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Sperm and sex peptide stimulate aggression in female *Drosophila*

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Female aggression towards other females is associated with reproduction in many taxa, and traditionally thought to be related to the protection or provisioning of offspring, such as through increased resource acquisition. However, the underlying reproductive factors causing aggressive behaviour in females remain unknown. Here we show that female aggression in the fruit fly *Drosophila melanogaster* is strongly stimulated by the receipt of sperm at mating, and in part by an associated seminal fluid protein, the sex peptide. We further show that the post-mating increase in female aggression is decoupled from the costs of egg production and from post-mating decreases in sexual receptivity. Our results indicate that male ejaculates can have a surprisingly direct influence on aggression in recipient females. Male ejaculate traits thus influence the female social competitive environment, with potentially far-reaching ecological and evolutionary consequences.

Female aggression directed towards other females (hereafter 'female aggression') can have important fitness consequences for females and their offspring[1–4]. In both vertebrate and invertebrate taxa, females act aggressively to acquire resources or mates, defend territories, and protect and provision eggs or offspring[5–10]. Females are expected to be more flexible than males in their use of aggression, with aggression heightened at particular stages of reproduction or life-history[11], and aggression is generally associated with maximizing the production or survival of their offspring[10]. One observed pattern is an elevation of female aggression after mating, whether it be during offspring development[6] or after egg production[12–14]. These increases in aggression typically occur in parallel with other notable changes in female behaviour and physiology, including alterations in sexual receptivity, feeding rates and hormone levels[4,15–17].

In many species, male ejaculate components can induce striking changes in female post-mating behaviour and physiology. For example, in insects such as the fruit fly, *Drosophila melanogaster*, male ejaculate components increase ovulation and egg laying, alter immunity and decrease female receptivity to re-mating[15,18]. Thus, ejaculate components are potential candidates for stimulating post-mating increases in female aggression. Male ejaculates could either act as a cue for females to optimally modify their behaviour, indicating upcoming physiological changes that require females to increase their levels of aggression, or could alter female behaviour for the male's benefit, which could either be in the interests of females or represent a source of conflict between the sexes[19].

There are two main pathways through which male ejaculates could stimulate post-mating aggression in females. First, by stimulating increased egg production[20], ejaculates increase the demand for resources, which could in turn lead to aggressive behaviour (Fig. 1a). Females lay eggs at high rates after mating and require more, or different, nutrients relative to virgin females[16,17,21–23], potentially increasing the motivation for females to compete aggressively over food. Another possibility is a more direct stimulation of female aggression by males during mating, with egg production as a separate, but simultaneous, pathway (Fig. 1b). Disentangling these alternatives is required to understand the physiological and molecular pathways that coordinate female aggression with reproduction, and thus to unravel the processes that drive the evolution of female aggressive behaviour.

In this study, we tested whether mating induces changes in female aggression in *D. melanogaster*. In the wild, this species aggregates on rotting fruit[24,25], and laboratory studies have shown that females will engage in fights when food and oviposition sites are limited[26,27]. Differential reproductive success may therefore result from variation in female ability to aggressively acquire and defend food and oviposition sites. Males and females both display aggressive behaviours in *D. melanogaster* in the laboratory, although there are significant sex differences in the duration of encounters and the types of behaviour observed[27,28]. There are also differences in aggressive behaviour between mated and virgin females, with mated females fighting for longer and potentially more likely to escalate to higher levels of intensity[27]. In addition, female *D. melanogaster* display other marked post-mating changes in reproductive behaviour, such as reductions in receptivity to re-mating, increases in feeding and reductions in sleep[15]. These identified post-mating responses are mediated by male seminal fluid proteins, some of which associate with sperm, such as the 'sex peptide'[29,30], suggesting that female aggression could also be influenced by male ejaculate components.

