

SUPERCOOLING AND ITS ECOLOGICAL IMPLICATIONS IN *COELOTES ATROPOS* (ARANEAE, AGELENIDAE)

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ABSTRACT. Field observations have shown *Coelotes atropos* to be winter-active and tolerant of a wide environmental gradient. This study suggests that low temperature tolerance is achieved by a combination of behavioral thermoregulation and physiological adaptation. It was found that the two populations studied, one living at 732 m elevation and the other at sea level, were not significantly different in their ability to supercool. However, a highly significant relationship between body weight and ability to supercool was demonstrated such that immature stages are far more tolerant of low temperatures than adults. Juvenile spiders were not only able to tolerate sub-zero temperatures, but also demonstrated an ability to cold acclimate. They were active in the supercooled state and capable of silk production at -5°C . Mechanisms which may account for the loss of supercooling ability are discussed as well as the implications of such a change for habitat utilization and life cycle strategy.

Poikilothermic arthropods living in northern temperate zones have evolved a variety of overwintering strategies to maximize their fitness. Spiders can be considered a model group in the study of winter ecology (Schaefer 1977, 1987), showing a variety of strategies of tolerating temporal low temperature stress. Five basic life cycle patterns can be distinguished: 1) Eurychronous species that mature after two or more years and therefore overwinter in various developmental stages; 2) Stenochronous species that reproduce in spring or summer and overwinter as immatures, (Theridiidae, Salticidae and Lycosidae); 3) Stenochronous species that lay eggs in autumn and overwinter as spiderlings inside the egg case (Araneidae); 4) Stenochronous species that reproduce during winter (Linyphiidae); and 5) Diplochronous species that reproduce both in spring and autumn and overwinter as adults.

Coelotes atropos (Walckenaer), an agelenid spider, appears to be annual (type two), the main overwintering stage being the juvenile spider. During this period mortality due to low temperature must be minimized. Thus one would expect cryoprotectant synthesis, which facilitates supercooling of tissues, to be strongly selected for in the juvenile spiders. Many passively overwintering insects accumulate polyhydric alcohols in their hemolymph (Kirchner & Kestler 1969;

Kirchner 1973, 1987). However, the increased osmotic pressure resulting from high polyol concentrations would require large scale physiological and biochemical changes, which may not be possible in a winter-active animal (Duman 1977) such as *Coelotes atropos*. Another factor implicated in freezing point depression is thermal-hysteresis protein (THP); these have been shown to occur in insects (Husby & Zachariassen 1980) and spiders (Duman 1979; Aunaas et al., 1983). Two more important parameters to consider when determining freeze tolerance are the type of gut contents (the presence of ice nucleators) and the level of dehydration (Sømme 1982; Zachariassen 1982; Cannon & Block 1988). This study was undertaken primarily to establish the supercooling point (SCP) of *Coelotes atropos*, to test whether this would differ between populations from two extremes of an altitudinal gradient, and to test the spiders' ability to cold acclimate. Further, I was interested to know whether adults were more or less cold tolerant than juvenile spiders (this question has a bearing on the phenomenon of the dead mother being cannibalized by her overwintering spiderlings (Bristowe 1954)).

METHODS

Study sites.—Two study sites were chosen for their degree of exposure to altitude (and therefore temperature) and wind effects. Both habitats had a plentiful supply of stones that were suitable for *C. atropos* retreats. *The Plynlimon site, Dyfed,*

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Wales UK: During January and February 1989, collections of spiders were made from rock scree on the summit of Plynlimon Fawr (National Grid Reference SN 789868) at an altitude of 752 m. Animals were taken from stones in unstable scree on the NW facing slopes and from more stable scree on the SE slope of the summit. Both habitats support a spider community which comprises *C. atropos*, *Robertus lividus* (Blackwall), *Poecilonea globosa* (Wider) and *Centromerus prudens* (O. P.-Cambridge). *The Arth valley, Aberarth, Dyfed:* During the same period animals were collected from the sheltered wooded valley of the River Arth (National Grid Reference SN 489625) at an altitude of 9.5 m, where stone-strewn slopes beneath *Quercus petraea* (Matushka) Liebl. were found to support large populations of *C. atropos*.

