GENETIC ANALYSIS OF CHROMOMERE 3D4 IN *DROSOPHILA MELANOGASTER*: THE *DUNCE* AND *SPERM-AMOTILE* GENES

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ABSTRACT

Both male and female Drosophila that are homozygous deficient for chromomere 3D4 are viable but sterile and lack detectable cAMP-specific phosphodiesterase activity. Two genes have been localized to this region: *sperm-amotile* (*sam*) and *dunce* (*dnc*). The *sperm-amotile* gene is required for male fertility, and the *dunce* gene is required for normal learning, female fertility, and cAMP-specific phosphodiesterase activity. The *sperm-amotile* gene maps 0.24 map units to the left of *dunce*. The expression of the *dunce* gene seems to be affected by a chromosomal break to the left of *sperm-amotile*. The fertility of *dunce* females varies according to changes in the genetic background and the presence or absence of an *X*-linked suppressor.

DROSOPHILA melanogaster flies have two forms of cyclic nucleotide phosphodiesterase, a cAMP specific phosphodiesterase and a cyclic nucleotide phosphodiesterase which hydrolyzes both cAMP and cGMP (DAVIS and KIGER 1980). The genome of Drosophila has been surveyed by segmental aneuploidy for sites that effect the activity of these phosphodiesterases (KIGER and GOLANTY 1977). Flies homozygous deficient for chromomere 3D4 on the *X*-chromosome lack the cAMP-specific phosphodiesterase (KIGER and GOLANTY 1979). Chromomere 3D4 does not contain any essential genes since flies deficient for it are viable. However, this region is required for normal female and male fertility (KIGER 1977). These phenotypes have permitted us to screen for mutants in this region. Two genes have been identified, *sperm-amotile* (*sam*), required for male fertility, and *dunce* (*dnc*), required for normal learning, cAMP-specific phosphodiesterase levels and female fertility. Biochemical analyses and learning performances of the *dunce* alleles have been reported previously (BYERS, DAVIS and KIGER 1981; DAVIS and KIGER 1981). The purpose of this paper is to report on the genetic analysis of these genes. Some of these results have been reported in summary form (KIGER *et al.* 1981).

MATERIALS AND METHODS

Stocks: The deficiency and duplication stocks used have been described by KIGER and GOLANTY (1977). The cytologies of these chromosomes are diagrammed in Figure 1. Unless otherwise mentioned LINDSLEY and GRELL (1968) have described the genetic markers and balancers employed.

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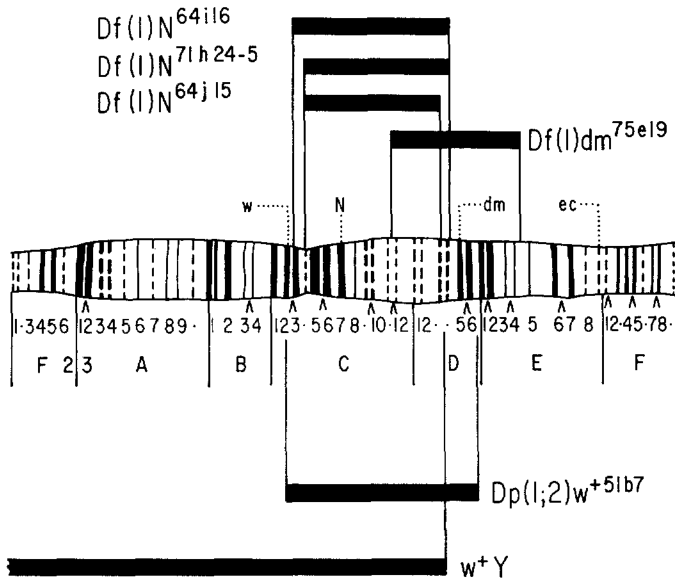

FIGURE 1.—A drawing of a segment of the salivary gland polytene *X*-chromosome of Drosophila showing the extent of the deficiencies and duplications discussed here. The numbering of the bands or chromomeres is that used in BRIDGES' revised map (LINDSLEY and GRELL 1968).

