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Male contributions to egg production: the role of accessory gland products and sperm in *Drosophila melanogaster*

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Drosophila melanogaster seminal fluid components, accessory gland proteins (Acps) and sperm, induce females to deposit high numbers of fertilized eggs for about 11 days. This high and sustained level of egg deposition requires that oogenesis be stimulated to provide the necessary mature oocytes. To investigate the relative timing and contributions of Acps and sperm in the egg-production process, we examined the rates of oogenic progression and egg deposition in females mated to genetically altered males that have seminal fluid deficient in Acps and/or sperm, and subjected these data to path analysis. We found that Acps and sperm are complementary stimuli necessary for inducing high rates of oogenic progression and rapid egg deposition. While egg deposition and oogenic progression can be induced by Acps alone, both Acps and sperm are required for maximum stimulation of oogenic progression and egg deposition immediately after mating.

Keywords: accessory gland proteins; sperm; vitellogenic oocyte development; egg production; egg laying

1. INTRODUCTION

Drosophila melanogaster females are stimulated by mating to deposit eggs at an elevated rate for up to 11 days (Manning 1962, 1967; Kalb *et al*. 1993; Xue & Noll 2000). Analysis of females one or more days after mating to males deficient in specific components of the seminal fluid demonstrates that male accessory gland proteins (Acps) and sperm are necessary for elevating this post-mating response. Females mated to males that transfer neither main-cell Acps nor sperm were not stimulated to deposit eggs (Kalb *et al*. 1993). In contrast, females mated to males that transfer Acps but not sperm deposit eggs for 24–48 h (Hihara 1981; Kalb *et al*. 1993; Xue & Noll 2000), while females mated to males that transferred sperm but not Acps deposit eggs for up to five days (Xue & Noll 2000). These observations indicate that Acps and sperm, individually, are necessary for stimulating egg deposition for a few days but that maintenance of elevated egg deposition for several days requires the receipt and storage of sperm by the female (the 'sperm effect'; Manning 1962, 1967; Kalb *et al*. 1993; Neubaum & Wolfner 1999).

To sustain this high level of egg deposition for several days, mature oocytes must be available. Each of the two ovaries is made up of 16 ± 1 ovarioles; each ovariole produces up to two mature oocytes daily. Several control points and feedback mechanisms regulate the production of a mature oocyte, a process that is divided into 14 stages (King *et al*. 1956; Cummings & King 1969; Margaritis 1985; reviewed in King 1970; Spradling 1993). During the first seven oogenic stages (pre-vitellogenic stages) the oocyte and its nurse cells grow at similar rates. During stages 8 through 12 (vitellogenic stages) the oocyte grows rapidly, incorporating yolk proteins from the haemolymph and follicle cells, and proteins and RNAs from the nurse cells. Yolk-protein uptake begins during stages 8–9; massive yolk-protein uptake occurs during stages 10A–12. During stages 13–14 there is no further increase in the volume of the oocyte and it is now covered by a vitelline membrane and chorion. A feedback mechanism prevents oocytes from entering the oviduct before completing oogenesis (reviewed in Spradling 1993) and oogenic progression appears to be regulated at stages 8–9, the start of yolk-protein uptake (Wilson 1982). Oocyte production is also influenced by egg deposition. If mature oocytes are not deposited, each ovariole accumulates two or three stage-14 oocytes, blocking the maturation of additional oocytes (King & Sang 1959). As a result, oocytes between stages 7 and 14 disappear (reviewed in Spradling 1993). In contrast, females during maximum fecundity show a constant distribution of oogenic stages, suggesting that oocytes are produced as fast as mature oocytes leave the ovariole (King & Sang 1959). Though egg deposition affects the production of additional oocytes, mating can stimulate oogenesis in the absence of egg deposition (Holzworth *et al*. 1974).

