Report

Current Biology

Juvenile Hormone Suppresses Resistance to Infection in Mated Female *Drosophila melanogaster*

Highlights

- Male Sex Peptide activates female JH synthesis and reduces resistance to infection
- Removal of the corpus allatum suppresses the post-mating reduction in resistance
- JH inhibits resistance through the receptor *germ cell-expressed* (*gce*)
- Hormonal pleiotropy could mediate the evolution of life history traits

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In Brief

Schwenke and Lazzaro identify the hormonal basis for post-mating immunosuppression in *Drosophila melanogaster* females. They find that transfer of Sex Peptide (Acp70a) during mating activates juvenile hormone (JH) synthesis, which suppresses resistance to bacterial infection through the receptor Germ Cell Expressed (GCE).

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Juvenile Hormone Suppresses Resistance to Infection in Mated Female *Drosophila melanogaster*

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SUMMARY

Hormonal signaling provides metazoans with the ability to regulate development, growth, metabolism, immune defense, and reproduction in response to internal and external stimuli. The use of hormones as central regulators of physiology makes them prime candidates for mediating allocation of resources to competing biological functions (i.e., hormonal pleiotropy) [1]. In animals, reproductive effort often results in weaker immune responses (e.g., [2–4]), and this reduction is sometimes linked to hormone signaling (see [5–7]). In the fruit fly, *Drosophila melanogaster*, mating and the receipt of male seminal fluid proteins results in reduced resistance to a systemic bacterial infection [8, 9]. Here, we evaluate whether the immunosuppressive effect of reproduction in female *D. melanogaster* is attributable to the endocrine signal juvenile hormone (JH), which promotes the development of oocytes and the synthesis and deposition of yolk protein [10, 11]. Previous work has implicated JH as immunosuppressive [12, 13], and the male seminal fluid protein Sex Peptide (SP) activates JH biosynthesis in female *D. melanogaster* after mating [14]. We find that transfer of SP activates synthesis of JH in the mated female, which in turn suppresses resistance to infection through the receptor *germ cell expressed* (*gce*). We find that mated females are more likely to die from infection, suffer higher pathogen burdens, and are less able to induce their immune responses. All of these deficiencies are rescued when JH signaling is blocked. We argue that hormonal signaling is important for regulating immune system activity and, more generally, for governing trade-offs between physiological processes.

RESULTS AND DISCUSSION

Under a model of antagonistic hormonal pleiotropy, reproduction and immunity are hypothesized to be interlinked by molecular cues that promote reproduction at the expense of immunity [1, 7]. Thus, we tested whether the endocrine signaling molecule juvenile hormone (JH) is responsible for post-mating immune suppression in females. We first tested whether application of a synthetic JH analog, methoprene, blocks immune system activation. Methoprene exposure (10^{-2} μg) suppressed the induction of antimicrobial peptides (AMPs) by unmated females after an inoculation with heat-killed Gram-negative bacteria, *Providencia rettgeri* (Tukey's honestly significant difference [HSD]; $p < 0.0001$), rendering them similar to untreated, mated females (Tukey's test; p = not significant [n.s.]; Figure 1A). Exposure to methoprene also significantly reduced the ability of virgin females to restrict the growth of live *P. rettgeri* (acetone versus methoprene across doses; t test; $t_{37} = 6.54$; $p < 0.0001$; Figure 1B) and survive the infection (log rank; $X^2_1 = 13.9$; $p < 0.0001$; Figure 1C). Application of methoprene to mated females also promoted fecundity, increasing the average number of eggs laid over the course of 5 days from 75.6 ± 24.3 to 95.4 ± 32.4 (t test; $t_{45} = 2.35$; $p = 0.0230$), again consistent with the general understanding of hormonal control of reproduction in *D. melanogaster* [11]. We conclude that JH facilitates reproduction but is immunosuppressive and that its application can phenocopy the immunosuppression observed in mated females (Figure 1A).

Next, we tested whether the immunosuppressive effects of JH stem from the receipt of the male seminal fluid protein Sex Peptide (SP). SP drives a large number of physiological changes in mated *D. melanogaster* females [15] and is important in post-mating immunosuppression [8]. We used mRNA expression levels of *JH acid methyltransferase* (*jhamt*), which encodes a key regulatory enzyme in the JH biosynthesis cascade [16], as an indirect indicator of JH activation. *jhamt* expression has been previously established as a proxy for JH titers in *D. melanogaster* (e.g., [17]). We evaluated *jhamt* expression levels in females mated to wild-type males (SP^{WT}), males lacking SP entirely (SP^{null}), or males lacking the N terminus of SP that has been previously shown to promote JH synthesis ex vivo ($SP^{\Delta 2–7}$) [14, 18]. We found that females mated to SP^{WT} males expressed significantly higher levels of *jhamt* than females mated to SP^{null} or $SP^{\Delta 2–7}$ males (Tukey's HSD; wild-type [WT] null, $p = 0.00977$; WT-Δ2–7, $p = 0.0158$; Figure 2A). Thus, we conclude that transfer and receipt of the N terminus of SP is required for JH production in mated females.

