

1 **Sex ratio and the evolution of aggression in fruit flies**

2

3 Eleanor Bath^{1,*}, Danielle Edmunds^{1,*}, Jessica Norman¹, Charlotte Atkins¹, Lucy Harper¹,

4 Wayne G. Rostant², Tracey Chapman², Stuart Wigby^{1,3}, Jennifer C. Perry^{1,2}

5

6 ¹Department of Zoology, 11a Mansfield Road, University of Oxford, Oxford, OX1 3SZ

7 ²School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ

8 ³Department of Evolution, Ecology, and Behaviour, Institute of Infection, Veterinary &

9 Ecological Sciences, University of Liverpool

10 * Joint first authors

11

12 **Corresponding author:** Eleanor Bath, eleanor.bath@zoo.ox.ac.uk, Department of Zoology,

13 University of Oxford, Oxford, UK

14

15 **Keywords:** Sexual selection, aggression, experimental evolution, *Drosophila melanogaster*,

16 sexual conflict, sex ratio

17

18

19

20 **Abstract**

21 Aggressive behaviours are among the most striking displayed by animals, and aggression
22 strongly impacts fitness in many species. Aggression varies plastically in response to the social
23 environment, but we lack direct tests of how aggression evolves in response to intrasexual
24 competition. We investigated how aggression in both sexes evolves in response to the
25 competitive environment, using populations of *Drosophila melanogaster* that we
26 experimentally evolved under female-biased, equal, and male-biased sex ratios. We found
27 that after evolution in a female-biased environment – with less male competition for mates
28 – males fought less often on food patches, although the total frequency and duration of
29 aggressive behaviour did not change. In females, evolution in a female-biased environment –
30 where female competition for resources is higher – resulted in more frequent aggressive
31 interactions among mated females, along with a greater increase in post-mating aggression.
32 These changes in female aggression could not be attributed solely to evolution either in
33 females or in male stimulation of female aggression, suggesting that co-evolved interactions
34 between the sexes determine female post-mating aggression. We found evidence consistent
35 with a positive genetic correlation for aggression between males and females, suggesting a
36 shared genetic basis. This study demonstrates the experimental evolution of a behaviour
37 strongly linked to fitness, and the potential for the social environment to shape the evolution
38 of contest behaviours.

39

40 **Introduction**

41 Aggressive contests occur in males and females across diverse animal taxa [1]. The nature of
42 aggressive contests often differs between the sexes: males largely compete for reproductive
43 opportunities and females largely for reproductive resources [2]. Because aggression
44 significantly impacts fitness in both sexes [3–5], aggressive contests form an important part
45 of reproductive competition [6–8]. Hence, the intensity of reproductive competition in a
46 population should determine the strength of sexual and social selection on aggressive
47 behaviours [2,9,10].

48 More intense reproductive competition is predicted to lead to heightened aggression [11].
49 This prediction has received empirical support. Comparative studies of chernetid false
50 scorpions and dung beetles have found that the presence and size of male weapons is
51 positively correlated with population density and degree of male bias in the sex ratio across
52 species [12,13]. Behavioural studies have reported increased aggression in the sex in excess
53 within populations in fish [14,15]. However, comparative studies cannot eliminate the
54 possibility that variation in aggression is due to other factors that covary with the intensity of
55 competition, such as conspecific density or resource distribution [16]. Likewise, behavioural
56 studies do not show how the competitive environment shapes diversity in aggression across
57 groups. Hence, direct tests of how aggression evolves in response to the intensity of
58 competition are lacking.

59 An additional challenge to studying adaptive variation in aggression is that male and female
60 aggression might be constrained by their shared genome, preventing either or both sexes
61 from reaching their optimum [17]. Indeed, intra-sexual aggression has sometimes been
62 considered a predominantly male trait, with female aggression assumed to arise as a by-

63 product of an intersex genetic correlation ([4], and references therein). Recently, female-
64 female aggression has gained attention as an adaptive strategy for maximising access to
65 resources required for reproduction [8,18], leading to improved reproductive success or
66 offspring survival [19–21]. However, we currently lack data on the independence of the
67 evolution of aggression in each sex.

68 Beyond constraints through the shared genome, female aggression might also depart from
69 the female optimum if female behaviour is subject to manipulation by males [22]. In
70 polygynous mating systems, the optimal level of female-female aggression will be higher for
71 males than for females whenever female aggression confers immediate reproductive benefits
72 that both mating partners experience, but incurs longer-term costs to females in lifetime
73 reproduction. Mating offers males an opportunity to influence female behaviour through
74 ejaculate transfer, and ejaculate-stimulated changes in female behaviour are well-
75 documented [23]. In several species, shifts in female aggression are associated with mating
76 [20,24,25]. Overall, because female aggression has been under-researched relative to male
77 aggression, key facets of the evolution of female aggression, including sexual conflict, the
78 intersex genetic correlation, and responses to intra-sexual competition, are not yet fully
79 understood.

80 Here, we used experimental evolution to ask how male and female aggression evolve in
81 response to the intensity of intra-sexual competition. We exposed replicate populations of
82 fruit flies, *Drosophila melanogaster*, to different competitive environments for >75
83 generations via manipulation of the population sex ratio, a common proxy for the intensity of
84 competition [11,26,27]. Aggression is heritable in *D. melanogaster* and can evolve rapidly
85 under laboratory conditions [28]. Both sexes engage in contests over food patches. For

86 females, food patches provide nutrition required for egg production [29]. For males, which
87 display limited adult feeding [30], food patches predominantly provide access to mates
88 [6,7,31,32]. Both sexes display aggressive behaviours including fencing, male lunging, and
89 female headbutting [7,33]. Mating increases female aggression [33,34] due to the effects of
90 sperm and seminal fluid proteins received at mating [35]. Therefore, evolved differences in
91 female aggression could represent a response to evolved differences in male stimulation of
92 aggression – mediated by sexual conflict – as well as the direct evolution of female behaviour.

93 We addressed the following questions: Does the evolutionary sex ratio drive the evolution of
94 male and female aggression? Does the evolutionary sex ratio affect the post-mating increase
95 in female aggression? Is there evidence for a genetic correlation between male and female
96 aggression? We predicted, first, that males and females evolving in a population biased
97 towards their sex would display heightened aggression. Second, if increased aggression after
98 mating is adaptive for females, then we expected a greater increase in aggression after mating
99 in females from female-biased populations. Third, if female aggression responds to the sex
100 ratio through female adaptation, then we expected that sex ratio effects would occur when
101 experimentally-evolved females mated with males from stock populations, whereas if female
102 aggression responds to the sex ratio through male adaptation to the sex ratio, then we
103 expected that experimentally-evolved males would induce altered aggression in female
104 mates from stock populations. Finally, if the sexes share a genetic basis for aggression, then
105 we expected congruent changes in aggression across populations.

106

107 **Methods**

108 ***Overview***

109 We conducted two experiments. First, we measured intra-sexual aggression in virgin females,
110 mated females, and mated males that had evolved under male-biased, equal and female-
111 biased evolutionary sex ratios (Experiment 1 - 'Coevolved'). In this experiment, all mated
112 individuals mated with partners from the same replicate population. We tested both virgin
113 and mated females because females show a distinct increase in aggression post-mating
114 [33,35], but tested only mated males because, to our knowledge, male aggression does not
115 change with mating (though there is some evidence for mate guarding [36]). We then
116 conducted a second, two-stage experiment to test whether differences in female aggression
117 among sex ratio treatments arise from the evolution of female aggression itself or of male
118 stimulation of female aggression. To do this, we mated experimentally-evolved females with
119 stock males (Experiment 2 - 'Evolved female'), and stock females with experimentally-evolved
120 males (Experiment 2 - 'Evolved male'), and measured female aggression before and after
121 mating. Stock individuals were derived from the same wild-type Dahomey background from
122 which experimentally-evolved populations were generated.

123 Experimentally-evolved flies were maintained in 3 independent replicate populations per sex
124 ratio (see supplementary methods and [37] for details). We assayed behaviour after 78
125 generations for the Experiment 1 and 92 generations for Experiment 2. Fly husbandry and
126 experiments were conducted at 25°C on a 12:12h light:dark cycle with uncontrolled humidity.

127

128 ***Generation of experimental flies***

129 We collected eggs from each of the 9 replicate populations and the stock population and
130 raised larvae at a standardized density on standard laboratory medium [38].