Supercooling point determination.—A Peltier unit (a thermopile) was set on a stage such that water cooled to 3 °C could circulate around a heat sink under the stage. In order to achieve sub-zero temperatures, it was necessary to cover the stage with an insulated cover. The freezing chamber consisted of two halves of an aluminum dish (45 mm diameter), the bottom half of which was in direct contact with the thermopile. Two thermocouples (Copper/Constantan) were used to record temperature, one connected to the chamber, the other to the spider's prosoma, thus allowing simultaneous monitoring of the animal's body temperature and the temperature of the chamber. After collection, juvenile and adult female spiders were stored in 5 cm × 2 cm vials at 5 °C prior to being anesthetized with CO₂, weighed, and placed in the freezing chamber. The thermocouple was attached to the prosoma with correcting fluid; however, for four of the largest spiders it had to be attached with cellophane adhesive tape. Thermocouples were connected to Comark electronic thermometers, which in turn were linked to a Bryan 2700 chart recorder. Convention in low temperature experiments is to decrease body temperature by 1 °C min⁻¹ (Salt 1961). This was achieved by use of a variable power control to the Peltier unit, while monitoring the falling temperature curve with a stop watch. Continual monitoring of the animal's falling body temperature enabled determination of the SCP exotherm, the sudden release of the latent heat of fusion when the animal freezes spontaneously.

Animals used in the cold acclimation experiment were placed in individual vials with small

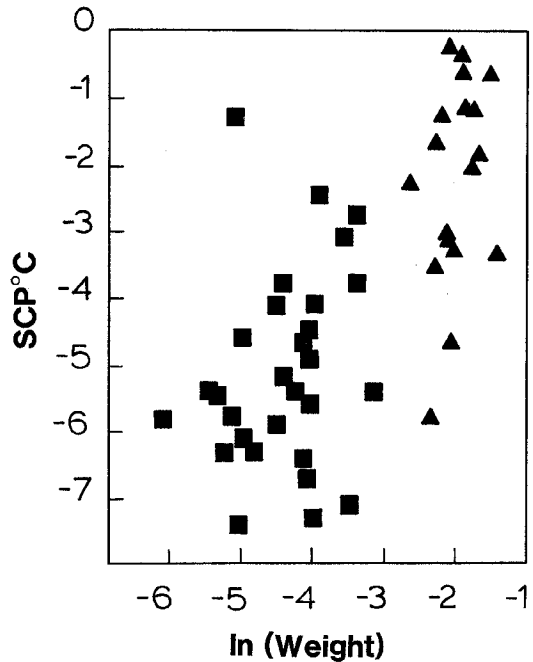


Figure 1.—Plot of supercooling points (SCP) against the natural logarithm of body weight (g): for adult ($n = 18$ [triangles]), and juvenile ($n = 29$ [squares]) *C. atropos*. Spiders were from both study sites.

amounts of moss as substrate. These were submerged in a water bath containing ethylene glycol and run at -5 °C to -2 °C (mean = -3.5 °C) for 14 days. Light regimes were L: 10: D: 14, closely following the ambient L/D cycle. Since a period of 14 days was found to be adequate for the spider *Clubiona* to acclimate (Duman 1979), a similar time scale was adopted for *C. atropos*. Throughout the experiment no animal was fed, and none underwent ecdysis. A total of 93 juvenile and 18 adult spiders were used for the statistical analyses using one-way ANOVA. Data from the samples shown in Fig. 1 were combined to test for differences in SCP between adults and juveniles. Tests to detect differences between the two populations of juveniles spiders were conducted on a separate sample ($n = 31$). Spiders used for the cold acclimation experiment were also from an independent sample ($n = 33$).

RESULTS

Supercooling experiments.—Fig. 1 shows a plot of SCP against the natural logarithm of body weight for animals from both Plynlimon and Arth sites. Adult spiders ($n = 18$) appear to have a much higher mean freezing point than juveniles ($n = 29$). Due to the paucity of adults at the

