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Report

Allocrine Modulation of Feeding Behavior by the Sex Peptide of *Drosophila*

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Summary

Mating elicits a dramatic reprogramming of female behavior in numerous insect species. In *Drosophila*, this postmating response (PMR) comprises increased egg-laying rate and reduced sexual receptivity and is controlled by the products of the male accessory glands, a family of ~80 small peptides transferred in the male seminal fluid [1–9]. Here, we show that copulation strongly stimulates female food intake. Remarkably, this change is abolished if the males lack a single, small seminal protein, the Sex Peptide (SP). Ectopic expression of SP in virgin females mimics the effect of mating on feeding behavior, demonstrating that SP is the main agent controlling this behavioral paradigm. Our observations identify enhanced feeding behavior as a novel component of the *Drosophila* PMR and suggest that SP represents a molecular link between energy acquisition and reproductive investment.

Results and Discussion

Nutrient availability plays a critical role in reproductive success [10–12]. Accordingly, changes in patterns of feeding behavior correlate with reproductive status in a wide range of organisms [13–15]. However, the mechanisms regulating this vital process are not well understood. To investigate this issue, we recorded adult food intake by allowing flies to feed on medium colored with a nonabsorbable, nonmetabolizable dye [16]. Visual inspection revealed a striking effect of mating status on female abdominal food accumulation. Mated females ingested substantially larger meals than age-matched virgins (Figure 1A). This disparity was both accentuated and accelerated if a 12 hr starvation period preceded the feeding trial. Spectrophotometric quantitation showed that, in these conditions, mated females consumed ~2.3 times as much food as virgins (Figure 1B). Other dyes of different colors and chemical compositions gave similar results (data not shown).

Drosophila feeding behavior can be monitored by radioactive labeling of the medium [17, 18]. An essential advantage of this method lies in its enhanced specificity and sensitivity, which make it possible to record steady-state food consumption in nonstarved flies. In addition, food intake can be measured over longer periods, avoiding short-term fluctuations and circadian variation. We recorded adult food ingestion over a 24 hr period by using food labeled with [α-^{32}P]dCTP. Averaged across multiple, independent trials, ad-libitum-fed, mated females showed a 56% elevation in radioactive tracer level when compared to virgins (Figure 1C). This result was reproducible with different isotopes ([α-^{32}P]dATP, [^{14}C]sucrose) and was conserved across several wild-type strains, including Canton-S, Oregon R, and Dahomey (data not shown). This observation cannot be explained simply by an enhanced total food capacity of mated animals, because isotope incorporation in both physiological states continued to increase up to at least 72 hr on labeled food (Figure S1 in the Supplemental Data available online). The 24 hr measurements shown in Figure 1C are therefore far from reaching saturation. Furthermore, the higher tracer levels in mated females are not a consequence of defective or delayed nutrient metabolization and/or excretion, given that the isotope level declined significantly faster in mated females than in virgins after the 24 hr pulse of labeled medium (Figure S2). This disparity in the rate of isotope elimination may reflect incorporation into developing oocytes in mated females. Together with the results obtained with dye-colored food (Figures 1A and 1B), these findings strongly suggest that our measurements reflect bona fide differences in volume of food ingestion between the virgin and mated states. In contrast to the situation in females, male feeding was not affected by mating status (Figure 1D). These results identify enhanced feeding behavior as a novel component of the *Drosophila* PMR.

Both previously described elements of the behavioral PMR—egg laying and rejection of secondary copulation—are regulated by the products of the male accessory gland [5]. We therefore asked whether the accessory-gland proteins (Acps) are also responsible for the feeding-behavior changes in mated females. Genetic ablation of the accessory-gland main cells can be achieved through expression of a modified form of diphtheria toxin subunit A (DTA) under the control of the main cell-specific promoter Acp95EF [5]. These DTA-expressing males produce only vestigial amounts of Acps (~1% of wild-type) and induce no egg-laying and only a slight, transient reduction of female receptivity [5]. Females mated to DTA males displayed no elevation of food intake, whereas isogenic control males lacking the DTA construct induced a normal response (Figure 2A), indicating that the physiological stimulation of feeding behavior requires the Acps.

