Laboratory and wild *Drosophila sechellia* have conserved niche specialization phenotypes

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Abstract

A major challenge to investigating the proximate causes of ecological adaptation is the difficulty of studying the phenotypes of organisms in their natural environments. By necessity, many studies seeking to determine the genetic and cellular basis of adaptation therefore investigate potentially adaptive phenotypes under laboratory conditions where organisms are more easily experimentally manipulated. For laboratory models, it remains unclear if organisms maintained long term under laboratory conditions are representative of relatives in their natural environment. In recent years, *Drosophila sechellia*, a specialist species endemic to the Seychelles, has emerged as a (neuro)genetic model for studying the molecular basis of ecological adaptation. A multitude of studies have investigated the genetic and cellular basis of various aspects of this species' specialization in a laboratory setting. However, the vast majority of these studies use laboratory strains of *D. sechellia* that were collected many decades ago, and have been maintained under conditions very

31 different from their natural niche. Thus, it remains unclear if and how these strains resemble
32 their wild counterparts. Here, we compare the phenotypes of these laboratory strains with
33 recently-collected wild *D. sechellia* to ask if laboratory strains display a loss or degradation
34 of phenotypes potentially involved in their specialization resulting from their long-term
35 laboratory maintenance. Across several behavioral and anatomical phenotypes, we find a
36 high degree of similarity between wild-caught and laboratory-maintained strains. Our results
37 suggest that studies of the molecular mechanisms underlying *D. sechellia*'s phenotypes
38 associated with specialization are likely representative of the evolution of these flies in the
39 wild.
40

41 **Introduction**

42 A significant challenge to studying the genetic and cellular underpinnings of ecological
43 adaptation is the necessity to remove individuals from their natural niche for laboratory
44 experimentation. This means that many mechanistic studies of the development, physiology
45 and behavior of a wide variety of organisms are traditionally performed in conditions that
46 differ, sometimes drastically, from an animal's natural environment. This issue is particularly
47 pertinent for laboratory model organisms, where strains are maintained for generations in
48 artificial environments, creating the potential for adaptation to laboratory conditions, which
49 results in uncertainty as to whether phenotypes measured in the laboratory are
50 representative of those in nature. For instance, a standard *Caenorhabditis elegans*
51 laboratory strain displays dramatic differences in social and feeding behaviors, growth, and
52 reproductive output compared to wild *C. elegans*, likely resulting from adaptation to
53 laboratory rearing conditions[1,2]. Moreover, several laboratory strains of *C. elegans,* and even
54 other *Caenorhabditis* species, have convergently evolved unnatural resistance to dauer-
55 inducing pheromones – a starvation-resistant stage of developmental arrest – which may be
56 an adaptation to laboratory culture at high densities[3]. Exacerbating this problem, it is
57 possible for the same genetic isolates that are maintained for years in different laboratories
58 to experience different selection pressures resulting from differences between these
59 environments (e.g., culture medium and rearing practices) creating laboratory-specific

60 phenotypic differences for the same original laboratory strain. For example, laboratory-
61 specific replicates of the commonly used *Drosophila melanogaster* isogenic strain, Canton-
62 S, display significant differences in locomotor behavior[4]. Likewise, in the mouse model, *Mus*
63 *musculus*, laboratory rearing environment influences phenotypic measures across
64 biological levels[5], in some cases resulting in the division of genetic isolates into substrains
65 with known phenotypic differences[6,7].

66 The issue of phenotypic divergence upon prolonged laboratory rearing is potentially more
67 extreme for specialist species, which display adaptation to a narrow ecological niche that is
68 not always easily replicated in a laboratory setting. One example is *Drosophila sechellia*,
69 which diverged from a common ancestor with *D. melanogaster* no more than 5 million years
70 ago[8]. Despite the phylogenetic proximity and high genomic and anatomical similarity of
71 these drosophilids[9], *D. sechellia* occupies a dramatically different ecological niche. While *D.*
72 *melanogaster* is a cosmopolitan species, feeding generally on rotting fruit[10], *D. sechellia* is
73 endemic to the Seychelles archipelago, where it specializes on the ripe noni fruit of the shrub
74 *Morinda citrifolia*[11] (Figure 1A), which is toxic to most other *Drosophila* species[12]. Moreover,
75 in its near-equatorial habitat, *D. sechellia* is exposed to tropical conditions year-round,
76 unlike globally-distributed *D. melanogaster*[8], which experiences higher degrees of seasonal
77 variation at high latitudes. As such, *D. sechellia* has been a focus of many comparative
78 studies aimed at describing the genomic, developmental, physiological, behavioral, and
79 neurological evolution that underlies its niche specialization (reviewed in[13]).

80 Most studies on *D. sechellia* have used laboratory adapted strains collected decades ago
81 (with approximately 25-30 generations per year). Additionally, in the laboratory, these strains
82 typically are not reared on their specific host fruit, but rather on standard *Drosophila* medium
83 sometimes supplemented with commercially available noni juice. This juice only partially
84 resembles noni fruit chemically[14], and has limited, at most, toxicity for other drosophilids
85 (unpublished observations). It is unclear how representative these laboratory strains of *D.*
86 *sechellia* are of those living in their specific niche today, or whether specialization has
87 degraded over the course of laboratory maintenance and adaptation. Recent field studies
88 have sought to address similar issues in *D. melanogaster* by observing the behaviors of

natural populations of flies to collect data that can be compared with laboratory generated data[15–17]. Here, we also sought answers to these questions by performing developmental, physiological, and behavioral laboratory assays on recently collected lines of *D. sechellia* from different islands of the Seychelles archipelago (Figure 1B). We measured multiple aspects of *D. sechellia*'s host specialization to compare the behavior of these non-laboratory adapted and laboratory strains.

Results

Wild *D. sechellia* collection

D. sechellia flies were collected in April 2023 during a field trip to three different islands in the Seychelles archipelago (Figure 1B) and used to found isofemale lines. Strain M1311 was obtained from the grounds of the National Biodiversity Center on Mahé Island. Strains A3312 and A3F311 were collected from a site on Aride Island. Strains P7611 and P7621 were collected from outside Vallee de Mai on Praslin Island. All strains except A3F311 were founded from single adult females collected directly from the surfaces of fallen noni fruits using a manual fly aspirator. Strain A3F311 was founded from a female that eclosed from a larval-infested fallen noni fruit collected in the field and brought back to the laboratory. Each strain was determined to be *D. sechellia* by molecular barcoding (see Methods).

Olfactory preference for ripe noni volatiles

D. sechellia displays a strong preference for ripe noni fruit and noni-derived acids over other substrates in open trap assays permitting olfactory and gustatory sampling both in the lab[18] and field[19]. In long-range and short-range laboratory assays that exclude gustatory sampling, *D. sechellia* displays increased olfactory-mediated preference for noni and noni volatiles[14,20,21]. This strong preference for noni has been attributed, in part, to evolution of the peripheral olfactory sensory system[20,22].

To compare the phenotypes of laboratory and wild *D. sechellia*, we first examined their olfactory preference given a choice between noni fruit juice and apple cider vinegar (a

118 fermentation product that is highly attractive to *D. melanogaster*[23]). We tested two
119 commonly-used laboratory *D. sechellia* strains, the five new wild-caught *D. sechellia* strains
120 and one *D. melanogaster* laboratory strain. In our trap design, flies could not physically
121 encounter the liquid prior to making a choice, limiting the possibility for gustatory sensation
122 (Figure 2A). All seven *D. sechellia* strains, both laboratory and recently-caught, displayed
123 significant preference for noni juice over apple cider vinegar, while *D. melanogaster*
124 displayed a slight but non-significant preference for apple cider vinegar (Figure 2B).
125 Importantly, there were no distinguishable differences when comparing the preferences
126 between *D. sechellia* strains, regardless of their collection date. However, all *D. sechellia*
127 strains displayed significantly different preference indices from *D. melanogaster*.
128
129 **Oviposition preference for noni substrates**
130 Upon successful location of a host fruit, flies will feed, engage in social behaviors with other
131 flies they encounter and reproduce[15]. *D. sechellia* consistently selects ripe noni or noni
132 media for oviposition over other substrates[18,19,24,25]. This inherent preference to oviposit on
133 ripe noni has been attributed to olfactory receptor tuning changes[24] and gustatory
134 preference differences[26].
135 To compare the oviposition preferences of our recently-caught and laboratory *D.*
136 *sechellia* strains, we replicated a single-fly oviposition choice assay[27 24]. Briefly, we offered
137 individual gravid females a choice between two oviposition media of equivalent texture,
138 made with noni juice or apple cider vinegar, over a 24-hour period. During this period, flies
139 were able to sample each media using tactile, gustatory, and olfactory cues (Figure 2C). In
140 this assay, all *D. sechellia* strains displayed a significant preference for ovipositing on noni
141 media over apple cider vinegar media, while our *D. melanogaster* laboratory strain displayed
142 a significant preference for ovipositing on apple cider vinegar media (Figure 2D). All *D.*
143 *sechellia* strains were statistically distinguishable from *D. melanogaster*. In this assay we
144 observed significant variation among our *D. sechellia* strains with respect to the strength of
145 their preference for ovipositing in noni media, but this did not reflect a consistent distinction
146 between laboratory and wild *D. sechellia* (Figure 2D).