We hypothesize that mating may stimulate aggression in females through two potential pathways, either indirectly through egg production or directly through ejaculate contents (Fig. 1). We tested for these pathways by assessing the levels of aggression in females with genetically reduced egg-laying or blocked vitellogenesis, and in females mated to males lacking specific ejaculate components, such as sperm and sex peptide.

Results

Mating elevates female aggression. We found that aggression was significantly elevated by mating: pairs with two mated females spent more time fighting over food (generalized linear model,

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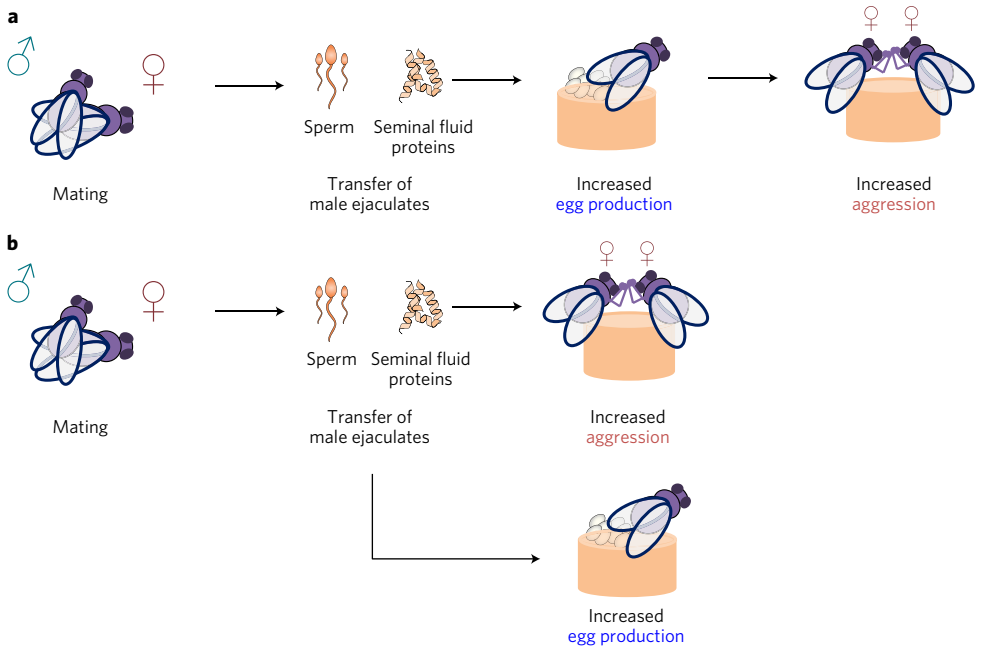

Figure 1 | Two proposed pathways for mating-induced female aggression. a, The transfer of male ejaculates stimulates increased egg production, which could in turn stimulate increased female aggression. **b**, Alternatively, the transfer of male ejaculates could stimulate increased female aggression directly, without requiring elevated egg production.

GLM: deviance $(Dev)_{1,240} = 26.01$, $P < 0.001$) than pairs with two virgins (compare MM and VV in Fig. 2a). Moreover, there was no significant difference in total contest duration between pairs with two mated females and pairs with one mated and one virgin female (Tukey test: $q_{2,20} = 0.81$, $P = 0.58$), whereas pairs with at least one mated female spent more time fighting than pairs with two virgin females (MM versus VV: $q_{3,24} = 4.81$, $P = 0.006$; MV versus VV: $q_{2,24} = 3.69$, $P = 0.015$; overall model: $Dev_{2,74} = 11.36$, $P = 0.004$; Fig. 2a). We next paired mated females with virgin females to test relative fighting ability. We found no evidence that mating status influenced which fly won or lost aggressive encounters ($\chi^2_{1,26} = 2.59$, $P = 0.41$; Fig. 2b). Thus, the presence of one mated female is sufficient to elevate the overall aggression level of a dyad, but mated females do not then win significantly more aggressive encounters.

