Drosophila melanogaster sex peptide regulates mated female midgut morphology and physiology

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Edited by David Denlinger, The Ohio State University, Columbus, OH, and approved November 10, 2020 (received for review September 2, 2020)

Drosophila melanogaster **females experience a large shift in energy homeostasis after mating to compensate for nutrient investment in egg production. To cope with this change in metabolism, mated females undergo widespread physiological and behavioral changes, including increased food intake and altered digestive processes. The mechanisms by which the female digestive system responds to mating remain poorly characterized. Here, we demonstrate that the seminal fluid protein Sex Peptide (SP) is a key modulator of female post-mating midgut growth and gene expression. SP is both necessary and sufficient to trigger post-mating midgut growth in females under normal nutrient conditions, and likely acting via its receptor, Sex Peptide Receptor (SPR). Moreover, SP is responsible for almost the totality of midgut transcriptomic changes following mating, including up-regulation of protein and lipid metabolism genes and down-regulation of carbohydrate metabolism genes. These changes in metabolism may help supply the female with the nutrients required to sustain egg production. Thus, we report a role for SP in altering female physiology to enhance reproductive output: Namely, SP triggers the switch from virgin to mated midgut state.**

Drosophila post-mating response | sex peptide | gut growth | digestive tract | transcriptome

Females invest large quantities of energy into reproduction. In the fruit fly, *Drosophila melanogaster*, female nutrition and egg production are tightly linked: Dietary protein is necessary for yolk protein synthesis (1, 2), and varying a female's protein intake can influence her fecundity (3–7). Furthermore, to support the metabolic demands of oogenesis, mated *Drosophila* females increase feeding and preferentially consume energy- and protein-rich diets (8–13). Given its essential role in digestion and nutrient absorption, the female midgut is an important modulator of changes in post-mating energy balance (14, 15). Signaling from the midgut to the ovaries and vice versa is essential for enhancing egg production after mating (16, 17).

The midgut is a regionalized structure divided into regions (Fig. 1*A*) that are morphologically and physiologically distinct (18, 19). The anterior midgut (red in Fig. 1*A*) primarily functions in digestion while the middle midgut (blue in Fig. 1*A*) functions similar to a stomach. The posterior midgut (magenta in Fig. 1*A*) is responsible for nutrient absorption (18). The midgut is an epithelial monolayer consisting of four cell types and sheathed by visceral muscles (VMs). Enterocytes (ECs), the most abundant cell-type making up the bulk of the midgut, absorb nutrients and secrete digestive enzymes. Secretory enteroendocrine cells (EEs) control peristalsis as well as excretory and digestive function through the release of neuropeptides (14). Intestinal stem cells (ISCs) renew ECs (20, 21) by differentiating into transient enteroblasts (EBs) whereas EEs are renewed through pre-EE progenitor cells (22).

The midgut is sexually dimorphic, with differences in physiology and gene expression (15, 23–25). In particular, female midgut ISCs express a unique branch of the female sex determination pathway, which influences female midgut reproductive plasticity (23). Moreover, virgin and mated female midguts are morphologically and physiologically distinct (15, 24). This mating responsiveness is essential for regulating female post-mating nutrition and egg production (15, 17). For instance, food travels more slowly through the guts of mated females, increasing time available for nutrient absorption and resulting in more concentrated excreta (24). Furthermore, genes involved in fatty acid metabolism are up-regulated in ECs after mating, which may promote fecundity (15). Through EE-derived Neuropeptide F release, the mated female midgut can stimulate gametogenesis, suggesting that the midgut plays a key role in the integration of physiological mating status and egg production (16).

Mating significantly increases female midgut size as a result of juvenile hormone (JHB3) and the ecdysteroid 20E (generated from ovarian-derived ecdysone) acting on ISCs to stimulate proliferation (15, 17). Despite the important connection between nutrition, gut physiology, and mating, little is known about how the midgut senses when mating has occurred and which processes are modulated to adjust midgut size and digestion to the demands of egg production.

During mating, males transfer sperm within a seminal fluid that contains a complex mixture of proteins and small molecules derived from male reproductive tract secretory tissues. Components of the male ejaculate trigger the switch between virgin and mated female states, which involves both behavioral and physiological changes (26), and occurs in two phases (27–29). Short-term post-mating responses occur during the first 24 h following mating, mediated by seminal fluid proteins (SFPs) and other nonsperm components of the ejaculate (26, 27). Thereafter, long-term responses last up to 2 wk after mating and require

Significance

We describe a role for the seminal fluid protein Sex Peptide (SP) in regulating the post-mating morphology and physiology of the female digestive tract. We show that females must receive SP to increase their midgut size following mating, a response that is important for female fecundity. Receiving SP is also important for post-mating changes in the expression of digestive enzyme and metabolic genes in the midgut. This is a significant step in our understanding of how female physiology shifts to cope with the metabolic demands of reproduction. Our study reveals another mechanism through which the male can alter female post-mating physiology to enhance reproductive output.

Author contributions: M.A.W., A.B., M.F.W., and N.B. designed research; M.A.W., A.B., and N.B. performed research; M.A.W., A.B., M.F.W., and N.B. analyzed data; and M.A.W., A.B., M.F.W., and N.B. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2018112118/-/DCSupplemental.

Published December 21, 2020.

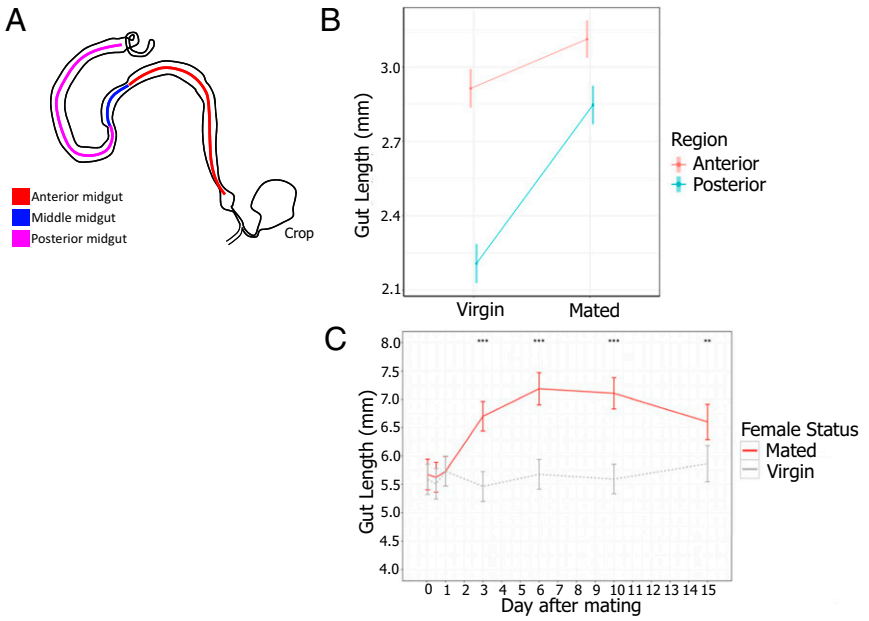

Fig. 1. Summary of the allometry and temporal dynamics of post-mating gut growth. (*A*) Schematic of the female midgut. The anterior midgut (containing regions 1 and 2, as defined in ref. 18), middle midgut (containing region 3, as defined in ref. 18), and posterior midgut (containing regions 4 and 5, as defined in ref. 18) are highlighted in red, blue, and magenta, respectively. (*B*) Interaction plot showing the effect of mating on posterior and anterior midgut length. Data are estimated marginal means ± a 95% CI from a linear mixed effects model (n_{virgin} = 104, n_{mated} = 99). (*C*) Time course of female midgut length after mating. Data are the estimated marginal means ± a 95% CI from a linear mixed effects model. Error bars represent a 95% CI. Midgut length of age-matched virgin and mated was measured at 7 post-mating time points: 1 h (n_{virgin} = 48, n_{mated} = 44), 12 h (n_{virgin} = 49, n_{mated} = 61), 1 d (n_{virgin} = 49, n_{mated} = 61), 3 d (n_{virgin} = 48, n_{mated} = 55), 6 d (n_{virgin} = 55, n_{mated} = 30), 10 d (n_{virgin} = 59, n_{mated} = 36), 15 d (n_{virgin} = 20, n_{mated} = 22). Data are derived from at least two biological replicates. ***$P < 0.0001$, **$P < 0.001$.

stored sperm (27, 29–31). The SFP Sex Peptide (SP) mediates both short-term and long-term post-mating responses (28, 29, 31). Once transferred to the female, a network of SFPs is required to bind SP to sperm via its N terminus within the female seminal receptacle (29, 32–34). To maintain long-term post-mating responses, the C-terminal domain of SP is continually cleaved from sperm (29). This C-terminal domain binds the SP receptor (SPR) (35) and potentially other unknown receptors (36) on reproductive tract sensory neurons expressing *fruitless*, *pickpocket,* and *doublesex*, a subset of which are also octopaminergic (35, 37–40).