147

148 **Olfactory sensory neuron population expansion and contraction**

149 The species-specific host location and oviposition behaviors of *D. sechellia* are thought to
150 be due in part to increases in the numbers of olfactory sensory neurons (OSNs) detecting
151 characteristic noni volatiles[28], namely hexanoic acid-sensing Ir75b OSNs in antennal
152 coeloconic 3I (ac3I) sensilla, and Or22a and Or85b OSNs in antennal basiconic 3 (ab3)
153 sensilla, which detect methyl esters and *2*-heptanone, respectively[14,29 20].

154 To determine whether the expansions described in laboratory strains are a conserved
155 phenotype of *D. sechellia* in the wild, we used immunofluorescence or RNA fluorescence *in-*
156 *situ* hybridization to label specific OSN populations in one laboratory strain each of *D.*
157 *melanogaster* and *D. sechellia*, as well as three of the wild-caught *D. sechellia* strains
158 (P7621, A3312, and M1311). Across both laboratory and wild-caught *D. sechellia* strains, we
159 observed a consistent two-fold increase in Ir75b OSNs relative to *D. melanogaster* (Figure
160 3A). Similarly, by labelling Or85b OSNs, we detected a consistent expansion of ab3 sensilla
161 (Figure 3B), with *D. sechellia* strains exhibiting a three-fold increase in OSN number
162 compared to *D. melanogaster*.

163 In addition to these OSN population expansions, several sensilla and/or OSN types
164 are reduced in *D. sechellia*. For example, ab1 sensilla, which house Or10a and Or42b
165 expressing OSNs, display a nearly 2-fold reduction in number relative to *D. melanogaster*[20,29].
166 In *D. melanogaster*, Or42b neurons are required for attraction to apple cider vinegar[23], so
167 their decrease in *D. sechellia* plausibly reflects a lower significance of this sensory stimulus.
168 Labelling of Or10a and Or42b neurons revealed a consistent reduction in all tested *D.*
169 *sechellia* strains compared to *D. melanogaster* (Figure 3C).

170

171 **Adult noni toxin resistance**

172 As part of its specialization on noni, *D. sechellia* has evolved a high resistance to octanoic
173 acid, an abundant noni chemical[30] that accounts for this fruit's toxicity for other
174 drosophilids[31,32]. The mechanistic basis of adult octanoic acid resistance in *D. sechellia* is
175 still poorly-understood, but genome-wide association and gene expression studies identify

176 a complex genetic architecture[33] highlighting candidates involved in cuticle formation[34–36],
177 enzymatic detoxification[37–39] and metabolism[40,41].
178 To compare the resistance to octanoic acid of our laboratory-adapted strains with
179 that of recent collections, we used a plate-based assay[33]. Adult males and females were
180 combined with 2 μl of pure octanoic acid in a Petri dish to ensure exposure to its volatiles
181 and scored every 5 min for loss of consciousness over 60 min (Figure 4A). When we calculate
182 the cumulative survival probability across 10 replicates for our *D. sechellia* and *D.*
183 *melanogaster* strains, we observe a consistent species difference: for all *D. sechellia* strains,
184 more than 60% of females (Figure 4B) and males (Figure S1A) remained conscious, while
185 only ~20% of *D. melanogaster* females and no males did. Comparing the proportion of
186 surviving flies at 60 min between strains for both females (Figure 4C) and males (Figure S1B),
187 all *D. sechellia* strains were significantly different from *D. melanogaster*. For females, we
188 detected some variation among our wild-caught *D. sechellia* strains (Figure 4C), but this
189 reflected slightly lower octanoic acid resistance levels compared to the laboratory strains.
190
191 **Fecundity and egg size**
192 In addition to adult resistance to noni toxins, *D. sechellia*'s eggs must be able to develop on
193 this toxic fruit. The eggs of *D. sechellia* are larger (~50% volume) than those of *D.*
194 *melanogaster*[42] and it has been hypothesized that this phenotype represents part of the
195 mechanism permitting their successful development on noni. This potential increase in
196 resource allocation toward individual eggs is further hypothesized by some to have resulted
197 in a trade-off with overall fecundity[32,33], due at least in part to a decrease in total ovariole
198 number[43], though it remains to be determined if and how this trait evolved during *D.*
199 *sechellia*'s specialization. While the molecular basis of reduced ovariole number is
200 unknown, association mapping studies have indicated a complex genetic architecture[44,45],
201 and developmental studies have identified differences in somatic gonadal precursor cell
202 number[46] and insulin signaling[47] that contribute to this trait.
203 To examine these phenotypes in wild-caught flies, we quantified reproductive output
204 for mated females, ovariole number and egg size. We used the single-fly oviposition assay

205 described above to determine the total reproductive output of a single female over a 24-h
206 period. Comparing the total eggs produced per female, irrespective of oviposition media, we
207 found that all *D. sechellia* strains were consistently distinguishable from *D. melanogaster,*
208 laying ~5-fold fewer eggs (all $p < 0.001$). Similarly, all *D. sechellia* strains, irrespective of
209 collection date, had fewer ovarioles (~2-fold reduction) than our *D. melanogaster* strain
210 (Figure 5B). Finally, all *D. sechellia* strains laid larger eggs than *D. melanogaster* (Figure 5C),
211 with the average volume of *D. sechellia*'s eggs being 45% greater than those of *D.*
212 *melanogaster* Canton-S. For these latter two phenotypes, we did not observe any significant
213 differences between any *D. sechellia* strain (Figure 5B-C).

214 Taken together, the decreased fecundity, decreased ovariole number and increased
215 egg size we document is similar to previously described reproductive divergence between
216 laboratory strains of *D. melanogaster* and *D. sechellia*[42,44,48], and consistent with the
217 hypothesis of an evolutionary trade-off between reproductive output and resource
218 allocation[26].

219

220 **Reduced circadian plasticity**

221 Finally, in addition to *D. sechellia*'s many adaptations to its host fruit, it was recently
222 discovered to be specialized to aspects of its equatorial environment, notably photoperiod
223 (day-length)[49]. Drosophilid flies typically display a bimodal pattern of circadian activity (with
224 some exceptions)[50], where individual flies time their peak morning and evening activity with
225 sunrise and sunset. Flies living at high latitudes display an ability to plasticly adjust the
226 timing of these peaks to match seasonal variation in day length, with the degree of plasticity
227 correlating with population latitude[49,51,52]. *D. sechellia* are found exclusively at the equator,
228 where day length varies minimally throughout the year[8]. As such, these flies are far less able
229 to delay the timing of their evening peak activity to match longer days that would be
230 experienced at higher latitudes[49]. This loss of plasticity has a polygenic basis, including
231 divergent regulation of the circadian neuropeptide, *Pigment dispersing factor*[49].

232 We used the *Drosophila* activity monitor system to compare the activity patterns of
233 our strains under both equatorial and high latitude summer conditions[53]. We first recorded

male flies of each strain for 7 days under 12:12 h LD (equatorial) conditions at 25°C (Figure 6A). All strains of both species displayed a characteristic peak of activity surrounding subjective dawn and dusk, separated by an afternoon period of reduced activity. We next took the average activity for each fly per strain during the last 4 days of this recording and annotated the timing of the evening activity peak (Figure 6B). *D. melanogaster* and all the laboratory and wild-caught *D. sechellia* strains displayed a high synchronicity of their evening peak time with the timing of subjective dusk (12 hours).

We next modified the photoperiod for these flies, recording them for an additional 7 days under 16:8 h LD (high latitude summer) conditions (Figure 6C). As before, we used the average activity from the last 4 days of this recording period from each fly to determine the timing of the evening activity peak under extended photoperiod conditions (Figure 6D). As previously described[49], we observed a clear species difference in evening peak plasticity, where our *D. melanogaster* laboratory strain was able to delay its evening peak time significantly longer than any of our *D. sechellia* strains. On average, *D. melanogaster* Canton-S was able to delay its evening peak to ~15 h, just 1 h shy of subjective dusk. By contrast, our *D. sechellia* strains' average evening peak time ranged from ~13-13.67 h. Comparing laboratory and wild-caught *D. sechellia* strains identifies a single wild-caught strain from Praslin Island (P7611) with a slightly elevated evening peak time relative to just one of our laboratory strains, *Dsec*07. There is therefore no consistent discernable difference in circadian plasticity between laboratory and wild-caught *D. sechellia* strains. This is unsurprising because, for this phenotype, standard laboratory fly culture conditions (12:12 h LD cycle at 25°C) more accurately reflect equatorial conditions than the variable conditions at high latitudes in the wild.

Discussion

Here, we sought to detect potential effects of laboratory adaptation on several phenotypes of *D. sechellia*. Our results indicate that *D. sechellia* laboratory strains do not display signs of a loss or degradation of the behavioral or anatomical phenotypes of this species, whose

evolution was likely influenced by its specialization to a specific ecological niche. We discuss these results on a phenotype basis below.