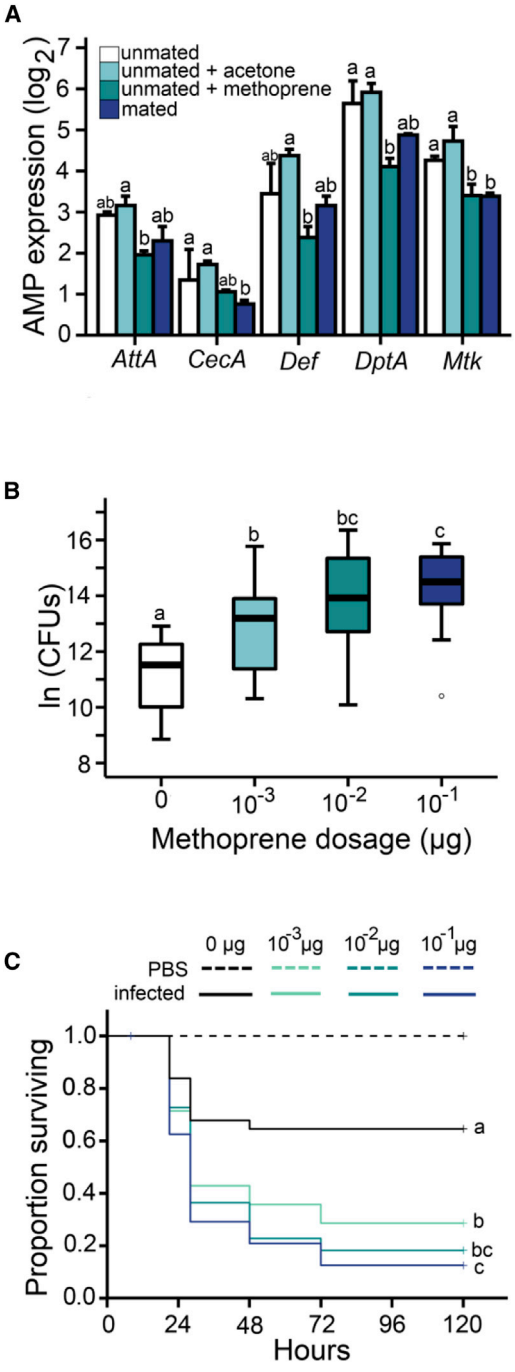


Figure 1. Juvenile Hormone Is Immunosuppressive

(A) mRNA expression of antimicrobial peptide genes 8 hr after an injection with heat-killed *P. rettgeri* relative to CO_2 controls. Unmated females were exposed to methoprene, acetone, or CO_2, and mated females were exposed to CO_2 (repeated-measures ANOVA; $p < 0.0001$; treatment group: $F_{3,52} = 27.24$; $p < 0.0001$). Tukey's HSD was performed within each gene. Means with the same letter are not significantly different ($p > 0.05$), and error bars represent SEM.

(B) Bacterial load of individual unmated females that received acetone or methoprene (ANOVA; treatment: $F_{3,\ 72} = 13.92$; $p < 0.0001$). Means with the same letter are not significantly different ($p > 0.05$), and error bars represent one SEM. None of the sterile wound treatments (dashed lines) experienced mortality events. n = 19 ± 1; two replicates.