Effects of egg production and male ejaculate components on female aggression. We found that mating-induced increases in aggression were not significantly different between sterile (ovo^{D1}) females and controls (mating status, $Dev_{1,126} = 17.32$, $P < 0.001$; sterile versus control, $Dev_{1,125} = 1.27$, $P = 0.12$; interaction, $Dev_{1,124} = 0.25$, $P = 0.49$; Fig. 3a). This shows that post-mating increases in female aggression are not restricted to females capable of vitellogenesis and egg laying, and are thus not driven by the energetic demands of egg production post-vitellogenesis.

Females mated to spermless males, which transfer seminal fluid proteins but no sperm to females[31], did not differ from virgin females in levels of post-mating aggression, and were significantly less aggressive than control mated females (overall model: $Dev_{2,68} = 14.32$, $P < 0.001$; control versus virgin: $q_{3,24} = 6.8$, $P < 0.001$; control versus mates of spermless males: $q_{2,24} = 5.3$, $P = 0.001$; virgin versus mates of spermless males: $q_{2,24} = 1.45$, $P = 0.32$; Fig. 3b). Sperm is therefore required for the mating-induced increase in female aggression.

Aggression in females mated to males that did not transfer the seminal fluid protein 'sex peptide' (SP null males) was significantly higher than in virgin females ($q_{2,30} = 3.4$, $P = 0.023$) but significantly lower than in mates of males that transferred SP ($q_{2,30} = 3.05$, $P = 0.04$; overall model $Dev_{2,127} = 11.26$, $P < 0.001$; Fig. 3c). The experiment was carried out in two blocks, but replicate block had no significant effect on total contest duration ($Dev_{1,126} = 1.225$, $P = 0.131$). These results suggest that SP contributes partially to increased post-mating female aggression, but that SP cannot fully explain the sperm effect on aggression. Females that lacked the receptor to SP (*SPR*-deficient females) did not significantly differ from wild-type females in their aggression response to mating (mating status, $Dev_{1,101} = 11.53$, $P < 0.001$; interaction mating status × SPR, $Dev_{1,99} = 0.05$, $P = 0.78$; Fig. 3d). *SPR*-deficient females also spent more time fighting than wild-type females overall, irrespective of their mating status ($Dev_{1,100} = 12.71$, $P < 0.001$).

Feeding behaviour responses to mating and egg production. In most of our experiments, the time that females spent in feeding posture (see Methods) was qualitatively similar to patterns of aggressive behaviour: for example, mated females generally spent more time in feeding posture than virgins (Supplementary Fig. 3). In the egg production experiment, there was a marginally non-significant trend for more time spent in feeding position in mated females ($Dev_{1,102} = 1.128$, $P = 0.056$; Supplementary Fig. 3a) but no significant effect of the ovo^{D1} mutation ($Dev_{1,101} = 0.15$, $P = 0.49$) or interaction ($Dev_{1,99} = 0.31$, $P = 0.97$). Therefore, ovo^{D1} females did not differ from control females in time spent in feeding posture, despite spending less time participating in aggressive encounters.

Virgin females and mates of spermless males did not differ in time spent in feeding posture ($q_{2,24} = 0.05$, $P = 0.97$), but both fed less than controls (control versus spermless: $q_{3,25} = 4.67$, $P = 0.008$; control versus virgin: $q_{2,24} = 4.62$, $P = 0.003$; Supplementary Fig. 3b). There was a non-significant trend for reduced feeding in mates of SP null males compared with controls ($q_{2,35} = 2.502$, $P = 0.086$), and mates of both SP+ and SP null males fed for longer than virgins (*SP*+ versus virgin, $q_{3,35} = 6.05$, $P = 0.0004$; SP null versus virgin; $q_{2,37} = 3.53$, $P = 0.02$; Supplementary Fig. 3c). However, time spent in feeding posture was not elevated in *SPR*-deficient females ($Dev_{1,100} = 0.14$, $P = 0.55$), and there was no significant effect of mating status ($Dev_{1,101} = 0.88$, $P = 0.13$; Supplementary Fig. 3d) or interaction ($Dev_{1,99} = 0.16$, $P = 0.52$), suggesting that the association

