021–0248.25, sec) fed a standard diet (sd) or morinda diet (md). $p = 0.0062$ and $p = 0.018$ using Student's *t* test *D. melanogaster* vs *D. sechellia* fed, respectively, a standard diet or morinda diet. (**C**) Western blots of total protein whole-fly extracts for TH-PLE, CATSUP, and α-TUBULIN as a loading control, in *D. melanogaster* wild-type Berlin (wtB) and *D. sechellia* (14021–0248.25, sec) fed a standard diet (sd) or morinda diet (md). The numbers under TH-PLE and CATSUP protein lanes indicate the relative protein levels (normalised to α-TUBULIN). (**D**) Ratios of L-3,4-dihydroxyphenylalanine (L-DOPA/tyr) ($N = 3$) and dopamine (DA/tyr) ($N = 3$) to tyrosine substrate in female *D. melanogaster* wild-type Berlin (wtB) and *D. sechellia* (14021–0248.25, sec) fed a standard diet (sd). **$p < 0.007$ and ***$p < 0.000007$ using Student's *t* test. (**E**) Drosophila CATSUP protein structure scheme showing a signal peptide (grey box) and six trans-membrane domains (black boxes). Deletions (dash) and exchanges (grey or white) of amino acids in *D. sechellia* (14021–0248.25, sec) compared to in *D. melanogaster* wild-type Berlin (wtB) and in *D. melanogaster* DGRP-357 (DGRP-357) CATSUP are indicated. (**F**–**H**) Dopamine (DA ng/fly) ($N = 3$) (**F**), relative rate of vitellogenesis (% >S8/<S8) ($N > 10$) (**G**), and egg-volume (% $mm^3(10^{-3})$) ($n > 10$) (**H**) in *D. sechellia* (14021–0248.25, sec), *D. melanogaster* wild-type Berlin (wtB) and *D. melanogaster* DGRP-357 (DGRP-357) fed a standard diet (sd). (**I**) Egg production (egg/female/day) ($N > 3$) in *D. melanogaster* wild-type Berlin (wtB), heterozygote flies (*Catsup^{1}/CyO*), *D. melanogaster* DGRP-357 (*Catsup^{In270Del}/Catsup^{In270Del}*), trans heterozygote flies (*Catsup^{In270Del}/Catsup^{1}*) and *D. sechellia* (14021–0248.25, sec), fed a standard diet (sd). Different letters denote significant differences ($p < 0.05$) using ANOVA followed by Tukey's test (**F**–**I**). Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.012

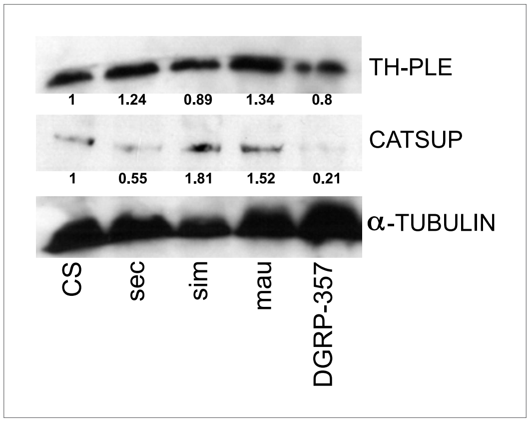

Figure 4—figure supplement 1. TH-PLE and CATSUP expression in Drosophila. Western blots of total protein whole-fly extracts for TH-PLE, CATSUP, and α-TUBULIN as a loading control in *D. melanogaster* Canton-S (CS), *D. sechellia* (14021–0248.31, sec), *D. simulans* (14021–0251.004, sim), *D. mauritiana* (14021–0241.01, mau) and *D. melanogaster* DGRP-357 (DGRP-357) fed a standard diet. The numbers under TH-PLE and CATSUP protein lanes indicate the relative protein levels (normalised to α-TUBULIN).

