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Glucose dehydrogenase is required for normal sperm storage and utilization in female *Drosophila melanogaster*

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Summary

Female sperm storage is a key factor for reproductive success in a variety of organisms, including *Drosophila melanogaster*. The spermathecae, one of the *Drosophila* sperm storage organs, has been suggested as a long-term storage organ because its secreted substances may enhance the quality of sperm storage. Glucose dehydrogenase (GLD) is widely expressed and secreted in the spermathecal ducts among species of the genus *Drosophila*. This highly conserved expression pattern suggests that this enzyme might have an important role in female fertility. Here, we examine the function of GLD in sperm storage and utilization using *Gld*-null mutant females. The absence of GLD reduced the amount of sperm stored in the spermathecae and led to a highly asymmetrical sperm distribution in the two spermathecal capsules of the mutant females. The storage defect was especially severe when the mutant females were crossed to a *Gld*-mutant male that had previously mated a few hours before the experimental cross. Under this mating condition, the mutant females stored in the spermathecae only one-third of the sperm amount of the wild-type control females. In addition, the mutant females used stored sperm at a slower rate over a longer period compared with wild-type females. Thus, our results indicate that GLD facilitates both sperm uptake and release through the spermathecal ducts.

Key words: sperm storage, sperm utilization, spermathecae, glucose dehydrogenase, *Drosophila melanogaster*.

Introduction

Sperm storage and usage directly affect the fitness of many organisms, including *Drosophila melanogaster*. Sperm that are transferred during mating are stored in specialized organs of *Drosophila* females and continuously used for 2–3 weeks after mating. Thus, female sperm uptake, storage and utilization play important roles in reproductive success.

In *D. melanogaster*, of the several thousands of sperm transferred to a female in one mating (Gilbert, 1981b), some are immediately used or stored for later use, but the majority are simply discarded. Because storage capacity is limited, females normally store only ~20–25% of the transferred sperm in two types of sperm storage organs: the spermathecae and the seminal receptacle (Lefevre and Jonsson, 1962; Fowler, 1973; Gilbert, 1981b). Immediately after mating, ~80% of the stored sperm are found in the seminal receptacle, a coiled tube-like organ (Lefevre and Jonsson, 1962; Fowler et al., 1968). The rest of the stored sperm (~20%) are kept in the spermathecae, a pair of capsules each connected to the anterior end of the uterus by a thin duct. Of the two types of organ, the spermathecae of *Drosophila* is considered as the long-term storage organ (Filosi and Perotti, 1975; Gilbert, 1981a). In contrast to the seminal receptacle, the spermathecae of all *Drosophila* are surrounded by glandular cells that secrete fluid into the lumen of the capsules (Filosi and Perotti, 1975). It has been suggested that the secretory fluid may enhance sperm longevity in spermathecae (Filosi and Perotti, 1975; Pitnick et al., 1999). Indeed, females lacking spermathecae have a shorter period of fertility and produce fewer progeny during the fertile period (Anderson, 1945; Boulétreau-Merle, 1977).

One of the molecules known to be required for sperm storage is Acp36DE, a male seminal fluid protein secreted in the male accessory gland and transferred to the female during mating (Neubaum and Wolfner, 1999). Females that mate with a male having a deficiency in accessory gland secretion store only 10% of normal storage levels in both the seminal receptacle and the spermathecae (Tram and Wolfner, 1999). However, the mechanisms of the female contribution to sperm storage are largely unknown. Here, we investigate the role of glucose dehydrogenase (GLD), one of the proteins secreted into the lumen of the spermathecal ducts of *D. melanogaster* females (Schiff et al., 1992).

GLD is expressed in a variety of arthropods. This enzyme is required for eclosion of adult fruit flies, and its expression in the reproductive tracts at the adult stage is conserved among 50 *Drosophila* species previously examined (Schiff et al., 1992). It has been suggested that the production of free radicals in the GLD-involved pathway may be responsible for weakening the puparium case during eclosion (Cox-Foster and

Stehr, 1994), but the catalytic mechanism of GLD in the reproductive organs is unknown. The expression level of *Gld* during development is regulated by ecdysone, which is released during larval molts (Murtha and Cavener, 1989). Throughout pre-adult development, *Gld* is expressed in many epidermally derived tissues, including some of the somatic reproductive organs (Cox-Foster et al., 1990). However, at the adult stage, *Gld* expression is completely absent in non-reproductive tissues and restricted to only a subset of those reproductive organs that express *Gld* at the pre-adult stage (Schiff et al., 1992; Keplinger et al., 2001).