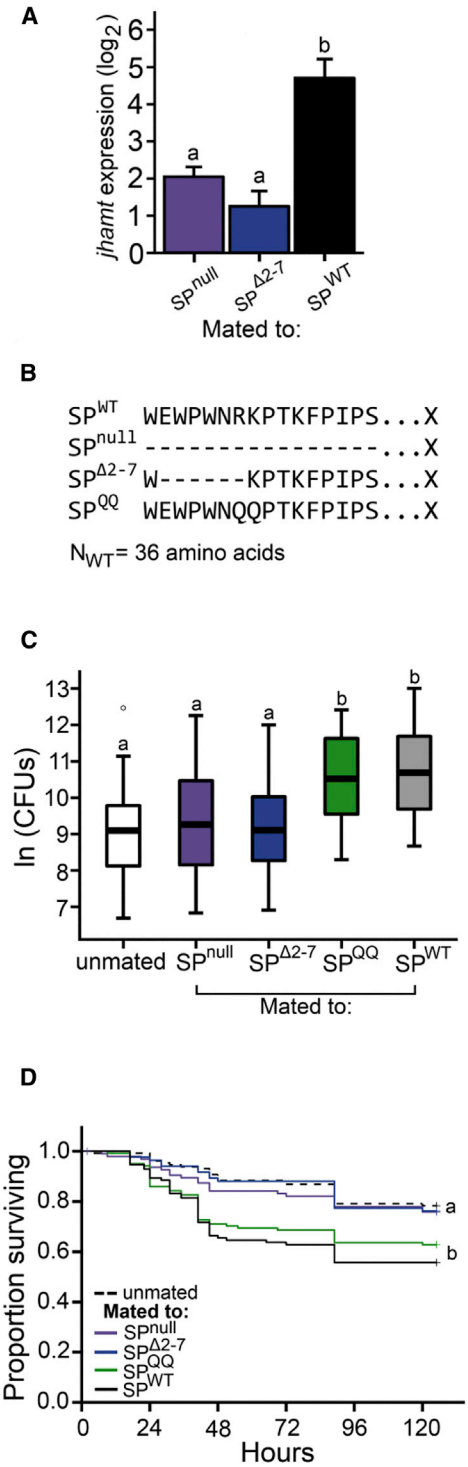
(C) Survivorship of unmated females subsequent to methoprene exposure and injection with sterile medium (PBS, dotted lines) or *P. rettgeri* (solid lines; Cox; chemical [infected only]: $X^2_3 = 35.98$; $p < 0.0001$). n = 50 ± 6; two replicates.

To test whether the inferred induction of JH leads to female immunosuppression, we mated females to several male genotypes expressing variant versions of SP (Figure 2B). SP is transferred to the female in the seminal fluid and binds to sperm tails by the N terminus after reaching the female reproductive tract [19]. Bound SP is cleaved from sperm tails at a trypsin cleavage site, providing females with a continued source of the C terminus [19]. Males with SP mutated at the trypsin cleavage site (SP^{QQ}) provide an intact N terminus during mating but deprive females of long-term access to the C terminus. Females mated to SP^{null} or $SP^{\Delta 2-7}$ males exhibited virgin levels of bacterial load and survivorship after mating. However, females mated to SP^{QQ} and SP^{WT} males exhibited significantly higher bacterial load and lower survivorship than virgins (Figures 2C and 2D). Therefore, the immunosuppressive effect of SP can be specifically attributed to the N terminus, which promotes JH synthesis.

To further substantiate the role of JH in post-mating immunosuppression, we tested whether blocking JH synthesis or receptor binding within the female would prevent the reduction in immunity after mating. JH is synthesized in the corpus allatum (CA). We used an inducible driver to overexpress either Diphtheria toxin (*DTI*) [20] or *NIPP1* [21] in the CA, partially ablating the tissue in late-stage pupae to avoid any early developmental defects caused by JH removal [22]. We found that females whose CA were partially ablated exhibited bacterial loads and infection survivorship that were no different than those of virgins (Figures 3A–3D). Thus, reduction of the CA was sufficient to prevent post-mating immunosuppression.

Because the CA was not fully ablated in our experiments, we performed a separate validation experiment to confirm that the observed partial ablation resulted in lower levels of JH activation after mating. First, we measured the expression of *jhamt* and two downstream targets of JH (*mnd* and *JHI-21*) [23] 10 hr after mating. Expression levels for all three genes were significantly reduced by 50% or more in both CA-ablation genotypes relative to their controls (t tests: $t_4 = 5.96-15.9$; $p < 0.01$). This is consistent with previous work showing that CA-ablated^{NIPP1} females have significant reductions in JH titer [21]. Additionally, CA-ablated^{DTI} and CA-ablated^{NIPP1} laid significantly fewer eggs (33.3 ± 29.5 and 51.6 ± 35.0) than control genotypes (130.9 ± 53.0 and 167.5 ± 82.5; Tukey's HSD comparisons; $p < 0.05$), also in concordance with prior findings [21]. Based on the full set of data, we conclude that CA-ablated females are deficient in JH synthesis and therefore exhibit reduced fecundity and virgin levels of resistance to bacterial infection.