131 At eclosion (day 1), we collected virgin flies under ice anaesthesia. Flies used in aggression
132 trials were housed singly. Males that were used as mates only (in Experiment 2) were housed

133 in pairs. We randomly assigned females to the virgin or the mated treatment. Females
134 assigned to the virgin treatment were housed singly and transferred to new vials on day 3
135 after eclosion (to mirror how mated females were handled). On day 3, we transferred pairs
136 of males and females (those assigned to the mating treatment) from the same replicate
137 population into fresh vials, recorded mating latency and duration, and separated pairs into
138 individual vials when copulation ended. We discarded pairs that did not mate within 3h.

139 ***Aggression Trials***

140 On day 4, we placed all flies singly into food deprivation vials containing only damp cotton
141 wool for 2h to increase aggressive motivation. We randomly assigned flies to a same-sex dyad,
142 with both flies in the dyad coming from the same replicate population and mating status
143 (N=10-29 per population; Tables S2-S4) to standardize the difference between competitors
144 within contests and to expose individuals to the type of competitor encountered in their
145 recent evolutionary history. We transferred dyads into observation chambers (20mm
146 diameter, 5mm depth) containing a central food cup (5mm diameter, standard laboratory
147 medium and live yeast paste). We randomly assigned dyads a trial time between 2-6h
148 Zeitgeber time and allowed 5 minutes acclimatisation before recording aggression trials of 15
149 minutes (Toshiba Camileo X400 cameras). We observed each dyad once and discarded flies
150 after trials.

151 ***Behavioural data extraction***

152 All videos were scored by observers blind to treatment using JWatcher v.1.0 (Macquarie
153 University & UCLA) and BORIS v.7.7.3 [39]. We recorded aggressive behaviours as described
154 in Table S1. To avoid pseudoreplication, the dyad was taken as the unit of replication, with
155 behaviour measures summed for the two individuals. Lunging, chasing and tussling (in males)

156 and headbutts (in females) represent high-intensity aggression and fencing in both sexes
157 represents low-intensity aggression [32]. We calculated a male high-intensity aggression
158 score by summing the amount of time each dyad spent lunging, chasing and tussling. Because
159 food patches can represent breeding territories for males [16,40], and attractive nutritional
160 resources for females [33,35], we calculated food patch occupancy as the average duration
161 the two flies in a dyad spent on the food patch so that we could assess the relationship
162 between aggression and patch occupancy. We recorded the sum of the duration the two flies
163 in a dyad spent walking to test for locomotor differences that might influence aggression. For
164 females, all videos were scored for headbutts as the main high-intensity aggressive behaviour.
165 A subset was also scored for female fencing so that we could assess whether differences
166 extended to low-intensity aggression.

167 ***Statistical analyses***

168 Statistical analyses were conducted in R version 3.6.2 (2019-12-12), using packages ‘MASS’
169 [41], ‘emmeans’ [42], ‘lme4’ [43], ‘survminer’ [44] and ‘coxme’ [45]. We identified outliers by
170 inspection of boxplots or, where data were non-normally distributed, adjusted boxplots [46].
171 We replaced points outside 1.5* the interquartile range with the value of the lower or upper
172 1.5*interquartile range (i.e., winsorization [47]).

173 For all experiments, we ran linear mixed effects models (LMMs; lme4 *lmer()* function) to test
174 the influence of evolutionary sex ratio on the number of lunges (in males) or headbutts (in
175 females), fencing duration, intense male aggression duration, locomotion duration and food
176 patch occupancy. We ran binomial general linear mixed effect models (GLMMs) to test the
177 influence of evolutionary sex ratio on the proportion of male total aggression (fencing,
178 chasing, lunging and tussling) or female headbutting performed on the food patch. For models

179 of female behaviour in EExperiment 1 – ‘Coevolved’ and EExperiment 2 – ‘Evolved female’,
180 we included evolutionary sex ratio, mating status, their interaction, and observer as fixed
181 factors. For models of male behaviour in the EExperiment 1 – ‘oevolved’Coevolved, we
182 included evolutionary sex ratio as a fixed factor (a single observer extracted male data). All
183 models included replicate population and day as random factors and Zeitgeber time as a
184 covariate, and models of female behaviour in EExperiment 1 – ‘Coevolved’ and EExperiment
185 2 – ‘Evolved female’ also included the interaction between replicate population and mating
186 status as a random effect. For EExperiment 2 – ‘Evolved male’, we had a single virgin female
187 treatment and three mated female treatments (i.e., stock females mated to males from each
188 sex ratio). We first assessed the effect of mating on aggression and food occupancy in an LMM
189 with mating status as a fixed factor. For mated females, we then ran a model including
190 evolutionary sex ratio as a fixed factor. Both models included replicate population and day as
191 random factors and Zeitgeber time as a covariate. We found no influence of evolutionary sex
192 ratio on mating latency or duration (Table S5), so we did not include mating behaviour as a
193 covariate in any models.

194 We examined model fit by inspection of diagnostic plots, and where necessary, applied
195 transformations. We analysed LMMs with Wald F tests with Kenward-Roger degrees of
196 freedom [48] (type III for models with significant interactions, type II for models without
197 significant interactions), and analysed binomial GLMMs with Wald χ^2 tests. In female models,
198 when we found a significant interaction between sex ratio and mating status, we re-ran
199 models separately for virgin and mated females to explore sex ratio effects within each group.
200 When sex ratio was significant, we explored the effect using post-hoc Tukey tests. For
201 females, we compared the magnitude of the post-mating changes in behaviours among sex
202 ratios using post-hoc effect size tests.

203 When we found an effect of evolutionary sex ratio on food patch occupancy, we investigated
204 the relationship between aggression and food patch occupancy. We used binomial general
205 linear mixed models as described above to test whether the individual that performs the
206 greatest proportion of total aggression (in males) or headbutts (in females) within a dyad also
207 spends the highest proportion of time on the food patch, and whether this relationship was
208 influenced by evolutionary sex ratio. Individuals that performed equal aggression (16 male
209 dyads, 24 female dyads) were excluded from this analysis. Full model output for all LMMs is
210 included in supplementary material.

211 To explore whether the evolution of sex-specific aggression might be constrained by a shared
212 genetic basis between the sexes, we assessed the correlation between the aggressive
213 behaviour of males and females that evolved in the same replicate population, using data
214 from EExperiment 1 – ‘Coevolved’. A positive correlation might arise from a shared genetic
215 basis, from similar effects of the time and day of behavioural observations in both sexes, or
216 from congruent evolution in response to the evolutionary sex ratio. To control for the
217 influence of time and day (and observer, for female data for which multiple observers were
218 involved) on variation in aggression among vials, we ran linear models of lunging, headbutting
219 and fencing against time and day (and observer, for female data), and used model residuals
220 to calculate a mean behaviour score for males, virgin females, and mated females for each
221 replicate population (N=9). We controlled for effects of the evolutionary sex ratio on variation
222 in aggression among replicate populations by extracting the residuals from linear models of
223 these 9 data points against evolutionary sex ratio. We used the residual values to test for
224 correlations in aggression (female headbutts and male lunges, and fencing in both sexes)
225 between males and virgin or mated females. We tested for a correlation between virgin and

226 mated female aggression to assess evidence for a shared genetic basis to female aggression
227 pre- and post-mating.

228

229 **Results**

230 ***Male aggression and food patch occupancy***

231 We detected no significant influence of the evolutionary sex ratio on the frequency of lunges
232 ($F_{2,6.0}=1.3$, $p=0.339$, square root-transformation; Fig. 1A), the duration of high-intensity
233 aggression (chasing, lunging and tussling; $F_{2,6.0}=1.4$, $p=0.322$, log-transformation), or the
234 duration of low-intensity fencing ($F_{2,6.0}=3.4$, $p=0.104$, square root-transformation).

235 We found that males from female-biased populations spent less time on the food patch
236 compared with male-biased and equal sex ratio populations ($F_{2,5.9}=14.0$, $p=0.006$ Fig. S1B).
237 Males from female-biased populations also performed a lower proportion of total aggression
238 on the food patch relative to males from the other treatments ($\chi^2_2=44.7$, $p<0.001$; Fig. 1B),
239 suggesting differences in resource defence. Aggressive behaviour was related to food patch
240 occupancy. Across all sex ratios, the individual that performed relatively more aggression
241 within a dyad spent relatively more time on the food patch ($\chi^2_1=56.5$, $p<0.001$), and this
242 relationship was weaker as the evolutionary sex ratio became more female-biased
243 ($\chi^2_2=113.8$, $p<0.001$, Fig. 2A). The reduction in food patch use by males from female-biased
244 populations was accompanied by a weak trend towards increased locomotion in these males,
245 relative to those from other sex ratios ($F_{2,6.0}=4.8$, $p=0.056$, Fig. S1A).

