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Pathogen dependence and inter-individual variability of post-infection reproductive fitness in *Drosophila melanogaster*

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ABSTRACT

In the experiments reported in this manuscript, we explore the effect of bacterial infections on the reproductive output of *Drosophila melanogaster* females. Canonical view of host-pathogen interactions supposes two possible outcomes. Because of immune defence being an energy/resource intensive function, an infected female reallocates resources away from reproductive processes and towards immune defence, therefore compromising its reproductive output. Alternatively, faced with impending mortality, an infected female increases its reproductive output to compensate for lost opportunities of future reproduction. We tested if pathogen identity, infection outcome (survival vs. death), and/or time of death determines the reproductive output of females infected with three bacterial pathogens. Our results show that pathogen identity is a reliable predictor of population level response of infected females but does not reliably predict the behaviour of individual females. Additionally, females succumbing to infection exhibit greater variability in reproductive output, compared to both survivors and controls, but this variability is not explained by either the time of death or the identity of the infecting pathogen. Furthermore, survivors of infection have reproductive output similar to control females.

Keywords: *Drosophila melanogaster*, bacterial infection, cost of immunity, terminal investment, fecundity compensation.

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20 # INTRODUCTION

21 Omnipresence of pathogens/parasites impose a strong selection pressure on hosts to evolve
22 mechanisms of defense. Such defense mechanisms go far beyond the canonical anatomical
23 and physiological defenses, and include behavioral strategies that either help alleviate risk of
24 infection or help mitigate the consequences. Fecundity compensation, that is the post
25 infection increase in reproductive effort of the host, is one such behavioral defense that helps
26 hosts maintain their evolutionary fitness [1]. Since increased reproductive effort maximizes
27 immediate reproductive output at the cost of future chance of reproduction [2], organisms
28 under benign conditions are expected to pace out their reproductive schedule so as to
29 maximize their life-time reproductive success [3, 4]. Under circumstances which lead to pre-
30 mature death, such as a lethal infection, future opportunities of reproduction are compromised
31 and organisms should, in theory, maximize their immediate reproductive effort [5, 6, 7].
32 Minchella and Loverde [8] first demonstrated this phenomenon in snails infected with
33 castrating trematode parasites, where hosts increased their immediate reproductive output in
34 response to parasitic infection.

35 An infection is also detrimental to the physiology of the host organism. One, mounting an
36 immune response requires investing energy and resources that could otherwise have been
37 utilized elsewhere, such as towards reproduction [9, 10, 11]. Two, infection leads to somatic
38 damage caused by the virulence factors produced by parasites/pathogens [12, 13]. And three,
39 the immune response mounted by the host often causes collateral somatic damage to the host,
40 leading to immunopathology [14]. Altogether this suggests that post-infection fitness of hosts
41 depends on its ability to restrict the systemic propagation of the parasite/pathogen, plus the
42 host's capacity to continue to maintain physiological functionality during and after recovery
43 from the infection [15]. Reallocating resources towards mounting an immune defense can
44 lead to reduced reproductive effort during acute infection [16], and lingering somatic damage

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45 can keep reproductive effort to a minimum even after recovery. Fecundity compensation, as
46 described above, therefore might not be the observed strategy in case of all hosts on every
47 occasion, and will depend on the features of the specific host-pathogen system being studied.

48 The choice of strategy is likely to depend on the balance between the actual risk of mortality
49 and the level of somatic damage incurred by the host. A greater risk of mortality should
50 induce a stronger fecundity compensation response, thereby increasing reproductive effort,
51 while reproductive effort should decline proportionately with increasing somatic damage.
52 This balance can vary at the level of individual hosts, causing the mean population behavior
53 to not be a true reflection of the individual variation in strategies. In fact, increased and
54 decreased reproductive effort can be viewed as two ends of a continuum – instead of a
55 dichotomous choice – with each individual host opting for an optimal level of reproductive
56 effort based on their proximate circumstances.

57 Furthermore, post-infection reduction in host reproductive effort may also be driven by
58 leeching of resources by the pathogen/parasite, damage to reproductive tissue, or
59 manipulation of the host physiology by virulence factors produced by the pathogen/parasite
60 [12]. Thus, post-infection reduction in host reproductive effort can also be a consequence of
61 the infection process (presence of pathogen), independent of the host response to infection.
62 To differentiate between post-infection phenotypes that are driven by pathogen manipulation
63 and those caused by host immune response, previous studies have often used attenuated
64 pathogens or pathogen-like proxies (bacteria-derived lipopolysaccharides, plastic beads, etc.;
65 viz. [17]), arguing that such proxies stimulate the host immune system without causing any
66 infection-related pathologies. While experiments with live pathogens may fail to tease apart
67 host's response to pathogens from pathogen's manipulation of the host, experiments with
68 pathogen-like proxies may not induce any fitness effects, both physiological and
69 reproductive. Furthermore, given that mounting an immune response is costly to the host,

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70 hosts are under pressure to evolve mechanisms that differentiate real infections from false
71 alerts. Thus, results obtained from experiments using attenuated pathogens and proxies are
72 difficult to interpret. The results of experiments are thus likely to depend upon the exact
73 biology of the interacting host and pathogen [18], on the physiological capability of the host
74 to modify its own reproductive effort, and on whether such modifications of reproductive
75 effort will materialize into benefits in terms of immune function [19], among various other
76 factors.

77 Previous studies exploring the effect of parasites and pathogens on reproductive behavior of
78 *Drosophila melanogaster* have reported diverse outcomes, depending partially upon the type
79 of infectious agent used in the experiments. Flies having successfully survived a parasitoid
80 attack as larvae have reduced fecundity as adults [20, 21]. Flies infected with Drosophila C
81 Virus exhibit genotype and infection route dependent increase or decrease in reproductive
82 output [22]. Infection with bacterial pathogens have been demonstrated to increase [23],
83 reduce [24, 25], or maintain fecundity at an unaltered level [26, 27]. The reasons for this
84 diversity of outcomes can be multiple, including host susceptibility to pathogens used [28],
85 infection route [29, 30], genotypic differences in host strains and possible interactions with
86 environmental factors [31, 32]. Another variable that can affect experimental outcomes is
87 whether reproductive effort is measured during the acute or the chronic phase of infection
88 [16]. Infection survivors continue to have a low level of systemic pathogen presence which
89 have life history consequences [33], although one study reported infection survivors have
90 similar fecundity as to the controls following recovery from a bacterial infection [34].

