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Developmental patterns of supercooling capacity in a subantarctic wingless fly

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Abstract. *Anatalanta aptera* is a wingless fly which lives in subantarctic islands, particularly in sea bird colonies. Developmental patterns of supercooling capacity were studied in an experimental population reared at 5 °C and fed ad libitum. Mean supercooling points of the eggs, second and third instar larvae, pupae, teneral and mature adults were −28.0 °C, −10.8 °C, −8.8 °C, 17.6 °C, −16.6 °C and −8.2 °C respectively. Low mean supercooling points were found for inactive stages (eggs, pupae) and, unexpectedly among Diptera, teneral adults. Mature adults had a combination of low supercooling ability, high lipid content and physogastry. The results are compared with those obtained in winged species from other families of Diptera.
Key words. Cold-hardiness; supercooling point; ontogenesis; winglessness; subantarctic fly; Diptera; Sphaeroceridae.

Cold-hardiness, the ability of an organism to survive at low temperature[1], has been studied in poikilotherms by means of the determination of the supercooling point (SCP) which is the temperature at which spontaneous freezing occurs[2]. The SCP may be considered the potential lower limit of survival for a given stage of a freezing-intolerant species. So an increased supercooling ability is an adaptive feature of paramount importance, especially for species which live in harsh habitats like polar and alpine regions or through a cold winter season[3].

Here we study the supercooling ability of different life stages of the wingless fly *Anatalanta aptera* Eaton (Diptera: Sphaeroceridae) (fig. 1), reared at 5 °C. This endemic fly is known from subantarctic islands of the South Indian Ocean (Crozet, Kerguelen and Heard) which are characterized by their geographic remoteness and by a cold and buffered oceanic climate (e.g. monthly mean temperatures of about 2 °C during winter and 7 °C during summer, and only 60 frosty days, mainly from July to October, at Alfred Faure, 140 m a.s.l., Iles Crozet). Adults and larvae are active all the year round and are associated with organic enrichments, particularly in sea bird colonies, from the seashore to 700 meters a.s.l. (fell-field)[4]. Availability and accessibility of food resources (faeces, carrion, feathers) are key factors which influence the duration of immature stages of *A. aptera*. Adult emergence periods are dependent on the seasonality of the trophic resources[5].

Previous studies have mainly documented ecological and physiological aspects of the adult life stage and have highlighted an exceptional longevity (nine months mean at 5 °C), striking fasting performances, and a moderate cold tolerance[6,7].

Materials and methods

Fly culture. A colony of the wingless fly *Anatalanta aptera* was maintained in the laboratory. Fly pupae, which originated from Ile de la Possession (Iles Crozet, 46°S 50°E), were sampled in December 1983, in a Royal Penguins (*Aptenodytes patagonicus*) rookery. Adults were then kept on coarse sand in an air-conditioned chamber at 5 °C (the annual mean temperature at Crozet) with a natural daily light:dark cycle. Adults

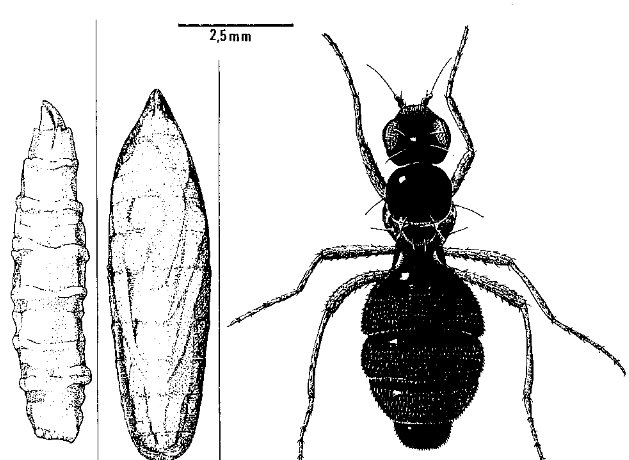

Figure 1. *Anatalanta aptera* Eaton 1875: third instar larva, pupa, mature male.

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Table. Means and standard deviation of supercooling point values (S.C.P.) (with coefficients of variation), fresh weight, water and fat contents (percentage of the fresh weight) for each life stage (except for mature adults of the parental generation and first instar larvae) of *Anatalanta aptera*.

Life stage	N	S.C.P. (°C)		Fresh weight (mg)	Water content (%)	Fat content (%)
Eggs	24	-28.0 ± 0.7	(2.5%)	0.070 ± 0.005	71.55 ± 3.50	7.24 ± 3.40
Second instar larvae	10	-10.8 ± 3.4	(31.5%)	3.876 ± 1.370	79.07 ± 3.76	9.23 ± 2.04
Third instar larvae	14	-8.8 ± 1.2	(13.6%)	8.819 ± 1.440	71.68 ± 2.74	13.17 ± 1.72
Pupae	19	-17.6 ± 1.0	(5.7%)	10.187 ± 1.648	72.09 ± 2.38	10.29 ± 2.13
Teneral males	10	-16.9 ± 2.4	(14.2%)	7.411 ± 0.752	73.25 ± 1.99	12.92 ± 1.79
Teneral females	12	-16.3 ± 1.3	(8.0%)	8.298 ± 0.894	74.05 ± 2.37	12.62 ± 2.46
Mature males	11	-8.6 ± 1.3	(15.1%)	12.630 ± 0.702	59.21 ± 3.10	22.73 ± 2.30
Mature females	13	-7.8 ± 1.2	(15.4%)	18.167 ± 2.060	57.60 ± 3.04	23.49 ± 2.32