246 ***Female aggression and food patch occupancy in Experiment 1 – ‘Coevolved’***

247 We found that mating status and evolutionary sex ratio interacted to influence female
248 headbutt frequency (interaction: $F_{2,6.1}=5.2$, $p=0.048$; mating status: $F_{1,5.3}=46.4$, $p<0.001$; sex
249 ratio: $F_{2,6.1}=2.0$, $p=0.213$; Fig. 3A). Headbutting increased after mating in all evolutionary sex
250 ratios, but females from female-biased populations increased headbutting twice as much
251 females from male-biased or equal sex ratio populations (Fig. 3A; Table S6). In virgin females,
252 we found no significant effect of evolutionary sex ratio on headbutt frequency ($F_{2,6.1}=2.7$,
253 $p=0.149$), but after mating, females from female-biased populations performed more
254 headbutts than females from male-biased populations ($F_{2,6.0}=5.1$, $p=0.050$; post-hoc male-
255 biased vs. female-biased comparison: $t=3.2$, $df=6.1$, adjusted $p=0.043$).

256 There was no evidence of an interaction between mating status and evolutionary sex ratio for
257 female fencing duration, nor evidence for a main effect of evolutionary sex ratio (interaction:
258 $F_{2,6.0}=2.8$, $p=0.142$, square root-transformation; sex ratio: $F_{2,5.8}=3.0$, $p=0.127$; Fig. S2A).
259 Fencing duration increased after mating within all evolutionary sex ratios (mating status:
260 $F_{1,6.0}=42.9$, $p<0.001$; Fig. S2A; Table S6).

261 We found no interaction between mating status and evolutionary sex ratio for food patch
262 occupancy, nor a main effect of evolutionary sex ratio (interaction: $F_{2,6.0}=1.1$, $p=0.382$; sex
263 ratio: $F_{2,6.0}=1.4$, $p=0.312$; Fig. S2C). Food patch occupancy increased post-mating in all
264 evolutionary sex ratios ($F_{1,5.8}=15.3$, $p=0.008$; Fig. S2C). As in males, the more aggressive mated
265 female within a dyad spent relatively more time occupying the food patch ($\chi^2_1=197.5$,
266 $p<0.001$), with the strongest positive correlation in mated females from male-biased sex
267 ratios (interaction: $\chi^2_2=28.4$, $p<0.001$; sex ratio: $\chi^2_2=27.3$, $p<0.001$; Fig. 2B). However, virgin
268 females showed the opposite pattern: more aggressive virgin females within a dyad spent
269 relatively less time occupying the food patch ($\chi^2_1=7.1$, $p=0.008$), with the strongest negative

270 correlation in male-biased sex ratios (sex ratio: $\chi^2_2=15.5$, $p<0.001$; interaction: $\chi^2_2=35.6$,
271 $p<0.001$; Fig. S3).

272 Mating reduced female locomotion ($F_{1,6.0}=33.6$, $p=0.001$, square root-transformation;
273 Fig.S2B), but we detected no influence of evolutionary sex ratio on locomotion, and no
274 interaction between mating and evolutionary sex ratio (evolutionary sex ratio: $F_{2,5.9}=2.5$,
275 $p=0.162$; interaction: $F_{2,6.0}=1.6$, $p=0.280$).

276

277 ***Female aggression and food patch occupancy in Experiment 2 – ‘Evolved female’***

278 In Experiment 1, the effect of sex ratio on female headbutting might have arisen from
279 evolutionary change in females, from changes in male stimulation of female aggression, or
280 from changes in both sexes. To test whether differences arose from females alone, we mated
281 experimentally-evolved females to stock males. As expected, mating caused a general
282 increase in headbutting ($F_{1,6.0}=10.0$, $p=0.019$). However, the evolutionary sex ratio did not
283 influence the magnitude of this post-mating increase (evolutionary sex ratio x mating
284 interaction: $F_{2,6.0}=0.1$, $p=0.947$, square root-transformation; Fig. 3B, Table S6). Females from
285 equal sex ratio populations tended to headbutt more, relative to female-biased and male-
286 biased females ($F_{2,6.0}=5.0$, $p=0.053$), regardless of mating status.

287 We observed no significant increase in fencing post-mating ($F_{1,6.1}=0.1$, $p=0.745$, log(constant-
288 x)-transformation; Fig. S4A), in contrast to results from the previous experiment. We found
289 no overall effect of evolutionary sex ratio on female fencing ($F_{2,5.9}=0.8$, $p=0.497$), nor an
290 interaction between evolutionary sex ratio and mating ($F_{2,6.0}=0.6$, $p=0.559$).

291 Similar to Experiment 1, we found no interaction between evolutionary sex ratio and mating
292 status for female food patch occupancy ($F_{2,6.0}=0.6$, $p=0.601$, Fig. S4C, nor a main effect of

293 evolutionary sex ratio ($F_{2,5.9}=1.5$, $p=0.307$), when evolved females mated with stock males.

294 Mating caused a general increase in food patch occupancy ($F_{1,6.1}=5.7$, $p=0.053$).

295

296 ***Female aggression and food patch occupancy in Experiment 2 – ‘Evolved male’***

297 To test whether the differences in female headbutting observed in Experiment 1 were due to

298 evolved differences in male stimulation of female aggression, we mated experimentally-

299 evolved males to stock females. All females showed a similar increase in headbutting post-

300 mating ($F_{1,7.9}=40.2$, $p<0.001$). There was no effect of male evolutionary sex ratio on headbutt

301 number post-mating ($F_{2,6.1}=0.4$, $p=0.706$, Fig. 3C).

302 Males did not stimulate a significant increase in fencing in stock females post-mating

303 ($F_{1,7.9}=0.4$, $p=0.553$), and we found no effect of male evolutionary sex ratio on female post-

304 mating fencing duration ($F_{2,6.1}=1.1$, $p=0.401$; Fig. S4B).

305 We detected no interaction between evolutionary sex ratio and mating status on food patch

306 occupancy when stock females mated with experimentally-evolved males. Regardless of

307 evolutionary sex ratio, all males stimulated increases in food patch occupancy in stock

308 females post-mating ($F_{1,7.8}=8.7$, $p=0.019$), but there was no significant effect of male

309 evolutionary sex ratio on female post-mating food-patch occupancy ($F_{2,6.1}=0.3$, $p=0.719$; Fig.

310 S4D).

311

312 ***The correlation between male and female aggression***

313 We found a positive correlation between the number of male lunges and female headbutts

314 across replicate populations (Spearman’s rank correlation, males and virgin females, $\rho=0.72$,

315 $S=34$, $p=0.037$; males and mated females, $\rho=0.63$, $S=44$, $p=0.076$; Fig. 4A,B), but found no

316 correlation in fencing duration between the sexes (males and virgin females, $\rho=-0.02$, $S=122$,
317 $p=0.982$; males and mated females, $\rho= -0.25$, $S=150$, $p=0.521$).

318

319 **The correlation between virgin and mated female aggression**

320 We found a positive correlation between pre- and post-mating female headbutting frequency
321 across replicate populations (Spearman's rank correlation, $\rho=0.70$, $S=36$, $p=0.043$, Fig. 4C),
322 but found no correlation in fencing behaviour ($\rho= 0.07$, $S=112$, $p=0.880$).

323

324 **Discussion**

325 We investigated how aggression evolves in response to the intensity of intra-sexual
326 competition by assaying aggression after experimentally manipulating the population sex
327 ratio for >75 generations. We predicted that males and females would evolve increased
328 aggression after evolution in populations biased towards their sex, and our results support
329 this prediction strongly in females and weakly in males. We observed a greater increase in
330 aggression after mating in females from female-biased populations, as predicted if higher
331 post-mating aggression is adaptive for females. Surprisingly, differences in the magnitude of
332 this increase among sex ratios occurred only after matings between experimentally-evolved
333 males and females, and not when experimentally-evolved individuals mated with stock flies.
334 These results suggest that differences in the post-mating increase in aggression do not arise
335 through evolution in either sex independently, but might depend on co-evolved interactions
336 between the sexes. We found positive correlations in aggression between the sexes,
337 consistent with a shared genetic basis for aggression. Our results suggest that the intensity of
338 competition can determine the strength of sexual and social selection on aspects of

339 aggression and food patch occupancy in both male and female *D. melanogaster*, shaping the
340 evolution of these behaviours.

