High sugar diets can increase susceptibility to bacterial infection in *Drosophila melanogaster*

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Abstract

Overnutrition with dietary sugar can worsen infection outcomes in diverse organisms including insects and humans, generally through unknown mechanisms. In the present study, we show that adult *Drosophila melanogaster* fed high-sugar diets became more susceptible to infection by the Gram-negative bacteria *Providencia rettgeri* and *Serratia marcescens,* although diet had no significant effect on infection by Gram-positive bacteria *Enterococcus faecalis* or *Lactococcus lactis.* We found that *P. rettgeri* and *S. marcescens* proliferate more rapidly in *D. melanogaster* fed a high-sugar diet, resulting in increased probability of host death. *D. melanogaster* become hyperglycemic on the high-sugar diet, and we find evidence that the extra carbon availability may promote *S. marcescens* growth within the host. However, we found no evidence that increased carbon availability directly supports greater *P. rettgeri* growth. *D. melanogaster* on both diets fully induce transcription of antimicrobial peptide (AMP) genes in response to infection, but *D. melanogaster* provided with high-sugar diets show reduced production of AMP protein. Thus, overnutrition with dietary sugar may impair host immunity at the level of AMP translation. Our results demonstrate that dietary sugar can shape infection dynamics by impacting both host and pathogen, depending on the nutritional requirements of the pathogen and by altering the physiological capacity of the host to sustain an immune response.

Author Summary

Diet has critical impact on the quality of immune defense, and high-sugar diets increase susceptibility to bacterial infection in many animals. Yet it is unknown which aspects of host and pathogen physiology are impacted by diet to influence infection dynamics. Here we show that high-sugar diets increase susceptibility to some, but not all, bacterial infections in *Drosophila*. We find that feeding on high sugar diet impairs the host immune response by reducing the level

of antimicrobial peptides produced. The expression of genes encoding these peptides is not affected, so we infer that protein translation is impaired. We further show that flies on high-sugar diets are hyperglycemic, and that some pathogens may use the excess sugar in the host to promote growth during the infection. Thus, our study demonstrates that dietary impacts on infection outcome arise through physiological effects on both the host and pathogen.

Introduction

Nutritional status and diet are important factors for immune defense in both mammals and insects (1–5). Hyperglycemia and diets excessively high in sugar have adverse effects on metabolic homeostasis and infection outcome (6–10). For example, human patients admitted to the intensive care unit with sepsis have a higher probability of death if they have elevated blood sugar (11) and non-diabetic hyperglycemia increases the severity of *Mycobacterium tuberculosis* infections in guinea pigs (1). A similar phenomenon is observed in the fruit fly *Drosophila melanogaster*, where adults reared on high sugar diets experience higher pathogen burden (3) and increased mortality from systemic bacterial infection (2,12). Despite these clear effects of diet and nutritional state on infection outcome, the molecular and physiological mechanisms by which high sugar impacts infections is often unclear. By uncovering these mechanisms, we can clarify the role that diet has on shaping organismal physiology during an active infection and identify strategies to alleviate consequences of infection that are exacerbated by obesogenic diets.

Drosophila is an excellent model to understand the mechanisms by which high-sugar diets affect infection dynamics, with extensive prior study on infection, diet, and metabolism (e.g., 13–16). Lacking adaptive immune systems, *Drosophila* rely on innate immune defenses that include humoral production and secretion into circulation of antimicrobial peptides (AMP) and cytokines secreted that share homologous function with innate immune defenses in mammals and other

72 invertebrate species (17,18). The Toll and the immune deficiency (IMD) pathways are the two
73 major regulators of the *Drosophila* humoral immune response to infection (14). Gram-positive
74 bacterial and fungal infections predominantly stimulate Toll activity (19,20) while Gram-negative
75 bacterial infections activate the IMD pathway (19,21). Once activated, both pathways lead to the
76 nuclear translocation of nuclear factor-κB (NF-κB) family transcription factors to drive expression
77 of hundreds of infection-responsive genes, including those encoding antimicrobial peptides
78 (22,23). Infection and/or constitutive activation of these pathways stimulates major shifts in
79 metabolic processes to support the immune response, including suppressed insulin signaling,
80 reduction in energetic stores like glycogen and triglycerides, and alterations in carbohydrate and
81 lipid metabolism (24–28).

82 Both high-sugar diets and infection alter metabolism and energetic usage in *Drosophila* (29).
83 In the absence of infection, high-sugar diets can also upregulate genes involved in the immune
84 response, including antimicrobial peptides (12,30), which is similar to high levels of dietary
85 sugar stimulating low-grade inflammation responses in humans (31). Additionally, uninfected
86 *Drosophila* larvae reared on high-sugar diets exhibit impaired melanization and reduced
87 phagocytic capacity of fungal spores (12,32). While there is evidence that high-sugar diets affect
88 the *Drosophila* immune response in uninfected states, it is less clear whether high-sugar diets
89 impact immune activity during a live infection. Resistance to infection can be measured as
90 immune system activity or control of pathogen burden (33). Feeding *Drosophila* high-glucose
91 diets results in higher pathogen burden after systemic infection with the Gram-negative bacteria
92 *Providencia rettgeri* (2,3) and *Pseudomonas aeruginosa* (6). This could suggest that high-sugar
93 diets impair host immune system activity, but *Pseudomonas aeruginosa* is additionally able to
94 use glucose in hyperglycemic hosts to establish higher pathogen burdens (6). Thus, it has been
95 difficult to discern whether elevated pathogen burden in *Drosophila* provided with high-sugar
96 diets is due to impaired host immune responses or to bolstered pathogen growth capacity.

97 These are not mutually exclusive alternatives. Furthermore, prior *D. melanogaster* studies that
98 investigated the effects of high dietary sugar on systemic infection outcome were performed
99 using flies that were reared throughout their entire development on high-sugar diets (2,3,12,32).
100 Rearing on elevated dietary sugar diets causes hyperglycemia, lipidemia, and reduced body
101 weight in both larvae and as adults, and prolongs larval developmental time (12,34). Thus, it is
102 difficult to determine in those experiments whether effects of diet on adult infection outcome
103 result from altered metabolism and immunity or from indirect consequences of altered
104 development.

105 In the present study, we specifically focus on the immediate effects of high dietary sugar on
106 infection dynamics in adult *D. melanogaster*. We do this by rearing *D. melanogaster* larvae on a
107 common base diet, then switching adults to diets that vary in sugar content prior to delivering
108 systemic bacterial infection. To determine whether adverse effects from high-sugar diets are
109 universal across pathogenic infections, we test infection with four different bacterial pathogens
110 representing Gram-positive and Gram-negative bacteria. We find that flies provided with high-
111 sugar diet as adults are particularly susceptible to Gram-negative bacterial infection, suffering
112 increased mortality and more rapid pathogen proliferation during the early hours of infection. We
113 find evidence that increased carbon availability in high-sugar hosts can accelerate the growth of
114 certain pathogens, and that high-sugar diets impaired immune system function at the level of
115 antimicrobial peptide translation without affecting transcription levels.

116

117 **Results**

118 **High-sucrose diets increase mortality from infection with some bacterial pathogens**

119 We first wanted to test whether high-sugar diets reduce immune defense in adult *D.*
120 *melanogaster* independent of any developmental consequences. We therefore reared larvae on

121 a yeast-cornmeal diet containing 4% sucrose, then split the population across the experimental
122 diets during the non-feeding pupal stage such that eclosing adults emerged onto experimental
123 diets that were 0%, 2%, 4%, 8%, 16%, or 24% (w/v) sucrose (Fig. 1A), which covers the range
124 used in prior studies (3,12,32). After 3-5 days on the experimental diets, adult female flies were
125 given a systemic infection with one of four bacteria: *Providencia rettgeri*, *Serratia marcescens,*
126 *Enterococcus faecalis, Lactococcus lactis*. These bacteria were chosen as natural pathogens of
127 *D. melanogaster* (35,36) and include both Gram-positive and Gram-negative bacteria, which are
128 respectively expected to predominantly activate the Toll and IMD immune response pathways
129 (19).

130 We found that flies provided with high-sucrose diets experienced higher mortality after
131 infection with the Gram-negative bacteria *S. marcescens* and *P. rettgeri* than flies fed low sugar
132 (Figs. 1B and 1C). This increased mortality occurred in a concentration-dependent manner
133 depending on the pathogen. Flies provided with 0%, 2%, or 4% sucrose experienced low
134 mortality and flies provided with 16% or 24% sucrose experienced high mortality after infection
135 with both pathogens. However, on the 8% sucrose diet, flies infected with *S. marcescens*
136 exhibited high survival (Fig. 1B), but flies infected with *P. rettgeri* exhibited low survival (Fig.
137 1C). Overall, there was no effect of diet on survivorship of infection with the Gram-positive
138 bacteria *L. lactis* and *E. faecalis* (Figs. 1D and 1E). Although flies provided with the 24%
139 sucrose diet experienced the highest mortality after *L. lactis* infection (Fig. 1D), we attribute this
140 to the diet being generally poor as sham-infected control flies fed on the 24% sucrose diet also
141 experienced elevated mortality (Fig. S1A). Varying the diet of the host can alter the composition
142 of the gut microbiota (37–39), which can yield effects on the host's metabolic status (40–42).
143 Flies provided with 16% sucrose in our experiments showed a reduced abundance of gut flora
144 compared to flies provided with 2% sucrose diet (Fig S2A). However, we found that high-sugar
145 diets increased the probability of death from systemic *P. rettgeri* infection even in axenic flies

with no microbiota (Fig S2B), suggesting that our observed effect of diet on immunity is not mediated by effects on gut flora.

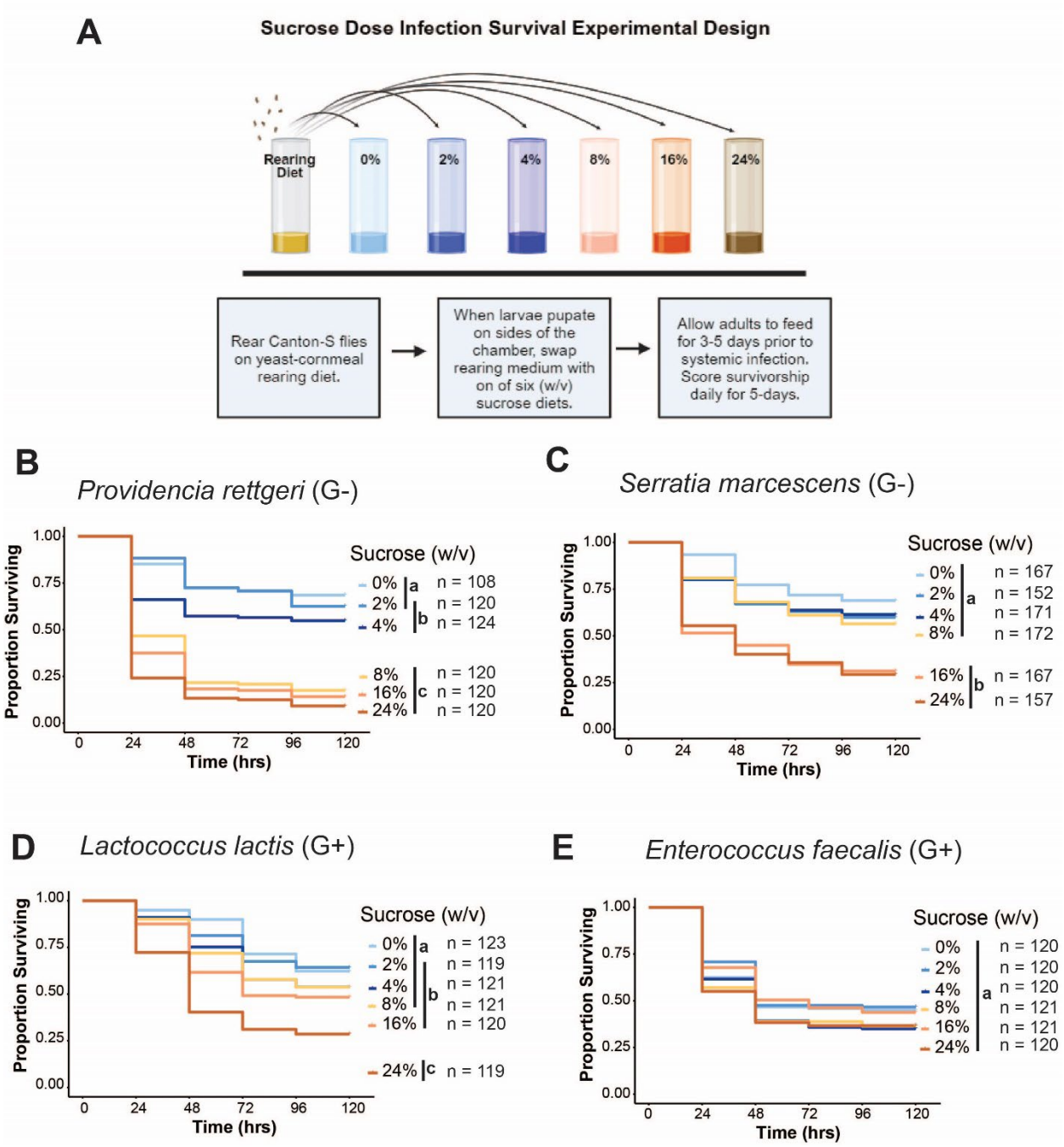
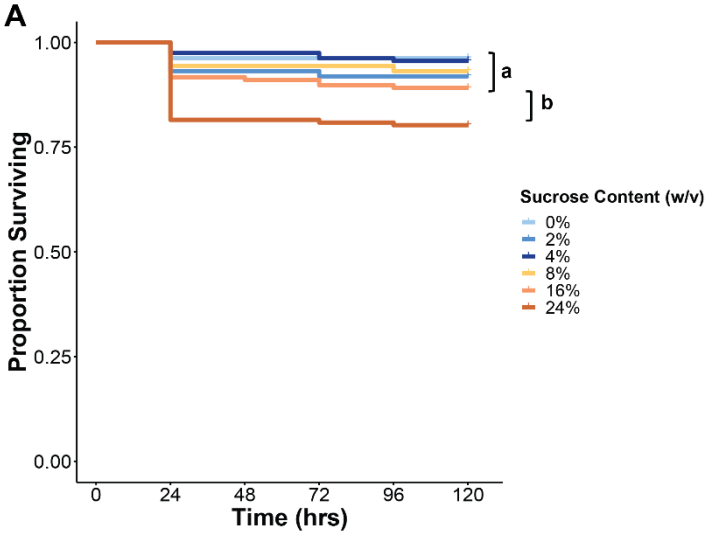
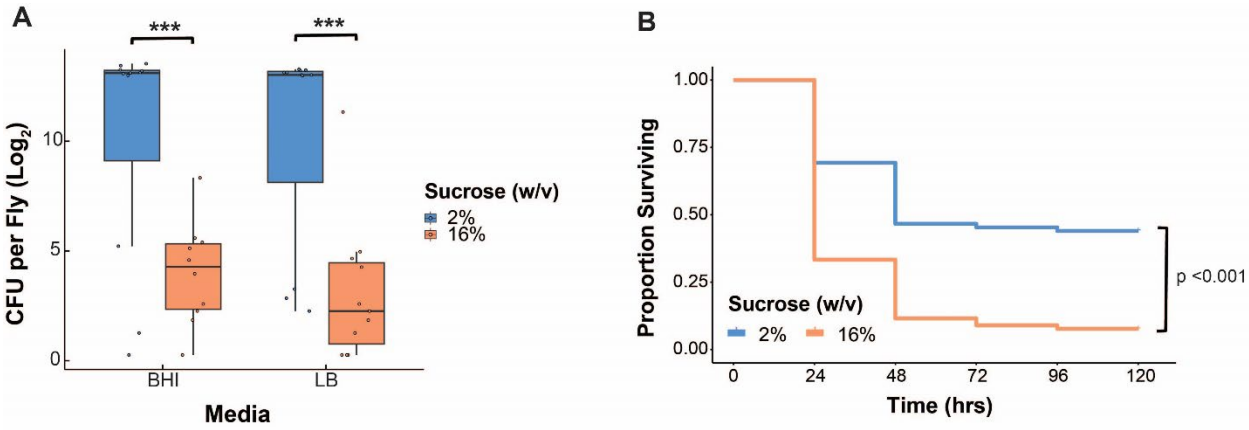


