1 **Sexual selection favours good or bad genes for pathogen resistance depending on males'**

2 **pathogen exposure**

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13

14 **Abstract**

15 Resistance to pathogens is often invoked as an indirect benefit of female choice, but
16 experimental evidence for links between father's sexual success and offspring resistance is
17 scarce and equivocal. Two proposed mechanisms might generate such links. Under the first,
18 heritable resistance to diverse pathogens depends on general immunocompetence; owing to
19 shared condition-dependence, male sexual traits indicate immunocompetence independently
20 of the male's pathogen exposure. In contrast, other hypotheses (e.g., Hamilton-Zuk) assume
21 that sexual traits only reveal heritable resistance if the males have been exposed to the
22 pathogen. The distinction between the two mechanisms has been neglected by experimental
23 studies. We show that *Drosophila melanogaster* males that are successful in mating contests
24 (one female with two males) sire sons that are substantially more resistant to the intestinal
25 pathogen *Pseudomonas entomophila* – but only if the males have themselves been exposed to
26 the pathogen before the mating contest. In contrast, sons of males sexually successful in the
27 absence of pathogen exposure are less resistant than sons of unsuccessful males. We detected
28 no differences in daughters' resistance. Thus, while sexual selection may have considerable
29 consequences for offspring resistance, these consequences may be sex-specific. Furthermore,
30 contrary to the "general immunocompetence" hypothesis, these consequences can be positive
31 or negative depending on the epidemiological context under which sexual selection operates.

32 **Keywords**: good genes, parasites, immunocompetence, Hamilton-Zuk hypothesis, female
33 choice, Drosophila

34

1. Introduction

The "good genes" hypothesis for sexual selection posits that traits enhancing male mating success are indicators that the male carries genetic variants improving non-sexual components of offspring fitness (relative to alternative alleles, i.e., "bad genes") [1]. In genetic terms, this means a positive correlation between a male's sexual traits and his breeding value for non-sexual fitness components [2, 3]. One fitness component often invoked in this context is resistance to pathogens and parasites: female preference for costly male display traits is hypothesized to bring indirect genetic benefits in terms of offspring resistance [4-7], and sexual selection is proposed to act in synergy with natural selection for improved resistance [8, 9] (Here we use resistance in a broad sense of reducing the impact of pathogen presence on host fitness, including behavioural avoidance, barriers to infection, immune defence and physiological tolerance of infection.) Despite its intellectual appeal and the research effort devoted to it, this idea remains controversial [3, 10-12]. In particular, very few studies experimentally tested the prediction that more sexually attractive or successful males actually do sire offspring more resistant to pathogens; their results are equivocal. In the three-spined stickleback, offspring of fathers with a stronger ornament (redder belly) became less heavily infected upon experimental exposure to a cestode parasite [13]. In contrast, in *Drosophila*, survival after a bacterial infection did not differ between offspring of sexually successful versus unsuccessful males [14]. Female mice mated to their preferred males did produce offspring more resistant to *Salmonella* than females mated to non-preferred males [15], but this appears mediated by MHC heterozygote advantage [16], and thus supports the "compatible genes" hypothesis [17] rather than the "good genes". In trout, offspring survival under conditions favouring opportunistic pathogens was positively correlated with father's melanin ornamentation, but negatively with carotene ornamentation; it is not clear which plays a greater role in female choice [18]. No relationship between father's attractiveness and measures of offspring immune response was found in scorpion flies [19, 20], whereas in ostrich one of several measures of plumage positively correlated with one of three measures of immune response [21]. Similarly mixed results about additive genetic correlations between sexually selected traits and resistance have emerged from quantitative genetic estimates [9, 22-26] and experimental evolution [27-30].

The study we report here suggests that those mixed results can be at least in part explained by a distinction between two ways in which a positive correlation between a male's sexual traits and his breeding value for pathogen resistance could be generated. The currently prevailing view is that variation in pathogen resistance relevant for sexual selection is largely due to general immunocompetence that determines resistance to a broad range of pathogens, and which depends on (or is an aspect of) the individual's physiological condition [6, 9, 31]. The condition is thought to be heritable because it captures a significant part of genetic variance for fitness maintained by mutation-selection balance and other mechanisms; sexual display traits evolve to be honest signals of condition [8, 32], and thus of immunocompetence [7, 9, 33].

An alternative scenario, first proposed by Hamilton and Zuk [4], assumes that variation in resistance is specific to pathogen species or genotypes, which undergo constant turnover; male sexual traits reveal heritable resistance to currently prevalent parasites and pathogens

78   (rather than general immunocompetence). This correlation is generated by differential
79   consequences of pathogen exposure for the health of males with different degrees of
80   resistance, and these health consequences are revealed by sexual display traits [4, 5, 34-38].
81   Thus, male sexual traits only "capture" variation in resistance to pathogens to which the males
82   have been exposed [38]. In the absence of any pathogen, resistant males are not expected to
83   be healthier and thus not more sexually attractive or successful [38]; they may be less
84   successful if resistance carries a physiological cost [5]. Thus, under this "specific resistance"
85   scenario the identity of "good genes" depends on the environmental context; offspring
86   resistance is an indirect benefit of mating choice only if both fathers and offspring are
87   exposed to the same pathogens [4, 5].

