Argyroneta aquatica (Clerck) over-wintering behaviour and super-cooling point

Clive Bromhall
Animal Ecology Research Group,
Department of Zoology,
University of Oxford,
South Parks Road, Oxford, OX1 3PS

Summary

Argyroneta, living in disused peat-workings, was found to live at all levels in the water during the summer and winter. The formation of ice in the waterways did not change the spider's distribution and *Argyroneta* was collected from both beneath and within ice. Spiders released from thawed blocks of ice were alive and unharmed.

The freezing-temperature, or super-cooling point (SCP) of *Argyroneta* was investigated for spiders frozen at a rate of 1°C/minute. The mean SCP of all the spiders tested was −9.2°C. There was no significant difference between the SCP's of (a) spiders collected in the summer and winter, (b) males and females, and (c) mature and immature spiders. There was also no significant relationship between weight and SCP. Premature freezing by 'ice-inoculation' did not occur when *Argyroneta* was frozen in contact with ice. None of the spiders, used in any experiment, survived freezing of their tissues.

Introduction

A remarkable feature of most temperate and arctic spiders is their ability to survive extremely cold winter conditions, some species even remaining active at temperatures below 0°C (Aitchison, 1978). Spiders, like any other invertebrate, can survive severe winters by avoiding lethal cold temperatures through behavioural adaptations, and/or by tolerating the cold through physiological adaptations. Of the 60 species, or so, of spiders tested, all have been found to freeze at temperatures well below 0°C (Kirchner, 1987); the freezing-temperatures, or 'super-cooling points' (SCP), of different species range from −4 to −34°C. However, the SCP of some spiders is lower in the winter than in the summer (Kirchner, 1973; Schaefer, 1976). Unlike some insects (Baust *et al.*, 1982), no spiders have yet been found which can tolerate freezing of their tissues (Kirchner, 1987). The survival of many species of spider over the winter thus depends on their possession of 'cryoprotectants', which depress the freezing point of the tissues below 0°C. Whereas a number of substances have been suggested as cryoprotectants in insects (see Duman *et al.*, 1982), only two possible cryoprotectants have been discovered in spiders. Glycerol was found to comprise 2-3% of the fresh body weight of *Araneus cornutus* Clerck in winter (Kirchner & Kestler, 1969), and a protein with cryoprotecting properties was found, in addition to glycerol, in species of *Philodromus* and *Clubiona* (Duman, 1979).

The SCP may depend on a number of factors other than the concentration of cryoprotectants. Both the type of gut-contents (Sømme, 1982; Zachariassen, 1982) and the level of dehydration (Sømme, 1982) have been found to affect the SCP in insects. In addition, water in contact with the animal's body may drastically reduce its ability to super-cool. Salt (1963, 1969) proposed that premature freezing occurs in some insects as a result of 'ice-inoculation'; he suggested that ice-crystals, forming on the outside of the animal, grow through the cuticle and thereby initiate freezing within the body. Many spiders overwinter in damp places (Schaefer, 1977) and these may therefore be susceptible to the putative ice-inoculation; however, none would be more at risk than the water-spider, *Argyroneta aquatica* (Clerck).

Of all the world's spiders, *Argyroneta* leads the most aquatic life-style (Bristowe, 1930, 1958). This spider needs to surface only to replenish the air, trapped by dense hairs, which envelops its body when it dives. Some of this air can be 'wiped off' the abdomen by the fourth pair of legs when the spider forms its underwater retreat. Once the air is released from the abdomen it is kept underwater by a small sheet of silk, which has previously been woven between vegetation, to form a 'diving-bell'. The spiders eat, mate and lay their eggs in the diving-bell, venturing out only to capture food, collect air, or to find a mate. During the winter, *Argyroneta* has not only been collected underwater (Ussing, 1912), but has also been found beneath ice (Nielsen, 1932). Furthermore, Wagner (1894) has described how *Argyroneta* hibernates in empty snail-shells which are secured underwater, and Wesenberg-Lund (1896) has reported finding the spider in snail-shells locked into ice.

This study set out to compare the summer and winter distributions of *Argyroneta* in a single site, to determine the spiders' SCP at different times of the year and to investigate the possibility of 'ice-inoculation' in freezing spiders.

Materials and Methods

Argyroneta aquatica was studied in a site on the English/Welsh border, near Shrewsbury. The spiders were living amongst *Sphagnum* moss in water-filled disused peat workings, the water being 10-40 cm deep with a pH of 4.9. This site was visited at various times of the year between September 1983 and June 1987. The spiders used for the freezing experiments were collected in the winter of 1984 (27 February) and in the summer of 1987 (10 June); mature and immature spiders of both sexes were collected during the winter, but only adult females during the summer.