and larvae were fed ad libitum with yolk of hen eggs (in the field, their natural food may be broken sea birds' eggs). Pupation took place in wet sand. Most of the adults emerged four to five months after the eggs were laid. Newly emerged adults are recognizable during about two weeks (the cuticle is then soft and clear) and sexual maturity is acquired around six weeks after emergence. The data reported here were obtained from March to August 1988, through a single generation. Eight mature adults of the parental generation (3 males and 5 females), 24 eggs, 24 larvae (10 larvae II and 14 larvae III), 19 pupae (pharate adults), 22 teneral adults (i.e. newly emerged adults less than one week old) (10 males and 12 females) and 24 two-months-old adults (11 mature males and 13 mature females) were surveyed. First instar larvae were not considered, owing to their fragility.

Supercooling points. Each individual was weighed with a Cahn 4700 electrobalance (sensitivity: 0.1 µg). The SCP was determined by attaching the insect to a nickel-chrome thermocouple (0.25 mm in diameter) introduced into an air-filled steel chamber (3 cm in diameter) which was cooled within a bath by a programmed cryostat at a cooling rate of 1 °C min^{-1}. The SCP was recorded as the lowest temperature at which the body fluids cease to be supercooled (i.e. when the latent heat of fusion is released).

Water and fat contents. Water contents were individually calculated as percentages of the fresh weight after drying the insect for four days in an oven at 50 °C and a week on silica gel at 0% R.H. and room temperature (c. 18 °C). Fat contents were individually determined as percentages of the fresh weight of the flies after immersion in a chloroform methanol bath (2:1) during two weeks.

Results

All the life stages were of the freezing susceptible type. Student's t-tests between male and female mean SCP values showed no significant differences. Therefore, male and female data have mostly been pooled. Mean SCP values for each of the life stages (except for first instar larvae) of *Anatalanta aptera* are given in the table.

The eggs had the lowest mean SCP and the mature adults the highest ones. Larvae and mature adults showed a weak ability to supercool (mean SCP values above to −11 °C). Pupae, teneral adults and eggs showed mean SCP values below −16 °C. In the course of the ontogenetic development, significant differences were noticed between the mean SCP values of mature adults and eggs, eggs and L II, L III and pupae, teneral adults and mature adults (t-tests, $p < 0.001$). SCP values of the two generations of mature adults did not differ statistically (-8.0 ± 1.9 °C, $n = 8$ vs. -8.2 ± 1.3 °C, $n = 24$).

Three phases in cold-hardiness were hence successively observed after the eggs were laid: 1) an important and progressive increase of SCP values from the eggs to the third instar larvae. During this phase, dry weight increase was considerable (from 0.02 to 2.37 mg, mean values); 2) a noticeable decrease of SCP values from the third instar larvae to the pupae. Supercooling ability seems to be not influenced by the adult eclosion process and SCP values of pupae and teneral adults do not statistically differ (-17.6 ± 1.0 °C, $n = 19$ vs. -16.6 ± 1.9 °C, $n = 22$); 3) an equivalent increase of SCP values from the teneral to the mature adults (i.e. identical SCP values for third instar larvae and mature adults). This decrease of supercooling ability was associated with an increase of mean fresh weight and fat content due to feeding (table). It amounts to a dramatic dry weight increase from 1.98 to 5.16 mg (males) and from 2.15 to 7.73 mg (females). A representation which associates individual SCP values and corresponding dry weights illustrates the evolution observed in the course of the second and third phases (fig. 2).

Except for teneral males, low mean SCP values were associated with a low variability of individual SCP values: the coefficients of variation were spread between 2.5% (eggs) or 5.7% (pupae) and 31.5% (second instar larvae) (table). A Spearman's rank correlation calculated between mean SCP values and coefficients