Although adult female expression patterns in different species vary in the oviduct, seminal receptacle and parovaria, the expression of *Gld* in the spermathecae is highly conserved (Schiff et al., 1992; Fig. 1). This may underlie a fundamental function of *Gld* for sperm storage in the spermathecae of the females. In addition, male flies of the melanogaster subgroup, including *D. melanogaster*, secrete high levels of GLD into the lumen of the ejaculatory duct and subsequently transfer it to females during copulation. Substances transferred to a female along with sperm have been shown to have diverse functions in insects. In addition to sperm storage (Neubaum and Wolfner, 1999; Tram and Wolfner, 1999), these functions include nutrient supplementation, reduction of a female's receptivity to subsequent mating, protection of females and eggs from predators, enhancement of oviposition (reviewed by Chapman, R. F., 1998; Chapman, T., 2001; Gillott, 2003) and sperm competition (e.g. Scott and Richmond, 1990; Harshman and Prout, 1994; Price et al., 1999; Gilchrist and Partridge, 2000). The conserved expression pattern in the spermathecae and the transfer of GLD to females in some species suggest that GLD may enhance female fertility by influencing sperm storage in the spermathecae.

We hypothesized that if GLD enhances sperm storage, then *Gld*-null mutant females would have reduced fertility. A preliminary experiment showed that there was no significant difference in fertility between *Gld*-null mutant and wild-type females when virgin males were used for crosses. However, consecutive mating might have a significant effect on female fertility due to a reduced amount of sperm and seminal proteins transferred from a male. For example, the complete recovery of the GLD level in males after one mating takes more than 24 h, and even at 12 h post-mating the GLD activity level in males is still half of the pre-mating level (Cavener and MacIntyre, 1983). We speculate that this probably applies to other seminal proteins transferred during mating and that GLD may facilitate sperm storage under conditions of low sperm load where the amount of sperm and seminal proteins transferred is not saturating the sperm storage organs of females. These conditions are probably common in the natural ecology of *Drosophila*.

Here, we examine sperm storage and usage in *Gld*-null mutant and heterozygous females upon mating to virgin males or once-mated males. We show that GLD may facilitate the sperm uptake and release through the spermathecal ducts. The sperm distribution in the two capsules of the spermathecae was highly asymmetrical in the absence of GLD, and the quantity of the stored sperm in the spermathecae was significantly reduced in the *Gld*-null mutant females when they were crossed with a previously mated male. In addition, the *Gld*-null mutant females used stored sperm at a slower rate over a longer period compared with wild-type females, suggesting that GLD might also be required for efficient release of sperm from the spermathecae.

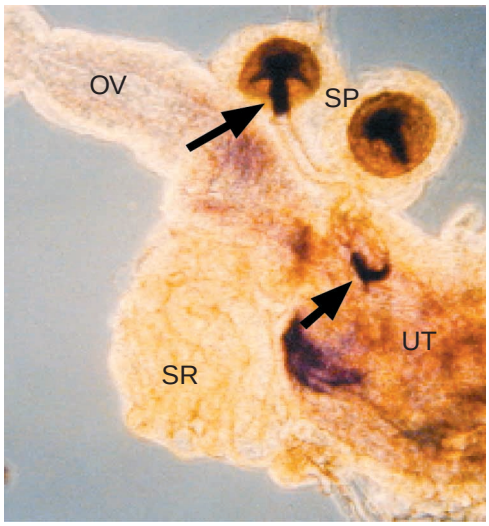

Fig. 1. A representative glucose dehydrogenase (GLD) expression pattern in adult *D. melanogaster* female reproductive organs. OV, oviduct; SP, spermathecae; SR, seminal receptacle; UT, uterus. Two arrows point to the GLD expression in the proximal and distal ends of the spermathecal ducts.