88   Both these scenarios have been originally invoked in the context of display traits targeted by
89   mate choice, but may apply as well to traits involved in intra-sexual competition for mates, as
90   these traits are also costly and likely condition-dependent, and often are the same traits as
91   those involved in mate choice [39]. The relative and absolute importance of these two
92   hypothetical scenarios linking pathogen resistance and sexual selection remains unresolved
93   [11]. Yet, the predictions about consequences of sexual selection differ between these
94   scenarios in a crucial way. Under the "general immunocompetence" scenario, fathers' sexual
95   success predicts offspring resistance to diverse pathogens irrespective of whether or not the
96   fathers have been exposed to any pathogens [38]. In contrast, under the "specific resistance"
97   scenario, sexually successful males sire offspring with higher resistance to a pathogen only if
98   the males have themselves been exposed to the pathogen while they were developing their
99   sexual traits; sexual success in the absence of pathogens does not predict offspring resistance
100   [38].

101   The aim of the present study was to test these distinct predictions. To our knowledge, the
102   distinction has not been experimentally addressed; in none of the experimental studies
103   summarized above were the fathers experimentally exposed to pathogens, although in some
104   [13, 18, 23] they might have been naturally exposed. We tested if sexually successful
105   *Drosophila melanogaster* males sire offspring more resistant to an intestinal pathogen
106   (*Pseudomonas entomophila*) than unsuccessful males, and, crucially, if this depends on
107   whether the males' success is determined after they have been exposed to the pathogen. This
108   pathogen causes substantial mortality in *Drosophila*, and fly populations harbour natural
109   genetic variation in resistance to this pathogen [40, 41]. This variation has been found
110   associated with differences in ROS production, tendency to lose gut wall integrity and activity
111   of gut repair [40, 41]. In contrast, genetically higher resistance to *P. entomophila* does not
112   seem to be mediated by greater expression of antimicrobial peptides or reduced ingestion of
113   the bacteria ([40, 41]), in spite of flies being able to learn to avoid this pathogen ([42]).

114   We staged mating contests in which two males (sires) from a single outbred population
115   competed for a female, where either both sires were earlier exposed to the pathogen or both
116   were sham-treated. *Drosophila* females have full control over mating, and although the
117   outcome of such contests is affected by male-male agonistic interactions, it contains a large
118   component of female choice [43]. Subsequently, we quantified pathogen resistance of
119   offspring sired by these winner and loser males before the infection treatment and the mating
120   contest. This excluded potential non-genetic effects of father's infection or contest outcome on
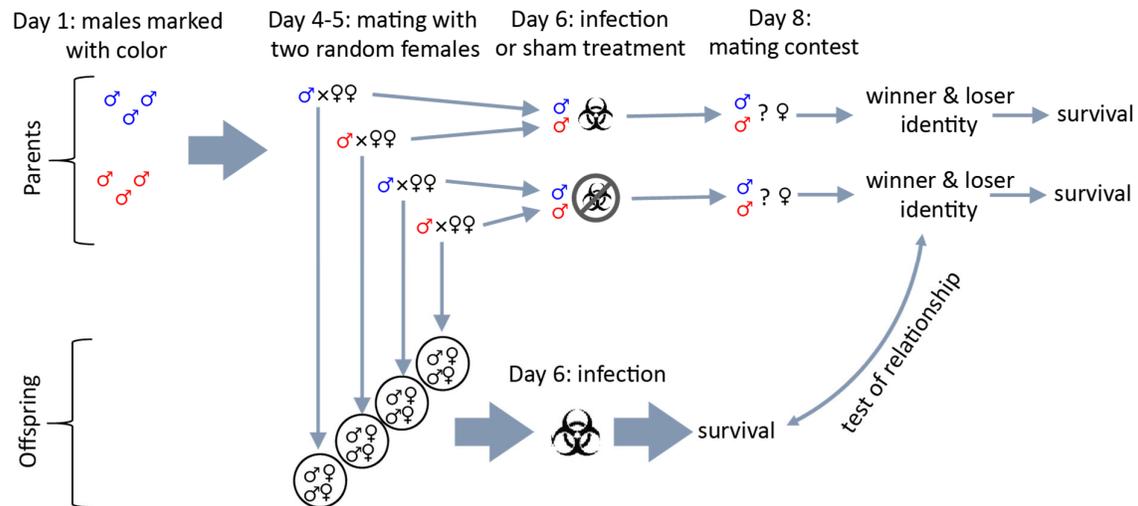
Figure 1. The design of the experiment to study the relationship between a sire's sexual success and his breeding value for resistance to P. entomophila. For explanations see Methods

121 offspring resistance, and prevented potential transmission of the pathogen from infected
122 fathers to offspring. Mean resistance of the offspring was thus an unbiased estimate of the
123 sire's breeding value (his "genetic quality") for that trait [2, 44], allowing us to test its
124 relationship with attractiveness.

