Sexual Hyperactivity and Reduced Longevity of *dunce* Females of *Drosophila melanogaster*

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ABSTRACT

The *dunce* gene of *Drosophila melanogaster* codes for a cyclic adenosine-3′,5′-monophosphate-specific phosphodiesterase. Mutations of *dunce* alter or abolish the activity of this enzyme, produce elevated cAMP levels, cause recessive female sterility, and produce learning deficiencies in both sexes. Aberrant male sexual behavior has also been associated with the memory defects of *dunce* mutants. Here we show that the longevity of *dunce* mutant females, homozygous for null-enzyme alleles, is reduced by 50% in the presence of males compared to control *dunce* females kept without males. Mutant *dunce* females mate on average every 14 hours whereas wild type revertants of *dunce*, and Canton-S females, mate every 22–24 hr. We propose a cause-effect relationship between mating and reduced longevity. Pheromones or peptides transferred during mating may activate adenylate cyclase and create an increase in cAMP levels that cannot be damped in *dunce* females. This increase may affect basic physiological functions and lead to reduced longevity.

THE *dunce* gene of *Drosophila melanogaster* is located in chromomere 3D4 of the *X* chromosome. Mutations of *dunce* alter or abolish cAMP-specific (form II) phosphodiesterase activity (BYERS, DAVIS and KIGER 1981; KIGER and SALZ 1985). The dnc^1 allele is responsible for the production of a thermolabile form II enzyme, and dnc^2 produces an enzyme with an altered K_m (DAVIS and KIGER 1981; KAUVAR 1982). These observations together with sequencing data of the *dunce* gene indicate that *dunce* is the structural gene for the form II phosphodiesterase (CHEN, DENOME and DAVIS 1986).

Mutant alleles (as well as duplications and deficiencies of *dunce*), cause measurable changes in the cAMP content of whole flies (DAVIS and KIGER 1978, 1981). Two abnormalities exhibited by mutant *dunce* flies immediately became objects of study: deficiencies in associative and nonassociative learning in both sexes and sterility in females (QUINN and GREENSPAN 1984; KIGER and SALZ 1985). Additional abnormalities involving male sexual behavior have been attributed to memory deficiency (GAILEY, JACKSON and SIEGEL 1984). The apparent absence of other overt defects accompanying what would seem to be a profound biochemical alteration, producing a five- to sixfold increase in cAMP concentration in the null mutants, is surprising (DAVIS and KAUVAR 1984; LIVINGSTONE, SZIBER and QUINN 1984; LIVINGSTONE 1985).

We have searched for other specific functions of the *dunce* gene and have identified at least five new defects in *dunce* mutants. Three of these defects underlie the female sterility phenotype. First, null mutant females are unable to lay eggs due to a somatic cell defect. Second, *dunce* females exhibit a germ line-specific maternal defect that blocks development of the zygote (H. BELLEN *et al.*, unpublished data). Both defects can be partially suppressed by mutations in the *rutabaga* gene affecting adenylate cyclase activity (LIVINGSTONE, SZIBER and QUINN 1984; H. BELLEN *et al.*, unpublished data). The cAMP concentrations in abdomens of females homozygous for a *dunce* allele and a *rutabaga* allele, *e.g.*, dnc^{M11} rut^1, are intermediate between wild type and *dunce*. Third, double mutant females lay eggs and produce a few progeny. About 20% of the progeny of these females exhibit severe morphological defects. These defects are the result of a maternal effect that is not rescuable by wild-type sperm. Hence, *dunce* plays, in addition to the somatic cell and germ line cell defects, an important role in embryogenesis (H. BELLEN *et al.*, unpublished data).

Two additional defects of *dunce* involve behavior and are the subject of this report. Mutant *dunce* females mate more frequently than normal females, and a single mating event significantly reduces the longevity of *dunce* females. We have measured the average time between copulations for *dunce* females and normal females, based on continuous observation for 7 days. Our results show that *dunce* females mate about twice as frequently as wild-type females. Repeated mating is proposed to be the cause of the reduction in lifespan of 50%. Our observations show that the *dunce* gene is required for several apparently unrelated physiological functions and that it plays an important role in longevity and female behavior.