Materials and methods

Drosophila strains

For the sperm storage comparison, we compared homozygous *Gld–/–* (*Gld[3689]/Gld[P4L]*) virgin females *versus* heterozygous *Gld+/–* (*Gld[3689]/TM3* or *Gld[P4L]/TM3*) virgin females mated to homozygous *Gld–/–* virgin or previously mated males. Both *Gld[P4L]* and *Gld[3689]*, which carry a point mutation and a P-element insertion, respectively, are loss-of-function mutations. The heteroallelic combination of these two mutations was used as the homozygous null (*Gld–/–*) flies in these experiments in order to mask other potential recessive traits on the mutagenized third chromosomes. To minimize environmental and developmental differences, our experimental design utilized sibling *Gld–/–* and *Gld+/–* females that were generated by mating between *Gld* mutant stocks balanced over the TM3 chromosome (which inhibits recombination and ensures the maintenance of recessive lethal *Gld* mutant alleles).

For the sperm usage comparison, virgin females of *Gld–/–* (*Gld[4.11]/Gld[P4L]*) and *Gld+/–* (*Gld[4.15]/Gld[P4L]*) were crossed with *Gld–/–* virgin males (*Gld[E1L]/Df dsx[M+R2]*). *Gld[4.15]* carries a copy of the *Gld* gene but shares the same genetic background as *Gld[4.11]*, which carries a null mutation

of *Gld*. The *Df dsx[M+R2]* mutation is a deletion of the entire *Gld* gene. The *Gld–/–* mutants, which are normally unable to eclose, were rescued by cutting open their puparium cases as late pharate adults. All flies used were 3–7 days old and thus fully competent for mating and reproduction.

Sperm storage comparison

In order to reduce the load of both sperm and seminal fluid proteins, we crossed the same *Gld* mutant male twice consecutively to different virgin females. Immediately after the first mating, the male was transferred to a fresh vial and placed with another virgin female for the second mating. The mean time interval between the first and second matings was 102 min, and the median was 70 min (see Results).

At the end of each mating, the male was removed from the vial and, at 1–2 h after mating, the seminal receptacle and spermathecae of the female were dissected in 60% acetic acid. Each organ was placed onto a slide (seminal receptacle was uncoiled) and stained with 2% orcein in 60% acetic acid (Lefevre and Jonsson, 1962; Gilbert, 1981b). The organs were pressed gently under a cover slip; the spermathecae were squeezed until the capsules were opened. The number of sperm was counted twice for each sample under a light microscope. Samples that had no sperm in any of the storage organs were eliminated from the analysis because it indicated that no sperm transfer had occurred during mating.

For the analysis of sperm distribution in the two spermathecal capsules, we divided the capsules into two classes for each female: a 'min' capsule (containing fewer sperm) and a 'max' capsule (containing more sperm). A χ^2 test was performed to test a departure of the sperm distribution in the two capsules from the expected 1:1 ratio. The females containing the small number of sperm in the spermathecae were pooled to achieve the appropriate expected frequency for a χ^2 test (>5).

Sperm usage comparison

Virgin females of *Gld–/–* and *Gld+/–* were crossed with virgin *Gld–/–* males. Immediately after mating, a female was transferred to a fresh vial and allowed to oviposit for 2 days. The females were continuously transferred to a fresh vial every other day until they completely stopped producing progeny. The number of fully developed adult flies was counted for each vial. The samples that had fewer than 100 total progeny were eliminated from the analysis because it may indicate that the sperm transfer was not complete or that the female reproductive organs might be defective and because low density of larvae in culture is correlated with the high mortality.

Results

Experimental design

To obviate the potential confounding effects of GLD transferred from males to females during mating, we exclusively used *Gld*-null mutant males. All of the *Gld*-null mutant females and males were homozygous for complete loss-of-function mutations (see Materials and methods). Female flies heterozygous for *Gld* (*Gld[3689]/TM3* or *Gld[P4L]/TM3*) were used as controls because they are phenotypically indistinguishable from wild-type (Cavener and MacIntyre, 1983; Cavener et al., 1986); we refer to these heterozygous females as wild-type throughout the rest of this study. No significant differences were observed in reproductive parameters (discussed below) between the *Gld[3689]/TM3* and *Gld[P4L]/TM3* heterozygous females and therefore data from them were pooled.