SP is known to initiate post-mating responses that enhance nutrition and fecundity, such as increased egg production, increased food intake, and changes in food preference (8–11, 35, 41, 42). In the midgut, SP, through SPR neurons, has previously been linked to increases in intestinal transit time and is known to stimulate Neuropeptide F release from EEs (16, 24, 43). Unbound SP can enter the hemolymph where its N-terminal domain stimulates release of JHB3 from the corpora allata (44–48). Since JHB3 is required for post-mating gut growth (15), it is possible that SP may indirectly trigger midgut enlargement through JHB3 signaling. SP could also initiate gut growth via SPR neuronal signaling and 20E. Both SP and neuronal SPR are needed for post-mating increases in ovarian 20E levels (49), and ovarian 20E is required for post-mating gut growth (17).

In this study, we characterize the role of SP in modulating both mated female midgut size and physiology. First, we demonstrate that, after mating, the posterior midgut grows more relative to the anterior region. Post-mating midgut growth occurs over the course of 6 d and persists at least 2 wk, a time frame consistent with long-term post-mating responses. We show that SP is both necessary and sufficient for post-mating midgut growth. Furthermore, long-term storage and release of SP from sperm is required for SP's effect on female midgut size, and SP's effect requires its receptor SPR. Finally, through whole-midgut transcriptome analysis, we show that mating triggers a change in midgut expression of digestive and metabolic enzymes, which is almost completely dependent on receipt of SP. Altogether, this study identifies a role for SP in the female post-mating response and advances our understanding of the mechanisms by which females address the nutritional demands of reproduction.

Results

The Midgut Grows Allometrically after Mating. To establish the scope of post-mating midgut growth, we examined regional changes in midgut size at 3 d after mating, a time point at which a mating effect on gut size had been previously reported (15). In addition to previously described (15) post-mating increases in total midgut length ($P < 0.0001$) (*SI Appendix*, Fig. S1*B*) and in posterior midgut width ($P < 0.0001$) (*SI Appendix*, Fig. S1*C*), we detected significant post-mating increases in anterior midgut width ($P < 0.0001$) (*SI Appendix*, Fig. S1*A*). Intriguingly, post-mating midgut length increases more in the posterior region than the anterior region (Fig. 1*B* and *SI Appendix*, Table S1). This demonstrates that mating triggers allometric growth of the midgut, with a greater relative length increase in the absorptive posterior region.

The midgut can grow and shrink in response to stimuli (50). This plasticity in size led us to ask how long the midgut remains enlarged after a single mating. We compared mated female gut lengths to those of virgins at seven time points after mating ranging from 1 h to 15 d. We observed a progressive increase in midgut length, which became significantly different from the virgin control after 3 d ($P < 0.0001$) (Fig. 1*C*). Midgut length continued to increase until 6 d after a single mating, plateaued between day 6 and day 10, and began to decrease. However, the midgut length 15 d post-mating remained significantly longer than that of virgin controls ($P < 0.001$) (Fig. 1*C*).

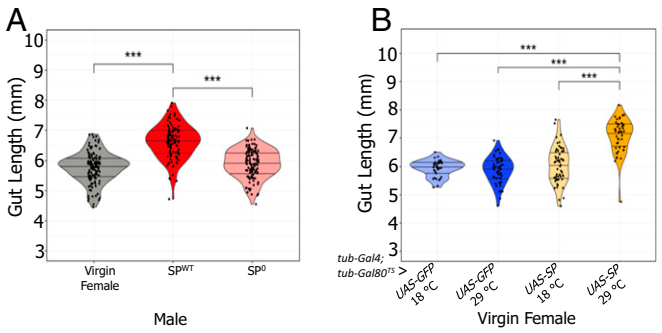

Fig. 2. SP is both necessary and sufficient for increasing post-mating midgut length under normal food conditions. (*A*) Midgut lengths of females mated to SP^0 males 3 d after mating ($n = 118$; pink) are no different in length from those of virgin females ($n = 127$; gray). Midguts of SP^{WT} mated females ($n = 100$; red) are significantly longer than those of both virgins ($P < 0.0001$) and SP^0 mated females ($P < 0.0001$). Data are derived from four biological replicates and analyzed with a linear mixed model. (*B*) Gut length of virgin females ectopically expressing SP (*tub-Gal4; tub-Gal80^{ts} > UAS-SP* 29 °C, $n = 44$, dark yellow) for 6 d compared to noninduced controls (*tub-Gal4; tub-Gal80^{ts} > UAS-SP* 18 °C, $n = 55$, light yellow) and GFP controls (*tub-Gal4; tub-Gal80^{ts} > UAS-GFP* 29 °C, $n = 53$, dark blue; *tub-Gal4; tub-Gal80^{ts} > UAS-GFP* 18 °C, $n = 35$, light blue). Data are combined from three biological replicates and analyzed using a linear mixed model. ***$P < 0.0001$.

SP Is Responsible for Post-Mating Midgut Growth. SP's stimulatory effect on egg production is known to persist for up to a week after mating (28), and midgut growth occurs along a similar timescale (Fig. 1*C*). Therefore, SP could be a key signal controlling gut resizing in response to mating. To test this hypothesis, we mated Canton S (CS) virgin females to either males unable to produce SP (SP^0) or control sibling males producing wild-type (WT) SP (SP^{WT}) (28). Receptivity assays confirmed that SP^0 males failed to stimulate female post-mating responses, affirming the integrity of our SP^0 lines (*SI Appendix*, Fig. S5*A*). As expected, the midgut lengths of females mated to SP^{WT} males were significantly longer than those of virgin females ($P < 0.0001$) while the midgut lengths of females mated to SP^0 males were no different from those of virgin females (Fig. 2*A*). This indicates that SP is required for mating-induced midgut growth.

We next investigated whether the SP signal alone is sufficient to induce changes in midgut length by ectopically expressing full-length SP in virgin females under the control of a temperature-sensitive driver (*tub-Gal4; tub-Gal80^{ts}*) (*SI Appendix*, Fig. S5*B*). After 6 d of ectopic SP expression, adult virgin females displayed significant lengthening of the midgut relative to virgin females of the same genotype kept at a nonpermissive temperature or virgin females ectopically expressing GFP ($P < 0.0001$ and $P < 0.0001$) (Fig. 2*B*). These changes are due to SP, and not differences in temperature because there was no difference in the midgut lengths of *UAS-GFP* flies at 18 °C and 29 °C (Fig. 2*B*).

Long-Term Storage and Release of SP Is Required for Midgut Growth, as Is SPR. SP is stored bound to sperm within the female reproductive tract for up to 10 d post-mating (29). Gradual release of SP's active C-terminal region by its cleavage from sperm allows SP effects to persist, creating a long-term post-mating response (LTR). Thus, the extended period of midgut growth observed after mating could be due either to short-term exposure to SP initiating a cell-autonomous growth cycle, or to continuous exposure to SP, as part of its LTR. Consequently, we examined mutations that perturb the ability of SP to bind to or be released from sperm. Release of the active C-terminal part of SP by cleavage from sperm was prevented by an SP with an inactivated cleavage site (SP-TG^{QQ}) (29). Likewise, sperm binding (and thus persistence) of SP was abolished using a deletion of SP's N-terminal sperm-binding region (SP-$TG^{\Delta 2\text{-}7}$) (29) or by using spermless (sons of *tudor* females) males that produced WT SP (13). In all three cases, midgut length was not significantly increased in mated females relative to virgin female controls, assessed at 3 d (Fig. 3). Thus, SP must both bind to and be released from sperm for long-term midgut growth.

Because SP's C-terminal domain is known to bind to its receptor, SPR (35, 37), we next investigated the involvement of SPR in post-mating midgut growth. We used a molecularly defined deficiency, *Df(1)Exel6234*, which removes SPR and four other genes (*frma, CG15784, CG4151*, and *CG32762*), to assess whether SPR is required for mating-induced midgut growth (51). Midguts of females homozygous for this deficiency did not grow in response to mating ($P > 0.05$) (Fig. 4*A*), suggesting that this gene region is needed for post-mating midgut growth. Since *CG15784* expressed in the gut (Dataset S1) and Frma is within the pathway required for SP's effect (33), to confirm a role for SPR in post-mating midgut growth, we tested a ubiquitous RNA interference (RNAi)-mediated knockdown of SPR (*SI Appendix*, Fig. S5*D*). Although there was a small increase in midgut size in mated SPR knockdown females ($P < 0.05$, Fig. 4*B*), the midguts of control females grew much more after mating than those of SPR knockdown females. Furthermore, we observed a significant interaction between female genotype and mating status on midgut size ($P < 0.01$) (*SI Appendix*, Table S4). Thus, SPR knockdown suppressed post-mating midgut growth. Altogether, our genetic results show that SPR and the portion of SP to which it binds are needed for normal post-mating midgut growth.

