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Supercooling ability, fat and water contents in a diapausing tropical beetle, *Stenotarsus rotundus* (Coleoptera: Endomychidae)

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Abstract. Traits of the diapause syndrome in the tropical beetle *Stenotarsus rotundus* were studied by measuring the supercooling point (SCP), and water and fat contents of the beetles in the period from deep diapause through to their activation. Diapausing beetles utilized body fat and accumulated water, the utilization rate being very low in untreated beetles and high in those treated with juvenile hormone analogue. Physiological and behavioural mechanisms were found to be involved in adaptation against drought.

SCP remained almost unchanged from December through February, at a level normal in diapausing insects from the temperate regions (–13°C). In April, after the termination of diapause, SCP rose to –7°C. Thus a decrease in the SCP seems to be a widespread trait of diapause syndrome, not restricted to temperate insects, and not necessarily connected with true cold tolerance.

INTRODUCTION

The inception of diapause is accompanied by a series of adaptations which are defined collectively as the diapause syndrome (De Wilde, 1970; Hodek, 1973; Tauber et al., 1986). The main physiological adaptations include accumulation of metabolic reserves, lowering of metabolism, and increased resistance to cold, heat and drought. The assumed origin of preadaptations for diapause in the tropics (Tauber et al., 1986) and similarities in the adaptations of both hibernating and aestivating insects suggest that some of the changes ascribed to cold hardiness may be general features of diapause syndrome (Hodková & Hodek, 1994). There are at least two features intrinsic to supercooling ability, the accumulation of polyols and an empty gut, displayed by many diapausing insects not exposed to cold. Although Denlinger (1991) considers summer diapause or diapause in the tropics as independent of cold hardiness, he admits that 'adaptations essential for survival during a hot, dry season may also confer protection against low temperature'. Thus, it is important to determine whether changes in SCP values correspond to changes in the diapause syndrome in tropical insects that never experience temperatures below zero, but have evolved adaptations against drought. We suggest that the parameters connected with the "diapause syndrome" are an adaptation to any unfavourable conditions.

Stenotarsus rotundus Arrow, 1920, is a handsome fungus beetle (Endomychidae) in which unusual ethological and physiological adaptations were found. On Barro Colorado Island (BCI), Panama, the beetles diapause in a large aggregation on a single palm tree, *Oenocarpus mapora*, from July through the rest of wet season and the whole dry season (until April). Flight muscles deteriorate, gonads remain undeveloped, the beetles do not feed and are relatively immobile. Male gonads start their development in January and flight muscles in both sexes and female gonads in March. After the first heavy rains in April, beetles mate and disperse (Wolda & Denlinger, 1984). From July to September, they accumulate glycerol and glucose (up to 4.5 mg/g, respectively 3.5 mg/g dry body weight) in the haemolyph and, from November to February, the concentration of these compounds decreases (to 1.5, respectively 1 mg/g dry weight, Pullin & Wolda, 1993). However, in comparison with temperate insects, the concentration of these two compounds is low. During deep diapause, the metabolic rate in beetles is extremely low: 22 µl O_2/h/g (Tanaka et al., 1988). Application of juvenile hormone analogue induces artificial termination in diapause (Tanaka et al., 1987b). Loss of water in the dry conditions is diminuted within large aggregation (Yoder et al., 1992).

MATERIAL AND METHODS

Beetles were sampled from an aggregation on the palm tree *O. mapora*, on Barro Colorado Island in Panama Canal Zone (9°9′N). Six males and six females of *S. rotundus* were removed from the aggregation for measurements each week from early December through late February (dry season) from both the upper part (1.5 m or higher) and the lower part near the base of the tree. Simultaneously, another sample was taken from a subpopulation maintained in an insectary, under natural photoperiod (near 12 h) and temperature (27°C) but in high humidity (sample called 'insectary'). In addition, other beetles maintained in the outdoor insectary were collected in January and treated with juvenile hormone analogue (JHA methoprene, 1 µg per beetle dissolved in 3 µl of acetone, topically applied, sample called 'JHA') and pure acetone (solvent control, called 'acetone'), as used in Tanaka et al. (1987a). Stocks of 'insectary' and 'JHA' were also taken to Czech Republic, placed in conditions similar to outdoor conditions in Panama (27°C, 12L : 12D) and measured after two and three months.

The supercooling point (SCP, the temperature at which spontaneous freezing of body fluids occurs) was measured by a thermocouple as described by Brunnhofer et al. (1991), under cooling rate 1K/min. Weights of the beetles were measured as: fresh weight (FW) before other measurements, dry weight (DW) after a 24 hour drying at 60°C, and lean weight (LW) after a 24 hour extraction in diethylether and further drying (Wolda & Denlinger, 1984). From these variables we computed water and fat ratios [W = (FW-DW)/FW, F = (DW-LW)/FW] and water and fat quotients [WQ = (FW-DW)/LW, FQ = (DW-LW)/LW]. LW is used in these quotients for size standardization to eliminate dependency of FW on water and fat (LW was constant over the entire period).

Statistical significance of correlations and ANOVA is marked as follows: NS : $p > 0.05$, * : $p \leq 0.05$, ** : $p \leq 0.01$, *** : $p \leq 0.001$, r and F values are not indicated.

RESULTS

Most of the measured variables differed according to sex of the beetles, treatment, and time. However, the upper and lower parts of the aggregation on the palm tree did not differ significantly from each other, except for one occasion (further in text). Thus, the results in these two samples were clumped.

The differences between sexes were significant (Tab. 1), but the results were also clumped, as we used an equal number of individuals of both sexes.