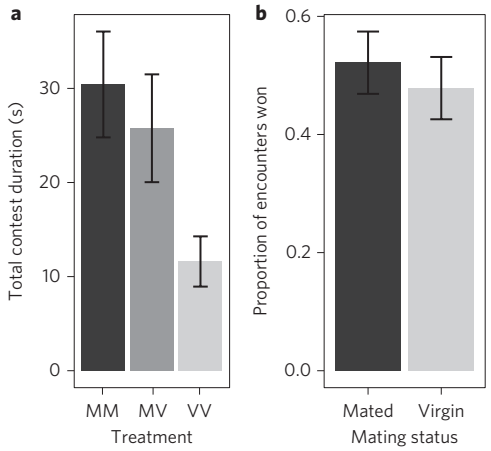

Figure 2 | Mated females spend more time fighting than virgins but do not win more fights. a, Contest duration in mated, virgin and mixed mating-status female dyads. MM, two mated females ($N=29$); MV, one mated female and one virgin female ($N=22$); VV, two virgin females ($N=26$). **b**, Proportion of encounters won by mated females and virgin females in the mixed treatment (MV in **a**). Dark grey bar, mated females ($n=45$); light grey bar, virgin females ($n=45$). Model estimate means and standard errors are shown.

between time spent engaging in feeding and aggressive behaviours is not obligatory. The proportion of encounters that an individual won was not associated with time spent in feeding posture in any experiment (mated versus virgin experiment: $Dev_{1,152}=0.08$, $P=0.6$ (results in Supplementary Fig. 4); *ovo*^{D1} experiment: $Dev_{1,100}=0.0006$, $P=0.97$; *tudor* experiment: $Dev_{1,69}=0.61$, $P=0.13$; SP experiment: $Dev_{1,112}=0.09$, $P=0.53$; SPR experiment: $Dev_{1,99}=0.16$, $P=0.52$).

Discussion

Our results show that mating strongly stimulates aggressive behaviour in female fruit flies. Mated females fought for more than twice as long as virgins, and the full increase in aggression requires the receipt of sperm and sex peptide in the male ejaculate, but not the ability of females to complete vitellogenesis or begin egg laying, nor the presence of the female sex peptide receptor (SPR)[32]. Sterile (*ovo*^{D1}) females displayed the same increase in aggression after mating as wild-type females, despite lacking the costs of egg production[20], while females that lacked the SPR gene (*SPR* deficient) spent more time fighting than wild-type females despite producing fewer eggs (Supplementary Fig. 2). These results indicate that the costs of egg production and the levels of aggression can be fully decoupled and are thus modulated by divergent pathways (Fig. 1b).

The receipt of SP was necessary for the full increase in post-mating aggression. It should be noted, however, that as we conducted tests of aggression 24 hours after mating, it is likely that only seminal fluid proteins that bind to sperm remained in the female; other seminal fluid proteins would no longer have been present in females at this time[33,34]. SP is known to bind to sperm, although it is unclear which other seminal fluid proteins also bind to sperm[35]. It is therefore possible that other seminal fluid proteins bound to sperm may also influence female aggression, or that proteins not bound to sperm could influence female aggression on a shorter timescale than the 24 hours that we measured.