Despite not being reared in the presence of noni fruit for hundreds of generations, *D. sechellia* laboratory strains show a strong retention of preference for noni-based substrates in both olfactory and oviposition assays, along with the increases/decreases in OSN number associated with these preferences. For this study, we maintained our *D. sechellia* strains on standard *Drosophila* media supplemented with a paste made with commercially available noni juice. It is possible that this approximation of noni fruit is sufficient to maintain noni preferences during laboratory culture. However chemical comparison of this juice with ripe noni fruit reveals differences in the relative proportions of specific chemicals, such as octanoic and hexanoic acids[14]. Our laboratory *D. sechellia* strains were originally sourced from the *Drosophila* Species Stock Center (DSSC), where they were previously maintained for years on a cornmeal media (DSSC Director Patrick M. O'Grady, *personal communication*). Given the many known examples of laboratory adaptation, that phenotypes can be experimentally evolved in far fewer generations[40,54,55], and the numerous examples demonstrating that such preferences are highly evolvable[14,21,26], why, then, have the *D. sechellia* laboratory strains studied here not evolved novel preferences to laboratory media? It is possible that these isogenic strains have simply not been reared in the laboratory for long enough to evolve altered preferences de novo. Alternatively, a laboratory environment might not select strongly for these preferences: in standard culture vials, flies have no need to forage and are not given a choice between media, and therefore have little option to exert (or need for) a preference. Thus, any chemosensory preferences might be effectively neutral in a laboratory setting, and such phenotypes would only degrade slowly over time via the accumulation of random mutations and genetic drift.

This hypothesis might also explain the persistence of octanoic acid resistance in laboratory populations. There is no obvious benefit to retaining octanoic acid resistance in the lab, as these flies never encounter it. However, as long as the cost of retaining octanoic acid resistance is not large, selection also would not favor its loss, rendering it a neutral phenotype. Alternatively, there might be hidden selective advantages. For example, cuticle

291 changes that confer octanoic acid resistance might also result in decreased desiccation
292 risk, which could still be favored in a laboratory setting. Thus, laboratory selection on one
293 effect of the phenotype might maintain the other.

294 We also show that the reduced fecundity and ovariole number previously described
295 in *D. sechellia* laboratory strains is indeed representative of the phenotypes of wild-caught
296 flies. Laboratory environments typically select for high fecundity through high-density
297 rearing practices[3]. Our laboratory strains do not appear to have responded to this selection
298 pressure. However, if the hypothesis of a trade-off in *D. sechellia* of reduced fecundity with
299 increased egg size[32,33] is correct, for laboratory strains to evolve increased fecundity, they
300 might also have to evolve reduced egg size, which presents a more complicated evolutionary
301 trajectory. In this respect, reduced fecundity of *D. sechellia* might represent a evolutionary
302 dead-end. Future studies exploring differences in maternal investment to directly test this
303 hypothesis seem warranted.

304 Laboratory *D. sechellia* strains have also retained their circadian specialization to an
305 equatorial environment. Considering most laboratories maintain flies under equatorial
306 conditions with respect to photoperiod and temperature, this is unsurprising. In this context,
307 it is notable *D. melanogaster* maintained at equatorial conditions have not lost their capacity
308 for photoperiodism, generally supporting the idea that laboratory strains maintain, at least
309 over these time scales, aspects of local adaptation to their collection site.

310 Finally, while it is most parsimonious to assume that the mechanisms underlying
311 species-specific traits are conserved between laboratory and wild-caught strains – as
312 supported here by the described expansions and contractions of OSN numbers – future
313 studies should further investigate in our wild-caught strains the previously-described
314 evolution of gene expression[37,38,40,49,56,57], the nervous system[14,20,21,24,26,49,57,58], and the
315 reproductive system[46,47]. Regardless, our data demonstrate robustness in *D. sechellia*'s
316 specialism-associated phenotypes, further supporting the power of this species as a model
317 system to uncover ecologically relevant genetic adaptations.

318

319 **Methods**

320

321 ***D. sechellia* wild-caught strain maintenance**

322 Following field collections in April 2023, isofemale *D. sechellia* lines were established and
323 maintained in the Extavour laboratory on *Drosophila* media (0.8% agar, 2.75% yeast, 5.2%
324 cornmeal, 11% dextrose) without noni juice at 25°C, 65% relative humidity, and a 12:12 h
325 light-dark cycle, from May-November 2023. From November 2023 onward, flies from each
326 strain were reared in the Benton laboratory on *Drosophila* media (0.6% agar, 5.8% yeast,
327 5.8% wheat, 10% fruit juice) supplemented with noni juice paste (Raab Vitalfood Bio noni
328 juice combined with instant *Drosophila* medium, Carolina #173210). From January 2025
329 onward, flies were reared in the Shahandeh laboratory on *Drosophila* media (0.9% agar, 2.6%
330 yeast, 6.25% cornmeal, 6.25% molasses) supplemented with noni juice (Healing Noni juice
331 combined with instant *Drosophila* medium, Carolina #173210). In both the Benton and
332 Shahandeh laboratories, flies were maintained in non-overlapping 2-week generations at
333 25°C, 50% relative humidity, and a 12:12 h light-dark cycle. To limit the potential for
334 laboratory adaptation to occur, all behavioral analyses reported herein were conducted
335 within a year of collection (by May 2024) in the Benton laboratory. Quantifications of ovariole
336 number, egg size (Shahandeh laboratory), and OSN number (Benton laboratory) were
337 conducted from July-December 2025.

338

339 ***Drosophila* laboratory strain maintenance**

340 The laboratory strain of *D. melanogaster* (Canton-S, RRID:BDSC_64349) was maintained on
341 a *Drosophila* media (recipes above) in the Benton and Shahandeh laboratories in non-
342 overlapping 2-week generations at 25°C, 50% relative humidity, and a 12:12 h light-dark
343 cycle. The laboratory strains of *D. sechellia* (*Dsec.*07, DSSC 140210248.07; *Dsec.*28, DSSC
344 140210248.28) were maintained under the same conditions on *Drosophila* media
345 supplemented with noni juice paste (as described above for each laboratory).

346

347 **Species identification by molecular barcoding**

348 Wild-caught flies were identified as *D. sechellia* using molecular barcoding of portions of the
349 *mitochondrial cytochrome c oxidase* (*mt:CoI*) gene, using COI-218F (5'
350 CAACATTTATTTTGATTTTTTGG 3') and COI-3037R (5' TYCATTGCACTAATCTGCCATATTAG 3')
351 primers, using standard methods. In brief, genomic DNA was extracted from individual flies,
352 amplified via PCR using OneTaq 2X Master Mix (includes 0.62 units Taq; New England
353 BioLabs #M0480), and purified using Monarch PCR & DNA Cleanup Kit (New England
354 BioLabs #T1030L). The resulting products were subject to Sanger sequencing (Bioscience
355 Inc), using COI-218F primer. Raw sequence data were analyzed using Chromas Lite v2.6.6
356 (Technelysium Pty Ltd. Australia) and sequences were compared with those in GenBank to
357 identify the species. Flies were identified as *D. sechellia* if the *mt:CoI* amplicon showed at
358 least 99.5% sequence identity with GenBank accession MK659840.1, which is the *mt:CoI*
359 sequence of *D. sechellia* voucher DSECH20161109 complete mitochondrial genome.
360 *mt:CoI* amplicon sequences from the strains described in this study were deposited in
361 GenBank (Accession numbers: M1311, PX502215; A3312, PX502212; A3F311, PX502211;
362 P7611, PX502214; P7621, PX502213).
363
364 **Olfactory trap assay**
365 The olfactory trap assay was replicated from previous studies investigating *D. sechellia*'s
366 short-range olfactory preference[14,21]. In brief, 25 3-5 day-old, mated females were
367 anesthetized with ice and added to a 12 cm circular plastic Tupperware containing two
368 olfactory traps prior to sealing the tops with a fine mesh secured by rubber band (Figure 2A).
369 Each trap contained 300 μl of either noni juice (Raab Vitalfood) or apple cider vinegar
370 (Denner brand) mixed with Triton-X (final concentration 0.2%). Flies can enter the traps
371 through a pipette tip puncturing the cap but are unable to find the opening to escape after
372 selecting an odor source. Traps were stored for 24 h at 25°C and ~60% humidity under red
373 light, after which the number of flies in each trap, or remaining untrapped animals (live and
374 dead), were counted. We excluded from our statistical analyses any traps where more than
375 25% of flies remained untrapped (Figure S2). We calculated preference indices as: (number
376 of flies in the noni juice trap − number of flies in the apple cider vinegar trap)/number of living

377 flies, both trapped and untrapped. We used a Wilcoxon rank-sum test to test individual
378 preference indices for differences from 0 (no preference), and pairwise Wilcoxon tests to
379 compare preference indices between strains. For both, p-values were corrected for multiple
380 comparisons using the Bonferroni method.
381
382 **Two-choice single-fly oviposition assay**
383 To measure individual oviposition preferences, we used a single-fly chamber assay where 30
384 3-5 day old mated females are housed individually in plexiglass cells where they can sample
385 two oviposition media[24,27] (Figure 2C). We provided a choice between two substrates: a
386 0.67% agar solution made with noni juice, or an agar solution of the same density prepared
387 with apple cider vinegar. Prior to assay, females were housed in high densities with males for
388 3 days to ensure they were gravid. The chambers were housed at 25°C and ~60% humidity
389 for 24 h under a 12:12 h light-dark cycle. At the end of the assay, flies were removed and the
390 number of eggs present on each substrate was recorded for each chamber. We calculated
391 preference indices as: (number of eggs laid on the noni media – the number of eggs laid on
392 apple cider vinegar)/total number of eggs laid. We used a Wilcoxon rank-sum test to test
393 individual preference indices for differences from 0 (no preference), and pairwise Wilcoxon
394 tests to compare preference indices between strains. For both, p-values were corrected for
395 multiple comparisons using the Bonferroni method.
396
397 **Histology and imaging**
398 Hybridization chain reaction RNA fluorescence in situ hybridization (HCR-FISH) was
399 performed as described in[59]. RNA probes for *Or10a* (accession number NM_078567.5),
400 *Or42b* (NM_078900.2) and *Or85b* (NM_079555.2), amplifiers, and buffers were purchased
401 from Molecular Instruments (HCR™ RNA FISH (v3.0)). Probe sequences correspond to *D.*
402 *melanogaster Or* genes, but have ≥95% identity with orthologous *Or*s in *D. sechellia*.
403 Immunofluorescence for Ir75b was performed as described previously (Auer et al.
404 2020), using anti-Ir75b (Prieto-Godino et al., 2016) diluted 1:500. Secondary anti-guinea
405 pig antibody conjugated to Alexa Fluor 488 (A11073, Invitrogen AG) was diluted 1:1000.