The mutations dnc^{1}, dnc^{2}, dnc^{M11} and dnc^{M14} were induced with ethylmethanesulfonate (EMS). The dnc^{1} and dnc^{2} alleles were induced in the *Canton-S* wild-type strain in the laboratory of S. BENZER (DUDAI *et al.*, 1976), while dnc^{M11} and dnc^{M14} were induced in an *X*-chromosome marked with *yellow* (*y*), *crossveinless* (*cv*), *vermilion* (*v*) and *forked* (*f*), by MOHLER (1977). The mutations dnc^{CK}, dnc^{ML} and *sam* were induced in our laboratory in *X*-chromosomes marked with *yellow*, *white* (*w*) and $forked^{36a}$: dnc^{ML} and *sam* were induced with EMS according to the method of LEWIS and BACHER (1968); dnc^{CK} was induced with X rays. For the isolation of *dnc* alleles, mutagenized males were mass mated to attached-*X* females *C(1)DX,y w f*. Surviving male progeny were mated individually to attached-*X* females to establish mutant stocks for screening. The isolation of dnc^{ML} involved assaying males from each of the above stocks for cAMP phosphodiesterase activity using the assays previously described by KIGER and GOLANTY (1977). The mutation dnc^{CK} was selected on the basis of female sterility in combination with $Df(1)dm^{75e19}$. The *dnc* alleles are now kept balanced with *FM7* (MERRIAM and DUFFY 1972).

A male-sterile mutation, *sam*, was isolated by mating mutagenized males to attached-*X* females carrying a duplication of chromomeres 3C2–3D6 in a 2nd chromosome balancer, *C(1)DX*, *y w f*; *SM1,Cy* $Dp(1;2)w^{+51b7}/+$, and then mating individual F_1 males carrying the duplication back to attached-*X* females to established stocks for screening. Males of the subsequent generation, not carrying the duplication, were tested for sterility. Only males whose sterility was covered by $Dp(1;2)w^{+51b7}$ but not by $w^{+}Y$ were maintained for further study. The *sam* stock is now maintained balanced by *FM7* or as *sam/sam*; *SM1,Cy* $Dp(1;2)w^{+51b7}/+$.

Salivary gland chromosomes of *sam*, and all the *dnc* alleles except dnc^{CK} are normal; dnc^{CK} is associated with a reciprocal translocation between the tip of *3L* and the *X*. The breakpoint on the *X* is just to the right of *3C* and to the left of *4*; the breakpoint on *3L* is at 63E.

Fertility tests: Female fertility tests were made by individually mating virgin females, one to three days old, with two to four *Canton-S* males in vials. Parent flies were removed from the vials on the sixth day. The numbers of progeny were scored on the 17th day. Tests of male fertility were done by mating individual males, one to three days old with two to four *Canton-S*

virgin females in vials. Parents were removed on the sixth day, and the vials were scored either as sterile or fertile on day seventeen. All tests were conducted at 25°.

Cyclic nucleotide phosphodiesterase assay: The assay has been previously described in DAVIS and KIGER (1980). Adult female flies were homogenized in 80 mM Tris-HCl pH 8.0, 20 mM $MgCl_2$ and 4 mM 2-mercaptoethanol at 0°. A 0.05 ml volume of homogenate was assayed with an equal amount of 0.2 mM ^{3}H-cAMP or 0.2 mM ^{3}H-cGMP substrate prepared in water. The separation of the unreacted substrate from the 5′-nucleoside monophosphate product of the phosphodiesterase was done by chromatography on Whatman 3 mm paper. Activity is calculated as picomoles of cyclic nucleotide hydrolyzed per minute and per milligram fly.

RESULTS

Selection of mutations in chromomere 3D4: To begin a mutational analysis of chromomere 3D4, a screen for male-sterile mutants was initiated since male sterility is a less variable phenotype than the reduction in female fertility originally observed by KIGER (1977). Sterile mutations were induced by EMS and selected on the basis of complementation by $Dp(1;2)w^{+51b7}$, but not by the w^+Y duplication; such mutations must lie between the endpoints of these duplications, in chromomeres 3D2–3D6. Because $Df(1)N^{64j15}/w^+Y$ males are fertile, and $Df(1)N^{64i16}/w^+Y$ males are sterile, any male-sterile mutation between the endpoints of w^+Y and $Dp(1;2)w^{+51b7}$ must be in chromomeres 3D4–3D6. The first mutation selected in this way was named for its phenotype which mimics the deficiency 3D4 phenotype, *sperm-amotile* (*sam*). Females homozygous for *sam* were found to be completely fertile (Table 1) and *sam* was found not to affect cAMP phosphodiesterase activity (KIGER *et al.* 1981), suggesting that additional mutants with different phenotypes might also be found in chromomere 3D4.