91 In this study we challenged *Drosophila melanogaster* females with three pathogenic bacteria,
92 (a) *Bacillus thuringiensis*, (b) *Pseudomonas aeruginosa*, and (c) *Seratia marscesens*, and
93 quantified their change in post-infection reproductive output, during the acute phase of
94 infection, compared to uninfected controls. The aim of the study was to identify the effect of

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95 (a) pathogen identity, (b) infection outcome, and (c) time of death, on post-infection
96 reproductive effort. Pathogen identity represents differences in pathogen virulence factors,
97 host defence mechanisms, associated costs and immunopathology. Therefore, we expect that
98 pathogen identity will be a strong determining factor for post-infection reproductive effort.
99 Infection outcome, that is survival versus death, is the ultimate determinant of fitness at the
100 level of individual hosts. Hosts that succumb to infection lose out on future opportunities to
101 reproduce, and therefore are expected to modulate their current reproductive effort differently
102 than hosts that recover from infection. And finally, individual hosts that die within a short
103 period following infection are expected to exhibit a greater increase in reproductive effort
104 compared to hosts that die relatively later.

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105 RESULTS

106 Using flies from a wild-type, outbred population of *Drosophila melanogaster* (BRB2, see
107 MATERIALS AND METHODS for more details) we tested for the effect of infection with
108 different entomopathogenic bacteria on female reproductive fitness. In the first experiment
109 (figure 1.A), we infected 4-5-day old, inseminated females with three bacteria: *Bacillus*
110 *thuringiensis* (hereafter *Bt*), *Pseudomonas aeruginosa* (hereafter *Pa*), and *Serratia*
111 *marcescens* (hereafter *Sm*); we maintained sham-infected and uninfected controls along with
112 the infected treatments. After infections, the females of each treatment were hosted in vials in
113 groups of 8, with 10 vials per treatment. The entire experiment was independently replicated
114 thrice. We monitored the mortality in these vials, every 2 hours, for 24 hours post-infection,
115 covering the acute phase of infection of all three pathogens. As a measure of reproductive
116 output, we counted the total number of eggs in each vial, laid by 8 females in 24 hours, and
117 also the total number of adult progeny that developed from the eggs. This provided us with an
118 additional measure of fitness: pre-adult viability (proportion of eggs that successfully
119 developed into adults) of the progeny produced by the infected females.

120 All three pathogens used imposed significant mortality upon the infected females compared
121 to the uninfected controls (figure 2.A). All females infected with *Sm* (hazard ratio, 95%
122 confidence interval: 31895.33, 4333.172-234773.08) and *Pa* (HR, 95% CI: 936.66, 130.79-
123 6707.86) died because of infection within 12 and 24 hours of infection, respectively, while
124 about half of all females infected with *Bt* (HR, 95% CI: 156.17, 21.80-1118.65) died of
125 infection within the observation period. Females that were sham-infected (HR, 95% CI: 7.98,
126 0.99-63.78) did not show significant difference in mortality compared to uninfected controls.

127 Since the lifespan of females in each treatment was different from one another, instead of
128 directly comparing the absolute number of eggs laid (or progeny produced), we divided the

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129 total number of eggs (or progeny) in each vial with the summation of the hours survived
130 (survival time right-censored at 24 hours post-infection for surviving females) by the females
131 in that vial (see MATERIALS AND METHODS for more details). We call this the
132 "standardised reproductive output" and use this value as the subject of analysis. This value is
133 essentially the measure of the number of eggs (or progeny) per female per hour, when the
134 females are either infected with pathogens, or sham-infected, or left uninfected.

135 Infection treatment had a significant effect on standardised reproductive output, in terms of
136 both eggs laid ($F_{4,147}$: 58.778, $p < 2.2$ e-16; figure 2.B) and progeny produced ($F_{4,147}$: 61.338,
137 $p < 2.2$ e-16; figure 2.C). Post-hoc pairwise comparison using Tukey's HSD indicated that
138 *Sm*-infected females (least-square mean, 95% CI: 1.580, 1.408-1.752) laid a significantly
139 greater number of eggs per female per hour compared to uninfected (LS mean, 95% CI:
140 0.641, 0.469-0.813), sham-infected (LS mean, 95% CI: 0.653, 0.481-0.825), *Bt*-infected (LS
141 mean, 95% CI: 0.783, 0.611-0.955), and *Pa*-infected (LS mean, 95% CI: 0.662, 0.490-0.834)
142 females; the other four experimental treatments did not differ from one another significantly
143 in terms of number of eggs laid (table S2.A). Similarly, *Sm*-infected females (LS mean, 95%
144 CI: 1.337, 1.207-1.467) produced a significantly greater number of progeny per female per
145 hour compared to uninfected (LS mean, 95% CI: 0.589, 0.458-0.719), sham-infected (LS
146 mean, 95% CI: 0.578, 0.448-0.708), *Bt*-infected (LS mean, 95% CI: 0.663, 0.533-0.794), and
147 *Pa*-infected (LS mean, 95% CI: 0.545, 0.414-0.675) females; the other four experimental
148 treatments did not differ from one another significantly in terms of number of progeny
149 produced (table S2.B).

150 Infection treatment had a significant effect on progeny pre-adult viability ($F_{4,150}$: 7.985, p =
151 7.304 e-06; figure 2.D). Post-hoc pairwise comparison using Tukey's HSD indicated that
152 progeny of *Bt*-infected (LS mean, 95% CI: 0.856, 0.830-0.881), *Pa*-infected (LS mean, 95%
153 CI: 0.826, 0.800-0.851), and *Sm*-infected (LS mean, 95% CI: 0.855, 0.829-0.880) females

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154 had significantly less pre-adult viability compared to progeny of uninfected females (LS
155 mean, 95% CI: 0.915, 0.889-0.940). There was no difference in viability between progeny of
156 sham-infected (LS mean, 95% CI: 0.888, 0.863-0.914) and uninfected females. Progeny of
157 *Pa*-infected females also had less viable compared to progeny of sham-infected females
158 (table S2.C).