One Acp in particular, the Sex Peptide (SP), is both necessary and sufficient to induce the PMR in virgins [6–9]. We therefore asked whether SP is the particular Acp responsible for stimulating female food intake. SP^0 males, which specifically lack SP as a result of a targeted chromosomal deletion, but normally express and transfer all remaining Acps and sperm [8], failed to

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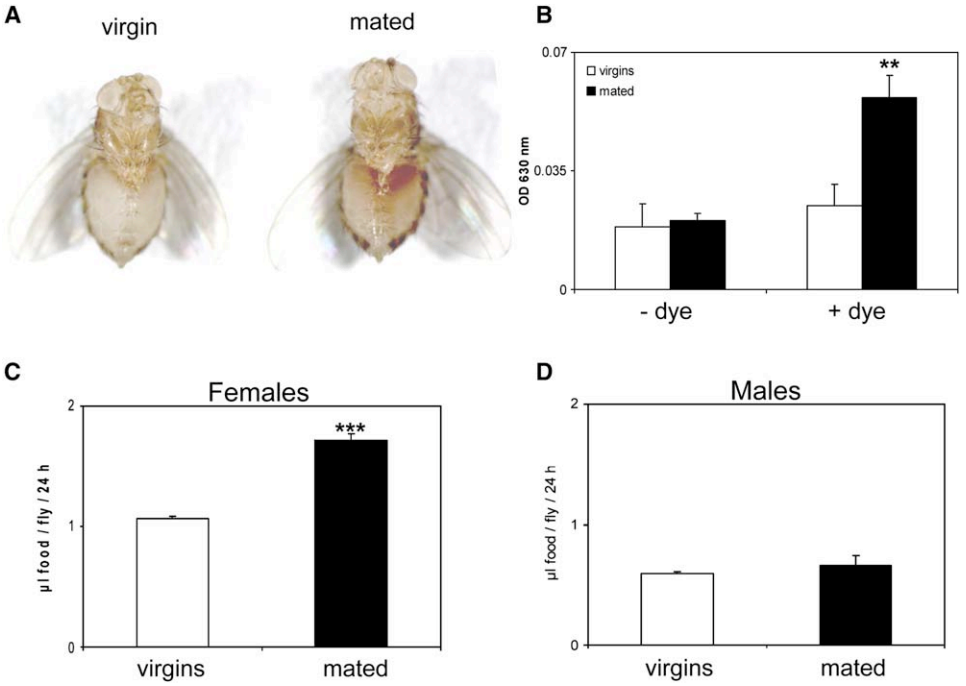

Figure 1. Mating Stimulates Female Food Intake

(A) Virgin and mated females after feeding on red-dyed medium for 2 hr.

(B) Feeding rate of virgin (− dye, n = 42; + dye, n = 39) and mated (− dye, n = 40; + dye, n = 36) females allowed to feed on medium with or without red dye for 30 min following 12 hr of wet (water-only) starvation. Shown is the average per fly ± standard deviation (SD) of three replicates. Y axis represents values of optical density (OD) of abdomen homogenate (assayed at 630 nm).

(C and D) Induction of feeding rate upon mating is female specific. Ingestion volume of [α-32P]dCTP-labeled food over a 24 hr period by ad-libitum-fed virgin and mated females (C) and males (D). Results are expressed as volume of food intake (in µl) over 24 hr averaged per fly ± SD of four replicates of 15 animals per condition. ** indicates $p < 0.01$; *** indicates $p < 0.0001$, two-tailed t test.

significantly induce feeding in females (Figure 2B). Both DTA and SP^0 males showed courtship and mating rates similar to those of the respective controls and successfully fertilized all females they were kept with, as assayed by scoring viable progeny of females kept in individual vials (data not shown). These results demonstrate that the main-cell Acps, and SP in particular, are required for stimulation of postcopulatory food ingestion in females.

We next tested directly the action of SP in regulating female feeding behavior. Ectopic expression of SP in the adult fat body of virgin females by means of a yolk protein 1 enhancer (*yp1*) has been shown to be sufficient to induce the two classical components of the PMR [7]. Females bearing the *yp1*-SP fusion construct exhibited a constitutively increased feeding rate that was not further elevated by mating (Figure 3A), suggesting that SP can, by itself, elicit a mated-like appetite in virgins. We tested this hypothesis further by expressing SP under the control of an upstream-activating system (UAS) promoter. Previous work has identified several independent galactose 4 (GAL4) insertion lines that, when used to drive SP, can elicit the PMR in virgin females [19]. Indeed, expression of SP under the control of either the 9Y- or C370-GAL4 driver lines [19] markedly stimulated virgin feeding rate (Figures 3B and 3C). Importantly, in neither case did copulation further increase food ingestion. Three additional GAL4 drivers gave identical results (data not shown). Although the central nervous system is the only area in common among the expression patterns of the five driver lines ([19], G.B.C. and S.B., unpublished data), the fact that SP is expressed as a secreted, diffusible molecule precludes a definite conclusion concerning its site of action. These findings demonstrate that SP modulates postcopulatory feeding in females, whereas sperm and the act of copulation per se do not play substantial roles.