DOI: 10.7554/eLife.03785.013

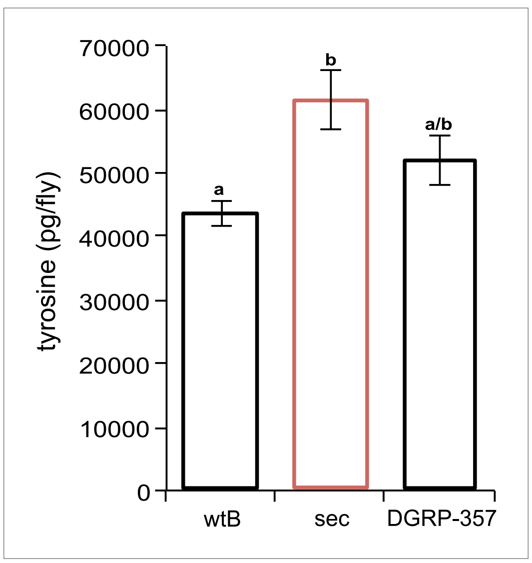

Figure 4—figure supplement 2. Tyrosine quantification in Drosophila. *D. sechellia* (14021–0248.25) females (sec) show higher tyrosine content (expressed as picogram per fly [pg/fly]), compared to *D. melanogaster* wild-type Berlin females (wtB) and *D. melanogaster* DGRP-357 (DGRP-357) females, fed a standard diet. $N = 3$. Different letters denote significant differences ($p < 0.05$) using ANOVA followed by Tukey's test. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.014

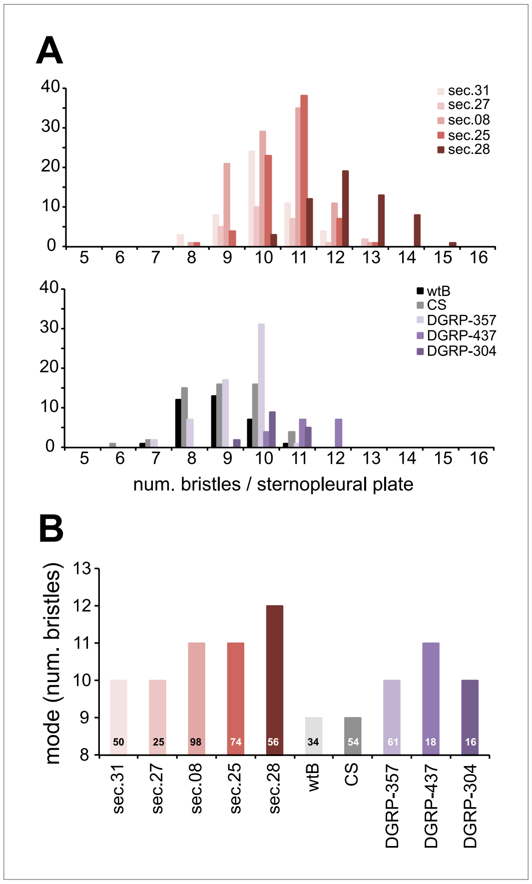

Figure 4—figure supplement 3. Sensory bristles. (**A**) Histograms of total (macro and micro quetas) number of bristles per sternopleural plaque and (**B**) graph of mode (*N* is indicated for each species) of female *D. melanogaster* wild-type Berlin (wtB), *D. melanogaster* Canton-S (CS), *D. melanogaster* DGRP-357 (DGRP-357), *D. melanogaster* DGRP-437 (DGRP437), *D. melanogaster* DGRP-304 (DGRP-304), and *D. sechellia* (sec) original from Praslin (14021–0248.31 (sec.31) and 14021–0248.08 (sec.08), Seychelles 14021–0248.27 (sec.27)) and Cousin (14021–0248.25 (sec.25) and 14021–0248.28 (sec.28)) islands.

DOI: 10.7554/eLife.03785.015

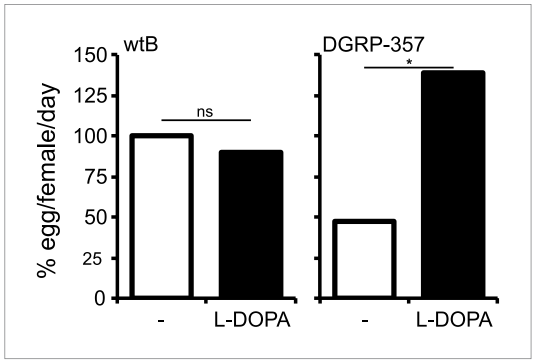

Figure 4—figure supplement 4. L-DOPA rescues diminished *Catsup*^{In270Del}/*Catsup*^{1} egg production. Relative egg production (% egg/female/day) (*N* = 3) in *D. melanogaster* wild type Berlin (wtB) and *D. melanogaster* trans heterozygote flies (*Catsup*^{In270Del}/*Catsup*^{1}) fed a non-supplemented (−) standard diet or a diet supplemented with L-3,4-dihydroxyphenylalanine (1 mg/ml, L-DOPA). ns = non-significant; *p < 0.015 using Student's *t* test to compare diets in each group.
DOI: 10.7554/eLife.03785.016