406 Images were acquired using a Zeiss confocal microscope LSM 710 with a 40×
407 objective and processed in ImageJ. Neurons were counted manually using the ImageJ
408 MultiPoint tool.
409
410 **OA resistance assay**
411 To measure adult resistance to octanoic acid, we used a previously-described plate
412 assay[33,40] (Figure 3A). In brief, ten male or female flies were briefly anesthetized with CO_2 and
413 placed in a 60 mm diameter × 15 mm high Petri dish to which 2 µl of pure octanoic acid had
414 been previously added to the center of the lid. Upon recovery from anesthesia, the flies were
415 scored every 5 min for loss of consciousness for 60 min. To test for differences between
416 strains, we compared the proportion of surviving flies at the final time point using pairwise
417 Wilcoxon tests followed by post-hoc correction for multiple comparisons.
418
419 **Ovary dissection and counting**
420 3-5-day-old, mated females (reared at 25°C, ~60% relative humidity, 12:12 h LD) were
421 anesthetized on CO_2 and sorted by sex. Whole flies were fixed for 2 h at room temperature in
422 4% paraformaldehyde in PBS containing 0.3% Triton X-100 (PBS-TX; 1 ml per ~30 flies) on a
423 nutator. Samples were washed 3 × 5 min in PBS-TX and ovaries were dissected in PBS-TX
424 under a stereomicroscope. To enhance contrast for ovariole visualization, each set of
425 dissected ovaries was briefly dipped in crystal violet solution (K1184, APExBio). Each ovary
426 was gently teased apart in PBS-TX on a ceramic slide using an eyelash probe to separate
427 ovarioles without rupturing the ovary. Ovarioles were identified as individual strings of
428 progressively developed egg chambers. Both ovaries from each female were counted and
429 averaged to obtain mean ovarioles per ovary.
430
431 **Egg collection and size measurements**
432 Oviposition plates were prepared as 5% sucrose, 2% agarose in distilled water and diluted
433 with noni juice or apple cider vinegar to a final concentration of 1%. Plates were poured into
434 60 mm diameter Petri dishes and cooled to room temperature. Groups of mated females (3-

435 5 days old; conditioned with males at high density for 3 days prior) were transferred to fresh
436 plates and allowed to oviposit for ~20 h at 25°C, ~60% relative humidity, 12:12 h light-dark
437 cycle. For imaging, a dissecting scope was used to locate eggs; individual eggs were placed
438 into a single file on the plate on their dorsal side. We photographed each plate along with a
439 ruler for scale using a Nikon Z50 DX 16-50mm on a tripod approximately 24 cm above the
440 plate's surface. Egg size was quantified in Fiji/ImageJ (https://imagej.net/ij/). For each egg,
441 length and width were measured using the straight-line tool. We calculated total egg volume
442 using a previously established formula[60]: $V = 1/6\pi W^2 L$ for ~30 eggs of each strain. Two
443 observers independently estimated egg volume to control for observer bias (Figure 5C and
444 Figure S3), as a completely blind study was not possible due to differences in the color of
445 substrate used for each species.
446
447 ***Drosophila* activity monitoring**
448 We used the *Drosophila* Activity Monitor system to record the daily activity patterns of our
449 strains under both equatorial (12:12 h LD) and high latitude summer (16:8 h LD) photoperiod
450 conditions[61], replicating experiments used to describe reduced circadian plasticity in *D.*
451 *sechellia*[49]. Briefly, we exposed individual 3-5 day old males first to 7 days of an equatorial
452 photoperiod, followed by 7 days of a high latitude summer photoperiod. We used a
453 previously developed algorithm to quantify the timing of each fly's evening peak[49] using the
454 last 4 days of each 7-day period, discarding the first three days as an acclimation period. We
455 compared evening peak time between our strains using pairwise Wilcoxon tests followed by
456 correction for multiple comparisons.
457 To estimate the circadian period of each strain, we acclimated flies to a 12:12 h LD
458 cycle for 4 days before subjecting them to 5 days of constant darkness conditions. We used
459 the constant darkness behavioral data to calculate the duration of each strain's free-running
460 period with the R package Rethomics, which was also used for all circadian data
461 visualization[62]. We compared periods across strains with pairwise Wilcoxon tests subjected
462 to post-hoc correction for multiple comparisons.
463

464 **Statistical analysis and data visualization**

465 All statistical analyses were conducted using the 'stats' package (v4.6.0) in R (v4.4.2)[63]. Data
466 were visualized using 'ggplot2'[64]. For boxplots in all figures, the bold line represents the
467 median value, with the length of the box displaying the interquartile range. Whiskers signify
468 the last quartiles excluding outliers.

469

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482

483 **References**

484 1. Andersen, E. C., Bloom, J. S., Gerke, J. P. & Kruglyak, L. A Variant in the Neuropeptide
485 Receptor npr-1 is a Major Determinant of *Caenorhabditis elegans* Growth and
486 Physiology. *PLoS Genet.* **10**, e1004156 (2014).
487 2. Zhao, Y. *et al.* Changes to social feeding behaviors are not sufficient for fitness gains of
488 the *Caenorhabditis elegans* N2 reference strain. *eLife* **7**, e38675 (2018).
489 3. McGrath, P. T. *et al.* Parallel evolution of domesticated *Caenorhabditis* species targets
490 pheromone receptor genes. *Nature* **477**, 321–325 (2011).
491 4. Colomb, J. & Brembs, B. Sub-strains of *Drosophila* Canton-S differ markedly in their
492 locomotor behavior. *F1000Research* **3**, 176 (2015).
493 5. Jaric, I. *et al.* The rearing environment persistently modulates mouse phenotypes from
494 the molecular to the behavioural level. *PLOS Biol.* **20**, e3001837 (2022).
495 6. Álvarez-López, A. I. *et al.* Differences of the 6N and 6J Substrains of C57BL/6 Mice in the
496 Development of Experimental Autoimmune Encephalomyelitis. *MedComm* **6**, e70228
497 (2025).

7. Mekada, K. & Yoshiki, A. Substrains matter in phenotyping of C57BL/6 mice. *Exp. Anim.* **70**, 145–160 (2021).
8. Lachaise, D. *et al.* Historical Biogeography of the *Drosophila melanogaster* Species Subgroup. in *Evolutionary Biology* (eds Hecht, M. K., Wallace, B. & Prance, G. T.) 159–225 (Springer US, Boston, MA, 1988). doi:10.1007/978-1-4613-0931-4_4.
9. Clark, A. G. *et al.* Evolution of genes and genomes on the *Drosophila* phylogeny. *Nature* **450**, 203–219 (2007).
10. McKenzie, J. A. & McKechnie, S. W. A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans*. *Oecologia* **40**, 299–309 (1979).
11. Markow, T. A. Host use and host shifts in *Drosophila*. *Curr. Opin. Insect Sci.* **31**, 139–145 (2019).
12. Legal, L., David, J. R. & Jallon, J. M. Toxicity and attraction effects produced by *Morinda citrifolia* fruits on the *Drosophila melanogaster* complex of species. *CHEMOECOLOGY* **3**, 125–129 (1992).
13. Auer, T. O., Shahandeh, M. P. & Benton, R. *Drosophila sechellia*: A Genetic Model for Behavioral Evolution and Neuroecology. *Annu. Rev. Genet.* **55**, 527–554 (2021).
14. Auer, T. O. *et al.* Olfactory receptor and circuit evolution promote host specialization. *Nature* **579**, 402–408 (2020).
15. Soto-Yéber, L., Soto-Ortiz, J., Godoy, P. & Godoy-Herrera, R. The behavior of adult *Drosophila* in the wild. *PLOS ONE* **13**, e0209917 (2018).
16. Silva-López, J., Godoy, P., Jara, L. & Godoy-Herrera, R. Interaction and integration among behaviors of adult *Drosophila* in nature. *PLOS ONE* **18**, e0278427 (2023).
17. Dukas, R. Natural history of social and sexual behavior in fruit flies. *Sci. Rep.* **10**, 21932 (2020).
18. Higa, I. & Fuyama, Y. Genetics of food preference in *Drosophila sechellia*. *Genetica* **88**, 129–136 (1993).
19. R'Kha, S., Capy, P. & David, J. R. Host-plant specialization in the *Drosophila melanogaster* species complex: a physiological, behavioral, and genetical analysis. *Proc. Natl. Acad. Sci.* **88**, 1835–1839 (1991).
20. Takagi, S. *et al.* Olfactory sensory neuron population expansions influence projection neuron adaptation and enhance odour tracking. *Nat. Commun.* **15**, 7041 (2024).
21. Prieto-Godino, L. L. *et al.* Evolution of Acid-Sensing Olfactory Circuits in Drosophilids. *Neuron* **93**, 661-676.e6 (2017).
22. Dürr, B. R. *et al.* Olfactory projection neuron rewiring in the brain of an ecological specialist. *Cell Rep.* **44**, (2025).
23. Semmelhack, J. L. & Wang, J. W. Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion. *Nature* **459**, 218–223 (2009).
24. Álvarez-Ocaña, R. *et al.* Odor-regulated oviposition behavior in an ecological specialist. *Nat. Commun.* **14**, 3041 (2023).
25. Amlou, M., Moreteau, B. & David, J. R. Genetic Analysis of *Drosophila sechellia* Specialization: Oviposition Behavior Toward the Major Aliphatic Acids of Its Host Plant. *Behav. Genet.* **28**, 455–464 (1998).

26. Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T. & Fuyama, Y. Odorant-Binding Proteins OBP57d and OBP57e Affect Taste Perception and Host-Plant Preference in *Drosophila sechellia*. *PLOS Biol.* **5**, e118 (2007).
27. Gou, B., Zhu, E., He, R., Stern, U. & Yang, C.-H. High Throughput Assay to Examine Egg-Laying Preferences of Individual *Drosophila melanogaster*. *J. Vis. Exp. JoVE* e53716 (2016) doi:10.3791/53716.
28. Benton, R. *et al.* An integrated anatomical, functional and evolutionary view of the *Drosophila* olfactory system. *EMBO Rep.* **26**, 3204–3225 (2025).
29. Dekker, T., Ibba, I., Siju, K. P., Stensmyr, M. C. & Hansson, B. S. Olfactory Shifts Parallel Superspecialism for Toxic Fruit in *Drosophila melanogaster* Sibling, *D. sechellia*. *Curr. Biol.* **16**, 101–109 (2006).
30. Wall, M. M., Miller, S. & Siderhurst, M. S. Volatile changes in Hawaiian noni fruit, *Morinda citrifolia L.*, during ripening and fermentation. *J. Sci. Food Agric.* **98**, 3391–3399 (2018).
31. Farine, J.-P., Legal, L., Moreteau, B. & Le Quere, J.-L. Volatile components of ripe fruits of *Morinda citrifolia* and their effects on *Drosophila*. *Phytochemistry* **41**, 433–438 (1996).
32. Legal, L., Chappe, B. & Jallon, J. M. Molecular basis of *Morinda citrifolia* (*L.*): Toxicity on drosophila. *J. Chem. Ecol.* **20**, 1931–1943 (1994).
33. Jones, C. D. The Genetic Basis of *Drosophila sechellia*'s Resistance to a Host Plant Toxin. *Genetics* **149**, 1899–1908 (1998).
34. Hungate, E. A. *et al.* A Locus in *Drosophila sechellia* Affecting Tolerance of a Host Plant Toxin. *Genetics* **195**, 1063–1075 (2013).
35. Andrade López, J. M. *et al.* Genetic basis of octanoic acid resistance in *Drosophila sechellia*: functional analysis of a fine-mapped region. *Mol. Ecol.* **26**, 1148–1160 (2017).
36. Ando, T. *et al.* Nanopore Formation in the Cuticle of an Insect Olfactory Sensillum. *Curr. Biol.* **29**, 1512-1520.e6 (2019).
37. Dworkin, I. & Jones, C. D. Genetic Changes Accompanying the Evolution of Host Specialization in *Drosophila sechellia*. *Genetics* **181**, 721–736 (2009).
38. Lanno, S. M. *et al.* Transcriptomic Analysis of Octanoic Acid Response in *Drosophila sechellia* Using RNA-Sequencing. *G3 GenesGenomesGenetics* **7**, 3867–3873 (2017).
39. Lanno, S. M. *et al.* Genomics Analysis of L-DOPA Exposure in *Drosophila sechellia*. *G3 GenesGenomesGenetics* **9**, 3973–3980 (2019).
40. Marconcini, M. *et al.* Intersecting experimental evolution and CRISPR screens to identify novel insecticide resistance loci. 2025.07.23.666417 Preprint at https://doi.org/10.1101/2025.07.23.666417 (2025).
41. Marconcini, M., Fragnière, C., Masuzzo, A. & Benton, R. Genome-wide association studies identify new candidate genes and tissues underlying resistance to a natural toxin in drosophilids. 2025.12.01.691102 Preprint at https://doi.org/10.64898/2025.12.01.691102 (2025).
42. Markow, T. A., Beall, S. & Matzkin, L. M. Egg size, embryonic development time and ovoviviparity in *Drosophila* species. *J. Evol. Biol.* **22**, 430–434 (2009).
43. Coyne, J. A., Rux, J. & David, J. R. Genetics of morphological differences and hybrid sterility between *Drosophila sechellia* and its relatives. *Genet. Res.* **57**, 113–122 (1991).

44. Orgogozo, V., Broman, K. W. & Stern, D. L. High-Resolution Quantitative Trait Locus Mapping Reveals Sign Epistasis Controlling Ovariole Number Between Two *Drosophila* Species. *Genetics* **173**, 197–205 (2006).
45. Jones, C. D. Genetics of egg production in *Drosophila sechellia*. *Heredity* **92**, 235–241 (2004).
46. Green, D. A. & Extavour, C. G. Convergent evolution of a reproductive trait through distinct developmental mechanisms in *Drosophila*. *Dev. Biol.* **372**, 120–130 (2012).
47. Green, D. A. & Extavour, C. G. Insulin signalling underlies both plasticity and divergence of a reproductive trait in Drosophila. *Proc. R. Soc. B Biol. Sci.* **281**, 20132673 (2014).
48. Lavista-Llanos, S. *et al.* Dopamine drives *Drosophila sechellia* adaptation to its toxic host. *eLife* **3**, e03785.
49. Shahandeh, M. P. *et al.* Circadian plasticity evolves through regulatory changes in a neuropeptide gene. *Nature* **635**, 951–959 (2024).
50. Hardeland, R. & Stange, G. Comparative studies on the circadian rhythms of locomotor activity of 40 *Drosophila* species. *J. Interdiscip. Cycle Res.* **4**, 353–359 (1973).
51. Beauchamp, M. *et al.* Closely Related Fruit Fly Species Living at Different Latitudes Diverge in Their Circadian Clock Anatomy and Rhythmic Behavior. *J. Biol. Rhythms* **33**, 602–613 (2018).
52. Rieger, D., Peschel, N., Dusik, V., Glotz, S. & Helfrich-Förster, C. The ability to entrain to long photoperiods differs between 3 *Drosophila melanogaster* wild-type strains and is modified by twilight simulation. *J. Biol. Rhythms* **27**, 37–47 (2012).
53. Chiu, J. C., Low, K. H., Pike, D. H., Yildirim, E. & Edery, I. Assaying Locomotor Activity to Study Circadian Rhythms and Sleep Parameters in *Drosophila*. *JoVE J. Vis. Exp.* e2157 (2010) doi:10.3791/2157.
54. Burke, M. K. & Rose, M. R. Experimental evolution with *Drosophila*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R1847-1854 (2009).
55. Turner, T. L., Miller, P. M. & Cochrane, V. A. Combining genome-wide methods to investigate the genetic complexity of courtship song variation in *Drosophila melanogaster*. *Mol. Biol. Evol.* **30**, 2113–2120 (2013).
56. McBride, C. S. Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. *Proc. Natl. Acad. Sci.* **104**, 4996–5001 (2007).
57. Lee, D., Shahandeh, M. P., Abuin, L. & Benton, R. Comparative single-cell transcriptomic atlases of drosophilid brains suggest glial evolution during ecological adaptation. *PLOS Biol.* **23**, e3003120 (2025).
58. Prieto-Godino, L. L., Schmidt, H. R. & Benton, R. Molecular reconstruction of recurrent evolutionary switching in olfactory receptor specificity. *eLife* **10**, (2021).
59. Mermet, J. *et al.* Multilayer regulation underlies the functional precision and evolutionary potential of the olfactory system. *Nat. Commun.* **16**, 9514 (2025).
60. Pischedda, A., Stewart, A. D., Little, M. K. & Rice, W. R. Male genotype influences female reproductive investment in *Drosophila melanogaster*. *Proc. Biol. Sci.* **278**, 2165–2172 (2011).
61. Pfeiffenberger, C., Lear, B. C., Keegan, K. P. & Allada, R. Locomotor activity level monitoring using the *Drosophila* Activity Monitoring (DAM) System. *Cold Spring Harb. Protoc.* **2010**, pdb.prot5518 (2010).

62. Geissmann, Q., Rodriguez, L. G., Beckwith, E. J. & Gilestro, G. F. Rethomics: An R framework to analyse high-throughput behavioural data. *PLOS ONE* **14**, e0209331 (2019).
63. R Core Team (2013) R A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. - References - Scientific Research Publishing. https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1787696.
64. Wickham, H. Data Analysis. in *ggplot2: Elegant Graphics for Data Analysis* (ed. Wickham, H.) 189–201 (Springer International Publishing, Cham, 2016). doi:10.1007/978-3-319-24277-4_9.