159 To have an estimate of the effect of infecting females with different pathogenic bacteria on
160 the fitness of their progeny, we measured the early-life fecundity of the progeny, beginning at
161 day 4-5 of adulthood till day 10-11 of adulthood. We pooled all progenies produced by all 80
162 females in each treatment (8 females × 10 vials) and randomly samples 60 males and 60
163 females, housing them in groups of 6 males and 6 females, setting up 10 vials per *maternal*
164 treatment. We counted the number of progenies produced by these flies over the next six
165 days, counting the progenies per day separately, and using that as the subject of analysis. The
166 day of count (age of the flies) had a significant effect on early-life fecundity of progeny flies
167 ($F_{1,897}$: 488.713, $p < 2.2$ e-16; figure S1). Progeny early life fecundity was also significantly
168 affected by *maternal* treatment ($F_{4,897}$: 31.427, $p < 2.2$ e-16; figure S1). Post-hoc pairwise
169 comparison using Tukey's HSD indicated that progeny of *Sm*-infected females (LS mean,
170 95% CI: 9.56, 8.52-10.06) had significantly lesser fecundity compared to progeny of
171 uninfected (LS mean, 95% CI: 12.08, 11.04-13.1), sham-infected (LS mean, 95% CI: 12.06,
172 11.02-13.1), *Bt*-infected (LS mean, 95% CI: 11.59, 10.55-12.6), and *Pa*-infected (LS mean,
173 95% CI: 11.79, 10.75-12.8) females; the other four *maternal* treatments did not differ from
174 one another significantly in terms of progeny fecundity (table S2.D).

175 In the above experiment, all females infected with *Sm* and *Pa* died of infection, while only
176 half of *Bt*-infected females died (figure 2.A). In the follow-up experiment (figure 1.B), we
177 tested if the outcome of infection (survival vs. death), and the time of death for individual
178 females, had any effect on reproductive fitness of the females. We housed infected and sham-

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179 infected females individually in food vials after infection, and monitored their mortality every
180 2 hours for 48 hours post-infection. The experiment was independently replicated thrice for
181 each bacterium used: *Bt*, *Pa*, and *Sm*. We counted the number of progeny produced by
182 individual females in the span of 48 hours (or till the time the female died) as a measure of
183 reproductive output. To account for differences in lifespan (number of hours survived by an
184 infected female; survival time right-censored at 48 hours post-infection for females that
185 didn't die within that time), we divided the number of progeny produced by a female by the
186 hours survived, and used this "standardised reproductive output" as subject of analysis. Since
187 in the first experiment, between treatment differences in standardised reproductive output did
188 not change based on whether we focused on the number of eggs or the number of progeny, in
189 this experiment we only counted the number of progeny produced.

190 Similar to the first experiment, only about half of *Bt*-infected females, while all of *Sm*- and
191 *Pa*-infected females, died due to infection (figure 3.A, 3.D, 3.G). For *Bt*-infected females,
192 infection outcome did not have a significant effect on mean standardised reproductive output
193 of the females ($F_{2,475}$: 1.4701, p = 0.2309); the infected-dead females (LS mean, 95% CI:
194 0.926, 0.683-1.17), the infected-alive females (LS mean, 95% CI: 0.895, 0.654-1.14), and the
195 sham-infected females (LS mean, 95% CI: 0.817, 0.577-1.06) had comparable mean
196 standardised reproductive output (figure 3.B). Infection outcome significantly affected the
197 variance in standardised reproductive output (Levene's test, $F_{2,475}$: 20.808, p = 2.174 e-09),
198 with infected-dead females exhibiting greater variance compared to both infected-alive and
199 sham-infected females (figure 3.B). Within infected-dead female, time-of-death had a
200 significant effect on standardised reproductive output ($F_{1,210}$: 6.3233, p = 0.01267), with
201 reproductive output having a mild negative correlation with time-to-death (coefficient, 95%
202 CI: -0.01733, -0.03109 – -0.00355; η^2, 90% CI: 0.03, 0.00-0.08; figure 3.C).

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203 For *Pa*-infected females (all of which died following infection; figure 3.D), infection
204 outcome had a significant effect on standardised reproductive output ($F_{1,474}$: 4.3739, p =
205 0.03703), with infected-dead females (LS mean, 95% CI: 0.743, 0.618-0.867) producing less
206 number of progeny compared to sham-infected females (LS mean, 95% CI: 0.851, 0.721-
207 0.981; figure 3.E). Infection outcome significantly affected the variance in standardised
208 reproductive output (Levene's test, $F_{2,474}$: 19.795, p = 1.075 e-05), with infected-dead females
209 exhibiting greater variance compared to sham-infected females (figure 3.E). Time-of-death
210 did not have a significant effect on standardised reproductive output ($F_{1,357}$: 0.511, p =
211 0.4503; figure 3.F).

212 For *Sm*-infected females (all of which died following infection; figure 3.G), infection
213 outcome had a significant effect on standardised reproductive output ($F_{1,408}$: 25.5, p = 6.684
214 e-07), with infected-dead females (LS mean, 95% CI: 1.28, 0.747-1.81) producing greater
215 number of progeny compared to sham-infected females (LS mean, 95% CI: 0.72, 0.207-1.23;
216 figure 3.H). Infection outcome significantly affected the variance in standardised
217 reproductive output (Levene's test, $F_{2,408}$: 40.875, p = 4.444 e-10), with infected-dead females
218 exhibiting greater variance compared to sham-infected females (figure 3.H). Time-of-death
219 did not have a significant effect on standardised reproductive output ($F_{1,290}$: 0.1505, p =
220 0.6983; figure 3.I).

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221 # DISCUSSION

222 Fecundity compensation (or, terminal investment) theory in its simplest form hypothesises
223 that an infected host facing impending death would increase its immediate reproductive effort
224 to compensate for the loss of future opportunities to reproduce [1, 6, 7]. This hypothesis can
225 be sub-structured into testable predictions, such as

226 (a) hosts infected with a lethal pathogen would exhibit increased reproductive effort
227 compared to hosts infected with a pathogen that does not kill all of the infected
228 individuals;
229 (b) in case of pathogens for which all hosts do not succumb to infection, hosts that die
230 due to infection would increase their reproductive effort compared to hosts that
231 survive the infection; and,
232 (c) among hosts that succumb to infection, there will be a negative correlation between
233 reproductive effort and time of death.