One approach taken to isolate additional mutants is to screen for female-sterile mutations. The fertility of homozygous deficient females is variable (KIGER 1977); however, by altering the genetic background the sterility phenotype can be enhanced. Deficiency females made by crossing $Df(1)dm^{75e19}/Basc$ females to $Df(1)N^{64i16}$; *SM1, Cy* $Dp(1;2)w^{+51b7}$ males have, on the average, 21 progeny—too many to make such a screen feasible. The $Df(1)dm^{75e19}/Basc$ stock was produced from a stock originally balanced over *FM7*. Deficiency females made by crossing $Df(1)dm^{75e19}/FM7$ females to the same males are much more infertile (17 females tested: one female produced eight female progeny, all others were sterile). It is evident that the genetic background is important in determining the fertility of deficiency females.

X-linked female-sterile mutants isolated by J. D. MOHLER (1977) that map between *y* and *cv* were screened for lack of complementation with $Df(1)N^{64i16}$ (3C3–3D4). Two of these mutants, *11–761* and *14–756*, renamed respectively *M11* and *M14*, were sterile in combination with this deficiency. Furthermore, both mutant flies lack the cAMP-specific phosphodiesterase (DAVIS and KIGER 1981). Subsequently, two additional mutants deficient in the cAMP-specific phosphodiesterase have been isolated, *ML* selected on the basis of low cAMP phosphodiesterase activity and *CK* selected on the basis of female sterility.

Independently of this work other alleles were found because of a different phenotype. The *dunce*$^+$ gene has been shown to be required for normal learn-

TABLE 1

Pairwise complementation tests of female fertility

	$dnc^1, Su(fs)$	dnc^1	dnc^2	dnc^{M14}	dnc^{M11}	dnc^{ML}	dnc^{CK}	*sam*
$dnc^1, Su(fs)$	48/48(59±3)	29/30(74±3)	50/50(48±2)	62/62(35±1)	44/51(39±2)	49/49(61±2)	49/49(39±1)	49/49(68±2)
dnc^1		6/30(5±2)	9/25(19±5)	2/19(3±2)	8/23(10±3)	18/29(55±8)	5/26(7±2)	....
dnc^2			2/44(11±6)	19/50(4±1)	22/37(7±1)	34/46(14±2)	32/53(7±1)	52/52(65±2)
dnc^{M14}				0/36	1/48(1)	5/50(3±1)	5/47(2±1)	45/45(57±2)
dnc^{M11}					0/33	6/46(3±1)	8/50(3±1)	43/43(55±2)
dnc^{ML}						13/64(8±2)	18/46(8±2)	34/34(56±3)
dnc^{CK}							1/21(2)	41/41(47±2)
sam								14/14(63±8)

The data recorded are the number of females producing progeny over the total number of females tested. The numbers in parentheses are the average number of progeny produced by the nonsterile females. Confidence intervals are one standard error of the mean.

The fertility of the appropriate controls are as follows: *vf/vf* 11/11 (51±5); ywf^{36a}/ywf^{36a} 34/34(65±3); *Canton-S/Canton-S* 50/50 (78±3); *y cv v f/y cv v f* 40/40(73±3); dnc^2/*Canton* 43/43 (32±2); dnc^{ML}/ywf^{36a} 45/45 (48±2); dnc^{CK}/ywf^{36a} 47/47 (47±2); sam/ywf^{36a} 40/40 (64±3); dnc^{M14}/*y cv v f* 34/35 (58±3); dnc^{M11}/*y cv v f* 40/41 (49±3).