Figures and legends

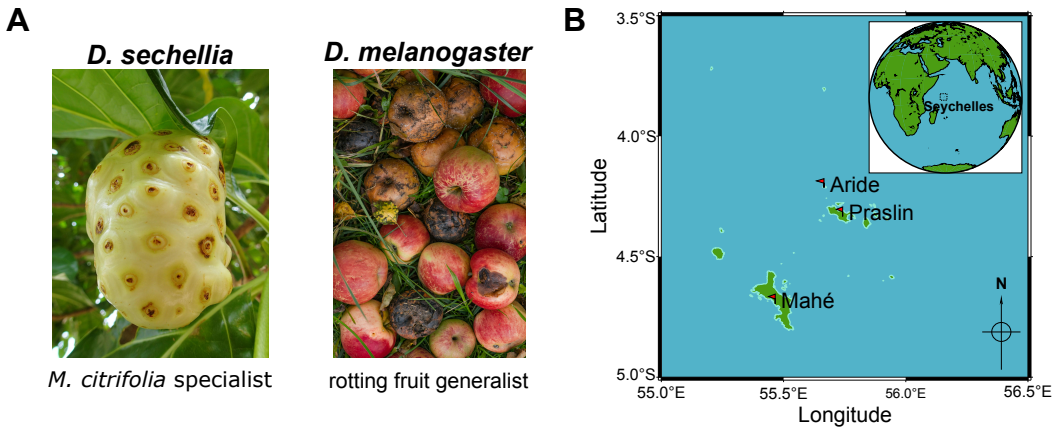


Figure 1. *D. sechellia* is a *M. citrifolia* specialist endemic to an equatorial island archipelago.

A. Host preferences of *D. melanogaster* and *D. sechellia*. **B.** A map of the Seychelles archipelago with specific coordinates of wild *D. sechellia* strain collection sites on three islands marked with red flags (Mahé Island coordinates: 4.69424° S, 55.46580° E, Aride Island coordinates: 4.21423° S, 55.66555° E, and Praslin Island coordinates: 4.33340° S, 55.74180° E). Upper-right inset shows the location of the Seychelles on the eastern hemisphere. The maps were generated using Generic Mapping Tools (GMT) version 6.6.0 (Wessel et al 2019).

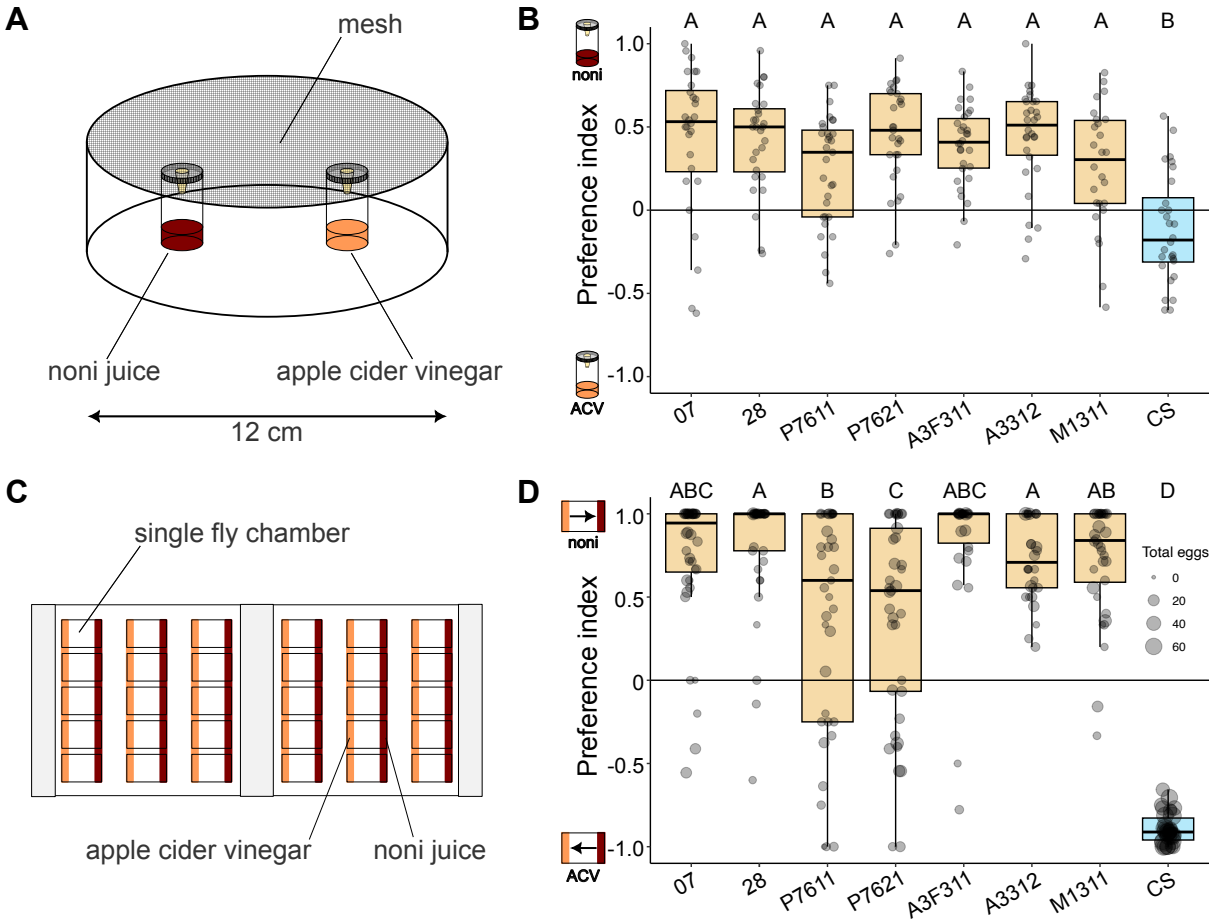


Figure 2. *D. sechellia* display strong olfactory preference for noni in short-range olfactory and oviposition assays.

A. Schematic of the short-range olfactory preference assay.

B. Short-range olfactory preference of wild and laboratory *D. sechellia* strains and *D. melanogaster* Canton-S (CS). All *D. sechellia* strains displayed a significant preference for noni juice over apple cider vinegar (all $p < 0.01$, Wilcoxon test), while CS displayed no significant preference. Sample sizes: 07 (28), 28 (28), P7611 (31), P7621 (29), A3F311 (30), A3312 (30), M1311 (26), and CS (28).

C. Schematic of the single-fly oviposition preference assay.

D. Single-fly oviposition preference for the same strains. Point size is scaled by the total number of eggs laid per female. All *D. sechellia* strains displayed a significant preference for noni juice over apple cider vinegar (all $p < 0.01$, Wilcoxon test), while CS displayed a significant preference for apple cider vinegar ($p < 0.001$, Wilcoxon test). Sample sizes: 07 (40), 28 (39), P7611 (37), P7621 (37), A3F311 (30), A3312 (30), M1311 (36), and CS (34).

For panels **B** and **D**, letters above boxplots indicate pairwise significant differences (all $p < 0.01$, Wilcoxon test followed by Bonferroni correction).

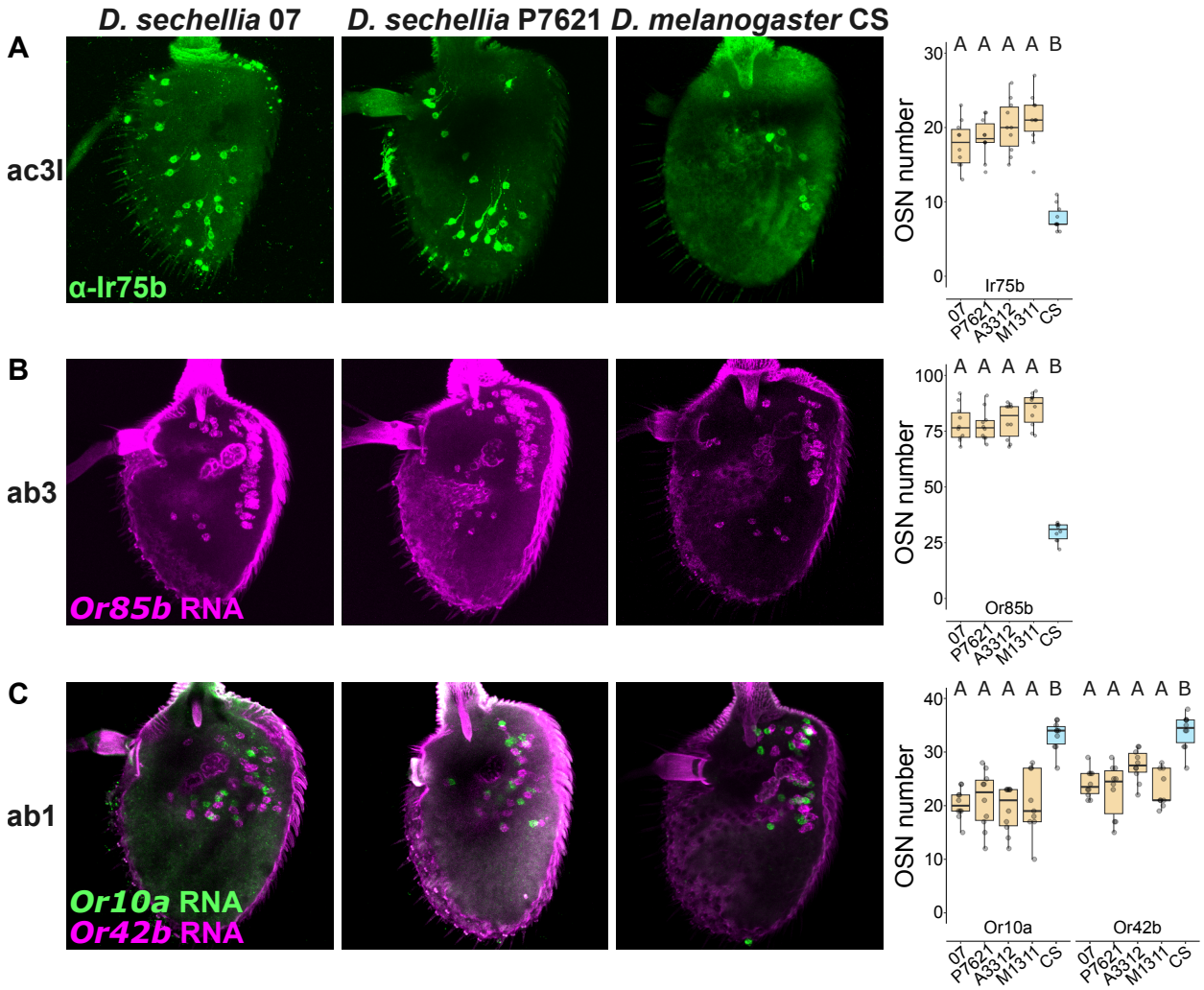


Figure 3. Olfactory sensory neuron population expansion and contraction in *D. sechellia*.