Finally, we sought to identify the receptor through which JH suppresses immunity in reproductively active females. Two recently duplicated paralogs are thought to be responsible for mediating the JH signal during development [24, 25]. Whereas *Methoprene tolerant* (*Met*) and *germ cell expressed* (*gce*) are partially redundant during development, it is unknown whether either or both of these receptors are required in post-mating immune suppression in adult females. We ubiquitously expressed



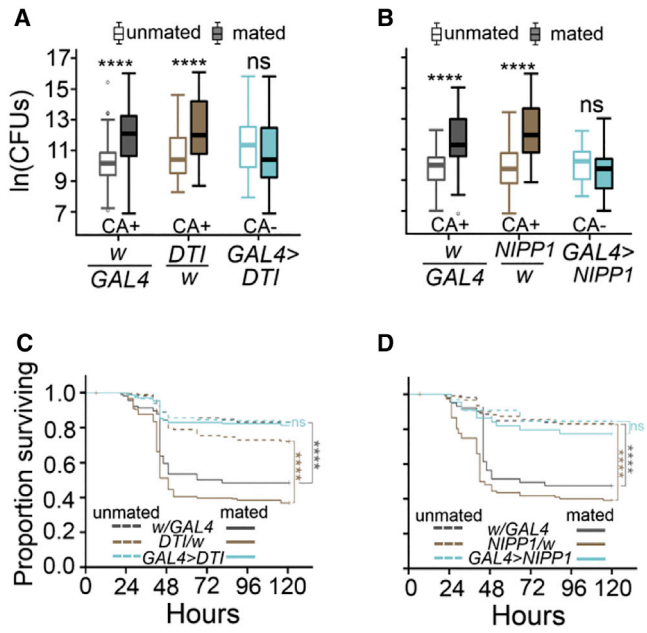


Figure 3. Genetic Ablation of JH Biosynthesis Rescues Virgin Levels of Resistance

(A and B) Bacterial load (colony-forming units, CFU) of individual CA+ and CA-ablated females subsequent to mating and infection. Mean bacterial load of unmated and mated females within a genotype were compared with a Wilcoxon test. (A) *DTI*; n = 60 ± 10; four replicates. (B) *NIPP1*; n = 30 ± 5; three replicates. Error bars represent one SEM.

(C and D) Survivorship of CA+ and CA− females subsequent to mating and infection. Survivorship was compared within a genotype using a log rank test. (C) *DTI*; n > 120; five replicates. (D) *NIPP1*; n = 48 ± 15; three replicates.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Figure 2. Transgenic Males Lacking the N Terminus of Sex Peptide Do Not Elicit Immunosuppression in Recipient Females

(A) *jhamt* mRNA expression in females 10 hr after mating to SP genotypes relative to unmated females (ANOVA; status: $F_{2,\ 6} = 24.87$; p = 0.00124). Bars represent the mean ± SEM. Means with the same letter are not significantly different (p > 0.05); three replicates.

(B) Amino acid sequences of Sex Peptide; SP^{QQ}: the R_7K_8 trypsin cleavage site has been changed to Q_7Q_8; $SP^{\Delta 2-7}$: N-terminal amino acids (E_2-R_7) deleted; SP^{WT}: wild-type.

an RNAi knockdown construct targeted against each gene in adult females. The respective knockdowns resulted in a 62% reduction in *gce* expression relative to the control genotype ($t_4 = 6.27$; p = 0.00330) and a 73.4% reduction in *Met* expression ($t_4 = 4.21$; p = 0.0136) relative to the control genotype as measured by qRT-PCR.

RNAi knockdown of *gce* significantly improved resistance to infection and eliminated post-mating immunosuppression (Figures 4B and 4D), whereas knockdown of *Met* had no effect on immune defense (Figures 4A and 4C). Specifically, bacterial loads within mated versus unmated females were not significantly different in the absence of *gce* (Wilcoxon; W = 494; p = n.s.; Figure 4B). In contrast, *Met* knockdown females continued to suffer from significantly higher bacterial loads as a consequence of mating (Wilcoxon; W = 610.5; p = 0.00796; Figure 4A). RNAi knockdown of *gce* improved female

(C) Bacterial load of individual females that had been infected with *P. rettgeri* subsequent to mating with males of different SP genotypes (ANOVA; mating status: $F_{4,\ 189} = 13.91$; p < 0.0001). Means with the same letter are not significantly different (p > 0.05), and error bars represent one SEM. n = 40 ± 6; three replicates.

(D) Infection survivorship subsequent to mating with males of different SP genotypes (Cox; mating status: $X^2_4 = 23.37$; p = 0.00011). Letters indicate levels of significance. n = 110 ± 15; three replicates.

