tures. In Fig. 2 this area is shown shaded and terminates at 4°W because Damotte *et al.* (*14*) cite evidence from Flexotir profiles along the tracks shown farther to the east that the continental slope is underlain by reflectors of Cretaceous and possibly Jurassic age. The absence of the Cretaceous reflector 1 on air-gun profiles from the 12° zone can be explained in two ways. It may not be present because that part of the crust is younger than Cretaceous. A second, equally plausible, explanation is that Cretaceous sediments were deposited there but are now too deeply buried by the highly reflecting Tertiary sediments to be detectable by the profiling system. Both interpretations conflict with the data of Watkins and Richardson. The latter precludes any Tertiary opening. The former allows only half the amount of rotation suggested by the paleomagnetic data (*5*). According to present information, the hypothesis of a post-Eocene but pre-Middle to Upper Miocene 12° rotation cannot be discarded. It is necessary to establish whether reflector 1 can be followed from the known Cretaceous outcrop at 45°04.5′N, 8°00.1′W across the 12° zone to Gascony Seamount with a seismic profiler capable of recording the total succession of the abyssal plain. However, there is no evidence for thinning of the sediments in the shaded segment in Fig. 2, which is inconsistent with a more recent age for that area. Indeed, the *C-9* profile reveals the thickest section observed on the air-gun records, showing at least 300 m of sediments below the lower turbidites. The remarkably constant depth of the lower turbidites (7.0 to 7.2 seconds of total reflection time) across the plain from the foot of the Spanish continental slope to Gascony Seamount also suggests that there is no major structural discontinuity along the southern edge of the 12° zone.

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References and Notes

1. A. Wegener, *Die Entstehung der Kontinente und Ozeane* (Vieweg, Braunschweig, 1929).
2. R. W. Girdler, *Nature* **207**, 396 (1965); *Ann. Geophys.* **24**, 695 (1968).
3. P. G. Van Dongen, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **3**, 417 (1967).
4. R. Van der Voo, *ibid.*, p. 393; *Tectonophysics* **7**, 5 (1969).
5. N. D. Watkins and A. Richardson, *Geophys. J.* **15**, 287 (1968).
6. D. H. Matthews and C. A. Williams, *Earth Planet. Sci. Lett.* **4**, 315 (1968).
7. E. J. W. Jones, *ibid.* **5**, 127 (1968); J. Cholet, B. Damotte, G. Grau, J. Debyser, L. Montadert, *Rev. Inst. Franc. Petrol. Ann. Combust. Liquides* **23**, 1029 (1968).
8. J. I. Ewing and R. Zaunere, *J. Geophys. Res.* **69**, 4913 (1964); E. J. W. Jones, *Inst. Electron. Radio Eng. Conf. Proc. No. 8* (1966), paper No. 23.
9. R. Brenot and L. Berthois, *Rev. Trav. Inst. Pêches Marit.* **26**, 221 (1962); D. Curry, E. Martini, A. J. Smith, W. F. Whittard, *Phil. Trans. Roy. Soc. London Ser. B* **245**, 267 (1962); M. L. Hadley, *Deep-Sea Res.* **11**, 767 (1964); A. A. Day, *ibid.* **5**, 249 (1959); B. M. Funnell, *Geol. Mag.* **101**, 421 (1964); M. Black, M. N. Hill, A. S. Laughton, D. H. Matthews, *Quart. J. Geol. Soc. London* **120**, 477 (1964).
10. A. S. Laughton, *Deep-Sea Res.* **15**, 21 (1968).
11. E. J. W. Jones and B. M. Funnell, *ibid.*, p. 701.
12. L. H. Burckle, personal communication.
13. B. M. Funnell, personal communication.
14. B. Damotte, J. Debyser, J. R. Delteil, L. Montadert, *Inst. Franc. Petrol. Ref. No. 16 182* (1968).
15. A. S. Laughton, *Deep-Sea Res.* **7**, 75 (1960).
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Freezing Tolerance in an Adult Insect

Abstract. *The adult carabid beetle* Pterostichus brevicornis *tolerates freezing under natural conditions. Laboratory tests confirm that winter beetles tolerate temperatures below −35°C, whereas summer beetles die if frozen at −6.6°C. Winter beetles can be cooled to about −10°C before freezing, and they thaw near −3.5°C. Summer beetles thaw at −0.7°C. To avoid freezing damage even in winter beetles, cooling rates must be near 20°C per hour or less.*

Of the multicellular animals that survive freezing, insects provide some of the most striking and best documented examples. However, freezing tolerance in insects has been thought to be almost exclusively confined to the immature forms. In fact, Asahina has stated that "in adult insects . . . bodily freezing always results in fatal injury, even at high subzero temperatures . . ." (*1*).