In numerous animal species, including humans, enhancing nutrient acquisition is a common strategy accompanying reproductive effort, and its pivotal role in ensuring reproductive success is well established [10–12]. *Drosophila* has found an elegant and effective way to couple reproductive investment to increased acquisition of energy resources—a single, small peptide transferred in the male ejaculate. Peptides play a central role in appetite control, both in insects and in higher organisms [20–24]. Remarkably, in this case, the molecule is produced by and regulates the feeding behavior of two separate individuals. Sexual allocrine mechanisms have also been described in vertebrates. For example, prostaglandins secreted in human semen can modulate female immune response [25], a role that has also been attributed to the SP of *Drosophila* [26]. How does SP orchestrate such a dramatic behavioral and physiological reprogramming? In the case of appetite modulation, a possible mechanism is suggested by the fact that SP binds to the subesophageal ganglion [27], a neuronal center previously implicated in taste recognition and feeding [28, 29]. Alternatively, SP may regulate food intake indirectly. Ex vivo, SP acts on the corpus allatum

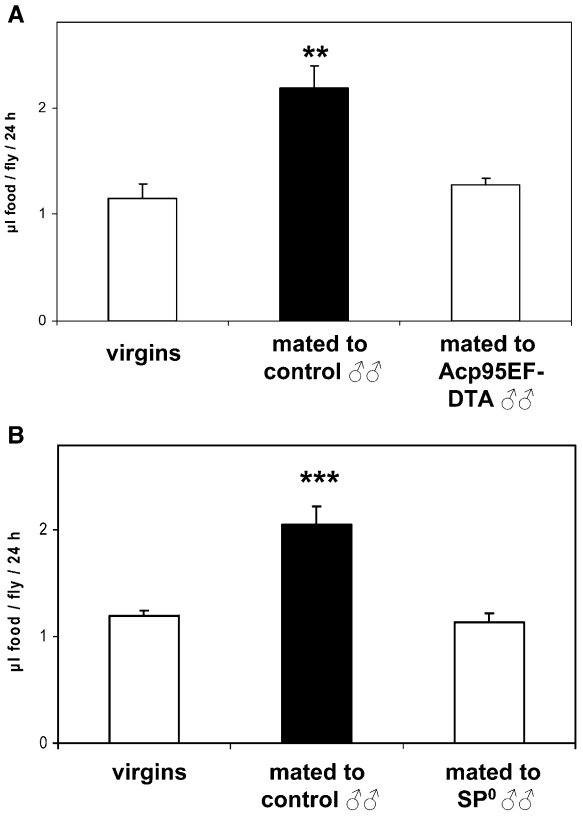

Figure 2. SP Is Necessary for Postcopulatory Induction of Female Food Intake

(A) Genetic ablation of male accessory-gland main cells abolishes stimulation of feeding behavior. Experimental males carry a construct in which the Acp95EF main-cell promoter is fused to the modified coding sequence of diphtheria toxin subunit A (DTA) [5]. Control males have identical genetic background but do not bear the DTA construct (one-way ANOVA, p = 0.0021).

(B) Males that lack SP (SP^0) but produce and transfer normal amounts of remaining Acps and sperm fail to stimulate female appetite. Experimental males carry the null mutant allele SP^0 (introduced by homologous recombination) over the Δ^{130} deletion, which uncovers the *SP* locus. Control males have identical genetic background but carry a wild-type copy of *SP* over Δ^{130} [8] (one-way ANOVA, p = 0.0001). All values are expressed as volume of food intake (in µl) over 24 hr averaged per fly ± SD of three replicates of 15 animals per condition.