MATERIALS AND METHODS

Mutations and balancer chromosomes are described in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM

TABLE 1

Stocks

Stocks	Comments	Source
Stocks with *dunce* null alleles		
y dnc^{M14} ec f/FM7		SALZ, DAVIS and J. KIGER (1982)
y w dnc^{M14} v f/FM7		H. K. SALZ
y dnc^{M11} cv v f/FM7		MOHLER and CARROL (1984)
y dnc^{M14} ec rut^{2}/FM7	*rut^{2}* is an EMS induced allele of *rutabaga*	H. BELLEN, B. GREGORY and J. KIGER (unpublished data)
y dnc^{M14} ec rut^{3}/FM7	*rut^{3}* is an EMS induced allele of *rutabaga*	H. BELLEN, B. GREGORY and J. KIGER (unpublished data)
Stocks with hypomorphic, revertant or wild-type alleles		
y w dnc^{1} v f/FM7	*dnc^{1}* refers to the *dnc^{1} cf^{1}* haplotype *dnc^{1}* allele encodes a PDE that exhibits about 50% of wild-type activity	SALZ and KIGER (1984)
y dnc^{+M14R1} ec f/ FM7	*dnc^{+M14R1}* is a fertile, spontaneous revertant isolated from the *y dnc^{M14} ec f/FM7* stock. Exhibits wild-type PDE activity	This study
y dnc^{+M14R2} ec f/ FM7	*dnc^{+M14R2}* is an EMS induced revertant, is fertile and exhibits 65% of wild-type PDE activity	This study
y dnc^{+} cv v f	Stock mutagenized to produce the *dnc^{M14}* and *dnc^{M11}* alleles	MOHLER (1977)

(1985). The stocks used are listed in Table 1. To relate abnormalities to the *dunce* locus itself, we used two revertant alleles of *dnc^{M14}* isolated from the *y dnc^{M14} ec f/FM7* stock: *dnc^{+M14R1}* and *dnc^{+M14R2}* (see Table 1).

All assays (except the mating frequency assay) were performed in incubators at 25 ± 1° with a 12-hr light, 12-hr dark cycle. CO_2 was used for the manipulations requiring anesthesia. In all assays females were scored as dead when no movement was observed for 30 seconds after shaking the vial.

Longevity Assays in the Absence of Males: One or five female virgins, 12–36 hr old, were kept in vials for 7 days in an incubator. At days 3 or 4 the flies were transferred to fresh food. The numbers of dead females were counted on day 7.

Longevity Assays in the Presence of Males. Single virgin females, 12–36 hr old, were placed with three Canton-S males, 3–5 days old, in a vial. At days 3–4, flies were transferred to fresh food. Dead females were counted at day 7 and surviving females were discarded. Vials were checked for the presence of eggs at day 7 and the progeny were counted on day 17.

Longevity after a Single Mating: Two-day-old, single *dunce* virgin females were placed with three Canton-S males in 8-dram vials. Males were removed immediately after mating. At the same time, vials containing a female that was courted but did not mate were cleared of males. These females served as controls.

Longevity Assays of Females with Varied Exposures to Males: We used a procedure similar to that described by HOFFMANN and HARSHMAN (1985). Five virgin females, 12–36 hr old, were put in a 20-ml scintillation vial three-quarters filled with food. The females were kept in the vial by a piece of cheesecloth fastened to the neck of the vial with a rubber band. The scintillation vial was fitted onto an 8-dram vial three-quarters filled with food, containing 15–20 males, 3–5 days old. The two vials were held together with elastic bands. The maximum distance between males and females was less than 3 cm. For some experiments a small hole was made in the cheese cloth. This hole allowed the flies to move freely between the vials. The numbers of dead females were scored at day 7. The survival rate of females kept on food previously used by Canton-S males (see legend Table 3) was determined as follows. Twenty Canton-S males were kept in an 8-dram vial for 24 hr and subsequently discarded. These vials were put on top of the scintillation vials which contained the females. A hole in the gauze allowed the females to come in contact with residual traces or marks of the males. This procedure was repeated every day. Dead females were counted at day 7.

Longevity Assays of Females in Presence of Canton-S Females: Five virgin *dunce* females, 12–36 hr old, were placed with nonvirgin, 3–5-day-old, Canton-S females in an 8-dram vial. As a control, five virgin *dunce* females were kept without Canton-S females. Approximately 90 females were assayed (not including controls). Dead females were counted at day 7.