341 **The evolution of male aggression with sex ratio**

342 We predicted that evolution under stronger sexual selection, through more intense
343 competition for mates in male-biased populations, should lead to increased male aggression,
344 mirroring plastic changes in response to sex ratio in a wide range of species [14,15,49]. The
345 results offer only weak support for this prediction. On the one hand, the absence of evolved
346 differences in the frequency and duration of male aggression in response to sex ratio does
347 not support the prediction. Two possible explanations for the absence of response are that
348 selection favours plasticity in aggression rather than fixed increases or decreases [50]; or that
349 changes in the strength of competition for mates with sex ratio are balanced by changes in
350 rival density and costs of fighting [10,51–53]. However, neither hypothesis accounts for our
351 observations of sex ratio effects on the evolution of female aggression and male aggression
352 in relation to food patches.

353 On the other hand, we observed the evolution of reduced food patch occupancy, a reduced
354 proportion of aggression performed on food, and a weaker relationship between aggression
355 and food occupancy, in males from female-biased populations relative to other males. The
356 function of male aggression in gaining access to food resources is supported both by our
357 finding that more aggressive males spend relatively more time occupying the food patch, and
358 by previous reports that aggressive male *D. melanogaster* win access to food patches [54,55],
359 which increases their access to mates [16,40,55]. Our results are consistent with weaker
360 selection for the use of aggression to attain access to food patches under female-biased
361 conditions, in which weaker competition for mates is expected to reduce the benefits of

362 dominating breeding sites [15,56]. An alternative hypothesis is that reduced male food patch
363 occupancy after evolution in female-biased populations might reflect reduced female
364 aggregation on food patches. However, females aggregate more, not less, on food patches in
365 our female-biased populations [37].

366

367 **The evolution of female aggression with sex ratio**

368 Females increase aggression after mating in many species [20,24,25,33,35]. Our results are
369 consistent with this pattern. Increased aggression post-mating might represent an adaptive
370 response that relates to the acquisition or defence of nutritional resources required for
371 reproduction, as the switch to a post-mating reproductive state increases female feeding and
372 protein requirements [29,57,58]. Our findings that females from all sex ratio treatments
373 display increased food patch occupation post-mating, and that aggression is positively related
374 to food occupancy in mated females, support this idea.

375 We found that the evolutionary sex ratio influences both the level of aggression in mated
376 females and the magnitude of the post-mating increase in aggression, with more headbutts
377 and a greater increase in headbutt frequency post-mating in females from female-biased
378 populations. The greater intensity of female competition in female-biased populations might
379 impose stronger selection favouring aggression in the nutritionally-demanding mated state.
380 Our results suggest that the intensity of intra-sexual competition can shape the evolution of
381 female aggression, and that this might relate to nutritional defence, although causality in this
382 relationship is unclear. Future work testing the relationship between female aggression,
383 defence of food, and reproductive success would improve understanding of the function of
384 aggression in this species.

385 Our findings are inconsistent with the hypotheses that evolution in either sex alone explains
386 the observed effect of sex ratio on the female post-mating increase in aggression. Previous
387 work has demonstrated that the receipt of male sperm and the seminal fluid protein ‘sex
388 peptide’ directly influence female aggression in *D. melanogaster* [35]. Moreover, some
389 properties of the male ejaculate such as sperm competitiveness and ejaculate expenditure
390 show evolvability in response to the sex ratio [27,59–61]. However, a male’s ability to
391 stimulate female aggression did not appear to evolve in the conditions of our experiment.

392 We are left with the hypothesis that the female post-mating behaviours observed when both
393 sexes had experimentally evolved reflect coevolved interactions between the sexes, such that
394 evolved changes occur only after matings between individuals from the same social
395 environment. Similar complex interactions between male and female genotypes are known
396 in *Drosophila*. For example, the effect of some male sex peptide alleles on sperm
397 competitiveness depends on the female sex peptide receptor allele [62]. Likewise, sperm
398 success can depend on interactions between male and female genotypes [63]. Although we
399 know that female post-mating aggression is linked to the receipt of male ejaculates [35], the
400 downstream mechanism within females remains elusive. Research into the post-mating
401 regulation of female aggression would help further evaluation of the co-evolution hypothesis.

402 **A positive correlation in aggression between the sexes**

403 Studying the evolution of male and female aggression simultaneously allowed us to evaluate
404 the hypothesis that aggression is genetically correlated between the sexes. This is especially
405 relevant because female aggression has sometimes been considered a non-adaptive by-
406 product of selection for male aggression [4,64] and has only recently been studied as an
407 adaptive female trait [21].

408 Our observation of a positive correlation between male lunging and female headbutting
409 across replicate populations is consistent with a shared genetic basis for aggression. There is
410 evidence that selection for aggression in male *D. melanogaster* results in correlated responses
411 in female aggression [65], supporting this idea. This suggests the possibility that genetic
412 constraints might impede the evolution of sex-specific optimal aggression. However, our
413 observation of divergent responses to sex ratio for males and females suggests that a genetic
414 correlation for aggression does not completely restrict its independent evolution in each sex.
415 Alternatively, a positive correlation could arise if aggression forms a behavioural syndrome
416 with other coevolving inter-sexual behaviours, such as male harassment of females and
417 female resistance. However, this seems unlikely because there is little evidence that
418 aggression covaries across contexts in *D. melanogaster* [66] and intra-sexual aggressive
419 behaviours are rarely directed at the opposite sex [67]. Furthermore, the positive correlation
420 between headbutting by virgin and mated females suggests a consistent genetic basis for
421 female aggression pre- and post-mating, such that females have a baseline level of aggression
422 that is enhanced by mating. In contrast, the absence of correlations in fencing behaviour
423 between males and females, and between virgin and mated females, across replicate
424 populations might reflect differences in the function of this low-intensity aggressive
425 behaviour between the sexes, and within females depending on their mating status. Fencing
426 is performed by both sexes, but there are distinct differences in the aggressive strategies of
427 males and females [33] and in females pre- and post-mating [35]. If there are distinct genetic
428 pathways underlying low- and high-intensity aggression, then the extent to which sex-specific
429 aggression is constrained by a shared genetic basis may vary for different aggressive
430 behaviours.

431 Our study provides evidence that the strength of sexual and social selection, mediated by
432 competition for mates and resources, can shape the evolution of aggressive behaviours in
433 both male and female *D. melanogaster*. These effects differ between the sexes, which might
434 reflect different routes by which aggression influences reproductive success [2]. The higher
435 energy demands of reproduction in females might result in greater reproductive costs from
436 energetically expensive aggression in females than in males, causing reduced female
437 aggression with greater sensitivity to the ecological setting.

438 Furthermore, although we found evidence consistent with a shared genetic basis for
439 aggression, our observation of divergent responses to sex ratio for males and females
440 suggests that a genetic correlation for aggression does not completely restrict its independent
441 evolution. Our study also highlights that increased female aggression in response to mating
442 might be sensitive to adaptations in both sexes. This underscores the value of future study of
443 the mechanisms underlying the female post-mating increase in aggression, and of studying
444 behaviour in both sexes.

445

446 **Acknowledgements**

447 We thank two anonymous reviewers for comments that improved the manuscript. EB was
448 funded by a fellowship from Christ Church College and grants from the John Fell Fund
449 (University of Oxford, ATD12830) and the Association for the Study of Animal Behaviour. DE
450 was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) Doctoral
451 Training Partnership. TC was funded by the Natural Environment Research Council (NERC;
452 NE/K004697/1). WGR was funded by a NERC grant to TC (NE/R010056/1). SW was funded by

453 a BBSRC David Phillips Fellowship (BB/K014544/1). JCP was funded by a NERC fellowship
454 (NE/P017193/1). The authors declare no conflict of interest.

455

456 **Author contributions**

457 EB, DE, SW, and JCP conceived the ideas and designed the methodology; JN, CA, and LH
458 assisted in designing methodology; TC and SW designed the experimental evolution protocol;
459 TC and WGR performed the experimental evolution; EB, DE, JN, CA, and LH collected the data;
460 EB, DE, and JCP analysed the data; EB, DE, and JCP drafted the initial version of the manuscript
461 and all authors contributed to later versions of the manuscript

462

463 **Data Accessibility**

464 Data are available from the Oxford University Research Archive (ORA). DOI:
465 <https://doi.org/10.5287/bodleian:9RpZgPGm5>

466

467 **Ethics statement**

468 No ethics approval was required for these experiments.

469

470

471 **References**

- 472 1. Clutton-Brock T. 2007 Sexual selection in males and females. *Science* **318**, 1882–1885.
- 473 2. Tobias JA, Montgomerie R, Lyon BE. 2012 The evolution of female ornaments and
474 weaponry: social selection, sexual selection and ecological competition. *Philosophical*
475 *Transactions of the Royal Society B: Biological Sciences* **367**, 2274–2293.
- 476 3. Haley MP. 1994 Resource-holding power asymmetries, the prior residence effect, and
477 reproductive payoffs in male northern elephant seal fights. *Behavioral Ecology and*
478 *Sociobiology* **34**, 427–434.