A. Left: example images of Ir75b OSNs in ac3I sensilla of the third antennal segment of laboratory *D. sechellia* (07), wild *D. sechellia* (P7612), and *D. melanogaster* (CS). Right: quantifications of Ir75b OSNs in laboratory *D. sechellia* (07), *D. melanogaster* (CS), and three wild *D. sechellia strains*, one from each island (P7612, A3312, M1311).

B. Left: example images of Or85b OSNs in ab3 sensilla for the same strains as in panel **A**. Right: quantifications of Or85b OSNs for the same strains as in panel **A**.

C. Left: example images of Or10a and Or42b OSNs labelled in ab1 sensilla for the same strains as in panel **A**. Right: quantifications of Or10a and Or42b OSNs for the same strains as panels **A**.

For **A**-**C**, letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction).10 antennae were imaged and quantified for each sensillum type per strain.

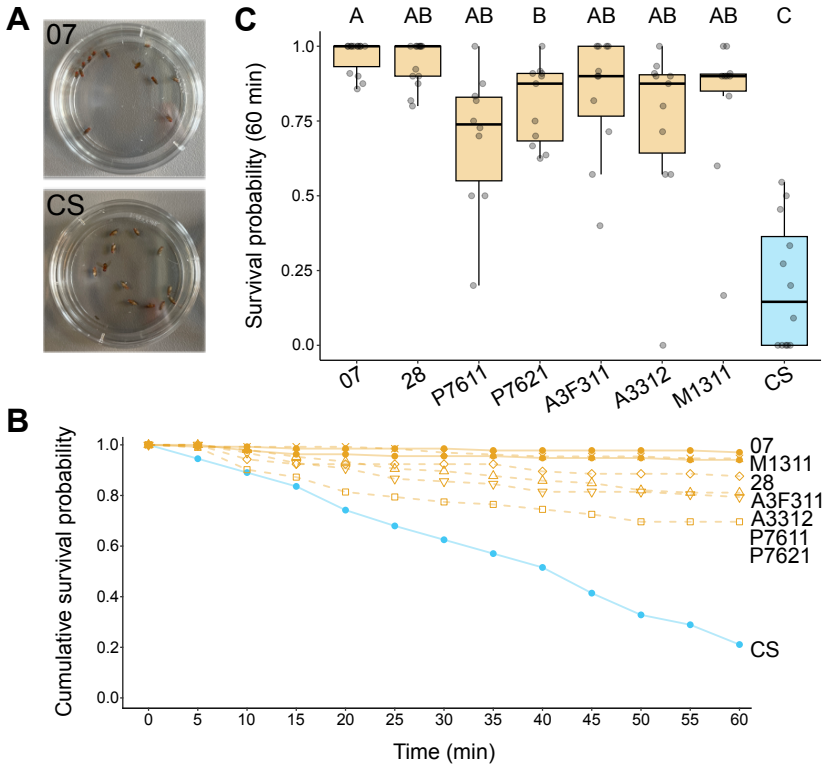


Figure 4. *D. sechellia* adult females display strong resistance to the noni toxin octanoic acid.

A. Photographs of a single replicate of laboratory *D. sechellia* (07) and *D. melanogaster* (CS) strains following 60 min of exposure to pure octanoic acid.

B. Cumulative survival probabilities (mean proportion of living flies across all replicates) of laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS is shown for 5 min increments over a 60-min exposure duration.

C. Final survival probability (proportion of living flies after 60 min of exposure to octanoic acid) for each replicate is shown for wild and laboratory *D. sechellia* strains and *D. melanogaster*. Letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction). Sample sizes: 07 (14), 28 (13), P7611 (10), P7621 (11), A3F311 (11), A3312 (11), M1311 (10), and CS (12).

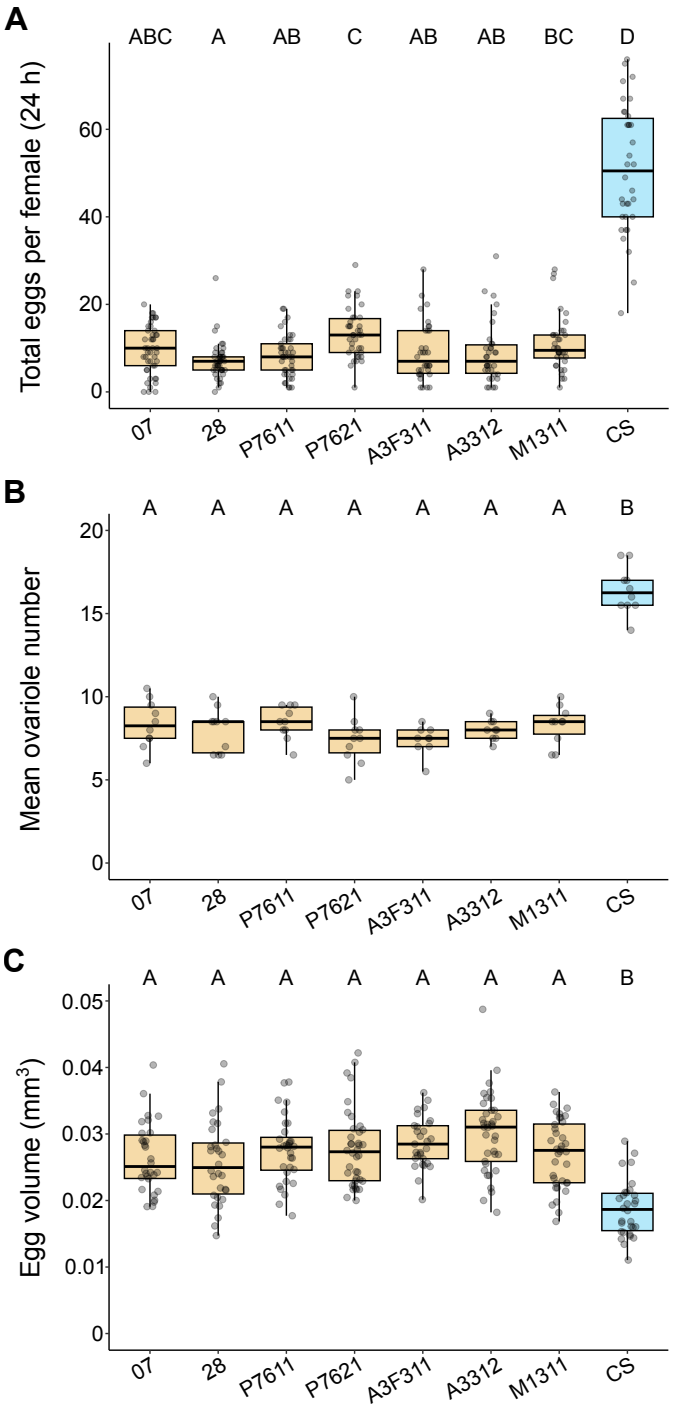


Figure 5. *D. sechellia* lay fewer, larger eggs, and display a reduced ovariole number compared to *D. melanogaster*.

A. The total eggs laid per female in a 24-h period is shown for laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS. Total eggs laid per female was obtained from the experiments shown in panels 2C-D.

B. The volume of individual eggs for the same strains as panel **A**. Sample sizes: 07 (30), 28 (30), P7611 (32), P7621 (38), A3F311 (29), A3312 (37), M1311 (36), and CS (30).

C. The mean number of ovarioles per ovary for individual adult female flies is shown for the same strains as panels **A** and **B**. Sample sizes: 07 (10), 28 (10), P7611 (10), P7621 (10), A3F311 (9), A3312 (9), M1311 (10), and CS (10).

For **A**-**C**, letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction).