125

2. Methods

127 (a) Fly maintenance

128 We used flies from a population collected in 2007 in the canton of Valais, Switzerland, and
129 maintained in the laboratory since at a population size of >1000 adults. Flies used in the
130 experiments were raised at 25°C, relative humidity 55% and 12:12 photoperiod on standard
131 yeast-cornmeal-sugar medium under density of about 250 larvae per bottle with 30 ml of food
132 (controlled by egg counting). Virgin flies of both sexes were collected within 12 h of
133 emergence. Virgin females were maintained in groups in food vials until used in the
134 experiment; their virginity was verified by the absence of larvae. All fly transfers were done
135 under light CO_2 anaesthesia.

136

137 (b) Father's sexual success and offspring resistance

138 The design of our main experiment is summarized in Figure 1. Immediately after being
139 collected, sires were dusted with red or blue powder (Sennelier), then maintained for 72 hours
140 in groups of about 50 in vials with food. Subsequently, each sire was placed with two virgin
141 females in a vial containing 10 ml of food and given 48 hours to mate before being removed
142 for the next step of the experiment. Females were given another 24 hours to lay eggs before
143 being removed from the vials; the vials were then kept until offspring collection.

144 After removal from the mating vials we haphazardly paired a red-dusted and a blue-dusted
145 sire; each sire duo was then subject to either the infection or the sham treatment (described

146   below) for 20 h. After the infection or sham treatment, each sire duo was transferred to a new
147   food vial divided by a removable longitudinal partition (Supplementary Figure 1). The sires
148   were placed on one side of the partition and a virgin female on the other side; they were
149   maintained so overnight to let them habituate and the CO_2 effect wear off. The next morning
150   (40 h after the beginning of the infection or sham treatment), we removed the partition,
151   bringing the two sexes together. We observed the flies until the first mating occurred; the
152   male that mated was defined as the "winner" and its less successful counterpart the "loser".
153   Replicates in which no mating occurred within 2 h or in which one or both males were dead
154   before the mating contest were discarded. Where mating occurred, flies were retained in the
155   vial and the survival of "winner" and "loser" males until 72 h post-infection was recorded.

156   To assess resistance of the offspring, 17 days after initial mating (4-6 days after adult
157   eclosion) we collected 10 female and 10 male offspring per sire. The offspring were orally
158   infected (in single sex groups) as described below and subsequently transferred to food vials;
159   the number of dead and alive flies was scored at 24, 48 and 72 hours from beginning of the
160   infection treatment.

161   This entire experiment was performed in three blocks spread over several months. Per block
162   and infection/sham treatment we assessed the resistance of offspring of five winner-loser duos
163   (3 blocks × 2 treatments × 5 duos × winner and loser × 2 sexes × 10 offspring = 1200
164   offspring in total). The design was paired in that we compared offspring of winner and loser
165   from the same duo, i.e., two sires that directly competed with each other (see section 2e). If
166   either sire of a duo failed to produce enough offspring, the entire duo was discarded to avoid a
167   sampling bias. To obtain this number of replicates, many more mating contests were set to
168   allow for sire mortality prior to contest, unresolved contests (i.e., no mating) and insufficient
169   number of offspring (i.e., fewer than 10 offspring of each sex for either sire of a winner/loser
170   pair). Thus, the number of replicate duos whose offspring's resistance was assessed was
171   smaller than the total number of mating contests.

172

173   (c) Bacterial culture and infection protocol

174   As the experimental pathogen we used *Pseudomonas entomophila*, a gram-negative bacterium
175   originally isolated from *D. melanogaster,* which is virulent upon intestinal infection at
176   sufficiently high doses [41, 45]. The *Pseudomonas entomophila* strain was originally provided
177   by Bruno Lemaitre [45] and maintained at –80°C. Cultures were first initiated on solid
178   medium (triptone, yeast, NaCL, agar and 5% milk). Milk was added to screen colonies for
179   protease activity, which is a marker of virulence and which will form a pale halo around the
180   colony [46]. A single colony from the plate was used to initiate culture in 50 ml of liquid
181   medium (with the same composition as the solid media but without agar and milk) for 24
182   hours at 28.5°C on a shaker at 190 rpm. The 50 ml of culture were then transferred into 200
183   ml of fresh medium and kept in the same conditions for another 24 hours. The content was
184   subsequently centrifuged for 20 minutes at 4°C and 3000 rpm. The pellet was resuspended in
185   0.9% NaCl solution to the optical density (OD) of 200 at 600 nm. For infection of the sires
186   and their male offspring, the final bacterial suspension was obtained by adding the same
187   volume of a 5% sucrose solution, reducing the final OD to 100. The same bacterial

concentration was used to infect the female offspring in the first experimental block; however, it resulted in over 90 % mortality for daughters of all sire categories. Aiming to reduce mortality and thus to increase the resolution of potential differences in daughter resistance, for the remaining two experimental blocks we halved the final concentration used to infect female offspring to OD 50. The infectious suspension was always prepared on the day when the flies were to be infected.