Both mutant and wild-type females were crossed either with a virgin male (first mating) or with a previously mated male within a few hours of the first mating (second mating). The latter cross was set up to examine the effect of reducing the amount of sperm and seminal proteins transferred to females. The second mating typically occurred within 2 h of the first mating [71% (15 of 21) in the *Gld–/–* and 64% (7 of 11) in *Gld+/–* females], and neither genotype group showed any correlation between the length of mating intervals and the amount of sperm stored in the second mating samples. Therefore, we included all second mating data in our analyses.

Sperm storage

In the first mating, the *Gld–/–* females stored a similar amount of sperm compared with the wild-type females (Table 1). In the second mating, although both genotypes showed a reduction in sperm storage to some extent, the spermathecal storage in the *Gld–/–* females was severely reduced by 76.7% on average compared with an average reduction in the wild-type females of only 19.8%. The number of sperm stored in the spermathecae of the *Gld–/–* females (mean ± S.E.M., 28±8 sperm) was significantly lower than the number observed in the wild-type females (89±13 sperm; *t*-test, P=0.00018; Table 1). It should be noted that the amount of sperm stored in the seminal receptacle was not significantly different between the *Gld–/–* and wild-type females. This indicates that the absence of GLD may significantly affect the spermathecal storage in the second mating.

Distribution of sperm stored in the two spermathecal capsules

When further examining the difference in spermathecal storage between the *Gld–/–* and *Gld+/–* females, we first noticed that there were many cases where one of the two capsules contained zero sperm in the *Gld–/–* females following the second mating whereas there was no such case found in the wild-type female of the second mating. After eliminating two cases that had zero sperm count in both capsules, 11 of 19 *Gld–/–* females following the second mating contained zero sperm in one of the two capsules. Remarkably, in three cases, no sperm were found in one capsule but more than 90 sperm were found in the other.

This indicated that the ratio of sperm storage between the two capsules of the *Gld–/–* females grossly departed from the expected 1:1 ratio. To analyze this storage asymmetry further, we performed a χ^2 test on each female with the expected ratio

Table 1. *Comparisons of sperm storage in* Gld–/– *and* Gld+/– *females from first and second matings*

	Mean number of sperm (± S.E.M.)				*P* value (*t*-test)	
	1st mating		2nd mating		*Gld–/– vs Gld+/–*	
	Gld–/–	*Gld+/–*	*Gld–/–*	*Gld+/–*	1st mating	2nd mating
Spermathecae	120±14	111±15	28±8	89±13	NS	0.00018 (*Gld–/–* ↓)
Seminal receptacle	475±26	386±17	369±35	357±24	0.046 (*Gld+/–* ↓)	NS
Total storage	594±36	497±25	397±39	446±32	NS	NS

The mean numbers of sperm counted in the spermathecae and seminal receptacle (± S.E.M.) are shown on the left side of the table. The samples are classified into four groups: *Gld–/–* from 1st mating (*N*=32); *Gld–/–* from 2nd mating (*N*=21); *Gld+/–* from 1st mating (*N*=13); *Gld+/–* from 2nd mating (*N*=11). The right side of the table shows the results of a Student's *t*-test, which was performed for a comparison of *Gld–/– vs Gld+/–* (within the same mating classes). The arrow (↓) indicates which group had a significant decrease at the shown *P* value. NS, not significant.

