RESEARCH PAPER

Genotypes and their interaction effects on reproduction and mating-induced immune activation in Drosophila melanogaster

Claudia Fricke1 | Sergio Ávila-Calero1,2 | Sophie A. O. Armitage1

1Institute for Evolution and Biodiversity, University of Münster, Münster, Germany
2Münster Graduate School of Evolution, University of Münster, Münster, Germany

Correspondence
Claudia Fricke, Institute for Evolution and Biodiversity, University of Münster, Hüfferstrasse 1, 48149 Muenster, Germany. Email: Claudia.Fricke@uni-muenster.de

Present address
Sophie A. O. Armitage, Institute of Biology, Freie Universität Berlin, Berlin, Germany

Funding information
This work was supported by the Deutsche Forschungsgemeinschaft (Heisenberg fellowships to CF: FR 2973/5-1, and to SAOA: AR 872/4-1) and the German Academic Exchange Service (DAAD Graduate School Scholarship Programme 2015-57145465) to SAC.

Abstract
Mating causes considerable alterations in female physiology and behaviour, and immune gene expression, partly due to proteins transferred from males to females during copulation. The magnitude of these phenotypic changes could be driven by the genotypes of males and females, as well as their interaction. To test this, we carried out a series of genotype-by-genotype (G × G) experiments using Drosophila melanogaster populations from two distant geographical locations. We expected lines to have diverged in male reproductive traits and females to differ in their responses to these traits. We examined female physiological and behavioural post-mating responses to male mating traits, that is behaviour and ejaculate composition, in the short to mid-term (48 hr) following mating. We then explored whether a sexually transferred molecule, sex peptide (SP), is the mechanism behind our observed female post-mating responses. Our results show that the genotypes of both sexes as well as the interaction between male and female genotypes affect mating and post-mating reproductive traits. Immune gene expression of three candidate genes increased in response to mating and was genotype-dependent but did not show a G × G signature. Males showed genotype-dependent SP expression in the 7 days following eclosion, but female genotypes showed no differential sensitivity to the receipt of SP. The two genotypes demonstrated clear divergence in physiological traits in short- to mid-term responses to mating, but the longer-term consequences of these initial dynamics remain to be uncovered.

KEYWORDS
Acp70a, antimicrobial peptide, female mating costs, genotype-by-genotype, immunogenic males, population divergence

INTRODUCTION

Reproduction is a fundamental biological process, where mating can cause significant post-mating changes in female physiology and behaviour. These changes are partly mediated by the receipt of male seminal fluid proteins. In Drosophila melanogaster, the suite of seminal fluid proteins transferred at mating is well described (Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011) with the sex peptide (SP)
being particularly well characterized. Overall, the receipt of SP enhances male reproductive success (Fricke & Chapman, 2017; Fricke, Wigby, Hobbs, & Chapman, 2009) by altering a variety of female responses. SP can increase egg-laying rates, decrease receptivity to other mates (Liu & Kubli, 2003) and alter feeding (Carvalho, Kapahi, Anderson, & Benzer, 2006) and activity patterns (Isaac, Li, Leedale, & Shirras, 2010). Seminal fluid-mediated female post-mating changes can also include immune system activation (Gioti et al., 2012; Peng, Zipperlen, & Kubli, 2005). Post-mating immune induction is known for many taxa, ranging from invertebrate to vertebrate species (Morrow & Innocenti, 2012), and has been well studied in D. melanogaster. Several immune genes are differentially expressed in mated compared to virgin females (Innocenti & Morrow, 2009; McGraw, Gibson, Clark, & Wolfnner, 2004). For instance, some antimicrobial peptide (AMP) genes showed higher levels of expression 1 hr post-mating with a peak between 2 and 4 hr following copulation (Delbare, Chow, Wolfnner, & Clark, 2017; Innocenti & Morrow, 2009; McGraw et al., 2004; Peng et al., 2005), highlighting the speed of these early female responses. Immune gene activation post-mating is a repeatable finding across studies that have used whole females (Innocenti & Morrow, 2009; McGraw, Clark, & Wolfnner, 2008; McGraw et al., 2004; Peng et al., 2005), female abdomens (Wigby, Domanitskaya, Choffat, Kubli, & Chapman, 2008) or only the reproductive tract (Kapelnikov et al., 2008; Mack, Kapelnikov, Heifetz, & Bender, 2006). However, despite the fact that mating can increase immune gene expression, it does not generally translate into an increased ability to reduce bacterial infection load compared to virgin females (McKean & Nunney, 2005; Short & Lazzaro, 2010; Wigby et al., 2008). From a broader taxonomic perspective, mating in insects generally has a negative effect on immune defences, although there are some exceptions (see review by Schwenke, Lazzaro, & Wolfnner, 2016). Mated females, therefore, do not appear to be able to fight an infection more effectively than virgin females, which may argue against the idea that mating-induced up-regulation of immune gene expression prepares females to fight sexually transmitted diseases (Lawniczak et al., 2007; Otti, 2015). Morrow and Innocenti (2012) argued that the observed immune activation could instead be a pleiotropic effect of sexually antagonistic coevolution. Sexually antagonistic coevolution occurs when the evolutionary interests of the sexes do not align during reproductive interactions. This can lead to opposing selection pressures on male and female traits, particularly when altering short-term versus long-term investments into reproduction. (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003; Parker, 2006). For example, males often manipulate females to invest more in short-term reproductive output at the expense of long-term investment because males will benefit most from female offspring production directly after mating, and before she remates. The arbitrary nature of sexually antagonistic coevolution can lead to divergence between populations (Arnqvist & Rowe, 2005; Holland & Rice, 1998). Besides being a potential mediator of sexually antagonistic coevolution (Fricke et al., 2009; Wigby & Chapman, 2005), SP is of particular relevance to this study, because it affects the up-regulation of immune genes (Gioti et al., 2012), including AMPs such as Metchnikowin (Mtk) (Domanitskaya, Liu, Chen, & Kubli, 2007; Peng et al., 2005). Conversely, SP is also involved in immunosuppression, for example the reduced ability to clear bacteria after mating (Short, Wolfnner, & Lazzaro, 2012). After mating, SP causes the activation of juvenile hormone (JH). The JH receptor (germ cell expressed) then reduces the ability of the female to lower the infection (Schwenke & Lazzaro, 2017). Hence, SP transfer and the female responses to it seem to be key in regulating female post-mating responses and the resulting reproductive outcomes.