234 Tests of theoretical predictions of change in investment towards reproduction, in response to
235 various intrinsic and extrinsic variables, hinge on accurate estimation of reproductive effort
236 (proportion of total available resources that is invested towards reproduction), which is often
237 difficult to measure [35]. Studies exploring infection induced changes in reproductive
238 investment subvert this problem using uninfected controls. The uninfected controls represent
239 an optimal reproductive output given a certain level of accessible resources and residual
240 reproductive value. Resultantly in such studies a change in reproductive output in infected
241 hosts compared to controls can be interpreted in light of the fecundity compensation/terminal
242 investment theory [1, 6, 7]. In this study we use '*standardised* reproductive output' (number
243 of eggs, or progeny, normalised by the post-infection time-to-death of the females) as a proxy
244 of reproductive effort. The lifespan of infected females in our experiments vary greatly

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245 depending upon the pathogen used for infection (figure 2.A), and thus a direct comparison of
246 absolute egg or progeny count is not suitable.

247 Briefly, in this study we investigated how infection with three entomopathogenic bacteria,
248 which differ from one another with respect to the level of mortality imposed on the host,
249 affect the reproductive output of female *Drosophila melanogaster*. Additionally, we explored
250 the effect of *maternal* infection on pre-adult viability and early-life fecundity of the progeny.
251 We further investigated if infection outcome (death vs. survival), and the time of death,
252 differentially affected the reproductive output of individual infected females. Our key
253 findings are as follows:

254 (a) The effect of infection on mean reproductive output is pathogen dependent (figures
255 2.B-C). Females infected with *Serratia marcescens* (hereafter *Sm*) produce a greater
256 number of eggs (and progeny) compared to uninfected control females, after
257 accounting for differences in post-infection lifespan. Females infected with *Bacillus*
258 *thuringiensis* (hereafter *Bt*) or *Pseudomonas aeruginosa* (hereafter *Pa*) have
259 reproductive output similar to controls.
260 (b) The effect of *maternal* infection on progeny life-history is different for each trait
261 measured. Progeny pre-adult viability was reduced by infection with all three
262 pathogens, with the greatest reduction seen in progeny of *Pa*-infected females (figure
263 2.D). On the other hand, progeny early-life fecundity was compromised only in case
264 of progeny of *Sm*-infected females; progeny of *Bt*- and *Pa*-infected females had
265 fecundity comparable to progeny of uninfected females (figure S1).
266 (c) Females that succumb to infection exhibit greater variability in reproductive output,
267 compared to control females and females that survive the infection, irrespective of the
268 pathogen used for infection (figures 3.B, 3.E, and 3.H). This variability in
269 reproductive output is not explained by time of death in *Pa*- and *Sm*-infected female

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270 (figures 3.F and 3.I); for *Bt*-infected females there is a negative correlation between
271 time of death and *standardised* reproductive output, but with a very low effect size
272 (figure 3.C). Females that survive the infection have reproductive output comparable
273 to controls in terms of both mean and variance (comparison possible for *Bt*-infected
274 females only).

275 Forbes [18] classified host-pathogen systems based on whether acute infection had any
276 negative effect on current reproduction (possibly due to somatic damage to the reproductive
277 tissue or leeching of resources) and future reproductive potential (brought about by host death
278 or permanent somatic damage) of the host. Increased reproductive output is the predicted
279 outcome only if future reproductive potential is compromised, but without any negative effect
280 on current reproduction [18]. An observed reduction in reproductive output during acute
281 infection can thus be because of (a) pathogen leeching resources from the host or
282 manipulating host physiology to reduce fecundity [12], (b) damage to reproductive tissue by
283 the pathogen [24] or by the host immune defence itself [14], or (c) rerouting of resources
284 meant for reproduction towards immune defence by the host [31]; although such reallocation
285 of resources in either direction may not always translate into greater fitness benefits [19].

286 Amongst the three pathogens used in this study, infection with two (*Pa* and *Sm*), is absolutely
287 lethal, while about half of females infected with *Bt* survive acute infection (figure 2.A).
288 Therefore, *Sm*- and *Pa*-infected females have zero future reproductive potential, while *Bt*-
289 infected females can continue to reproduce post-recovery, assuming that there is no lingering
290 somatic damage. *Drosophila melanogaster* flies never clear out infecting pathogens from
291 their system completely [36], and a chronic, low level of pathogens continue to persist with in
292 the fly body, which requires some investment into immune function on part of the host to
293 keep in check [33]. It is therefore a possibility that *Bt*-infected females may never regain
294 uninfected levels of reproduction even post-recovery, but given that *Bt*-infected females that

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295 survive the infection continue to reproduce at levels of control females even during acute
296 infection period (figure 3.B), this is an unlikely possibility. *Bt*-infected females should
297 therefore invest towards immune defence and not towards increasing immediate reproductive
298 output, to maximise chances of survival and opportunity of future reproduction, as we see in
299 the results from our experiment (figure 2.B and 2.C).

300 Based on the arguments outlined above it is expected that females would increase their
301 reproductive effort after being infected with *Sm* and *Pa*, but we observe an increase in
302 reproductive output only in case of *Sm*-infected females (figure 2.B and 2.C). The absence of
303 any change in reproductive output of *Pa*-infected females may be driven by many possible
304 reasons, including damage to reproductive tissue, and exploitation or manipulation of host by
305 the pathogen; we rule out resource reallocation driven costs since we have argued above that
306 when infection guarantees lethality, investment away from reproduction is counter-
307 productive. Since we directly did not measure damage to reproductive tissue, we cannot
308 choose with sanguinity between the different possibilities listed above based on the data at
309 hand.

310 Progeny of infected females, independent of the infecting pathogen suffered from reduced
311 pre-adult viability; progeny of *Pa*-infected females exhibited the greatest reduction (figure
312 2.D). Perrin et al [37] have proposed that when *maternal* infection compromises progeny
313 viability, increasing progeny production is not a suitable strategy for an infected host; this
314 may be another explanation for why *Pa*-infected females do not increase reproductive output
315 despite of guaranteed lethality due to infection. Reduced viability of progeny of Pa-infected
316 females have been reported in other previous studies (viz. [38]; but see [23]). Reduced
317 progeny viability can lead to a progeny quantity vs. progeny quality trade-off [2], making
318 investment into progeny quality, instead of increasing progeny number, a potential strategy
319 for *Pa*-infected females.

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320 Infection with *Pa* has been previously demonstrated to both increase [23] and suppress [25]
321 reproduction in females. A host's response to the same pathogen can change because of the
322 route of infection [22, 29], which can be a possible reason behind different observations in
323 different studies: Hudson et al [23] infected flies via oral route, while in this study and in that
324 of Linder and Promislow [25] flies were infected via septic injury to the thorax. A systemic
325 infection is more likely to reach the reproductive tissues, via the haemolymph, than an oral
326 infection, which first has to colonise the gut and breach the gut lining to enter into circulation.