Oogenesis, ovulation and egg deposition are part of a multi-step continuous process. Though the regulation of egg deposition by Acps and sperm in the first days after mating is well documented (Manning 1962, 1967; Kalb *et al*. 1993; Xue & Noll 2000), early time-points have not been examined. It is likely that Acps and/or sperm have important effects much earlier than 24 h, since both are detectable in the female within 10 min of the start of mating (Lung & Wolfner 1999) and Acp-dependent changes in ovulation are detectable by 1.5 h after mating (Heifetz *et al*. 2000). Females mated to males lacking Acp26Aa show a low initial ovulation rate, indicating that Acp26Aa regulates the release of mature oocytes by the ovary (Heifetz *et al*. 2000). Acp70A (sex peptide),

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when injected into unmated females, stimulates oocytes to progress to stage 10, indicating that it can override the control point between stages 9 and 10 (Soller *et al.* 1997, 1999). While injected Acp70A can stimulate oogenic progression, it is not clear what roles Acps play in initiating the production of additional mature oocytes and in increasing oogenic-progression rate after a normal mating. The role of sperm in stimulating oogenesis has not been addressed. To address these issues, we analysed the oocyte-accumulation pattern in the ovaries and the egg-deposition rate of females mated to males that lack Acps and/or sperm. We found that both Acps and sperm are required to stimulate a high rate of oogenic progression and rapid, maximal egg deposition. In the absence of sperm, females exhibit a lower oogenic-progression rate and delay their egg deposition, even shortly after mating.

2. MATERIAL AND METHODS

(a) *Flies*

Flies were maintained, and aged to three days for experiments, as described previously (Heifetz *et al.* 2000). Females used in all the assays came from wild-type Canton S stocks. Males deficient in Acps ('DTA-E') came from a transgenic stock (Kalb *et al.* 1993). These males make no Acps from their main cells (96% of the accessory gland) and no sperm. They make secondary cell, ejaculatory bulb and ejaculatory duct proteins. Their $rosy^{506}$ non-transgenic brothers served as the wild-type controls in all experiments using DTA-E males. Spermless males were the sons of Canton S males and *bw sp* tud^{1} females (Boswell & Mahowald 1985).

(b) *Bioassays and analyses*

The distribution of vitellogenic oocytes from unmated and mated females was analysed. At the end of mating, females were aspirated into fresh vials and held singly for 1.5, 3, 6, 10, 18, or 24 h before their ovaries were removed. Ovaries were fixed, washed and examined as described in Verheyen & Cooley (1994).

The oocyte-accumulation pattern is defined by the number and type of vitellogenic oocytes present in the ovary. Vitellogenic oocytes were classified into three categories (Holzworth *et al.* 1974): (i) stages 8–9, when yolk comprises one-third or less of the oocyte; (ii) stages 10A–12, in which yolk comprises one-half or more of the oocyte; and (iii) stages 13–14, in which dorsal appendages are present. Since no significant difference in the distribution of oocyte stages between the left and the right ovaries was found (data not shown), data from the left and right ovaries were pooled and analysed as number of oocytes per female.

For each female whose ovaries were examined, the number of eggs deposited in the holding vials was also counted.

Path analysis was performed (Wright 1968; Li 1975; Heifetz *et al.* 2000) to evaluate the effects of the act of mating and/or other components in the male seminal fluid, Acps, sperm and time after mating on oogenic progression and egg deposition. The path is shown as a diagram that presents the combined relationship between the predictors (male genotype (DTA-E; spermless and wild-type), time after mating and the preceding developmental stage) and the criteria (number of oocytes at stages 8–9, stages 10A–12, stages 13–14, and the number of deposited eggs). The coefficients that estimate the strengths of the relationships were determined by linear regression (SPSS 8.0 for Windows; SPSS, Inc., Chicago, IL, USA). For the path analysis, four regression equations were estimated.

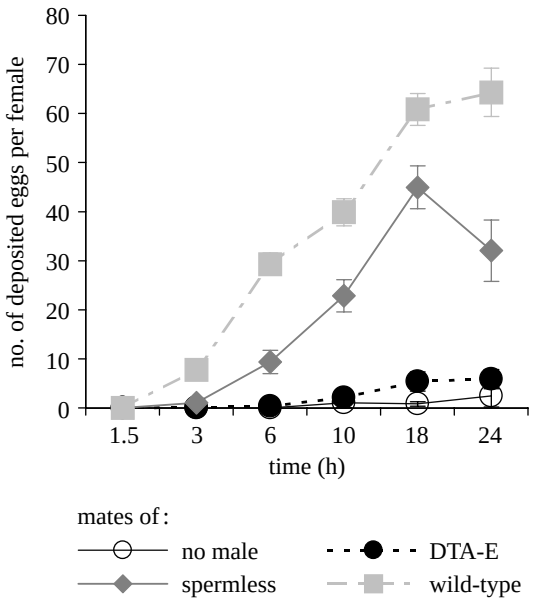


Figure 1. Acps and sperm are required for the stimulation of egg deposition. The number of eggs deposited by virgin females and females mated to DTA-E, spermless or wild-type males at each time-point (1.5, 3, 6, 10, 18 and 24 h) are plotted as mean ± s.e.m. For each treatment at each time-point, 18–40 females were examined.