Given that mated females can show reduced protection against a bacterial pathogen compared to virgin females (Short et al., 2012), the post-mating immune response could further contribute to the cost of mating, which is one hallmark of sexual conflict (Fricke et al., 2009; Wigby & Chapman, 2005). Sexual conflict theory predicts that rapid coevolutionary cycles drive divergence between allopatric populations (Arnqvist & Rowe, 2005; Holland & Rice, 1998). Hence, males could cause different post-mating responses in allopatric compared to coevolved females (Knowles & Markow, 2001; Rice, 1996). Evidence for population by population effects of mating comes from the ant Cardiocondyla obscurior, where queens showed higher immune gene expression and lower survival after mating with males from an allopatric compared to a sympatric population (Schrempf et al., 2015). However, a different picture emerges from D. melanogaster, where in the 1–3 hr following mating with a male from an allopatric or sympatric North American population, the males’ genotype did not influence the females’ transcriptomic profile (McGraw, Gibson, Clark, & Wolfnner, 2009). Furthermore, a G × G experiment showed that variation in post-mating bacterial load was not dependent upon the male partner, suggesting that genotype interactions were not driving post-mating immune responses (Short & Lazzaro, 2010). More recently, however, transcriptomic analyses 5–6 hr post-mating between five divergent lines showed not only that genotypic interactions affected transcriptional responses of immune genes, but that they also affected fecundity traits such as egg production and egg hatchability (Delbare et al., 2017). G × G interactions also affected pre- and post-mating behavioural phenotypes in two North American populations, without changing transcriptional profiles (McGraw et al., 2009). Hence, the extent to which G × G interactions impact on mating traits, and in particular whether variation in female post-mating responses can be explained by diverging male signals (e.g., immunogenic ejaculatory proteins), divergence in female sensitivity to these signals or the combination of both, is still unresolved puzzles.

Using populations from two distant geographical locations (Benin and Germany), we tested whether there are G × G interaction effects on mating behaviour, offspring production and immune gene expression, and then investigated whether differences in SP expression in males or female responses to SP might explain our observed responses to mating. If sexually antagonistic coevolution is driving variation in the traits we measured, our general prediction was that we would find G × G interaction effects on our measured traits. For example, over time, reciprocal adaptations between male seminal fluid proteins and female receptors that respond to these proteins will have happened within...
a population. We expect that such reciprocal adaptations will have occurred along separate trajectories for the two distinct populations, which we predict may lead to G × G interaction effects. Compared to subsequent matings, the first mating is particularly important because there is no reversal possible to the virgin state. The profound changes in female physiology shortly after this first mating might have long-term consequences, and determine subsequent investment into reproduction versus somatic maintenance. We therefore here focus on the early period (0–48 hr) after the first mating in order to capture these physiological changes. In experiment 1, we first asked what are the short-term (6 hr post-mating) to mid-term (6–48 hr post-mating) effects of genotype of mating partner on offspring production. In experiment 2, we then focussed on premating and the short-term effects post-mating, to ask how genotype of mating partner affects behaviour and female immune gene expression. Given that we found that SP affects female immune gene expression (Domanitskaya et al., 2007; Liu & Kubli, 2003), in experiment 3 we asked whether the two male genotypes differed in their expression of the SP gene over 7 days post-adult eclosion. We predicted that the male genotype eliciting the stronger female immune response in experiment 2 would also have higher SP expression. Lastly, in experiment 4, we addressed whether there are short- or mid-term post-mating differences between the two genotypes in the sensitivity of females to SP by assaying fecundity after allowing females to copulate with genetically manipulated males that either could, or could not, produce the SP protein. We predicted that if there were antagonistic physiological adaptations by the females within a population to the level of male-produced SP, then this would be expressed as differential female sensitivity to SP. We measured this as the difference in phenotypic responses (here fecundity) to the presence and absence of transferred SP.

2 | MATERIALS AND METHODS

2.1 | Drosophila melanogaster wild-type populations and genetically modified lines

We used two wild-type populations: the Dahomey (Dah) population was collected in the 1970s in Dahomey, West Africa (now Benin), and the Münster (Ms) population was collected in 2008 in Münster, Germany (Kutzer & Armitage, 2016). We refer interchangeably to these populations as genotypes, because even though we are aware that these populations harbour genetic variation and hence a number of genotypes, we expect them to be distinct from each other. Both populations were maintained in population cages, with overlapping generations and kept at 25°C, 70% relative humidity on a 12:12-hr light–dark cycle. We generated males that do not produce sex peptide (SP) and their genetically matched controls (SP+). To do this, we collected virgin females from a line having a deletion at the genomic location where the sex peptide gene resides, delta130/TM3,Sb,ry, whereby the 3rd chromosome balancer (TM3,Sb,ry) suppresses recombination and the phenotypic markers coded within allow for verification of the genotype. Virgin females were crossed with males with a mutated sex peptide gene (SP/TM3,5b,ry) or with a genetically matched control male with an additional functioning SP copy (SP+;SP+/TM3,5b,ry). The resulting delta130/SP+ (SP+) males do not produce SP, whereas delta130/SP0 (SP0) males serve as SP-transferring controls (Liu & Kubli, 2003). To increase vigour, the delta130/TM3,5b,ry line had previously been backcrossed into the Dah genetic background for three generations, whereas the other two lines had been backcrossed to the same genetic background for four generations. To produce SP+ or SP0 male offspring, three females and three males of the respective parental lines were kept in glass vials (25 × 100 mm) containing 7 ml of standard sugar, yeast, agar medium (SYA medium) (Bass et al. 2007). To verify the success of the crosses, we performed a bioassay testing female remating and egg-laying behaviour (Appendix S1).

2.2 | Production of experimental flies

Experimental flies were reared at constant larval density: grape juice agar plates (4.5% agar, 54.5% red grape juice, 3.8% nipagin and water) were smeared with a thin layer of active yeast paste and placed inside each of the population cages for egg-laying, and then retrieved approximately 20 hr later. The plates carrying eggs were incubated overnight at 25°C and 70% humidity; then, larvae were collected and placed into SYA medium-containing vials at a density of 100 larvae per vial. After adult eclosion, virgin females and males were collected on ice and housed in vials for 6 days in single sex groups of 20 individuals.

