Seminal fluid causes temporarily reduced egg hatch in previously mated females

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In *Drosophila*, male accessory gland fluid (seminal fluid) has multiple effects on the female's reproductive efficiency. Here, we show the effect of seminal fluid on rate of egg hatch immediately following mating. Singly mated females were remated to two classes of sterile males, one with seminal fluid and one without seminal fluid. Transfer of seminal fluid results in a strong reduction in egg hatch shortly after the mating. Also, it is shown that remating with normal males causes an immediate reduction of egg hatch followed by recovery to normal egg hatch. In all cases, unhatched eggs contained no sperm. These results are consistent with a role for seminal fluid in sperm competition, mediated by incapacitation or inefficient use of resident sperm.

Keywords: *Drosophila*; sperm competition; seminal fluid

1. INTRODUCTION

Sexual selection in the form of sperm competition has been studied in many organisms including many insects, fishes, mammals and birds (Birkhead & Møller 1998). In general, sperm competition in *Drosophila* and other organisms results in the second male's sperm having an advantage over the first male's sperm in fertilization success (Prout & Bundgaard 1977). In *Drosophila melanogaster* the proportion of fertilizations by the second male's sperm (*P*₂) is approximately 0.80, but this figure varies considerably among strains of males (Clark *et al.* 1995) and of females (Clark & Begun 1998). The mechanism of high *P*₂ or 'sperm displacement' is known in only a few cases (reviewed in Harshman & Prout 1994). There is evidence in *Drosophila* that seminal fluid from the second male reduces the female's subsequent fertility when the second male transmits no sperm. Apparently the seminal fluid somehow incapacitates the first male's sperm, either by direct incapacitation or by making the female avoid use of that sperm (Harshman & Prout 1994). In this paper we confirm and extend this result by determining the egg hatch rate using two kinds of sterile males with and without seminal fluid.

2. MATERIAL AND METHODS

Two strains of spermless males that transmit seminal fluid were used. XO males were sons of females from a wild-type third-chromosome extracted line from Beltsville, MD, USA crossed to attached XY males. These XO males have accessory glands that produce normal seminal fluid, but they produce no motile sperm and are completely sterile (Kiefer 1966). In addition, male offspring of *tudor* females fail to produce germline cells, and are likewise spermless but produce normal seminal fluid (Schüpbach & Wieschaus 1986). The results using these males were compared with the results of remating with sterile DTA (which bear a diphtheria toxin transgene) males, which lack an accessory gland and thus produce no seminal fluid proteins (Kalb *et al.* 1993). A third-chromosome extracted line

(B3–09) from Beltsville, was used as a control with normal fertility.

In the experiments, each female was first mated to a B3–09 male and then kept in a vial with ten females. Three days later the singly mated females were divided into five treatments. Treatment 1 consisted of no further mating. In treatment 2 the females were placed singly into vials with two B3–09 males and watched until matings were completed, at which time the females were transferred to egg-collecting medium. Treatments 3, 4 and 5 were the same as treatment 2, except that females were allowed to remate with XO, *tudor* or DTA males, respectively. Within 1 h of mating, females were transferred into vials for egg collection. Eggs were collected on small plastic spoons with grape juice–yeast egg-collection medium (Ashburner 1989). Females were transferred to fresh egg-collecting spoons the next morning (18 h later) and allowed to oviposit for an additional 12 h. When females were removed, eggs were counted, and the hatch rate was determined by observation at 36–40 h post-laying. The entire experiment was repeated on two days that were two months apart.

In addition another experiment was done with rematings to wild-type males. The egg hatch rate was determined immediately after remating and again one or two days later. This experiment was done twice; once at Pennsylvania State University (Penn State) using B3–09 females, and again by H. Kross at University of California at Davis using a freshly caught wild-type line.

We determined whether the eggs that failed to hatch had been fertilized by placing unhatched eggs on a glass microscope slide immersed in PMS buffer, and examining them under Nomarski optics (S. Pitnick, personal communication). The refractive properties of the sperm tail make it readily apparent if present within the egg, and the sperm tail does not degrade (Pitnick & Karr 1998).

3. RESULTS

There were large differences in the mean egg hatch rates among the five treatments (table 1). Analysis of variance of arcsine square-root-transformed data showed that there was a significant variation in egg hatch when the same B3–09 females remated with males of the different treatments (table 2). The Tukey–Kramer method

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Table 1. Mean egg-hatch rates of B3-09 females following different mating protocols

treatment	N	hatch ($\pm$ s.e.)
no remate	78	0.945 $\pm$ 0.008
remate B3-09	47	0.885 $\pm$ 0.016
remate DTA	40	0.812 $\pm$ 0.032
remate tudor	58	0.611 $\pm$ 0.032
remate XO	71	0.558 $\pm$ 0.034

Table 2. Analysis of variance for angular-transformed egg-hatch rate

source	d.f.	MS	F	p
day	1	0.0012	0.01	0.904
treatment	4	3.1087	36.71	0.000
day $\times$ treatment	4	0.1351	1.59	0.176
error	284	0.0847	—	—
total	293	—	—	—

for unplanned posterior contrasts (Sokal & Rohlf 1995) showed that females that were singly mated, and those that remated with B3-09 or DTA males, had the same high hatching rate, females that had remated with tudor or XO males (spermless males that transmit seminal fluid) had a reduced hatch rate, and that within the group (singly mated, B3-09, DTA) and within the group (tudor, XO) there were no differences in egg hatch. This result implies that there was no reduction in egg hatch following remating either to the same strain or to males without seminal fluid, but reduced egg hatch is seen following mating to males with normal seminal fluid. In no case did an egg that failed to hatch have visible sperm inside, so the failure in egg hatch is caused by failure to fertilize the eggs.