ing (DUDAI *et al.* 1976). Two mutants had been isolated on the basis of learning deficiency; dnc^1, which was reported to be female-fertile, and dnc^2 which is female-sterile. dnc^2 does not complement the female infertility of *M11* and *M14* which were also found to be learning-deficient (BYERS, 1979). Both dnc^1 and dnc^2 are deficient in the cAMP-specific phosphodiesterase (BYERS, DAVIS and KIGER 1981; DAVIS and KIGER 1981). These results suggest that there is a single locus in chromomere 3D4 required for normal learning, normal cAMP-specific phosphodiesterase activity and female fertility. The other mutants have been renamed dnc^{M11}, dnc^{M14}, dnc^{CK} and dnc^{ML} because of the priority of description of the *dunce* gene. All the *dunce* mutants are fertile in males, therefore there must be another gene in chromomere 3D4 that is required for male fertility (DAVIS 1979).

Complementation studies: Complementation studies of the effects of the *dunce* alleles on female fertility are presented in Table 1. Since the sterility of homozygous alleles is not complete, two components are considered: the number of females producing progeny and the average number of progeny produced by these females. *dnc* females can be considered sterile because the majority of females produce no progeny, and the few females that are not sterile have very few progeny, whereas each dnc^+ female produces at least 50 progeny. The number of homozygous mutant females producing progeny is affected by changing the genetic background of a *dnc* stock (data not shown); therefore, the data given in Table 1 are specific for a given allele in a given genetic background.

Suppressor of Female Sterility: The original dnc^1 allele is female-fertile when homozygous or in combination with any other *dnc* allele. However such mutant females can be rendered sterile by replacing the base of the *X*-chromosome. The fertile, unmarked dnc^1 chromosome was combined with a multiply-marked *X*-chromosome *y cho cv v f*. Using cho^+ as a marker for *dnc*, single cho^+ male recombinants were isolated and tested in combination with $Df(1)dm^{75e19}$ for female sterility. Some, but not all the recombinants that contained *forked* were sterile; no other recombinants were found to be sterile. The anomolous female fertility of dnc^1 (compared to the other *dunce* alleles) can be explained by the presence of dominant suppressor at the base of the *X*-chromosome. The original mutant chromosome is therefore designated dnc^1, *Su(fs)* and the sterile derivative dnc^1, $Su(fs)^+$ or just dnc^1. The data are summarized in Table 2. These data cannot be used to determine a more exact location of *Su(fs)* because the

TABLE 2

Removal of Su(fs) *from* dnc^1, Su(fs)

cho^+ recombinants	Number collected	Number female sterile
cv v f	27	6
v f	172	46
f	135	17
cv v	3	0
v	3	0
y	17	0

use of $Df(1)dm^{75e19}$ resulted in an underestimation of the number of dnc^1, $Su(fs)^+$ chromosomes recovered. The genetic background of $Df(1)dm^{75e19}$ is such that it allows $dnc^1/Df(1)dm^{75e19}$ females to produce some progeny (Table 4) and only those females that produced no progeny were scored as dnc^1, $Su(fs)^+$.

dnc^1 does not complement any of the other *dunce* mutants (Table 1), with the exception that about two-thirds of the dnc^{ML}/dnc^1 females tested were fertile and produced about 55 progeny. Since neither dnc^{ML} nor dnc^1 complement the other *dnc* alleles it is possible that this increase in fertility is due to some other cause than complementation. One possibility is that the dnc^{ML} chromosome carries *Su(fs)*, but the dnc^{ML} allele is not affected by *Su(fs)*. To test this possibility the base of the *X*-chromosome was replaced with that carrying $Su(fs)^+$ on ten different dnc^{ML} bearing chromosomes. Each stock was tested *en masse* for fertility over dnc^1. Eight of ten had progeny over dnc^1. Moreover, on retesting individual virgin females, two-thirds had progeny (33/55 females with progeny, the mean number of progeny was 22.8 ± 3.2). Although the average number of progeny produced by those females was not quite half of wild-type, these females are still fertile compared to dnc^1 or dnc^{ML} homozygotes. While these observations are not definitive, it appears that the dnc^{ML} chromosome does not carry an allele of *Su(fs)*. The increase in fertility of dnc^{ML}/dnc^1 heterozygotes may instead be due to intraallelic complementation or to another suppressor somewhere else in the genome.