2.3 | Experiment 1: Short- and mid-term effects of genotype on offspring production

In this experiment, we established whether there are effects of mating partner genotype on offspring production in terms of egg production and the number of offspring surviving until adulthood, which allowed us to calculate egg-to-adult survival. This was measured for the eggs produced within 6 hr of mating (short-term effects) as well as for eggs produced 24–48 hr post-mating (mid-term effects). We predicted G × G interaction effects if the populations had diverged in these traits.

2.3.1 | Mating assay

In a fully reciprocal, no-choice design, single Dah and single Ms females were allowed to mate with either a Dah or a Ms male. Approximately 18 hr before mating, 80 Dah and 80 Ms virgin females were placed without anaesthesia into individual vials containing a blob of active yeast paste. The next morning, after lights on, each female was combined with a male of either genotype (n = 40 per combination). We noted latency and duration for each ensuing copulation. Each pair was given a maximum of 3 hr to start mating, after which unsuccessful pairs were excluded from further analyses. The mating assay was performed in a climate-controlled
room (25°C and 70% humidity). Any flies mating for less than 5 min were also discarded, as this likely means that sperm had not yet been transferred (Gilchrist & Partridge, 2000). Directly after mating, males were removed from vials to prevent remating. The sample sizes for successfully mated pairs were as follows (female–male genotype): Dah-Dah, n = 33; Dah-Ms, n = 38; Ms-Dah, n = 34; Ms-Ms, n = 36.

2.3.2 | Egg and adult offspring counts

Females were kept in individual vials and allowed to lay eggs for 6 hr (i.e. 0–6 hr post-mating). After 6 hr, all females were transferred to new individual vials and allowed to lay eggs for a further 18 hr (i.e. 6–24 hr post-mating) and then for a subsequent 24 hr (i.e. 24–48 hr post-mating). We counted the number of eggs laid and measured egg-to-adult survival for a random subset (Dah-Dah, n = 20; Dah-Ms, n = 29; Ms-Dah, n = 25; Ms-Ms, n = 23) of 6- to 24-hr post-mating replicates. This was done to verify that there are not genetic incompatibilities between the genotypes. All vials from the three egg-laying intervals were kept until eclosion and then frozen at -20°C to subsequently count the number of all eclosed adults. Sample sizes, in terms of the number of vials from which the number of adult offspring, but this time only considering the eggs from which the sample sizes of successfully mated pairs were as follows (female–male genotype): Dah-Dah, n = 30; Dah-Ms, n = 38; Ms-Dah, n = 34; Ms-Ms, n = 36.

2.4 | Experiment 2: Short-term effects of genotype on mating, offspring production and immune gene expression

In this experiment, we focussed only on the short-term effects (up to 6 hr post-mating) of genotype on reproduction and immune gene expression. Consistent with experiment 1, we again assessed the number of adult offspring, but this time only considering the eggs laid between 0 and 3 hr (hereafter 3 hr post-mating) or between 0 and 6 hr (hereafter 6 hr post-mating) after mating. Novel to this experiment was the question of whether mating affected immune gene expression at either 3 or 6 hr post-mating, and whether there were effects of mating partner genotype on gene expression in females. If there is divergence due to separate coevolutionary histories, where males differ in their immunogenicity and females differ in their immune response to mating, we predicted G × G interaction effects. Alternatively, if parasite history has shaped female immune gene expression, we expected to find mostly female genotype effects.

2.4.1 | Mating assay

The experimental design was the same as in 2.3.1, with initial sample sizes of 120 pairs for each of the four mating combinations. Similarly to experiment 1, we calculated mating latency and copulation duration, and each pair was given a maximum of 3 hr to start mating after which unsuccessful pairs were excluded from further analyses. The sample sizes of successfully mated pairs were as follows (female–male genotype): Dah-Dah, n = 98; Dah-Ms, n = 92; Ms-Dah, n = 115; Ms-Ms, n = 99. We also had a virgin treatment, for which Dah (n = 110) and Ms (n = 110) females were treated in the same way as the mated females except for access to a mate.

2.4.2 | Female collection and adult offspring counts

Females were sampled either 3 or 6 hr after the end of mating; time-matched virgin females were collected at the same times. Sampling was done by placing nonanaesthetized females individually in 1.5-ml microcentrifuge tubes, immediately flash-freezing them in liquid nitrogen and storing them at -80°C until further processing. For each genotype and mating treatment, females were evenly allocated to either the 3- or 6-hr group. The resulting numbers of females frozen were as follows (female–male genotype, 3 hr/6 hr): Dah-Dah = 48/46; Dah-Ms = 45/45; Ms-Dah = 56/56; Ms-Ms = 50/47; Dah/- = 54/55; Ms/- = 54/53. The vials that had contained the females before sampling were retained until all offspring had eclosed, and offspring were counted as described in 2.3.2.

2.4.3 | AMP expression

Seven flies were pooled directly before RNA extraction, resulting in six pools per genotype/mating treatment/time point. These groups of flies were a random subset of all frozen flies, the rest served as backup. For details of RNA extraction, reverse transcription and qPCR conditions, see Appendix S1. qPCR analyses were performed using three target AMP genes: Diptericin A (DiptA), Drosomycin (Drs) and Metchnikowin (Mtk) (Table 5). Table S5. These three genes were chosen because their expression responds to mating and SP (Peng et al., 2005: Dipt [no suffix given], Drs and Mtk; Domanitskaya et al., 2007: DiptA and Mtk). For standardization of expression levels, we used two reference genes: Ribosomal protein L32 (RpL32) and Ribosomal protein L13a (RpL13a) (Table S1).

2.5 | Experiment 3: Male sex peptide gene expression

Given the hypothesis that the transfer of SP transfer during mating might elicit a sexually antagonistic up-regulation of immune genes (Gioti et al., 2012; Peng et al., 2005), we predicted that the degree of up-regulation in female immune gene expression should be correlated with the level of SP expression in the genotype of the mating partner. In this experiment, we therefore compared relative SP gene expression of virgin males from the Dah and Ms genotypes for 7 days after eclosion.
2.5.1   Male collection

Dah and Ms flies were produced as in section 2.2., except that only males were collected using brief ice anaesthesia of freshly eclosed adults. For each of the two genotypes, we randomly assigned males to one of five age categories (0, 1, 3, 5 and 7 days post-eclosion) to test SP expression changes during male sexual maturation. The 0-day-old category of males was collected within 2 hr of eclosion and immediately snap-frozen. For all other time points, males were distributed in groups of 10 per vial and snap-frozen in liquid nitrogen on the corresponding day ± 2 hr from the collection time. All freezing took place at the same time of day (12:00 p.m.) to control for potential variation in gene expression due to circadian rhythm. All samples were stored at −80°C until further processing.