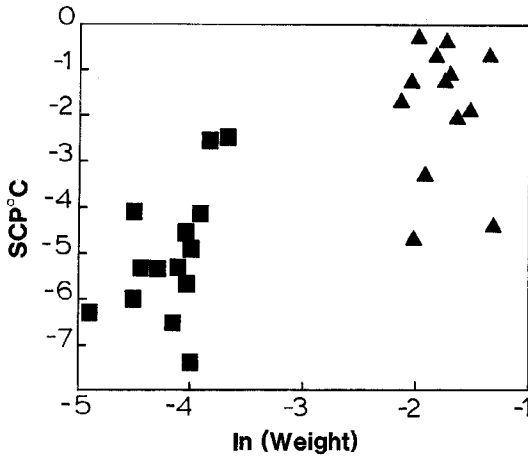


Figure 2.—Plot of supercooling points (SCP) against the natural logarithm of body weight (g): for adult ($n = 13$ [triangles]), and juvenile ($n = 14$ [squares]) *C. atropos*. Spiders were from sea level, Arth site.

Plynlimon site ($n = 5$), data from the Arth site (Fig. 2) were used to test for a difference in SCP between adults > 100 mg ($n = 13$) and juveniles < 100 mg ($n = 14$). It proved to be highly significant (One-Way ANOVA: $F_{(1,25)} = 46.66$, $P < 0.001$). Consequently, adults and juveniles were considered separately in population comparisons.

A further test showed there to be no significant difference between the SCP of the Plynlimon ($n = 17$) and Arth ($n = 14$) juvenile samples (One-Way ANOVA: $F_{(1,29)} < 1$, NS; Fig. 3). Therefore, ability to cold acclimate was tested using juveniles from both populations. Cold acclimation to -3.5 °C for 14 days resulted in a significantly enhanced mean SCP. Cold acclimated animals mean SCP = -6.1 °C, $n = 33$; whereas the mean SCP for field fresh animals = -4.4 °C, $n = 39$ (One-Way ANOVA: $F_{(1,70)} = 17.49$, $P < 0.001$; Fig. 4). A test for an effect of location (Arth or Plynlimon) on ability to cold acclimate proved to be non-significant ($P > 0.05$).

Two observations of interest made during the cold acclimation experiment related to the adults' inability to survive sub-zero temperatures and to silk production by the juveniles. Three adult female spiders subjected to sub-freezing temperatures (-3.5 °C) died after 24 hours exposure. All juvenile spiders ($n = 33$) not only survived for the duration of the experiment (14 days), but exhibited normal movements in the supercooled state; silk synthesis continued apparently unhindered and webs were fashioned on the substrate.

Natural History Observations.—In Britain the

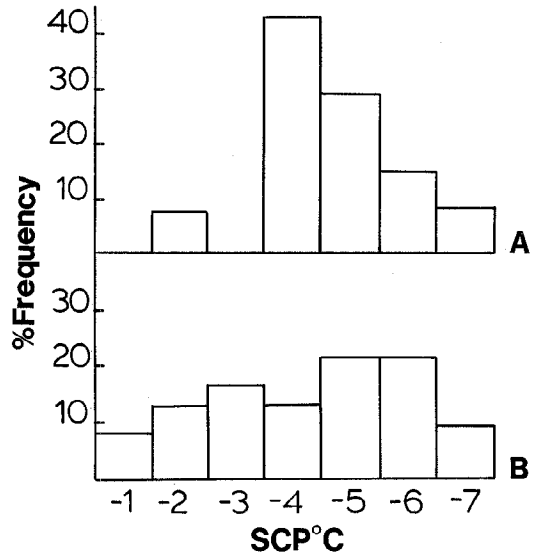


Figure 3.—Frequency distribution histograms of supercooling points (SCP) for juvenile spiders. A) Arth sample ($n = 14$). B) Plynlimon sample ($n = 17$). There was no significant difference between the populations in their ability to supercool body fluids.

genus *Coelotes* contains two species; *C. atropos* and *Coelotes terrestris* (Wider). Whereas their distribution overlaps in southern counties, *C. atropos* is much more common in the North and West (Lockett et al. 1974) where it is particularly associated with high ground. However, its range does not extend much into Scotland, even though it is able to tolerate extremes of climate associated with mountain tops. While the *C. atropos*

