Table 2. Emergence data on C. amoena larvae after subzero treatment.

	Days at specified t°					Numbers			Days		
Source	10°C	-2°C	-5/6°C	-2°C	10°C	l	p	i	l-p	p-i	T
E.J.	7	7				2	2	1	5	10	15
E.J.	7	8	5	8	1	7	6	4	5-10	11-15	16-20
G.R.	-	-	22h	-	-	5	1	0	no emergence		
G.R.	3	-	5	8	1	6	0	0	reactive		
G.R.	7	1	5	8	1	5	0	0	reactive		

As shown in Table 2, successful emergence after -5/6°C requires at least several days preconditioning at 10°C and -2°C beforehand. This is similar to the previous discovery that storage at -2°C a week or longer requires preconditioning at 10°C and is therefore equivalent to what botanists call "hardening". Whatever changes are occurring are necessary to withstand successive lower temperatures, which in Michigan may be well below -6°C when there is no snow cover.

After both -2°C and -5/6°C larvae require at least 5 days to reach the pupal stage again. Emergence data are comparable, 15-16 days, and compare favorably to emergence data for C. amoena from spring-collected apples kept in the laboratory at 22°C.

As a check that the 3rd or late instar is the overwintering stage for C. amoena, apples were collected from a nearby orchard in early March after a period of very cold weather in a winter of little snow cover. They were held at 10°C overnight, then inspected for C. amoena larvae. Five were found; one 2nd instar was dead, three of those actively mobile and feeding were late instars, and one was borderline late instar in size. The apples were quite soggy after defrosting, which suggests that C. amoena larvae may be freeze tolerant rather than freeze-susceptible with a very low supercooling point (Zachariassen and Hammel 1976a,b).

References: Zachariassen, K.E. and H.T. Hammel 1976a, Norw. J. Zool. 24:349-352; ____ and ____ 1976b, Nature 262:285-287.

Beck, A.K., R.R. Racine and F.E. Würgler.
Institute of Toxicology, Swiss Federal Institute of Technology, and University of Zürich, Switzerland. Primary nondisjunction frequencies in 7 chromosome substitution stocks of D. melanogaster.

In continuation of the work by Racine, Beck and Würgler (1979) a number of chromosome substitution stocks were studied for the frequency of primary nondisjunction in females. The balancer stock contained the following chromosomes: Cy = In(2LR)SM5, al^2 Cy lt^v cn^2 sp^2; Pm = In(2LR)bw^{V1}; Ubx = In(3LR)Ubx^{130}; Sb = In(3R),Sb; pol = spa^{pol}. H indicates unmarked chromosomes from a Hikone-R stock, P unmarked autosomes from a stock containing the attached-XY chromosome Parker 110-8 and A the autosomes with inversions (balancer stock). Since all chromosome substitution stocks contain identical X-chromosomes (from the Hikone-R stock) and identical 4th chromosomes (pol from the A stock) the abbreviations used indicate only the stock constitution with respect to chromosomes 2 and 3 (see the table).

In the nondisjunction tests we studied the meiotic segregation of the sex-chromosomes in the females of the chromosome substitution stocks. We crossed 1-2 day old males of the genotype $Y^SX.Y^L$, In(1)EN, y B to 1 day old females. Three pairs were used per vial. After 3 days the parents were discarded. The progeny were classified according to sex and phenotype. Two types of males could not be distinguished by phenotype: $Y^SX.Y^L$, In(1)EN,y B/0 (resulting from primary nondisjunction in XX females) and $Y^SX.Y^L$, In(1)EN, y B/Y (resulting from secondary nondisjunction in XXY females). These males were crossed to C(1)DX, y f / y^+ Y B^S; bw; st pP females and surviving C(1)DX progeny indicated the presence of a free Y chromosome in the male tested, because C(1)DX, y f contains a Y-suppressed lethal.

Female	Total	Normal progeny		Nondisjunctional progeny		
stock	progeny	F	M	F	M	ND %
AA	48577	18994	29575	4	4	0.02
HA	20383	9645	10722	9	7	0.08
AH	22787	9591	13183	8	5	0.06
HH	24139	10007	14073	3	56	0.24
PA	24056	9573	14456	12	15	0.11
AP	25207	10339	14834	17	17	0.13
PP	22022	8434	13573	9	6	0.07

F = females, M = males, ND % = nondisjunctional progeny in percent of total progeny.

The results with the numbers of progeny scored and the observed frequencies of primary nondisjunction are shown in the table. With all crosses more male than female progeny were obtained. The frequencies of primary nondisjunction show some variation. In particular the HH stock displayed an unexplained high number of exceptional males. But all the nondisjunction frequencies are within the range found with wild type stocks. This demonstrates that no meiotic mutants are present in the chromosome substitution stocks studied.