Average Lifetime Assays: Three hundred virgin females were subdivided into six equal groups and individual females were put in 8-dram vials. The first group of females received one male per vial, the second group two males per vial, and so forth. The sixth group did not receive males. The presence of dead females was recorded every 12 hr until 50% of the females kept in the absence of males had died. Thereafter, observations were made every 24 hr until all the females had died. The flies were transferred to fresh vials when the food started to retract from the vial wall.

Remating Frequency Assay: Virgin females, 3–5 days old, were put into 8-dram vials together with Canton-S males. The males were removed within 30 min after the end of mating. Two days later, four to six nonvirgin Canton-S males were introduced to the individual females and the flies were observed during a 3-hr period. The number of mating females was recorded and the males were removed after copulation. This procedure was repeated at day 4. The procedure is similar to one described by GROMKO and PYLE (1978).

Mating Frequency Assay. Nine different sets of flies, six of which are shown in Table 4, were assayed. All females were virgins and 1–3 days old. All males were *XO* or Canton-S and 2–3 days old. One virgin was put in an 8-dram numbered vial containing three males or no males (not shown in Table 4). All the vials were randomized and set up in an isolated room kept at 25 ± 1°. A light-dark cycle was simulated with red light. The dark period was matched with the real night period. As the assay was performed in the beginning of July in Davis, California, the dark cycle was from 9:30 pm to 6:00 am. Flies were observed every 15

min for 7 consecutive days (and nights). We scored as "mating" a female mounted by a male that did not dissociate upon a light tap against the vial. The tap interrupted mating in some cases and may have disrupted mating behavior, but we found this the most efficient way of scoring, since observation alone cannot easily differentiate between mating and mounting. Flies stuck in the food or dead were also recorded. Number of females mating, day and hour, were recorded for each 15-min period. Flies were transferred to fresh food when the food started to retract from the vial wall.

Flies were recorded as mating once if they were mating in a single scoring round or in consecutive scoring rounds. Occasionally a single mating observation was missing in otherwise consecutive scoring rounds. We assumed that the missing observation was an error, due to the magnitude of the experiment, and recorded the matings before and after the missing observation as a single mating event. We thus tried to avoid overestimating the number of copulations. The average time between copulations (ATBC) was computed as follows: ATBC = period of observation/number of matings observed. The period of observation ended either when the fly died, was stuck in the food (such flies usually died within a period of 12 hr) or at the end of the assay for those flies that remained alive.

Courtship Index (CI) and Number of Courtship Interactions: Single homozygous virgin *y dnc^{M14} ec f* and *y dnc^{+M14R1} ec f* females, 0–12 hr old, were transferred to 8-dram vials. Two days later three Canton-S males were transferred to the vials and flies were allowed to mate. Males were removed immediately after mating. Two days after the first mating, one to five nonvirgin Canton-S males were added to the individual females. After a 5-min equilibration period we measured the total time that the males courted the females and the number of courtship interactions occurring in a 10-min period. Females that mated within the 10-min period were not included for the computation of the CI or for the number of courtship interactions. The CI is defined as follows: CI = average time male(s) courted the female/10 min (CI, *e.g.*, TOMPKINS, HALL and HALL 1980). We assayed 100 *dnc^{M14}* and 50 *dnc^{+M14R1}* females.

RESULTS

The survival rates of females homozygous for *X*-chromosome genotypes producing three phenotypic categories were measured in the absence or presence of males. The phenotypic categories are based on fertility characteristics of females: category 1, no eggs are laid; category 2, eggs are laid but only a few percent hatch; category 3, many eggs are laid and most of the eggs hatch. In Table 2, it can be seen that (in the absence of males) females of category 1 (*dnc^{M14}* and *dnc^{M11}*) show a significantly reduced survival rate compared to the other categories (for all genotypes $\chi^2_8 = 32.8$, $P < 0.0001$, for category 1 $\chi^2_2 = 1.7$, $P = 0.44$, for category 2 and 3 $\chi^2_5 = 8.4$, $P = 0.13$). Within category 1 (in the absence of males) the differences between different genotypes are not significant. In the presence of wild-type males (Canton-S) the survival rate of females of the three categories is significantly different ($\chi^2_8 = 305$, $P < 0.0001$). There are significant differences in survival rate between females kept with males within category 1 ($\chi^2_2 = 10.3$, $P < 0.01$). However, the presence of males significantly decreases the survival rate by 36, 42 and 48% (respectively $\chi^2_1 = 35.2$, 28.6 and 48.4, all $P < 0.0001$) when compared to females kept in the absence of males. Females in category 2 carry an allele of *rutabaga* and a *dunce* null allele or have a *dnc^1* allele that encodes a phosphodiesterase that exhibits 50% of wild-type activity. These females show a 18, 25 and 29% decrease (respectively $\chi^2_1 = 3.9$, 7.6 and 17.5, all $P < 0.05$) in survival rate. Females of category 3 show almost no decrease in survival rate in the presence of males.