Prior to infection flies were first starved for 2 hours in empty vials to increase their consumption of bacteria. For the infection treatment, the flies were transferred to vials with a filter paper disc soaked with 100 µl of bacterial mix placed on top of agarose and left there for 20 hours. Subsequently, they were transferred to vials with food and monitored for survival until 72 h from the onset of infection. Based on previous studies [40, 41, 45], comparing survival at 72 h post-infection offers good resolution of differences between treatments in resistance to *P. entomophila*. For the sham treatment, sires were manipulated in the same way as sires in the infection treatment except that the paper disk was infused with 100 µl of 50:50 mixture of 0.9 % NaCl and 5 % sucrose.

(d) Infection and the ability to mate

In order to verify if our infection treatment impaired males' ability to mate in the absence of male-male competition or mate choice, in a separate experiment we performed mating trials that excluded these factors. Virgin males (raised and handled as in the main experiment except not being dusted with colour powder) were either infected with *P. entomophila* or sham-treated as described above. Thereafter a single male and a virgin female were placed on opposite sides of a vial divided by a partition, as in mating contests described above and left to habituate overnight. The next day, the partition was removed and the mating trial started and we scored whether mating occurred within the 2 h period. Replicates in which the male was dead or immobile before the trial were discarded, leaving 29 males in the infection treatment and 50 in the sham treatment.

(e) Statistical analysis

All statistical analyses were performed using R (version 3.5.1) and the RStudio plugin (version 1.1.463). Colour of the powder used to mark males had no detectable effect on their probability of winning ($p = 0.37$, binomial test), in agreement with our previous unpublished results. We focused on offspring resistance in terms of the likelihood of surviving 72 h post-infection. Using survival until 48 h post-infection led to the same conclusions; statistics for both time points are reported in Supplementary Table S1. With offspring survival as the binary response variable, we used the glmer function of R package *lme4* to fit generalized mixed models with logit link and binomial error distribution. Mating outcome (winner or loser), treatment (infection or sham) and offspring sex (where both sexes were analysed together) were the fixed effects. The main unit of replication – winner-loser duo – was included as a random explanatory variable; block was also treated as a random variable (an alternative analysis with block treated as a fixed factor resulted in the same conclusions). To

229   test directly if survival odds ratios differed between sons and daughters of sires of the two
230   treatments, we also fitted generalized mixed models separately for infected and sham-treated
231   sires and tested for the interaction between contest outcome and offspring sex with the
232   likelihood ratio test. Marginal means were estimated with *emmeans*; pairwise contrasts were
233   performed with pairs function of the *emmeans* package. A further analysis was performed
234   with father's success in the mating contest and father survival (as a binary variable: the fathers
235   were either dead or alive after 72 hours) as fixed factors, only including data from the infected
236   treatment. Because the infectious dose used for female offspring in blocks 2 and 3 was
237   reduced compared to block 1 (see above), we repeated all analyses involving female offspring
238   with data from blocks 2 and 3 only. None of the conclusions were affected; thus, we only
239   report the analysis including all the blocks.

240

241   **3. Results**

242   (a) Father's sexual success predicts sons' resistance

243   The relationship between a sire's winning versus losing the mating contest and resistance of
244   his offspring to *P. entomophila* depended on offspring sex (contest outcome × sire infection
245   treatment × offspring sex interaction: $\chi^2_1 = 7.4$, $p = 0.0067$, likelihood ratio test, GLMM on
246   probability of surviving 72 h post-infection; for detailed analysis see Supplementary Table
247   S1a). This justified splitting the analysis by offspring sex.

248   The relationship between father's success and pathogen resistance of his male offspring had
249   opposite signs depending on whether or not the contest took place after pathogen exposure
250   (contest outcome × sire infection treatment interaction: $\chi^2_1 = 38.6$, $p < 0.0001$, Supplementary
251   Table S1b). When the fathers were infected prior to the contest, the odds of surviving 72 h
252   post-infection were five times greater for sons of winners than for sons of losers (Figure 2a,c;
253   odds ratio 5.1, $z = 5.83$, $p < 0.0001$). The opposite was the case for sham-treated sires – here
254   the winners' sons were half as likely to survive infection than losers' sons (Figure 2a,c; odds
255   ratio 0.49, $z = 2.6$, $p = 0.007$). These differences were consistent among three experimental
256   blocks performed weeks apart, despite considerable variation among blocks in overall
257   mortality (Supplementary Figure S2a).