Nutrient Deprivation Suppresses Post-Mating Midgut Growth. We also examined whether post-mating gut remodeling can occur during nutrient stress, a condition under which investing energy in organ growth and reproduction may be detrimental to survival (52). Females were placed on a 10-fold (0.1×) diluted diet at eclosion. At 3 d old, females were either mated to males or kept virgin, and half of each mating treatment was transferred to a 1× diet while the other half remained on the diluted food. Females were dissected 5 d after mating (*SI Appendix*, Fig. S6*A*). Females

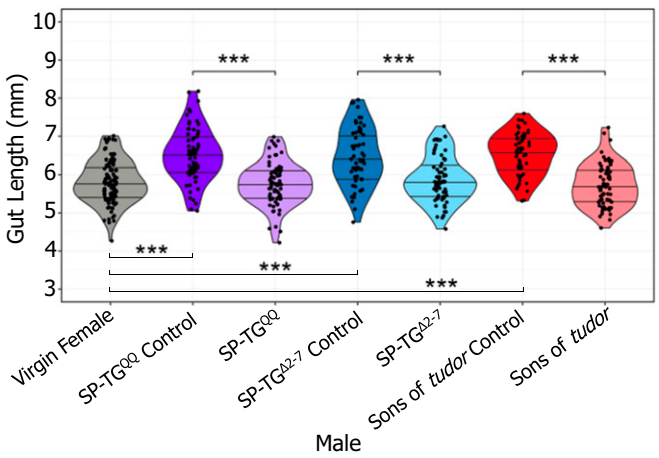

Fig. 3. Long-term storage and release of SP is needed for post-mating midgut enlargement. At 3 d post-mating females mated to control males that transferred WT SP and sperm (SP^{QQ} control, $n = 66$, dark purple; $SP^{\Delta 2\text{-}7}$ control, $n = 58$, dark blue; sons of *tudor* control, $n = 60$, dark red) had midguts significantly longer than those of virgin females (gray); mates of males mutant for SP storage or release from sperm (SP^{QQ}, n = 76, light purple; $SP^{\Delta 2\text{-}7}$, n = 68, light blue; sons of *tudor*, $n = 66$, light red) failed to elicit a midgut growth response in the female. Data are derived from three biological replicates. ***$P < 0.0001$.

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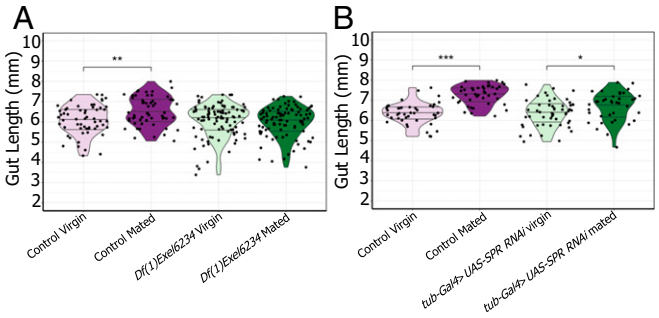

Fig. 4. SPR plays a role in post-mating midgut growth. (*A*) Midguts of homozygous *Df(1)Exel6234* females (n_{mated} = 94, n_{virgin} = 97) do not grow after mating while those of control [*Df(1)Exel6234/FM7c*] females do (n_{mated} = 56, n_{virgin} = 57). (*B*) Midguts of SPR knockdown females (*tubGal4-UAS SPR RNAi*, n_{mated} = 38, n_{virgin} = 52) grow less after mating compared to control (*UAS SPR RNAi;TM3,* n_{mated} = 41, n_{virgin} = 43). ***$P < 0.0001$, **$P < 0.001$,*$P < 0.05$.

placed on 1× yeast-sucrose food after 3 d on diluted food exhibited post-mating midgut growth ($P < 0.0001$) (*SI Appendix*, Fig. S6*B*). Female flies kept on a 10-fold diluted diet show no significant difference between the gut lengths of virgin and mated females 5 d after mating ($P > 0.05$) (*SI Appendix*, Fig. S6*B*). Additionally, the midgut lengths of both virgin and mated females kept on diluted food were significantly smaller than those of virgin females on 1× food ($P < 0.001$ and $P < 0.05$) (*SI Appendix*, Fig. S6*B*). We also found a significant interaction between the mating status of the female and diet ($P < 0.01$) (*SI Appendix*, Table S5), indicating that female post-mating midgut growth is dependent on nutrition and that a poor nutritional state can suppress SP's effect on post-mating midgut growth.

Mating Reshapes the Midgut Transcriptome. To ascertain the impact of mating and SP on digestive physiology, we characterized the transcriptome of whole midguts of CS virgin females, CS females mated to SP^{WT} males at 2 d post-mating by 3′ end RNA sequencing (RNA-seq). The 2-d post-mating time point was chosen because midguts are actively growing at this time (Fig. 1*C*). Differential expression analysis revealed 502 genes significantly differentially expressed in the midgut in response to mating, comparing virgin females to females mated to SP^{WT} males (false discovery rate [FDR] ≤ 0.05) (Dataset S1). At a twofold expression change threshold, 65 genes were up-regulated, and 104 genes were down-regulated in mated females compared to virgin females. Gene Ontology (GO) analysis revealed a significant enrichment in the down-regulated genes for carbohydrate metabolism (Fig. 5*A* and Datasets S2 and S3): Three of the four most significantly enriched GO process terms included "carbohydrate metabolic process" (FDR q < 0.0001), "monosaccharide metabolic process" (FDR q < 0.0001), and "hexose metabolic process" (FDR q < 0.0001) (*SI Appendix*, Fig. S2). These genes included *Amyrel*, an α-amylase (53), *tobi*, an α-glucosidase linked to insulin signaling (54), and *Rpi*, a component of the pentose-phosphate pathway. Additionally, we detected post-mating down-regulation of genes involved in sugar digestion, including seven of the eight *maltase A* family genes and multiple genes involved in glucose (*zw*, *fbp*, and *pgi*), and galactose metabolism (*Galk* and *Gal*) (Fig. 5*A*). We also detected enrichment for genes involved in "glutathione metabolic process" (FDR q < 0.01) (Dataset S1); notably, seven glutathione-S-transferases were down-regulated in the midgut upon mating (Fig. 5*A*).

We examined the 65 genes that were up-regulated upon mating to WT males and found enrichment of genes with "transmembrane transport" function (FDR q < 0.0001) (Datasets S2 and S3): the amino acid transporters *NAAT1* and *slif,* tetracycline resistance gene and sugar transporter *rtet*, and peroxisomal membrane protein transporter *pmp-70*. "Serine-type endopeptidase activity" genes, including five members of the Jonah family of proteases and one trypsin family protease (*deltaTry*), were also enriched (FDR q < 0.001). We also found mild up-regulation of genes involved in fatty acid and lipid metabolism (Fig. 5*A*). For instance, *Bgm* is a long chain fatty acid CoA ligase involved in fatty acid synthesis while *bmm* is a lipase that regulates the mobilization of fat stores (55). Altogether, these data suggest that mating induces a shift in midgut physiology from carbohydrate metabolism to protein and lipid digestion.

To gain insight into how post-mating transcriptional changes may be regulated downstream of SP, we used i-cisTarget (56) to examine whether any transcription factor binding motifs were enriched among the genes differentially expressed between virgin and SP^{WT} mated females (*SI Appendix*, Fig. S4). This program identifies enriched regulatory features among a gene set and uses this information to infer regulatory networks (56). Among the top candidates were motifs for the binding of *caudal*, a homeobox transcription factor. *Caudal* has previously been implicated in gut development and in repressing the gut immune response by inhibiting the immune-activating transcription factor NF-κB (57). We also detected enrichment in motifs for *GATAe* binding (*SI Appendix*, Fig. S4).

SP Influences Post-Mating Changes in the Midgut Transcriptome. To ascertain SP's effect on the mated female gut transcriptome, we also performed RNA-seq on the midguts of females mated to SP^0 males 2 d after mating. Principal component analysis (PCA) demonstrated that virgin females and females mated to SP^0 males cluster together away from females mated to SP^{WT} males (Fig. 5*B*). Therefore, most of the variation in midgut gene expression upon mating can be explained by the receipt of SP. Our RNA-seq analysis revealed only 11 genes differentially expressed between virgin females and females mated to SP^0 males, none of which met the twofold expression change cutoff. The low number of differentially expressed genes and the clustering of SP^0 mated females and virgin females in the PCA show that the guts of SP^0 mated females are transcriptionally similar to those of virgin females. In addition, hierarchical clustering of differentially expressed genes reveals that these samples cluster together, indicating that changes in midgut RNA species induced by mating are almost completely dependent on SP (Fig. 5*C* and *SI Appendix*, Fig. S2).