- 479 4. Cain KE, Ketterson ED. 2012 Competitive females are successful females; phenotype,
480 mechanism, and selection in a common songbird. *Behavioral Ecology and Sociobiology*
481 **66**, 241–252.
- 482 5. Clutton-Brock T, Huchard E. 2013 Social competition and its consequences in female
483 mammals. *Journal of Zoology* **289**, 151–171.
- 484 6. Hoffmann AA. 1987 A laboratory study of male territoriality in the sibling species
485 *Drosophila melanogaster* and *D. simulans*. *Animal Behaviour* **35**, 807–818.
- 486 7. Hoffmann AA. 1987 Territorial encounters between *Drosophila* males of different sizes.
487 *Animal Behaviour* **35**, 1899–1901.
- 488 8. Stockley P, Campbell A. 2013 *Female competition and aggression: interdisciplinary*
489 *perspectives*. The Royal Society.
- 490 9. Fitze PS, Le Galliard J-F. 2008 Operational sex ratio, sexual conflict and the intensity of
491 sexual selection. *Ecology Letters* **11**, 432–439.
- 492 10. Weir LK, Grant JW, Hutchings JA. 2011 The influence of operational sex ratio on the
493 intensity of competition for mates. *The American Naturalist* **177**, 167–176.
- 494 11. Kvarnemo C, Ahnesjö I. 1996 The dynamics of operational sex ratios and competition for
495 mates. *Trends in Ecology & Evolution* **11**, 404–408.
- 496 12. Zeh DW. 1987 Aggression, density, and sexual dimorphism in chernetid pseudoscorpions
497 (Arachnida: Pseudoscorpionida). *Evolution* **41**, 1072–1087.
- 498 13. Pomfret JC, Knell RJ. 2008 Crowding, sex ratio and horn evolution in a South African
499 beetle community. *Proceedings of the Royal Society B: Biological Sciences* **275**, 315–
500 321.
- 501 14. Kvarnemo C, Forsgren E, Magnhagen C. 1995 Effects of sex ratio on intra-and inter-
502 sexual behaviour in sand gobies. *Animal Behaviour* **50**, 1455–1461.
- 503 15. Grant JW, Foam PE. 2002 Effect of operational sex ratio on female female versus male
504 male competitive aggression. *Canadian Journal of Zoology* **80**, 2242–2246.
- 505 16. Hoffmann AA, Cacoyianni Z. 1990 Territoriality in *Drosophila melanogaster* as a
506 conditional strategy. *Animal Behaviour* **40**, 526–537.
- 507 17. Bonduriansky R, Chenoweth SF. 2009 Intralocus sexual conflict. *Trends in ecology &*
508 *evolution* **24**, 280–288.
- 509 18. Rosvall KA. 2011 By any name, female–female competition yields differential mating
510 success. *Behavioral Ecology* **22**, 1144–1146.
- 511 19. Ketterson ED, Nolan Jr V, Sandell M. 2005 Testosterone in females: mediator of
512 adaptive traits, constraint on sexual dimorphism, or both? *the american naturalist* **166**,
513 S85–S98.

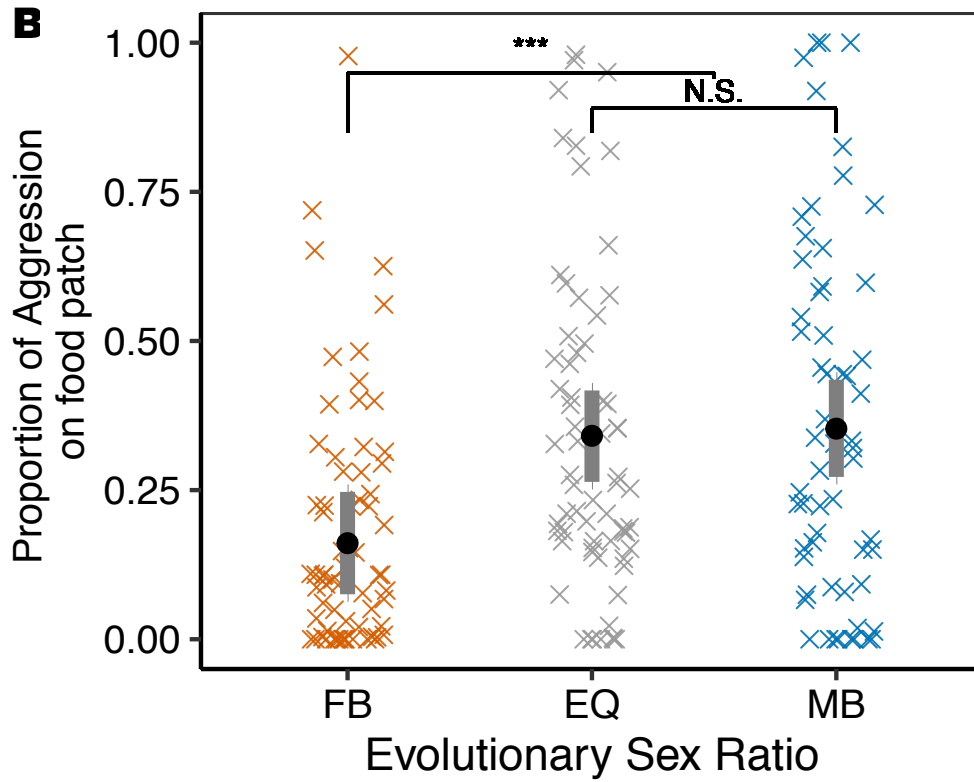
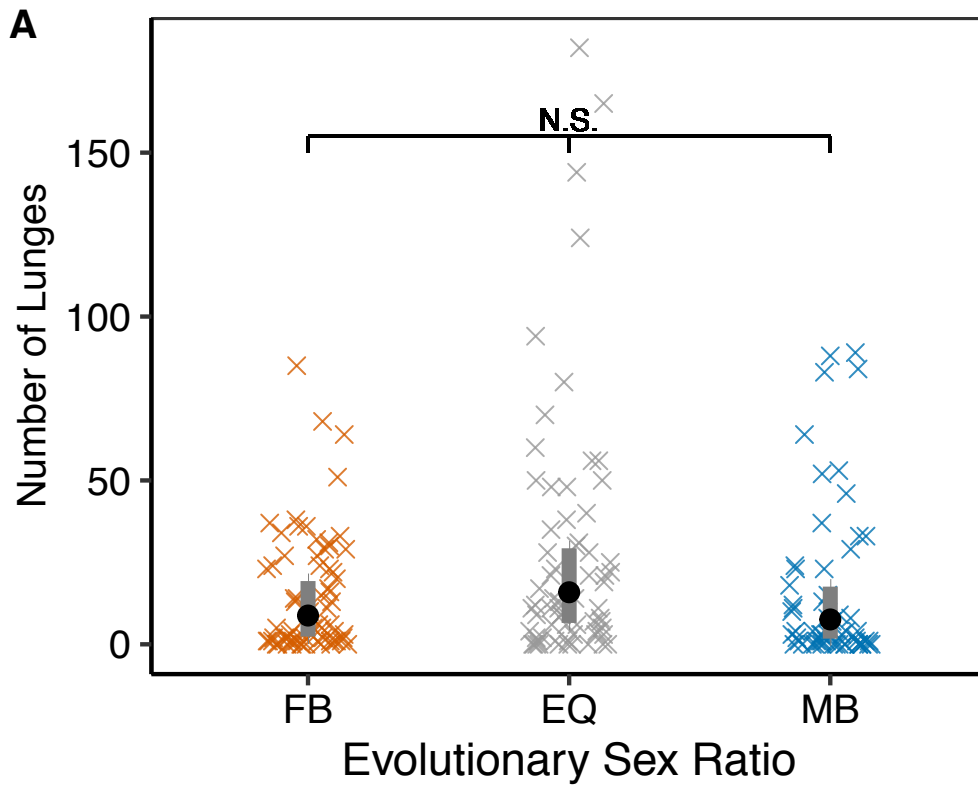
- 514 20. Sinn DL, While GM, Wapstra E. 2008 Maternal care in a social lizard: links between
515 female aggression and offspring fitness. *Animal Behaviour* **76**, 1249–1257.
- 516 21. Stockley P, Bro-Jørgensen J. 2011 Female competition and its evolutionary consequences
517 in mammals. *Biological Reviews* **86**, 341–366. (doi:<https://doi.org/10.1111/j.1469-185X.2010.00149.x>)
518
- 519 22. Perry JC, Rowe L. 2015 The evolution of sexually antagonistic phenotypes. *Cold Spring
520 Harbor perspectives in biology* **7**, a017558.
- 521 23. Gillott C. 2003 Male accessory gland secretions: modulators of female reproductive
522 physiology and behavior. *Annual review of entomology* **48**, 163–184.
- 523 24. Mainardi D, Re L, Palanza P, Parmigiani S, Brain PF. 1996 Male and female competitive
524 strategies of wild house mice pairs (*Mus musculus domesticus*) confronted with intruders
525 of different sex and age in artificial territories. *Behaviour* **133**, 863–882.
- 526 25. Seebacher F, Ward AJW, Wilson RS. 2013 Increased aggression during pregnancy comes
527 at a higher metabolic cost. *Journal of Experimental Biology* **216**, 771–776.
- 528 26. Kvarnemo C, Simmons LW. 1999 Variance in female quality, operational sex ratio and
529 male mate choice in a bushcricket. *Behavioral Ecology and Sociobiology* **45**, 245–252.
- 530 27. Linklater JR, Wertheim B, Wigby S, Chapman T. 2007 Ejaculate depletion patterns
531 evolve in response to experimental manipulation of sex ratio in *Drosophila melanogaster*.
532 *Evolution* **61**, 2027–2034.
- 533 28. Dierick HA, Greenspan RJ. 2006 Molecular analysis of flies selected for aggressive
534 behavior. *Nature genetics* **38**, 1023–1031.
- 535 29. Jensen K, McClure C, Priest NK, Hunt J. 2015 Sex-specific effects of protein and
536 carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. *Aging
537 cell* **14**, 605–615.
- 538 30. Carvalho GB, Kapahi P, Anderson DJ, Benzer S. 2006 Allocrine modulation of feeding
539 behavior by the sex peptide of *Drosophila*. *Current Biology* **16**, 692–696.
- 540 31. Dow MA, von Schilcher F. 1975 Aggression and mating success in *Drosophila
541 melanogaster*. *Nature* **254**, 511–512.
- 542 32. Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA. 2002 Fighting fruit flies: a model
543 system for the study of aggression. *Proceedings of the National Academy of Sciences* **99**,
544 5664–5668.
- 545 33. Nilsen SP, Chan Y-B, Huber R, Kravitz EA. 2004 Gender-selective patterns of
546 aggressive behavior in *Drosophila melanogaster*. *Proceedings of the National Academy of
547 Sciences* **101**, 12342–12347.
- 548 34. Bath E, Biscocho ER, Easton-Calabria A, Wigby S. 2020 Temporal and genetic variation
549 in female aggression after mating. *PloS one* **15**, e0229633.