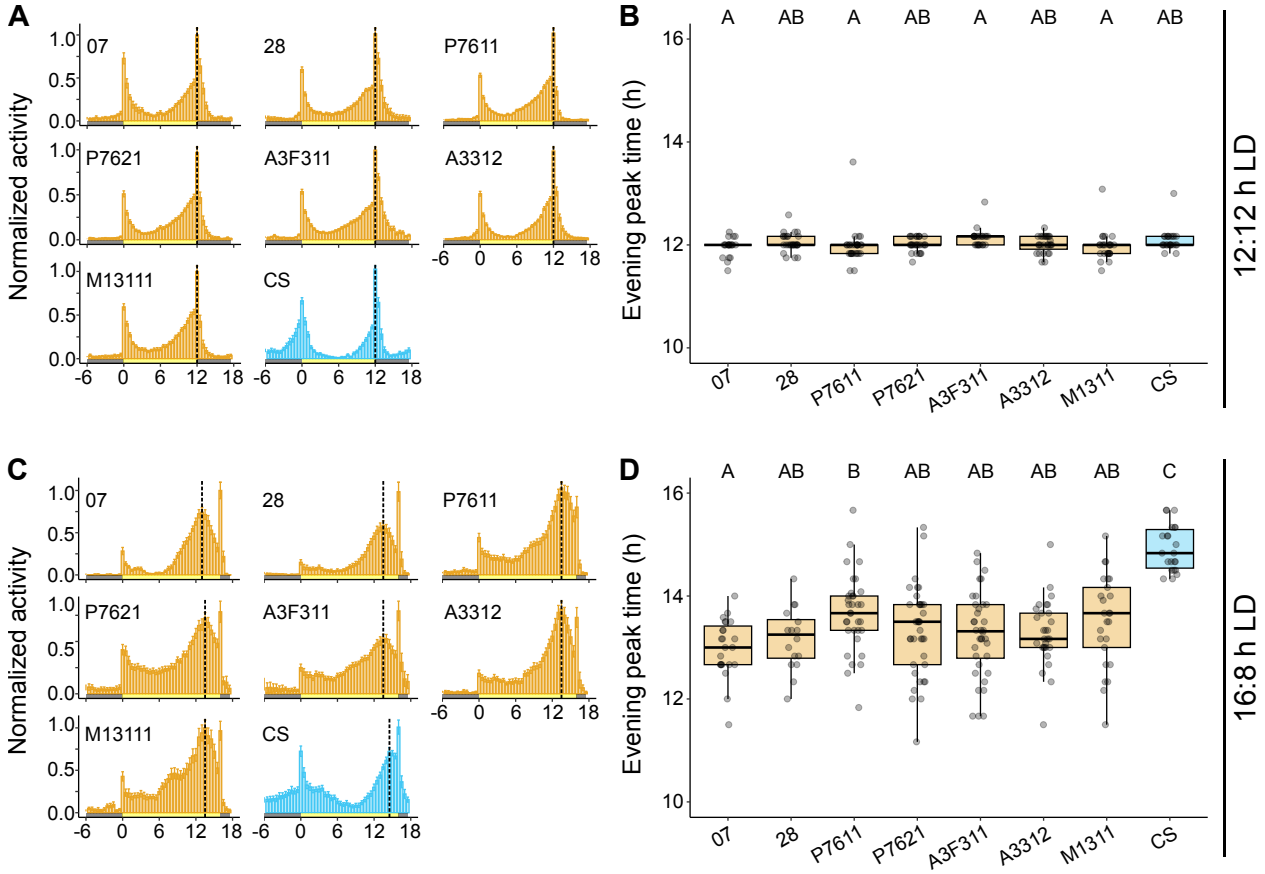


Figure 6. *D. sechellia* displays reduced circadian plasticity during long days.

A. Maximum normalized average activity plots for laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS under a 12:12 h photoperiod.

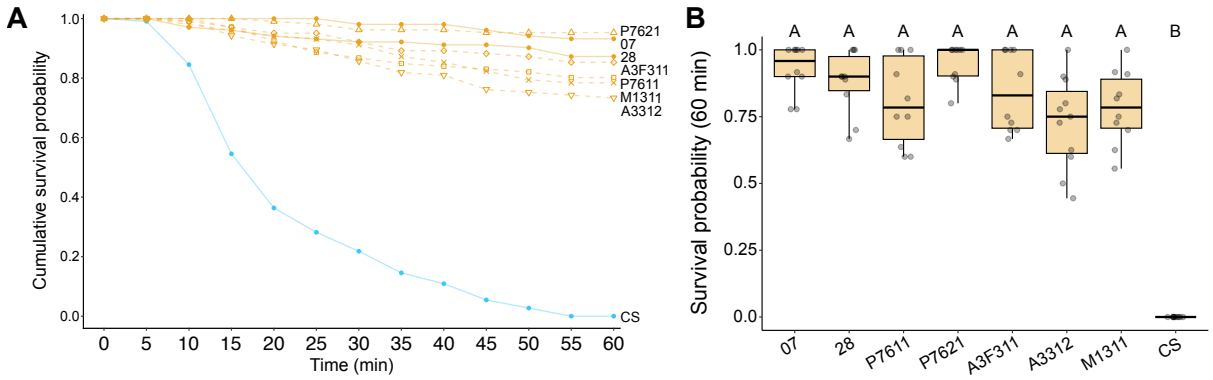
B. Mean evening peak time for the last 4 days of a 7-day period under 12:12 h photoperiod conditions is shown for individual flies for the same strains as panel **A**. Sample sizes for panels **A** and **B**: 07 (21), 28 (28), P7611 (29), P7621 (28), A3F311 (24), A3312 (27), M1311 (26), and CS (24).

C. Maximum normalized average activity plots for the same strains as panels **A** and **B** under a 12:12 h photoperiod.

D. Mean evening peak time for the last 4 days of a 7-day period under 16:8 h photoperiod conditions is shown for individual flies for the same strains as panels **A**-**C**. Sample sizes for panels **C** and **D**: 07 (23), 28 (16), P7611 (34), P7621 (37), A3F311 (36), A3312 (29), M1311 (25), and CS (22).

For **A** and **C**, histograms display maximum normalized activity of flies in 30-min bins across a 24-h period. Error bars denote standard error of the mean. Grey and yellow bars along the x-axis represent lights-off and lights-on in the incubator, respectively. Vertical dashed lines represent the average evening peak time.

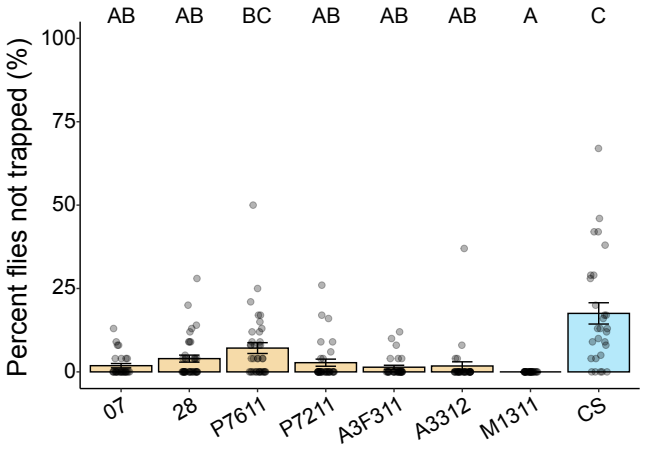
For **B** and **D**, letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction).



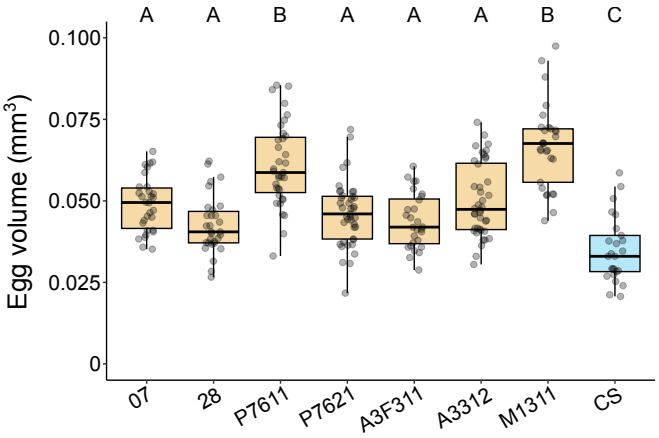
Supplemental Figure 1. *D. sechellia* adult males display strong resistance to the noni toxin octanoic acid.

A. Cumulative survival probabilities (mean proportion of living flies across all replicates) of laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS is shown for 5-min increments over a 60-min exposure duration.

B. Final survival probability (proportion of living flies after 60 min of exposure to octanoic acid, y-axis) for each replicate is shown for wild and laboratory *D. sechellia* strains and *D. melanogaster* (x-axis). Letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction). Sample sizes: 07 (10), 28 (10), P7611 (10), P7621 (10), A3F311 (10), A3312 (11), M1311 (10), and CS (10).



Supplemental Figure 2. Percent of flies not trapped during short-range olfactory trap assay. The percent of flies that were found in neither the noni juice nor apple cider vinegar trap for each replicate for laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS. Bar plots represent mean values and error bars denote standard error of the mean. Letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction). Sample sizes are provided in Figure 2 legend.



Supplemental Figure 3. Egg volume quantified by a second observer confirms *D. sechellia* lays larger eggs than *D. melanogaster*. The volume of individual eggs is displayed for laboratory *D. sechellia*, wild *D. sechellia*, and *D. melanogaster* CS. Letters above boxplots indicate pairwise significant differences (all $p < 0.05$, Wilcoxon test followed by Bonferroni correction). Sample sizes: 07 (30), 28 (30), P7611 (35), P7621 (41), A3F311 (28), A3312 (40), M1311 (30), and CS (25).