To determine if these effects require the presence of live males, or if they might be due to a diffusible or a nondiffusible substance from males, we compared females kept in close proximity to males but separated by a porous cheesecloth, with others in which the cheesecloth contained a hole through which flies could pass. As shown in Table 3, there is an obvious difference in survival rate of *dnc^{M14}* females kept under these two conditions. The *dunce* females kept separated from the males with a cheesecloth have the same survival frequency as *dunce* females kept in the absence of males (see Tables 2 and 3), suggesting that a diffusible substance (or sight and sound) is not the cause of male induced death ($\chi^2_1 = 14$, $P < 0.001$). In addition we observed very little effect on the survival rate of *dnc^{M14}* females kept in the presence of many dead males or in vials with residues previously deposited by males (see legend Table 3). Moreover, five *dnc^{M14}* females kept in the presence of 15 Canton-S females exhibit higher survival rates than *dnc^{M14}* females kept in groups of five (*dunce* females) or alone. This difference is however not statistically significant ($\chi^2_1 = 0.86$, $P = 0.35$). Hence, an airborne substance or a residual substance produced by males or females is not the responsible agent, and crowding does not affect the survival rate of *dunce* females. Therefore, the decreased longevity of *dunce* females is likely to be related to sexual behavior.

Several aspects of sexual behavior might be responsible. These include: contact with males not involving mating; physical exhaustion associated with repeated courtships and attempted copulations by males; exhaustion due to mating itself; or a substance transferred from males to females during mating. The following observations help distinguish among these possibilities.

First, we observed that the number of courtship interactions elicited by, and the CI of, a single *dunce* female kept in the presence of one to five males are roughly proportional to the number of males present in the vial, if the number of males is small. The average number of courtship interactions varied from 1.8 (SEM = 0.5) in the presence of one male to 6.0 (SEM = 1.5) in the presence of five males. The CI varied from 0.09 (SEM = 0.03) in the presence of one

TABLE 2

Longevity of homozygous *dunce* females

Phenotypic category	Genotype females	Survival rate[a] In absence of males Percent (No. tested)	Survival rate[a] In presence of males Percent (No. tested)	Percent females laying eggs	Average progeny (SEM)
1	*y dnc^{M14} ec f*	72 (92)	24 (124)	0	0 (0)
	y w dnc^{M14} v f	78 (135)	42 (121)	0	0 (0)
	y dnc^{M11} cv v f	70 (50)	28 (167)	0	0 (0)
2	*y dnc^{M14} ec rut^{2}*	90 (30)	72 (60)	57	6.4 (1.8)
	y dnc^{M14} ec rut^{3}	97 (30)	72 (61)	61	2.7 (0.5)
	y w dnc^{1} v f	98 (50)	69 (71)	30	2.2 (1.0)
3	Canton-S	96 (80)	96 (140)	100	78.2 (2.1)
	y dnc^{+M14R1} ec f	88 (139)	83 (190)	92	63.1 (2.3)
	y dnc^{+M14R2} ec f	85 (80)	84 (90)	92	23.1 (1.0)

[a] Survival rate: percentage of females alive at the end of the assay period.

TABLE 3

Survival rate of females with varied exposure to males

Female genotype	Male genotype (%)		*P* value
(Barrier)	Canton-S	*y dnc^{M14} ec f*	
y dnc^{M14} ec f (intact)	70	80	>0.05
y dnc^{M14} ec f (hole)	32	44	>0.05
Canton-S (hole)	98	100	>0.05

Fifty females were assayed in each experiment. Survival rates in the absence of males, 82%; survival rates in the presence of 15–20 dead Canton-S males, 78%; survival rates of females kept on food previously used by Canton-S males, 72%. *P* values indicate that there is no significant difference between Canton-S and *y dnc^{M14}ec f* males.