(c) *Statistics*

Duncan's post-hoc multiple range test ($p < 0.05$, SPSS 8.0 for Windows) was used to rank the number of oocytes at each vitellogenic developmental stage and the number of deposited eggs for the different male genotypes and specific times after mating. A measure of the linear association between two variables (e.g. each developmental stage and the number of deposited eggs) was determined by Pearson correlation (SPSS 8.0 for Windows).

3. RESULTS

(a) *Acps and sperm are required for the rapid induction of egg deposition*

We examined the egg-deposition rate during the first 24 h after mating of females mated to wild-type, spermless or DTA-E males to determine the earliest time-point at which the effects of Acps and sperm on egg deposition were detectable. The number of eggs deposited by females mated to wild-type males increased rapidly and significantly over the first 18 h, from 7.8 ± 1.6 eggs at 3 h to 60.8 ± 3.3 eggs at 18 h after mating ($p < 0.05$); egg-deposition levels remained constant from 18 to 24 h after mating (figure 1, squares). Though egg deposition was also stimulated in females mated to spermless males, it began later (6 h versus 3 h after mating; figure 1, diamonds) and was at a consistently lower level (e.g. 32% at 6 h) than that of females mated to wild-type males (figure 1). Over the 24 h time-course, mates of wild-type males deposited twice as many eggs as mates of spermless males. In contrast, females mated to DTA-E males began to deposit eggs only at 10 h after mating (2.1 ± 1 eggs) and deposited 5.9 ± 2 eggs over the 24 h period, a level not significantly different from that seen for virgin females (figure 1, closed and open circles, respectively). The difference observed in egg-deposition kinetics between mates of

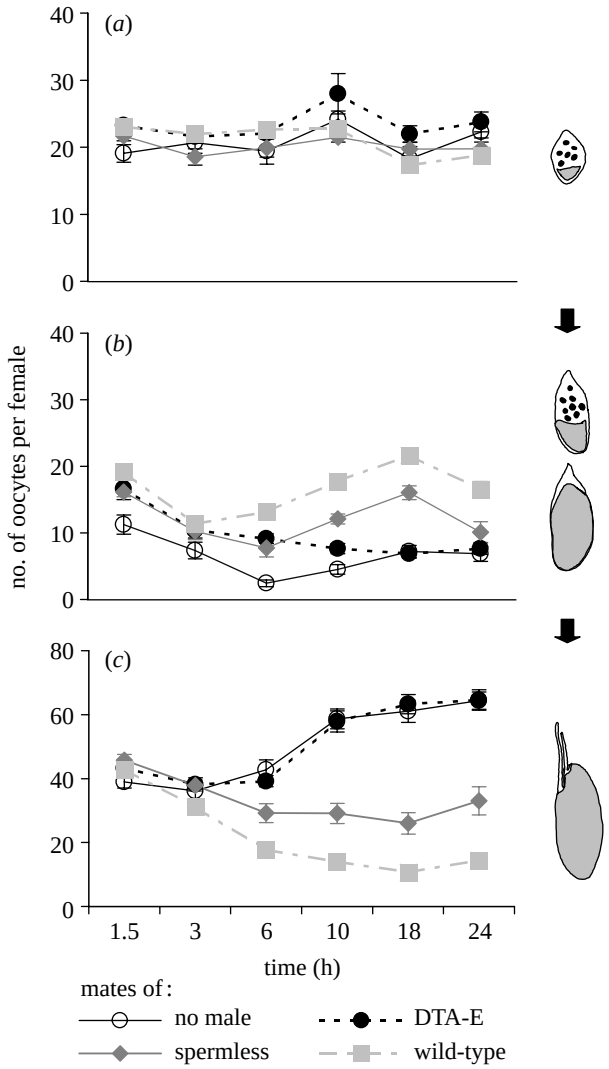


Figure 2. Both Acps and sperm are required to stimulate an increase in oogenic-progression rate as early as 3 h after mating. The number of oocytes at (*a*) stages 8–9, (*b*) stages 10A–12 and (*c*) stages 13–14 in ovaries of virgin females and females mated to wild-type, DTA-E or spermless males are plotted as mean ± s.e.m. for the different time-points examined. Eighteen to 41 pairs of ovaries were examined for each treatment at each time-point. Tracings of typical egg chambers at stages 9, 10A, 12 and 14 are shown at the right. The yolk protein-containing oocyte is shown in dark grey; nurse cells are shown in white, with black nuclei.

spermless and wild-type males indicates that both Acps and sperm are required for the full and rapid initiation of egg deposition.