2.5.2   Sex peptide gene expression

From each vial of 10 males, we took a random subset of five males. We removed their abdomens whilst keeping them on dry ice, and pooled the abdomens together in one reaction vial. Each group of five abdomens was termed a replicate, and abdomens from both male genotypes and their five age categories were processed on the same day, one or two replicates per age category/genotype at a time. The pools were stored at −80°C until RNA extraction the following day. We produced five replicates per genotype and age category, except for the 3-day-old Dah, and 0- and 1-day-old Ms, which each had four replicates. For details of RNA extraction, reverse transcription and qPCR conditions, see Appendix S1. Quantitative PCR analyses were performed using SP as the target gene (Table S1) and the same reference genes as in Section 2.4.3.

2.6   Experiment 4: Short- and mid-term effects of sex peptide on mating and offspring production

SP transfer can increase female fecundity (Liu & Kubli, 2003). We here measured female responses to receipt of SP by assessing fecundity of females that had received SP during mating compared to females that did not receive SP during mating. By using standard SP+/SP− males, we could isolate the effects of SP receipt from potential variation introduced by the males of Dah and MS genotype. Our prediction was that if females from the population with the higher male SP expression have evolved resistance to SP, then they would differ in the strength of their response when mated to males with, compared to without, SP.

2.6.1   Mating assay and egg and adult offspring count

To assess the short- and mid-term effects of the transfer of sex peptide on offspring production by the two genotypes, 80 SP+ and 80 SP− males were allowed to mate individually with a single virgin Ms or Dah female (n = 40 per combination). The mating assay followed the same methods as in Section 2.3.1, including the calculation of mating latency and copulation duration. The sample sizes of successfully mated flies were as follows (female–male genotype): Dah-SP+, n = 34; Dah-SP−, n = 36; Ms-SP+, n = 35; Ms-SP−, n = 31. To assess fecundity, we subsequently counted the number of eggs and adult offspring resulting from the crosses as described in section 2.3.2.

FIGURE 1   Experiment 1: Egg and adult offspring production. (a) The effect of female genotype on the number of eggs laid in the 6- to 24-hr period post-mating and (b) the proportion of eggs laid between 6 and 24 hr after mating that became adult (egg-to-adult survival). (c) The number of adult offspring that were produced in the three time intervals following mating. (d) The cumulative number of adult offspring produced up to 48 hr post-mating. Grey dots indicate the values from single females, and the dots are jittered to aid visualization. Standard errors are shown. Stars indicate statistical significance: *p < .05
2.7 | Statistical analyses

Statistical analyses were performed using RStudio version 1.1.383 (RStudio Team, 2016) and R version 3.4.3 (R Core Team, 2018). In general, our models tested for the effects of female genotype (Dah or Ms) and male genotype (Dah, Ms, SP1 or SP2) and their interactions on our response variables. We first assessed the full models, including the G × G interaction term and then performed model simplification (Appendix S1 and Table S2). For rationale behind using model simplification, see Appendix S1 and Crawley (2007). The reduced models are represented throughout the results section, and a comparison with the full models is given in Appendix S2. Where the results of the full and reduced models differ, it is mentioned in the main results text.

3 | RESULTS

3.1 | Experiment 1: Short- and mid-term effects of genotype on offspring production

Between 6 and 24 hr post-mating, Ms females laid more eggs than Dah females (LR $\chi^2 = 4.68, df = 1, p = .030$; Figure 1a). Model simplification revealed female genotype as the only significant explanatory variable. Egg-to-adult survival was higher in allopatric matings ($F_1 = 7.30, p = .008$; Figure 1b). Because of this significant interaction, we do not interpret the significant main effect of female genotype ($F_1 = 4.46, p = .037$; note that this main effect was nonsignificant in the full model, see Appendix S2). Ms females started producing offspring more quickly than Dah females (female × interval, $\chi^2 = 7.43, df = 2, p = .024$, Figure 1c). This difference early in the reproductive output resulted in Ms females producing cumulatively marginally more offspring over the entire 48-hr post-mating period ($F_1 = 4.14, p = .044$; Figure 1d). Unsurprisingly, the length of the egg-laying interval affected the number of offspring produced ($\chi^2 = 36.49, df = 2, p < .0001$). Neither mating latency nor copulation duration affected any of the variables here reported; therefore, they were dropped through model simplification.

3.2 | Experiment 2: Short-term effects of genotype on mating, offspring production and immune gene expression

Not only did matings start earlier when mating with Dah compared to Ms males, but the latency period was about 50% shorter when a Dah male mated with a Ms female compared to a Dah female (Figure 2a; female × male genotype: $F_{1,397} = 22.11, p < .0001$). There were also significant main effects of female genotype ($F_{1,397} = 9.58, p = .002$) and male genotype ($F_{1,397} = 36.39, p < .0001$) on latency. In contrast, the only factor that affected copulation duration was male genotype, where matings with Dah males were approximately two and a half minutes shorter than matings with Ms males (Figure 2b; $F_{1,397} = 18.92, p < .0001$; there was a marginally nonsignificant male × female genotype interaction: $F_{1,396} = 3.42, p = .065$).

Matings with Dah males were more likely to produce offspring than matings with Ms males ($F_{1,392} = 4.84, p = .028$; Figure 2c). Conversely, a higher proportion of Ms females produced offspring compared to Dah females ($F_{1,392} = 20.16, p < .0001$; Figure 2d). Longer latencies were less likely to result in offspring (Figure S1a; $F_{1,392} = 5.64, p = .018$; Appendix S1). As expected, females were more likely to produce offspring within six, compared to within 3 hr after mating ($F_{1,392} = 137.25, p < .0001$). When we used a hurdle model (to account for zero-inflation, see Zuur, Ieno, Walker, Saveliev, & Smith, 2009) to analyse the fraction of females producing no offspring 6 hr post-mating, the logistic part of the model revealed similar results to the overall data set. A higher proportion of Dah females produced no adult offspring ($\chi^2 = 8.25, df = 1, p = .004$; Figure S1b), and there was a negative relationship between offspring presence and latency ($\chi^2 = 5.79, df = 1, p = .016$; Figure S1c). However, unlike the previous results, there was no influence of male genotype on offspring numbers. The count part of the hurdle model (dealing with numbers of offspring produced) showed that Ms females produced more adult offspring within 6 hr compared to Dah females ($\chi^2 = 7.94, df = 1, p = .005$; Figure 2e).