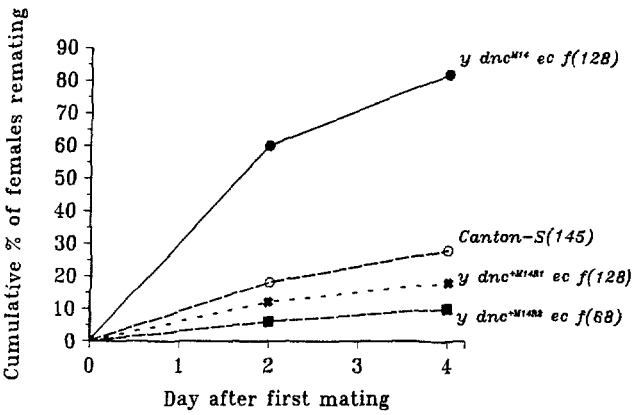

FIGURE 1.—Cumulative percent females remating on day 2 and day 4 after the first mating. The numbers between parentheses indicate the number of females tested.

male to 0.27 (SEM = 0.08) in the presence of five males. In the presence of two, three or four males, the number of courtship interactions and the CI are between these values. The wild-type *dunce* revertant females did not exhibit significant differences in number of courtship interactions and CI as a function of varying number of males (data not shown).

Second, we measured the lifespan of *dunce* females exposed to 0, 1, 2, 3, 4 and 5 males (see MATERIALS AND METHODS). The average lifespan of *dunce* females in the absence of males (347 hr, SEM = 30) is significantly greater than the average lifespan of *dunce* females kept in the presence of one to five males (range 158–188 hr, maximum SEM = 16; one way analysis of variance: $F = 16.4$, $P < 0.0001$). There is no statistical difference between the mean lifespans in the presence of one to five males. Female lifespan is reduced by 50% in the presence of one or more males. Hence, physical exhaustion of females due to intense courtship is unlikely to decrease the life expectancy unless the courtship resulting from the presence of one male is sufficiently exhausting to reduce *dunce* female lifespan.

Third, we observed that mutant females mate more frequently than normal females. This was determined in two different paradigms. In the first, we followed a procedure similar to one described by GROMKO and PYLE (1978) (see MATERIALS AND METHODS). As shown in Figure 1, 63% of the surviving *y dnc^{M14} ec f* females remate within 2 days compared to control females which remate at a frequency of only 8–18%, in agreement with data presented in GROMKO and PYLE (1978). Similar observations were made for another *dunce* strain: *y dnc^{M11} cv v f* females remate at a significantly higher frequency at day 2 and day 4 when compared to control *y cv v f* females (day 2, $\chi^2_1 = 22.8$, $P < 0.0001$ and day 4, $\chi^2_1 = 29.7$, $P < 0.0001$).

In a second experiment we measured the average time between copulations (ATBC) for *dnc^{M14}* females and control females in the presence of Canton-S and *XO* males. We observed the flies continuously for seven days and recorded the number and time of matings. As shown in Table 4, *dnc^{M14}* females remate on average every 14 hr, whereas the revertant females and the Canton-S females remate approximately every 22–24 hr. Figure 2 shows the histograms for the ATBC for the different genotypes. Note that each genotype shows a wide range. Some flies mate very frequently (ATBC less than 4 hr) whereas very few

TABLE 4

Mating frequency assay

Genotype female	Genotype males	No. assayed	ATBC (2 SEM)[a]
y dnc^{M14} ec f	Canton-S	110	14 (1.5)
	XO	60	26 (3.6)
y dnc^{+M14R1} ec f	Canton-S	94	22 (3.5)
	XO	45	28 (3.9)
Canton-S	Canton-S	91	24 (2.6)
	XO	46	20 (2.3)

[a] ATBC = average time between copulations (in hours); 2 SEM = two standard error of mean.

TABLE 5

Survival rate in the presence of *XO* males

Female genotype	No. assayed	Survival rate (%) in presence of *XO*	Survival rate (%) in presence of Canton-S	Survival rate (%) in presence of No males	χ^2 values *XO vs.* Canton-S
y dnc^{M14} ec f	96	42	25	69	6.0 ($P < 0.05$)
y w dnc^{1} v f	58	81	52	93	11.2 ($P < 0.05$)
y dnc^{+M14R1} ec f	48	83	73	83	1.52 ($P = 0.22$)

The survival rates were determined in a single independent experiment and differ somewhat from the survival rates in Table 1.

flies mate less than once in 2 days. This agrees with previous observations which suggested that females mate frequently when kept in the presence of males (PROUT and BUNDGAARD 1977; GROMKO and PYLE 1978). The mean of the ATBC for the *dunce* flies (14 hr) is significantly lower than the means of *dunce* revertant females and Canton-S females (Kruskal-Wallis value = 28.5, $P < 0.0001$ and 45.6, $P < 0.0001$, respectively). The *dunce* revertant and Canton-S ATBCs are not significantly different at the 1% level but are significant at the 5% level (Kruskall-Wallis value = 4.33, $P = 0.04$). These results confirm that *dunce* females remate more often than wild-type females. The observation that mated Canton-S and *dunce* revertant females rarely remate in the first paradigm contrasts with their behavior in the continuous presence of males in the second paradigm.