- 550 35. Bath E, Bowden S, Peters C, Reddy A, Tobias JA, Easton-Calabria E, Seddon N,
551 Goodwin SF, Wigby S. 2017 Sperm and sex peptide stimulate aggression in female
552 *Drosophila*. *Nature ecology & evolution* **1**, 1–6.
- 553 36. Baxter CM, Barnett R, Dukas R. 2015 Aggression, mate guarding and fitness in male
554 fruit flies. *Animal Behaviour* **109**, 235–241. (doi:10.1016/j.anbehav.2015.08.023)
- 555 37. Rostant WG, Mason JS, de Coriolis J-C, Chapman T. 2020 Resource-dependent
556 evolution of female resistance responses to sexual conflict. *Evolution letters* **4**, 54–64.
- 557 38. Clancy DJ, Kennington WJ. 2001 A simple method to achieve consistent larval density in
558 bottle cultures. *Drosoph. Inf. Serv* **84**, 168–169.
- 559 39. Friard O, Gamba M. 2016 BORIS: a free, versatile open-source event-logging software
560 for video/audio coding and live observations. *Methods in Ecology and Evolution* **7**,
561 1325–1330.
- 562 40. Markow TA. 1988 Reproductive behavior of *Drosophila melanogaster* and *D.*
563 *nigrospiracula* in the field and in the laboratory. *Journal of Comparative psychology* **102**,
564 169.
- 565 41. Venables WN, Ripley BD. 2002 *Modern applied statistics (Fourth S., editor) New York.*
566 Springer. ISBN 0-387-95457-0
- 567 42. Lenth R (2020). emmeans: Estimated Marginal Means, aka, Least-Squares Means. R
568 package version 1.5.1. <https://CRAN.R-project.org/package=emmeans>
- 569 43. Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting linear mixed-effects models using
570 lme4. *Journal of Statistical Software*, *67(1)*, 1-48.
- 571 44. Kassambara A, Kosinski M and Biecek P (2019). survminer: Drawing Survival Curves
572 using 'ggplot2'. R package version. 0.4.6. [https://CRAN.R-](https://CRAN.R-project.org/package=survminer)
573 [project.org/package=survminer](https://CRAN.R-project.org/package=survminer)
- 574 45. Therneau TM (2020). coxme: Mixed Effects Cox Models. R package version 2.2-16.
575 <https://CRAN.R-project.org/package=coxme>
- 576 46. Hubert M, Vandervieren E. 2008 An adjusted boxplot for skewed distributions.
577 *Computational statistics & data analysis* **52**, 5186–5201.
- 578 47. Wilcox R. 2005 Trimming and winsorization. *Encyclopedia of biostatistics* **8**.
- 579 48. Arnqvist G. 2020 Mixed Models Offer No Freedom from Degrees of Freedom. *Trends in*
580 *Ecology & Evolution* **35**, 329–335. (doi:10.1016/j.tree.2019.12.004)
- 581 49. Gaskin T, Futerman P, Chapman T. 2002 Increased density and male–male interactions
582 reduce male longevity in the medfly, *Ceratitis capitata*. *Animal Behaviour* **63**, 121–129.
- 583 50. Nandy B, Dasgupta P, Halder S, Verma T. 2016 Plasticity in aggression and the
584 correlated changes in the cost of reproduction in male *Drosophila melanogaster*. *Animal*
585 *Behaviour* **114**, 3–9. (doi:10.1016/j.anbehav.2016.01.019)

- 586 51. Grant JW. 1993 Whether or not to defend? The influence of resource distribution. *Marine*
587 *& Freshwater Behaviour & Phy* **23**, 137–153.
- 588 52. Knell RJ. 2009 Population density and the evolution of male aggression. *Journal of*
589 *Zoology* **278**, 83–90.
- 590 53. Kilgour RJ, McAdam AG, Betini GS, Norris DR. 2018 Experimental evidence that
591 density mediates negative frequency-dependent selection on aggression. *Journal of*
592 *animal ecology* **87**, 1091–1101.
- 593 54. Hoffmann AA. 1988 Heritable variation for territorial success in two *Drosophila*
594 *melanogaster* populations. *Animal Behaviour* **36**, 1180–1189.
- 595 55. Lim RS, Eyjólfsson E, Shin E, Perona P, Anderson DJ. 2014 How food controls
596 aggression in *Drosophila*. *PLoS one* **9**, e105626.
- 597 56. Aronsen T, Berglund A, Mobley KB, Ratikainen II, Rosenqvist G. 2013 Sex Ratio and
598 Density Affect Sexual Selection in a Sex-Role Reversed Fish. *Evolution* **67**, 3243–3257.
599 (doi:10.1111/evo.12201)
- 600 57. Barnes AI, Wigby S, Boone JM, Partridge L, Chapman T. 2008 Feeding, fecundity and
601 lifespan in female *Drosophila melanogaster*. *Proceedings of the Royal Society B:*
602 *Biological Sciences* **275**, 1675–1683.
- 603 58. Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, Taylor PW, Soran N,
604 Raubenheimer D. 2008 Lifespan and reproduction in *Drosophila*: new insights from
605 nutritional geometry. *Proceedings of the National Academy of Sciences* **105**, 2498–2503.
- 606 59. Nandy B, Gupta V, Sen S, Udaykumar N, Samant MA, Ali SZ, Prasad NG. 2013
607 Evolution of mate-harm, longevity and behaviour in male fruit flies subjected to different
608 levels of interlocus conflict. *BMC evolutionary biology* **13**, 212.
- 609 60. Nandy B, Chakraborty P, Gupta V, Ali SZ, Prasad NG. 2013 Sperm competitive ability
610 evolves in response to experimental alteration of operational sex ratio. *Evolution* **67**,
611 2133–2141.
- 612 61. Chechi TS, Syed ZA, Prasad NG. 2017 Virility does not imply immensity: Testis size,
613 accessory gland size and ejaculate depletion pattern do not evolve in response to
614 experimental manipulation of sex ratio in *Drosophila melanogaster*. *Journal of insect*
615 *physiology* **98**, 67–73.
- 616 62. Chow CY, Wolfner MF, Clark AG. 2010 The genetic basis for male \times female
617 interactions underlying variation in reproductive phenotypes of *Drosophila*. *Genetics* **186**,
618 1355–1365.
- 619 63. Clark AG, Begun DJ, Prout T. 1999 Female \times male interactions in *Drosophila*
620 sperm competition. *Science* **283**, 217–220.
- 621 64. Bateman AJ. 1948 Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368.
- 622 65. Edwards AC, Rollmann SM, Morgan TJ, Mackay TF. 2006 Quantitative genomics of
623 aggressive behavior in *Drosophila melanogaster*. *PLoS Genet* **2**, e154.