Regarding the dynamics of immune gene expression, Dah females had consistently higher expression of all three immune genes (relative to control genes) compared to Ms females (Table S3; Figure 3a–c). Matings resulted in up-regulation of Mtk in females of both genotypes (Table S3; Figure 3d–f) and of DiptA in Ms females only (interaction term Table S3; Figure 3g). The response of Drs to mating was time-dependent, with expression increasing from 3 to 6 hr after mating. Time-matched virgins in comparison show a decline in expression with time, indicating that the increase in mated females was related to mating status rather than time of day (interaction term in Table S3; Figure 3h).

To test the effect of male genotype on immune gene expression due to mating, we here only consider gene expression changes in mated females. Male genotype affected the degree of up-regulation of Mtk in a time-dependent manner. Mating with a Ms male up-regulated female Mtk gene expression 6 hr post-mating, but this pattern was not apparent 3 hr post-mating (significant interaction term in Table S4 after removing two influential points; with those two data points included, the interaction is nonsignificant [$p = .508$; Figure 3i]). Consistent with the data set containing virgin and mated females, Dah females had higher relative immune gene expression compared to Ms females (Table S4; Figure 3j–l). Drs showed a dynamic expression pattern, whereby expression was higher at 6 hr (mean = 0.079, SE = 0.007) compared to 3 hr (mean = 0.070, SE = 0.007) after mating (see mated females in Figure 3h). The full model for DiptA also showed a significant effect of time (Appendix S2), but this became nonsignificant after correcting for multiple testing (Table S4).

3.3 | Experiment 3: Male sex peptide gene expression

Overall, Ms males had a higher relative SP gene expression than Dah males ($F_{1,41} = 4.77, p = .035$; Figure 4a). SP expression varied
with time ($F_{4,41} = 4.36, p = .005$; Figure 4a). It rose between eclosion and day 1, and then remained relatively constant over the following days, with a tendency to decrease over the 7-day period (Table S5).

### 3.4 | Experiment 4: Short- and mid-term effects of sex peptide on mating and offspring production

Considering female mated with either SP<sup>0</sup> or SP<sup>+</sup> males, there was no effect of female or male genotype on the number of eggs laid between 6 and 24 hr post-mating. Egg-to-adult survival did not depend on male genotype, but it did depend on female genotype ($F_1 = 6.33, p = .014$; Figure 4b). Increased mating latency had a weak negative effect on the probability of eggs developing into adults ($F_1 = 4.81, p = .032$; Figure S2a). Note that there was no significant effect of female genotype or mating latency on egg-to-adult survival in the full model (Appendix S2; $p < .08$ in both cases). Again, the number of adult offspring varied over the three time intervals (0–6, 6–24 and 24–48 hr) post-mating ($\chi^2 = 169.58$, $df = 2, p < .0001$; Figure S2b) and by female genotype ($\chi^2 = 12.55$, $df = 1, p = .0004$; Figure S2c). However, the ability of males to transfer sex peptide did not have an effect on these dynamics or the total number of offspring produced. When we analysed cumulative offspring production, in the subset of the data where females produced offspring (count part of the hurdle model), SP did not affect the total number of offspring that females produced in the 48 hours post-mating. Although there was a non-significant trend for an interaction between female and male genotypes ($\chi^2 = 2.96, df = 1, p = .085$; Figure 4c). When we analysed the fraction of females producing no offspring, the logistic part of the model showed that a higher proportion of Dah females produced adult offspring ($\chi^2 = 14.12, df = 1, p = .0002$; Figure 4d), but male genotype had no effect.

### 4 | DISCUSSION

Given the potential for female–male coevolution within a population, we had predicted that we would uncover G × G interactions for ejaculate-elicited post-mating traits, such as egg production and mating-induced immune gene expression. Instead, we found
strong genotype effects, largely in the absence of interactions. Our results indicated that either female or male genotype alone explained a significant amount of variation in reproductive traits and AMP gene expression. G × G interactions, however, did influence mating latency and egg-to-adult survival. As we had predicted, AMP expression was increased in response to mating. Ms
males in particular induced increased Mtk expression, a SP responsive gene. These results led us to the prediction that Ms males would therefore have higher relative SP gene expression compared to Dah males, which was indeed the case. Despite the fact that the Ms males had higher SP expression, for the reproductive traits that we measured, females from our two genotypes did not differ in their responses to the receipt of SP. Our findings indicate that sexually antagonistic coevolution might not explain differences in mating-induced immune gene expression here. Below, we discuss G × G interactions first as they are our main focus, and then, we discuss our other results ordered by experiment.

In contrast to our predictions, we uncovered few G × G interactions. Egg-to-adult survival was one of the few traits where we found G × G interactions. Mating with an allopatric male was advantageous in terms of increasing egg-to-adult survival (Figure 1b). This genotypic difference in female fecundity might be explained by a faster maturation of oocytes, or increased egg-laying rate and/or egg-to-adult survival for Ms females. Although we did not count the eggs laid in the first 6 hr, Ms females indeed laid more eggs in the six to 24 hr post-mating compared to Dah females (Figure 1a). Interestingly, a mere 6-hr male–female interaction period could lead to relevant differences in female fitness and potentially lay the foundation for female costs of mating.

Given that the difference in number of adult offspring produced by the two genotypes was particularly pronounced in the first 6 hr post-mating, in experiment 2 we focussed on the short-term (3- to 6-hr) post-mating responses. Apart from differing in the number of eggs produced (experiment 1), Ms females were faster to start ovipositing fertilized eggs than Dah females (Figure 2d). Incidentally, overall, 21% of females from both genotypes produced adult offspring in the first 3 hr post-mating and by 6 hr this value had increased considerably, thereby maximizing the probability of finding a signature of G × G interactions.