A total of 39 spiders were used for the freezing experiment. After collection from the field site, the spiders were kept for 3 days, without food and at the same temperature as that at the collection site, before being tested. None of the spiders moulted in the 3 days preceding freezing. Their SCP was measured using a cooling-rate of 1°C/minute, this being achieved by placing the spiders inside an insulated container (Fig. 1a) which was then lowered into a −70°C freezer. In order to maintain the same cooling-rate for all experiments, the amount of insulation was reduced in those experiments when the spider-chamber contained water. The SCP of the spiders was determined using the latent heat of fusion released by the liquids of the spiders as they froze. The descending temperatures of living, but restrained, spiders were continuously recorded with a thermocouple, a 3 to 8°C rise occurring when the spider froze. A Portec (P.I.8013) NiCr/NiAl thermometer and a Washington 400 MD2C chart-recorder were used to monitor the spider's temperature. To restrain the spiders, they were first anaesthetised with CO_2 and then held down onto a perspex plate by placing "Plasticene" over their legs. A thermocouple was fixed to the dorsal surface of the prosoma using latex glue ("Copydex"). The spiders were then placed in a glass bottle and either kept dry with silica gel, or wet by mist-spraying into the bottle (Fig. 1b). Whilst the rate of freezing was carefully controlled, the spiders thawed rapidly when they were removed from the insulated box and exposed to room temperature. Statistical methods throughout this paper follow Steel & Torrie (1981).

Results

During the summer and winter, *Argyroneta* was found at all levels in the *Sphagnum*, with diving bells being most common at a depth of 2-6 cm below the water-surface. In the winter of 1983, when the surface of the water was frozen, *Argyroneta* was also found at all levels in the water, and spiders were collected from both beneath and within the ice. The spiders collected from beneath the ice were active when brought above water. On the other hand, eight *Argyroneta* were found within the ice and these spiders, rather than being encapsulated in pockets of air, had their legs spread out so that all parts of their bodies were held by ice. These spiders were brought back to the laboratory in blocks of ice and, after melting the ice, were found to be alive and unharmed. The only other animals to be released from the ice were small, unidentified, beetles.

The mean SCP of all the spiders tested was −9.2°C (n = 39, SD = 2.0), values ranging from −5.7 to −15.8°C. *Argyroneta* collected in the winter, and frozen under dry conditions, were found to have a mean SCP of −9.8°C (n = 13, SD = 2.9). The spiders collected in the summer, when frozen under dry conditions, had a mean SCP of −9.0°C (n = 12, SD = 0.8). There was no significant difference between the SCP's of winter- and summer-collected spiders (one-way ANOVA: $F_{2,25} = 0.78, p > 0.3$). The spiders collected in the winter included males and females at different stages of maturity; there was no significant difference between the SCP of the two sexes (one-way ANOVA: $F_{2,13} = 0.82, p > 0.3$) or between mature and immature spiders (one-way ANOVA: $F_{2,13} = 1.24$, $p > 0.2$). The summer-collected spiders were used to test for possible ice-inoculative effects; spiders frozen under wet conditions had a mean SCP of −8.9°C (n = 14, SD = 1.5). There was no significant difference between the SCP's of summer-collected dry- and wet-frozen spiders (one-way ANOVA: $F_{2,26} = 0.131, p > 0.7$). When the SCP's of all the spiders tested were plotted against their respective weights, there was no significant relationship between SCP and weight (two-tail t-test using correlation coefficient: $p > 0.3$). None of the spiders, used in any experiment, survived being frozen.

Discussion

Argyroneta has been shown to be capable of staying underwater at all times of the year, regardless of the water conditions. Indeed, the spiders would be at considerably more risk if they ventured out of the water, where they would be exposed to the more extreme air-temperatures. During the winter, the air-temperature at the study site regularly falls below the SCP of *Argyroneta*; air-temperatures in Shrewsbury have fallen below −10°C at least once per year between