(b) *Acps and sperm cause a rapid change in the oocyte accumulation pattern*

Since a sustained increase in egg deposition requires a continued elevated rate of oogenesis, we compared the oocyte-accumulation patterns in sexually mature virgin females to those of females mated to DTA-E, spermless or wild-type males at different times during the first day after mating (figure 2). Within the 24 h time-course examined, the ovaries of unmated females showed a progression towards the accumulation of stage 13–14 oocytes (figure 2*c*). The number of oocytes at stages 10A–12 showed an initial decrease during the first 6 h (figure 2*b*) followed by an increase back to the levels found at 3 h, by 24 h. These changes seen in the late oogenic stages indicate that oogenesis is still progressing in three-day-old virgin females. This contrasts with the situation in older unmated females (e.g. five-day-old females, see Soller *et al.* (1997, 1999)), which have accumulated many mature oocytes and have largely arrested oogenesis.

The oocyte-accumulation pattern of females mated to DTA-E males was similar to that of virgin females; they showed an overall accumulation of stage 13–14 oocytes (figure 2*c*) and a decrease in the number of oocytes at stages 10A–12 (figure 2*b*). These observations indicate that the act of mating itself and/or seminal-fluid components other than main-cell Acps and sperm did not have a significant effect on late-stage oogenic development in the ovary.

In contrast, females mated to either spermless or wild-type males showed a continuous decrease in the number of stage 13–14 oocytes (figure 2*c*) and a rapid decrease followed by an increase in the number of oocytes at stages 10A–12 (figure 2*b*). Thus, during egg deposition, stage 13–14 oocytes are released from the ovaries and an increased oogenic-progression rate is detectable in the increased number of oocytes at stages 10A–12. Though the oocyte-accumulation pattern of females mated to spermless males was similar to that of females mated to wild-type males, the magnitudes of changes in the numbers of oocytes at stages 10A–12 and stages 13–14 were different in these two classes of females. Mates of spermless males, for example, exhibited a decrease in stage 13–14 oocytes that was only 45% of that observed in mates of wild-type males. The fact that the responses of mates of spermless and wild-type males are similar, but differ in magnitude, indicates that both Acps and sperm are necessary for inducing the changes in the distribution of late vitellogenic stages.

(c) *By 10 h after mating, Acps and sperm stimulate an increase in oogenic-progression rate*

During the first 10 h after mating, significant changes in the numbers of stage 8–9 oocytes were not observed. At 18 h after mating a significant decrease in the numbers of stage 8–9 oocytes was observed in all females ($p < 0.05$; figure 2*a*). A significant increase in the number of oocytes at stages 10A–12 was also observed at this time-point, but only in females mated to wild-type or spermless males; however, the response observed in females mated to spermless males was approximately 33% lower than in those mated to wild-type males ($p < 0.05$; figure 2*b*). These changes indicate that an increase in the rate of oogenic progression from stages 8–9 to stages 10A–12 first occurs between 10 and 18 h after mating. The decrease in the number of stage 8–9 oocytes observed in virgin females supports the suggestion in § 3(b) that unmated three-day-old females continue to produce oocytes at a low rate. Comparison of the changes observed in mated females indicates that while Acps can stimulate an increase in the rate of oogenic progression from stages 8–9 to stages 10A–12 between 10 and 18 h after mating, both sperm and Acps are required to induce the maximal response. The fact that virgin females exhibit a low oogenic rate, and that there is a threefold increase in the number of oocytes at stages 10A–12 in

Table 1. *Linear regression coefficients relating to the path diagram in figure 3*

	dependent variable							
	stages 8–9		stages 10A–12		stages 13–14		deposited eggs	
independent variable	β	p	β	p	β	p	β	p
constant[a]	20.42	0.0001	5.47	0.0001	39.18	0.0001	8.25	0.0001
stages 8–9	—	—	0.05	0.1144	0.57	0.0001	0.15	0.0232
stages 10A–12	—	—	—	—	−0.37	0.0001	0.78	0.0001
stages 13–14	—	—	—	—	—	—	−0.53	0.0001
time: short (< 6 h)/long (> 10 h)	0.45	0.4267	0.30	0.5706	3.48	0.0058	21.29	0.0001
mates of DTA-E males	2.76	0.0011	2.66	0.0007	−0.42	0.8242	−0.64	0.6632
mates of spermless males	−0.40	0.6370	5.38	0.0001	−15.27	0.0001	4.64	0.0031
mates of wild-type males	0.52	0.5429	9.90	0.0001	−25.67	0.0001	10.04	0.0001