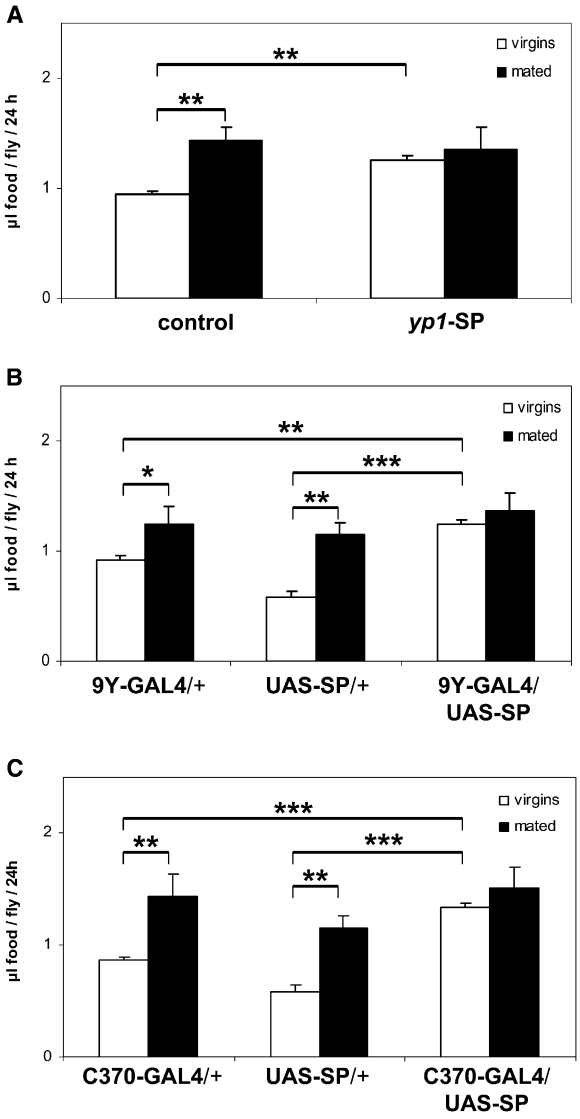

Figure 3. Ectopic Expression of SP in Virgin Females Mimics the Effect of Copulation

(A) Constitutive fat-body expression of SP in virgins by means of the yolk protein 1 (*yp1*) enhancer stimulates feeding to mated-like levels. Isogenic control strain is *cinnabar; rosy* (*cn; ry*).

(B and C) Effect on feeding behavior of expression of a UAS-SP construct driven by the 9Y- (B) or C370- (C) GAL4 driver lines. All values are expressed as volume of food intake (in µl) over 24 hr averaged per fly ± SD of three replicates of 15 animals per condition. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.0001$, two-tailed t test.

to stimulate the secretion of Juvenile Hormone (JH) [30], which plays a crucial role in sexual maturation and oogenesis in *Drosophila* females [31–33]. Induction of oogenesis and vitellogenesis by JH may in turn induce female food intake. In this regard, it will be interesting to investigate whether appetite modulation requires intact reproductive activity.

Our findings raise another intriguing question. Mating drastically reduces the lifespan of *Drosophila* females [34], a phenomenon that has been attributed to the action of the Acps [35], and to SP in particular [36]. Given the link between increased food consumption and shortened lifespan in many organisms, it is conceivable that the reduced longevity of mated females may somehow relate to their accrued nutrient ingestion. Further study on the biology of Acps should help elucidate this intriguing aspect of animal reproduction.

Experimental Procedures

Drosophila *Strains*

Unless otherwise stated, flies were in the *w1118* background. Acp95EF-DTA (mc/DTA-D) was kindly provided by M. Wolfner. SP^0 and control stocks were kindly provided by E. Kubli. UAS-SP was kindly provided by T. Aigaki. *yp*1-SP (Yp1-hsp70-SPgene) and control stocks were obtained from the Bloomington Stock Center.

Culturing Conditions

Flies were raised on Lewis medium [37]. All experiments were conducted at 25ºC on a 12 hr:12 hr light:dark cycle.

Feeding Assays

Flies were collected under brief (<2 min) CO^2 anesthesia. Mature (4-day-old) virgin females were divided in two groups, "virgins" (20 females/vial) and "mated" (15 females + 5 males/vial) and kept for 3 days, with fresh food being provided on the second day. Assays were conducted immediately after the mating period. In the case of females expressing SP, and thus exhibiting enhanced rejection behavior, the flies were kept as "virgins" (20 females/vial) or "mated" (10 females + 10 males per vial). All the above conditions consistently resulted in the insemination of 100% of females in the "mated" group.

The dye assay was performed as follows: For visualization (Figure 1A), ad-libitum-fed flies were allowed to feed on red-colored (FD & C Red 40) Lewis medium for 2 hr, anesthetized, and imaged. For quantitation (Figure 1B), flies were allowed to feed on colored medium for 30 min following 12 hr of starvation in vials containing moist filter paper. Abdomens were isolated and homogenized in 1 × Phosphate Buffer Saline, and OD was recorded at 630 nm in a Benchmark Plus microplate spectrophotometer (BioRad).

The radioactive assay was carried out essentially as described [18]: Flies were allowed to feed for 24 hr on medium containing 0.35 nCi/μL [α-^{32}P]dCTP (MP Biomedicals), switched to empty shell vials and allowed to groom for 30 min, anesthetized by cold, transferred to scintillation vials, and covered with 10 mL scintillation fluid (Research Products International). Scintillation was recorded with a Beckman LS 5000 TA Liquid Scintillation System. Background signal (defined as scintillation counts recorded from a sample fed nonradioactive food) was subtracted from raw values. Each trial included two [α-^{32}P]dCTP calibration samples, which were used to convert scintillation counts to ingestion volume.

Statistics

Graphpad Prism software package was utilized for all statistical analyses.

Supplemental Data

Supplemental Data include two figures and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/7/692/DC1/.

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