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Journal: Proceedings. Biological sciences

Year: 2019 May 15

Issue: 286

Volume: 1902

Pages: 20190226

DOI: 10.1098/rspb.2019.0226

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

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Proceedings of the Royal Society Series B-Biological Sciences 286:20190226 (2019)
http://dx.doi.org/10.1098/rspb.2019.0226

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Abstract

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

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

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

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

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

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

We staged mating contests in which two males (sires) from a single outbred population competed for a female, where either both sires were earlier exposed to the pathogen or both were sham-treated. *Drosophila* females have full control over mating, and although the outcome of such contests is affected by male-male agonistic interactions, it contains a large component of female choice [43]. Subsequently, we quantified pathogen resistance of offspring sired by these winner and loser males before the infection treatment and the mating contest. This excluded potential non-genetic effects of father's infection or contest outcome on

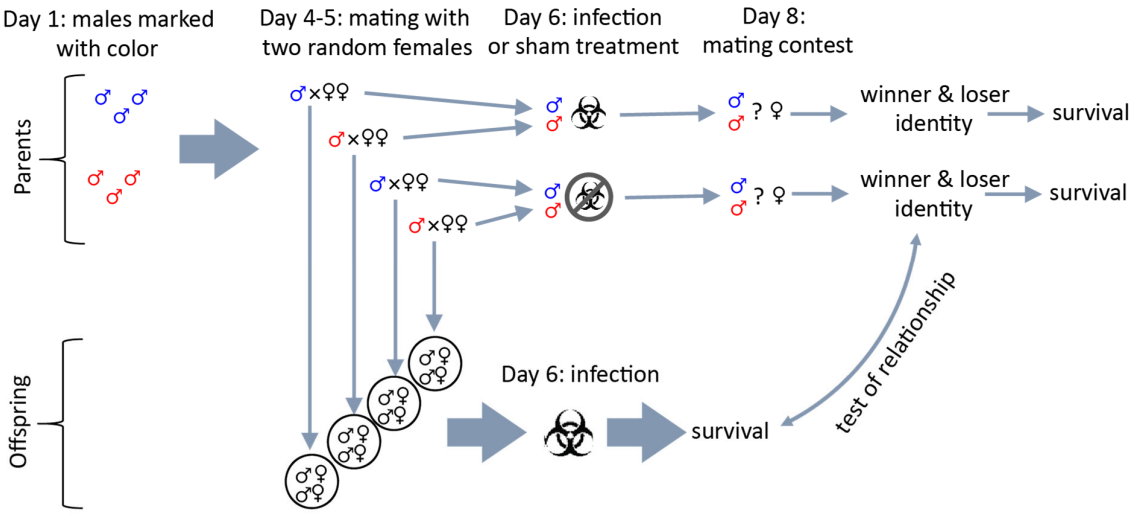

Figure 1. The design of the experiment to study the relationship between a sire's sexual success and his breeding value for resistance to P. entomophila. For explanations see Methods

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

2. Methods

(a) Fly maintenance

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

(b) Father's sexual success and offspring resistance

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

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

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

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

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

(c) Bacterial culture and infection protocol

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

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

3. Results

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

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

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

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

To compare these survival odds ratios for daughters with those for sons, we tested for an interaction between mating outcome and offspring sex separately for infected and sham-treated sires. Although this test was not significant for sham-treated sires ($\chi^2_1 = 0.5$, $p = 0.48$),