[a] The major part of the 'constant' effect is due to virgin females.

mates of wild-type males compared to virgin females, indicate that Acps and sperm received during mating induce an increase in oogenic-progression rate rather than initiating oogenic progression.

To determine whether oogenic progression from stages 8–9 to stages 10A–12 is mediated by feedback from egg deposition, we investigated whether there was a correlation between the number of stage 8–9 oocytes, or the number of oocytes at stages 10A–12, and the level of egg deposition throughout the entire experimental period. The number of stage 8–9 oocytes was not correlated with levels of egg deposition in mates of wild-type, spermless or DTA-E males ($r = -0.13$, $r = 0.04$ and $r = 0.02$, respectively) while the number of oocytes at stages 10A–12 was loosely correlated with levels of egg deposition ($r = 0.4$, $r = 0.46$ and $r = 0.09$, respectively, as above). This indicates that the progression from stages 8–9 to stages 10A–12 that we observed was due to the presence of Acps and sperm.

(d) *Acps and sperm are the main factors that stimulate oogenic progression*

Oogenesis and egg deposition are part of a multi-step process. Though the steps are interconnected, each step may also be regulated individually. To evaluate the magnitude of the effects that mating and male seminal fluid components have on oogenic progression and egg deposition, and to determine which stage they affect, we quantitatively monitored each of these steps independently but simultaneously using path analysis (Wright 1968; Li 1975; Heifetz *et al*. 2000). In path analysis, each dependent variable (oogenic stage and egg deposition) is regressed on each independent variable that is predicted to affect it (male genotype and time after mating) and may also be regressed on some of the other dependent variables (figure 3 and table 1).

In females mated to wild-type or spermless males, the number of stage 8–9 oocytes did not differ significantly from that in virgin females, while the numbers of oocytes at stages 10A–12 and stages 13–14 and the number of eggs deposited did. The presence of Acps and sperm in the seminal fluid increased the number of oocytes at stages 10A–12 by 9.9 oocytes per mated female ($p < 0.0001$), decreased the number of stage 13–14 oocytes by 25.7 oocytes per female ($p < 0.0001$) and increased the

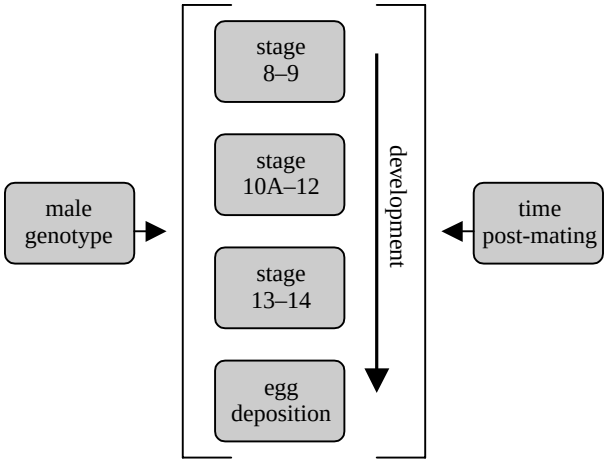
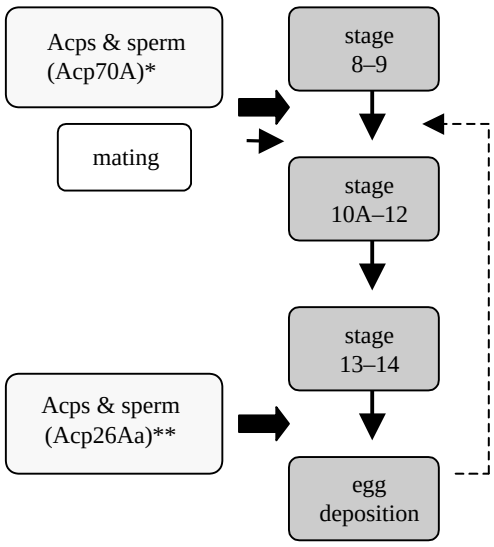