327 Infected females that died of infection, irrespective of the pathogen used for infection,
328 exhibited greater inter-individual variability in reproductive output compared to control
329 (sham-infected) females and females that survived the infection (figures 3.B, 3E, and 3.H).
330 The observed difference in the mean reproductive outputs of the infected and control females
331 remained consistent across both experiments, suggesting that pathogen identity is a reliable
332 predictor of post-infection reproductive output at the population level. Females that survived
333 the infection with *Bt* had reproductive output similar to that of controls (figure 3.B), in terms
334 of both population mean and inter-individual variability, suggesting that infected females
335 may be able to judge their own prognosis and invest into reproduction accordingly. Since no
336 females infected with either *Pa* or *Sm* survived the infection, we cannot conclude if this
337 observation is generalizable for all other pathogens for which mortality is less than hundred
338 percent. What seems puzzling therefore is why don't females that succumb to infection,
339 irrespective of pathogen identity, increase their reproductive output?

340 Based on the earlier discussion, individual females that succumb to infection are expected to
341 increase their reproductive output. This increase should happen irrespective of pathogen
342 identity, driven only by the risk of mortality, except in a case where infection compromises
343 current reproductive capacity. Contrary to this expectation, we see that the reproductive
344 output of infected-dead females for each pathogen ranges from zero to extremely high values;

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345 with some females reproducing less compared to the controls and other reproducing far more
346 in excess (figures 3.B, 3E, and 3.H). Therefore, in canonical sense, we see some females
347 exhibiting 'cost of immunity' while other females exhibiting 'fecundity compensation' when
348 infected with the very same pathogen. This variation in reproductive output seems to be
349 independent of both pathogen identity and time of death.

350 The observed inter-individual variability in reproductive output of females that die of
351 infection may purely be stochastic, without any consequence in terms of evolutionary
352 outcomes [39]. Alternatively, the heterogeneity may reflect variation in individual female
353 quality [40] and physiological state [41]. The physiological state of an individual is a potent
354 predictor of its residual reproductive value, and all else being equal, can therefore influence
355 infection-induced changes in reproductive effort [42]. A third possibility is that the
356 heterogeneity is a consequence of host variation, genetic or otherwise, in response to
357 infection, in terms of both resistance and tolerance [15, 27, 32, 43]. Further empirical
358 exploration is necessary to disentangle these potential causes of inter-individual variation.

359 To summarize, we find that lethal infections do not always induce an increased investment
360 towards immediate reproduction in female *Drosophila melanogaster*; females infected with
361 only one out of two pathogens that imposed hundred percent mortality increased their
362 reproductive effort. Furthermore, females dying of infection do not have greater reproductive
363 effort compared to females that survive the infection, and reproductive effort had a negative
364 correlation with time of death in case only one out of three pathogens used in this study.
365 These findings suggest that the mechanistic interaction between a host and a pathogen has a
366 greater influence on host reproductive effort, compared to infection status, infection outcome,
367 and mortality risk on the host by the pathogen. Additionally, our results suggest that pathogen
368 identity is a reliable predictor of bacterial infection induced change in reproductive effort of
369 the females at the level of population means, but pathogen identity does not predict

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370 reproductive output of individual females. Females infected with all three pathogens used in
371 this study have overlapping range of reproductive output. Furthermore, maternal infection
372 can affect progeny life-history traits, but the effect is specific to individual traits. In
373 conclusion, dichotomy of 'cost of immunity' versus 'fecundity compensation' is too narrow
374 in scope to account for all nuances involved in post-infection change in reproductive effort.

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375 # MATERIALS AND METHODS

376 **Host population and general handling**

377 Flies from BRB2 population - a large, lab adapted, out-bread population of Drosophila
378 melanogaster - was used for the experiments reported in this paper. The Blue Ridge Baseline
379 (BRB) populations were originally established by hybridizing 19 wild-caught iso-female lines
380 [44], and has been maintained since then as an outbred population on a 14-day discrete
381 generation cycle with census size of about 2800 adults each generation. Every generation,
382 eggs are collected from population cages (plexiglass cages: 25 cm length × 20 cm width × 15
383 cm height) and dispensed into vials (25 mm diameter × 90 mm height) with 8 ml banana-
384 jaggery-yeast food medium, at a density of 70 eggs per vial. 40 such vials are set up; the day
385 of egg collection is demarcated as day 1. The vials are incubated at 25 °C, 50-60% RH, 12:12
386 hour LD cycle; under these conditions the egg-to-adult development time for these flies is
387 about 9-10 days. On day 12 post egg collection all adults are transferred to population cage,
388 and provided with fresh food plates (banana-jaggery-yeast food medium in a 90 mm Petri
389 plate) supplemented with *ad libitum* live yeast paste. On day 14, the cage is provided with
390 fresh food plate, and 18 hours later eggs are collected from this plate to begin the next
391 generation.

392

393 **Pathogen handling and infection protocol**

394 Three bacterial pathogens were used in this study for infecting the flies: *Bacillus*
395 *thuringiensis* (*Bt*; obtained from DSMZ, Germany, catalogue number: DSM2046),
396 *Pseudomonas aeruginosa* (*Pa*; obtained from MTCC, India), and *Serratia marcescens* (*Sm*;
397 [29]). All three pathogens are maintained in the lab as glycerol stocks, and are cultured in
398 Luria Bertani broth (Himedia, M1245); cultures are incubated at 30 °C for *Bt*, and 37 °C for

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399 *Pa* and *Sm*. Overnight culture of bacteria grown from glycerol stocks was diluted (1:100) in
400 fresh LB medium and incubated till confluency (optical density OD_{600} = 1.0-1.2). The
401 bacterial cells were pelleted down by centrifugation and re-suspended in sterile 10 mM
402 $MgSO_4$ buffer at OD_{600} = 1.0. Flies were infected by pricking them at the dorsolateral side of
403 the thorax with a fine needle (Minutien pin, 0.1 mm, Fine Science Tools, CA, item no.
404 26001-10) dipped in bacterial suspension under light CO_2 anesthesia. Flies for sham-
405 infections were similarly treated, but pricked with needle dipped in sterile 10 mM $MgSO_4$
406 buffer. Uninfected control flies were only subjected to CO_2 anesthesia.