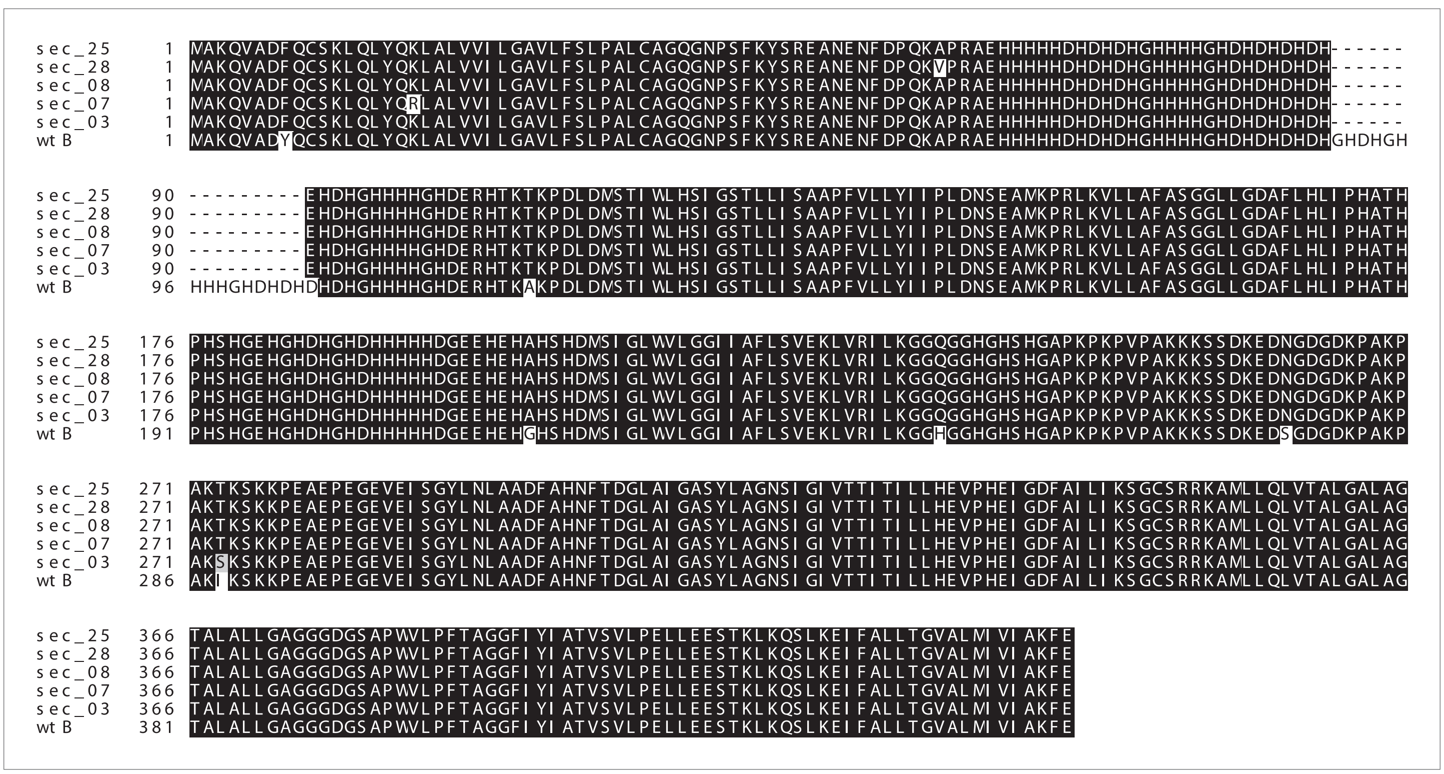

Figure 4—figure supplement 5. Conserved *Catsup* sequence in *D. sechellia*. Drosophila CATSUP amino acids sequence showing deletions (dash) and exchanges (grey or white) of amino acids in *D. sechellia* (14021–0248.03 (sec_03), 14021–0248.07 (sec_07), 14021–0248.08 (sec_08), 14021–0248.25 (sec_25), 14021–0248.28 (sec_28)) compared to in *D. melanogaster* wild-type Berlin (wtB).
DOI: 10.7554/eLife.03785.017