Fig 1. High-sugar diet increases mortality after infection with some pathogens. (A) Experimental setup for assaying effects of high-sugar diets on adult survival of infection. The six experimental diets used have the same yeast and cornmeal content, but they varied in sucrose (w/v). Diets with 0%, 2%, and 4% are represented in shades of blue while diets with 8%, 16%, 24% sucrose are represented in shades of orange. The rearing diet contains 4% sucrose. (B) Flies fed 8%, 16%, and 24% sucrose diets exhibit

154 significantly higher mortality after *Providencia rettgeri* infection than flies given 0%, 2%, and 4% sucrose
155 diets (p<0.0001; Cox Mixed Effects Model). There was no difference in survival among flies provided with
156 the three high-sugar diets, while flies provided with the 4% sucrose diet exhibited significantly lower
157 survivorship than flies provided with 0% added sucrose (p = 0.035; Cox Mixed Effects Model). (C) Flies
158 fed 16% and 24% sucrose diets exhibit significantly higher mortality after *Serratia marcescens* than flies
159 given diets with 8% sucrose or lower (p<0.0001; Cox Mixed Effects Model). (D) Flies fed the 24% sucrose
160 diet have the highest mortality after *Lactococcus lactis* infection compared to all other diets (p<0.001, Cox
161 Mixed Effects Model). Flies fed 16% sucrose died significantly faster than flies fed 0% sucrose diets (p =
162 0.047; Cox Mixed Effects Model) but there were no other significant pairwise differences in survivorship
163 among flies provided the 16% sucrose diet or lower. (E) There was no effect of diet on survivorship after
164 infection with *Enterococcus faecalis*. Letters denote significant pairwise differences between diets
165 (p<0.05).



166 **Fig. S1**. A) Survivorship of PBS injury controls across all infection experiments pooled together.
167 Flies fed 24% (w/v) sucrose diet have higher proportion of death after sham-infection with sterile
168 PBS compared to all other diets (p<0.05; Cox Proportional Hazards Mixed effects model).



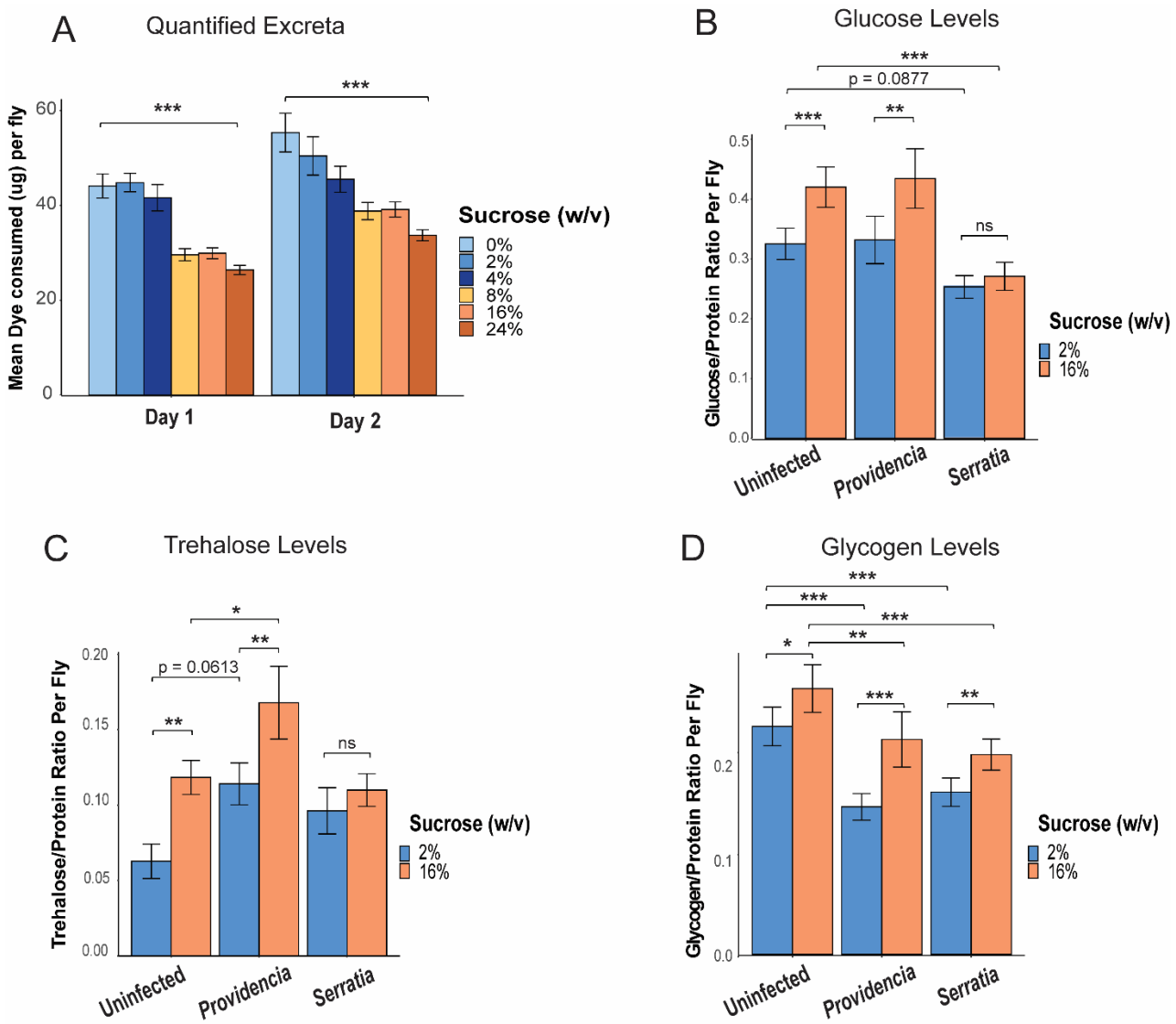
169
170 **Fig. S2**. A) To measure the abundance of the endogenous gut microbiota in uninfected flies fed on
171 the 2% (w/v) sucrose or 16% sucrose diets, 10 whole flies were pooled and homogenized in 250 µl
172 of PBS, and 50 µl of the homogenate was plated on lysogeny broth (LB) or brain heart infusion (BHI)
173 agar. Flies fed on the 2% diets exhibit higher microbiota loads on LB (p<0.001, n = 15 pools of 10
174 flies per diet) and BHI (p<0.001, n = 15 pools of 10 flies per diet) plates than flies fed 16% sucrose.
175 C) Axenic flies were infected with *P. rettgeri*, and flies fed 16% sucrose diets exhibited higher
176 mortality than flies given 2% sucrose (p<0.001; Cox Proportional Hazards model).

177 **Flies fed on high-sucrose diets become hyperglycemic despite eating less**

178 *D. melanogaster* will modulate feeding behavior depending on the content of their diet (43–
179 45), and the nutritional composition of the diet influences metabolic state (3,34,46). We
180 therefore first assessed whether varying sucrose levels in the diet impacts feeding rate. We
181 performed an excreta quantification assay (ExQ) (47) on uninfected females to measure feeding
182 across the six experimental diets shown in Figure 1A. Flies were housed in ExQ chambers over
183 a 2-day period with experimental diets containing the dye erioglaucine (1% w/v) and their
184 excreta was collected every 24-hours. We found that flies ate significantly more of the three
185 diets with the lowest sucrose (0%, 2%, and 4%) than the three diets with the highest sucrose
186 (8%, 16%, 24%; $p < 0.0001$, Fig. 2A). There was no significant difference in feeding rate among
187 the three lowest-sucrose diets or among the three highest-sucrose diets. In subsequent
188 experiments, we focus on the 2% and 16% sucrose diets as representative the low- and high-
189 sugar diets.

190 To assess the effects of diet on the metabolic status of adults, we measured glucose,
191 trehalose, glycogen, and soluble protein in the whole body of uninfected flies provided with
192 either 2% or 16% sucrose diets. We found that uninfected flies fed the high-sucrose diet exhibit
193 higher levels of glucose ($p < 0.001$, Fig. 2B), trehalose ($p = 0.001$, Fig. 2C), and glycogen (Fig.
194 2D, $p= 0.0479$) than flies fed the low-sugar diet (Fig. 2B-D). Given that infection can stimulate
195 shifts in carbohydrate levels (26,48), we also tested whether flies fed high-sugar continue to
196 exhibit elevated carbohydrate levels after infection. We indeed found that flies fed the high-
197 sucrose diet exhibited higher levels of glucose ($p<0.01$, Fig. 2B), trehalose ($p < 0.01$, Fig. 2C),
198 and glycogen ($p =0.019$, Fig. 2C) than flies fed the low-sucrose diet at 24 hours after *P. rettgeri*
199 infection. Interestingly, there was no difference in glucose content (Fig. 2B) or trehalose content
200 (Fig. 2C) between flies fed low-sucrose or high-sucrose at 24 hours after infection with *S.*
201 *marcescens*. However, flies given a high-sucrose diet exhibited higher glycogen stores after *S.*

202 *marcescens* infection than flies given low sucrose (p = 0.035 Fig. 2D). Despite reduced feeding
203 on high sugar, flies on the high-sucrose diet still sustain higher levels of carbohydrate stores in
204 both uninfected and infected states.



205
206 **Fig. 2.** Flies fed high sugar sustain higher carbohydrate stores despite reduced feeding. (A) Excreta
207 quantification of uninfected female flies fed on diets ranging from 0%-24% sucrose was measured
208 over two days. Flies feeding on the 8%, 16%, and 24% diets consumed significantly lower dye than
209 those fed on the 0%, 2%, 4% sucrose diets (p < 0.001). (B) Uninfected flies fed on the 16% diet had
210 higher glucose levels compared to flies fed on 2% sucrose (p < 0.001; ANOVA with Tukey HSD
211 post-hoc). After 24 hours of *P. rettgeri* infection, flies fed on high sugar continued to exhibit higher
212 glucose levels compared to flies fed 2% sucrose (p < 0.01; ANOVA with Tukey HSD post-hoc).
213 However, there was no difference in glucose levels between flies provided with 2% and 16% diets
214 24-hours after *S. marcescens* infection (p=0.658). Flies fed 16% sucrose exhibit significantly
215 reduced glucose levels after *S. marcescens* infection (p<0.001), and there was a nearly significant
216 decrease in glucose levels of flies fed 2% sucrose after *S. marcescens* infection (p = 0.0877) (C)
217 Trehalose levels were significantly higher in flies fed 16% sucrose than in flies fed 2% sucrose when

the flies were uninfected ($p<0.001$) or when they were infected with *P. rettgeri* ($p<0.01$). However, *S. marcescens* infection had no effect on trehalose levels between flies given the 2% and 16% diets ($p = 0.38$). *P. rettgeri* infection led to higher trehalose levels in flies on both 2% ($p = 0.0554$) and 16% ($p = 0.0446$) sucrose diets compared to uninfected flies, while *S. marcescens* had no effect on trehalose levels on either diet. (D) Glycogen levels were significantly higher in flies on 16% versus 2% diets when flies were uninfected ($p = 0.0479$), infected with *P. rettgeri* ($p = 0.019$), or infected with *S. marcescens* ($p = 0.035$). On both diets, infection with both pathogens led to reduced glycogen levels compared to uninfected (2% *Providencia*, $p<0.01$; 16% *Providencia,* $p = 0.036$; 2% *Serratia*, $p<0.01$; 16% *Serratia*, $p < 0.01$). Legend for panel figure: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Feeding on high sucrose increases pathogen load in the early stages of infection

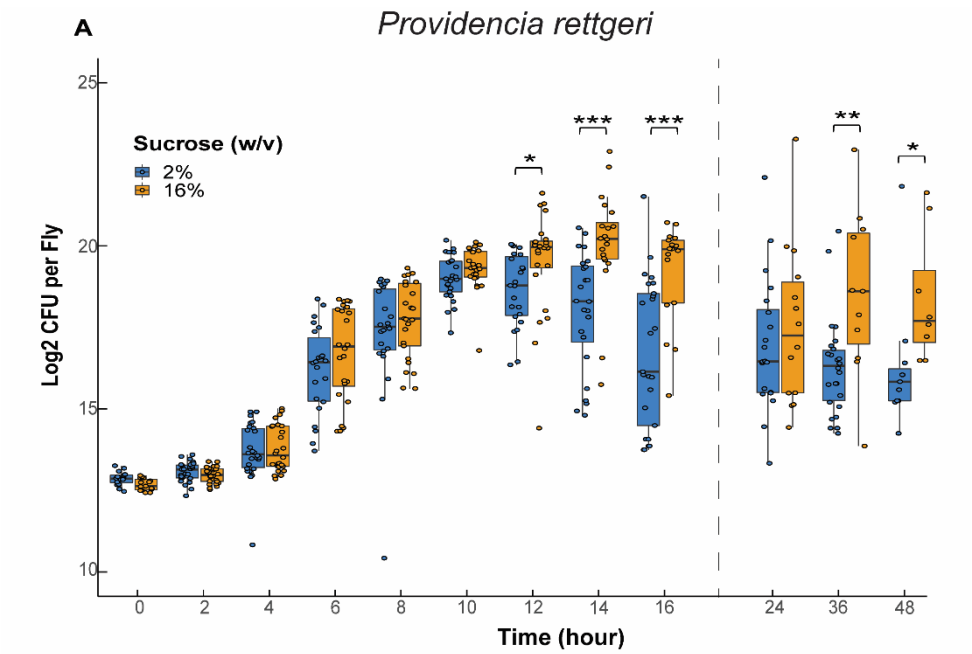
High pathogen load is associated with higher mortality from infection (49,50). Since we observed that high-sugar diets lead to higher mortality during infection with the Gram-negative bacteria *S. marcescens* and *P. rettgeri*, we wanted to establish whether this high mortality arises from higher bacterial load. To test whether flies fed on high sugar have higher pathogen loads, we performed an *in vivo* bacterial growth assay where we sampled infected flies at two-hour intervals from 0-16 hours post-infection and at 24, 36, and 48 hours post-infection. At each time of sampling, individual flies were homogenized and plated on agar plates to estimate the number of live bacterial cells in the fly.