Seminal fluid proteins (including SP) can, under certain conditions, lower female lifetime survival and reproductive output[36–38], raising the possibility that ejaculate-stimulated female aggression could contribute to these costs and thus represent an arena of sexual conflict rather than cooperation. However, the net fitness costs and

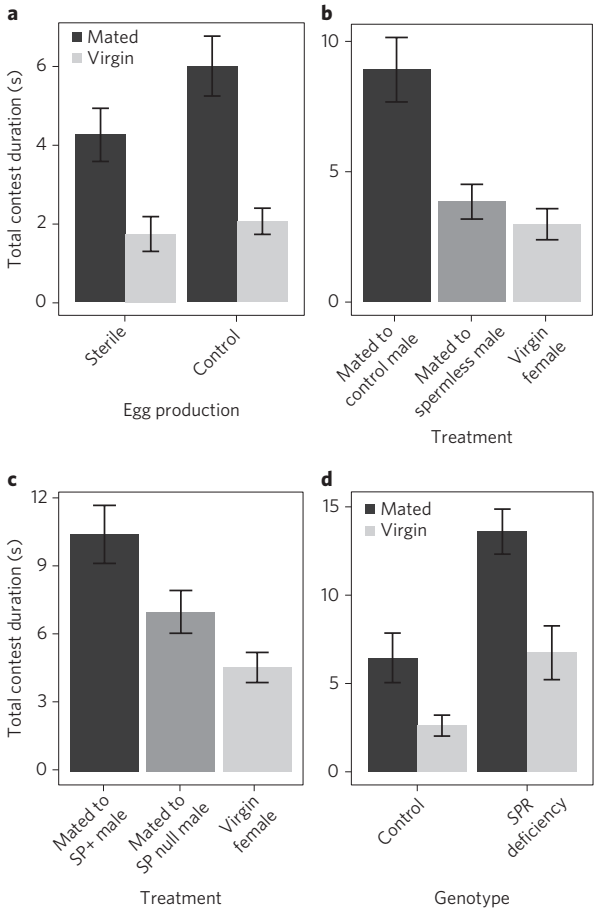

Figure 3 | Effects of male ejaculate components, female egg production and sex peptide receptor on total contest duration. a, Total contest duration of sterile females (*ovo*^{D1}) and control females. Number of pairs, *N*: mated sterile (*ovo*^{D1}) = 33, virgin sterile (*ovo*^{D1}) = 31, mated control = 31, virgin control = 33. **b**, Effect of sperm transfer on female contest duration. Number of pairs, *N*: control = 26, spermless = 25, virgin = 25. The experiment was carried out in two blocks, but results are pooled in this figure. **c**, Effect of sex peptide transfer on female contest duration. Number of pairs, *N*: SP + = 39, SP null = 42, virgin = 49. **d**, Effect of sex peptide receptor (SPR) on female contest duration. Number of pairs, *N*: mated control = 25, virgin control = 24, mated *SPR*-deficient = 29, virgin *SPR*-deficient = 25. Bars represent means, and error bars indicate standard errors.

benefits of female aggression, and the role of males in determining this, remain an area ripe for exploration. Surprisingly, females lacking the SPR displayed full increases in post-mating aggression, suggesting that SPR or one of the other deleted genes may affect aggressive behaviour, that SP may act through alternative pathways to stimulate female aggression, or that SP may act indirectly through association with other seminal proteins[39]. In addition to acting through the SPR, SP stimulates juvenile hormone production in the *corpora allata*, although the mechanism of this stimulation is unknown[40,41]. Juvenile hormone stimulates vitellogenesis in *D. melanogaster*, playing a crucial role in reproduction[42]. The amount of juvenile hormone present in the haemolymph has also been linked to aggression in both sexes in other insect species, such as burying beetles, paper wasps and cockroaches[43–45]. It is therefore possible that SP acts to increase female aggression by stimulating juvenile hormone production, although this has yet to be tested.

Male ejaculates could potentially stimulate post-mating aggression through inducing increased feeding, although our data are not fully consistent with this idea. Results from the sperm and SP

experiments suggest an association between feeding and aggression, but results from the *ovo*^{D1} and *SPR*-deficiency experiments show weaker or no associations. Future work directly manipulating food consumption would help to establish to what extent the two behaviours are linked. In addition, although using *ovo*^{D1} females allows us to conclude that aggression is not tied to the costs of reproduction (as *ovo*^{D1} females do not suffer the lifespan costs of egg production)[20,46], it is possible that eggs do still play a role in inducing female aggression. Further investigation using females that do not possess a germline may clarify the role of egg production in stimulating female aggression.