To test the effect of *Su(fs)* on the other *dnc* alleles the base of the dnc^1 *Su(fs)* chromosome was exchanged with that of the other chromosomes bearing *dnc* alleles. Since *Su(fs)* is located close to the *forked* locus, *forked*$^+$ was used as a marker for the suppressor. In each case the stocks were made with a single *dnc*, f^+ male. Homozygous females from each stock were then tested, *en masse*, for *fertility*. Those stocks that were not sterile were then retested, this time using single homozygous virgin females as described in MATERIALS AND METHODS. The disadvantage to using this selection scheme is that there is a simultaneous selection for a genetic background that allows higher fertility. A small increase in fertility may be due either to partial activity of *Su(fs)* or to some other factors in the genome. Because we cannot distinguish between these two possibilities, *Su(fs)* is considered to be active only if it restores full fertility to each *dnc* allele. The data are summarized in Table 3.

The suppressor was first recombined with dnc^{M14}. 100 recombinant stocks were tested—none of which were fully fertile, although most had some progeny. When testing individual females from one of those stocks that produced the most progeny, female fertility was not significantly increased, although there were more females with progeny, possibly due to a change in genetic background. To test whether the suppressor was actually present in the stock, the base of each test chromosome was returned to the dnc^1 chromosome. 84 stocks were made homozygous for independently isolated recombinant *X*-chromosomes. Three to four single nonvirgin females were individually tested in vials for fertility. In 74 of these stocks full fertility was restored. This confirms that the suppressor is indeed present on the dnc^{M14} chromosomes and that it does map close to, but separable from, *forked*. The suppressor, although it does not act

TABLE 3

Effects of Su(fs) *on the female fertility of dunce alleles*

	dnc^x/dnc^x	dnc^x, $Su(fs)/dnc^x$, $Su(fs)$	dnc^1, $Su(fs)/dnc^x$	dnc^x, $Su(fs)/dnc^1$	dnc^x/dnc^1
dnc^1	6/30(5±2)	48/48(59±3)	29/30(74±3)	29/30(74±3)	6/30(5±2)
dnc^{M14}	0/36	12/39(5±2)	62/62(35±1)	32/51(31±3)	2/19(3±2)
dnc^{M11}	0/33	11/24(8±4)	44/51(39±2)	58/91(29±2)	8/23(10±3)
dnc^2	2/44(11±6)	19/36(26±4)	50/50(48±2)	70/97(40±2)	9/25(19±5)
dnc^{CK}	1/21(2)	31/55(9±1)	49/49(39±1)	66/99(10±1)	5/26(7±2)

The data recorded are the number of females producing progeny over the total number of females tested. The numbers in parentheses are the average number of progeny produced by the non-sterile females. Confidence intervals are on standard error of the mean.

on dnc^{M14}, can act in *trans* on dnc^1; dnc^{M14}, $Su(fs)/dnc^1$ females are semifertile (32/51; 31±3). Although these females are not as fertile as dnc^1, $Su(fs)/dnc^{M14}$ the difference is more likely to be due to different backgrounds rather than a *cis/trans* effect.

The suppressor was then recombined with the other *dnc* alleles. In no case did the presence of the suppressor dramatically increase the fertility of females carrying the other *dnc* alleles. The suppressor of female sterility was found not to affect significantly the phosphodiesterase activity in whole fly homogenates. dnc^1, $Su(fs)$ homogenates hydrolyze 133 ± 14 units of cAMP per mg fly and 125 ± 30 units of cGMP per mg fly compared to dnc^1 homogenates which hydrolyze 124 ± 8 units of cAMP per mg fly and 175 ± 20 units of cGMP per mg fly (confidence intervals are 95%). The suppressor appears to be relatively specific for the dnc^1 allele, perhaps because dnc^1 retains more cAMP-specific phosphodiesterase activity than other mutant alleles (DAVIS and KIGER 1981). However, the relation between enzyme activity and female fertility is complex. Even the homozygous deficiency can exhibit some fertility in certain backgrounds.