Table 2. *Comparisons of the number of sperm stored in the two spermathecal capsules*

	1st mating				2nd mating			
	Gld–/–		*Gld+/–*		*Gld–/–*		*Gld+/–*	
No. of sperm	Min capsule	Max capsule	Min capsule	Max capsule	Min capsule	Max capsule	Min capsule	Max capsule
0–20	16	4	3	2	21	12	2	0
21–50	7	3	5	1	0	6	7	7
51–100	7	16	5	8	0	2	2	4
101–150	2	8	0	2	0	1	0	0
151–200	0	1	0	0	0	0	0	0
Total	32	32	13	13	21	21	11	11
No. of test groups departed from the 1:1 ratio	22/29 (0.76)		4/12 (0.33)		9/11 (0.82)		3/11 (0.27)	

The two capsules of the same fly were divided into two classes: 'min' (capsule containing fewer sperm) and 'max' (capsule containing more sperm). When both capsules contain a similar number of sperm, the frequency distributions of the sperm amount should be similar between the two capsules. The table shows the number of females that contained the indicated range of sperm number for each capsule class as well as the results of χ^2 tests on the ratio of sperm counts in the two capsules. The χ^2 results are shown as (no. of female flies or groups whose spermathecal storage had a significant departure from the expected 1:1 ratio at $P \leq 0.05$)/(no. of total test females or groups), and the number in parentheses is a frequency of the females that showed a departure from 1:1. Females that contained fewer than total 10 sperm in the spermathecae were pooled as one group to achieve an appropriate expected frequency of a χ^2 test (>5).

of 1:1. In both first and second matings, more than 75% of the *Gld–/–* females had a significant departure from the 1:1 ratio in the spermathecal storage at $P=0.05$ (Table 2). By contrast, only ~30% of the wild-type females showed a significant departure from the 1:1 ratio.

Although the *Gld–/–* females exhibited a marked asymmetry in spermathecal storage following the first mating, the sperm amounts in both capsule classes (min and max) were not significantly different from those in the wild-type females. However, in the second mating, both capsule classes had a significant reduction in the *Gld–/–* females (min capsule mean, 3±1 sperm; max capsule mean, 28±8 sperm) as compared with the wild-type females (min, 35±7 sperm; max, 53±8 sperm). Thus, GLD affects the sperm distribution between the two spermathecal capsules in both first and second matings, whereas the reduction in the quantity of stored sperm is significant only in the second mating.

Sperm utilization

Gld–/– mutant *Drosophila* males and females are fertile (if rescued), and female mutants do not exhibit any reduction in the number of stored sperm after mating to a virgin male. However, reproductive success may also be impacted by sperm utilization, which is reflected in the number of progeny resulting from a mating. To examine further the role of GLD in sperm usage of the females following the first mating, we counted the number of progeny produced by the mutant and wild-type females in 2-day periods after mating until the stored sperm was completely exhausted. Interestingly, we found that, on average, the mutant females produced offspring over a

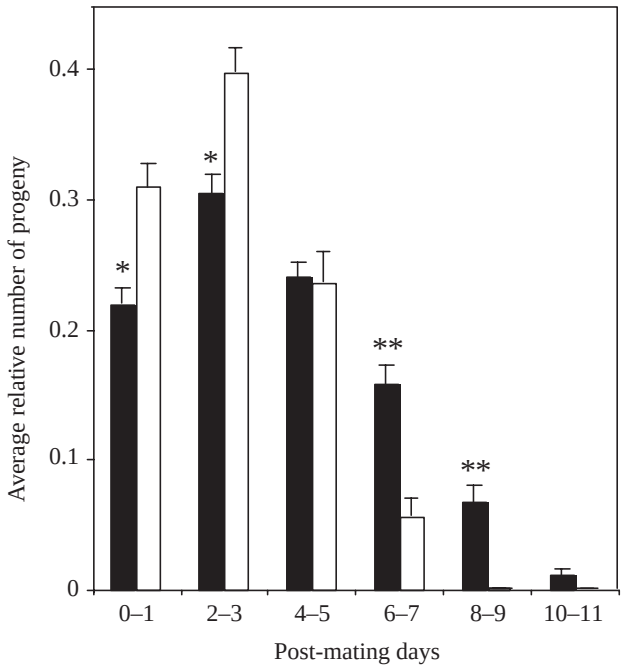

Fig. 2. Temporal distributions of progeny production by *Gld*–/– and *Gld*+/– females following the first mating. The *y*-axis shows the average relative number of progeny produced in a two-day period [the average of (no. of progeny produced in a two-day period)/(total no. of progeny produced over 11 days)]. The difference between the two genotypes was tested by *t*-test at each time point (*P<0.001; **P<0.0001). Filled bars, *Gld* mutant (N=24); open bars, wild-type control (N=23). The averages of the total progeny number are 410 (*Gld*–/–) and 291 (*Gld*+/–).

longer period (11 days) than the wild-type females (7 days) due to a reduced rate of sperm usage in the mutant females (Fig. 2). This suggests that, in addition to its role in sperm storage in the spermathecae, GLD may enhance the release of sperm from the spermathecae.