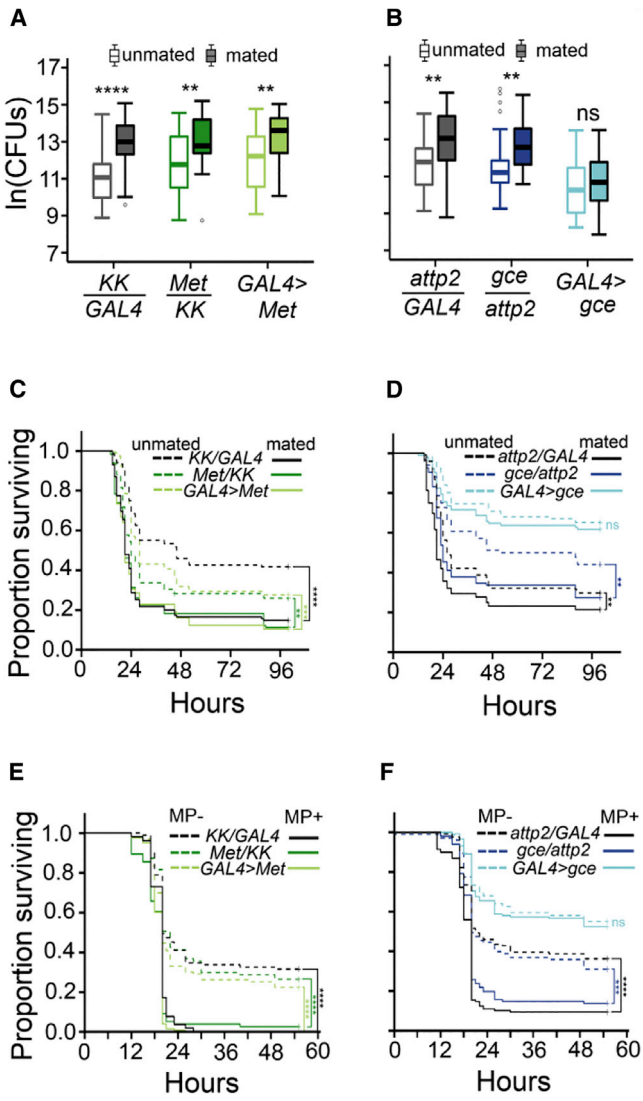


Figure 4. RNAi-Mediated Knockdown of *gce*, a JH Receptor, Mediates the Effect of JH on Immunity

(A and B) Bacterial load (CFU) within *Met*-RNAi or *gce*-RNAi females and their controls, respectively. Mean bacterial load of unmated and mated females within a genotype were compared with a Wilcoxon test. Error bars represent one SEM. n = 29 ± 2; three replicates.

(C and D) Infection survivorship of *Met*-RNAi or *gce*-RNAi females and their controls, respectively. Survivorship was compared within a genotype using a log rank test. n = 105 ± 15; four replicates.

(E and F) Infection survivorship of unmated *Met*-RNAi or *gce*-RNAi females exposed to methoprene (MP+) or acetone (MP−). Survivorship was compared within a genotype using a log rank test. In the absence of infection, methoprene did not impact survivorship. (E) *Met*; n = 80 ± 15; three replicates. (F) *gce*; n = 120 ± 20; three replicates.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

survivorship after mating and infection, with mated and unmated females experiencing similar rates of mortality (log rank; $X^2_1 = 0.5$; p = n.s.; Figure 4D). On the contrary, *Met* knockdown females remained immunologically sensitive to mating and experienced higher levels of infection-induced mortality as a consequence of mating (log rank; $X^2_1 = 23.7$; p < 0.0001; Figure 4C). Interestingly, a reduction in *gce* expression significantly improved survivorship relative to background controls as well (Tukey's HSD; p < 0.05), suggesting that even basal levels of JH in unmated females may negatively influence immune defense. We predicted that if *gce* expression mediates resistance to infection via JH signaling, then *gce* knockdown females should be resistant to the immunological effects of methoprene. We tested this and found that methoprene application increased infection-induced mortality in all genotypes except for *gce*-RNAi (log rank; $X^2_1 = 11.3–37.6$; p < 0.0001; Figures 4E and 4F). Thus, we conclude that GCE is the receptor that mediates the post-mating reduction in resistance driven by JH and SP, and we have solidified a role for JH as a central mediator of the physiological trade-off between reproduction and immunity in *D. melanogaster*.

Our finding that *gce* alone regulates post-mating immune suppression highlights the intricate nature of the molecular action of JH. Whereas MET and GCE have apparent redundancies [24], new evidence posits a divergence in the functionality of the two basic-helix-loop-helix with PER-ARNT-SIM homology domain (bHLH-PAS) transcription factors [26–28]. For example, Reiff et al. [26] demonstrated that the effect of JH on enterocyte growth and concomitant increases in reproduction are mediated largely by GCE. It is worth noting that, whereas the duplication of the JH receptor is specific to Dipterans, *gce* is the ancestral gene [28, 29], suggesting that JH-mediated immunosuppression might occur via a similar mechanism in other taxa.