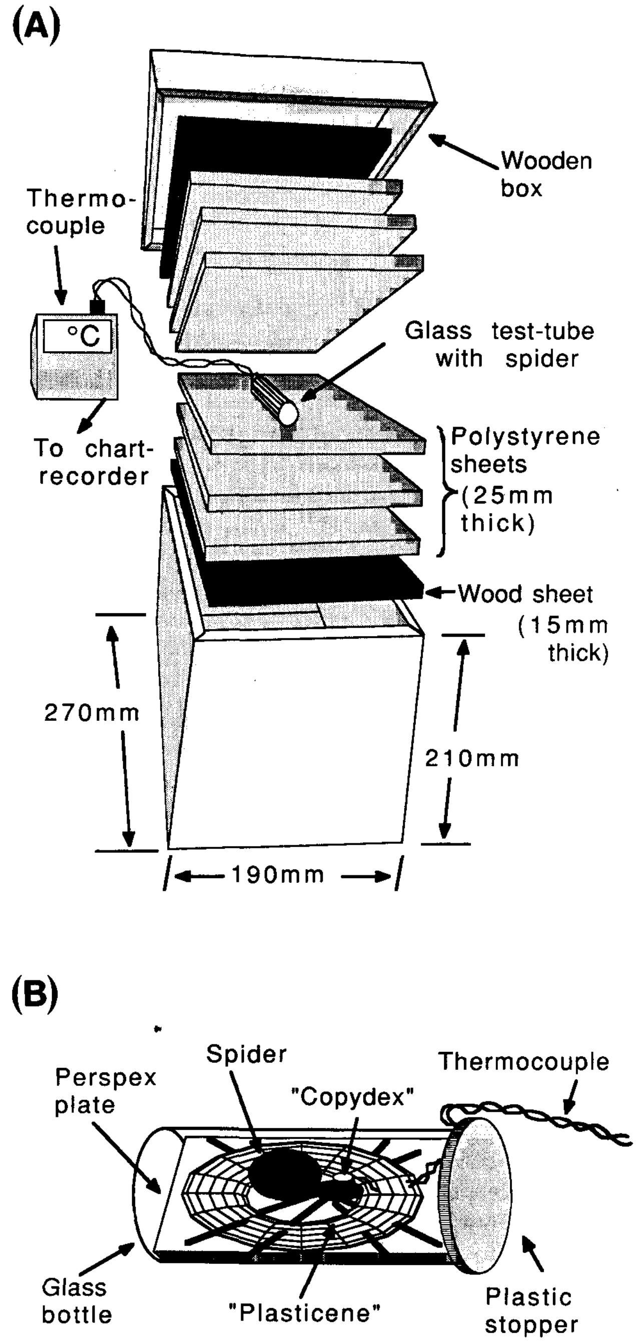

Fig. 1: Apparatus used to measure the freezing point of *Argyroneta*. **(A)** Insulated box, used to achieve a spider cooling-rate of 1°C/min when lowered into a −70°C freezer; **(B)** method of restraint, allowing temperature-recording of the spider.

1983 and 1987 (Meteorological Office Records). On the other hand, by remaining underwater, *Argyroneta* is buffered against the fluctuations of the air-temperature by the thermal capacity of the water- or ice-mass. Even during the severest winter, the deeper waterways are unlikely to be frozen for their whole depth and thus some unfrozen water will nearly always be available to some spiders.

The behaviour of *Argyroneta* during the winter appears to depend on the local conditions. Clearly, the high acidity of the peat-workings renders this site unsuitable for snails and thus precludes the behaviour described by Wagner (1894) and Wesenberg-Lund (1896) in which *Argyroneta* hibernates in snail-shells. That *Argyroneta* does not necessarily need the protection of a snail-shell to avoid being crushed by the ice was shown by the survival of the spiders released from the blocks of ice. *Argyroneta* collected from beneath the ice were capable of movement at temperatures just above 0°C. The spiders may therefore maintain some activity throughout the winter, that is, unless they become encased in ice. Like most other overwintering spiders (Kirchner, 1987), it is unlikely that *Argyroneta* needs to feed much, if at all, during the winter. Kirchner (1973) found that the oxygen consumption of *Araneus cornutus* in winter is less than half that in summer, when individuals from both seasons were tested at 25°C. Furthermore, the food requirement of *Argyroneta* is likely to be even lower than most other spiders since this species has a metabolic rate of about a half that of nearly all other spiders studied (Braun, 1931). There may, however, be selective advantages in maintaining growth during the winter, particularly for males since *Argyroneta* is one of the few spider species in which mature males can attain a larger size than mature females (Vollrath, 1980).