Overall, the bacteria proliferated more quickly in flies fed on the high-sugar diet after both *P. rettgeri* (Fig. 3A) and *S. marcescens* (Fig. 3B) infection. However, the divergence in load between the two diets occurs at different times for the two pathogens. *S. marcescens* infections start to exhibit a higher pathogen burden as soon as 6 hours into the infection, while *P. rettgeri* burden becomes higher by 12 hours into the infection. Flies that died during the sampling period were not included in this assay, which may mean that pathogen burdens are underestimated if the surviving flies at any given timepoint tend to be the ones with lower pathogen burdens. At 10 hours and later post-infection with *S. marcescens*, we observed a lower proportion of surviving flies on the high-sugar diet than on the low-sugar diet (Fig. 3B), which may explain the apparent absence of difference in pathogen burden 10-16 hours into the infection. We similarly observe a

248 lower proportion of flies provided with high-sugar diet surviving *P. rettgeri* infection 10 hours and

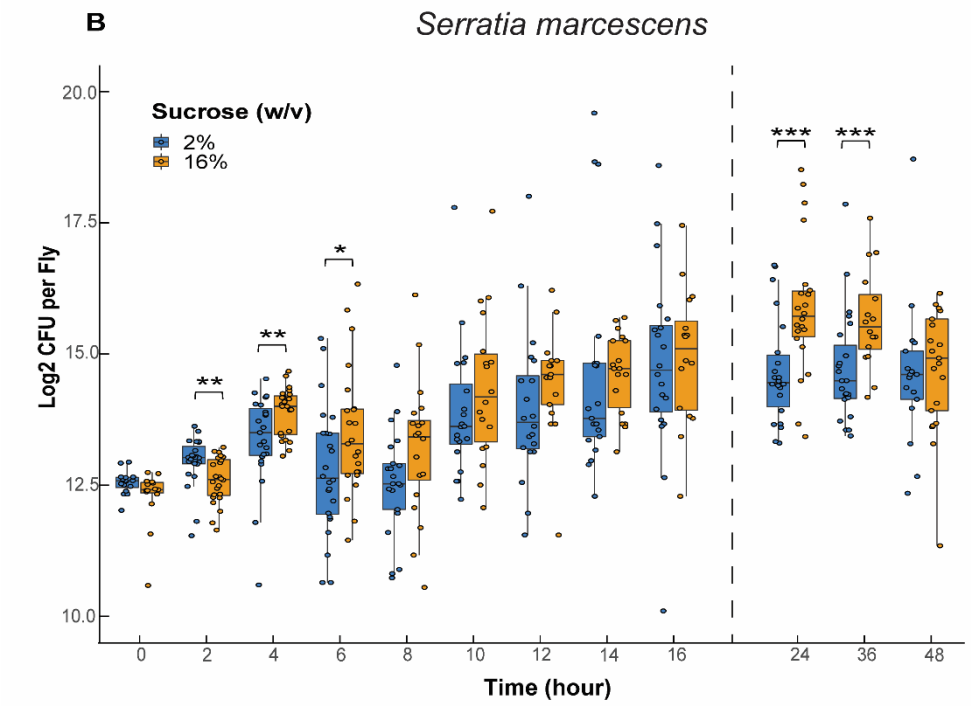
249 later, although the difference in pathogen burden between flies on the two diets remains

250 significant (Fig. 3A).



Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	0.97	0.93	0.93	1.0	0.93	0.90	0.90	0.97	0.76	0.76	0.71
16%	1.0	0.97	1.0	0.89	1.0	0.83	0.85	0.81	0.59	0.47	0.29	0.29



Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	1.0	1.0	0.96	0.90	0.93	0.85	0.90	0.83	0.80	0.74	1.0
16%	1.0	1.0	0.93	1.0	0.83	0.69	0.76	0.67	0.63	0.62	0.42	0.44

252

253 **Fig 3.** Pathogens proliferate faster in flies fed high sugar. (A) Pathogen load was assayed at 2-hour
254 intervals from 0-16 hours post-infection and at 24, 36, and 48 hr post-infection in flies fed 2% and
255 16% sucrose diets. After *P. rettgeri* infection, flies provided with high-sugar diets have higher
256 pathogen loads at 12, 14, 16, 36, and 48 hr than flies given low-sugar diet (12 hr, $p = 0.031$; 14 hr, p
257 < 0.001, 16 hr, $p < 0.001$; 36 hr, $p < 0.01$; 48 hr, $p = 0.022$; Generalized Least Square Means (GLS)
258 with Tukey HSD post-hoc for pairwise comparisons). Flies given high-sugar diets have consistently
259 lower survival to time of sampling at hour 10 and onward. (B) After *S. marcescens* infection, flies
260 given high-sugar diets have higher pathogen loads than flies given low-sugar diet at 4 hr, 6 hr, 24 hr,
261 and 36 hr post-infection (4 hr, $p = 0.011$; 6 hr, $p = 0.044$, 24 hr, $p < 0.001$; 36 hr, $p < 0.01$;
262 Generalized Least Square Means (GLS) with Tukey HSD post-hoc for pairwise comparisons). Flies
263 given high-sugar diets have consistently lower survival to time of sampling at hour 8 and beyond. At
264 2 hours post-infection, flies given low-sugar diets have higher pathogen load than flies given high-
265 sugar diets ($p < 0.01$). Each dot represents the pathogen burden of an individual fly. Legend for
266 panel figure: * = $p<0.05$, ** = $p<0.01$, *** = $p <0.001$.

267

268 **Nutrient availability promotes *S. marcescens* proliferation within hosts on high-sugar**

269 **diets**

270 Having determined that *D. melanogaster* provided with high-sucrose diets are both more
271 susceptible to infection and sustain higher internal carbohydrate stores, we next sought to
272 understand whether the increased susceptibility to infection on the high-sugar diet is due to
273 impaired immune system activity or to an altered nutritional environment experienced by the
274 infecting pathogen. To test this, we measured pathogen proliferation during *in vivo* infection of
275 immune compromised *D. melanogaster* on the low- and high-sugar diets. We predicted that the
276 effect of diet on sensitivity to infection would be eliminated in immunocompromised flies if the
277 dietary effect is due to impaired immunity, but it would remain if the dietary effect were due to
278 altered nutritional environment for the pathogen. We used the *D. melanogaster* strain ΔAMP10
279 (51), which has intact Toll and IMD signaling but is missing 10 genes encoding major infection-
280 inducible antimicrobial peptides. Antimicrobial peptides are small, cationic peptides that are
281 secreted from the insect fat body in response to a systemic infection, and they are critical for
282 killing pathogens by disrupting function of specific microbial processes (52–55). The ΔAMP10

283 flies have mutations that eliminate *Diptericin A*, which is critical for controlling *P. rettgeri*
284 infection (51,56), and the four *Attacin* genes, three of which are critical for the control of *S.*
285 *marcescens* infection (P. Nagy, N. Buchon, and B.P. Lazzaro, unpublished). We can use these
286 flies to monitor pathogen growth in response to diet without any interference from the humoral
287 immune response. There was no difference in the rate of *P. rettgeri* proliferation in ΔAMP10
288 hosts provided with either low or high sucrose (Fig. 4A), indicating that the typically observed
289 effect of diet on sensitivity to this infection may be a consequence of diet-dependent immune
290 impairment. In contrast, *Serratia marcescens* continued to proliferate faster in ΔAMP10 flies fed
291 high sucrose than in those fed low sucrose (Fig. 4B), indicating an effect of diet beyond direct
292 mediation of the immune system.

293 As an independent, albeit indirect, test of whether *S. marcescens* might utilize excess
294 available carbon to grow faster in hosts provided with high-sugar diets, we measured *in vitro*
295 growth curves of *P. rettgeri* and *S. marcescens* in minimal medium supplemented with either a
296 carbon source or a nitrogen source. We performed an 18-hour growth curve assay in M9
297 minimal media supplemented with different combinations of casamino acids, sucrose, and
298 glucose. We found that supplementation with 1% casamino acids enabled *P. rettgeri* growth, but
299 there was no further change in the growth trajectory or final OD with supplementation with 1%
300 glucose, 1% sucrose, or no additional sugar ($p > 0.05$, Fig. 4C). These data indicate that *P.*
301 *rettgeri* is nitrogen-limited but not carbon-limited in minimal media. The addition of either
302 glucose or sucrose dramatically increased the growth of *S. marcescens* in minimal media and
303 resulted in a significantly higher OD at the end of the assay ($p<0.01$, Fig. 4D), indicating a
304 greater dependence on environmental carbon under these growth conditions. While we do not
305 know precise bioavailability of carbon to bacteria infecting *D. melanogaster*, the data in Figure 2
306 show that flies on high-sugar diets have higher levels of circulating and stored sugars, and in
307 combination these data offer conceptual support to the hypothesis that the increased

308 proliferation of *S. marcescens* within hosts provided with a high-sugar diet may arise in part
309 from increased availability of carbon to the infecting bacteria.

310 We had observed that flies provided with high-sucrose diets show elevated glucose and
311 trehalose levels after infection with *S. marcescens* (Fig. 2B, 2C) despite reduction in glycogen
312 stores in both diets (Fig. 2D), leading us to hypothesize that *S. marcescens* might consume
313 excess circulating sugar within the host. We measured the change in carbohydrate levels after
314 infection with either *P. rettgeri* or *S. marcescens* relative to uninfected flies on both diets using
315 the data in Fig. 2B-D. We found that flies infected with *S. marcescens* have a significantly higher
316 loss in glucose levels compared to flies infected with *P. rettgeri* on both the low-sugar ($p < 0.01$)
317 and high-sugar ($p<0.001$, Fig. 4E) diets, although they exhibit similar relative loss in glycogen (p
318 > 0.05, Fig. 4F). Interestingly, we observed significantly higher loss of glycogen in flies provided
319 with low-sugar compared to high-sugar diets after both *P. rettgeri* ($p = 0.0178$) and *S.*
320 *marcescens* infection ($p = 0.029$, Fig. 4F). There was no significant difference between the
321 infections in proportional change in trehalose levels on either diet (Fig. 4G). Our observation
322 that both infections stimulate loss in glycogen stores but only *S. marcescens* infection results in
323 a significant reduction in glucose levels is consistent with *S. marcescens* consumption of free
324 sugars during infection.

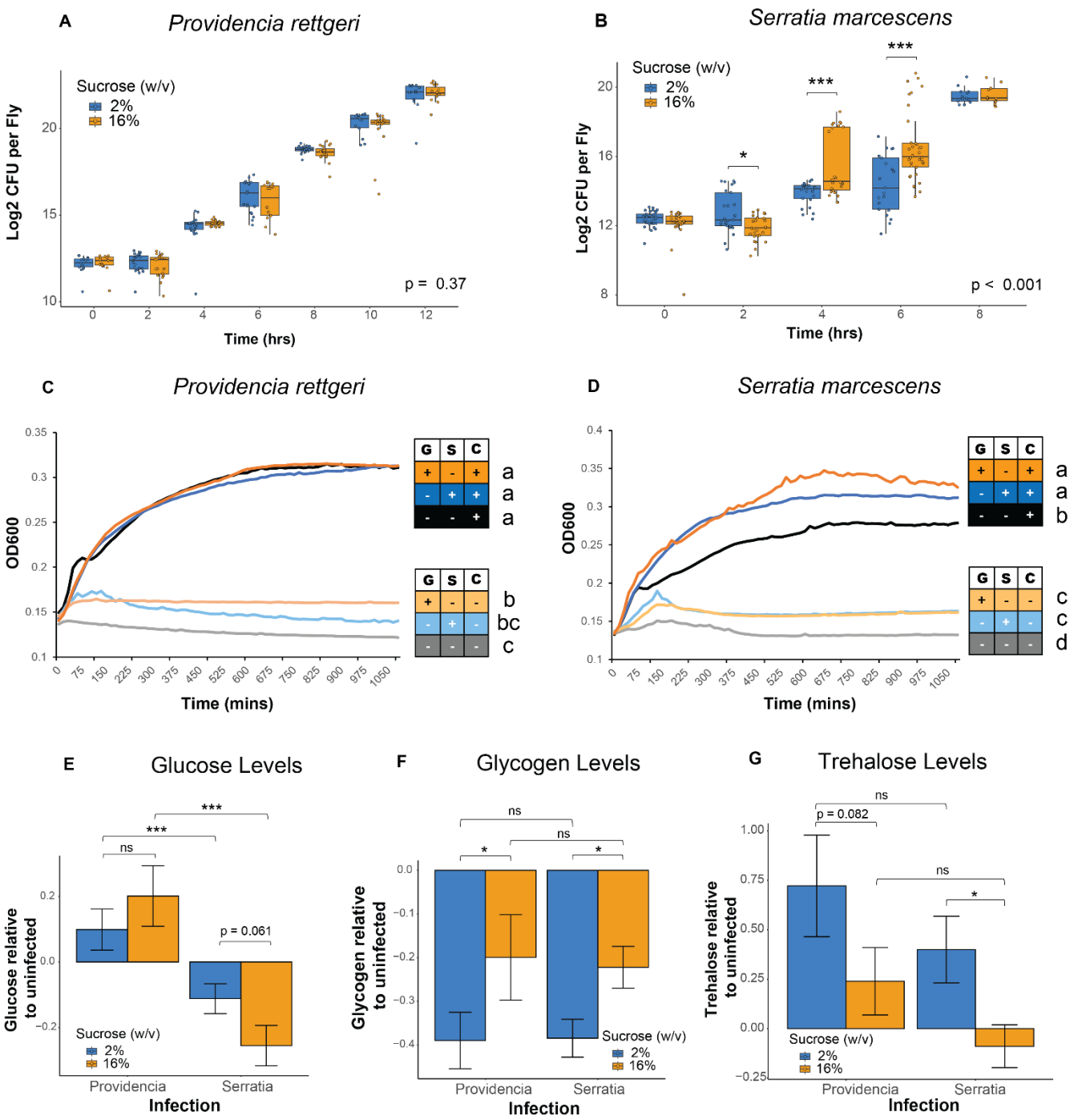


Fig 4. Nutrient availability promotes *Serratia marcescens* proliferation within hosts on high-sugar diets. (A) Diet did not affect the proliferation of *P. rettgeri* in flies lacking 10 major inducible antimicrobial peptides (p> 0.05 time*diet; GLS model). (B) *S. marcescens* proliferate significantly more rapidly in ΔAMP10 flies fed high sugar (p<0.001 time*diet; GLS model). Two hours into the infection, flies fed high sugar exhibit a lower *Serratia* burden (p < 0.01; GLS model followed by Tukey HSD post-hoc), but flies fed high sugar exhibit significantly higher loads by hours by hours 4 (p<0.001) and 6 (p<0.0001; GLS model followed by Tukey HSD-post-hoc). (C) There was no effect of