Figure 3. The path diagram showing the possible combined effects that mating and seminal fluid components and time after mating have on the number of oocytes at each oogenic stage, and on egg deposition. Male genotype: DTA-E or spermless wild-type. Time after mating was examined as two categories: short post-mating periods (1.5–6 h after mating), which include the beginning of egg deposition, and long post-mating periods (10–24 h after mating), when females are continually depositing eggs (all the other combinations of time categories that have been tested show the same result).

number of eggs deposited by ten eggs per mated female ($p < 0.0001$) over the 24 h examined (table 1). Thus, a high oogenic-progression rate is detectable in females mated to wild-type males as an increase in the number of oocytes at stages 10A–12 and a decrease in the number of oocytes at stages 13–14. The highest rate is seen in females that receive both Acps and sperm. Females that received Acps but not sperm showed twofold lower oogenic-progression and egg-deposition rates than females that received both. This observation supports the results in § 3(a) and § 3(c) that sperm are needed for enhanced egg-deposition and oogenic-progression rates.

Although females mated to DTA-E males had an oocyte-accumulation pattern similar to virgin females, path analysis revealed that the numbers of oocytes at stages 8–9 and stages 10A–12 in females mated to DTA-E



*Soller *et al*. 1997, 1999; **Heifetz *et al*. 2000

Figure 4. A model for mating and seminal fluid component regulation of oogenic-progression rate in a three-day-old mated female.

males were significantly different from those found in virgin females ($p < 0.0011$ and $p < 0.0007$, respectively, table 1). Mating to males that lack Acps and sperm increased the number of stage 8–9 oocytes by 2.76 oocytes per female and increased the number of oocytes at stages 10A–12 by 2.66 oocytes per female over the 24 h examined. In contrast, the number of stage 13–14 oocytes and the number of eggs deposited did not differ between the two groups. These results suggest that the act of mating and/or seminal fluid factors other than main-cell Acps and sperm can induce an initial enhancement in oogenic-progression rate. The number of stage 8–9 oocytes in DTA-E mated females is also greater than that found in mates of wild-type or spermless males (table 1). However, an increased number of oocytes at stages 10A–12 and a decreased number of stage 13–14 oocytes, both indicative of a high oogenic-progression rate, were not observed in mates of DTA-E males, suggesting that in the absence of Acps and sperm the initial enhancement is not maintained.

We also used path analysis to determine how each stage of oogenesis affected the subsequent stages in mated females over the 24 h examined (table 1). The number of stage 8–9 oocytes did not affect the number of oocytes at stages 10A–12, but had a small effect on the number of oocytes at stages 13–14 ($\beta = 0.6$, $p < 0.0001$) and increased slightly the number of eggs deposited ($\beta = 0.15$, $p < 0.023$). In contrast, the number of oocytes at stages 10A–12 and stages 13–14 had highly significant effects on the number of eggs deposited ($\beta = 0.78$ and $\beta = -0.53$, respectively, $p < 0.0001$). The fact that the number of stage 8–9 oocytes does not affect the number of oocytes at stages 10A–12 suggests that the number of stage 8–9 oocytes present in the ovarioles is unrelated to the initial increase in oogenic-progression rate, consistent with the idea that Acps, including Acp70A (Soller *et al*. 1997, 1999), have their initial effect on the control point between stages 8–9 and stages 10A–12. But a high or low oogenic-progression rate, as reflected in the number of oocytes at stages 10A–12, makes a significant contribution to egg deposition.

Time after mating had different effects on the numbers of oocytes in each oogenic stage. Time after mating had no effect on the number of oocytes at stages 8–9 and stages 10A–12 in all mated females (table 1). However, in all mated females, the number of stage 13–14 oocytes was significantly higher ($\beta = 3.48$, $p < 0.006$) at longer than at shorter post-mating periods. At longer post-mating periods females deposited 21.29 eggs per female ($p < 0.0001$) more than at shorter post-mating periods.

Taken together our data suggest that Acps and sperm are the main factors mediating enhanced oogenic progression. Egg deposition is also regulated by Acps and sperm but, in addition, it is influenced by time after mating and the number of mature oocytes.