In the winter of 1966–67 several species of adult ground beetles (Carabidae) were found overwintering in large numbers in partially decayed tree stumps in the vicinity of Fairbanks, Alaska (65°N). When warmed to room temperature, the beetles soon became active and appeared to function in a normal, coordinated manner. Since the adult beetles were apparently surviving exposure at subzero temperatures, a series of studies was initiated to investigate the lower limits of lethal temperature, susceptibility to freezing damage, supercooling points, and whole body thawing points. In addition to these characteristics, the water content of the whole body was determined. *Pterostichus brevicornis* proved to be the most common species and the work reported here pertains to this beetle.

To determine possible seasonal changes, beetles were collected at intervals during the year. Specimens were tested within a few days to a week after collection. Initial work indicated that *P. brevicornis* collected in the winter underwent rapid changes in temperature tolerance and body chemistry when warmed to any temperature above freezing. Because of this fact, until the beetles were tested, they were stored at temperatures close to the average environmental situation at time of collection.

Determination of lower lethal temperature took into account the effect of variable cooling rate, final temperature reached, and time at final temperature. Various warming rates were tried in the initial studies (*2*).

Temperatures were measured with a fine (36 gauge) copper-constantan thermocouple affixed to the dorsal abdomen with a small bit of wax. Specimens were cooled or warmed in insulated vials in a regulated (± 0.3°C) bath. Water contents were determined during the year on representative specimens by drying to constant weight at 90° or 100°C. Studies of temperature and snow cover were also made to determine exposure in the natural situation.

The criteria for survival of lower lethal temperature tests were as follows: the specimen, when warmed to room temperature (23°C), must be capable of directed, coordinated activity such as walking, feeding, and avoidance response, and no paralysis or erratic behavior must be evident. These criteria had to be met for at least 4 days after testing for survival to be judged complete.

Results of cooling tests on beetles from different seasons are given in Table 1. Supercooling points decreased with the onset of cold weather in October and remained near −10°C during the coldest months (December,

Table 1. Seasonal summary of lethal temperatures, supercooling points (SCP), and thawing points (TP) in *Pterostichus brevicornis.*

Period	No.	SCP (°C)*	TP (°C)*	Lethal temperatures (°C)
15 Sept.–15 Oct.	120	− 8.0° ± .13		− 5° to −10°
1 Dec.–15 Mar.	48	−10.0° ± .17	−3.5°	Below −35°
15 April–25 May	62	− 7.4° ± .19		−25° to −15°
June	20	− 6.6° ± .35	−0.7°	Above −6.6°

* Mean plus or minus standard error.

Table 2. Lethal temperature tests, 1967–68 winter beetles.

No.	Cooling rate (°C per hour)	Minimum temperature	Time (hour)*	Survival† (%)
6	20	−10°	1	100
6	20	−30°	1	100
6	20	−30°	1	83
8	~300	−30°	1	38
7	~300	−30°	1–4	14
8	~300	−10°	1	100
8	200–1200	−15°	1	100
8	200–1200	−20°	1	50
24	3	−50°	2	33
15	3	−50°	8	50
8	3.5	−60°	2	75
20	4	−70°	2	90
17	4	−87°	5	67

* Refers to length of time kept at minimum temperature. † Refers to percentage of test beetles that were alive and apparently normal 3 days after testing.

January, and February). They rose during the spring and reached a maximum near −6.6°C in the summer. Thawing points are available only for summer and winter beetles, but they show a seasonal change similar in magnitude and direction to changes in supercooling points.

