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Delayed desemination by low temperature exposure in two strains of D. melanogaster.

The following longitudinal study of D. melanogaster was undertaken in order to determine the paternity of offspring produced by one female mated sequentially with two males. Two strains were used, a Sevelen line and a mutant line, wmei-41^{D5} (Boyd et al. 1976, Genetics 84:485-506), maintained in our laboratory for 10 years and 2 years, respectively.

Twenty-nine 1-3 day old Sevelen females, randomly collected from a population cage, were mated overnight with similarly aged Sevelen males. Each mated pair was housed separately in 80 ml plastic vials with standard yeasted medium. The next day, following Novitski and Rush (Biol.Bull. 97:150-7, 1949), females housed individually in plastic vials without medium were "frozen" at -11±1°C for 20 min. After recovery (3-4 hr at room temperature) the females were mated with 8-12 day old Basc males for the duration of the experiment. These separately housed pairs were transferred to fresh media every day or every other day, as indicated in Tables 1 and 2. No flies were etherized at any time during the experiment.

The Muller-5 test was used to ascertain the paternity of female offspring produced by all post-freeze (Sevelen by Basc) matings.

Table 1. Summary data on fertility and desemination effects of exposure to -11±°1C for 20 minutes, Sevelen strain.

	pre-treatment	day 1 post-treatment	day 2 post-treatment	days 3-4 post-treatment	days 5-6 post-treatment	days 7-18 post-treatment
#pairs	29	28	28	28	28	28
Fertility ($\bar{X}$±s.d.)	8.45±7.62	0.43±1.37	2.21±2.85	35.14±23.09	38.21±27.64	37.18±30.76
% ♀offspring sired by 2nd ♂(N)		0%(0/8)	53.1%(17/32)	93.6%(479/512)	100%(546/546)	99.6%(460/462)
# fertile pairs with 100% ♀offspring sired by 2nd ♂(%)		0	5(45%)	21(81%)	21(100%)	18(90%)

As Table 1 demonstrates, it is not until the 6th day post-freezing that 100% of the female offspring were sired by the second (i.e., Basc) male. Apparently not all of the sperm from the first mating (Sevelen by Sevelen) stored by the female were rendered immotile (DIS 36:86) by exposure to low temperature. Expected levels of fertility were not observed until days 3-4 post-freezing (cf. DIS 36:86).

During freezing there was one death among the 29 Sevelen females housed individually and no deaths among 271 same-aged Sevelen males and females housed in 4 vials and frozen simultaneously. At 25 and 30 minutes duration, freezing at -11±1°C resulted in substantial mortality and markedly reduced fertility from which the female Sevelens did not recover (see Table 2).

Table 2. Mortality and infertility effects of three durations exposure to low temperature, Sevelen strain.

		% Infertile matings (N)					
Treatment @ -11±°1C	%Mortality (N)	pre-treatment	day 1 post-treatment	day 2 post-treatment	days 3-4 post-treatment	days 5-6 post-treatment	days 7-18 post-treatment
20 min	0%(1/301)	14%(4/28)	82%(23/28)	50%(14/28)	7%(2/28)	25%(7/28)	25%(7/28)
25 min	22%(52/234)	12%(3/25)	67%(16/24)	83%(15/18)	67%(12/18)	65%(11/17)	54%(6/11)
30 min	48%(118/245)	13%(3/23)	90%(19/21)	100%(9/9)	67%(6/9)	60%(3/5)	33%(1/3)

The same protocol was followed with the wmei-41^{D5} strain. Thirteen 1-2 day old females were mated for two days with same-aged wmei males, each mated pair housed separately. The next day individually housed females were "frozen" at -11±1°C for 20 min along with 368 2-4 day old wmei males and females. After one hour at room temperature, 94.2% (359/381) of the flies recovered. Wmei females were then mated with same aged (3-4 day old) Sevelen males for 7 days.

The paternity of female offspring produced by post-freeze matings was ascertained by scoring eye color, with 100% red-eyed daughters indicating complete desemination.

Table 3. Summary data on fertility and desemination effects of exposure to -11±1C for 20 minutes, wmei-41^{D5} strain.

pre-treatment	day 1 post-treatment	day 2 post-treatment	day 3 post-treatment	day 4 post-treatment	day 5 post-treatment	day 6 post-treatment	day 7 post-treatment
# pairs:							
13	13	13	13	13	13	13	13
fertility ($\bar{X}$±s.d.):							
7.62±10.29	11.62±13.82	14.23±10.85	15.69±8.81	16.92±9.66	11.69±10.98	9.69±9.07	16.00±13.90
% ♀offspring sired by 2nd ♂ (N):							
	14.1% (12/85)	88.3% (83/94)	99.0% (96/97)	100% (95/95)	100% (70/70)	100% (62/62)	100% (106/106)
# fertile pairs with 100% ♀offspring sired by 2nd ♂(%):							
	1(10%)	8(67%)	11(92%)	12(100%)	11(100%)	11(100%)	12(100%)

As Table 3 demonstrates, 100% of the female offspring are sired by the second (i.e., Sevelen) male beginning with the fourth day post-freezing. The more rapid desemination of the wmei strain as opposed to the Sevelen strain (4 vs 6 days post-freezing) may be related to the reduced DNA repair capacity of the former (Boyd & Setlow 1976, Genetics 84:596-26). Moreover, fertility is not reduced after exposing wmei females to low temperature (Table 3), unlike the situation with Sevelens (Table 1).

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Mazar-Barnett, B. & E.R. Munoz. Comision Nacional de Energia Atomica, Buenos Aires, Argentina. Dominant lethal tests with nipagin in Drosophila melanogaster.

A certain decrease in egg hatchability has been observed both in Drosophila melanogaster and Dacus olea cultures when nipagin (p-hydroxybenzoic acid methyl ester) is added to the food as fungicide. Since this drug is of current use, an investigation was started to study its action on the developing germ cells of D. melanogaster as a contribution to establishing the causes of the observed increase in embryonic lethality.

We report here preliminary results of dominant lethal tests performed to determine the effect of nipagin administered by injection (thus circumscribing the analysis to its direct effect on the male and female germinal cell lines) in motile sperm and mature oocytes.

	Unhatched/ total eggs	% embryonic death
controls	81/642	10.39
treated ♂♂ x untreated ♀♀	67/645	10.39
treated ♀♀ x untreated ♂♂	69/525	13.14

The flies were raised in acid medium, without nipagin and treated when 7 days old. To study the effect on motile sperm, Samarkand males were injected intraabdominally with nipagin at a concentration of 1.67% in NaCl 0.4%. The treated males were then pair mated with untreated cn bw, e females in empty vials. After one observed mating the males were discarded and the females transferred to oviposition chambers (Munoz & Mazar 1978) for two 24 h periods. The eggs were counted and the