Argyroneta has a rather low resistance to cold, in terms of the SCP, compared with most other spiders studied (reviewed by Kirchner, 1987). As would be expected, SCP and overwintering behaviour/temperature have been found to be related when comparing species (Kirchner, 1987); for example, araneids which overwinter in vegetation have been found to have SCP's of about −20°C, whereas agelenids which overwinter under rocks have SCP's of about −6°C. The limited ability of many spiders to super-cool down to temperatures commonly recorded in the air in their locality, shows the importance of low-temperature avoidance. Schaefer (1977) found that only 6.9% of the species in northern Germany spend the winter unprotected in the vegetation. The majority of spiders use the insulating properties of vegetation, soil, stone, or even snow to protect themselves from the extremes of the air temperatures. The inability of *Argyroneta* to super-cool down to low temperatures shows that this spider relies on the insulating properties of water and ice for its survival during the winter.

As with all other spiders tested, *Argyroneta* did not survive freezing; however, the spiders were subjected to freezing and thawing rates very much faster than would normally be experienced in nature. It still remains possible that some spiders are freeze-tolerant if subjected to much slower changes in temperature. Whether or not *Argyroneta* can survive temperatures much below 0°C, regardless of whether freezing has occurred or not, was not studied. Cold injury has been shown to occur well before the SCP has been reached in both insects (Danks, 1978) and spiders (Kirchner, 1973). The duration of the cold period has also been shown to affect survival (Kirchner & Kestler, 1969; Schaefer, 1976).

The SCP of *Argyroneta* might, however, vary with the region from which the spiders are collected or with the prevailing temperatures at the time of collection. Geographical variation in the SCP of a single species has been found in insects (Sømme, 1982) and mites (MacPhee, 1961), whilst cold-temperature acclimation has been shown to lower the SCP of collembolans (Sømme, 1981) and mites (Young & Block, 1980; Block & Sømme, 1982). Seasonal changes in SCP have also been shown to occur in some spiders (Kirchner, 1987). In this study, the greater variation in the SCP's of winter-collected spiders, compared with summer-collected spiders, could indicate temperature-induced differences; for example, the spiders with relatively low SCP's may have lived in shallow water and experienced colder temperatures than spiders living in deeper water. However, the lack of any significant difference between the SCP of spiders collected, and maintained, at the winter and summer temperatures, shows that the SCP of *Argyroneta* is unaffected by temperature-acclimation. Kirchner (1987) has suggested that spiders which have constant SCP's throughout the year are typically those with 'low' or 'medium' resistance to cold (he defines resistances as: 'low' for SCP's of −4 to −8°C, 'medium' for SCP's of −8 to −16°C, and 'high' for SCP's of −16°C or lower). In accordance with this scheme, *Argyroneta* was found to have a 'medium' resistance to cold and no seasonal change in SCP.

The causes of intra-specific variation in spider SCP's have been little investigated. As with all other spiders studied (Kirchner, 1987), there was no difference between the SCP's of males and females in *Argyroneta*. There was also no significant relationship between weight and SCP, however, no previous spider weight/SCP relationships can be found for comparison. Dehydration is known to affect the SCP of some insects (Sømme, 1982), and the water content of spiders has been shown to vary with developmental stage, lipid content (which increases in gravid females), physiological state (e.g. state of feeding and moulting), age, humidity and temperature (Pulz, 1987). Developmental stage has been found to be related to differences in SCP in some species (Kirchner & Kullmann, 1975; Schaefer, 1976, 1977); however, in *Argyroneta* there was no significant relationship between maturity and SCP. Both feeding and moulting differences were minimized by starving all the spiders for 3 days before freezing and ensuring that none had moulted during this time. Whilst the possibility of gut-contents causing premature freezing, by ice-nucleation, was reduced by starving the spiders, predators feeding on a liquid diet, such as spiders, are unlikely to contain ice-nucleators in their gut (Block & Sømme, 1982; Kirchner, 1987).

Under the experimental conditions of the 'wet' freezing experiment, ice formation around the outside of the spider did not cause premature freezing by inoculation. Using similar experimental conditions, Danks (1971) found that chironomid larvae in contact with ice froze at −2 to −3°C, compared with the SCP's of −6 to −11°C for dry larvae. However, ice-inoculation is a time-dependent process (Sømme, 1982), and it is therefore possible that ice-inoculation would occur in *Argyroneta* under different experimental conditions. Sømme (1982) has suggested that a correlation exists between sensitivity to desiccation and ice-inoculation. Since the desiccation-rate of *Argyroneta* is very high compared with other spiders studied (Nemenz, 1954), its cuticle could be expected to be more permeable to ice than those of most other species. However, the dense layer of water-repelling hairs, rather than the cuticle's impermeability to ice, may be important in preventing ice-inoculation in *Argyroneta*.

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