258   In contrast to sons, we did not detect any relationship between the father's winning versus
259   losing the mating contest and his daughters' survival upon infection (contest outcome $\chi^2_1 =$
260   0.02, $p = 0.89$; contest outcome × sire infection $\chi^2_1 = 3.0$, $p = 0.083$). The pattern of
261   survivorship differences did resemble that for sons (Fig 2b), but was not consistent among
262   blocks (Supplementary Figure S2b); odds ratio for daughters of winners versus losers was
263   1.44 for infected sires ($z = 1.14$, $p = 0.25$) and 0.65 for sham-treated sires ($z = 1.35$, $p = 0.18$).
264   Daughters suffered higher mortality than sons ($\chi^2_1 = 303.5$, $p < 0.0001$), and this was
265   consistent across the three experimental blocks (Supplementary Figure 2), despite daughters
266   in blocks 2 and 3 being infected with a reduced dose of the pathogen (see Methods).

267   To compare these survival odds ratios for daughters with those for sons, we tested for an
268   interaction between mating outcome and offspring sex separately for infected and sham-
269   treated sires. Although this test was not significant for sham-treated sires ($\chi^2_1 = 0.5$, $p = 0.48$),
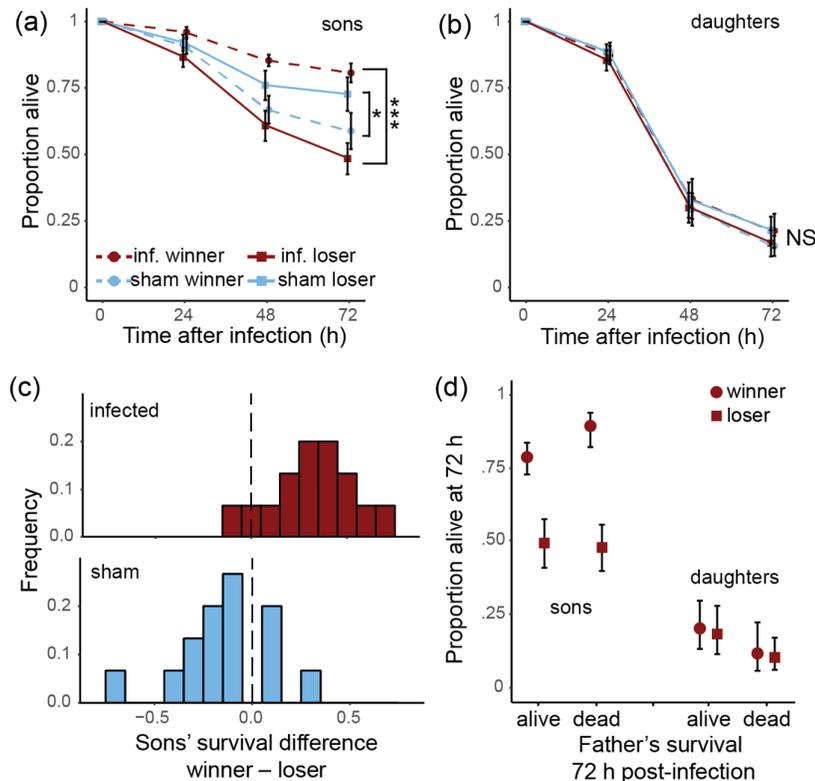
Figure 2. The relationship between the father's sexual success and offspring resistance to *P. entomophila*. (a) Post-infection survival curves of sons and (b) of daughters of sires that won versus lost a mating contest, depending on whether the sires were themselves exposed to the pathogen prior to the mating contest (inf.) or not (sham). (c) The distribution of pairwise differences in the proportion of sons' surviving 72 h post infection for each winner/loser sire duo, depending on the sire's treatment. (d) The proportion of offspring of each sex surviving 72 h post infection broken down by sire's winner/loser status and his own survival 72 h post-infection (only for sires subject to the infection treatment prior to mating contest). Symbols in (a), (b) and (d) are means ± SE.

270   it was for infected sires ($\chi^2_1 = 9.8$, $p = 0.0017$). Thus, even if daughters of infected winners
271   might have been somewhat more resistant than daughters of infected losers, father's success
272   made less difference to their odds of surviving the infection than it did to that of the sons.

273   We monitored the survival of sires after the mating contest. Only four out of 50 sham-treated
274   sires died within 72 h. As expected, mortality was higher among infected sires. Infected
275   winners had a higher likelihood than losers of surviving until 30 h after the end of the contest
276   (i.e., 72 h post-infection). Among all replicates in which contest between infected sires was
277   resolved, 26 out of 32 winners and 11 out of 32 losers survived ($p = 0.0003$, Fisher's exact
278   test); for the subset of sires whose offspring resistance was assayed, 13 out of 15 winners and
279   7 out of 15 losers survived ($p = 0.05$). This demonstrates that, unsurprisingly, fathers that
280   were phenotypically more resistant in terms of mortality were more likely to win the mating
281   contest. However, when father's survival 72 h post-infection was added to the statistical
282   model as a binary explanatory variable, it was not associated with differences in sons' survival
283   upon infection ($\chi^2_1 = 1.2$, $p = 0.26$; Supplementary Table 2). In other words, both among
284   winners and among losers, sires that died had sons as susceptible as the sons of sires that

285   survived the infection (Figure 2d). This shows that sons' survival upon infection was better
286   predicted by the father's success in the mating contest than by the father's own survival.