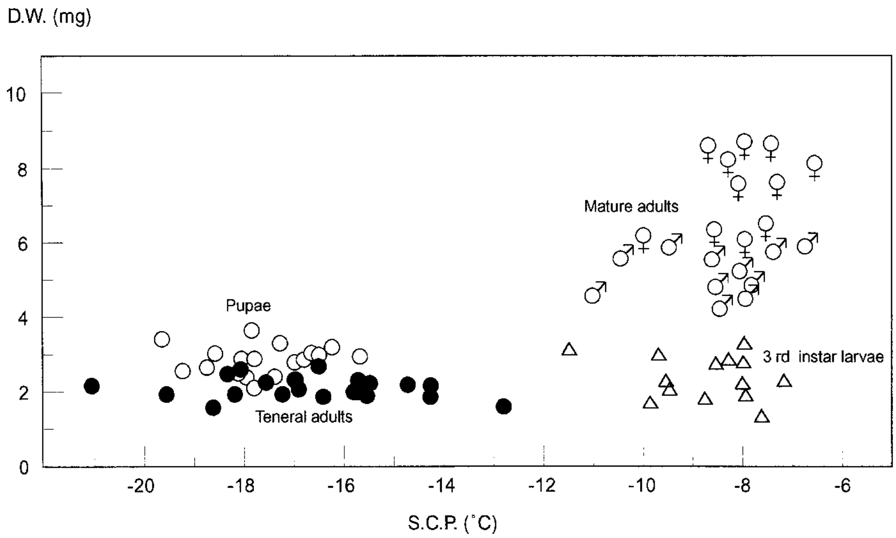

Figure 2. Individual supercooling points (S.C.P.) and dry weights (D.W.) for third instar larvae, pupae, teneral adults, and mature males and females of *Anatalanta aptera*.

of variation gave a significant coefficient of 0.762 ($p < 0.05$).

At any life stage, from the eggs to teneral and mature adults (males and females), no relationship was observed between fresh weights, water contents or fat contents on the one hand and supercooling capacities on the other. It also appears that throughout ontogenesis, light stages supercooled to lower temperatures than heavier ones. Moreover, for the different life stages, a positive correlation linked mean SCP values and mean lipid contents (Spearman's rank correlation: $r = 0.833$, $p < 0.05$) (table).

Discussion

Several studies have been devoted to the description of developmental patterns of cold-hardiness in Diptera[3,8], but, the number is small in comparison with the specific richness of the order (c. 10^5 species). Among Sphaeroceridae, an Acalypterate family of more than 700 species[9], only the cold tolerance of the wingless fly *Anatalanta aptera* has previously been investigated[7]. Developmental patterns of cold-hardiness have mainly been described in Calypterate flies like Muscidae[10,11] or Calliphoridae[12,13].

We have not investigated in this work whether the supercooling capacity of a given stage is a reliable indicator of cold-hardiness in field conditions. Several studies, not only among Diptera, have shown that in fact the SCP may not be a reliable indicator, and that cold injury mortality is the key factor in the field[14–20]. SCP data may however be useful for comparison between stages of the life cycle or between species[15]. We have found here significant differences in supercooling ability among the different instars of a species reared at a constant temperature and fed ad libitum.

As a general rule, lower mean SCP values are found in insect eggs than in other stages[3]. This is the case for the eggs of *A. aptera* which have the greatest capacity to supercool (−28.0 °C). During the second and third larval instars, supercooling ability declines, with no significant difference between the mean SCP values of each instar (−10.8 °C vs. −8.8 °C) (fig. 2). Mean SCP values of the eggs, second and third larval instars of *Musca domestica*, *Lucilia sericata*, *Calliphora vicina* or *C. vomitoria* are of the same order[10–13]. In *M. domestica*, larval age differences in cold tolerance seem to depend on differences in size[10]. Among Drosophilidae (Acalypterate flies), multiple overwintering mechanisms have been described in *Chymomyza amoena*, with a polymorphic larval population of both freeze-tolerant and freeze-sensitive larvae[21,22].

In the case of pupae, the mean SCP decreases (−17.6 °C), and makes this stage the one next to the egg with respect to ability to supercool, a trend shared with *M. domestica* and *L. sericata*, but not *C. vicina* nor *C. vomitoria*[10–13]. The newly emerged adults of *A. aptera* keep a low mean SCP (−16.6 °C), with no significant difference between observed values for these teneral adults and the pupae. This result is unusual as, in Diptera, adult emergence generally goes with a noticeable loss of supercooling ability[10–13]. In contrast, a drastic increase of mean SCP (−8.2 °C) characterizes the two-months-old mature adults of *A. aptera*. It is striking that in *M. domestica* or *L. sericata*, SCP values of mature adults are slightly lower than those of freshly emerged flies[10–12], the same trend being also noteworthy for *Drosophila melanogaster*[23]. The SCP values of *A. aptera* mature adults observed in this work are of the same order as in previous studies[7,24].

The fly teneral adults only keep the pharate adult supercooling ability until a spectacular weight increase takes place, due to feeding, which is associated with a decrease of the supercooling ability. Winglessness facilitates such a process of increase in weight, thanks to physogastry and the use of the thorax, which is free from flight muscles and allows a complementary storage of reserves[6]. As adults and larvae are active all the year, teneral adults (low SCP), mature adults and larvae (high SCP) experience the same temperatures in the field. This seems to exclude the possibility that the SCP has here an adaptive significance. In the course of development, a high SCP in larvae and mature adults might simply be related to feeding. However, the mean SCP value is still higher in two-months starved adults than in fed ones[7]. This unusual feature might be associated with the striking fasting performances of this wingless fly[6].

In the subantarctic environment, the preservation of a high supercooling ability in teneral adults may be a physiological heterochronic event associated with some developmental flexibility in *A. aptera* populations. This question needs additional data on survival responses to temperature not only under experimental conditions but also in contrasted natural environments[25,26].

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