333 sugar supplementation on *P. rettgeri* growth over 1080 mins in M9 minimal media supplemented with
334 1% casamino acids (CAS; p> 0.05; ANOVA followed by Tukey HSD post-hoc). CAS supplementation
335 significantly increased OD at 1080 minutes compared to media without casamino acids (p<0.001;
336 ANOVA). (D) *S. marcescens* grew significantly more in minimal media supplemented with the
337 combination of casamino acids and glucose (p<0.001; ANOVA with Tukey HSD post-hoc) or sucrose
338 (p < 0.01, ANOVA with Tukey HSD post-hoc) than in media supplemented with casamino acids alone.
339 *S. marcescens* OD at 1080 mins was significantly lower in media supplemented only with glucose (p
340 < 0.001; ANOVA with Tukey HSD). Each curve represents 6-8 replicates per treatment, pooled.
341 Letters in figures denote significant pairwise differences with p <0.05. Legend: G = glucose, S =
342 sucrose, C = casamino acids. Colors for *in vitro* curves: 1% glucose (light orange), 1% sucrose (light
343 blue), 1% casamino acids (black), or 1% glucose & casamino acids (dark orange), and 1% sucrose &
344 casamino acids (dark blue). (E) Glucose levels declined significantly more after infection with *S.*
345 *marcescens* but increased in flies infected with *P. rettgeri* on both 2% (p < 0.01; ANOVA followed by
346 Tukey HSD post-hoc) and 16% (p< 0.0001; ANOVA followed by Tukey HSD post-hoc) sucrose diets.
347 There was no significant difference in change in glucose levels after *P. rettgeri* infection between
348 diets (p = 0.24; ANOVA followed by Tukey HSD post-hoc). There was a trend for increased relative
349 loss of glucose after *S. marcescens* infections in flies fed the 16% sucrose diet compared to 2%,
350 however this was not statistically significant (p = 0.0601, ANOVA followed by Tukey HSD post-hoc).
351 (F) Glycogen levels were reduced after infection with both *P. rettgeri* and *S. marcescens*. Flies fed
352 2% sucrose had significantly higher loss of glycogen stores compared to flies fed 16% sucrose after
353 infection with both *P. rettgeri* (p = 0.0178, ANOVA with Tukey HSD post-hoc) and *S. marcescens* (p =
354 0.0291, ANOVA with Tukey HSD post-hoc). (G) There was no significant difference in change upon
355 infection in trehalose levels between *P. rettgeri* and *S. marcescens* on either the 2% diet (p = 0.197)
356 or the 16% sucrose diet (p = 0.215). There was a significant reduction in trehalose levels of flies
357 infected with *S. marcescens* on 16% sucrose diets compared to flies fed 2% sucrose (p= 0.0372,
358 ANOVA with Tukey HSD post-hoc). There was a trend toward reduced trehalose in flies provided with
359 16% sucrose diet after *P. rettgeri* infection, but it was not statistically significant (p = 0.0816, ANOVA
360 with Tukey HSD post-hoc). Legend for panel figure: * = p<0.05, ** = p<0.01, *** = p <0.001.

361

362 ***Drosophila* on high sugar diets produce less AMP peptide after infection**

363 We inferred that high-sugar diets increase sensitivity to *P. rettgeri* infection by impairing the
364 immune system because the effect of diet was eliminated in immunocompromised flies.
365 However, the inference that *S. marcescens* may consume excess carbohydrates present in flies
366 on high-sugar diets does not preclude the possibility that those flies might also have reduced
367 immune responses. To directly test whether high-sugar diets impair the immune response, we
368 first measured mRNA transcripts of a panel of genes encoding six *D. melanogaster*
369 antimicrobial peptides (AMPs). Gene expression was measured in flies fed low or high-sugar
370 diets at 8-hours after infection with either *S. marcescens* or *P. rettgeri*. We found that diet had
371 no effect on the infection-induced expression levels of *Diptericin A, Attacin A, Cecropin A1,*

372 *Drosocin, Drosomycin,* or *Defensin* after either *P. rettgeri* and *S. marcescens* infection (Figs 5A
373 and 5B).

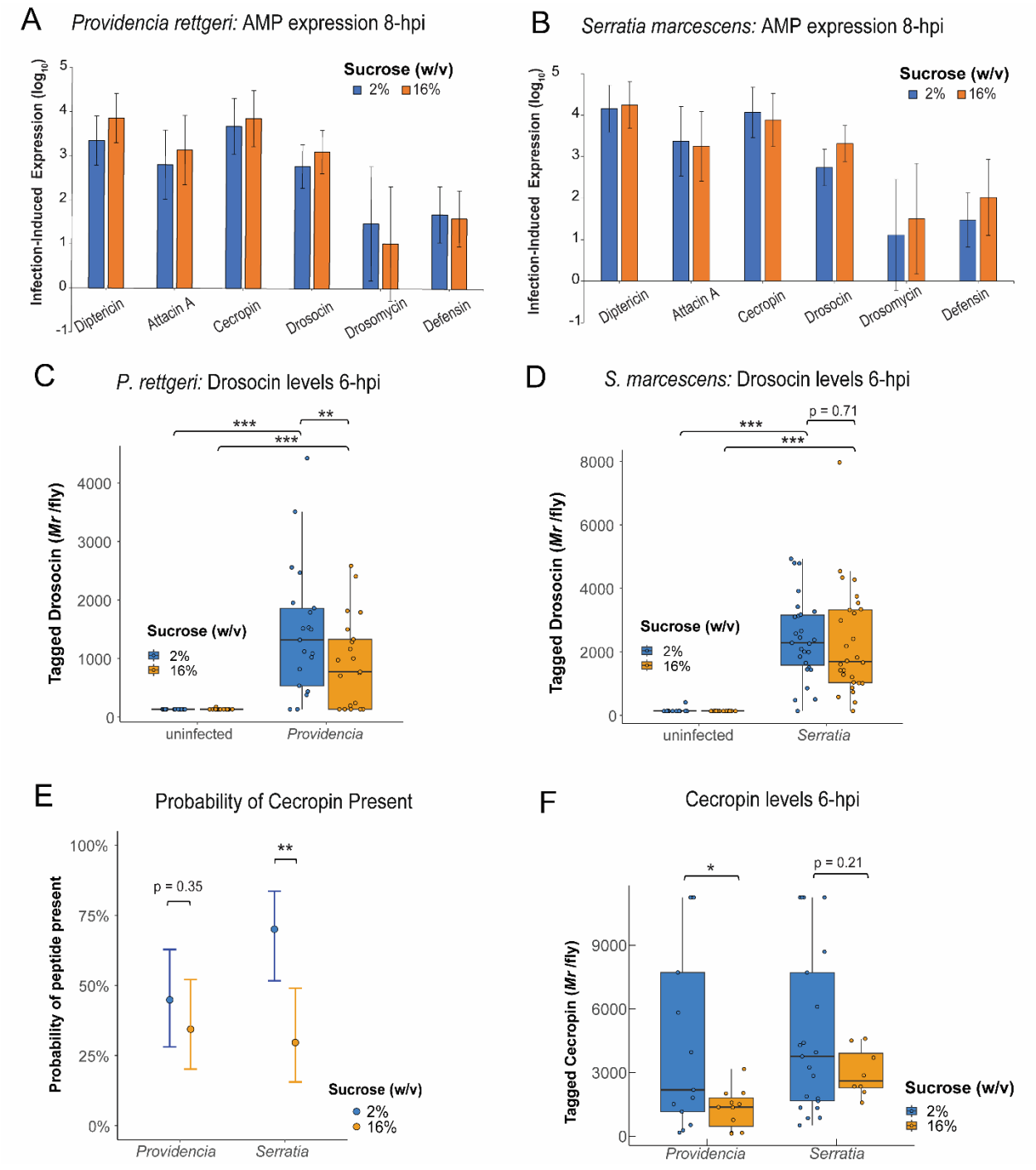
374 Measuring the amount of mRNA transcript does not directly demonstrate the abundance of
375 translated peptide (57), and translation can be impaired by cellular stress during the *D.*
376 *melanogaster* response to infection (58). To test whether high-sugar diets impact AMP protein
377 levels in response to infection, we used sandwich ELISA quantify Drosocin and Cecropin A1
378 from flies that have hemagglutinin (HA) and FLAG epitopes tagged to the endogenous AMPs.
379 Infection-induced production of tagged AMP peptides is detectable as early as 3 hours post-
380 infection (Fig. S3A, S3B). We quantified tagged AMPs from individual flies at 6-hours after
381 infection with either *P. rettgeri* or *S. marcescens.* Flies fed on the high-sugar diet produced
382 significantly lower levels of tagged Drosocin than flies fed on the low-sugar diet after *P. rettgeri*
383 infection ($p = 0.008$, Fig. 5C). However, we observed no effect of diet on Drosocin levels in flies
384 infected with *S. marcescens* (Fig. 5D). An unexpectedly high number of flies did not have
385 detectable levels of tagged Cecropin peptide after infection with either *P. rettgeri* or *S.*
386 *marcescens* (Fig. S4A, S4B). We performed a hurdle model to statistically test for differences in
387 Cecropin abundance. The hurdle model first tests the probability that detectable levels of tagged
388 Cecropin are present using a binomial logistic regression, and subsequently tests whether there
389 are quantitative differences in peptide levels conditional on having observed a detectable level
390 of peptide. While the probability of detecting Cecropin was equivalent flies infected with *P.*
391 *rettgeri* on both diets (Fig. 5E), flies infected with *S. marcescens* had lower probability of
392 expressing detectable Cecropin when fed the high-sugar diet ($p < 0.01$, Fig. 5E). In the flies for
393 which Cecropin was detectable, peptide abundance was significantly lower in the flies on the
394 high-sugar sucrose diet than in the flies on the low-sugar diet after infection with *P. rettgeri* ($p =$
395 0.023, Fig. 5F). Diet had no effect on the abundance of detectable Cecropin after *S.*
396 *marcescens* infection ($p = 0.21$, Fig 5F). Overall, flies provided with 16% sucrose diets produce

397 less Drosocin and Cecropin after infection with *P. rettgeri* and have a lower probability of

398 detectable Cecropin 6 hours post-infection with *S. marcescens*. Thus, the translational

399 production of AMPs seems to be impaired in flies on high-sucrose diets even when there is no

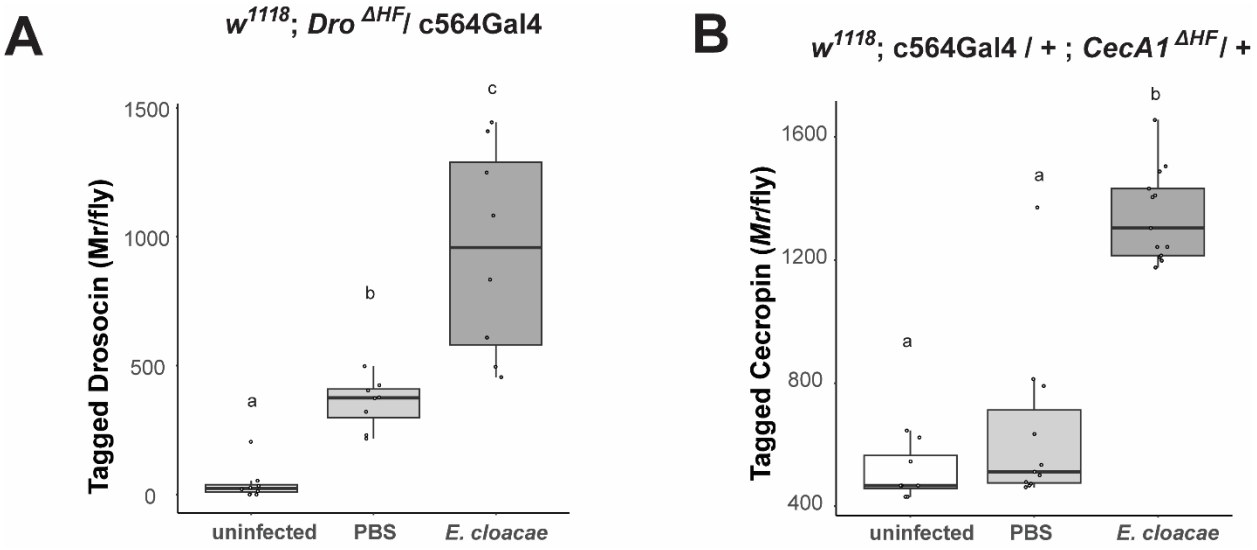
400 decrease in transcription level.



401

402 **Fig 5.** High sugar diets reduce production of AMP peptides in response to infection without affecting
403 AMP gene expression. (A) There was no effect of diet on expression of genes encoding the AMPs
404 Diptericin, Attacin A, Cecropin A1, Drosocin, Drosomycin, and Defensin at 8-hours post-infection with
405 *P. rettgeri* or (B) *S. marcescens* ($p > 0.05$). (C) *P. rettgeri* infection resulted in significantly higher
406 levels of tagged Drosocin than in uninfected flies on both 2% ($p < 0.0001$) and 16% ($p = 0.0002$)
407 diets. Flies on 16% sucrose diet had significantly lower amounts of tagged Drosocin six hours after *P.*
408 *rettgeri* infection than flies on 2% sucrose diet ($p = 0.008$). (D) Tagged Drosocin levels were higher at
409 6 hours after *S. marcescens* infection than in uninfected flies on both 2% ($p < 0.001$) and 16% ($p <$
410 0.001) diets, but there was no difference between the diets after *S. marcescens* infection ($p = 0.71$).
411 (E) The probability of detectable Cecropin peptide was similar after *P. rettgeri* infection on both diets
412 ($p = 0.45$), while flies fed the 2% diet were significantly more likely to have detectable peptide present
413 after *S. marcescens* infection than those fed the 16% diet ($p = 0.003$). (F) Of the flies with detectable
414 Cecropin levels, there were significantly higher peptide levels in flies fed the 2% diet compared to
415 16% diet after both *P. rettgeri* ($p = 0.0229$) and *S. marcescens* ($p = 0.211$) infections.

416



417

418 **Fig S3.** Demonstration that HA- and FLAG-tagged Drosocin and Cecropin can be detected by
419 sandwich ELISA following bacterial infection. (A) Tagged Drosocin levels are significantly higher 3-
420 hours after injection with PBS ($p = 0.0455$) or ~6.0×10^4 cells of the Gram-negative bacterium
421 *Enterobacter cloacae* ($p<0.001$) than they are in uninjured flies. *E. cloacae* infection results in
422 significantly higher Drosocin levels than PBS injection ($p<0.001$; linear model with Tukey post-hoc).
423 (B) Tagged Cecropin levels are significantly higher 3-hours after infection with *E. cloacae* than after
424 PBS injection ($p<0.001$) or in uninjured flies ($p<0.001$). There was no detectable difference in
425 Cecropin levels between uninjured and PBS-injected flies ($p = 0.32$). Letters denote pairwise
426 difference $p<0.05$.

A

	Providencia detected	Providencia undetected	Proportion in linear range
2%	13	16	0.45
16%	11	21	0.34

B

	Serratia detected	Serratia undetected	Proportion in linear range
2%	21	9	0.70
16%	8	19	0.30

427

428 **Fig. S4.** (A) The number of flies fed the 2% and 16% diets that have, or do not have, detectable
429 tagged Cecropin six hours after *P. rettgeri* infection. (B) The number of flies fed the 2% and 16%
430 sucrose diets that have, or do not have, detectable tagged Cecropin six hours after *S. marcescens*
431 infection.