Comparing the midgut of females mated to SP^0 males and females mated to SP^{WT} males, we identified 398 differentially expressed genes. Of these, 339 were also identified as differentially expressed between virgin females and females mated to SP^{WT} males. This suggests that SP is regulating many of the genes that respond to mating (*SI Appendix*, Fig. S2) and that the midguts of females mated to SP^0 males are virgin-like. At a threshold of twofold expression change, 104 genes are differentially expressed in response to mating alone, 99 of which reach the same threshold in response to mating and WT SP (*SI Appendix*, Fig. S3). GO terms significantly enriched in the genes that are down-regulated in SP^{WT} mated females compared to SP^0 mated females include "carbohydrate metabolic process" (FDR q < 0.0001), "oxidation reduction process" (FDR q < 0.001), and "monocarboxylic acid metabolic process" (FDR q < 0.01) (Datasets S2 and S3). Many of these GO terms were also significantly enriched in the list of genes at least twofold down-regulated in the midgut after mating to SP^{WT} males (Dataset S1). Overall, given the requirement of SP to initiate many of the post-mating midgut gene expression changes, these results suggest that SP is a key regulator of the post-mating switch in the midgut transcriptome.

A

Gene Symbol	Expression Values (TPM)			Log$_2$ fold induction		
	Virgin	SP^0 mated	SP^{WT} mated	SP^{WT} mated vs. Virgin	SP^0 mated vs. Virgin	SP^{WT} mated vs. SP^0 mated
Amino Acid Transport						
dmGlut	77.1299	106.0279	279.7759	1.8589	0.4591	1.3998
slif	52.3319	64.8553	129.8747	1.3114	0.3095	1.0018
NAAT1	7.6271	8.4056	15.7905	1.0498	0.1402	0.9096
Chitin Metabolism						
Cht9	27.842	31.739	61.137	1.135	0.189	0.946
CG32284	10.555	16.673	37.175	1.816	0.660	1.157
CG7298	77.862	145.032	265.038	1.767	0.897	0.870
Serine-Type Endopeptidase						
deltaTry	167.899	175.172	487.291	1.537	0.061	1.476
Jon65Aiii	7251.087	8826.084	17177.337	1.244	0.284	0.961
Jon25Bii	2425.845	2580.916	5421.771	1.160	0.089	1.071
Jon65Aiv	10007.960	11363.978	21710.856	1.117	0.183	0.934
Lipid Metabolism						
bmm	43.514	51.685	77.229	0.828	0.248	0.579
bgm	46.502	45.783	82.330	0.824	-0.022	0.847
Lpin	113.993	125.458	137.430	0.270	0.138	0.131
Glucose Metabolism						
Zw	109.892	77.899	53.280	-1.044	-0.496	-0.548
fbp	71.857	56.885	24.951	-1.526	-0.337	-1.189
Pgi	167.683	116.003	57.683	-1.540	-0.532	-1.008

Gene Symbol	Expression Values (TPM)			Log$_2$ fold induction		
	Virgin	SP^0 mated	SP^{WT} mated	SP^{WT} mated vs. Virgin	SP^0 mated vs. Virgin	SP^{WT} mated vs. SP^0 mated
Galactose Metabolism						
Gal	718.925	632.329	285.717	-1.331	-0.185	-1.146
Galk	33.298	26.622	14.094	-1.240	-0.323	-0.918
Galt	106.353	94.241	63.823	-0.737	-0.174	-0.562
Glutathione-S-Transferase						
GstD8	26.632	17.732	6.694	-1.992	-0.587	-1.405
GstE7	151.223	100.521	51.798	-1.546	-0.589	-0.957
GstT4	412.433	359.743	153.571	-1.425	-0.197	-1.228
Maltase						
Mal-A7	1755.161	1543.820	336.000	-2.385	-0.185	-2.200
Mal-A8	580.446	494.725	118.409	-2.293	-0.231	-2.063
Mal-A6	3542.888	3117.695	1000.981	-1.824	-0.184	-1.639
Carbohydrate Metabolism						
Rpi	262.052	188.184	108.829	-1.268	-0.478	-0.790
tobi	4600.033	3997.561	868.261	-2.405	-0.203	-2.203
Amyrel	275.031	164.804	20.306	-3.760	-0.739	-3.021
Idh	475.097	412.313	300.120	-0.663	-0.204	-0.458
Glucose Transport						
CG8249	34.974	33.212	13.100	-1.417	-0.075	-1.342
CG1208	75.802	46.466	15.063	-2.331	-0.706	-1.625
CG6484	1411.603	1306.765	481.978	-1.550	-0.111	-1.439
CG1213	501.552	475.950	297.561	-0.753	-0.076	-0.678

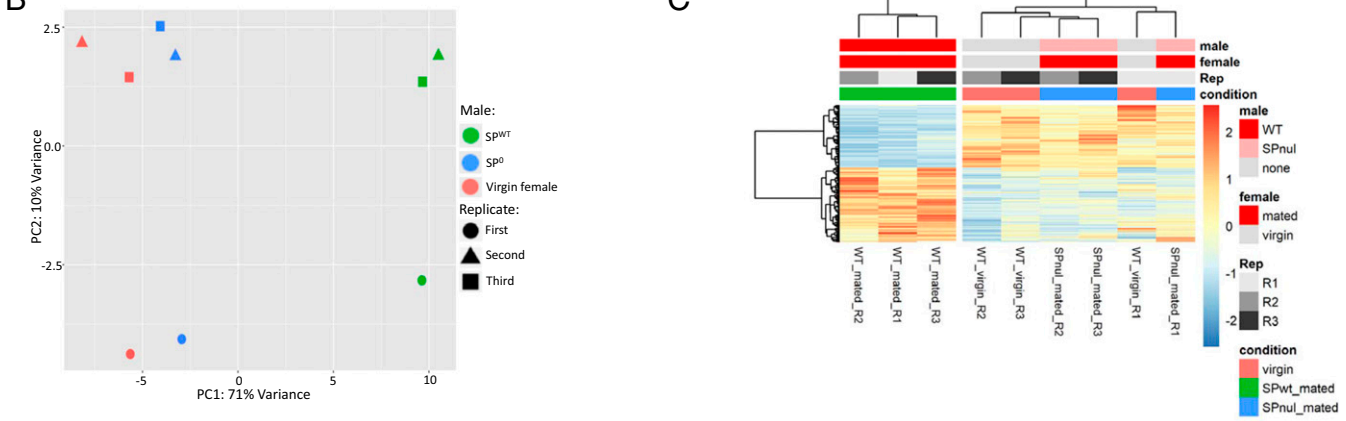

Fig. 5. SP influences the midgut post-mating transcriptome. (*A*) Table of genes representative of several GO terms significantly enriched in the list of genes differentially expressed in response to mating (*SI Appendix*, Table S5). Expression values are expressed in transcripts per kilobase million (TPM). (*B*) PCA performed using DEseq2. (*C*) Hierarchical clustering of significantly differentially expressed genes.

Discussion

Production of progeny requires a significant female energy investment. Consequently, mated females alter aspects of nutrient intake and digestion to maintain energy homeostasis (58). In *Drosophila*, such changes help sustain egg production (2, 11, 15, 23, 24). Given the important role of these responses in supporting reproduction, understanding their mechanisms is crucial to understanding the determinants of reproductive success. In this study, we identified female receipt of the seminal SP as a central signal that triggers post-mating shifts in mated female midgut size and gene expression.

SP Mediates Post-Mating Size and Transcriptome Changes in the Midgut. Previous studies have shown that males can modulate female physiology and behavior to enhance reproductive output via SP. These SP-induced post-mating responses that influence female nutrition include increasing female food intake, shifting nutrient preference, and concentrating female excreta (9, 11). In mated females, SP also regulates levels of JHB3 and 20E, both of which are necessary for midgut resizing (15, 17). We show here that SP received during mating is both necessary and sufficient for enlargement of the mated female's midgut and thus that SP is the sole male-derived signal needed to mediate the switch between a female's virgin and mated midgut size. Other SP-related ligands likely play a minimal role in the initiation of post-mating midgut growth. The SP paralog Dup99B (59) is transferred at normal levels within the seminal fluid of SP^0 males (28). Since the midgut lengths and transcriptomes of SP^0 mated females were no different from those of virgin females, it is unlikely that Dup99B contributes significantly to stimulating midgut growth. For similar reasons, while any post-mating midgut growth role of myoinhibitory peptides (MIPs), the ancestral ligands of SPR (60, 61), is unknown, any effect that MIPs might have is likely downstream of SP.