It is possible that mating results in a reduction of general social tolerance in females towards other individuals, as females are both less receptive to re-mating with males and more aggressive towards other females. However, the behaviours involved in rejection of males are very different from those involved in incidents of female aggression[27,47]. In addition, rejection behaviours and aggressive behaviours seem to be activated through different pathways, as rejection behaviours are primarily stimulated through the SPR, but our results show that female aggressive behaviours are not.

Our results fit into a broader trend, across taxa, of mating leading to increased female aggression. For example, gestating and lactating mammals, and gestating fish, display higher levels of aggression towards conspecifics[6,12,48,49]. However, our results suggest that, in *D. melanogaster*, it is possible to decouple aggression from increased energetic demands of reproduction after mating. Instead, we have shown that female aggression in *D. melanogaster* is stimulated more directly by male ejaculates, with downstream indirect effects on other females, whereby post-mating aggression affects the wider female competitive environment. For example, the levels of aggression experienced by females may depend not only on the abundance of resources and rivals in their environment, but also on the seminal characteristics of their mates and mates of their rivals.

These findings have potential implications for a wide range of sexually reproducing animals. In many insect species, females compete aggressively[43,50], and male accessory gland products induce striking changes in female behaviour and physiology other than female aggression[18,51,52]. Females of some mammal species also display increased aggression after mating[53], and, in some mammal species, ejaculate components have been shown to influence female physiology[54,55]. Thus, it is intriguing to speculate that ejaculate-induced female aggression may occur in mammals, analogous to what we have shown here for *D. melanogaster*. However, further studies are required to verify whether ejaculate-mediated effects on female aggression occur in other species. Further key areas of focus for future research include identifying the neuronal mechanisms producing increased post-mating aggression, and understanding the fitness implications for individual females, their competitors and their mates.

Methods

We conducted five experiments to test our main hypotheses about the causes of female aggression:

1. **Effects of mating**: we measured aggression in pairs of mated females, virgin females, and mated versus virgin females to test for effects of mating on aggression.
2. **Effects of egg production:** to examine whether any increase in female aggression after mating is mediated by the demands and costs of egg-production[20,56], we used the *ovo*^{D1} mutation, which blocks oogenesis prior to vitellogenesis[57]. The presence of the *ovo*^{D1} mutation in females abolishes the mortality costs associated with egg production: if these same costs drive female aggression, any differences in post-mating aggression between mated and virgin females should be abolished in *ovo*^{D1} flies.
3. **Effects of sperm:** to test whether sperm are necessary to stimulate female post-mating aggression, we measured aggression in females mated to spermless *son-of-Tudor* and control males[31].
4. **Effects of (i) the male seminal fluid protein 'sex peptide', and (ii) the female 'sex peptide receptor':** to determine whether the seminal fluid protein SP is involved in stimulating increased aggression after mating, we compared aggression in females mated to SP null males with that of control-mated and virgin females. We also used females that lacked the receptor to SP (SPR) to examine the downstream pathway through which SP could potentially stimulate female aggression.

Fly stocks and culture. All flies were kept and experiments conducted at 25 °C on a 12:12 light:dark cycle. We used the Dahomey genetic background as our stock population, and genetic mutations were backcrossed into this background for more than generations where appropriate.

Production of wild-type females. Wild-type Dahomey females[58] were reared in bottles at standardized larval density[59]. Virgins were collected within 8 hours of eclosion, and sexes were housed separately (females individually) in vials containing standard fly food media[60], with no live yeast. For the experiments using mated versus virgin flies, *ovo*^{D1}, and *SPR* knockout females, Dahomey males were used as mates.