432

433

434 **Discussion**

435 In this study, we show that *D. melanogaster* provided with high-sugar diets exhibit higher
436 mortality and elevated pathogen load after infection with the Gram-negative bacteria
437 *Providencia rettgeri* and *Serratia marcescens*, even when the high-sugar diet is provided only to
438 the adult stage after development is complete. *S. marcescens* may be able to take a growth
439 advantage from the excess carbohydrates available in hosts provided with high-sugar diets, and
440 the effect of diet on sensitivity to both infections seems to be mediated by impaired host immune
441 response on high-sugar diet. Interestingly, we saw no effect of diet on susceptibility to infection
442 with the Gram-positive bacteria *Enterococcus faecalis* and *Lactococcus lactis*. As Gram-
443 negative (*S. marcescens* and *P. rettgeri*) infections should primarily activate the IMD signaling
444 pathway whereas Gram-positive (*E. faecalis* and *L. lactis*) infections should primarily activate
445 the Toll pathway (50,59), the much larger effect of diet on sensitivity to Gram-negative infections
446 may implicate an interaction with the IMD pathway.

447 Our approach of feeding flies on diets that range from 0%-24% sucrose was based on
448 previous studies of systemic infection outcome that defined “high” dietary sugar as ranging from
449 10% glucose (3) to 24% (w/v) sucrose (12). We predicted that there would be a concentration-
450 responsive decrease in host survival post-infection as dietary sugar increased. However, what
451 we observed was a threshold response where quantitatively variable low-sucrose diets (0%, 2%
452 and 4%) were statistically equivalent to each other but different than high-sucrose diets (16%
453 and 24%) with respect to host survivorship of infection. However, the precise threshold

454 depended on the pathogen, with 8% sucrose being a functionally high-sugar diet for a *P. rettgeri*
455 infection but low-sugar for a *S. marcescens* infection.

456 Our observation of immunological effects of diet that vary among pathogens is consistent
457 with previous reports in the literature. For example, Ayres and Schneider (2009) (60)
458 demonstrate that dietary restriction at 24 hours prior to infection of adult *D. melanogaster* is
459 protective against a *Salmonella typhimurium* infection, decreases resistance to *Listeria*
460 *monocytogenes*, and has no effect on *E. faecalis* infection (60). Similarly, Singh et al. (2022)
461 (61) found that *D. melanogaster* reared on a calorie-restricted diet were more susceptible to
462 *Pseudomonas entomophila* infection while diet had no effect on mortality after *E. faecalis*
463 infection. Our finding that diet had no effect on *E. faecalis* infection outcome is corroborated by
464 several studies (60–62) that suggest negligible impact of diet or relevant host physiology on
465 defense against this pathogen. Our data in combination with these previous studies suggest that
466 the effects of diet on immune outcome are in part dependent on the unique physiology of the
467 infecting pathogen.

468 We observed that *S. marcescens* and *P. rettgeri* pathogen burdens are both higher in *D.*
469 *melanogaster* fed high sugar. However, the increased pathogen load arises differently for the
470 two pathogens. The effect of diet on defense against *P. rettgeri* is mediated by the host immune
471 system, as flies provided with 16% sucrose diet produce less Drosocin after infection with *P.*
472 *rettgeri* and the effect of dietary sugar on *P. rettgeri* proliferation is eliminated in *D.*
473 *melanogaster* lacking a functional immune response. However, while flies infected with *S.*
474 *marcescens* produced less Cecropin when provided with 16% sucrose diet, we also observed
475 that *S. marcescens* continued to proliferate more rapidly in flies provided with 16% sucrose diets
476 even when those flies lack most inducible AMPs. We found that high-sugar diet increases the
477 level of circulating sugar within the fly, and that *S. marcescens* benefits more than *P. rettgeri*
478 from carbon supplementation when growing *in vitro.* These data suggest *S. marcescens* may

479 perhaps gain a growth benefit from the hyperglycemia of *D. melanogaster* feeding on high-sugar
480 diets. A similar phenomenon has been observed with the Gram-negative opportunistic pathogen
481 *Pseudomonas aeruginosa* infecting hyperglycemic mice. Wild-type *P. aeruginosa* proliferates
482 more quickly in hyperglycemic mice, but mutants for glucose uptake and metabolism genes
483 (*oprB, gltK, gtrS*, *glk)* exhibit similar growth in control and hyperglycemic mice (6). More detailed
484 future study of *S. marcescens* mutants deficient for glucose uptake or metabolism infecting *D.*
485 *melanogaster* mutant for key factors in metabolism and immune response could probe the
486 relationship between diet, host physiology, and pathogen physiology as determining infection
487 outcome.

488 Interestingly, we noticed that both wild-type and ΔAMP10 *D. melanogaster* genotypes had
489 lower *S. marcescens* burdens at 2 hours after infection on high-sugar diet than on low-sugar
490 diet (Figs. 3B and 4B). Phagocytosis is known to play a role in controlling *S. marcescens*
491 infection in some contexts (63) and infection has previously been shown to increase aerobic
492 glycolysis in *Drosophila* phagocytes (64). Since ΔAMP10 flies still have intact cellular immune
493 systems, it is possible that the elevated dietary sugar provides energy for phagocytes to use in
494 clearing pathogens sensitive to phagocytosis, although this process alone is not sufficient to
495 manage the infection delivered in our experimental context.

496 We found that elevated dietary sugar does not impact the transcription of antimicrobial
497 peptides genes (Fig. 5A, 5B), but it does reduce translation of the encoded peptides Drosocin
498 and Cecropin A1 (Fig. 5C-5E). A similar transcriptional effect was observed in larvae of the beet
499 army worm (*Spodoptera exigua*) and fall army worm (*Spodoptera frugiperda*), where infection-
500 induced expression of the AMPs *attacin* and *gloverin* transcripts were equivalent between a low
501 protein and control diet despite reduced resistance to infection on the low-quality diet (65). This
502 suggests that dietary impacts on the immune system may not occur at the transcriptional level
503 but at the level of translation. A recent proteomics study on *D. melanogaster* found that naive

504 flies subjected to dietary restriction exhibit lower peptide levels of the AMPs Attacin A and
505 Cecropin C (66), although those authors did not measure the effect of diet on production of
506 antimicrobial peptides in the context of a systemic infection.

507 Antimicrobial peptides are critical for controlling Gram-negative infections in *D.*
508 *melanogaster*. At the transcriptional level, there is a high degree of co-regulation of genes
509 encoding AMPs (19,67), and we would anticipate corresponding similarity in translational
510 efficiency (68). Given that high dietary sugar reduced production of Drosocin and Cecropin, we
511 anticipate it would also reduce production of other AMPs. It should also be noted that the IMD
512 and Toll immune regulatory pathways do more than simply stimulate production of AMPs.
513 Activation of these pathways alters host metabolism and is influenced by host metabolic status
514 (13), and even stimulates host sequestration of micronutrients to limit pathogens from accessing
515 them (69). Future experiments will be necessary to establish the mechanistic details of how
516 high-sugar diets impact the IMD and Toll pathways to shape resistance to infection.

517 Our present work establishes that adverse effects of high-sugar diets on infection outcome
518 are due to the combination of impaired host immune function as well as, in some cases, faster
519 proliferation of the infecting pathogen in a hyperglycemic environment. Notably, the immune
520 impairment is not necessarily at the level of transcriptional activation of the immune response
521 but appears to be due to reduced translation of antimicrobial peptides. It is also notable that
522 dietary effects on immune defense are pathogen-specific, and there is not one universal
523 immunological consequence of high-sugar diets. Our findings illustrate the importance of
524 understanding the nutritional requirements of the infecting pathogens as well as the
525 physiological intertwining of host metabolism and immune responses when interpreting effects
526 of diet on infection outcome. We expect this principle to be general across host-pathogen
527 infection systems.

528

529 **Methods**

530 **Fly stocks and husbandry**

531 All flies were reared and maintained at 25 °C on a 12-hour light/dark cycle. Mated females of
532 the wild-type strain Canton S (CS) were used for all experiments unless otherwise indicated.
533 Females were cohoused with males in culture chambers and were aged to 3-5 days post-
534 eclosion prior to infection for all experiments. Flies were anesthetized on CO_2 pads for collection
535 and during infection.

536 **Generation of Tagged AMP Flies**

537 Flies carrying tagged Cecropin A1 or Drosocin were generated using CRISPR-facilitated
538 homology-directed repair. In brief, guide sequences for *CecA1* or *Dro* were cloned into pCFD5
539 (*CecA1* guides: guide 1: CCATTGGACAATCGGAAGCT; guide 2:
540 ATAATTATAAATAATCATCG; *Dro* guides: guide 1: CAAAAACGCAAGCAAGCAGC; guide 2:
541 CAATCAATTGTGACACAATG) (70). Homology-directed repair cassettes were cloned into pHD-
542 Scarless-dsRed, encoding the replacement of endogenous *CecA1* and *Dro* with peptides that
543 included FLAG and HA tags (predicted sequences of mature peptides: $CECA1^{HF}$,
544 GWLDYKDDDDKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATYPYDVPDYAR; DRO^{HF},
545 GDYKDDDDKPRPYSPRPTSHPRPIYPYDVPDYARV) (71). These two plasmids were then
546 injected together into embryos of the genotype y^1 *P(nos-cas9, w-) M(RFP-.attP)ZH-2A w**. Male
547 flies emerging from this injection were crossed with females of the genotype w^{1118}; *If /* SM6a or
548 w^{1118}; TM2 / TM6c, Sb^1, as appropriate. Males expressing dsRed in the eye were then selected
549 and crossed to flies carrying *CyO, Tub-PBac* to enable excision of the 3xP3-dsRed cassette.
550 The final structure of each locus was verified by PCR.

551 **Experimental Diets**

552 All flies were reared from egg to pupation in rearing chambers with interchangeable food plates
553 on a standard base sucrose-yeast-cornmeal diet (per liter: 60 g yeast, 60 g cornmeal, 40 g

554 sucrose, 7 g *Drosophila* Agar, 0.5 mL phosphoric acid, 5 mL propionic acid, and 6 g
555 methylparaben dissolved in 26.5 mL 100% ethanol). *D. melanogaster* pupated on the chamber
556 walls and, prior to eclosion, the food plates containing the standard rearing media were
557 swapped with one of the experimental diets. Emerging adult flies were aged on the experimental
558 media for 3-5 days post-eclosion prior to infection for all experiments. Experimental diets were
559 identical to the rearing diet aside from the concentration of sucrose. Experimental diets
560 contained either 0%, 2%, 4%, 8%, 16%, and 24% (w/v) of sucrose.

561 **Infection Survival Assays**

562 Flies were anesthetized on CO_2 and injected in the thorax with either a bacterial suspension or
563 sterile PBS (as injury control) using a pulled glass capillary needle and nano-injector (72). The
564 following bacteria were injected in at least four experimental blocks: *Providencia rettgeri* strain
565 Dmel, *Serratia marcescens* strain 2698B, *Enterococcus faecalis* strain Dmel, *Lactococcus lactis*
566 strain Dmel. All bacteria were originally isolated as natural infections of *D. melanogaster* (36,73)
567 except *S. marcescens* 2698B, which is a clinical isolate (74). Cultures for *P. rettgeri* and *S.*
568 *marcescens* were started from a frozen glycerol stock then cultured overnight in liquid lysogeny
569 broth (LB) at 37 °C on a shaker, then subcultured the next morning in fresh LB to achieve
570 growth phase. *L. lactis* was cultured overnight from a frozen glycerol stock in liquid Brain Heart
571 Infusion (BHI) at 37 °C with shaking, then subcultured the next morning in fresh BHI to achieve
572 log-phase. *E. faecalis* was cultured from a frozen glycerol stock at room temperature (~24 °C) in
573 BHI. All bacteria were resuspended in 1x phosphate-buffered saline (PBS), diluted to A_{600} = 0.1,
574 and injected at a volume of 23 nL per fly. There were ~4000 *P. rettgeri* cells, ~3000 *S.*
575 *marcescens* cells, ~1000 cells *E. faecalis* cells injected, or ~ 1000 *L. lactis* cells injected per fly.
576 Flies were housed in groups of ten and each experimental block contained 3-4 vials of flies per
577 experimental diet. Survival curves were assessed using a Cox proportional hazards mixed effect
578 model (coxme) with the “CoxMe” package in R.

579 Model A: coxme(Surv(status, time) ~ diet + (1|block)

580 Diet was treated as a main effect and experimental block was treated as a random factor in our
581 model. This model was used to perform all pairwise comparisons using the “emmeans” package
582 in R with a Tukey p-value correction ($p<0.05$).

583 **Bacterial Load Trajectory Assay**

584 To assay bacterial proliferation during *in vivo* infection, flies were infected with *P. rettgeri* or
585 *S. marcescens* as described above, then collected for measurement of bacterial load at 0, 2, 4,
586 6, 8, 10, 12, 14, 16, 24, 36, and 48 hours post-infection. Single, live flies were homogenized in
587 500 µL sterile 1x-PBS with a metal bead and 50 µL of the homogenate was plated onto LB agar
588 using a spiral plater (Whitley Automated Spiral Plater-2). To ensure there was no overgrowth of
589 bacteria on the plates, fly homogenates were diluted with PBS at time points where we
590 anticipated higher bacterial growth. For wild-type CS flies, fly homogenates from timepoints 0, 2,
591 and 4 hours were directly plated, homogenates from timepoints 6-12 were diluted 1:10, and
592 homogenates from timepoints 14-48 were diluted 1:100. For ΔAMP10 mutants, fly homogenates
593 were directly plated for time points 0, 2, and 4, diluted 1:10 at hour 6, and 1:100 at time points 8,
594 10 and 12. Agar plates were incubated at 37°C overnight. The resulting colonies were counted
595 using PROTOCOL3 software to estimate the number of colony forming units (CFU) per mL of fly
596 homogenate. We then used the CFU/mL value to approximate the number of bacteria in the
597 individual fly at the time of sampling by using the following formula:

598 CFU/fly = CFU/mL * (dilution factor) * (0.5 mL/fly of original homogenate)

599 Two experimental blocks were completed for *P. rettgeri* and *S. marcescens* in CS flies, and
600 three experimental blocks were completed for ΔAMP10 mutants with each pathogen. A
601 generalized least squares (GLS) model from the “nlme” package in R was used to determine the
602 effect of diet on pathogen loads over time. Fixed effects in the model include time point sampled
603 (T) and diet (D). The function weights = varIdent(form = ~1|time point) from the “nlme” package

604 was incorporated into the model to account for unequal variances across time points, which
605 arises as the pathogen grows from the initial injection time point. The emmeans function was
606 used for pairwise comparisons between the experimental diets within each time point using the
607 Tukey method to determine p-values ($p<0.05$)

608 Model B: gls($\log_2$(pathogen load) ~ T * D, weights = varIdent(form = ~ 1|T))

609 **Excreta Quantification (ExQ) to measure feeding**

610 Methods for the ExQ assay are further detailed in Wu (2020) (47). Briefly, food was prepared by
611 melting down experimental diets and adding 1% (w/v) of dry erioglaucine powder (Sigma #
612 861146). The dyed food mixture was then dispensed into microcentrifuge tubes caps filled up to
613 50 µL volume. ExQ chambers were prepared from 50 mL conical vials (Cell Treat # 229421). Air
614 holes were poked along the sides of tubes and the lid using a pushpin (~0.5 mm diameter). A
615 precision knife was used to cut a hole in the lid of the conical vial to fit the diameter of the food
616 caps. Naïve flies were anesthetized with CO_2 and sorted into ExQ chambers in groups of 8-10.
617 Every 24 hours, the flies were transferred to new chambers with fresh food. After 48 hours, flies
618 were emptied from chambers and 3 mL of 1x-PBS was used to collect the excreta. Total dye
619 concentration was determined by using a spectrophotometer to measure absorbance of the dye
620 excreted from flies. A standard curve was prepared from a stock solution of erioglaucine by
621 dissolving 10 mg of powder dye in 10 mL of PBS. A serial 2-fold dilution was then created from
622 this initial stock, and the final range of standards used to create the standard curve was 312.5
623 µg/mL to 5 µg/mL. Absorbance was measured at 630 nm in a 96-well plate on a spectrometer
624 (Molecular Biosciences Spectra Max Series Plus 384). Standards and samples were run in
625 duplicates with 100 µL per well and then averaged for data analysis. Dye consumed per fly
626 within a single ExQ chamber was determined using the following equation:

627 Dye (µg) per fly = [(Absorbance – Slope)]/(Intercept * # flies)

628 A two-way Analysis of Variance (ANOVA) in R was used to test for differences in dye
629 consumed by flies on each diet on Day 1 and Day 2 of feeding. Then the emmeans function in R
630 was used for pairwise comparisons between diets within each day using the Tukey test to
631 determine p-values ($p<0.05$).