4.1 | Experiments 1 and 2: Female and male genotype main effects influenced pre- and post-mating reproductive traits

Overall, Ms females produced more adult offspring than Dah females in the short to mid-term, that is 48 hr, post-mating (Figure 1d). In our more detailed timeline, we showed that these genotypic fecundity differences are largely derived from the first 6 hr post-mating, where Ms females produced over 30% more adult offspring (Figure 1c). This genotypic difference in female fecundity might be explained by a faster maturation of oocytes, or increased egg-laying rate and/or egg-to-adult survival for Ms females. Although we did not count the eggs laid in the first 6 hr, Ms females indeed laid more eggs in the six to 24 hr post-mating compared to Dah females (Figure 1a). Interestingly, a mere 6-hr male–female interaction period can negatively affect female survival rates (Civetta & Clark, 2000). Therefore, early post-mating processes can have long-lasting effects on fitness and slight differences in the onset of female reproduction could lead to relevant differences in female fitness and potentially lay the foundation for female costs of mating.

Although Ms females produced more adult offspring than Dah females in the short to mid-term, that is 48 hr, post-mating (Figure 1d). In our more detailed timeline, we showed that these genotypic fecundity differences are largely derived from the first 6 hr post-mating, where Ms females produced over 30% more adult offspring (Figure 1c). This genotypic difference in female fecundity might be explained by a faster maturation of oocytes, or increased egg-laying rate and/or egg-to-adult survival for Ms females. Although we did not count the eggs laid in the first 6 hr, Ms females indeed laid more eggs in the six to 24 hr post-mating compared to Dah females (Figure 1a). Interestingly, a mere 6-hr male–female interaction period can negatively affect female survival rates (Civetta & Clark, 2000). Therefore, early post-mating processes can have long-lasting effects on fitness and slight differences in the onset of female reproduction could lead to relevant differences in female fitness and potentially lay the foundation for female costs of mating.
around 78%. These percentages are consistent with a previous estimate (20%) for another genotype, the Canton-S D. melanogaster genotype 4 hr post-mating (Bubis, Degreen, Unsell, & Tompkins, 1998; note that they did not test 3 hr post-mating). Hence, females from the two populations have diverged in oviposition timing and investment. Both populations were reared under similar conditions in the laboratory, and despite the common garden conditions, they show phenotypic differences. These differences could reflect a signature of local adaptation, but they could also be shaped by male–female coevolution. Indeed, we found that the likelihood of producing offspring was lower when mating with a Ms male (Figure 2c), indicating that there are also male-specific effects affecting mating behaviour.

4.2 | Experiment 2: Mating and genotype affect AMP expression

We tested whether the divergence in short-term post-mating responses was driven by a signature of sexually antagonistic coevolution. One of the early post-mating signatures is differential expression of immune genes (e.g., Innocenti & Morrow, 2009; Lawniczak et al., 2007; McGraw et al., 2004). Morrow and Innocenti (2012) hypothesized that this gene expression might be antagonistically manipulated by the male. In agreement with previous work, we found that two of the three analysed AMP genes increased in expression when females switched from a virgin to the mated state. Mtk expression increased significantly for both genotypes after mating and has previously been demonstrated to increase expression also in response to the receipt of sex peptide (Peng et al., 2005). Unexpectedly, DiptA showed contrasting effects for the two genotypes, as its expression increased after mating in Ms females but not in Dah females. Mating-responsive modulation of AMP expression is therefore to some degree population-specific. This could be due to differences in sensitivity to seminal proteins transferred at mating (see below), the degree of wounding caused by mating (Kamimura, 2007) and/or the transfer of microbes (Knell et al., 2012), or the transfer of microbes (Knell et al., 2012). Drs expression was also modulated insofar as mated females increased expression over three to 6 hr post-mating, whereas virgins reduced expression in this time frame. These data show that all of the tested AMP genes were mating-responsive, but that the response is context-specific.

Overall, Dah females had a higher constitutive relative expression of all three AMP genes compared to Ms females. The question arises as to whether this translates into increased resistance against pathogens. Across four inbred D. melanogaster genotypes, AMP expression 24 hr after experimental bacterial infection varied according to genotype but expression did not correlate with the bacterial load (Kutzer, Kurtz, & Armitage, 2019). However, the expression in uninfected animals was not examined, so the above possibility remains to be tested. Lastly, male genotype affected female gene expression. Ms males, but not Dah males, induced a higher expression of Mtk at six compared to 3 hr post-mating. This hints at male genotype differences in inducing female immunological responses.

Overall, experiments 1 and 2 showed that Ms females produced more eggs and adult offspring and did so earlier after mating. On the other hand, Dah females had consistently higher constitutive immune gene expression than Ms females and this was consistent across changes in mating status. This would broadly support the idea of a trade-off between reproduction and immune defence (Schwenke et al., 2016), whereby the higher baseline immune expression of Dah females might trade-off with investment in reproduction, therefore resulting in less offspring produced. However, this idea is speculative and would need experimental testing.

4.3 | Experiments 3 and 4: Male SP expression is genotype-dependent but female genotypes are not differentially sensitive to SP transfer

Given that Ms males induced a post-mating rise in female Mtk transcription and that SP transfer can elicit female Mtk expression (Peng et al., 2005), we hypothesized that SP might differ in expression between males from the two genotypes. As predicted, we found that Ms males had higher SP gene expression compared to Dah males across the first 7 days post-adult eclosion (Figure 4a). Males reach sexual maturity by 4 days post-eclosion as shown by an increase in accessory gland size (Ruhmann, Wensing, Neuhalfen, Specker, & Fricke, 2016). The maximal expression of SP at 24 hr post-eclosion found here will contribute towards the filling of the male SP stores before they reach maturity. If higher SP expression is a signature of sexually antagonistic coevolution, then our last prediction was that, as a result of the increased SP expression in Ms males, Ms females would show differential sensitivity to the protein compared to Dah females. However, there was only a nonsignificant trend for an interaction between female genotype and male genotype (SP+ or SP−). Ms females tended to react more strongly to SP receipt, that is to produce more offspring in the presence of SP and less offspring in the absence of SP (Figure 4c), that is they showed little potential for resistance to SP effects. This would match our above finding that Ms females were quicker to start egg-laying after mating and that the presence of SP is potentially driving this. However, in general, the females from the two populations were not differentially affected by the presence or absence of SP. We note that the SP knockout was backcrossed into the Dah genetic background and not the Ms genotype; hence, behaviour and the remaining seminal fluid proteins resemble the Dah male phenotype.