Fourth, we observed that mutant *dunce* females elicit more courtship than *dunce* revertant females. We measured the CI of *dunce* females and revertant females in the presence of Canton-S males. The CI of *dunce* females is significantly greater than the CI of *dunce* revertant females (CI *dunce* = 0.20, SEM = 0.03 and CI revertant = 0.07, SEM = 0.02. Mann-Whitney test value = 1261, $P < 0.0001$). This increased mating activity is associated with abnormal behavior on the

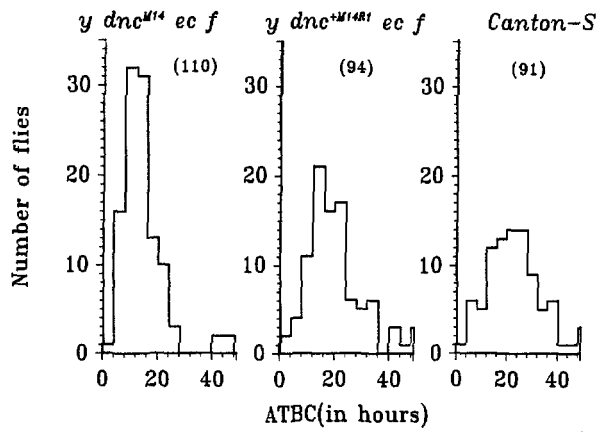

FIGURE 2.—Histograms of the average time between copulations (ATBC) in the presence of three Canton-S males. The small bars at 48 hr indicate females which mated less frequently than every 48 hr. The number of females for each genotype is in parentheses.

part of both wild-type males and their *dunce* partners. Like previously mated wild-type females (SPIETH 1974), previously mated *dunce* females display avoidance behavior that includes decamping, kicking, abdomen elevation or depression, wing fluttering and ovipositor extrusion (although the ovipositor extrusion is incomplete). This behavior is, however, less intense than that of wild-type females. The *dunce* females also abandon avoidance behavior sooner than do revertant females. These observations are substantiated by the fact that significantly more successful copulations were observed with *dunce* females compared to revertant females in the 10-min assay period used to determine the CI (data not shown).

To determine if time of death is correlated with frequency of mating we computed the Spearman rank correlation between the ATBC and the time at which the females died. The value equals 0.22. This value may be artificially low and not truly reflective of a stronger relationship between mating frequency and death for the following reasons. First, 25–30% of the females die in the absence of males. Second, the tap against the vial to ensure that the flies were mating (see MATERIALS AND METHODS), may have disrupted matings during which substantial amounts of male substances were already transferred. Both of these occurrences would tend to obscure a correlation between mating frequency and death. We therefore measured the effect of a single mating event on the survival rate. Once-mated *dunce* females exhibit a significantly lower survival rate than *dunce* females that do not mate ($\chi^2_1 = 6.2$, $P < 0.02$). However, the decrease in survival rate of *dunce* females, allowed to mate only once, is less pronounced than that of *dunce* females kept in the continuous presence of males for 7 days, indicating that more than one mating event is necessary to decrease the survival rate dramatically.

All the aforementioned observations indicate that mating, and not crowding, reduces the longevity of *dunce* females. Additional evidence, supporting a negative effect of mating on survival rate, is provided by experiments employing *XO* males. As shown in Table 5, the survival rate of *dnc^{M14}* and *dnc^{1}* females is significantly higher in the presence of *XO* males than

