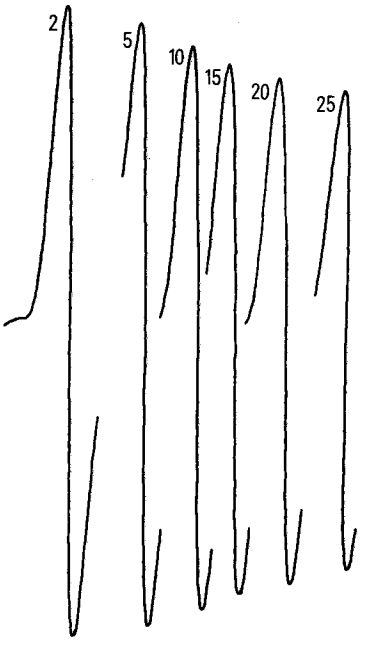
suspension reflects the rate of permeation of the red cell membrane by these compounds[11].

In a parallel experiment, blood was taken from 10 patients with trisomy 21 (karyotpye 47,XX,21+ or 47,XY,21+) and 8 age-matched healthy controls, and transport of both labels into non-fractionated erythrocytes of both groups of donors was compared.

Results and discussion. Reduction of TEMPO by erythrocyte suspension illustrating the principle of the method is shown in the figure. Within the time period employed this reduction obeyed simple exponential kinetics: $c = c(O) \exp(-kt)$ where: c-concentration of a label in the sample (proportional to the height of the ESR signal), t - time, k - penetration constant.



Reduction of TEMPO by human erythrocyte suspension. Intensity of the midfield peak of ESR spectrum as a function of time after introduction of the label (indicated in min).

Comparison of penetration constants of the labels for different age fractions of human red cells (table 1) confirms the results obtained for bovine erythrocytes[8] a decrease in the rate of penetration with increasing cell age. This effect is more pronounced under TEMPO than under TEMPOL.

Comparison of penetration constants for erythrocytes of normal donors and patients with Down's syndrome (table 2) reveals a significant reduction in the penetration rate of TEMPO and an insignificant decrease in the penetration rate of TEMPOL in Down's syndrome. These results are in agreement with the hypothesis[6] proposing that there is accelerated erythrocyte aging in Down's syndrome at the membrane level.

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Multiple overwintering mechanisms in *Chymomyza amoena* larvae (Diptera: Drosophilidae) and laboratory induction of freeze tolerance

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Summary. Chymomyza amoena larvae in apples in summer were found to be either potentially freeze tolerant or to supercool. By late September only potentially freeze tolerant larvae were recovered from apples. Larvae from walnut husks in winter supercooled to avoid freezing. However, freeze tolerance could be induced in laboratory grown larvae by placing them in apples around the seed area and maintaining them at chilling temperatures for several weeks. Overwintering mechanisms employed by *C. amoena* larvae in Michigan appear to depend upon larval feeding site.

Chymomyza amoena in Michigan has been breeding in apples since 1891 in association with primary pests[1,2]. Band and Band[3] found that fly larvae were overwintering in apples and using proteins to achieve cold-hardiness. Hemolymph osmolarity, 665 mOsm, was in agreement[4]. The watery condition of apples collected in March, 1981, after defrosting, suggested that larvae might be freeze tolerant[5]. Freeze tolerant organisms usually have an elevated freezing point, above $-10\,^{\circ}C$, which promotes rapid freezing. Freezing and supercooling points are the same and cold acclimated larvae are able to recover. In summer only potential freeze tolerance is expressed and winter-collected organisms also lose freeze tolerance if kept at room temperature[6-8]. Organisms that rely on supercooling to achieve cold-hardiness remain freeze sensitive though they may produce additional antifreeze agents to lower the freezing point as the season changes from summer to autumn to winter[9]. Unpublished data on larvae grown on chymomyzid medium indicated that *C. amoena* larvae supercooled.

C. amoena larvae feeding in apples in summer and fall, 1981, enabled determination of the mechanisms of cold-hardiness and laboratory reared larvae were later used to test induced recoverability expected of freeze tolerant organisms. Larvae obtained from walnut husks in January,

Freezing point (FP) and supercooling point (SCP) in °C of *C. amoena* 3rd instar larvae in apples in Michigan in summer and autumn, 1981. Apples collected in October and November kept in a 4 °C refrigerator until measured. All apples collected were on the ground