5 | CONCLUSIONS

We here found G × G interactions for mating latency and egg-to-adult survival. The remaining post-mating traits were predominantly influenced by the male or female genotype alone and were not necessarily maximized by mating with a sympatric male (e.g., Schrempf et al., 2015). The early dynamics reported here and...
elsewhere (e.g., Gioti et al., 2012; McGraw et al., 2004; Morrow & Innocenti, 2012) might set the stage for subsequent dynamics commonly observed such as female lifespan reduction and reduced lifetime reproductive output in D. melanogaster as a result of increased male exposure and elevated mating rate (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995; Edward, Fricke, Gerrard, & Chapman, 2011; Rice et al., 2006; Wigby & Chapman, 2005). The genotypes differed in their baseline expression of immune genes, which indicates genetic differences likely due to differences in past selection pressures. Also, male genotypes differed in SP expression, but transfer of this peptide to females did not result in differential female genotype responses. However, we did not test whether SP transfer differentially affected female immune gene expression. We have demonstrated divergence between our two populations, but we found little signature of sexually antagonistic coevolution to explain this divergence. Therefore, understanding the drivers of this divergence is less obvious. It is predicted coevolution to explain this divergence. Therefore, understanding the drivers of this divergence is less obvious. It is predicted that sexually antagonistic selection pressures might be important (Morrow & Innocenti, 2012), as mating-induced increased immune gene expression does not seem to protect females against a bacterial infection (Short et al., 2012). However, we found no clear evidence for sexual antagonism, and it is possible that mating-induced increased immune gene expression could be protective against infections other than those tested by Short et al. (2012)

ACKNOWLEDGMENTS

We would like to thank Dolors Amoros Moya, Kathrin Brüggemann, Barbara Hasert, Kathrina Kleinlein, Mareike Koppick, Joachim Kurtz, Megan Kutzer, Hanna Ruhmann and Kristina Wensing for their advice or technical support. We thank the editor Christoph Haag and two anonymous reviewers for their insightful comments.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

CF, SAC and SAOA designed the study; all three performed experiments and collected data; SAC and SAOA analysed the data. CF and SAOA wrote the manuscript with input from SAC.

ORCID

Claudia Fricke https://orcid.org/0000-0002-0691-6779
Sophie A. O. Armitage https://orcid.org/0000-0002-5561-9543

REFERENCES

Arnvist, G., & Rowe, L. (2005). Sexual conflict. Princeton, NJ: Princeton University Press.
Avila, F. W., Sirot, L. K., LaFlamme, B. A., Rubinstein, C. D., & Wolfner, M. F. (2011). Insect seminal fluid proteins: Identification and function. Annual Review of Entomology, 56, 21–40. https://doi.org/10.1146/annurev-ento-120709-144823
Bass, T. M., Grandison, R. C., Wong, R., Martinez, P., Partridge, L., & Piper, M. D. W. (2007). Optimization of Dietray Restriction Protocols in Drosophila. Journal of Gerontology: Biological Sciences, 62(A10), 1071–1081.
Bubis, J. A., Degreen, H. P., Unsell, J. L., & Tompkins, L. (1998). Temporal manipulation of ejaculate components by newly fertilized Drosophila melanogaster females. Animal Behaviour, 55, 1637–1645. https://doi.org/10.1006/anbe.1997.0723
Carvalho, G. B., Kapahi, P., Anderson, D. J., & Benzer, S. (2006). Alloecrine modulation of feeding behavior by the sex peptide of Drosophila. Current Biology, 16, 692–696. https://doi.org/10.1016/j.cub.2006.02.064
Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003). Sexual conflict.

Trends in Ecology and Evolution, 18, 41–47. https://doi.org/10.1016/S0169-5347(02)00004-6
Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in Drosophila melanogaster females is mediated by male accessory gland products. Nature, 373, 241–244.
Civetta, A., & Clark, A. G. (2000). Correlated effects of sperm competition and postmating female mortality. Proceedings of the National Academy of Sciences of the United States of America, 97, 13162–13165. https://doi.org/10.1073/pnas.230305397
Crawley, M. J. (2007). The R book. Chichester, UK: John Wiley & Sons.
Delbare, S. Y. N., Chow, C. Y., Wolfner, M. F., & Clark, A. G. (2017). Roles of female and male genotype in post-mating responses in Drosophila melanogaster. Journal of Heredity, 108, 740–753. https://doi.org/10.1093/jhered/esx081
Domanitskaya, E. V., Liu, H., Chen, S., & Kubli, E. (2007). The hydroxyproline motif of male sex peptide elicits the innate immune response in Drosophila females. FEBS Journal, 274, 5659–5668.
Edward, D. A., Fricke, C., Gerrard, D. T., & Chapman, T. (2011). Quantifying the life-history response to increased male exposure in female Drosophila melanogaster. Evolution, 65, 564–573. https://doi.org/10.1111/j.1558-5646.2010.01151.x
Fricke, C., & Chapman, T. (2017). Variation in the post-mating fitness landscape in fruit flies. Journal of Evolutionary Biology, 30, 1250–1261. https://doi.org/10.1111/jeb.13090
Fricke, C., Wigby, S., Hobbs, R., & Chapman, T. (2009). The benefits of male ejaculate sex peptide transfer in Drosophila melanogaster. Journal of Evolutionary Biology, 22, 275–286. https://doi.org/10.1111/j.1420-9101.2008.01638.x
Gilchrist, A. S., & Partridge, L. (2000). Why it is difficult to model sperm displacement in Drosophila melanogaster: The relation between sperm transfer and copulation duration. Evolution, 54, 534–542. https://doi.org/10.1111/j.0014-3820.2000.tb00056.x
Gioti, A., Wigby, S., Wertheim, B., Schuster, E., Martinez, P., Pennington, C. J., … Chapman, T. (2012). Sex peptide of Drosophila melanogaster males is a global regulator of reproductive processes in females. Proceedings of the Royal Society B-Biological Sciences, 279, 4423–4432.
Holland, B., & Rice, W. R. (1998). Perspective: Chase-away sexual selection: Antagonistic seduction versus resistance. Evolution, 52, 1–7. https://doi.org/10.1111/j.1558-5646.1998.tb05132.x
Innocenti, P., & Morrow, E. H. (2009). Immunogenic males: A genome-wide analysis of reproduction and the cost of mating in Drosophila melanogaster females. Journal of Evolutionary Biology, 22, 964–973.
Isaac, R. E., Li, C., Leedale, A. E., & Shirras, A. D. (2010). Drosophila male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female. Proceedings of the Royal Society B-Biological Sciences, 277, 65–70.
Kamimura, Y. (2007). Twin intromittent organs of Drosophila for traumatic insemination. Biology Letters, 3, 401–404.
Kapelnikov, A., Zelinguer, E., Gottlieb, Y., Rhrissorrakrai, K., Gunsalus, K. C., & Heifetz, Y. (2008). Mating induces an immune response and developmental switch in the Drosophila oviduct. Proceedings of the National Academy of Sciences of the United States of America, 105, 13912–13917. https://doi.org/10.1073/pnas.0710997105
Knell, R. J., & Webberley, K. M. (2004). Sexually transmitted diseases of insects: Distribution, evolution, ecology and host behaviour. *Biological Reviews of the Cambridge Philosophical Society*, 79, 557–581.