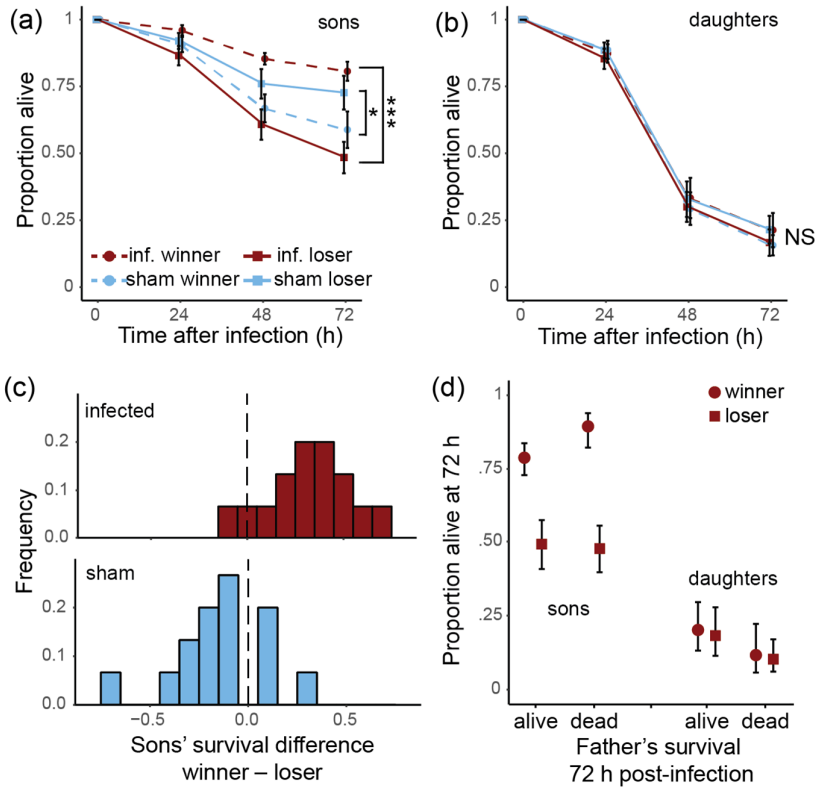

Figure 2. The relationship between the father's sexual success and offspring resistance to *P. entomophila*. (a) Post-infection survival curves of sons and (b) of daughters of sires that won versus lost a mating contest, depending on whether the sires were themselves exposed to the pathogen prior to 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.

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

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

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

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

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

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

395 **Funding**

396 This research was supported by funding from the University of Lausanne.

397 **Competing interests**

398 The authors declare no competing interests.

399 **Acknowledgements**

400 We thank B. Erkosar and R. Vijendravarma for advice, and B. Hollis, M. Kapun and D.
401 Shuker for discussions and comments on an earlier version of the paper.

402

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

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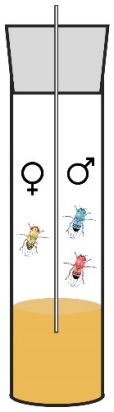
Proceedings of the Royal Society B, DOI:10.1098/rspb.2019-0226

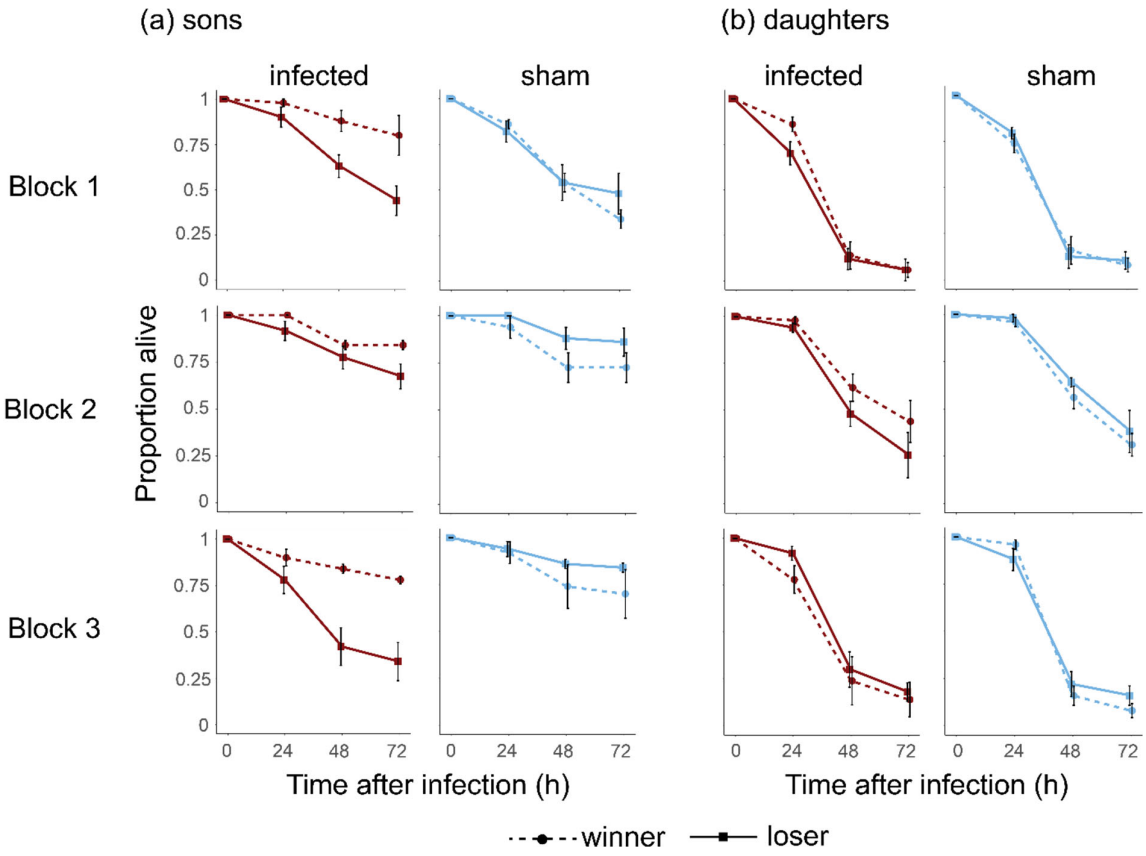
Supplementary material

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.

	Sons		Daughters	
Factor	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