We show several additional ways that SP modifies the midgut, beyond the roles that were described above. We find that midgut size peaks at 6 d post-mating and persists for at least 15 d after mating. This is consistent with the time frame of other SP-mediated long-term post-mating responses, such as reduced receptivity to remating, which persists for ∼10 d (28, 31). Additionally, our experiments utilizing males that transfer SP defective for either sperm binding or release from sperm (29) demonstrate that post-mating midgut resizing requires both long-term storage of SP and the gradual release of its C-terminal domain from sperm.

Consistent with our finding that release of SP's C terminus is necessary for post-mating midgut growth, we find that its receptor

SPR plays a role in post-mating midgut growth as well. The midguts of females homozygous for a deletion that removes SPR and four other genes did not exhibit post-mating midgut growth (Fig. 4*A*). To determine whether their lack of post-mating midgut growth was due to loss of SPR, rather than to loss of any of the other four genes, we also examined post-mating midgut grown in SPR-knockdown females. Although the knockdown females still had small amounts of residual SPR expression (*SI Appendix*, Fig. S5*D*), they exhibited suppressed post-mating midgut growth (Fig. 4*B*). Taken together, our data indicate that SPR is needed for post-mating midgut growth. SP acting via SPR neuronal signaling has previously been linked to midgut post-mating responses, such as stimulating neuropeptide F release from EEs (16) and increasing intestinal transit time (24). Additionally, our RNA-seq analysis identified SPR expression in the midgut (Dataset S1), suggesting the possibility that SP could act directly on the gut to stimulate growth. Investigating where SPR acts to stimulate post-mating gut growth is an intriguing avenue for future research.

The mechanisms by which SP stimulates post-mating midgut growth are important areas for future investigation. For example, SP could act indirectly, through its regulation of the hormones JHB3 and 20E. SP acts via neuronal SPR to increase 20E synthesis in the ovaries (49), and ovarian 20E is necessary for post-mating gut growth (17), together suggesting that SP could stimulate midgut growth by raising ovarian 20E levels. SP's stimulation of JHB3 could also contribute to post-mating midgut growth. During the early hours after mating (short-term response), SP's N-terminal domain (unbound to sperm) can stimulate JHB3 release from corpora allata (44), potentially inducing midgut growth. However, while JHB3 release may initiate growth in the midgut, this is not sufficient for SP's persistent, long-term effect on midgut growth because we did not observe midgut growth after 3 d in females that had mated to SP-TG^{QQ} or spermless males, both of which deposit SP with an intact N terminus (Fig. 3) and thus should initiate SP's short-term responses. Moreover, the requirement for SPR implicates SP's C terminus (as opposed to its JHB3-inducing N terminus) in the extended post-mating midgut growth. Thus, any early effects of SP on JHB3 that potentially impacted midgut growth cannot be sustained without activity of SP's C-terminal region, released long-term from sperm.

Additionally, we find SP-induced gut morphological changes are dependent on the female's nutritional state. Previous studies have shown that sterile *Ovo*^{D} females, who do not increase food intake post-mating, still undergo post-mating gut growth (15). We show that post-mating gut growth is not entirely independent of female nutrition. Midguts of mated females fed a nutrient-poor diet do not grow after mating, despite receiving WT SP from males. This could be the result of nutritional deficiencies negatively regulating production of JHB3 in the corpora allata. Under stress conditions, such as starvation, increased levels of 20E have been proposed to negatively regulate juvenile hormones (62–64). Additionally, arrested vitellogenesis of nutritionally deprived females can be rescued by treating them with the juvenile hormone analog methoprene (65), and insulin receptor mutants exhibit reduced JHB3 synthesis (66). These findings suggest that signals of nutritional state can affect JHB3 synthesis, and poor nutritional state may suppress SP's effect on JHB3.

In addition to the effects of SP on post-mating midgut morphology, SP alters midgut physiology by reshaping the intestinal transcriptome. Gioti et al. (67) and Domanitskaya et al. (68) profiled transcriptomic responses to SP using microarrays to assay the effects of SP on the head and abdomen at 3 to 6 h after mating (67, 68). Both studies demonstrated SP-induced changes in the transcriptome, such as induction of genes involved in immune responses. Here, we show that SP modulates the messenger RNA (mRNA) complement of a single tissue and demonstrate that SP's effect on midgut transcription can persist for at least 2 d after mating, consistent with the timescale of SP's long-term response (43). We found that SP underlies the vast majority of transcriptional changes in the midgut: Only 11 genes that were significantly differentially expressed between the guts of virgin females and females mated to SP^{0} males, and none of those genes exhibited a greater than twofold difference in expression between virgin and SP^{0}-mated females. Additionally, the genes differentially regulated between females mated to SP^{WT} and those mated to SP^{0} males largely overlap with those differentially expressed between virgin and mated females. In other words, the switch from a virgin to a mated state at the RNA level does not occur without SP, analogous to our finding that post-mating midgut size is regulated by SP.

Male proteins transferred during mating may also trigger enteric changes in other insects. In mosquitoes, extracts from male accessory glands can stimulate post-mating responses in females (69). In the dengue vector *Aedes aegypti*, male-derived substances can increase blood meal size and promote blood meal digestion in the female (70, 71). Additionally, post-mating transcriptional changes have been observed in the guts of *Anopheles gambiae* females (72). In *Anopheles coluzzii* females, post-mating transcriptomic changes in the gut are evoked by male transfer of the ecdysteroid hormone 20E, suggesting that male-derived molecules triggering gut remodeling may be common in insects (73). Given the link between nutrition and egg production, understanding how male mosquitoes influence the female midgut may lead to new strategies for vector control.

Shifts in Midgut Transcriptome Parallel Different Nutritional States of Virgin and Mated Females. The observed post-mating changes in midgut transcriptome parallel post-mating dietary shifts. Mated females showed down-regulation of genes involved in carbohydrate metabolism, such as the maltase-encoding genes and those linked to galactose and glucose metabolism, in the midgut. In conjunction, there was up-regulation of genes required for protein digestion and lipid metabolism. Despite differences in experimental design and execution, other studies examining mating-induced transcriptional changes in whole females or female abdomens have detected similar gene expression changes to those that we report here (67, 74, 75). Zhou et al. (74) observed down-regulation of maltase genes in the whole-organism transcriptome of 3- to 5-d-old mated females, and Fowler et al. (76) also found down-regulation of carbohydrate metabolic genes in female abdomens at 3 h after mating. Both Gioti et al. (67) and Zhou et al. (74) detected post-mating up-regulation of *jonah* family serine-type endopeptidase genes. McGraw et al. (75) also found up-regulation of several proteases, and Fowler et al. (76) found up-regulation of proteolysis-related genes in female abdomens 3 h after mating. Reiff et al. (15) used qPCR to detect up-regulation of several fatty acid metabolic genes in the midgut after mating. Our results confirm that *bgm* expression is induced upon mating and add *bmm* to the list (15) although we do not find induction of some other genes (*SREBP, Acsl, FAS,* and *ACC*) detected by Reiff et al. as up-regulated after mating (15). Discrepancies in exact complements of differentially expressed genes likely reflect methodological differences. We also found down-regulation of genes with GST activity involved in detoxification. Their down-regulation after mating could have consequences for the female's ability to deal with toxic dietary compounds and oxidative stress (77). Indeed, post-mating gut growth increases a female's propensity to develop life span-shortening gut dysplasia (17). This may reflect potential trade-offs between the demands of reproduction and somatic maintenance (78, 79).

These results may reflect a post-mating increase in protein and lipid digestion to help sustain egg production. Previous studies

have shown that food intake increases after mating, and sufficient dietary protein and lipids are essential for yolk protein production and female fecundity (1, 80). However, the up-regulation of protein and lipid metabolic genes is likely not simply a consequence of increased food intake since we saw a coincident down-regulation of carbohydrate metabolic genes. Rather, it is probable we observed the mated female midgut altering digestive parameters to adapt to new nutritional demands.

Although the molecular pathways underlying post-mating midgut transcriptomic changes remain unknown, we detected enrichment of several transcription factor binding motifs among genes regulated by mating, including those for *caudal* and *GATAe*. *GATAe* has been previously linked to regulating ISC maintenance and differentiation and also plays a key role in maintaining gut homeostasis (81–83), suggesting that *GATAe* could play a role in the increase in ISC proliferation observed after mating, as well as the altered expression of digestive enzymes (15, 84).

In addition to observing mating-induced changes in the midgut transcriptome, we found that regions of the midgut display varying degrees of morphological plasticity. Although all regions of the midgut increase in length after mating, we found that the length of the posterior midgut, a region involved in nutrient absorption, grows more in proportion to total midgut length than any other region (18). This may be the result of egg production increasing nutrient demand. Alternatively, it could be an indirect result of the proximity of the posterior midgut to the ovaries, or mechanical stress exerted from the ovaries toward the midgut (16).