287

288   (b) Infection does not impair the ability to mate

289   While the above results are consistent with the "specific resistance" hypothesis, how confident
290   can one be that they are mediated by sexual selection, in particular in the case of infected
291   males? The mating contests took place 40 h after the onset of infection (Figure 1), when
292   mortality had already started to occur; about 40% of replicates set up for the mating contests
293   were discarded because at least one of the two males was dead or inactive. One could thus
294   question whether the winner/loser outcome for infected males reflects male-male competition
295   or female choice rather than the losers simply being too morbid to court and mate. Based on
296   qualitative observations, all males involved in the mating contests were active and courted at
297   least some of the time. Furthermore, if a substantial number of infected males had indeed been
298   unable to mate, we should have seen more cases of mating failure during the contest between
299   infected than between sham-treated males. This was not the case; in both treatments about
300   25% of contests did not produce mating within the 2 h of contest duration (11/43 between
301   infected versus 17/71 between sham-treated, $p = 1.0$, Fisher's exact test).

302   As a further test of the infected males' ability to mate, we performed a separate experiment in
303   which a single infected or sham-treated male was allowed to interact with two virgin females
304   for 2 h, in the same time frame as in the mating contests. In this setting, the proportion of
305   males that failed to mate was not significantly different between treatments (6/29 = 21% for
306   infected, 16/50 = 32% for sham treated; $p = 0.31$, Fisher's exact test). These results show that,
307   in spite of pathogen virulence, our infection treatment did not impair the males' ability to mate
308   within the time frame of the mating contests. Thus, the outcome of the mating contests can be
309   attributed to the relative sexual competitiveness/attractiveness of the males.

310

311   **4. Discussion**

312   We found that fathers that are more successful in a mating contest sire sons that are more
313   resistant to *P. entomophila* – if the contest takes place after the fathers have been exposed to
314   the pathogen. In contrast, males that win the contest in the absence of *P. entomophila*
315   exposure sire sons that are less resistant to the pathogen. These differences in resistance are
316   manifested, respectively, as five- and two-fold differences in odds of surviving 72 h post-
317   infection. The experimental design allowed us to exclude non-genetic paternal effects of
318   winning versus losing or of pathogen exposure (such as transgenerational immune priming
319   [47]) on offspring resistance. Thus, our results are most parsimoniously interpreted as
320   mediated by additive effects of genes passed on by the sires, as postulated under the "good
321   genes" hypothesis.

322   These results demonstrate that the relationship between male traits under sexual selection and
323   the males' breeding value ("genetic quality") for resistance to a pathogen can depend strongly
324   on the epidemiological context under which competition for mates and mate choice take
325   place. They support the scenario envisioned by Hamilton and Zuk [4] and Adamo and Spiteri

326   [37], under which male sexual traits reflect health as determined by their interactions with the
327   pathogen, and thus can only reveal the male's breeding value for resistance if the male has
328   been exposed to the pathogen. They do not support the "general immunocompetence"
329   scenarios, which postulate a positive genetic correlation between sexual success and
330   resistance to pathogens irrespective of pathogen exposure, mediated by shared condition-
331   dependence of sexual traits and immunocompetence [6, 9, 31, 33].

332   This conclusion is consistent with findings in ecological genetics of pathogen resistance in
333   *Drosophila*. If variation in pathogen resistance were mainly mediated by a condition-
334   dependent general immunocompetence, resistance to different pathogens should be highly
335   positively correlated. Yet, in *Drosophila* natural genetic variation in resistance seems largely
336   uncorrelated across different pathogens [48, 49]. Even variation in resistance to the same
337   pathogen may have different genetic bases depending on the route of infection: experimental
338   populations that evolved high resistance to oral infection with *P. entomophila* showed no
339   changes in resistance to systemic infection and vice versa [49]. Furthermore, flies raised on a
340   nutrient-poor larval diet show similar resistance to *P. entomophila* as flies raised on standard
341   diet, despite being only half the normal body weight [41], suggesting that resistance to this
342   pathogen is largely condition-independent.