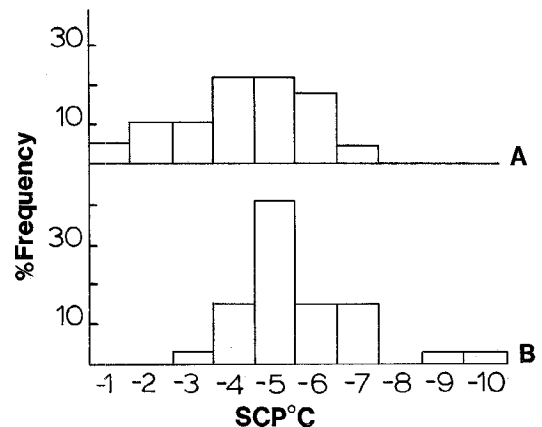


Figure 4.—Frequency distribution histograms of supercooling points (SCP) for juvenile *C. atropos* samples from both sites, juvenile spiders only. A) field fresh spiders ($n = 39$). B) spiders after 14 days acclimation at -3.5 °C ($n = 33$).

population from Plynlimon Fawr (752 m) was sampled during this study, the species has also been recorded from several other Welsh mountain habitats including Snowdon (> 920 m), Cader Idris (893 m), and the Brecon Beacons (887 m) (Bristowe 1938).

C. atropos is cryptozoic, and the web, often in the form of a tube, is built under stones and logs. A collar surrounds the opening of the retreat, while the proximal end often bifurcates. Eggs are laid in June and spiderlings eclose after a month or so (Bristowe 1954). Petto (1990) reports that populations of *C. terrestris* in Germany are biennial; mating occurs in the autumn and both juveniles and adult females overwinter. Bristowe (1954) states that in Britain *C. atropos* mates during spring or early summer suggesting an annual life-cycle; the loss of supercooling ability demonstrated to occur in adult female *C. atropos* is certainly consistent with such a strategy. After emergence the spiderlings remain together for a considerable period, often several months, during which time they are fed and guarded by the mother (Bristowe 1954). In casual observations of *C. atropos* web sites over several seasons, I have often observed dead adult females being consumed by their spiderlings. This has been observed by other authors (Bristowe 1954; Tretzel 1961) and should facilitate offspring survival until the spring.

Whereas immature stages of *C. atropos* often feed on various stages of Collembola (pers. obs.), adults and sub-adults feed largely on Coleoptera (Bristowe 1954; Tretzel 1961). Prey remains (elytra) found in webs at the Plynlimon site indicate that adult *C. atropos* feed largely on the following predatory ground beetles: Family Carabidae, *Pterosticus madius* F., *Carabus problematicus* Herbst, *Carabus arvensis* Herbst, and *Calathus melanocephalus* L.; Family Elateridae, *Ctenicera cuprea* F.

DISCUSSION

Kirchner (1973) recognized three main categories of spider SCP distributions which clearly reflect the animals' overwintering microhabitat. These range from the low SCP of *Theridion notatum* (Clerck) (= *Enoplognatha ovata* (Clerck)) (mean = -26.1 °C) which overwinters in open vegetation, to the high SCP of *Meta menardi* (Latreille) (mean = -4 °C) which lives in caves that are subject to little temperature fluctuation. A German population of *Coelotes terrestris* (also cryptozoic) was shown to have a mean SCP of

-6.2 °C: this Kirchner placed in a medium-to-low category of cold tolerance. The equally high SCP (mean = -4.4 °C, $n = 39$) exhibited by *C. atropos* in this study appears to be consistent with its cryptozoic behavior.

Ability to withstand the lowest winter temperatures that occur annually in a habitat will be strongly selected for. Consequently, geographical variation in supercooling ability and behavioral thermoregulation (or both) should be expected in populations with wide geographical or altitudinal ranges (Sømme 1982), and should be most strongly expressed in species that overwinter in exposed conditions. Whereas differences in supercooling abilities have been detected between separate populations in other arthropod species (Macphee 1961; Hansen 1978), no significant difference could be detected between the two populations of *C. atropos* with regard to their ability to supercool. Cryptozoic thermoregulatory behavior seems to be a vital component in allowing *C. atropos* to utilize hostile environments (i.e., mountain summits). Perhaps the effectiveness of such behavior might explain why the species as a whole has such a high SCP, and why high elevation populations have not evolved a lower SCP. *Coelotes atropos* responded to sub-zero temperature acclimation by enhancing its ability to supercool. Such cold acclimation has been shown to occur in many species of insects and mites (Schenker 1983; Cannon 1986; Cannon & Block 1988). However, Kirchner & Kullman (1975) showed that supercooling ability in the spiders *Theridion sisyphium* (Clerck) and *T. impressum* (L. Koch), both of which overwinter in unprotected vegetation, did not appear to be affected by warm or cold acclimation. Whereas the end products of cold acclimation, such as increased levels of glycerol and other cryoprotectants, are easily demonstrable in insects, the precise ecophysiological mechanisms of acclimation are little understood. Indeed, only recently has a start been made to elucidate the neural basis of thermal reception and perception in spiders (Pulz 1986).