407

408 **Generation of experimental flies**

409 Eggs were collected from BRB2 population cages and distributed into food vials with 8 ml of
410 standard food medium at a density of 70 eggs per vial. These vials were incubated as per
411 general maintenance. Twelve days post egg-laying flies were flipped into fresh food vials and
412 hosted for two more days before experimentation. This ensured all focal females were 4-5
413 day old, sexually mature and inseminated, at the time of infections. Flies were again flipped
414 into fresh food vials 6 hours before being subjected to experimental treatments (as described
415 below).

416

417 **Experimental design**

418 **Experiment 1.** Focal females were randomly distributed into five treatments: (a) infected
419 with *Bacillus thuringiensis* (*Bt*), (b) infected with *Pseudomonas aeruginosa* (*Pa*), (c) infected
420 with *Serratia marcescens* (*Sm*), (d) sham-infected controls, and (e) uninfected controls. The
421 entire experiment was independently replicated thrice. Flies were placed in fresh food vials
422 after being subjected to respective treatments. For each treatment 10 vials were set up, each

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423 with 8 females for oviposition; each vial was used as a unit of replication. The vials were
424 monitored every 2 hours to record any mortality, for 24 hours post-infection, divided into two
425 consecutive 12-hour windows. Flies alive at the end of first 12-hour window were flipped
426 into fresh food vials (one-to-one mapping of vial identity), and flies alive at the end of 24
427 hours were discarded (censored). The number of eggs in each vial was counted at the end of
428 respective 12-hour windows. The vials were then incubated under standard maintenance
429 conditions for the eggs to develop into adults, and 12 days after the oviposition period, all
430 adult progeny were counted under light CO_2 anesthesia and transferred to fresh food vials.

431 *Standardized reproductive output* of females in each vial was calculated as,

432 $$Standardized\ egg\ count = \frac{Total\ number\ of\ eggs\ layed\ over\ 24\ hours}{Summation\ of\ lifespan\ of\ all\ females\ in\ the\ vial}$$, and

433 $$Standardized\ progeny\ count = \frac{Total\ number\ of\ adult\ progeny\ produced}{Summation\ of\ lifespan\ of\ all\ females\ in\ the\ vial}.$$

434 Standardization was carried out to account for the differences in post-infection survival time
435 of females in various treatments (see RESULTS for more details). *Progeny pre-adult viability*
436 for each vial was calculated as,

437 $$Pre\ adult\ viability = \frac{Total\ number\ of\ adult\ progeny\ produced}{Total\ number\ of\ eggs\ laid}.$$

438 Following progeny counts, 4-5-day old adult progeny were pooled together according to
439 treatments and distributed to fresh food vials with 5 females and 5 males in each vial; 10 such
440 vials were set up per *maternal* treatment per replicate. These flies were allowed to oviposit
441 for six consecutive days (by flipping them into fresh food vials every day) to obtain an
442 estimate of *offspring early-life fecundity*. These vials were incubated at standard maintenance
443 conditions, and the number of progeny in these vials were counted 12 days post-oviposition.

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444 **Experiment 2.** Focal females were randomly distributed into two treatments: (a) infected
445 with bacteria, and (b) sham-infected controls. For infected treatment, 120 females were
446 individually hosted in vials for oviposition, while for sham-infected controls 40 females were
447 hosted individually. The experiment was replicated thrice with each pathogen. (Due to a
448 handling accident, one replicate with Sm had sample size of 60 and 30 females for infected
449 and sham-infected treatments, respectively.) The vials were monitored every 2 hours for any
450 mortality, for 48 hours post-infection, after which the alive flies were discarded. The vials
451 were then incubated under standard maintenance conditions for the eggs to develop into
452 adults, and 12 days later the number of adult progeny was counted for each individual female.

453 *Standardized reproductive output* for each individual female was calculated as,

454 $$Standardized\ progeny\ count = \frac{Number\ of\ offspring\ produced}{Lifespan\ of\ the\ female\ in\ the\ vial}.$$

455

456 **Statistical analysis**

457 All analyses were carried out using R statistical software (version 4.1.0 [45]), using various
458 functions from the *survival* [46], *coxme* [47], *lmerTest* [48], *emmeans* [49], and *car* [50]
459 packages. Graphs were created using the *ggplot2* [51] and *survminer* [52].

460 **Experiment 1.** Survival data was analyzed using mixed-effects Cox proportional hazards
461 model, with 'Treatment' as a fixed factor and 'Replicate' as a random factor. Reproductive
462 output, progeny viability, and progeny early-life fecundity was modeled using linear models
463 (as described below) and subjected to significance testing using type III analysis of variance
464 (ANOVA). Post-hoc pairwise comparisons, wherever necessary, was carried using Tukey's
465 HSD method.

466 Standardized egg count ~ Treatment + (1|Replicate)

467 Standardized progeny count ~ Treatment + (1|Replicate)

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468 Progeny pre-adult viability ~ Maternal treatment + (1|Replicate)

469 Progeny early-life fecundity ~ Day + Maternal treatment + (1|Replicate)

470 Significance tests for random effects are tabulated in table S1.

471 **Experiment 2.** Reproductive output data was modeled using a linear model (as described
472 below) and subjected to significance testing using type III ANOVA. Post-hoc pairwise
473 comparisons, wherever necessary, was carried using Tukey's HSD method.

474 Standardized progeny count ~ Category + (1|Replicate)

475 'Category' denoted the combination of infection status and infection outcome, and consists of
476 three levels: sham-infected females, infected-alive females, and infected-dead females. Effect
477 of time on death on reproductive output of infected-dead females was similarly analyzed with
478 type III ANOVA using the following linear model:

479 Standardized progeny count ~ Time of death + (1|Replicate)

480 Significance tests for random effects are tabulated in table S1. Comparison of variances
481 across 'category' was carried out using Levene's test after pooling data from all three
482 replicates for each pathogen.