Knowles, L. L., & Markow, T. A. (2001). Sexually antagonistic coevolution of a post-mating-prezygotic reproductive character in desert *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 8692–8696. https://doi.org/10.1073/pnas.151123998

Kutzer, M. A. M., & Armitage, S. A. O. (2016). The effect of diet and time after bacterial infection on fecundity, resistance, and tolerance in *Drosophila melanogaster*. *Ecology and Evolution*, 6, 4229–4242.

Kutzer, M. A. M., Kurtz, J., & Armitage, S. A. O. (2019). A multi-faceted approach testing the effects of previous bacterial exposure on resistance and tolerance. *Journal of Animal Ecology*, 88, 566–578. https://doi.org/10.1111/1365-2656.12953

Lawniczak, M. K. N., Barnes, A. I., Linklater, J. R., Boone, J. M., Wigby, S., & Chapman, T. (2007). Mating and immunity in invertebrates. *Trends in Ecology and Evolution*, 22, 48–55. https://doi.org/10.1016/j.tree.2006.09.012

Liu, H., & Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9929–9933. https://doi.org/10.1073/pnas.1631700100

Mack, P. D., Kapelnikov, A., Heifetz, Y., & Bender, M. (2006). Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 10358–10363. https://doi.org/10.1073/pnas.0604046103

McGraw, L. A., Clark, A. G., & Wolfner, M. F. (2008). Post-mating gene expression profiles of female *Drosophila melanogaster* in response to time and to four male accessory gland proteins. *Genetics*, 179, 1395–1408.

McGraw, L. A., Gibson, G., Clark, A. G., & Wolfner, M. F. (2004). Genes regulated by mating, sperm, or seminal proteins in mated female *Drosophila melanogaster*. *Current Biology*, 14, 1509–1514.

McGraw, L. A., Gibson, G., Clark, A. G., & Wolfner, M. F. (2009). Strain-dependent differences in severable reproductive traits are not accompanied by early postmating transcriptome changes in female *Drosophila melanogaster*. *Genetics*, 181, 1273–1280.

McKean, K. A., & Nunney, L. (2005). Bateman’s principle and immunity: Phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution*, 59, 1510–1517. https://doi.org/10.1111/j.0014-3820.2005.tb01800.x

Morrow, E. H., & Innocenti, P. (2012). Female postmating immune responses, immune system evolution and immunogenic males. *Biological Reviews of the Cambridge Philosophical Society*, 87, 631–638.

Otti, O. (2015). Genital-associated microbes in insects. *Insect Science*, 22, 325–339.

Parker, G. A. (2006). Sexual conflict over mating and fertilization: An overview. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 361, 235–259. https://doi.org/10.1098/rstb.2005.1785

Peng, J., Zipperlen, P., & Kubli, E. (2005). *Drosophila* sex-peptide stimulates female innate immune system after mating via the Toll and Imd pathways. *Current Biology*, 15, 1690–1694. https://doi.org/10.1016/j.cub.2005.08.048

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna Austria: R Foundation for Statistical Computing.

R Core Team (2008). *R: A language and environment for statistical computing*. Vienna Austria: R Foundation for Statistical Computing.

R Core Team (2016). *RStudio: Integrated development for R*. Boston, MA: RStudio, Inc.

Ruhmann, H., Wensing, K. U., Neuhalfen, N., Specker, J.-H., & Fricke, C. (2016). Early reproductive success in *Drosophila* males is dependent on maturity of the accessory gland. *Behavioral Ecology*, 27, 1859–1868.

Schrempf, A., Von Wyschetzki, K., Klein, A., Schrader, L., Oettler, J., & Heinze, J. (2015). Mating with an allopatriac male triggers immune response and decreases longevity of ant queens. *Molecular Ecology*, 24, 3618–3627. https://doi.org/10.1111/mec.13267

Schwenke, R. A., & Lazzaro, B. P. (2017). Juvenile Hormone suppresses resistance to infection in mated female *Drosophila melanogaster*. *Current Biology*, 27, 596–601.

Schwenke, R. A., Lazzaro, B. P., & Wolfner, M. F. (2016). Reproduction-immunity trade-offs in insects. *Annual Review of Entomology*, 61, 239–256. https://doi.org/10.1146/annurev-ento-010715-023924

Short, S. M., & Lazzaro, B. P. (2010). Female and male genetic contributions to post-mating immune defence in female *Drosophila melanogaster*. *Proceedings of the Royal Society London B*, 277, 3649–3657.

Short, S. M., Wolfner, M. F., & Lazzaro, B. P. (2012). Female *Drosophila melanogaster* suffer reduced defense against infection due to seminal fluid components. *Journal of Insect Physiology*, 58, 1192–1201. https://doi.org/10.1016/j.jinsphys.2012.06.002

Wigby, S., & Chapman, T. (2005). Sex peptide causes mating costs in female *Drosophila melanogaster*. *Current Biology*, 15, 316–321. https://doi.org/10.1016/j.cub.2005.01.051

Wigby, S., Domanitskaya, E. V., Choffat, Y., Kubli, E., & Chapman, T. (2008). The effect of mating on immunity can be masked by experimental piercing in female *Drosophila melanogaster*. *Journal of Insect Physiology*, 54, 414–420. https://doi.org/10.1016/j.jinsphys.2007.10.010

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Fricke C, Ávila-Calero S, Armitage SAO. Genotypes and their interaction effects on reproduction and mating-induced immune activation in *Drosophila melanogaster*. *J Evol Biol*. 2020;33:930–941. https://doi.org/10.1111/jeb.13625