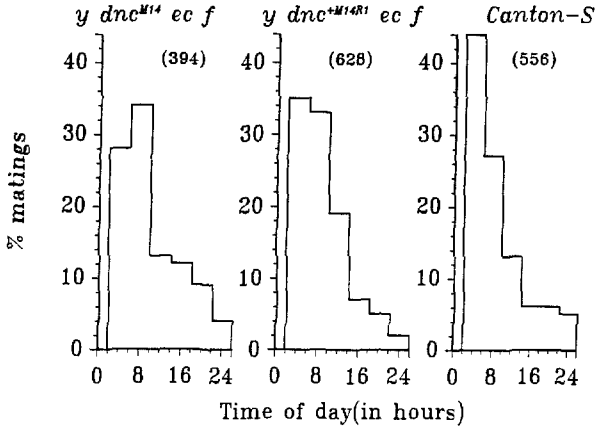

FIGURE 3.—Diurnal mating rhythm. The relative frequencies of matings observed during the period between day 2 and the end of the assay. The number of matings for each genotype is in parentheses.

Canton-S males. Since *dunce* females mate less frequently with *XO* males (ATBC = 26 hr) than with Canton-S males (ATBC = 14 hr), the increase in survival rate may be due to the decreased mating frequency. Alternatively, *XO* males may produce less of a substance that decreases longevity.

We wondered if the abnormally high mating frequency of *dunce* females would alter the sexual behavior of males or females such that they would lose their known diurnal rhythmicity (HARDELAND 1972). As shown in Figure 3, the distributions of matings during a 24-hr cycle are similar for *dunce*, *dunce* revertant and Canton-S females. Hence, there is conservation of diurnal rhythmicity in *dunce* females paired with Canton-S males.

DISCUSSION

The results of these studies show that *dunce* mutations have at least two distinct effects on longevity. First, the survival rates of *dunce* females in the absence of males are 15–25% lower than those of the controls (see Table 2). Almost identical survival rates were obtained for *dunce* males (data not shown). These survival rates indicate that loss of the cAMP-specific phosphodiesterase has an effect on longevity that is not sex-specific. Second, the male-specific effect on female longevity is investigated in detail here.

Three aspects of mating that may directly affect *dunce* female longevity are transfer of seminal fluid, transfer of a male contact pheromone and physical stimulation. The first two possibilities imply that chemical signals are transferred from the male to the female during mating and that *dunce* females are unable to cope with these signals. The third possibility implies that *dunce* females are unable to cope with sensory stimulation.

Chemical signals released by males, *e.g.*, pheromones, peptides or other hormones, could bind receptors that activate female adenylate cyclase and hence stimulate cAMP production. Olfactory signals and peptide hormones have been shown to be potent adenylate cyclase activators in other eukaryotic species (PACE *et al.* 1985; for review see GILMAN 1984). Alternatively, sensory stimulation may raise cAMP levels in specific neurons. Adenylate cyclase activity has been reported in all body parts of the fruitfly so far examined (LIVINGSTONE, SZIBER and QUINN 1984; DUDAI and ZVI 1985), and adenylate cyclase activity has been shown to be present in the blowfly labellar chemoreceptors (FELT and VANDE BERG 1977). Mutant *dunce* females are defective in cAMP-specific phophodiesterase. Hence, the increase in cAMP levels resulting from adenylate cyclase activation may not be damped normally. This increase in cAMP level could alter basic physiological functions which would lead to a decreased longevity.

This hypothesis is supported by the following observations. First, *XO* males reduce the longevity of *dunce* females to a lesser extent than Canton-S males. *XO* males have no sperm but do transfer accessory gland secretions during copulation. These include at least one antiaphrodisiac pheromone and a peptide that enhances egg-laying (JALLON 1984; CHEN 1984). Some of these subtances enter the hemolymph rapidly and may act upon a number of targets (CHEN 1984). The pattern of protein synthesis in the accessory glands of *XO* males appears to be normal (CHEN 1984). The observation that *XO* males mate less frequently with *dunce* females than do Cantons-S males (see Table 4), may explain why the survival rate of *dunce* females is higher in the presence of *XO* males. Alternatively, *XO* males may transfer less of chemical substances that could activate adenylate cyclase.