Why has JH evolved an immunosuppressive function, and is post-mating immunosuppression adaptive? Under the immunopathology-avoidance hypothesis [30], the risk of damage from autoimmunity is potentially greater than the risk associated with immune system functionality (i.e., being immunocompromised). If immune activation disrupts reproductive tissues and output [31, 32], such processes would be strongly selected against due to their fitness consequences. Under this hypothesis, JH may act to suppress immune signaling to prevent instances of autoimmunity, especially in cases where reproductive tissues may be targeted. Thus, immunosuppression could occur to support reproductive output.

A perhaps more likely explanation is that the trade-off stems from a simple competition for resources. Both immune function and reproduction are resource intensive [33–35]. Under the resource limitation hypothesis [30], JH may operate as the molecular cue for the investment in reproduction rather than immunity. Whereas evidence for reallocation of a specific nutrient to antibacterial immunity has not been demonstrated in *Drosophila*, protein and specific amino acids strongly influence both reproduction and immunity in insects (e.g., [36, 37]). Recently, JH was shown to increase reproductive output through enhanced lipid metabolism, with sterile females storing more triacylglycerides [26]. Sterile females have also been shown to be resistant to the effects of mating on immunity [8]. The fat body is a tissue that drives systemic immunity, regulates central metabolism and allocation to egg provisioning, and stores lipid [38] and thus may be the organ regulating the trade-off. Whether this trade-off operates as a simple function of resource availability or whether there is a more direct antagonism remains to be conclusively demonstrated. Altogether, our work demonstrates an

unambiguous role for JH in suppressing immunity in mated females, thus providing a mechanism for a classic life history trade-off and supporting the hormonal theory of pleiotropy.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.01.004.

AUTHOR CONTRIBUTIONS

R.A.S. conducted the research; R.A.S. and B.P.L. designed the experiments and wrote the manuscript.

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Supplemental Experimental Procedures

Fly stocks and crosses

Canton-S (CS) flies (Bloomington #1) were used as a generic wild-type genotype for all experiments unless otherwise specified. SP^{null} males were created by crossing $SP^{\Delta 130}/TM3$, *Sb ry* and $SP^{0}/TM3$, *Sb ry* [S1]. Males lacking the N-terminus of SP ($SP^{\Delta 2\text{-}7}$) were generated by crossing *w*; SP-$TG^{\Delta 2\text{-}7}$/SP-$TG^{\Delta 2\text{-}7}$; $SP^{\Delta 130}/TM3$, *Sb ry* and $SP^{0}/TM3$, *Sb ry* stocks [S2]. Similarly, SP trypsin cleavage mutants (SP^{QQ}) were obtained by crossing *w*; SP-TG^{QQ}/SP-TG^{QQ}; $SP^{\Delta 130}/TM3$, *Sb ry* and $SP^{0}/TM3$, *Sb ry* [S2]. To genetically ablate the corpus allatum (CA), ablation construct lines, *w; GAL80^{ts}*; *UAS-DTI* [S3] or *w; UAS-NIPP1/TM3* [S4], were crossed to the *Aug21-GAL4*, *UAS-EGFP*/*CyO* driver at 18°C; control genotypes were produced by crossing construct and driver lines to w^{1118}. Vials with white pupae were shifted to 29°C to activate tissue ablation and unmated females were housed at 29°C for 5 days and then shifted to 25°C for the remainder of the experiment. For receptor knockdowns, the *gce*-RNAi line (y^{1} v^{1}; P{TRiP.JF02097}attP2) (Bloomington #26323) and the *Met*-RNAi line (P{KK104562}VIE-260B) (VDRC #10638) were crossed to *GAL80^{ts}*; *da-GAL4*, *Dpt-lacZ* females at 18°C; control genotypes were produced by crossing RNAi construct and driver lines to the attP2 background (Bloomington #36303) for *gce*-RNAi or to the KK background (VDRC #60100) for *Met*-RNAi. White pupae were shifted to 29°C and remained there throughout the entire experiment.

Fly husbandry

All flies were reared on a glucose medium (8.3% glucose, 8.3% Brewer's yeast, 1% agar, 0.04% phosphoric acid and 0.4% propionic acid). Except for the CA ablation and receptor RNAi crosses, all flies were housed continuously at 25°C.