Conclusions. We find that the seminal peptide SP is a key regulator of both female post-mating midgut size and transcription of metabolic pathways. Our data show that, in well-nourished females, SP is both necessary and sufficient for post-mating midgut enlargement. Additionally, post-mating midgut growth is a component of SP's long-term response, requiring both long-term SP storage and release from sperm, as well as SPR. Mating also causes a shift in the transcriptome of the midgut, a change due almost completely to SP. The post-mating midgut increased transcription of genes involved in lipid and protein metabolism, while decreasing mRNA levels of sugar metabolic genes, and genes involved in detoxification. These results provide insight into SP's role as a regulator of mated female nutritional homeostasis, by helping the female meet the energetic demands of egg production. Overall, these findings illustrate the dynamic nature of the *Drosophila* midgut, demonstrating how the male can alter female internal morphology and physiology to enhance reproduction.

Materials and Methods

Fly Husbandry and Strains. Flies were reared at 25 °C on a 12:12 light/dark cycle on either a dextrose yeast diet or a sucrose yeast diet (for 1 L of diet, 50 g of yeast, 60 g of yellow cornmeal, 40 g of sucrose, 7 g of agar, 26.5 mL of moldex, 5 mL of propionic acid, 0.498 mL of phosphoric acid [85%]). Dietary conditions were kept constant for each experiment. Virgin females and males were collected within 8 h of eclosion and aged in separate vials for 3 to 5 d. An SP null mutant line (0325/TM3, *Sb*, *ry*) was crossed to a deficiency line (Δ130/TM3, *Sb*, *ry*) to generate SP^0 males (0325/Δ130) and control SP^{WT} males (0325/TM3, *Sb*, *ry* or Δ130/TM3, *Sb*, *ry*) (28). Crossing the SP^{QQ} cleavage mutant line (*w*/Y; $SP\text{-}TG^{QQ}$/$SP\text{-}TG^{QQ}$; Δ130/TM3) to the SP null mutant line (0325/TM3) generated cleavage mutant males (*w*/Y; $SP\text{-}TG^{QQ}$; 0325/Δ130) (29). Crossing the $SP^{\Delta 2\text{-}7}$ N-terminal deletion line (*w*/Y; $SP\text{-}TG^{\Delta 2\text{-}7}$/ $SP\text{-}TG^{\Delta 2\text{-}7}$; Δ130/TM3) to the SP null mutant line (0325/TM3, *Sb*, *ry*) produced N-terminal deletion males (*w*/Y; $SP\text{-}TG^{\Delta 2\text{-}7}$; 0325/Δ130) (29). Spermless males were the sons of *tud*^{1}, *bw*, *sp* females mated to CS males; the maternal effect *tudor* mutation in these females eliminates the germline (85). The *tud*^{1}, *bw*, *sp*/CyO sisters of *tudor* females crossed to CS males produced control males (*tud*^{1}, *bw*, *sp*/+) that were genetically identical to the spermless males but had a normal germline.

Females ectopically expressing SP (*w*; *tub-Gal80*^{ts}/*UAS-SP*; *tub-Gal4*/+) were generated by mating a temperature-sensitive *tub-Gal4*; *tub-Gal80*^{ts} flies (*w*; *tub-Gal80*^{ts}/*tub-Gal80*^{ts}; *tub-Gal4*/TM3) to a SP ectopic expression line (*w*; *UAS-SP*/*UAS-SP*) (86). Control flies expressing GFP (*w*; *tub-Gal80*^{ts}/+; *tub-Gal4*/*UAS-GFP*) were made by mating the *tub-Gal4*; *tub-Gal80*^{ts} line to a GFP ectopic expression line (*w*; +/+; *UAS-GFP*/TM3). To prevent developmental phenotypes, we used the Gal4-Gal80^{ts} system to temporally restrict transgene expression (87). Adult *tub-Gal4*; *tub-Gal80*^{ts}>*UAS-SP* and *tub-Gal4*; *tub-Gal80*^{ts}>*UAS-GFP* virgin females reared at 18 °C were placed at 29 °C for 6 d to induce ectopic expression. To control for the effect of temperature on gut size, additional groups of *tub-Gal4*; *tub-Gal80*^{ts}>*UAS SP* and *tub-Gal4*; *tub-Gal80*^{ts}>*UAS-GFP* virgin females were kept at 18 °C. Ectopic GFP expression was verified by fluorescence microscopy. After 6 d of transgene induction, guts were dissected and processed as described in *Midgut Length Measurements*. The SPR deficiency line *Df(1)Exel6234* was obtained from the Bloomington *Drosophila* Stock Center (Line 7708). Control females were *Df(1)Exel6234/FM7c* (51). SPR knockdown females were made by crossing SPR short hairpin RNA (shRNA) line 106804 (VDRC) to *tub-Gal4*. Experimental females were *tub-Gal4*>*UAS-SPR RNAi* while control females were *UAS-SPR RNAi; TM3*.

Midgut Length Measurements. Single-pair matings were conducted between 3- to 5-d-old virgin females and 3- to 5-d-old males. Matings were monitored, and females who mated for less than 15 min were discarded. Males were discarded upon completion of mating. After mating, females were aged in cohorts of 10 to 15. Age-matched virgin female control groups were also maintained. Guts were dissected in phosphate-buffered saline (PBS) at the stated time point (1 h, 12 h, 1 d, 3 d, 6 d, 10 d, 15 d) after mating and fixed with 4% paraformaldehyde solution in PBS (Electron Microscopy Sciences). Samples were stained with DAPI in PBS-0.01% Triton X-100 (1:50,000; Sigma Aldrich) and mounted on slides. All images were obtained using a Zeiss LSM 700 fluorescence-confocal inverted microscope at 10× magnification, using a tiling Zeiss algorithm, and quantification was performed using ImageJ. Gut length was measured as the length of a spline curve drawn down the midline. Gut anterior and posterior region width was measured as the longest line drawn across the region.

RNA-Seq. Female midguts were collected 2 d after mating to SP^0 or SP^{WT} males, and from virgin females. RNA was isolated from 60 midguts per sample per replicate, using a modified TRizol extraction protocol (Life Technologies). Following RNA extraction, 3′end RNA-seq libraries were prepared using the QuantSeq 3′ mRNA-Seq library prep kit, according to the manufacturer's instructions (Lexogen). Sample quality was evaluated at the RNA level and on the library using fragment analyzer (Advanced Analytical). Libraries were sequenced on two lanes of the Illumina Nextseq 500 platform, using standard protocols for 75-base pair (bp) single-end read sequencing, at the Cornell Life Sciences Sequencing Core.

On average, 6 million reads per sample were sequenced at their 3′ termini. Raw reads were quality-filtered with fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (version 0.11.3) and trimmed using Trimmomatic (version 0.32) (88). Trimmed reads were mapped to the *D. melanogaster* reference transcriptome (genome version 6.80) using the STAR RNA-seq aligner (version 2.4.1a) (89). Read depth for each transcript was then estimated using htseq (version 0.6.1). PCA and extraction of the first two principal components (PCs) was performed in R with the DEseq2 package. Genes differentially regulated were identified using DEseq2 with an FDR of 5%. Genes represented below 1.2 counts per million were filtered out prior to differential expression analysis. GO enrichment analysis was performed using GORILLA (cbl-gorilla.cs.technion.ac.il/). I-cisTarget (https://gbiomed.kuleuven.be/apps/lcb/i-cisTarget/) was used to identify transcription factor binding sites enriched in genes differentially expressed between virgin and mated females. RNA-seq data can be downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive with accession number PRJNA668203.

Data Analysis. All statistical analyses were performed in R version 3.1.2 (https://www.r-project.org). For experiments measuring the effects of SP on gut size, experimental replicates were combined and analyzed using a linear mixed model, with replicate included as a random effect. If the F statistic was significant, notable pairwise comparisons were selected, and the *P* value was recalculated to correct for multiple testing using the Bonferroni method. Time course experiments were fit to a linear mixed effects model with time point after mating, mating status, and their interaction as fixed effects, and experimental replicate as a random effect. If the interaction term between time point and mating status was significant, post hoc pairwise comparisons between mating status groups were made at each time point, and *P* values were adjusted for multiple testing with a Bonferroni correction. Clustering

was performed using pheatmap and WardD2 scaling. For experiments manipulating SPR, linear mixed models included an interaction term for genotype by mating status interactions. For all models, residual analyses were performed to ensure model assumptions of normality and homogeneity of variances were met.

Data Availability. RNA-seq data have been deposited in the NCBI Sequence Read Archive (accession no. PRJNA668203). All other study data are included in the article, *SI Appendix*, and Datasets S1–S3.