632 Model C: aov(dye ~ diet + day)

633 **Quantitative real-time PCR (RT-qPCR) for Antimicrobial Peptide Gene Expression**

634 Female flies provided with 2% and 16% sucrose diets were injected with ~3000 CFU of log-
635 growth *S. marcescens* or *P. rettgeri* then sampled at 8 hours after injection for quantification of
636 gene expression. Flies were pooled in groups of 5 and frozen at -80 °C for subsequent RNA
637 extraction. In 1.5 mL microcentrifuge tubes, RNA was isolated and purified using a TRIzol
638 extraction procedure according to the manufacturer's instructions (Zymo Kit direct-Zol RNA kit
639 #R2050). Briefly, flies were homogenized in TRIZoL (Invitrogen #15596018) using a motorized
640 pestle in a microcentrifuge tube and RNA was then extracted using the Zymo Spin Column
641 (Zymo Research #C1078). Samples were then treated with the kit's DNAse and RNA was
642 dissolved 50 µL of molecular grade water. RNA concentration was determined using a Qubit4
643 Broad Sense RNA kit (Thermo #Q10210). cDNA was generated using 500 ng of total RNA with
644 the iSynthesis cDNA kit (Bio-Rad #1708891). RT-qPCR was performed on a CFX Connect
645 Real-Time Detection System (Bio-Rad) using PerfeCTa SYBER Green fast mix (VWR #101414-
646 270). Expression of the AMPs *Drosocin, Defensin, CecropinA, Dipterin, Attacin A, Drosomycin*
647 was normalized to the expression of the housekeeping gene *Actin 5C*. Uninfected controls were
648 not subject to injury. Primer sequences used are listed in Supplementary Table 1. A linear
649 model was used to test the contributions of diet and infection status to expression of each AMP
650 gene, standardized to the housekeeping gene (75).

651 Model D: lm(AMP_CT ~ *Actin5C*_CT + Diet + Infection Status * Diet)

652 Diet and Infection Status were fixed effects in the model. Estimated marginal mean CT values
653 for the Diet x Infection interaction terms were extracted using the emmeans package in R. Then
654 we subtracted the estimated marginal mean CT value for uninfected flies from the estimated
655 marginal means from the infected flies for each diet. This difference in estimated marginal mean
656 CT between uninfected and infected values indicates infection-induced change in AMP gene
657 expression on a $\log_2$ scale. Using the emmeans function, we compared infection-induced
658 expression values between experimental diets with Tukey's corrections test for p-values
659 ($p<0.05$).

660

661

Gene	Forward Primer	Reverse Primer
Actin-5C	AGCGCGGTTACTCTTTCACCAC	GTGGCCATCTCCTGCTCA AAGT
Attacin A	CGTTTGGATCTGACCAACG	AAAGTTCCGCCAGGTGTGAC
CecropinA1	CTCTCATTCTGGCCATCACC	TCTTGAGCGATTCCCAGTC
Defensin	GCGGATCATGTCCTGGTGCAT	TCGCTTTGGCGGCTATG
Diptericin A	GCGGCGATGGTTTTGG	CGCTGGTCCACACCTTCTG
Drosocin	TTTTCCTGCTGCTTGCTTGC	GGCAGCTTGAGTCAGGTGAT
Drosomysin	CTGCCTGTCCGGAAGATACAA	TCCCTCCTCCTTGCACACA

662 **Supplementary Table 1:** Primer sequences for AMP and housekeeping genes ('5-3').

663 **Sandwich ELISA to quantify tagged AMPs**

664 HA/FLAG tagged AMPs were quantified 6 hr post-infection. The fly line w^{1118} ; Dro^{HF}/Dro^{Hf}
665 was used to quantify tagged Drosocin and w^{1118} ; $Cec^{HF}/+$ was used to quantify tagged
666 Cecropin. Maxisorp Nunc 96-well ELISA plates (Thermo Scientific 232698) were coated with 2.5
667 µg/mL of anti-FLAG antibody (Sigma-Aldrich F1804) in coating buffer (0.2 M sodium

668 carbonate/bicarbonate buffer) overnight at 4 °C, then the plates were blocked for 2 hours at
669 room temperature with 2% bovine serum albumin (Sigma-Aldrich A2153) prior to use. At the
670 time of sampling, flies were homogenized in 200 µL of 0.2% Triton-X buffer and centrifuged at
671 10,000 RPM for 5 mins to pellet fly debris. Drosocin samples were diluted 1:20 and Cecropin
672 samples were diluted 1:10 so that they fell within linear range of a HA/FLAG peptide standard
673 curve (sequence NH2-DYKDD DDKGG GGGSY PYDVP DYA-NH2 made from AAPTec). The
674 standard curve was prepared by performing a 2-fold serial dilution starting from a stock solution
675 of 2 ng/mL of HA/FLAG peptide to yield 14 standard concentrations. For sample incubation, 50
676 µL of each standard and sample were added to the ELISA plate and then incubated overnight.
677 Any unused wells were filled with 50 µL of 0.2% Triton-X buffer to serve as blanks. The next
678 day, anti-HA diluted 1:5000 in 0.2% Triton-X was added to each well for another overnight
679 incubation at 4 °C. The following morning, 100 µL of room temperature 1-Step Ultra TMB-ELISA
680 Substrate Solution (ThermoFisher #34028) was added to each well and the plate was incubated
681 at 25 °C for 30 min. After incubation, 100 µL of 2M sulfuric acid was added to each well to stop
682 the reaction. Plates were then read on a spectrometer at 450 nm to measure absorbance. The
683 amount of peptide per fly was determined from the HA/FLAG standard curve with the following
684 equation:

685 Peptide per fly = [(Absorbance-Slope) * Dilution Factor]/ (Intercept * Fly Homogenate)

686 To determine whether diet affects Drosocin peptide levels, a linear mixed effects model was
687 applied, with diet and infection status as main factors and experimental block as a random
688 factor. The emmeans function in R was used to compare peptide yield from flies on each diet
689 within infection status.

690 Model E: lmer(Dro_levels ~ diet * infection + diet + (1|block))

691 To determine effects of diet on Cecropin peptide levels, a hurdle model was applied. In the first
692 step, a binomial generalized linear model (GLM) was used to test whether diet and infection
693 status (*Serratia* or *Providencia* infection versus uninjured) affected the probability of detecting
694 Cecropin peptide in the samples.

695 Model F: glm(Cec_present ~ diet * infection + diet, family = binomial)

696 In the second step, a GLM was performed on samples with detectable peptide to test
697 whether diet and infection resulted in quantitative differences in peptide abundance.

698 Model G: glm(Cec_level ~ diet *infection + diet)

699 The significance of the contrast between diets within each infection was determined with a
700 post-hoc Tukey test ($p<0.05$) using the emmeans package in R.

701 **Measurement of nutritional indices**

702 Nutritional indices were measured in naïve flies and in flies 24 hours post-infection with
703 either *P. rettgeri* or *S. marcescens.* Flies were homogenized in pools of three in 150 µL of cold
704 lysis buffer (10 mM Tris, 1 mM EDTA, pH 8.0 with 0.1% (v/v) Triton-X-100) with a motorized
705 pestle in 1.5 mL microcentrifuge tubes. Homogenates were centrifuged for 1 minute at 13,000
706 rpm at 4°C to pellet fly debris. The supernatants were transferred to fresh 1.5ml microfuge
707 tubes, and 10 µL of homogenized sample was removed for protein quantification. The remaining
708 samples were placed in a 72°C water bath for 20 minutes to denature endogenous enzymes.
709 The tubes were stored at -80 °C for later use.

710 <u>Protein measurements:</u> Protein was measured to normalize all carbohydrate measures. Non-
711 heated fly homogenates were diluted to 1:8 in lysis buffer. Protein was quantified according to
712 Bio-Rad Assay DC Protein Assay Kit instructions. Briefly, 5 µL of standard and sample were
713 incubated with 25 µL reagent A and 200 µL of reagent B. Bovine serum albumin was serially
714 diluted to generate a standard curve that was then used to determine the quantity of protein in

715 each sample. Samples and standards were all run in duplicates. Samples were incubated at
716 room temperature for 15 minutes and then read on a spectrometer plate reader at 750 nm.

717 Glucose, trehalose, and glycogen level measurements: Glucose, trehalose, and glycogen
718 levels were measured using reagents from the Sigma Aldrich Glucose (GO) Assay Kit
719 (GAGO20-1KT) and processed according to manufacturer's instructions. The same fly
720 homogenates were used across all nutrients assayed. Glucose was measured by mixing 5 μL of
721 standard or 5 μL of sample with 150 μL of glucose assay reagent. A glucose standard curve
722 was generated from 2-fold serial dilution from a 1 mg/mL glucose stock concentration. Each
723 sample and standard were run in duplicate. The plate was incubated for 30 minutes at 37°C,
724 then 150 μL of 12M sulfuric acid was added to each well to stop the reaction. Plates were then
725 measured for absorbance at 544 nm on a spectrometer. For trehalose measurements, 5 μL of
726 sample and standards were incubated with 2.5 μL of trehalase overnight at 37 °C. For each
727 sample, two replicates were treated with trehalase enzyme and two were not. Trehalose
728 standard curves were generated from a glucose standard curve, trehalose standards treated
729 with trehalase, and trehalose standards without enzyme. Glucose liberated from trehalose
730 digestion was quantified the next day using the procedure described above, and the abundance
731 of trehalose in the standard was inferred from the difference between the samples that were
732 treated with trehalase and those that were not. Glycogen measurements were conducted
733 similarly, except 5 μL of standard and 5 μL of sample were incubated overnight with
734 amylogucosidase instead of trehalase. The glycogen standard curve was prepared from a
735 glycogen stock solution over the range 1 mg/mL to 0.1 mg/mL. All estimates of carbohydrate
736 levels were normalized to measured protein level for each sample to correct for potential
737 differences in the size of the fly. To calculate change in metabolite level after infection, we
738 subtracted the average post-infection value of the metabolite level from the average level in
739 uninfected samples within an experimental block. A linear mixed-effects model was performed

740 to test for the main effects of diet and infection on carbohydrate/protein ratios with experimental
741 block as a random factor. The emmeans package was used to perform a post-hoc Tukey's test
742 to determine the significance of pairwise comparisons between diet and infection ($p<0.05$).

743 **Bacteria growth curves *in vitro***

744 *Serratia marcescens* and *Providencia rettgeri* were measured for *in vitro* growth using the
745 kinetic cycle in a spectrometer (Molecular Biosciences Spectra Max Series Plus 384) at room
746 temperature. Bacteria were first cultured overnight with shaking in liquid LB at 37°C. The next
747 morning, bacterial cells were resuspended in 1X M9 minimal media (M9) to achieve A_{600} = 1.0,
748 then further diluted to A_{600} = 0.20 in one of six different experimental M9 media conditions. The
749 combinations of M9 media used were 1% glucose, 1% sucrose, 1% casamino acids (CAS), 1%
750 casamino acids and 1% glucose, 1% casamino acids and 1% sucrose, and a M9 blank. CAS
751 was added to the media to provide a nitrogen source while glucose and sucrose provide a
752 carbon source. In a sterile 96-well plate with a clear flat bottom, 100 µL of bacteria resuspended
753 in the experimental media were pipetted into individual wells in 3-fold replicate. Two wells of
754 uninoculated experimental media were included to monitor for contamination. The plate was
755 covered with a lid and inserted into the spectrometer to measure A_{600} at 15-minute intervals over
756 18 hours at 37°C. We used a linear mixed effect model to test for the main effects of CAS and
757 sugar with block as a random factor to test whether the addition of CAS and/or sugar led to
758 increased bacterial growth, measured as a higher final A_{600}. A post-hoc Tukey HSD was
759 performed using the emmeans package in R to test pairwise comparisons ($p<0.05$) between
760 media containing sugars and CAS.

761 Model H: lmer(OD600 at 18 hours ~ sugar *CAS + sugar + (1|block))

762

763

764 **Preparation of Axenic Flies**

765 Axenic flies were prepared by embryo bleaching. Conventionally reared (microorganism
766 associated) flies were fed on lab standard yeast cornmeal diet (4% sucrose w/v), and
767 approximately 100-150 flies were placed into an egg laying chamber and allowed to lay eggs on
768 grape juice agar plates. Embryos were collected after 20 hours and removed from agar plates
769 with a clean paint brush into a cell strainer. In a six-well tissue culture plate, embryos were
770 washed within the cell strainer first in 10% bleach twice for 1.5 minutes each, followed by two
771 30-second washes in 70% ethanol. Lastly, embryos were washed twice for 10 seconds with
772 autoclaved 1X PBS. Embryos were continuously mixed throughout the process to prevent them
773 from sticking to each other or the cell strainer. Once sterilized, embryos were transferred with a
774 sterile brush onto autoclaved yeast-cornmeal rearing media in groups of 50. During pupation,
775 the rearing media was replaced with autoclaved 2% and 16% sucrose diets for adult flies to
776 eclose onto.