Mating procedure

Females were collected as virgins and housed in cohort sizes of 10–15 individuals. Virgin males were housed in groups of 30 individuals. Five days after eclosion (9 days in the RNAi experiments), females were transferred to new food vials and males were added or transferred and left unmated. Vials were visually inspected to ensure copulation was occurring; within 5-30 minutes, all females were typically paired with males. Males were removed after 4–5 hours when injections began.

Infection procedure

Providencia rettgeri, a Gram-negative bacterium isolated from wild-caught *D. melanogaster* [S5], was used for all infection experiments. Cultures were grown for 20 ± 2 hours in Luria Broth (LB) in a shaking incubator at 37°C. Cultures were spun down at 3000 RPM at 4°C and resuspended in sterile PBS. Cultures were diluted to $OD_{600} = 0.05$ with PBS, which corresponds to 1000 (±200) viable cells per individual. CO_2-anesthetized females received an injection of 9.3 nl of media in the thorax 3–4 hours after mating (or as virgins) using a pulled capillary needle mounted on a Nanoject II apparatus (Drummond Scientific). After injection, flies were placed into new food vials in a cohort size of 10–15 per vial.

Survival Assays

Survival was recorded daily for 5 days. PBS-injection rarely resulted in mortality (<1%). Females that did not recover from the injection within 8 hours were censored from the dataset as their death was due to handling rather than infection. Flies that did not die during the course of the experiment were censored (i.e. right-censored data). The effect of mating or hormonal application was assessed using a Cox proportional hazard model in R [S6].

Bacterial load

To assay bacterial load 20 hours after infection, single flies were homogenized in 500 ul of sterile PBS using a linear motion homogenizer (OPS Diagnostics). A 1:100 dilution of the samples was performed and 50 ul of the homogenates were plated on LB agar plates using a WASP2 spiral plater (Microbiology International). Plates were incubated overnight at 37°C and colonies were counted using a ProtoCOL plate counting system (Microbiology

International). Control flies were injected with sterile PBS, and the plates from these individuals never yielded any colonies. We modeled the effect of mating to transgenic Sex Peptide males on bacterial load as:

$\log_e(\text{CFU per female}) = \text{MatingStatus}_{fixed} + \text{ExperimentalReplicate}_{random}$.

The effect of mating was compared within a genotype for CA-ablation and RNAi knockdown experiments using Chi-square tests.

Hormonal treatment

Methoprene (Sigma-Aldrich) was suspended in acetone and ectopically applied using a pulled glass capillary mounted on a Nanoject II apparatus. Females were anesthetized on CO_2 and received 50.6nl of the carrier or the methoprene suspension to their ventral, abdominal cuticle. All females were unmated except in the egg laying assay, in which females were had been mated recently before receiving a dose of methoprene. Dosages ranged from 10^{-1} to 10^{-3} µg and are based on prior studies [S7, S8]. We selected 10^{-2} µg as a mid-range dosage to test subsequent phenotypes.

RT-qPCR

Total RNA was isolated using a TRIZOL (Invitrogen)-chloroform extraction protocol and was resuspended in RNAase-free water. Purity was verified and RNA amounts were quantified using a NanoDrop 2000 (Thermo Scientific) spectrophotometer. Approximately 1000 ng of nucleic acid from each sample was treated with DNase (Promega) and M-MLV reverse transcriptase (Promega). We used SsoAdvanced SYBR Green Supermix (Bio-Rad), and RT-qPCR reactions were performed using the CFX Connect Real-Time Detection System (Bio-Rad). All amplifications were performed with in a two-step amplification cycle that consisted of a 5 second denaturation at 95ºC and a 30 second annealing at 58ºC. Primer sequences, amplification efficiencies and amplicon lengths are given in the "RT-qPCR Primer Sequences" section below. Genes of interest were compared to the reference gene *RpL32* (also known as *rp49*) which is highly reliable even across hormonal treatment [S9, S10] and has been shown to exhibit constant expression after mating and infection [S11]. We saw no significant variation in RpL32 expression across treatments conditions in the present experiment. Gene expression analysis was performed using the ΔΔCt method [S12]. Each RT-qPCR experiment consisted of three biological replicates and two technical replicates.

To test whether mating and hormonal application negatively impacted the induction of antimicrobial peptides, we measured *Attacin A, Cecropin A, Defensin, Diptericin A,* and *Metchnikowin* induction in Canton-S females. Unmated females received the hormone treatment (or acetone-carrier) and heat-killed bacteria ($OD_{600} = 1.0 \pm 0.2$), and were compared to mated and unmated injected females (no acetone or methoprene application) 8 hours after injection with heat-killed bacteria (i.e. 10 hours after hormonal treatment or mating). Three pools of 15 females were frozen and stored at -80°C until processing. Induction of AMPs was determined relative to CO_2-exposed flies.