Localization of dunce *to chromomere 3D4*: In order to confirm their cytological locations, the six *dunce* mutants were tested for complementation of female-sterility with several deficiency chromosomes (see Table 4). All alleles failed to complement either $Df(1)N^{64i16}$ (3C3–3D4), $Df(1)N^{71h24-5}$ (3C4–3D4), or $Df(1)dm^{75e19}$ (3C12–3E4) although there appears to be variations in the number of females producing progeny, probably due to a change in genetic background. Therefore the *dunce* locus must reside between 3C12 and 3D4. The mutants were then tested for complementation with $Df(1)N^{64j15}$ (3C4–3D3 or nicks 3D4). Complementation for female fertility was not complete. The data suggest that the right breakpoint of $Df(1)N^{64j15}$ may affect the expression of the *dunce* locus but does not remove it. This conclusion is supported by biochemical evidence: $Df(1)N^{64j15}/w^+Y$ males possess the cAMP-specific phosphodiesterase but exhibit reduced cAMP phosphodiesterase activity (KIGER and GOLANTY 1979; KIGER *et al.* 1981). Therefore, $Df(1)N^{64j15}$ does not remove the $dunce^+$ gene, which must in consequence be located in chromomere 3D4.

Localization of sperm-amotile *to chromomere 3D4*: KIGER (1977) showed that chromomere 3D4 is required for male and female fertility. This suggests that

TABLE 4

Complementation of deficiencies with the dnc *alleles*

	$Df(1)N^{64j15}$	$Df(1)N^{64i16}$	$Df(1)N^{71h24-5}$	$Df(1)dm^{75e19}$
dnc^1	3/27(51±8)	0/28	2/22(8±7)	4/28(10± 3)
dnc^2	17/28(53±5)	7/50(9±4)	1/23(1)	7/45(28±10)
dnc^{M14}	39/58(24±2)	0/29	4/56(1±1)	5/46(2± 1)
dnc^{M11}	17/57(16±3)	0/39	1/48(8)	2/35(12± 1)
dnc^{ML}	19/47(31±5)	4/43(4±2)	6/47(5±2)	9/36(19± 2)
dnc^{CK}	13/47(12±4)	0/50	4/31(4±2)	25/58(18± 4)
Canton-S	50/50(67±2)	39/39(69±4)	32/45(37±5)	4/14(76± 3)
$y\ w\ f^{36a}$	58/58(80±2)	50/50(74±2)	44/45(73±2)	53/54(90± 3)
y cv v f	50/50(58±2)	61/61(62±2)	43/46(63±3)	41/41(67± 2)

The data recorded are the number of females producing progeny over the total number of females tested. The numbers in parentheses are the average number of progeny produced by the nonsterile females. Confidence intervals are one standard error of the mean.

there is another gene in 3D4 that is required for male fertility. Because, like the deficiency, *sam* lacks motile sperm, and is located in 3D4–3D6, it may be this gene. Therefore, *sam* was mapped with respect to dnc^{M14} to determine its position relative to the *dunce* locus (see Table 5 for scheme and results). Male progeny recombinant in the *white-echinus* region produced by mothers of the genotype $y\ w\ sam + f^{36a}/y + dnc^{M14}\ ec\ f$ were individually crossed to attached-*X* females (*C(1)DX,y f*) to establish stocks of sam^+ recombinants. Each of these recombinant stocks was then tested for the presence of dnc^{M14} by backcrossing recombinant males to $dnc^{M14}/FM7$ females, and testing the fertility of nonbalanced female progeny. The order of the *sam* and the *dnc* genes was determined by the phenotype of the sam^+dnc^+ recombinants. We found that all the sam^+ dnc^+ recombinants were w^+ec^+, indicating the gene order *w sam dnc ec*. This result proves that *sam* is located in chromomere 3D4 and not in 3D5 or 3D6.