This work was supported by the Swiss National Science Foundation, project no. 3.1560.77.

Reference: Racine, Beck and Würgler 1979, Mutation Res. 63:87-100.

Batterham, P. and G.K. Chambers*. Monash University, Clayton, Victoria, Australia; *Australian National University, Canberra, A.C.T., Australia. The molecular weight of a novel phenol oxidase in D. melanogaster.

PHOX, a newly discovered form of phenol oxidase (O-diphenol: O_2 oxidoreductrase E.C. 1.10.3.1.) encoded by the Phox locus (II 80.6) in D. melanogaster has been described by Batterham and McKechnie (1980). To firmly establish the novelty of this enzyme it was important to devise a test to distinguish it from A component phenol oxidases (see Seybold et al. 1974). We report here determination of the molecular weight of this new enzyme by Sephadex G-150 gel filtration. D. melanogaster pupae (48 hours old) from the Silvan (Victoria) population were homogenized (6 g pupae/4 ml buffer) in ice cold 50mM Tris/HCl buffer pH 8.3 containing 10% (w/v) sucrose and 2M urea. The homogenate was centrifuged at 10,000g for 30 min at 4°C. The supernatant (6.0 ml) was applied to a Sephadex G-150 column (5.0 x 75 cm) equilibrated with homogenization buffer lacking sucrose and urea. Fractions (15 ml) were collected at a flow rate of 40-45 ml/hr. Effluent was monitored for absorbance at 280mn, MDH activity (malate dehydrogenase: internal standard) after McReynolds and Kitto (1970) and polyacrylamide gel electrophoresis to detect phenol oxidase (after Batterham and McKechnie, 1980). The column was calibrated with chymotrypsinogen (α-CT: 25,000), ovalbumin (OA:45,000) and bovine serum albumin (BSA monomer: 68,000; BSA dimer 136,000).

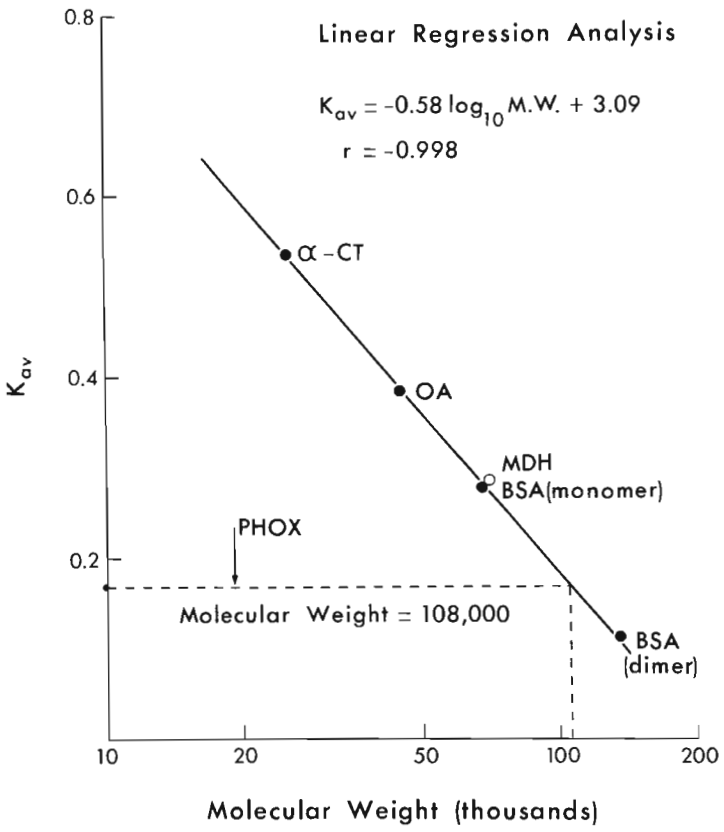

Fig. 1. Calibration of Sephadex G-150 column and calculation of the molecular weight of PHOX.

The molecular weight of MDH was taken as 71,500 (G.K. Chambers unpublished). The elution position of the PHOX enzyme was judged to be 44.5±0.5 fractions, from which we calculated a molecular weight of 108,000 ± 4,000 for the PHOX oligomer--see Fig. 1. Electrophoretic evidence (Batterham 1980) suggests that the Phox gene product is a dimer and hence we deduce the subunit molecular weight to be 54,000. From such evidence we cannot discount the possibility of higher order aggregates (e.g., a tetramer that hybridizes as pairs of dimers in heterozygotes). However, it is certain that PHOX is non-identical to the Al phenoloxidase components described by Seybold et al. (1974) as a monomer of subunit molecular weight 77,000.