Production of sterile (ovo^{D1}*) females.* To produce sterile *ovo*^{D1} females, we crossed males carrying the dominant *ovo*^{D1} mutation to Dahomey virgin females[46]. Sterile *ovo*^{D1} females have ovaries that degenerate prior to S5, meaning that vitellogenesis is blocked and females cannot produce eggs[57]. Dahomey females, from the stock into which the *ovo*^{D1} flies were crossed, were used as controls. We tested the efficacy of the mutation by counting offspring collected from overnight vials following mating. No *ovo*^{D1} females produced offspring, confirming their complete sterility. Previous research has found mixed evidence for the effects of the *ovo*^{D1} mutation on feeding behaviour[46], so we tested the effect of the mutation on feeding behaviour in this study (see Results).

Production of spermless males. To produce males that did not produce sperm, we used *tudor*[31]. The *tudor* mutation is a maternal-effect mutation which prevents germ plasm assembly. Sons of homozygous *tudor* females have no germline and so do not produce sperm[31,61]. We collected the male offspring of homozygous *tudor* females mated to wild-type Dahomey males as our spermless males. Males of the same genetic background, but with mothers that did not possess the *tudor* mutation, were used as controls.

Production of sex-peptide-less males. To produce males that did not produce sex peptide (SP null) and their controls (SP+), we used stocks created by Liu and Kubli[29]. SP null males carry one non-functional SP gene, and a deletion (Δ130) that removes SP (ref.[29]). The SP+ control males are genetically matched with one non-functional SP gene and one functional SP gene, and show wild-type SP expression. To verify the phenotype of SP null and SP+, we counted offspring from a subset of female overnight vials. Females mated to SP+ males produced more eggs and offspring than females mated to SP null males, confirming that our mutants were acting in the expected way (that is, SP null males were not transferring SP). In a GLM with quasi-Poisson distribution, in block 1, we counted offspring only and females mated to SP+ males produced more offspring than those mated to SP null males: $\chi^2_{1,109}=82.917$, $P=0.009$. In block 2, we counted the number of eggs produced overnight. Females mated to SP+ males produced more eggs than those mated to SP null males or virgins: SP+ versus SP null: Tukey test-statistic $q_{2,46}=11.089$, $P<0.001$; SP+ versus V: $q_{3,46}=9.711$, $P<0.001$ (Supplementary Fig. 1). The SP experiment was carried out over two blocks.

Production of females lacking sex peptide receptor. To produce *SPR*-deficient females, we used the genetic deficiency *Df(1)Exel6234*, which deletes the *SPR* gene and four other genes of unknown function[32,62]. *SPR*-deficient females do not produce the SPR. As expected, *SPR*-deficient females did not display reduced receptivity after re-mating, nor did they show elevated levels of offspring production relative to virgin females (GLM on offspring production: $\chi^2_{1,101}=111.22$, $P<0.001$; Supplementary Fig. 2)[32]. *SPR*-deficient females have also previously been shown to have slightly shorter copulation duration than wild-type females[63].

Experimental design. Virgin females, 3 days post-eclosion, were marked with acrylic paint (red or yellow) on the thorax to allow individual identification[27], and returned to individual vials. Twenty-four hours later, females in the 'mated' treatments were placed individually with one male, and a single copulation was observed. After each female had mated exactly once, males were discarded, and all females (both mated and virgins) were individually transferred to fresh vials, again containing standard media and no live yeast.

The following morning (5 days post-eclosion), females were individually placed in vials containing damp cotton wool and no food for 2 hours, after which pairs of females were simultaneously aspirated from these vials into the contest arena. The arena was a single well of a 12-well plate, containing an Eppendorf tube cap filled with standard fly food media and a ~2-µl drop of yeast paste, providing a limited resource to compete over[27]. Females were either both mated, both virgin, or (to test the relative competitive ability of mated versus virgin females) one of each. Females were allowed 5 minutes to acclimatize, and then behaviour was recorded for 30 mins using Toshiba Camileo X400 HD video cameras (short sample videos are available in Supplementary Information).