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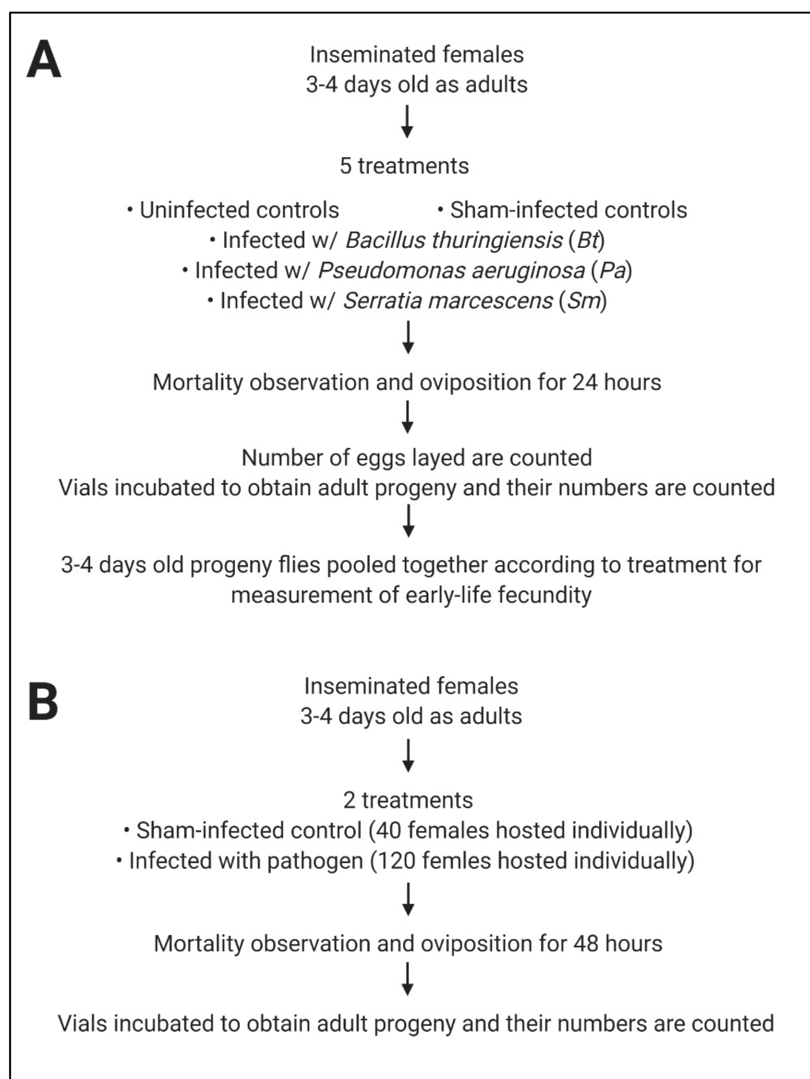
483 FIGURES

484 **Figure 1.** Experimental design for testing the effect of (A) pathogen identity, and (B)
485 infection outcome and individual variability, on post-infection reproductive output of
486 *Drosophila melanogaster* females.



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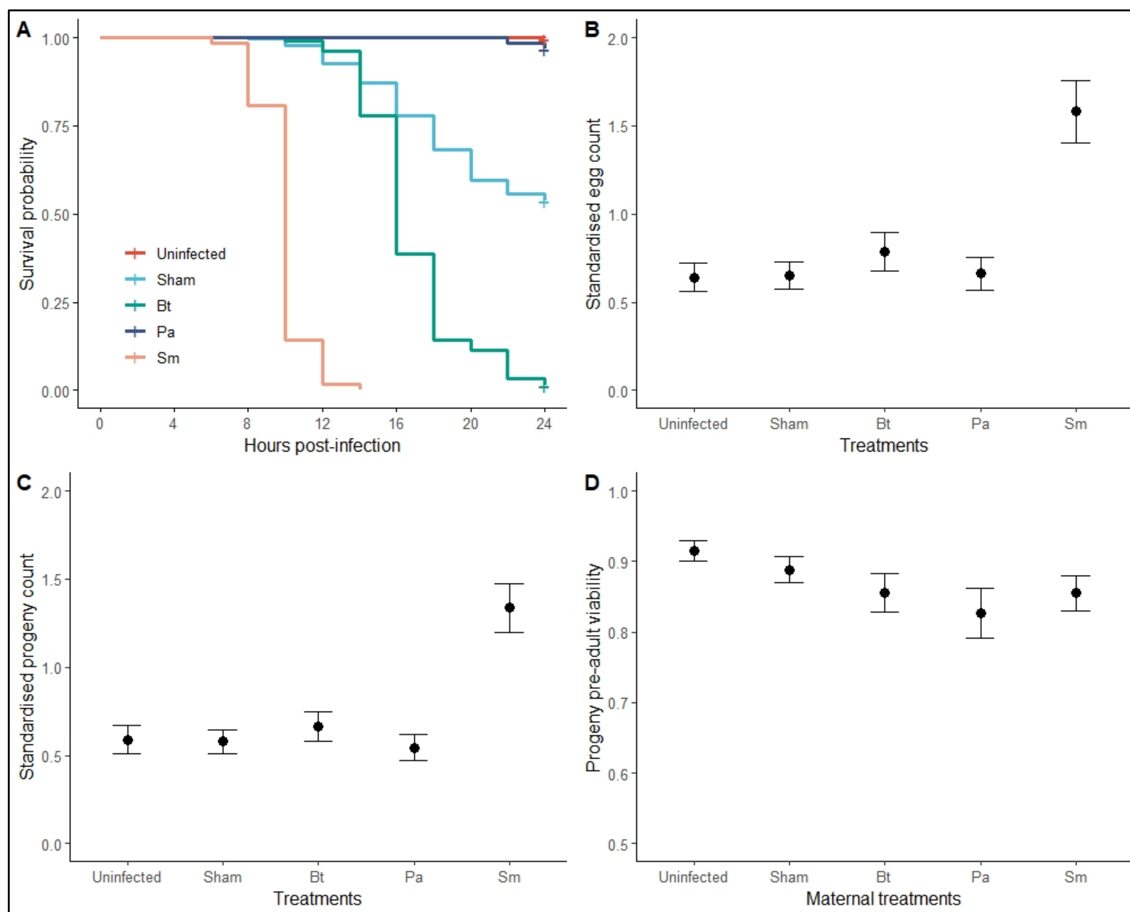
488 **Figure 2.** Effect of infection with different pathogens on (A) survival, (B) number of eggs
489 produced, (C) number of progeny produced, and (D) pre-adult viability of progeny, of
490 *Drosophila melanogaster* females.