Another factor implicated in freezing point depression is dehydration. Although the required degree of desiccation was probably not reached during the cold acclimation experiment reported here, it must be borne in mind as a possible contributory factor. Water loss can increase the solute concentration of the hemolymph, thus depressing freezing point, without necessarily requiring further cryoprotectant synthesis. Finally,

certain spiders have been shown to possess THP in their hemolymph (Duman 1979; Husby & Zachariassen 1980), and under natural conditions, THP production should be strongly selected for in a winter-active animal.

The remarkable ability of all juvenile spiders ($n = 33$) to synthesize silk and construct webs on the frozen substrate during the cold acclimation experiment warrants further study. Throughout the fourteen day period (at -3.5°C) the animals exhibited normal coordinated movements, with no sign of chill coma. Such observations are supported by Aitchison (1987) who observed winter-activity in juvenile spiders of several families in temperatures as low as -8°C , and Hågvar (1973) who reported copulation in *Bolyphantes index* (Thorell) at sub-freezing temperatures. Whereas overwintering juvenile spiders may display both inhibition of ecdysis and low metabolic rates (Schaefer 1987), ability to move normally and produce silk at sub-freezing temperatures might confer selective advantage if it allowed food capture and consumption during periods when temperatures rose above 6°C . *C. atropos* is capable of high food consumption at 8°C and 10°C but exhibits an arrested development at 6°C (Aitchison 1981). If, as the evidence seems to suggest, *C. atropos* does consume food during the winter, it seems likely that THP will be synthesized in the midgut, thus preventing inoculative freezing. However, there is some debate concerning the effectiveness of the filtering process as a method of removing ice nucleators during the feeding process in spiders in general and Kirchner (1987) has suggested that most nucleators may be removed by the process. Ramsey (1964) has shown THP to occur in the midgut of insects, but its occurrence in the midgut of spiders has yet to be shown. Loss of supercooling ability in adult females may result from physiological changes associated with oogenesis, or if THP is involved, its synthesis may be mediated by the presence of juvenile hormone (JH). THP regulation by JH does occur in certain insects (Horwath & Duman 1983; Hamilton et al. 1986), and recent evidence (Carrel et al. in press), has shown for the first time that spiders do utilize JH to regulate development. The precise mechanism notwithstanding, loss of cold tolerance after maturity should result in strong selection pressure towards an annual life cycle. In *Argyroneta aquatica* (Clerck) (Bromhall, 1988) and in some lycosids (Schaefer 1977), where overwintering occurs in both adult and juvenile

stages, no loss of supercooling ability occurs during either stage. Conversely, Kirchner & Kullman (1975) found that supercooling ability in *Theridion* spp. where overwintering occurs mainly in the juvenile stages, did, as in *C. atropos*, vary with age.

Ontogenetic loss of supercooling ability in *C. atropos* combined with temporal and spatial climatic fluctuations may result in a change of life cycle strategy. At the Plynlimon site only five adult females were encountered during a total of four collecting trips (cf juveniles $n = 37$), whereas at the Arth site, presumably as a consequence of temperature amelioration by the nearby sea, adult females were plentiful throughout the sample period (during January and February 1988, temperatures at the Arth site fell below freezing on only 9 occasions, reaching a low of -2.2°C , with a mean of -1.1°C for those days when the temperature fell below zero). If there are severe low temperatures early in the winter, a female may die and be digested by (and thereby contribute to the survivorship of) her spiderlings. If, however, the winter is unusually mild (as during the period of the study 1988-1989), then adult females are able to survive and possibly reproduce for a second time the following spring. Such a strategy together with cannibalism of the dead mother by her overwintering spiderlings, provide the animal with a "bet hedging" system well able to contend with most climatic eventualities.

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