Behavioural analysis. Videos were scored blind with respect to treatment. Fighting behaviours (head-butt, leg 'fencing', shove, retreat[27]) and feeding behaviours were recorded using the program JWatcher + Video[64]; leg fencing was later excluded because of difficulties in accurately quantifying the behaviour. Specifically, we quantified the number and duration of aggressive encounters, the identity of females initiating each encounter, and the outcome of each encounter (win, lose, draw). An encounter began when one female head-butted or shoved the other female and continued until the females separated or stopped interacting (for example, when they were still within touching distance but both resumed eating). We used total duration of aggressive encounters as our primary response variable, which was measured as the sum of time spent in all encounters. We took total duration to be the best indicator of overall aggression as it took into account both the number of encounters and the time spent in encounters, providing a more accurate indicator of aggression than number of encounters alone. We also quantified the time that females spent in a feeding posture: that is, the time for which females were standing on the food cap with their heads tilted down towards the food in a position consistent with feeding.

Statistical analysis. The response variables (duration of aggressive encounters, and time spent in feeding posture) were continuous and most closely fitted a gamma distribution. Thus, we used GLMs with a gamma error distribution to test the effects of mating status, egg production status and various ejaculate components on total aggressive contest duration and time spent in feeding posture. The models in R followed the format below, with the model from the *ovo*^{D1} experiment given as an example:

$$\text{glm(Fight duration} + 1 \sim \text{Mating status} * \text{Egg production, family} = \text{Gamma(link} = \text{"log"))}$$

Fight duration + 1 was the response variable measured in seconds, and mating status and egg production were the explanatory variables. The explanatory variables differed depending on which experiment was being analysed.

For feeding posture analyses, we additionally included proportion of encounters won as an explanatory variable. The models then followed this format:

$$\text{glm(Feeding duration} + 1 \sim \text{Treatment} * \text{Proportion of encounters won, family} = \text{Gamma(link} = \text{"log"))}$$

Because gamma error distributions use a logistic function, we added 1 to all scores of total contest duration before transformation to include replicates with scores of 0. We tested for outliers using the Grubbs outlier test and excluded outliers below a threshold of $P = 0.001$. We excluded one outlier in the egg production experiment in the control mated treatment (Grubbs $G = 6.15$, $P < 0.0001$). Winsorizing the data did not qualitatively alter the results (in the winsorized analysis, mating status had a significant effect on fighting duration ($Dev_{1,127} = 13.413$, $P < 0.001$), egg production capability was marginally non-significant ($Dev_{1,126} = 1.364$, $P = 0.069$), and there was no interaction between egg production capability and mating status ($Dev_{1,125} = 0.124$, $P = 0.583$). We excluded the outlier in our final analysis even though the results did not qualitatively change, as it seemed that even when winsorized, the one outlier was still exerting undue leverage on the results. To allow for non-independence of individuals, we used each dyad as the unit of replication in the GLMs, rather than individual flies. For treatments with one mated and one virgin female, we randomly chose one focal individual from each dyad and analysed the proportion of encounters won using a GLM with a quasi-binomial distribution. For multiple comparisons within experiments, we used Tukey tests. All models were run in the R environment[65] version 3.0.1.

Data availability. Data will be made publicly available on the Oxford University Research Archive (ORA: https://ora.ox.ac.uk).

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Author contributions

E.B., S.W. and N.S conceived the project. E.B. and S.W. designed the experiments, with S.F.G. providing additional advice on experimental design for later experiments. E.B., S.B., C.P. and A.R. performed the behavioural experiments and scored the behavioural data. E.B. analysed the data and wrote the manuscript. S.W., S.F.G., N.S., E.E.-C. and J.A.T. discussed the results and contributed to the manuscript.

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