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780

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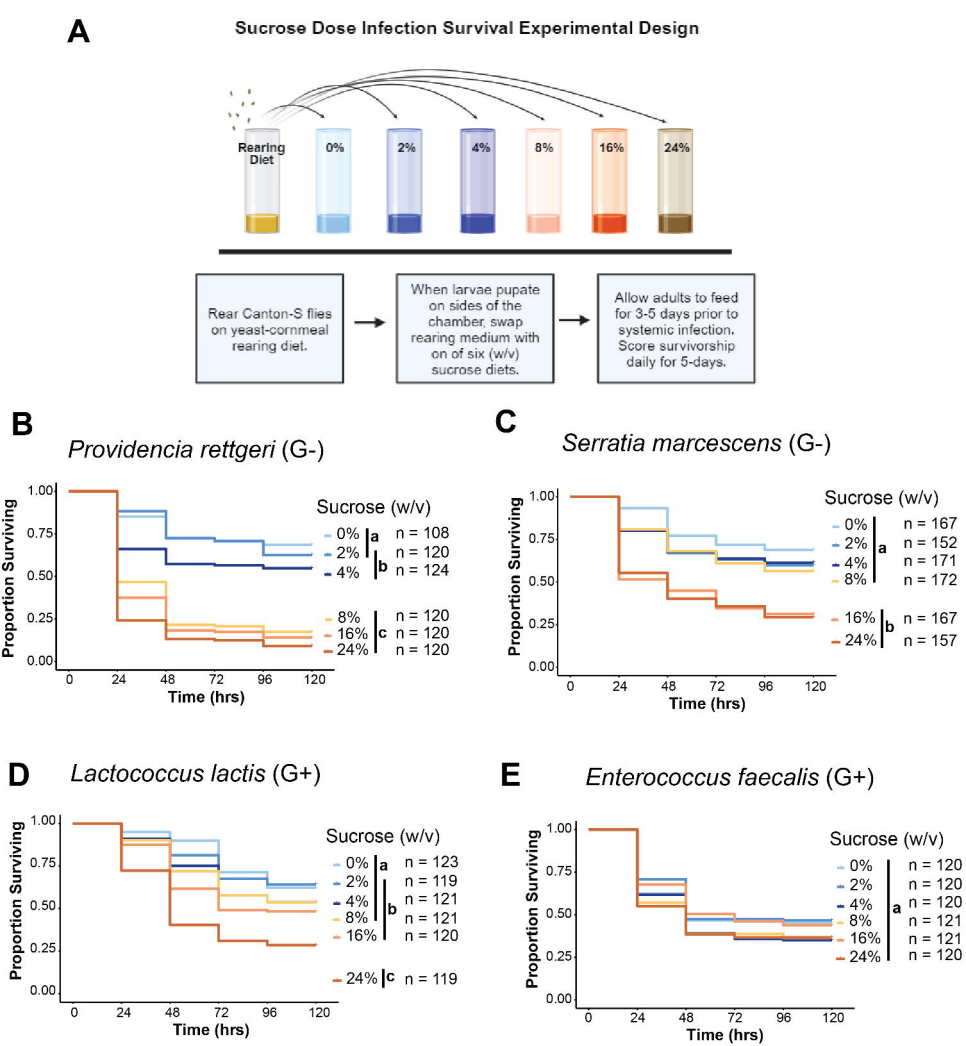
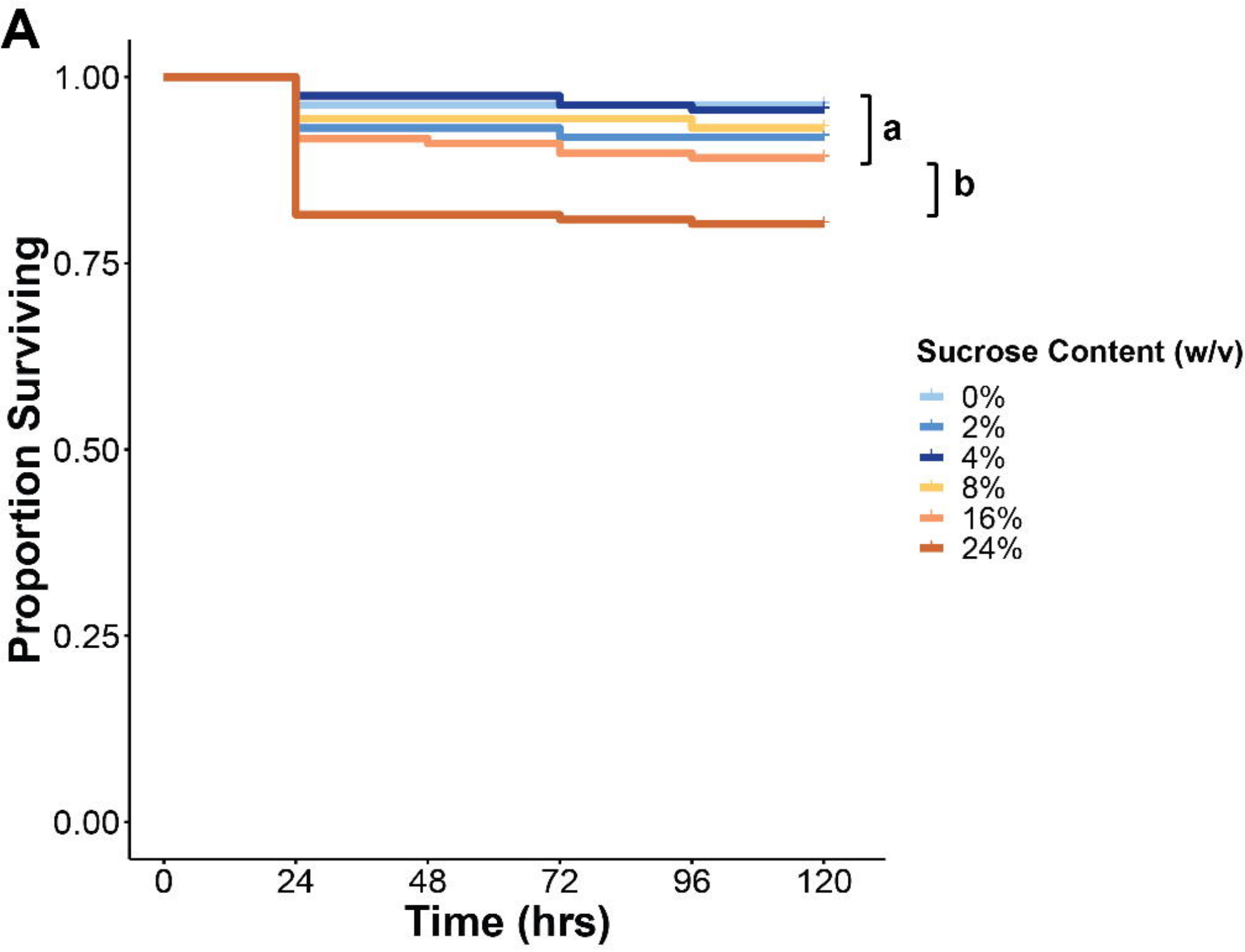
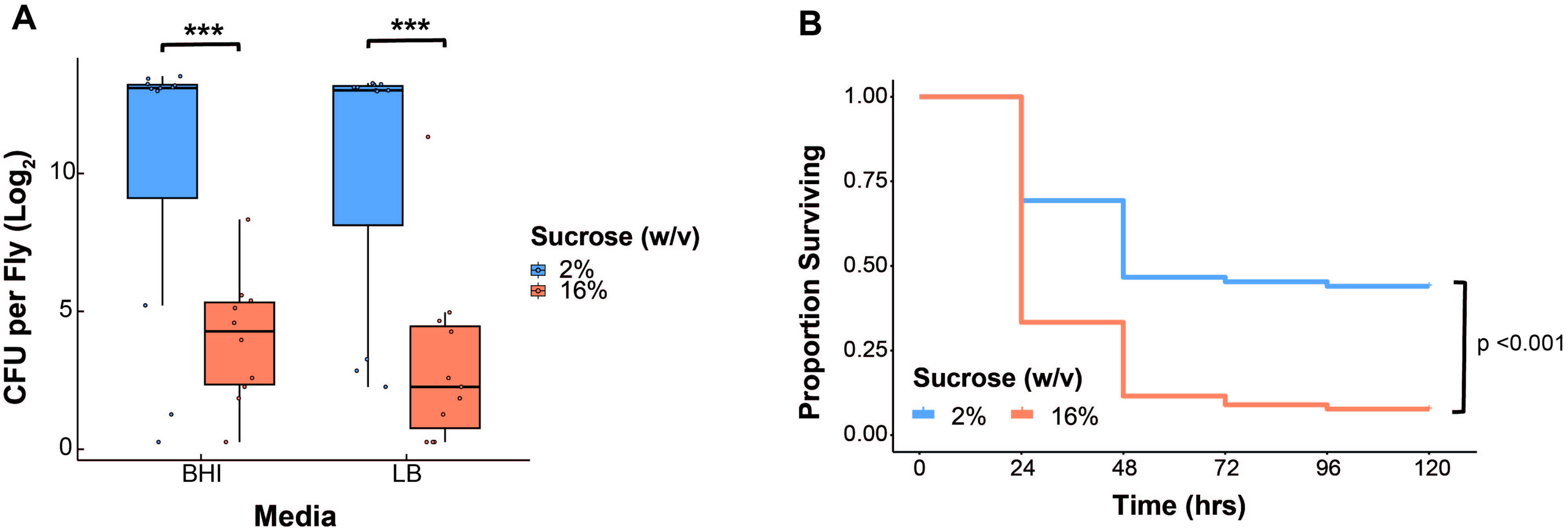
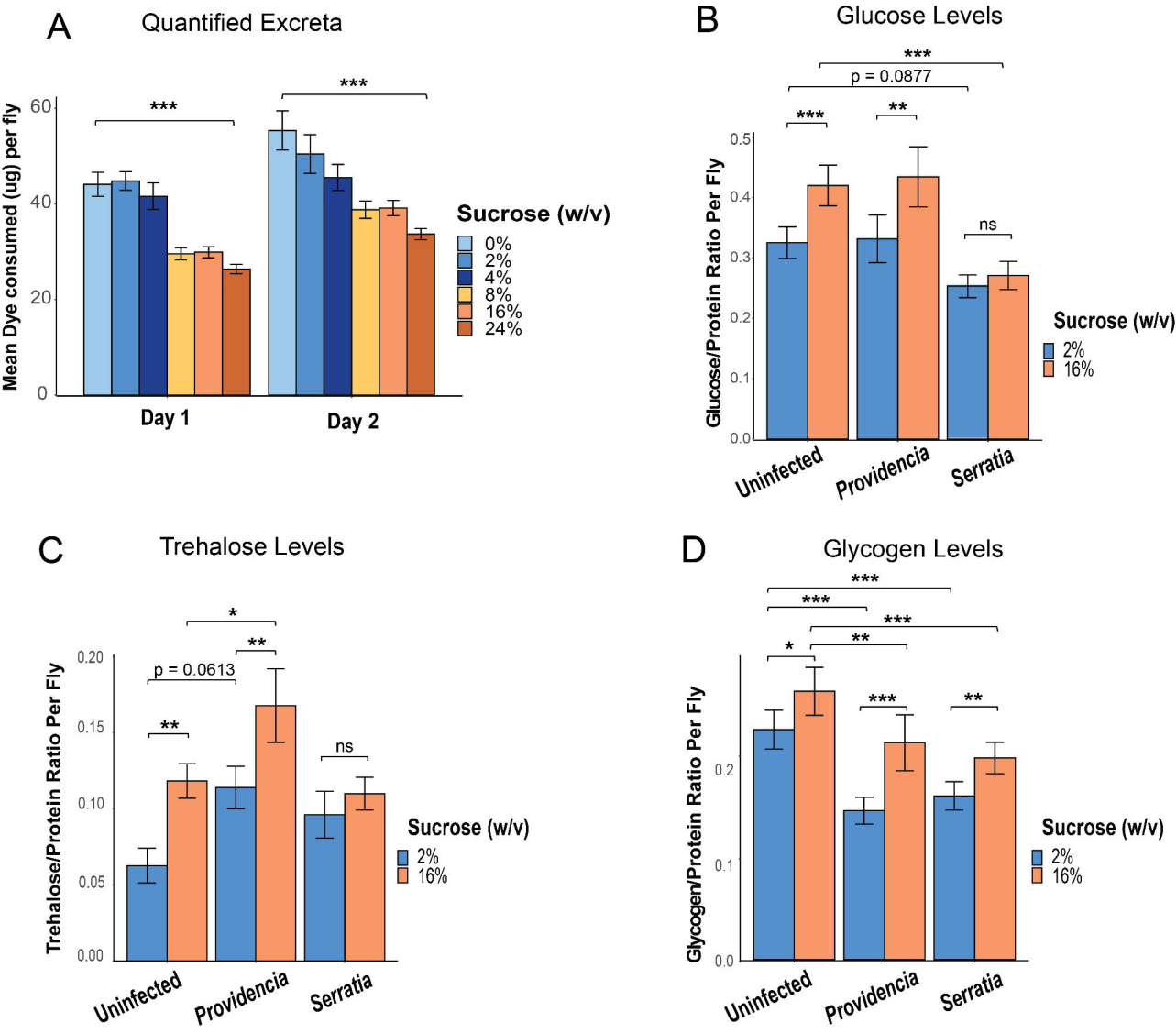
983 reduction of immune defense in mated female *Drosophila melanogaster.* Journal of Insect
984 Physiology. 2022 Jul 1;140:104414.

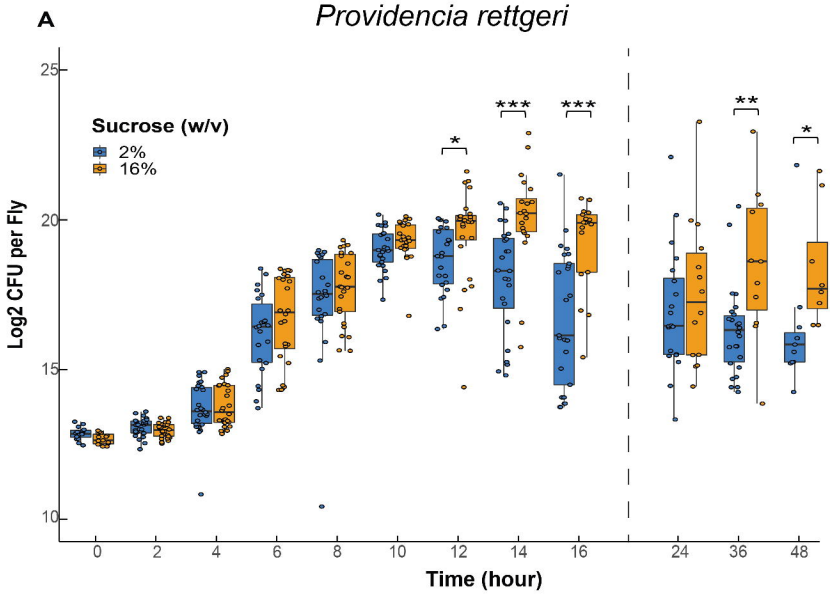
985


A
Sucrose Dose Infection Survival Experimental Design
Rearing Diet
0%
2%
4%
8%
16%
24%
Rear Canton-S flies on yeast-cornmeal rearing diet.
When larvae pupate on sides of the chamber, swap rearing medium with on of six (w/v) sucrose diets.
Allow adults to feed for 3-5 days prior to systemic infection. Score survivorship daily for 5-days.
B
Providencia rettgeri (G-)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 108
2% b n = 120
4% n = 124
8% c n = 120
16% n = 120
24% n = 120
C
Serratia marcescens (G-)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 167
2% n = 152
4% n = 171
8% n = 172
16% b n = 167
24% n = 157
D
Lactococcus lactis (G+)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 123
2% n = 119
4% b n = 121
8% n = 121
16% n = 120
24% c n = 119
E
Enterococcus faecalis (G+)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% n = 120
2% n = 120
4% a n = 120
8% n = 121
16% n = 121
24% n = 120


A
1.00
0.75
0.50
0.25
0.00
Proportion Surviving
0
24
48
72
96
120
Time (hrs)
a
b
Sucrose Content (w/v)
0%
2%
4%
8%
16%
24%

