**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. 1A) that are morphologically and physiologically distinct (18, 19). The anterior midgut (red in Fig. 1A) primarily functions in digestion while the middle midgut (blue in Fig. 1A) functions similar to a stomach. The posterior midgut (magenta in Fig. 1A) 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 enterocendrinc 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 ISC 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 ecysdine) 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 non-sperm components of the ejaculate (26, 27). Thereafter, long-term responses last up to 2 wk after mating and require 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.

**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.

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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, pick-pocket, 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. S1B) and in posterior midgut width (P < 0.0001) (SI Appendix, Fig. S1C), we detected significant post-mating increases in anterior midgut width (P < 0.0001) (SI Appendix, Fig. S1A). Intriguingly, post-mating midgut length increases more in the posterior region than the anterior region (Fig. 1B 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 and advances our understanding of the mechanisms by which females address the nutritional demands of reproduction.
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. 1C). 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 (SP0) or control sibling males producing wild-type (WT) SP (SPWT) (28). Receptivity assays confirmed that SP0 males failed to stimulate female post-mating responses, affirming the integrity of our SP0 lines (SI Appendix, Fig. S5A). As expected, the midgut lengths of females mated to SPWT males were significantly longer than those of virgin females (P < 0.0001) while the midgut lengths of females mated to SP0 males were no different from those of virgin females (Fig. 2A). 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-Gal80Δ) (SI Appendix, Fig. S5B). 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) (Fig. 2B). 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. 2B).

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-TGQC) (29). Likewise, sperm binding (and thus persistence) of SP was abolished using a deletion of SP’s N-terminal sperm-binding region (SP-TGQΔ2-7) (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, Di(1)Eel6234, 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. 4A), 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. S5D). Although there was a small increase in midgut size in mated SPR knockdown females (P < 0.05, Fig. 4B), 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 (0.1x) 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 1x diet while the other half remained on the diluted food. Females were dissected 5 d after mating (SI Appendix, Fig. S6D). Females in organ growth and reproduction may be detrimental to survival (52).
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 SP0 males 2 d after mating. Principal component analysis (PCA) demonstrated that virgin females and females mated to SP0 males cluster together away from females mated to SPWT males (Fig. 5B). 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 SP0 males, none of which met the twofold expression change cutoff. The low number of differentially expressed genes and the clustering of SP0 mated females and virgin females in the PCA show that the guts of SP0 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. 5C and SI Appendix, Fig. S2).

Comparing the midguts of females mated to SP0 males and females mated to SPWT males, we identified 398 differentially expressed genes. Of these, 339 were also identified as differentially expressed between virgin females and females mated to SP0 males, none of which met the twofold expression change cutoff. The low number of differentially expressed genes and the clustering of SP0 mated females and virgin females in the PCA show that the guts of SP0 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. 5C and SI Appendix, Fig. S2).

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 SPWT 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. 1C). Differential expression analysis revealed 502 genes significantly differentially expressed in the midgut in response to mating, comparing virgin females to females mated to SPWT 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. 5A 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. 5A). 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. 5A).

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 NAA11 and slf, tetracycline resistance gene and sugar transporter rtt, and peroxyisomal membrane protein transporter pmp-70. “Serine-type endopeptidase activity” genes, including five members of the Jonah family of proteases and one trypsin family protease (deltaTn), were also enriched (FDR q < 0.001). We also found mild up-regulation of genes involved in fatty acid and lipid metabolism (Fig. 5A). For instance, Bgm is a long chain fatty acid CoA ligase involved in fatty acid synthesis while bmu 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 SPWT 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-kB (57). We also detected enrichment in motifs for GATAe binding (SI Appendix, Fig. S4).
performed using DEseq2. (Previous studies have shown that males can modulate
midgut. SP Mediates Post-Mating Size and Transcriptome Changes in the
central signal that triggers post-mating shifts in mated female
this study, we identified female receipt of the seminal SP as a
to understanding the determinants of reproductive success. In
Drosophila melanogaster sex peptide regulates mated female midgut morphology and
physiology

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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+ males (28). Since the midgut lengths and transcriptomes of SP+ 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. 4A). 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 growth in SPR-knockdown females. Although the knockout females still had small amounts of residual SPR expression (SI Appendix, Fig. S5D), they exhibited suppressed post-mating midgut growth (Fig. 4B). 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 neuromodulation 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-TGQ9 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 via sperm.

Additionally, we find SP-induced gut morphological changes are dependent on the female’s nutritional state. Previous studies have shown that sterile OvoD 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. Giotti 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 SP0 males, and none of those genes exhibited a greater than twofold difference in expression between virgin and SP0-mated females. Additionally, the genes differentially regulated between females mated to SP0 or those mated to SP0 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 depositing gut remodelling 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 Giotti 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 bhm 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 cadoal 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 immediately. Alternatively, it could arise as 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 gene expression 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 mol dex, 3 mL of propionic acid, 0.498 mL of phosphoric acid [85%]). 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 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 (http://cbl-test.wlv.ac.uk/cgi-bin/cbl-gorilla.cgi) and i-cisTarget (https://ghoamended.kuleuven.be/biosoft/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 block 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

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was performed using phetmap and WardD2 scaling. For experiments ma-
nipulating SPR, linear mixed models included an interaction term for
nutotype 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.

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