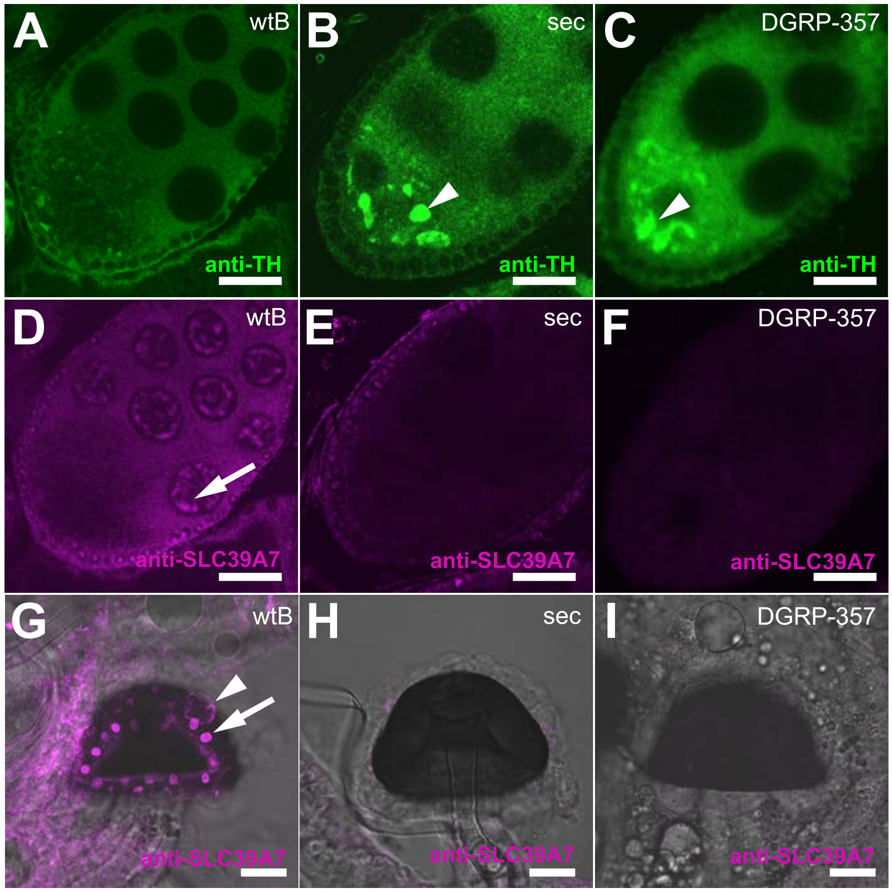

Figure 5. Expression of TH-PLE and CATSUP in Drosophila female reproductive system. (**A**–**C**) Confocal images showing striking accumulation (arrowhead) of TH-PLE (green, anti-TH) in *D. sechellia* (14021–0248.25) (**B**) and *D. melanogaster* DGRP-357 (**C**), compared to in *D. melanogaster* wild-type Berlin (wtB) (**A**) oocytes. (**D**–**F**) Confocal images showing CATSUP (magenta, anti-SLC39A7) expressed in the nurse cells (arrow) of *D. melanogaster* wild-type Berlin (wtB) oocytes (**D**) and absent in the nurse cells of *D. sechellia* (14021–0248.25, sec) (**E**) and *D. melanogaster* DGRP-357 (DGRP-357) (**F**) oocytes. (**G**–**I**) Confocal image showing CATSUP expressed in the nuclei (arrow) and the membrane (arrowhead) of *D. melanogaster* wild-type Berlin (wtB) spermatheca secretory cells (**G**), and absent from *D. sechellia* (14021–0248.25) (**H**) and *D. melanogaster* DGRP-357 (DGRP-357) (**I**). Scale bar 20 μm.
DOI: 10.7554/eLife.03785.018

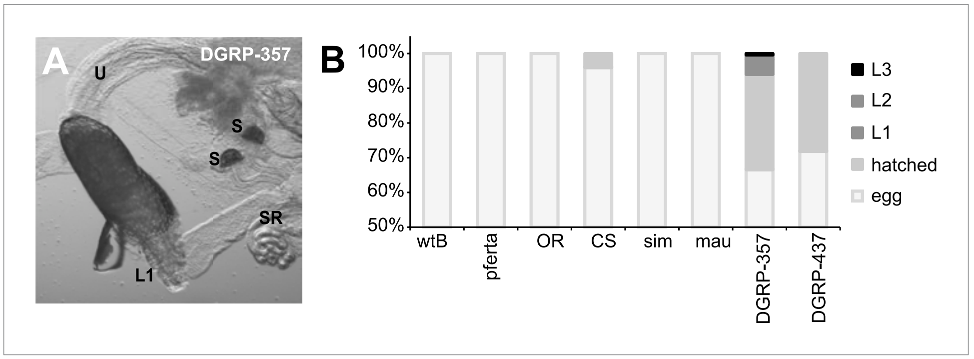

Figure 5—figure supplement 1. Egg hatching in morinda. (**A**) *D. melanogaster* DGRP-357 first-instar larvae (L1) hatching inside the female reproductive system. U: uterus; S: spermatheca; SR: seminal receptacle. Scale bar 20 μm. (**B**) Proportion of eggs hatched or moulted to larva 1 (L1) larva 2 (L2) or larva 3 (L3), in *D. melanogaster* wild-type Berlin (wtB), *D. melanogaster* pferta (pferta), *D. melanogaster* Oregon-R (OR), *D. melanogaster* Canton-S (CS), *D. melanogaster* DGRP-357 (DGRE-357) *D. melanogaster* DGRP-437 (DGRP-437), *D. simulans* (14021–0251.004, sim) and *D. mauritiana* (14021–0241.01, mau).