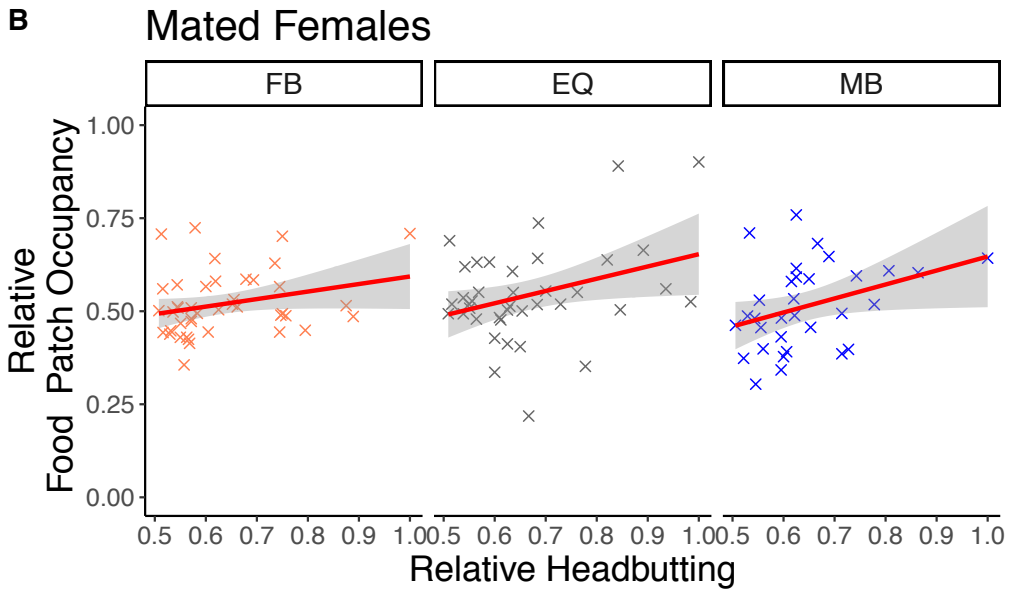
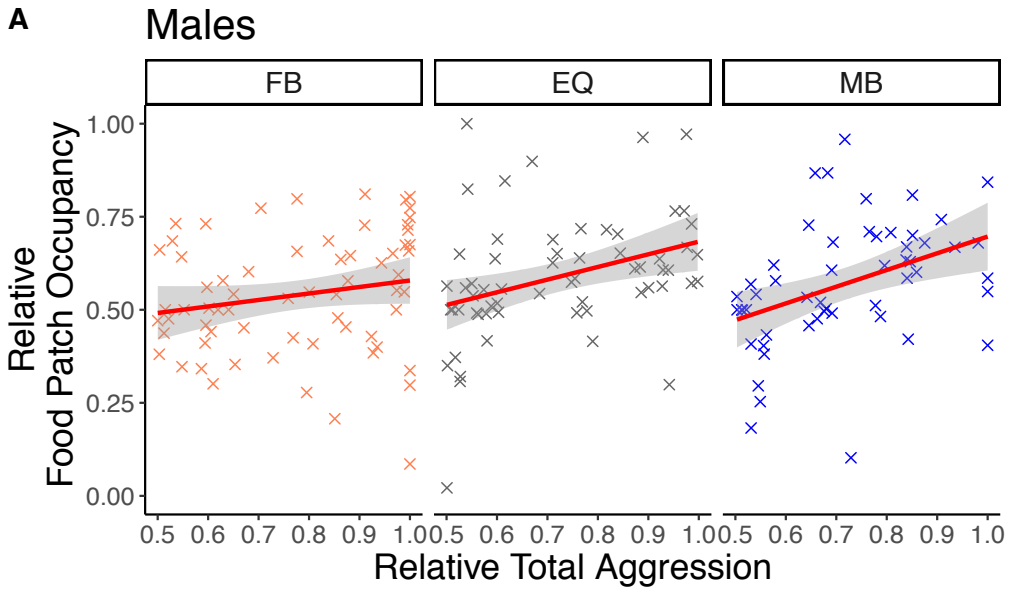
- 624 66. Baxter CM, Dukas R. 2017 Life history of aggression: effects of age and sexual
625 experience on male aggression towards males and females. *Animal Behaviour* **123**, 11–
626 20. (doi:10.1016/j.anbehav.2016.10.022)
- 627 67. de la Paz Fernández M, Chan Y-B, Yew JY, Billeter J-C, Dreisewerd K, Levine JD,
628 Kravitz EA. 2010 Pheromonal and behavioral cues trigger male-to-female aggression in
629 *Drosophila*. *PLoS Biol* **8**, e1000541.

Figures



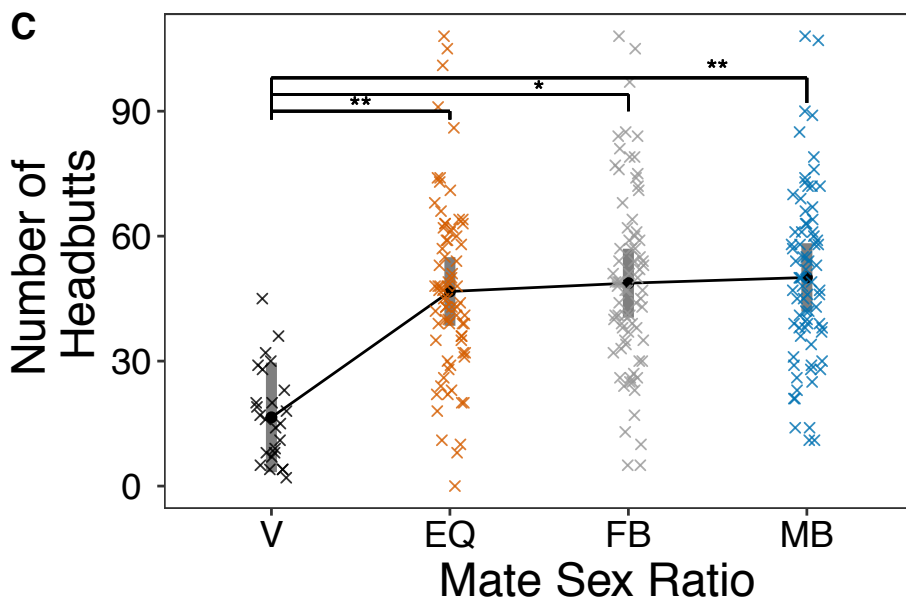
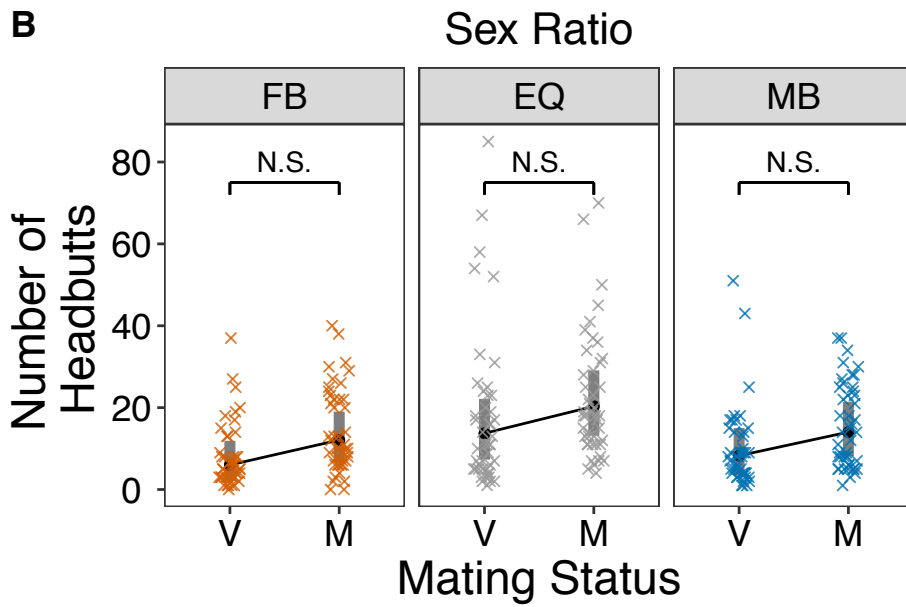
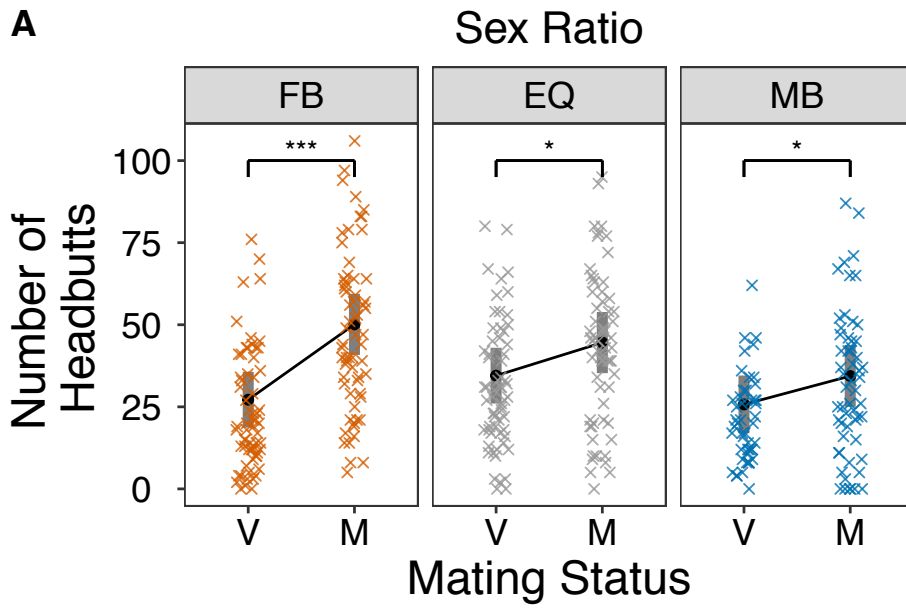
630 **Figure 1: Male aggressive behaviour in Experiment 1 – ‘coevolved’**