ACKNOWLEDGMENTS. We thank Dr. Andrew G. Clark for suggesting this study and for helpful comments on this manuscript. We thank Matt Meiselman for help with dissections and imaging. We thank Lynn Johnson and the Cornell Statistical Consulting Unit for helpful discussions on data analysis. SP-TG^{QQ} and SP-TG$^{\Delta 2\text{-}7}$ flies were a kind gift of Eric Kubli. *UAS-SP* flies were a kind gift of Toshiro Aigaki. We thank Philip Houtz, Sofie Delbare, Andrea Vogel, Dashiell Massey, and two anonymous reviewers for comments on this manuscript. We also thank NIH Grants R01-HD038921 and R37-HD038921 (to M.F.W.) and Cornell University startup funding and NSF Grant IOS1656118 (to N.B.) for support of this work. For part of this study, M.A.W. was supported by NIH Training Grant T32-GM07617.

1. M. Bownes, Expression of the genes coding for vitellogenin (yolk protein). *Annu. Rev. Entomol.* **31**, 507–531 (1986).
2. M. Bownes, A. Scott, A. Shirras, Dietary components modulate yolk protein gene transcription in Drosophila melanogaster. *Development* **103**, 119–128 (1988).
3. D. Drummond-Barbosa, A. C. Spradling, Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis. *Dev. Biol.* **231**, 265–278 (2001).
4. J. Terashima, M. Bownes, Translating available food into the number of eggs laid by Drosophila melanogaster. *Genetics* **167**, 1711–1719 (2004).
5. T. J. Bradley, F. H. Simmons, An analysis of resource allocation in response to dietary yeast in Drosophila melanogaster. *J. Insect Physiol.* **43**, 779–788 (1997).
6. J. N. Schultzhaus, G. E. Carney, Dietary protein content alters both male and female contributions to Drosophila melanogaster female post-mating response traits. *J. Insect Physiol.* **99**, 101–106 (2017).
7. M. D. W. Piper *et al*., A holidic medium for Drosophila melanogaster. *Nat. Methods* **11**, 100–105 (2014).
8. G. B. Carvalho, P. Kapahi, D. J. Anderson, S. Benzer, Allocrine modulation of feeding behavior by the Sex Peptide of Drosophila. *Curr. Biol.* **16**, 692–696 (2006).
9. C. Ribeiro, B. J. Dickson, Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in Drosophila. *Curr. Biol.* **20**, 1000–1005 (2010).
10. M. A. Vargas, N. Luo, A. Yamaguchi, P. Kapahi, A role for S6 kinase and serotonin in postmating dietary switch and balance of nutrients in D. melanogaster. *Curr. Biol.* **20**, 1006–1011 (2010).
11. A. I. Barnes, S. Wigby, J. M. Boone, L. Partridge, T. Chapman, Feeding, fecundity and lifespan in female Drosophila melanogaster. *Proc. Biol. Sci.* **275**, 1675–1683 (2008).
12. V. M. Corrales-Carvajal, A. A. Faisal, C. Ribeiro, Internal states drive nutrient homeostasis by modulating exploration-exploitation trade-off. *eLife* **5**, e19920 (2016).
13. M. F. Camus, C. C. Huang, M. Reuter, K. Fowler, Dietary choices are influenced by genotype, mating status, and sex in Drosophila melanogaster. *Ecol. Evol.* **8**, 5385–5393 (2018).
14. B. Lemaitre, I. Miguel-Aliaga, The digestive tract of Drosophila melanogaster. *Annu. Rev. Genet.* **47**, 377–404 (2013).
15. T. Reiff *et al*., Endocrine remodelling of the adult intestine sustains reproduction in Drosophila. *eLife* **4**, e06930 (2015).
16. T. Ameku *et al*., Midgut-derived neuropeptide F controls germline stem cell proliferation in a mating-dependent manner. *PLoS Biol.* **16**, e2005004 (2018).
17. S. M. H. Ahmed *et al*., Fitness trade-offs incurred by ovary-to-gut steroid signalling in Drosophila. *Nature* **584**, 415–419 (2020).
18. N. Buchon *et al*., Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. *Cell Rep.* **3**, 1725–1738 (2013).
19. C. A. Micchelli, N. Perrimon, Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* **439**, 475–479 (2006).
20. B. Ohlstein, A. Spradling, The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* **439**, 470–474 (2006).
21. X. Zeng, S. X. Hou, Enteroendocrine cells are generated from stem cells through a distinct progenitor in the adult Drosophila posterior midgut. *Development* **142**, 644–653 (2015).
22. A. Marianes, A. C. Spradling, Physiological and stem cell compartmentalization within the Drosophila midgut. *eLife* **2**, e00886 (2013).
23. B. Hudry, S. Khadayate, I. Miguel-Aliaga, The sexual identity of adult intestinal stem cells controls organ size and plasticity. *Nature* **530**, 344–348 (2016).
24. P. Cognigni, A. P. Bailey, I. Miguel-Aliaga, Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. *Cell Metab.* **13**, 92–104 (2011).
25. B. Hudry *et al*., Sex differences in intestinal carbohydrate metabolism promote food intake and sperm maturation. *Cell* **178**, 901–918.e16 (2019).
26. F. W. Avila, L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, M. F. Wolfner, Insect seminal fluid proteins: Identification and function. *Annu. Rev. Entomol.* **56**, 21–40 (2011).
27. J. M. Kalb, A. J. DiBenedetto, M. F. Wolfner, Probing the function of Drosophila melanogaster accessory glands by directed cell ablation. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8093–8097 (1993).
28. H. Liu, E. Kubli, Sex-peptide is the molecular basis of the sperm effect in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9929–9933 (2003).
29. J. Peng *et al*., Gradual release of sperm bound sex-peptide controls female postmating behavior in Drosophila. *Curr. Biol.* **15**, 207–213 (2005).
30. A. Manning, A sperm factor affecting the receptivity of Drosophila melanogaster females. *Nature* **194**, 252–253 (1962).
31. T. Chapman *et al*., The sex peptide of Drosophila melanogaster: Female post-mating responses analyzed by using RNA interference. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9923–9928 (2003).
32. K. R. Ram, M. F. Wolfner, A network of interactions among seminal proteins underlies the long-term postmating response in Drosophila. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 15384–15389 (2009).
33. G. D. Findlay *et al*., Evolutionary rate covariation identifies new members of a protein network required for Drosophila melanogaster female post-mating responses. *PLoS Genet.* **10**, e1004108 (2014).
34. A. Singh *et al*., Long-term interaction between Drosophila sperm and sex peptide is mediated by other seminal proteins that bind only transiently to sperm. *Insect Biochem. Mol. Biol.* **102**, 43–51 (2018).
35. N. Yapici, Y. J. Kim, C. Ribeiro, B. J. Dickson, A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. *Nature* **451**, 33–37 (2008).
36. I. U. Haussmann, Y. Hemani, T. Wijesekera, B. Dauwalder, M. Soller, Multiple pathways mediate the sexpeptide- regulated switch in female Drosophila reproductive behaviours. *Proc. Biol. Sci.* **280**, 20131938 (2013).
37. Y. Chung-Hui *et al*., Control of the postmating behavioral switch in Drosophila females by internal sensory neurons. *Neuron* **61**, 519–526 (2009).
38. M. Häsemeyer, N. Yapici, U. Heberlein, B. J. Dickson, Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. *Neuron* **61**, 511–518 (2009).
39. C. Rezával *et al*., Neural circuitry underlying Drosophila female postmating behavioral responses. *Curr. Biol.* **22**, 1155–1165 (2012).
40. C. Rezával, T. Nojima, M. C. Neville, A. C. Lin, S. F. Goodwin, Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in Drosophila. *Curr. Biol.* **24**, 725–730 (2014).
41. M. Soller, M. Bownes, E. Kubli, Control of oocyte maturation in sexually mature Drosophila females. *Dev. Biol.* **208**, 337–351 (1999).
42. M. Soller, M. Bownes, E. Kubli, Mating and sex peptide stimulate the accumulation of yolk in oocytes of Drosophila melanogaster. *Eur. J. Biochem.* **243**, 732–738 (1997).
43. J. Apger-McGlaughon, M. F. Wolfner, Post-mating change in excretion by mated Drosophila melanogaster females is a long-term response that depends on sex peptide and sperm. *J. Insect Physiol.* **59**, 1024–1030 (2013).
44. P. Moshitzky *et al*., Sex-peptide activates juvenile hormone biosynthesis in the Drosophila melanogaster corpus allatum. *Arch. Insect Biochem. Physiol.* **32**, 363–374 (1996).
45. M. Ottiger, M. Soller, R. F. Stocker, E. Kubli, Binding sites of Drosophila melanogaster sex peptide pheromones. *J. Neurobiol.* **44**, 57–71 (2000).
46. Z. Ding, I. Haussmann, M. Ottiger, E. Kubli, Sex-peptides bind to two molecularly different targets in Drosophila melanogaster females. *J. Neurobiol.* **55**, 372–384 (2003).
47. R. A. Schwenke, B. P. Lazzaro, Juvenile hormone suppresses resistance to infection in mated female Drosophila melanogaster. *Curr. Biol.* **27**, 596–601 (2017).
48. G. Bontonou, H. A. Shaik, B. Denis, C. Wicker-Thomas, Acp70A regulates Drosophila pheromones through juvenile hormone induction. *Insect Biochem. Mol. Biol.* **56**, 36–49 (2015).
49. T. Ameku, R. Niwa, Mating-induced increase in germline stem cells via the neuroendocrine system in female Drosophila. *PLoS Genet.* **12**, e1006123 (2016).
50. L. E. O'Brien, S. S. Soliman, X. Li, D. Bilder, Altered modes of stem cell division drive adaptive intestinal growth. *Cell* **147**, 603–614 (2011).
51. A. L. Parks *et al*., Systematic generation of high-resolution deletion coverage of the Drosophila melanogaster genome. *Nat. Genet.* **36**, 288–292 (2004).
52. M. L. Wayne, U. Soundararajan, L. G. Harshman, Environmental stress and reproduction in Drosophila melanogaster: Starvation resistance, ovariole numbers and early age egg production. *BMC Evol. Biol.* **6**, 57 (2006).
53. G. Claisse, G. Feller, M. Bonneau, J. L. Da Lage, A single amino-acid substitution toggles chloride dependence of the alpha-amylase paralog amyrel in Drosophila melanogaster and Drosophila virilis species. *Insect Biochem. Mol. Biol.* **75**, 70–77 (2016).
54. S. Buch, C. Melcher, M. Bauer, J. Katzenberger, M. J. Pankratz, Opposing effects of dietary protein and sugar regulate a transcriptional target of Drosophila insulin-like peptide signaling. *Cell Metab.* **7**, 321–332 (2008).
55. S. Grönke *et al*., Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. *Cell Metab.* **1**, 323–330 (2005).
56. C. Herrmann, B. Van de Sande, D. Potier, S. Aerts, i-cisTarget: An integrative genomics method for the prediction of regulatory features and cis-regulatory modules. *Nucleic Acids Res.* **40**, e114 (2012).
57. J. H. Ryu *et al*., Innate immune homeostasis by the homeobox gene Caudal and commensal-gut mutualism in Drosophila. *Science* **319**, 777–782 (2008).
58. J. Roa, M. Tena-Sempere, Connecting metabolism and reproduction: Roles of central energy sensors and key molecular mediators. *Mol. Cell. Endocrinol.* **397**, 4–14 (2014).
59. P. Saudan *et al*., Ductus ejaculatorius peptide 99B (DUP99B), a novel Drosophila melanogaster sex-peptide pheromone. *Eur. J. Biochem.* **269**, 989–997 (2002).