343   Without prior exposure to the pathogen, males that sired more resistant sons were less
344   successful in the mating contests, although the magnitude of the difference was smaller than
345   between the offspring of infected winner and loser males. This is interesting because two
346   independent experimental evolution studies failed to detect any costs of improved *P.*
347   *entomophila* resistance in terms of larval fitness traits, larval competitive ability, stress
348   resistance or reproductive output [50, 51]. This suggests that traits under sexual selection are
349   more sensitive to subtle trade-offs of resistance than life history traits under natural selection.
350   Interestingly, the success of an infected father in the mating contest predicted his sons'
351   resistance better than the father's own post-infection survival. Both of these findings are
352   consistent with the notion that sexually selected traits are particularly sensitive to heritable
353   differences in the physiological condition of the organism [8, 32, 33] – with the twist that in
354   the absence of pathogens the resistant individuals may actually be in lower condition because
355   of physiological trade-offs of resistance.

356   An unexpected aspect of our results is the apparent sex-specificity of the relationship between
357   father's sexual success and offspring resistance. Although the effects on daughters tended in
358   the same direction as those on sons, they were not significant; the mating outcome × offspring
359   sex interaction indicates that they were significantly smaller (in terms of odds ratio) than on
360   sons. Although not generally the case for *P. entomophila* infections in *D. melanogaster* [52],
361   in our study females showed a substantially lower post-infection survival than males. Halving
362   the bacterial concentration used to infect daughters (in the last two experimental blocks, see
363   Methods) did little to change this. Possibly, the effect of genes passed on by winner versus
364   loser fathers on offspring resistance vanishes as the overall virulence of the infection
365   increases, which could explain the absence of detectable effects on daughters' survival.
366   Alternatively, alleles that differentiate winners from losers may have truly sex-specific effects
367   on offspring resistance. This possibility is supported by increasing evidence that natural
368   genetic variation may affect pathogen resistance in sex-specific or even sexually antagonistic

369   way [53, 54]. Under this interpretation, the indirect genetic benefits of sexual selection in
370   terms of pathogen resistance could be largely limited to male offspring.

371   This study demonstrates that consequences of sexual selection for offspring pathogen
372   resistance can be large and strongly context-dependent. It implies that sexual selection will
373   promote the evolution of pathogen resistance when the pathogen is prevalent in the
374   population, but will oppose it when the pathogen is absent. Females that mate with successful
375   males will benefit in terms of offspring fitness if both generations are exposed to the pathogen
376   (because their offspring will be more resistant) or if both experience no pathogen pressure
377   (because the offspring will be genetically less resistant and thus avoid paying the pleiotropic
378   costs of resistance). However, "good genes" may become "bad genes" if the epidemiological
379   situation changes radically between the generations, as inherent in the Hamilton-Zuk [4, 34]
380   and Adamo-Spiteri [5, 37] models. It remains to be tested to what degree sexual selection in
381   the presence of *P. entomophila* affects offspring resistance to other pathogens and vice versa.
382   Nonetheless, it is clear that in this system and under the type of mating competition
383   implemented here, male sexual success is not an unconditional predictor of offspring
384   resistance. The hypothesis that sexually selected traits reveal the breeding value for general
385   immunocompetence independently of pathogen exposure may well still apply to other species
386   and other pathogens. However, our results support the call for a greater experimental effort to
387   test hypotheses assuming that the link between heritable pathogen resistance and sexual traits
388   is generated by interactions of males with specific pathogens [10, 11].

389

390   **Data accessibility**

391   Data are available as electronic supplementary material.

392   **Authors' contribution**

393   TJK and PJ designed the study, PJ carried out the experiments, both authors analysed the data
394   and wrote the manuscript.

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397   **Competing interests**

398   The authors declare no competing interests.

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402

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541

Sexual selection favours good or bad genes for pathogen resistance depending on males' pathogen exposure

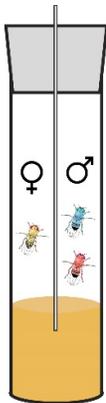Patrick Joye and Tadeusz J. Kawecki

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Supplementary material

Supplementary Figures S1 and S2.

Supplementary Tables S1 and S2.