Discussion

Sperm storage defects in the spermathecae of Gld-null females

Drosophila deficient for GLD are unable to eclose from their puparium case (Cavener and MacIntrye, 1983) but, if rescued, they are nonetheless fertile. Moreover, we show herein that *Gld*–/– mutant females are capable of storing normal levels of sperm in their seminal receptacles and spermathecae under conditions of saturating sperm and seminal protein concentrations associated with mating to virgin males. However, the number of sperm stored in the spermathecae of the mutant females decreased by 76.7% when crossed to once-mated males, ostensibly lowering the concentration of sperm that could be potentially stored. By contrast, only a 19.8% reduction was seen in wild-type females following such matings. No substantial differences between genotypes were observed in the number of sperm stored in the seminal receptacles in the second mating compared with the first. The restricted impact on the spermathecae of ablating GLD expression is expected because the GLD enzyme is present in the proximal and distal ends of the spermathecal ducts in mature adult females whereas it is absent in the seminal receptacle. The total number of sperm stored in both organs is not significantly different owing to the predominance of the much larger sperm storage capacity of the seminal receptacle. Although this would seemingly argue that the observed genotypic differences in the spermathecae are unimportant, these two types of organs may differ substantially with respect to sperm utilization and longevity (Filosi and Perotti, 1975; Pitnick et al., 1999). Moreover, the spermathecae are considerably more conserved in structure across the *Drosophila* genus and related genera compared with the seminal receptacle (Schiff et al., 1992; D. R. Cavener, unpublished observations), arguing that the spermathecae play a central role in sperm storage, at least in *Drosophila*.

Asymmetry of sperm storage in the two spermathecal capsules

GLD may also facilitate an equal distribution of sperm into the two spermathecal capsules. Asymmetry in the number of sperm stored in the two capsules has been investigated in Mediterranean fruit flies *Ceratitis capitata* (Taylor et al., 2001). According to this study, the degree of asymmetry in the Mediterranean fruit flies becomes more apparent when the total sperm storage is smaller. However, in our study of *D. melanogaster*, there is no correlation between total sperm storage and the percent difference in the sperm amount stored in the two capsules of the *Gld* mutant females. In fact, asymmetry was observed even in the mutant females from the first mating, when the quantity of both spermathecal and total sperm storage was at a level comparable with that of wild-type females. Therefore, we conclude that the observed asymmetrical distribution of sperm is due to the absence of GLD and is not a secondary effect of the level of total sperm storage. We propose that GLD may facilitate the initial catalysis of sperm uptake, and thus, in the absence of GLD, the probability that sperm uptake would be initiated in a particular spermathecae would be dramatically decreased. Moreover, we propose that this initiation event is controlled by the localized expression of GLD in the distal end of each spermathecal duct adjoining the uterus, and therefore initiation of sperm uptake in the two spermathecae would be independent of each other, giving rise to the observed asymmetry in sperm storage in the *Gld*–/– mutant female.

Effects of consecutive mating

GLD may be the main factor regulating sperm distribution into the two spermathecal capsules because we observed the asymmetrical storage in both first and second matings in the absence of GLD. By contrast, the quantity of spermathecal storage was affected when females mated with males that had previously mated within a few hours. This indicates that GLD may enhance sperm uptake in a synergistic way with other factors affected by consecutive mating, such as the amount of sperm and seminal fluid proteins transferred from the male to