As a proxy for JH activity, we measured *jhamt, mnd*, and *JHI-21* expression levels 10 hours after mating relative to unmated females. Three pools of 15 females were frozen and stored at -80°C until processing. To validate the knockdown efficiency of the constructs, unmated *da-GAL4>gce* and *da-GAL4>Met* females were collected and housed at 29°C for 9 days. Gene expression in knockdown females was compared to background expression levels in *da-GAL4*/+ females. Three pools of 20 females were collected and stored at -80°C until processing.

RT-qPCR Primer Sequences (5' - 3') and DRSC FlyPrimerBank [S13] Identifiers

Target Gene: *RpL32*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 177 bp
rp49F	AGGCCCAAGATCGTGAAGAA	rp49R	GACGCACTCTGTTGTCGATACC
Target Gene: *gce*	DRSC FlyPrimerBank Identifier: PP31774		Amplicon length: 158 bp
gce-F3	AGCTGCGTATCCTGGACACT	gce-R3	TCGAGAGCTGAAACATCTCCAT
Target Gene: *Met*	DRSC FlyPrimerBank Identifier: PP33609		Amplicon length: 88 bp
Met-F2	GCCTCAAGGGAACGGGTATG	Met-R2	AGCAGTTGCATTAGAGTGTCC
Target Gene: *JHI-21*	DRSC FlyPrimerBank Identifier: PP16896		Amplicon length: 104 bp
JHI-21F	TCAAGCGGAAGCTAACACTCA	JHI-21R	TTCGGTGTAAATAAAGACTCCCG

Target Gene: *mnd*	DRSC FlyPrimerBank Identifier: PP36903		Amplicon length: 87 bp
mnd-F	CTCCGGCTCCATAGGACAATC	mnd-R	CCCAATTCGGCGTAGCATAGG
Target Gene: *Jhamt*	DRSC FlyPrimerBank Identifier: PP25470		Amplicon length: 77 bp
Jhamt-2F	CTCCCGATTCGTGGACAACTG	Jhamt-2R	CGCTGGTAATGCTTACTGGCA
Target Gene: *AttA*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 167 bp
AttA-F	CGTTTGGATCTGACCAAGG	AttA-R	AAAGTTCCGCCAGGTGTGAC
Target Gene: *CecA*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 188 bp
CecA-F	CTCTCATTCTGGCCATCACC	CecA-R	TGTTGAGCGATTCCCAGTC
Target Gene: *Def*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 66 bp
Def-F	GAGGATCATGTCCTGGTGCAT	Def-R	TCGCTTCTGGCGGCTATG
Target Gene: *DptA*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 54 bp
DptA-F	GCGGCGATGGTTTTGG	DptA-R	CGCTGGTCCACACCTTCTG
Target Gene: *Mtk*	DRSC FlyPrimerBank Identifier: n/a		Amplicon length: 111 bp
Mtk-F	AACTTAATCTTGGAGCGATTTTTCTG	Mtk-R	ACGGCCTCGTATCGAAAATG

Egg laying assays

Females were housed as virgins in groups of 10−15 individuals and provided with 30 CS males when 5 days old. Methoprene (or acetone) was applied to CS females within 5 hours of mating and females were placed singly into food vials. Eggs were counted daily for four days. Samples sizes were 24 and 23, respectively.

In CA ablation egg-laying experiments, females were housed as virgins and provided with 20 CS males. Males were removed and mated females remained together within a vial. Females were transferred to new vial every day for 4 days and eggs were counted daily. Egg counts represent the average number of eggs per female. Vials had 5−12 females and three independent replicates were collected; n = 3. Cohort sizes were similar across treatments and we did not detect a significant impact of number of co-housed females on egg output ($F_{1,13} = 2.37$, $p = 0.147$).

Statistical analyses

All analyses were performed in R [S6]. All sample sizes refer to the total number of individuals within each treatment across experimental replicates. Survivorship data was fitted to a Cox proportional hazard model and treatments were tested pairwise with a Log-rank test. Multiple comparisons were tested with Tukey's HSD. When bacterial load data violated assumptions of normality, a Mann-Whitney-Wilcoxon test was performed. In our analysis of AMP expression, we performed a Repeated-measures ANOVA because we used the same pools of individuals across the five AMPs. We declared 'Treatment group' as fixed effect and included an error term that had "Treatment group" nested within "Gene". Tukey's HSD was used to test for differences among experimental groups within a single gene.

Supplemental References

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