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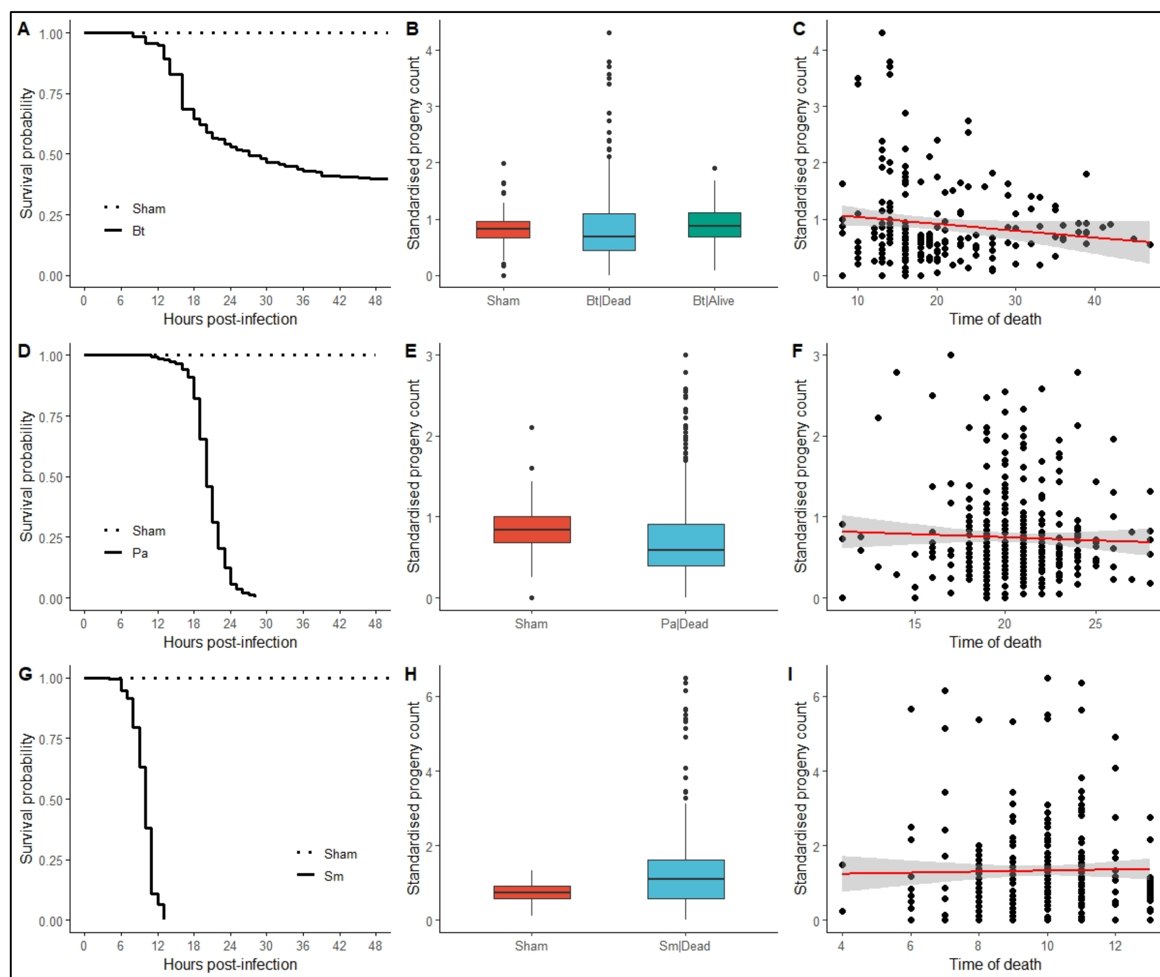
493 **Figure 3.** Effect of infection outcome on post-infection reproductive output of *Drosophila*
494 *melanogaster* females, infected with *Bacillus thuringiensis* (A: survival, B: effect of infection
495 outcome on progeny count, C: effect of time of death on progeny count), *Pseudomonas*
496 *aeruginosa* (D: survival, E: effect of infection outcome on progeny count, F: effect of time of
497 death on progeny count), and *Serratia marcescens* (G: survival, H: effect of infection
498 outcome on progeny count, I: effect of time of death on progeny count).



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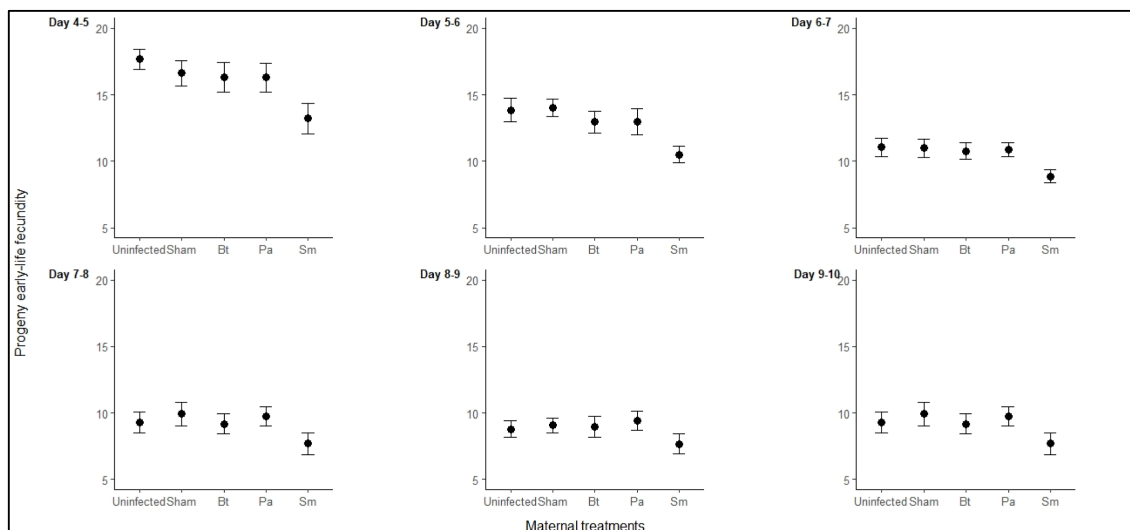
625 SUPPLEMENTARY FILES

626 **Figure S1.** Effect of *maternal* infection treatment on progeny early-life fecundity; fecundity

627 measured on each day shown separately.



628

629 **Table S1.** Significance tests for random factors included in various type-III ANOVA

630 reported in the 'Results' section. See 'Materials and Methods' for full details on statistical

631 analysis.

632 **Table S2.** Post-hoc pairwise comparisons using Tukey's HSD for significant effects reported

633 for fixed factors in various type-III ANOVA reported in the 'Results' section. See 'Materials

634 and Methods' for full details on statistical analysis.