DOI: 10.7554/eLife.03785.019

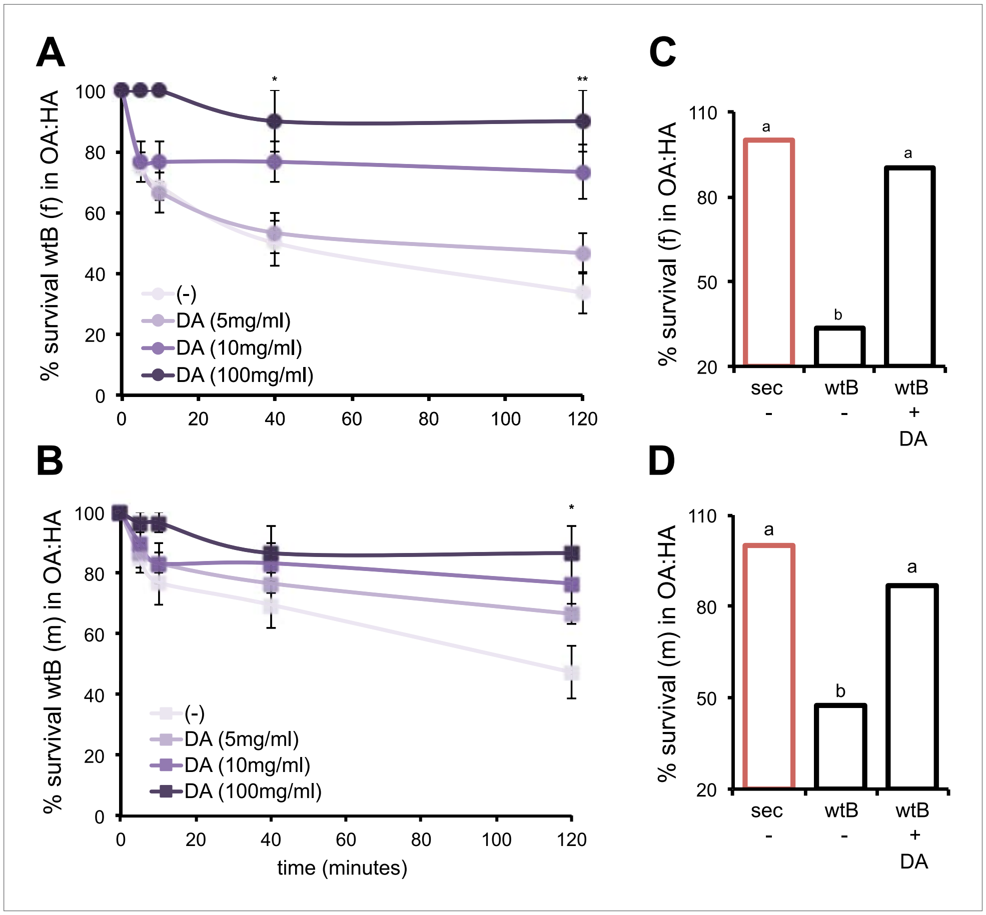

Figure 6. DA contributes to the behavioral resistance to morinda carboxylic acids. (**A**–**B**) Survival kinetic curves for *D. melanogaster* wild-type Berlin (wtB) (**A**) females (f, *N* > 3) and (**B**) males (m, *N* > 3) exposed to morinda carboxylic acids (OA:HA) and fed a standard diet supplemented with either no (–), or increasing doses of DA (5 mg/ml, 10 mg/ml and 100 mg/ml). *p < 0.02 and **p < 0.002 using Student's *t* test. (**C**–**D**) Survival (%) (*N* > 3) of *D. sechellia* (14021–0248.25, sec) and *D. melanogaster* wild-type Berlin (wtB) (**C**) females (f) and (**D**) males (m), fed a non-supplemented (–) or DA (+DA, 100 mg/ml) supplemented standard diet upon 2 hr exposure to morinda carboxylic acids (OA:HA). Different letters denote significant differences (p < 0.01) using ANOVA followed by Tukey's test. Error bars represent s.e.m.

DOI: 10.7554/eLife.03785.020