Additional evidence for this hypothesis is provided by the observation that *rutabaga* mutations, which affect adenylate cyclase activity, suppress some of the defects displayed by *dunce* females. Indeed, homozygous double mutant females, *e.g.*, dnc^{M14} rut^{2} have higher survival rates than the parent dnc^{M14} females (see Table 2). In the absence of males, double mutant females exhibit survival rates similar to those of wild type flies, suggesting that the double mutant females are healthier than the dnc^{M14} females. The difference in survival rate in the presence and absence of males of double mutant females is less than that of dnc^{M14} females, *i.e.*, about 25% compared to 45%. Thus, the presence of males has an effect on longevity of double mutant females and this effect is reduced because of the *rutabaga* mutation. Similar observations pertain to the dnc^{1} mutant (Table 2) that has 50% of the wild-type cAMP-specific phosphodiesterase and exhibits lower cAMP levels than dnc^{M14} or dnc^{M11} flies (BYERS, DAVIS and KIGER, 1981). The dnc^{1} females have high survival rates in the absence of males but their survival rate is decreased by 35% in the presence of males (see

Tables 2 and 5). These data substantiate the hypothesis that an aberrant cAMP signal is responsible for female death in the presence of males.

Wild-type mated females display avoidance behavior when courted. This behavior alone is normally insufficient to repel the males. Wild-type females produce substances when displaying this behavior that are necessary to repel males (JALLON 1984; TOMPKINS 1984). Two substances are thought to be active as repellents. First, *cis*-vaccenyl acetate is present in seminal fluid and acts as an antiaphrodisiac in the first hours after mating (TOMPKINS and HALL 1981; JALLON 1984; TOMPKINS 1984). Second, an unknown antiaphrodisiac may be made by females after mating (TOMPKINS 1984). 7-tricosene may be this antiaphrodisiac (D. SCOTT, personal communication). Some of the repellent pheromones may be dispersed on males during the extrusion of the ovipositor (SPIETH 1974; JALLON 1984). Females also produce another substance that may play a role in avoidance behavior. Virgin females produce a courtship-stimulating substance, heptacosadiene. It has been observed that mated females make less heptacosadiene a few days after mating (JALLON 1984). The aforementioned substances may have a long-term effect since mated females are relatively unattractive for up to 8 days after mating (JALLON 1984; TOMPKINS 1984).

Our data show that mated dnc^{M14} females induce more male courtship than dnc^{+M14R1} females. We propose that *dunce* females may have a defect that does not allow complete ovipositor extrusion and therefore does not allow efficient dispersion of an antiaphrodisiac; alternatively, *dunce* females may either produce less of a repelling substance or more of an aphrodisiac. Either or both possibilities may explain why *dunce* females stimulate more courtship than do dnc^{+M14R1} females.

Mated dnc^{M14} and dnc^{M11} females also remate more frequently than the controls. We suggest that the male signals transferred during the first mating have weakened the *dunce* females such that they cannot reject the males. Since *dunce* females also induce more courtship than the wild-type revertant females they will be more likely to remate.

The observation that Canton-S females remate on average every 24 hr in the continuous presence of wild-type males suggests that whatever chemical signals exist, they are effective for a period of less than a day under these assay conditions. Long duration effects of chemical signals involved in rejection of males by mated females have been cited (for review see JALLON 1984 and TOMPKINS 1984). We question the existence of such signals of long duration since they seem to be observed only when the experimental design focuses on the temporary presence of males.

Diurnal rhythmicity in courtship activity has led to the design of laboratory experiments in which females are confined with males only once a day (see GROMKO and PYLE 1978). On the basis of their experiments GROMKO and PYLE concluded that remating does not occur before most of the sperm of a previous mating has been depleted. Therefore, they concluded that there is no opportunity for selection among male genotypes through sperm competition as has been proposed by PROUT and BUNDGAARD (1977). Our findings seem to contraindicate this type of experiment because diurnal rhythmicity in courtship (HARDELAND 1972) and mating is conserved in our laboratory conditions. This implies that sperm competition may play an important role for selection among male genotypes.

It is now evident that the cAMP-specific phosphodiesterase of *Drosophila melanogaster* is involved in several physiological processes whose interrelationships are presently unknown. Moreover, the *dunce* mutants will probably be useful in understanding other, so far unknown, physiological processes associated with cAMP metabolism, some of which may underly the shortened longevity of *dunce* male and female flies. The sexual hyperactivity of females, and the similarly aberrant sexual behavior of *dunce* males (GAILEY, JACKSON and SIEGEL 1984), may be a consequence of an underlying memory defect, *i.e.*, a failure to remember the last mating act (see also KYRIACOU and HALL 1984). The evidence presented here also suggests that pheromonal response and/or regulation may be altered in *dunce* females. The observation that mating reduces the lifespan of *dunce* females and that male substances may be involved suggests an important role for cAMP metabolism in communication between males and females. Altering this metabolism might prove an interesting way to affect reproduction in some insect species.

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