Site	Date	Measured	'Freeze tolerant' FP = SCP	N	Supercooling FP	SCP	N
Lansing	VII/8	VII/28	− 7.1 ± 0.25	4			
	VII/27	VII/27	− 7.4 ± 1.7	5	− 11.9 ± 1.4	− 12.4 ± 1.3	4
	VII/31	VII/31	− 7.0 ± 0	2			
	IX/14	IX/15	− 12.0 ± 2.8	2	− 9.5	− 11	1
	IX/20	IX/22	− 15.2 ± 2.1	2			
	X/10	X/14	− 8.25 ± 1.8	2			
St. Johns	XI/12	XI/16	− 4.0 ± 3.5	3			
E.J.*, D	VIII/16	VIII/17	− 7.0	1	− 10.8 ± 2.5	− 12.2 ± 2.8	3
	VIII/20	VIII/21	− 12.2 ± 3.9	5			
	X/10	X/14	− 6.5 ± 5.4	3			
E.J., V	VIII/29	VIII/31	− 7	1	− 10.9 ± 1.6	− 12.0 ± 2.8	2
E.J., S	X/10	X/23	− 7.25 ± 0.4	2	− 8	− 9	1

* E.J., East Jordan. D, V and S are separate sites. D is across the road from S and V which are about 22 m apart. All are locations of 1 or more old but still producing apple trees.

1982, confirmed the fact that in this species both supercooling and freeze tolerance are employed as overwintering mechanisms.

Apples collected at 1 site in Lansing, MI and 2 sites at East Jordan, MI provided the *C. amoena* larvae. Only larvae at least 4-5 mm in length were measured since both *Drosophila algonquin* and *D. melanogaster* shared the East Jordan sites with *C. amoena* in 1981. Larvae of the 3 species may be distinguished by the posterior spiracular region; in case of doubt larvae were compared to larvae in laboratory cultures of the 3 species.

Measurements were made in a freezing chamber cooling to − 25 °C. Larvae were attached by vaseline to the probe of a Model YS14SC telethermometer connected to a Sargent Recorder Model SF. Apples containing *C. amoena* larvae were collected in July in Lansing, August in East Jordan, in September again in Lansing and in October at both locations. In November apples were collected in a commercial orchard near Lansing. Results are shown in the table.

Larvae in apples were found to be either potentially freeze tolerant or to be relying on supercooling mechanisms. Larvae near the seed or in heavily parasitized apples tended to be potentially freeze tolerant. July to mid-August, these values ranged around − 7 °C. Larvae in apple flesh tended to supercool; average SCP was around − 12.2. Values of − 12 and − 15 °C were later attained by potentially freeze tolerant larvae.

In October and November there was again an increase in the freezing point among freeze tolerant larvae, above − 10 °C. Baust and Lee[10] have found similar decreases and increases in populations of freeze tolerant *Eurosta solidagensis* larvae. In October and November no *C. amoena* larvae registered as supercooling when apples were kept for 4 days at 4 °C before freezing point determinations were made. If there was no recovery, neither were there 2nd blips at − 12 or − 15 °C as found by Ring in one arctic species[11].

To demonstrate recoverability, 3rd instar larvae grown on chymomyzid medium were placed near the seeds in apples from the commercial orchard that lacked larvae. 2 such apples were placed in a 15 °C incubator and 2 were placed in a 4 °C refrigerator.

After 2 weeks larvae were tested for freeze tolerance. 2 of 3 at 15 °C recovered after − 7 °C but died within a week. 3 at 4 °C after 2 weeks did not recover. 2 at 4 °C after 30 days recovered after − 7 and − 14 °C respectively, and 1 pupated and emerged, was mated to successive laboratory-reared females but proved infertile. Unfortunately nothing may be inferred about the conditions needed to assure fertility since males from natural population breeding sites are not always fertile either[2].

Larvae collected from walnut husks in January were found to be supercooling to avoid freezing: FP = − 12.5 ± 0.71; SCP = − 14.0 ± 1.4. Baust et al.[12] have found that different organisms inhibiting the same hibernacula may show different mechanisms of overwintering. Here, the type of overwintering mechanism expressed by a larva seems to depend upon feeding site.

Whether the polymorphism is merely an expression of phenotype-environmental interaction or has a genetic basis currently is not known. The prolonged time at low non-freezing temperatures required to achieve recoverability at − 7 °C is in agreement with Duman's findings that proteinaceous compounds are produced slowly[13,14] while the recoverability induced in laboratory grown larvae is in agreement with Zachariassen and Hammel[7] that cold acclimated freeze tolerant organisms recover after being subjected to subzero temperatures equal to their freezing point. The data also indicate that overwintering mechanisms inferred about drosophilidids grown on laboratory media may not be wholly accurate. *D. algonquin* larvae on laboratory medium were observed to supercool; larvae from apples in August registered only potential freeze tolerance[15].

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