60. Y. J. Kim *et al*., MIPs are ancestral ligands for the sex peptide receptor. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 6520–6525 (2010).
61. J. Poels *et al*., Myoinhibiting peptides are the ancestral ligands of the promiscuous Drosophila sex peptide receptor. *Cell. Mol. Life Sci.* **67**, 3511–3522 (2010).
62. M. Meiselman *et al*., Endocrine network essential for reproductive success in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E3849–E3858 (2017).
63. M. R. Meiselman, T. G. Kingan, M. E. Adams, Stress-induced reproductive arrest in Drosophila occurs through ETH deficiency-mediated suppression of oogenesis and ovulation. *BMC Biol.* **16**, 18 (2018).
64. J. Terashima, K. Takaki, S. Sakurai, M. Bownes, Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in Drosophila melanogaster. *J. Endocrinol.* **187**, 69–79 (2005).
65. M. Bownes, G. Reid, The role of the ovary and nutritional signals in the regulation of fat body yolk protein gene expression in Drosophila melanogaster. *J. Insect Physiol.* **36**, 471–479 (1990).
66. M. P. Tu, C. M. Yin, M. Tatar, Mutations in insulin signaling pathway alter juvenile hormone synthesis in Drosophila melanogaster. *Gen. Comp. Endocrinol.* **142**, 347–356 (2005).
67. A. Gioti *et al*., Sex peptide of Drosophila melanogaster males is a global regulator of reproductive processes in females. *Proc. Biol. Sci.* **279**, 4423–4432 (2012).
68. E. V. Domanitskaya, H. Liu, S. Chen, E. Kubli, The hydroxyproline motif of male sex peptide elicits the innate immune response in Drosophila females. *FEBS J.* **274**, 5659–5668 (2007).
69. G. B. Craig, Mosquitoes: Female monogamy induced by male accessory gland substance. *Science* **156**, 1499–1501 (1967).
70. A. E. R. Downe, Internal regulation of rate of digestion of blood meals in the mosquito, Aedes aegypti. *J. Insect Physiol.* **21**, 1835–1839 (1975).
71. V. Adlakha, M. K. K. Pillai, Rôle of male accessory gland substance in the regulation of blood intake by mosquitoes. *J. Insect Physiol.* **22**, 1441–1442 (1976).
72. D. W. Rogers *et al*., Molecular and cellular components of the mating machinery in Anopheles gambiae females. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19390–19395 (2008).
73. F. A. Dahalan, T. S. Churcher, N. Windbichler, M. K. N. Lawniczak, The male mosquito contribution towards malaria transmission: Mating influences the Anopheles female midgut transcriptome and increases female susceptibility to human malaria parasites. *PLoS Pathog.* **15**, e1008063 (2019).
74. S. Zhou, T. Mackay, R. R. H. Anholt, Transcriptional and epigenetic responses to mating and aging in Drosophila melanogaster. *BMC Genomics* **15**, 927 (2014).
75. L. A. McGraw, G. Gibson, A. G. Clark, M. F. Wolfner, Genes regulated by mating, sperm, or seminal proteins in mated female Drosophila melanogaster. *Curr. Biol.* **14**, 1509–1514 (2004).
76. E. K. Fowler, T. Bradley, S. Moxon, T. Chapman, Divergence in transcriptional and regulatory responses to mating in male and female fruitflies. *Sci. Rep.* **9**, 16100 (2019).
77. C. P. D. Tu, B. Akgül, Drosophila glutathione S-transferases. *Methods Enzymol.* **401**, 204–226 (2005).
78. T. Chapman, L. F. Liddle, J. M. Kalb, M. F. Wolfner, L. Partridge, Cost of mating in Drosophila melanogaster females is mediated by male accessory gland products. *Nature* **373**, 241–244 (1995).
79. Y. Wang, A. B. Salmon, L. G. Harshman, A cost of reproduction: Oxidative stress susceptibility is associated with increased egg production in Drosophila melanogaster. *Exp. Gerontol.* **36**, 1349–1359 (2001).
80. E. Parra-Peralbo, J. Culi, Drosophila lipophorin receptors mediate the uptake of neutral lipids in oocytes and imaginal disc cells by an endocytosis-independent mechanism. *PLoS Genet.* **7**, e1001297 (2011).
81. T. Okumura, A. Matsumoto, T. Tanimura, R. Murakami, An endoderm-specific GATA factor gene, dGATAe, is required for the terminal differentiation of the Drosophila endoderm. *Dev. Biol.* **278**, 576–586 (2005).
82. N. Buchon, N. A. Broderick, B. Lemaitre, Gut homeostasis in a microbial world: Insights from Drosophila melanogaster. *Nat. Rev. Microbiol.* **11**, 615–626 (2013).
83. D. Dutta *et al*., Regional cell-specific transcriptome mapping reveals regulatory complexity in the adult Drosophila midgut. *Cell Rep.* **12**, 346–358 (2015).
84. T. Okumura *et al*., GATAe regulates intestinal stem cell maintenance and differentiation in Drosophila adult midgut. *Dev. Biol.* **410**, 24–35 (2016).
85. R. E. Boswell, A. P. Mahowald, tudor, a gene required for assembly of the germ plasm in Drosophila melanogaster. *Cell* **43**, 97–104 (1985).
86. S. Nakayama, K. Kaiser, T. Aigaki, Ectopic expression of sex-peptide in a variety of tissues in Drosophila females using the P[GAL4] enhancer-trap system. *Mol. Gen. Genet.* **254**, 449–455 (1997).
87. J. L. Mueller, J. L. Page, M. F. Wolfner, An ectopic expression screen reveals the protective and toxic effects of Drosophila seminal fluid proteins. *Genetics* **175**, 777–783 (2007).
88. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
89. A. Dobin *et al*., STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).