The map distance between *sam* and dnc^{M14} is 0.24 ± 0.08 map units, assuming the standard map distance of four map units between *white* and *echinus*. However, only 1.3% recombination was observed between *white* and *echinus* in this cross. In contrast 2.5% recombination was observed if neither the *sam* nor dnc^{M14} chromosomes were present. To determine which mutant chromosome is responsible for the reduction in recombination, the crosses were repeated with either the *sam* or dnc^{M14} chromosome alone. The *sam* chromosome does not significantly reduce the amount of recombination between the two markers, but the

TABLE 5

Mapping of sam *and* dnc

Maternal genotype $\dfrac{y\quad w\quad sam\quad +\quad +\quad f^{36a}}{y\quad +\quad +\quad dnc^{M14}\quad ec\quad f}$

	Classification of recombinants between *white* and *echinus*			
	Number collected	Number sam^+	Number sam^+ tested	Number of dnc^+
w ec	204	72	70	0
+ +	133	42	39	8

dnc^{M14} chromosome does (see Table 6 for summary of results). It is unclear why the dnc^{M14} chromosome reduces recombination in this region. The salivary gland chromosomes of dnc^{M14}/dnc^{+} females show no visible inversion or deletion. If there is a small deletion, it cannot include either the *sam* locus, since dnc^{M14} is male fertile, or the *diminutive* locus in 3D5 (LEFEVRE 1976) since dnc^{M14}/dm^{1} females are fertile (48/48; 81 ± 2). Because recombination is detected between the *dnc* and the *sam* loci, if there is a small inversion, it cannot include the *sam* locus.

DISCUSSION

Chromomere 3D4 is required for normal learning, male and female fertility and cAMP phosphodiesterase activity. Two genes have been found to be responsible for these phenotypes: sam^{+} and dnc^{+}. sam^{+} is responsible for male fertility; dnc^{+} is responsible for female fertility, normal learning and cAMP-specific phosphodiesterase activity. The female-sterility of the *dnc* mutants is affected by variation in the genetic background. The female-sterility of one of the *dnc* alleles, dnc^{1}, is also suppressible by *Su(fs)*, located at the base of the *X*-chromosome. *Su(fs)* is dominant, and has no effect on cAMP or cGMP hydrolysis in whole fly homogenates. Nor does the suppressor restore the learning ability of dnc^{1} since the original dnc^{1} mutant was isolated with the suppressor. The mechanism by which the suppressor acts is not clear. However, because dnc^{1} is a hypomorph (DAVIS and KIGER 1981), it is possible that the suppressor is acting directly on the cAMP-specific phosphodiesterase, in such a way that an increase in enzyme activity is not detectable in our assay. If the increase is small, or tissue-specific, the increase would not be detected in whole fly homogenates.

Another factor that affects the expression of the *dunce* gene is the breakpoint of $Df(1)N^{64j15}$. $Df(1)N^{64j15}/w^{+}Y$ males, although they retain chromomere 3D4,

TABLE 6

Amount of recombination between white *and* echinus

Maternal genotype	Progeny genotype				% recombination
	w +	+ *ec*	*w ec*	+ +	
y w + + + *f* / *y* + + + *ec f*	1477	1406	35	39	2.5
y w sam + + *f* / *y* + + + *ec f*	642	666	16	12	2.1
y w + + + *f* / *y* + + dnc^{M14} *ec f*	535	398	3	4	0.75
y w sam + + *f* / *y* + + dnc^{M14} *ec f*	979	1149	14	14	1.3

The amount of recombination between *w* and *ec* with and without *sam* is not significantly different as determined by a contingency Chi square test (χ^2=3.1, df=3; 0.3<P<0.5). The amount of recombination between *w* and *ec* with and without dnc^{M14} is significantly different (χ^2=530; df=3 P<0.001).

show a reduction in c*AMP* phosphodiesterase activity, yet retain the cAMP-specific phosphodiesterase (KIGER and GOLANTY 1979; KIGER *et al.* 1981). The breakpoint of $Df(1)N^{64j15}$ also appears to affect the fertility of *dnc* females since $Df(1)N^{64j15}/dnc$ females are not completely fertile. However the breakpoint of $Df(1)N^{64j15}$ can not be at the *dnc* gene because *sam* maps to the left of *dnc* and is to the right of $Df(1)N^{64j15}$ because $Df(1)N^{64j15}/w^{+}Y$ males are fertile. In order to better understand this region, more *sam* mutants are being isolated, and fine structure mapping of both *sam* and *dnc* is underway.

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