The results of the two experiments with females remated to wild-type males comparing early hatch (day 1) with subsequent hatch (days 2 and 5) and with egg hatch rate of non-remating females are shown in table 3. The significance of results in table 3 were tested by treating the data as a $2 \times 2 \times 2$ contingency table for the Penn State data, and as a $3 \times 2 \times 2$ table for the Davis data, where the categories are day (1, 2 or 3), remating (yes or no), and hatching (yes or no). When hatching was considered as the response variable, the day $\times$ remating interaction was significant in both the Penn State ($\chi^2=3.94$, 1 d.f., $p<0.047$) and Davis data ($\chi^2=16.06$,

Figure 1. Egg hatch rates for each replicate of all five treatments. Small horizontal lines represent treatment means.

1 d.f., $p<0.0001$). Dropping day 3 from the Davis data, the day $\times$ remating interaction remains significant ($\chi^2=4.64$, 1 d.f., $p<0.031$). A consistent result from these two independent experiments using different strains of flies was a significant decrease in egg hatch immediately after remating, followed by a recovery in egg-hatch rate.

4. DISCUSSION

It is clear that the seminal fluid causes females to lay a greater proportion of unfertilized eggs, because males that transmit seminal fluid induce a temporary reduction in egg hatch, whereas males that lack seminal fluid do not. This may be either from ‘mistakes’ made by the female in her use of stored sperm, or the resident sperm may have been incapacitated. Examination of sperm within the unhatched eggs shows that the effect is not post-zygotic. These results are consistent with those of Gilchrist & Partridge (1995), who remated females to irradiated males a week after irradiation and which presumably had no sperm but had seminal fluid. Gilchrist & Partridge (1995) observed a sharp reduction in egg hatch which persisted.

These results also support the findings of Harshman & Prout (1994) who showed that rematings with two of these sterile males produced fewer offspring when remating with tudor compared with DTA. They also found that interrupting matings after 2 and 4 min, when only seminal fluid is transferred, caused a reduction in the number of offspring. Our data show that these results are caused by egg hatch. The immediate reduction in egg hatch when remating with normal males might be due to the early transfer of seminal fluid, which is then counteracted by the incoming fresh sperm.

The results here contradict those of Price *et al.* (1999), who found a reduction in offspring when females were

Table 3. Egg-hatch rate of females remating or not with wild-type males

location	strain	remate	day 1		day 2		day 3	
			N	hatch	N	hatch	N	hatch
Penn State	B3-09	yes	37	0.79	47	0.92	—	—
		no	35	0.96	74	0.94	—	—
Davis	wild	yes	784	0.87	496	0.95	672	0.96
		no	807	0.96	597	0.97	841	0.95

remated to XO males seven days after the first mating, but no reduction when remating to XO after two days. The reduced egg hatch here was found after three days. This difference could well be due to differences in female strains used, since large genetic differences among females in response to competing sperm have been reported (Clark & Begun 1998). Genetic differences among females in response to XO males should be studied. At this time we cannot reject the hypothesis that these laboratory differences are environmental or nutritional.

The slight initial reduction found in egg hatch after remating to normal males may result from an initial seminal fluid effect which is then overwhelmed by the abundance of fertile sperm. An important question yet to be answered is how the new sperm avoids the effect of its own seminal fluid. Gilchrist & Partridge (1995) showed there is no 'self-recognition' process because there was no difference in egg hatch between females remated to the same initial males compared with females remated to different males.

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REFERENCES

- Ashburner, M. 1989 *Drosophila: a laboratory manual*. p. 402. Cold Spring Harbor, NY: Cold Spring Harbor Press.
- Birkhead, T. R. & Möller, A. P. 1998 *Sperm competition and sexual selection*. San Diego, CA: Academic Press.
- Clark, A. G. & Begun, D. J. 1998 Genetic variation in female components of sperm displacement. *Genetics* **149**, 1487–1493.
- Clark, A. G., Aguadé, M., Prout, T., Harshman, L. G. & Langley, C. H. 1995 Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* **139**, 189–201.
- Gilchrist, A. S. & Partridge, L. 1995 Male identity and sperm displacement in *D. melanogaster*. *J. Insect. Physiol.* **41**, 1087–1092.
- Harshman, L. G. & Prout, T. 1994 Sperm displacement without sperm transfer in *Drosophila melanogaster*. *Evolution* **48**, 758–766.
- Kalb, J. M., DiBenedetto, A. J. D. & Wolfner, M. F. 1993 Probing the function of *Drosophila* accessory glands by directed cell ablation. *Proc. Natl Acad. Sci. USA* **90**, 8093–8097.
- Kiefer, B. 1966 Ultrastructural abnormalities in developing sperm of XO *Drosophila melanogaster*. *Genetics* **54**, 1441–1452.
- Pitnick, S. & Karr, T. L. 1998 Paternal products and by-products in *Drosophila* development. *Proc. R. Soc. Lond. B* **265**, 821–826.
- Price, C. S., Coyne, J. & Dyer, K. A. 1999 Sperm competition in *Drosophila melanogaster* involves displacement and incapacitation of stored sperm. *Nature* **400**, 449–452.
- Prout, T. & Bundgaard, J. 1977 Population genetics of sperm displacement. *Genetics* **85**, 95–124.
- Schüpbach, T. & Wieschaus, E. 1986 Germline autonomy of maternal-effect mutations altering the embryonic body pattern of *Drosophila*. *Dev. Biol.* **113**, 443–448.
- Sokal, R. R. & Rohlf, J. 1995 *Biometry*, 3rd edn, pp. 251–252. New York: Freeman.