4. DISCUSSION

We showed that seminal fluid proteins and sperm are both required to stimulate oogenic progression and egg deposition in *D. melanogaster* shortly after the start of mating. This extends the understanding of how mating affects oogenic progression in several ways. First, after mating, Acps can increase the oogenic-progression rate in females that were continually producing eggs at a low rate (this study) in addition to initiating oogenic progression in females that had largely arrested oogenesis (Soller *et al*. 1997, 1999). Second, by analysing for the first time the events during the first 24 h after mating, we demonstrated that both Acps and sperm exert their effects as early as 3 h after mating and that both sperm and Acps are required for maximal response at this time. Third, we showed that sperm, as well as Acps, are necessary to stimulate oogenesis.

Acps and sperm may directly affect the oogenic-progression rate, or may mediate it indirectly via feedback from their effect on oocyte release and egg deposition. Since we did not detect a correlation between the number of stage 8–9 oocytes and levels of egg deposition, our results strongly suggest that the progression from stages 8–9 to stages 10A–12 is not primarily mediated by feedback from egg deposition. Rather, Acps and sperm stimulate this progression. Path analysis allowed us to dissect further the effect of stage 8–9 oocytes on egg deposition, and we detected a very small (though significant) interaction between the number of stage 8–9 oocytes and the level of egg deposition. This implies that in addition to the primary effect of Acps and sperm on oogenic-progression rate, effects of Acps and sperm on oocyte-release or egg-deposition rates have a small contribution to oogenic-progression rate (figure 4). The act of mating and/or mating components other than main-cell Acps and sperm also appear to cause a small, transient enhancement of oogenic-progression rate, which is dependent upon Acps and sperm for maintenance.

Acps and/or sperm might regulate oogenesis by modulating juvenile hormone or ecdysteroid levels in mated females. *In vivo*, juvenile hormone titres are associated with effects on oogenic progression: while high titres of juvenile hormone appear to initiate oogenic progression (Bownes & Rembold 1987; Sliter *et al*. 1987), lower titres are required for it to continue (Bownes & Rembold 1987).

Acp70A can elevate juvenile hormone III-bisepoxide levels *in vitro* (Moshitzky *et al*. 1996). A role for ecdysteroids is suggested by the observation of Harshman *et al*. (1999) that the post-mating increase in whole-body titres of ecdysteroids is abolished in the absence of sperm and Acps. Soller *et al*. (1999) proposed that the balance between juvenile hormone and 20-hydroxyecdysone controls the initiation of oogenic progression. Further studies are required to determine whether juvenile hormones and ecdysteroids mediate the increase in oogenic-progression rate as well as maintaining a high oogenic-progression rate.

It is not known how sperm stimulate oogenic progression. Sperm transferred to *Drosophila* females enter special storage organs where they can be stored for up to two weeks until used for fertilization (reviewed by Gromko *et al*. 1984). Females mated to wild-type males can finish storing sperm by 1 h after mating (Tram & Wolfner 1999). We first detected an effect of sperm on oocyte-accumulation patterns and egg deposition by 3 h after mating. Our data cannot determine whether the 'sperm effect' is mechanical or is mediated via the innervation of the sperm-storage organs, via some substance released into the haemolymph from neurosecretory cells located in the sperm-storage organs or in their genital tract vicinity (Manning 1962, 1967; Davey 1965; Ruegg 1981), analogous to the 'spermathecal factor' of *Rhodnius prolixus* (Davey 1965; Ruegg 1981), or via release of molecules bound to sperm as proposed by Kubli (1992).

In summary, in a normal mating, sperm and main-cell Acps play important and complementary roles in mediating oogenic progression and egg deposition, and both Acps and sperm affect oogenic progression as early as 3 h after mating.

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REFERENCES

Boswell, R. E. & Mahowald, A. P. 1985 tudor, a gene required for the proper assembly of the germ plasm of *Drosophila melanogaster*. *Cell* **43**, 97–104.

Bownes, M. & Rembold, H. 1987 The titer of juvenile hormone during the pupal and adult stages of the life cycle of *Drosophila melanogaster*. *Eur. J. Biochem.* **164**, 709–712.

Cummings, M. R. & King, R. C. 1969 The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster*. I. General staging characteristics. *J. Morphol.* **128**, 427–441.

Davey, K. G. 1965 Copulation and egg-production in *Rhodnius prolixus*: the role of the spermathecae. *J. Exp. Biol.* **42**, 373–378.

Gromko, M. H., Gilbert, D. G. & Richmond, R. C. 1984 Sperm transfer and use in the multiple mating system of *Drosophila*. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 371–426. Orlando, FL: Academic Press.