Changes in lethal temperature during the year were even more striking. During the summer *P. brevicornis* could not withstand any freezing. If the supercooling point was reached and freezing occurred, the beetles were immediately killed or badly injured. In contrast, winter beetles consistently withstood temperatures below −35°C, and one group of 17 showed 67 percent survival without apparent injury after 5 hours at −87°C (Table 2). At very low temperatures (−50°C or colder) rather wide variations in mortality occurred from one group to the next, even with very slow cooling. It is probable that rate of warming becomes important at such low temperatures because of recrystallization processes.

In winter beetles, freezing survival was dependent to a considerable extent on cooling rate (Table 2). Survival was greatest at rates of 20°C per hour or less. At rates of 200°C per hour or over, freezing mortality increased markedly if the minimum temperature reached was below −15°C.

To test for the possibility that beetles in their natural, undisturbed situation were not frozen, but only supercooled, several experiments were done. By visual observation, beetles in stumps at −20°C or below appeared to be brittle. Beetles collected at stump temperatures below −20°C were placed in insulated containers and brought into the laboratory unthawed for determination of possible supercooling points and thawing point curves. No supercooling point curves were seen upon cooling from −20°C to −40°C, but when warmed from −20°C to 0°C, small negative heat of fusion curves were invariably seen near the expected thawing point. The lack of a supercooling point and presence of a thawing point curve both indicate that the beetles were frozen while in the stumps.

Environmental observations confirmed the laboratory findings with respect to freezing survival. Temperatures near −30°C have been measured in the center of snow-covered stumps in winter. In years of light snowfall, stump temperatures must frequently approach air temperature, and during prolonged cold spells the beetles are undoubtedly exposed to −40°C or below. Yet in early April and May the beetles show increasing movement in their natural habitat, and by the end of May only a few individuals remain in the winter hibernaculum.

The question thus arises as to what mechanisms are involved in freezing survival in *Pterostichus brevicornis.* Since freezing points and supercooling points change from summer to winter, paralleling changes in freezing tolerance, it is reasonable to suspect that changes in composition of body fluids confer protection against freezing. Similar changes, some even more marked, have been noted in a variety of immature insects (*3–5*). In many of these, changes in supercooling and freezing points may be accounted for on the basis of changes in concentrations of glycerol or other polyhydric alcohols. However, immature insects have been found which do not contain glycerol, yet are tolerant of freezing (*6, 7*). To add to the confusion, there are a number of insect larvae that cannot survive freezing even though they contain large amounts of glycerol (*3, 4, 6*).

Two possible mechanisms of freezing tolerance have been investigated in the present species. Seasonal changes in hemolymph glycerol in *P. brevicornis* vary from 0 percent in summer to about 25 percent in winter (*8*). In addition, whole body water content, determined on a seasonal basis, ranges from 54 ± 1 percent in winter to 65 ± 1 percent in summer. Such a relatively small difference in water content between the two seasons could not begin to account for changes in freezing point. However, the freezing point depression during winter could be accounted for by the glycerol present.

The fact that even winter beetles do not tolerate rapid cooling favors the idea that intracellular freezing occurs during fast cooling, and that beetles survive under natural conditions because they are in a situation where cooling is relatively slow and freezing is extracellular. These results are thus compatible with the calculations of Mazur (*9*), namely, that cooling velocities below 1°C per minute favor survival in many cells.

The finding that adult insects can survive freezing implies that the limiting factor in freezing survival, at least in insects, is not the level of tissue organization, as postulated (*1*). Although glycerol may be a major factor enabling *P. brevicornis* to tolerate freezing, in view of the conflicting information relating glycerol and freezing resistance, additional information is needed.

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References and Notes

1. E. Asahina, in *Cryobiology*, H. T. Meryman, Ed. (Academic Press, New York, 1966), p. 463.
2. H. T. Meryman, in *ibid.*, p. 70.
3. E. Asahina, in *ibid.*, pp. 470–471.
4. R. W. Salt, *Can. Entomol.* **89**, 491 (1957).
5. L. Somme, *Can. J. Zool.* **43**, 765 (1965).
6. ———, *ibid.* **42**, 87 (1964).
7. I. Takehara and E. Asahina, *Low Temp. Sci.* **B18**, 57 (1960).
8. J. Baust, *Proc. Alaskan Sci. Conf. 19th* (1968).
9. P. Mazur, *J. Gen. Physiol.* **47**, 347 (1963).
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