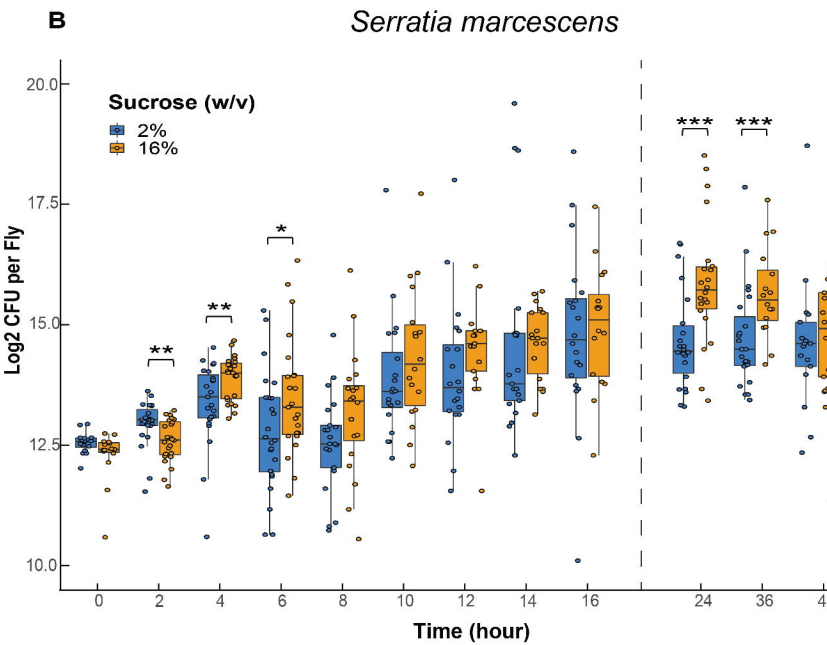

A
***
***
CFU per Fly (Log2)
10
5
0
BHI
LB
Media
Sucrose (w/v)
2%
16%
B
Proportion Surviving
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
Time (hrs)
Sucrose (w/v)
2%
16%
p <0.001


A
Quantified Excreta
Mean Dye consumed (ug) per fly
60
40
20
0
***
***
Day 1
Day 2
Sucrose (w/v)
0%
2%
4%
8%
16%
24%
B
Glucose Levels
Glucose/Protein Ratio Per Fly
0.5
0.4
0.3
0.2
0.1
0.0
***
p = 0.0877
***
**
ns
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%
C
Trehalose Levels
Trehalose/Protein Ratio Per Fly
0.20
0.15
0.10
0.05
0.00
*
**
p = 0.0613
**
ns
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%
D
Glycogen Levels
Glycogen/Protein Ratio Per Fly
0.2
0.1
0.0
***
***
***
**
*
***
**
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%



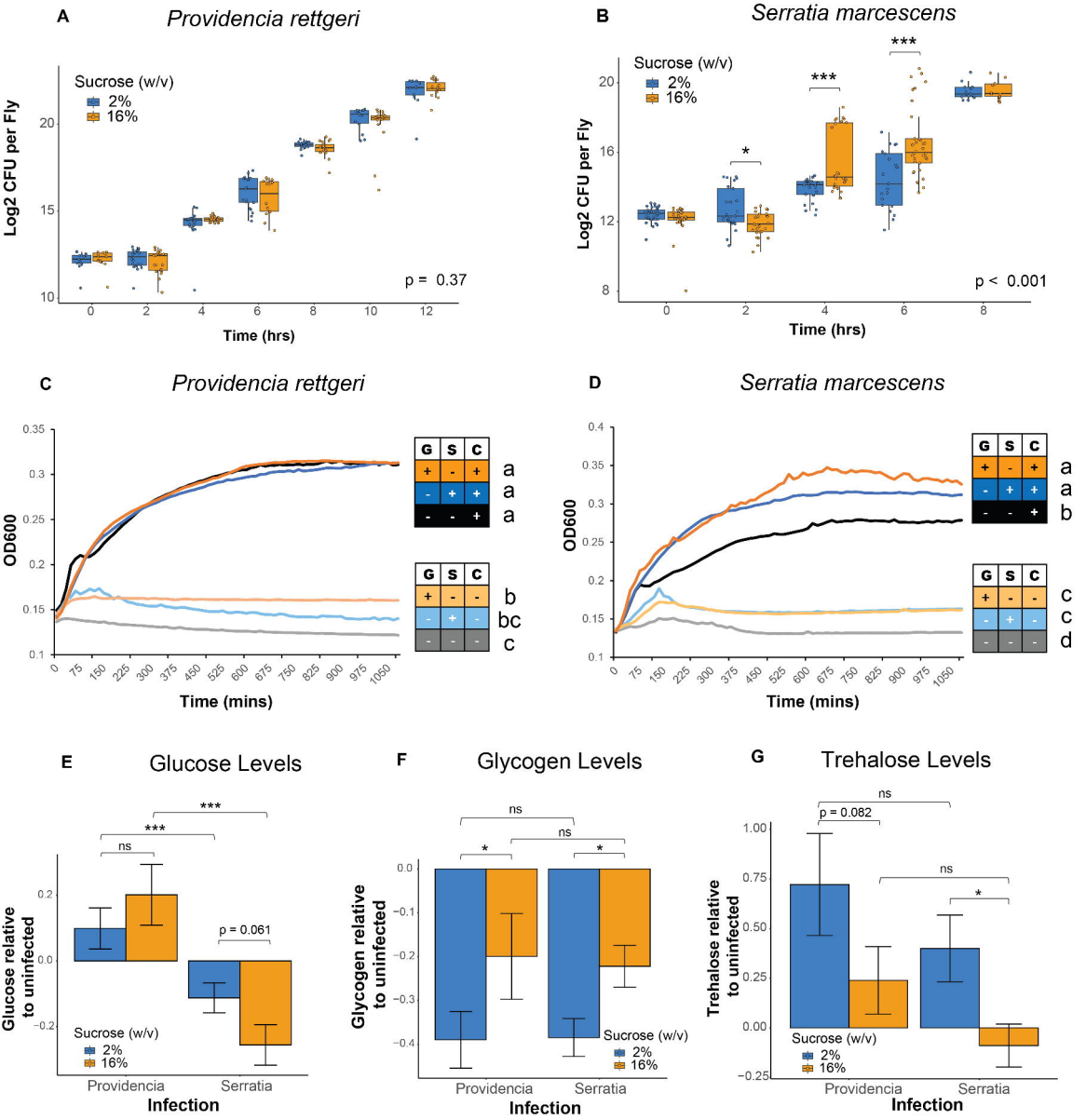
Proportion of flies alive at each time point

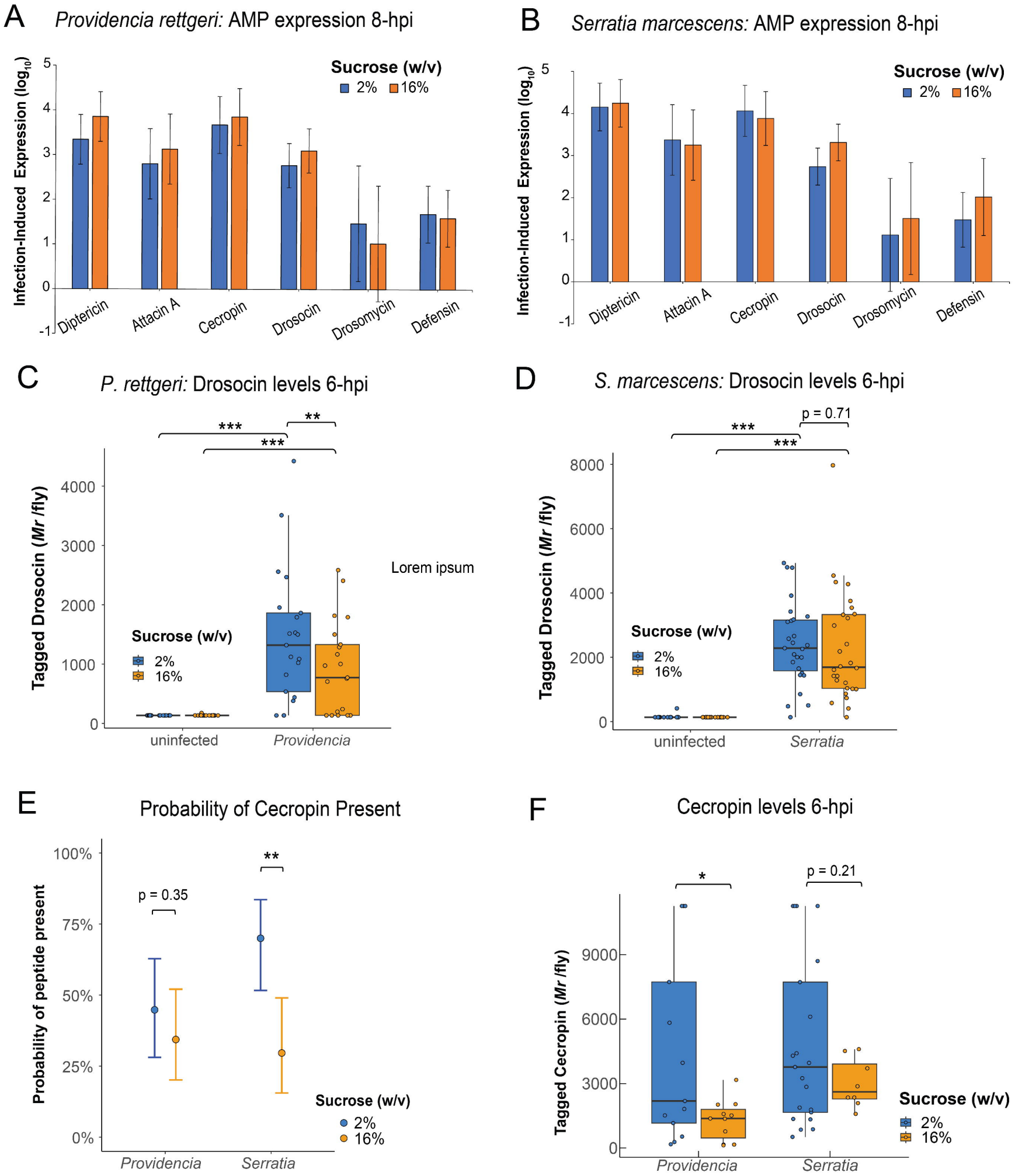
Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	0.97	0.93	0.93	1.0	0.93	0.90	0.90	0.97	0.76	0.76	0.71
16%	1.0	0.97	1.0	0.89	1.0	0.83	0.85	0.81	0.59	0.47	0.29	0.29

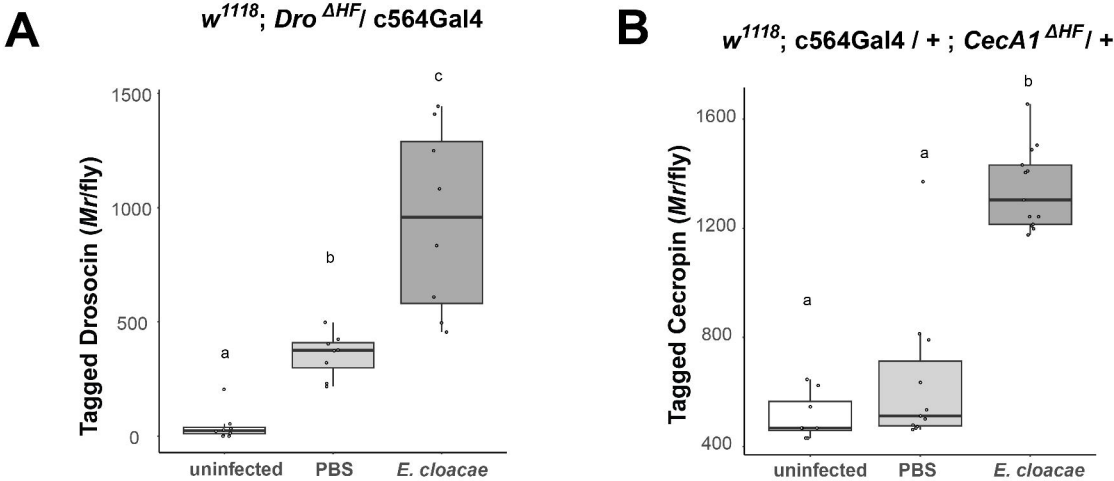


Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	1.0	1.0	0.96	0.90	0.93	0.85	0.90	0.83	0.80	0.74	1.0
16%	1.0	1.0	0.93	1.0	0.83	0.69	0.76	0.67	0.63	0.62	0.42	0.44


A
Providencia rettgeri
Sucrose (w/v)
2%
16%
Log2 CFU per Fly
Time (hrs)
p = 0.37
B
Serratia marcescens
Sucrose (w/v)
2%
16%
Log2 CFU per Fly
Time (hrs)
p < 0.001
C
Providencia rettgeri
OD600
Time (mins)
G S C
a
a
a
b
bc
c
D
Serratia marcescens
OD600
Time (mins)
G S C
a
a
b
c
c
d
E
Glucose Levels
Glucose relative to uninfected
Sucrose (w/v)
2%
16%
ns
***
***
p = 0.061
Providencia
Serratia
Infection
F
Glycogen Levels
Glycogen relative to uninfected
Sucrose (w/v)
2%
16%
ns
ns
*
*
Providencia
Serratia
Infection
G
Trehalose Levels
Trehalose relative to uninfected
Sucrose (w/v)
2%
16%
ns
p = 0.082
ns
*
Providencia
Serratia
Infection


A
Providencia rettgeri: AMP expression 8-hpi
Infection-Induced Expression (log10)
Sucrose (w/v)
2%
16%
Diptericin
Attacin A
Cecropin
Drosocin
Drosomycin
Defensin
B
Serratia marcescens: AMP expression 8-hpi
C
P. rettgeri: Drosocin levels 6-hpi
Tagged Drosocin (Mr /fly)
Lorem ipsum
uninfected
Providencia
D
S. marcescens: Drosocin levels 6-hpi
p = 0.71
Serratia
E
Probability of Cecropin Present
Probability of peptide present
p = 0.35
F
Cecropin levels 6-hpi
Tagged Cecropin (Mr /fly)
p = 0.21


A
w1118; Dro ΔHF/ c564Gal4
Tagged Drosocin (Mr/fly)
1500
1000
500
0
a
b
c
uninfected
PBS
E. cloacae
B
w1118; c564Gal4 / + ; CecA1 ΔHF/ +
Tagged Cecropin (Mr/fly)
1600
1200
800
400
a
a
b
uninfected
PBS
E. cloacae

Gene	Forward Primer	Reverse Primer
Actin-5C	AGCGCGGTTACTCTTTCACCAC	GTGGCCATCTCCTGCTCA AAGT
Attacin A	CGTTTGGATCTGACCAACG	AAAGTTCCGCCAGGTGTGAC
CecropinA1	CTCTCATTCTGGCCATCACC	TCTTGAGCGATTCCCAGTC
Defensin	GCGGATCATGTCCTGGTGCAT	TCGCTTTGGCGGCTATG
Diptericin A	GCGGCGATGGTTTTGG	CGCTGGTCCACACCTTCTG
Drosocin	TTTTCCTGCTGCTTGCTTGC	GGCAGCTTGAGTCAGGTGAT
Drosomycin	CTGCCTGTCCGGAAGATACAA	TCCCTCCTCCTTGCACACA