Harshman, L. G., Lobe, A. M. & Johnson, B. A. 1999 Ecdysteroid titers in mated and unmated *Drosophila melanogaster* females. *J. Insect Physiol.* **45**, 571–577.

Heifetz, Y., Lung, O., Frongillo Jr, E. A. & Wolfner, M. F. 2000 The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Curr. Biol.* **10**, 99–102.

Hihara, F. 1981 Effects of the male accessory gland secretion on oviposition and remating in females of *Drosophila melanogaster*. *Zool. Mag.* **90**, 303–316.

Holzworth, K. W., Spector, C. & Gottlieb, F. J. 1974 Control of oocyte production, development, and release in *Drosophila melanogaster*. *Wilhelm Roux'Arch. Dev. Biol.* **174**, 260–266.

Kalb, J. M., DiBenedetto, A. J. & Wolfner, M. F. 1993 Probing function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proc. Natl Acad. Sci. USA* **90**, 8093–8097.

King, R. C. 1970 Cellular interactions during the development of the egg chamber. In *Ovarian development in* Drosophila melanogaster, pp. 149–188. New York: Academic Press.

King, R. C. & Sang, J. H. 1959 Oogenesis in adult *Drosophila melanogaster*. VIII. The role of folic acid in oogenesis. *Growth* **23**, 37–53.

King, R. C., Rubinson, A. C. & Smith, R. F. 1956 Oogenesis in adult *Drosophila melanogaster*. *Growth* **20**, 121–157.

Kubli, E. 1992 The sex-peptide. *Bioessays* **144**, 779–784.

Li, C. C. 1975 *Path analysis—a primer*. Pacific Grove, CA: Boxwood Press.

Lung, O. & Wolfner, M. F. 1999 *Drosophila* seminal fluid proteins enter the circulatory system of the mated female fly by crossing the posterior vaginal wall. *Insect Biochem. Mol. Biol.* **29**, 1034–1052.

Manning, A. 1962 A sperm factor affecting the receptivity of *Drosophila melanogaster* females. *Nature* **194**, 252–253.

Manning, A. 1967 The control of sexual receptivity in female *Drosophila*. *Anim. Behav.* **15**, 239–250.

Margaritis, L. H. 1985 Structure and physiology of the eggshell. In *Comprehensive insect physiology, biochemistry and pharmacology* (ed. L. I. Gilbert & G. A. Kerkut), pp. 153–230. Oxford, UK: Pergamon Press.

Moshitzky, P., Fleischmann, I., Chaimov, N., Saudan, P., Klauser, S., Kubli, E. & Applebaum, S. W. 1996 Sex-peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. *Arch. Insect Biochem. Physiol.* **32**, 363–374.

Neubaum, D. M. & Wolfner, M. F. 1999 Mated *Drosophila melanogaster* females require a seminal fluid protein, Acp36DE, to store sperm efficiently. *Genetics* **153**, 845–857.

Ruegg, R. P. 1981 Factors influencing reproduction in *Rhodnius prolixus* (Insecta: Hemiptera). PhD thesis, York University, Canada.

Sliter, T. J., Sedlak, B. J., Baker, F. C. & Schooley, D. A. 1987 Juvenile hormone in *Drosophila melanogaster*: identification and titer determination during development. *Insect Biochem.* **17**, 161–165.

Soller, M., Bownes, M. & Kubli, E. 1997 Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. *Eur. J. Biochem.* **243**, 732–738.

Soller, M., Bownes, M. & Kubli, E. 1999 Control of oocyte maturation in sexually mature *Drosophila* females. *Dev. Biol.* **208**, 337–351.

Spradling, A. C. 1993 Developmental genetics of oogenesis. In *The development of* Drosophila melanogaster, vol. 1 (ed. M. Bate & A. Martinez-Arias), pp. 1–70. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Tram, U. & Wolfner, M. F. 1999 Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. *Genetics* **153**, 837–844.

Verheyen, E. & Cooley, L. 1994 Looking at oogenesis. *Methods Cell Biol.* **44**, 545–561.

Wilson, T. G. 1982 A correlation between juvenile hormone deficiency and vitellogenic oocyte degeneration in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **191**, 257–263.

Wright, S. 1968 *Genetic and biometric foundations. Evolution and the genetics of populations*. University of Chicago Press.

Xue, L. & Noll, M. 2000 *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. *Proc. Natl Acad. Sci. USA* **97**, 3272–3275.