631 Male aggressive behavior after experimental evolution at female-biased (FB), equal (EQ), or
632 male-biased (MB) sex ratios: lunging (A, back-transformed data) and the proportion of
633 aggression performed on food patches (B). Circles indicate means. Grey bars indicate 95%
634 confidence intervals. *** indicates $p < 0.001$, * indicates $0.01 < p < 0.05$, N.S. (not significant)
635 indicates $p > 0.05$.



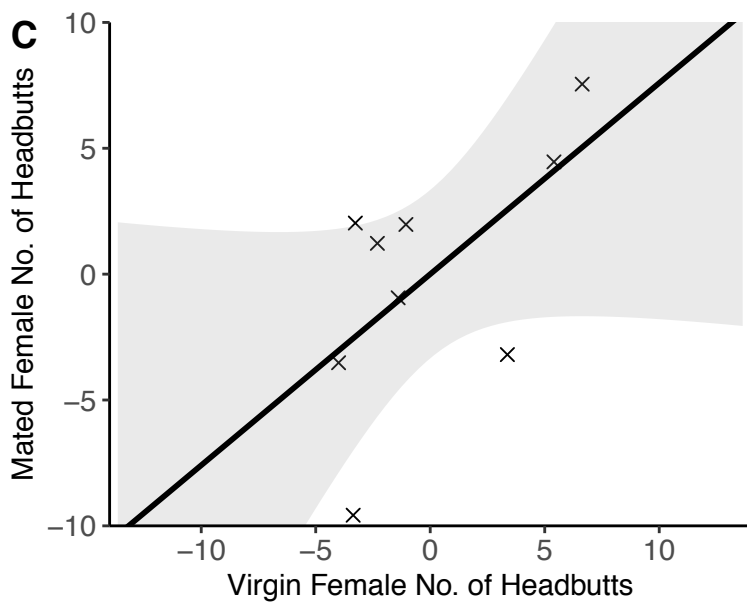
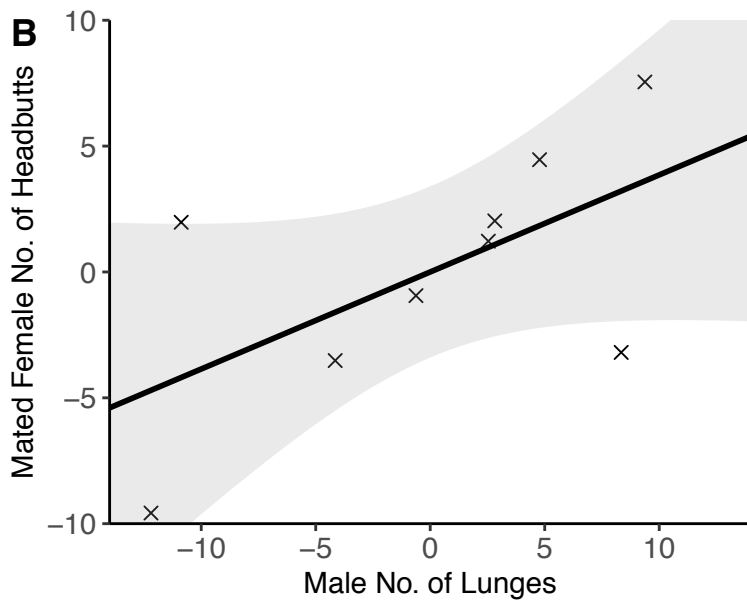
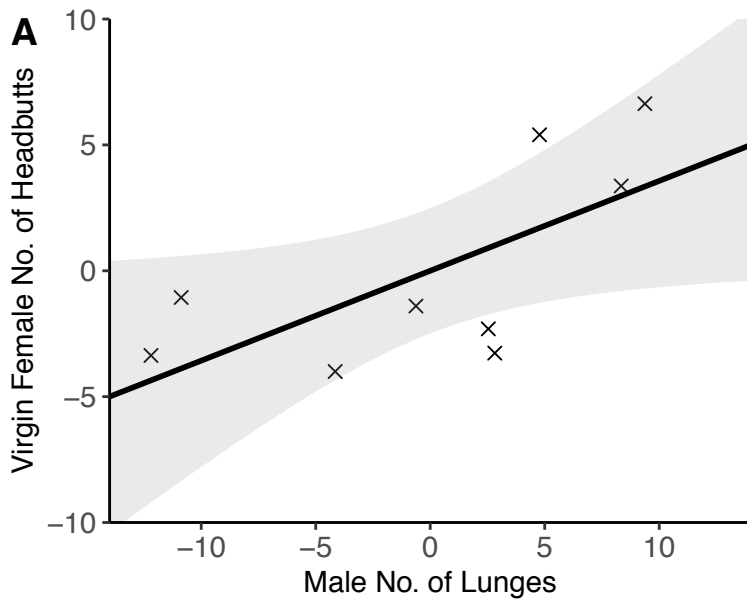
636 **Figure 2: The relationship between aggression and food patch occupancy within dyads**

637 The relationship between the proportion of aggression (male total aggression and female
638 headbutts) performed by the most aggressive individual in a pair and the proportion of food
639 patch occupancy for that individual, for males (A) and mated females (B) at female-biased
640 (FB), equal (EQ), or male-biased (MB) sex ratios. Grey shading indicates 95% confidence
641 intervals.



642 **Figure 3: Female headbutting**

643 Female headbutting after experimental evolution at female-biased (FB), equal (EQ), or male-
644 biased (MB) sex ratios, for virgin (V) or mated (M) females. Female headbutting was measured
645 when experimentally-evolved females mated with experimentally-evolved males (A;
646 Experiment 1 – ‘Coevolved’), when experimentally-evolved females mated with stock males
647 (B; Experiment 2 – ‘Evolved female’; back-transformed data), and when stock females mated
648 with experimentally-evolved males (C; Experiment 2 – ‘Evolved male’). Circles indicate means.
649 Grey bars indicate 95% confidence intervals. *** indicates $p < 0.001$, ** indicates
650 $0.001 < p < 0.01$, * indicates $0.01 < p < 0.05$, N.S. (not significant) indicates $p > 0.05$.



651 **Figure 4: Correlations between male and female aggressive behaviours**

652 The relationship between male and female aggressive behaviour (male lunges and headbutts
653 by virgin (A) or mated females (B)) and between virgin and mated female headbutts (C). Points
654 are residual values from models controlling for day, time and sex ratio. Lines indicate the
655 monotonic fit from Spearman's correlation; grey shading indicates the 95% confidence
656 interval.

657