Supplementary Figure 1. A scheme of the vial used for mating contests. Flies of the two sexes are placed on opposite sides of a cardboard divider and let acclimatize. The assay is initiated when the separator is removed through the slit in the plug.
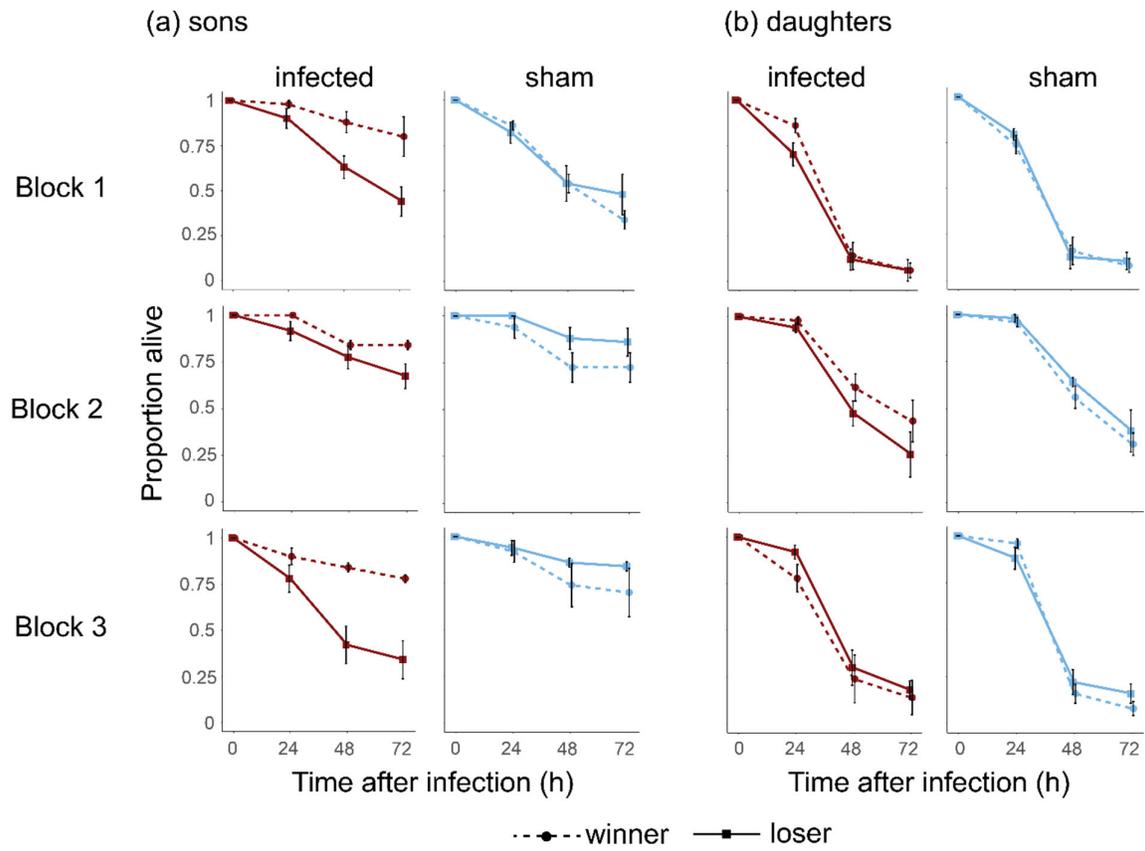
Supplementary Figure S2. Post-infection survival curves of offspring of winner and loser fathers (the same data as in figure 2a,b) split by experimental block. (a) sons and (b) daughters. Symbols are means ± SE.

Supplementary Table 1. The results of likelihood ratio tests of factors included in the generalized mixed model fitted to offspring survival until 48 h and until 72 h from the beginning of infection.

(a) Joint analysis of offspring of both sexes

Factor	48 h post-infection		72 h post-infection	
	χ^2_1	p	χ^2_1	p
Mating outcome (winner/loser)	2.8	0.091	2.1	0.14
Treatment (infection/sham)	0.3	0.58	0.02	0.89
Mating outcome × treatment	17.2	< 0.0001	28.2	< 0.0001
Offspring sex	230.3	< 0.0001	303.5	< 0.0001
Mating outcome × offspring sex	3.0	0.081	3.0	0.081
Treatment × offspring sex	0.1	0.74	0.1	0.81
Mating outcome × treatment × offspring sex	8.0	0.0046	7.4	0.0067
Block (random)	22.9	< 0.0001	15.7	< 0.0001
Winner-loser duo (random)	3.8	0.051	10.1	0.0015

(b) Separate analysis for offspring of each sex

Factor	Sons 48 h		Daughters 48 h		Sons 72 h		Daughters 72 h	
	χ^2_1	p	χ^2_1	p	χ^2_1	p	χ^2_1	p
Mating outcome (winner/loser)	5.3	0.021	0.00	0.98	5.6	0.018	0.02	0.89
Treatment (infection/sham)	0.3	0.59	0.03	0.86	0.01	0.92	0.01	0.96
Mating outcome × treatment	22.9	< 0.0001	0.9	0.33	38.6	< 0.0001	3.0	0.083
Block (random)	1.2	0.27	22.9	< 0.0001	4.2	0.041	10.2	0.0014
Winner-loser duo (random)	6.3	0.012	1.7	0.19	15.4	< 0.0001	10.8	0.0010

Supplementary Table 2. Likelihood ratio test of father's success versus father's post-infection survival as predictors of offspring survival 72 h post-infection (GLMM with binomial error distribution and logit link). Only sires subject to the infection treatment are included.

Factor	Sons		Daughters	
	χ^2_1	p	χ^2_1	p
Mating outcome (winner/loser)	33.9	<0.0001	0.1	0.75
Father's survival (dead/alive)	1.2	0.26	2.6	0.10
Mating outcome × father's survival	1.8	0.18	0.0	0.98
Block (random)	4.5	0.033	15.3	< 0.0001