the females. However, Lefevre and Jonsson (1962) reported that the numbers of progeny produced from the first and second consecutive matings were similar and that a significant reduction in progeny can be first observed at the third consecutive mating. Their data are consistent with our results that the total sperm storage was not significantly different in the wild-type females following first and second matings. There are two possible explanations for little difference between the first and second matings. One is simply that the transfer load is not different between the first two matings. The other possibility is that the transfer load does change but that the change is not reflected in the amount of stored sperm or resultant progeny due to a limited sperm storage capacity (the average storage is ~1000 sperm; Gilbert, 1981b; Tram and Wolfner, 1999). Because a male transfers 3000–5000 sperm to a female in one mating (Kaufmann and Demerec, 1942; Gilbert, 1981b), even a 50% reduction in the number of sperm transferred does not impact on the amount of sperm stored in a wild-type female. Therefore, it is possible that transfer load decreases in the second consecutive mating to a level that is still beyond the maximum storage capacity. Moreover, the complete recovery of seminal proteins in males after one mating may take hours, as it takes at least 24 h for the male GLD level to be completely recovered after one mating (Cavener and MacIntyre, 1983). Thus, the 'invisible' reduction of sperm and also a possible great reduction of seminal fluid proteins in a consecutive mating could make sperm storage significantly less efficient in the absence of GLD. We speculate that this level of reduction in transfer load is common in nature and that GLD may be critical to maintain the efficiency of reproduction in *Drosophila*.

Males of *D. melanogaster* transfer GLD to females upon mating. Therefore, we would expect that crossing a *Gld*-null female to a wild-type male would restore the spermathecal storage level and storage symmetry. It would be interesting to see if consecutive mating has the same effect on sperm storage following this cross. Because consecutive mating decreases the amount of GLD transferred to a female, spermathecal storage might still be affected in *Gld*-null females following the second or third matings as compared with the control females that express GLD.

The slower rate of sperm utilization in Gld-null females

The second process that may be enhanced by GLD is sperm release from the spermathecae. The total numbers of resultant progeny and initially stored sperm following the first mating were not substantially different between the mutant and wild-type females, but the sperm usage pattern in fertilization was distributed over a longer period of time in the mutant females from the first mating. This suggests that GLD may control the timing of sperm usage by enhancing the release of sperm from the spermathecae, independent of a synergistic effect with transfer load. It is likely that this control of timing could occur only for the sperm stored in the spermathecae because GLD is not expressed in the seminal receptacle in adult females of *D. melanogaster* (Schiff et al., 1992). Our study showed that the mutant females produced 41% more progeny on average than wild-type females, and the observed delay in the exhaustion of sperm in the mutant females could be explained by taking a longer time to use up a larger amount of sperm. However, when we limited the data to a subset that had 200–400 progeny in total, we still observed the same trend in which the mutant females (N=9) took 11 days on average to exhaust stored sperm whereas the wild-type females (N=19) took only 7 days. Thus, we conclude that the observed slower rate in sperm usage is due to the absence of GLD rather than the size of total storage. We propose that the release of sperm from the spermathecae may be controlled or enhanced by the GLD expressed in the proximal end of the spermathecal ducts, while the aforementioned sperm uptake event is controlled by the GLD in the distal end of the ducts. Changes in sperm utilization in wild populations could obviously impact reproductive success given the temporal and spatial uncertainties in finding mates and suitable ovipositioning sites.

GLD contains a highly conserved signal peptide (Krasney et al., 1990; K. Iida and D. R. Cavener, unpublished data), and its expression and function indicate that GLD participates in important extracellular catalysis. The enzymatically active form of GLD is detected in molting fluid of pupa (Cox-Foster et al., 1990) and in the lumen of the spermathecal ducts and of the male ejaculatory duct (Schiff et al., 1992). GLD in molting fluid is required to weaken a puparium case structurally in advance of eclosion, which relies upon the imago to force open the operculum. Cox-Foster and Stehr (1994) suggest that GLD is involved in generating free radicals that help degrade the old curricular matrix. However, the biochemistry of GLD in the adult reproductive organs is unknown. We speculate that it alters the extracellular environment of the spermathecal ducts to affect a change in sperm motility. An analogous claim has been made for *esterase-6*; a null mutation of *esterase-6* causes a slower rate of sperm loss and sperm storage (Gilbert, 1981a), which is similar to the phenotype observed in *Gld*-null mutants. Further experiments are required to elucidate the physiological mechanism of GLD function in sperm trafficking.

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