In the 'insectary' sample, sexes differed less in all variables, thus this sample is used in more analyses. Fat content decreased more in this sample (Fig. 1) and water content increased (Fig. 2). Thus total weight remained unchanged. SCP values were slightly higher than in 'palm' samples (mean −11.7°C ± 1.7K, n = 108), the change with time from December through February was not significant.

TABLE 1. Differences between sexes in measured variables. All significant on level 0.001 (***).

Variable (mean ± SD)	Females	Males
Fresh weight (mg)	21.5 ± 3.9	18.3 ± 4.0
Dry weight (mg)	9.0 ± 1.8	8.0 ± 1.8
Lean weight (mg)	6.0 ± 1.1	5.2 ± 1.1
Water ratio	0.579 ± 0.036	0.564 ± 0.040
Supercooling point (°C)	−12.6 ± 2.1	−13.7 ± 2.1

Individuals in the 'acetone' sample responded similarly to those in the 'insectary' sample. Fat content decreased, water content varied without a clear trend and freezing curve characteristics remained unchanged. The beetles treated with JHA showed an increase in metabolic processes. Total weight and fat content decreased very rapidly (Fig. 1), water content increased (Fig. 2). Within this period, SCP remained similar to 'palm' samples.

The rate of fat utilization was converted to values of oxygen consumption (as in Tanaka et al., 1988). Beetles sitting on the palm consumed 30 µl O_2/g/h, the 'insectary' 59 µl, the 'acetone' 102 µl, and the 'JHA' 196 µl (that corresponds to the slopes of lines in Fig. 1).

Regardless of sex, relative water content (W) was higher in smaller individuals, while relative fat content (F) was higher in larger ones. Thus, water percentage was correlated strongly negatively to the percentage of fat (***, n = 108). Even water and fat quotients (WQ and FQ), variables independent on fresh weight changes caused by water and fat themselves, showed this correlation (***, n = 108, Fig. 3).

Over the period of observation on Barro Colorado Island, individuals of *S. rotundus* sitting on the palm tree moved gradually to lower parts of the trunk and into crevices in the soil. One remarkable migration in late January was accompanied with changes of water

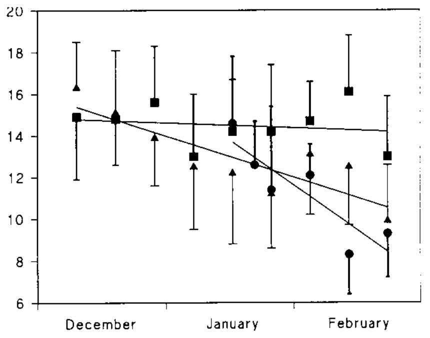

Fig. 1. Changes in fat content (% of fresh weight) within experimental period in samples of *S. rotundus*. Squares = 'palm', each point represents mean of 24 individuals, triangles = 'insectary', n = 12, circles = 'JHA', n = 12, bars associated with each point represent one standard deviation. Linear regression is plotted for each data set.

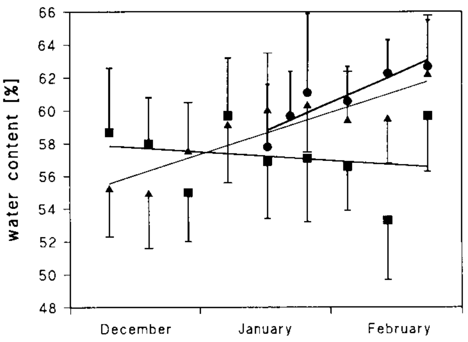

Fig. 2. Changes in water content (% of fresh weight) within experimental period in samples of *S. rotundus*. Squares = 'palm', each point represents mean of 24 individuals, triangles = 'insectary', n = 12, circles = 'JHA', n = 12, bars associated with each point represent one standard deviation. Linear regression is plotted for each data set.

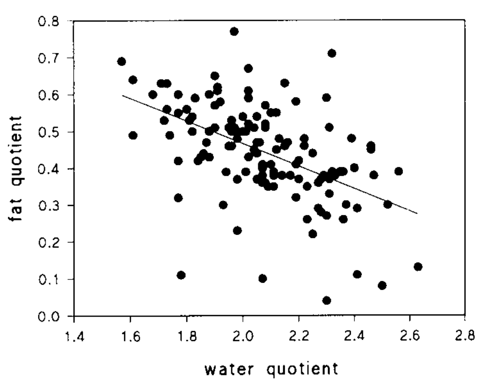

Fig. 3. Relationship between fat and water quotients (see methods) in 'insectary' sample of *S. rotundus*. Linear regression: $y = -0.31 * x + 1.08$, $r = 0.51$, $n = 120$.

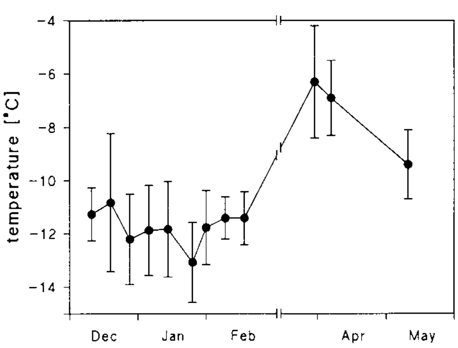

Fig. 4. Temporal changes in supercooling point in 'insectary' sample of *S. rotundus*. Each point represents mean of 12 individuals, associated bars one standard deviation.

quotient: it increased in the upper part of the palm tree, while it decreased in the lower part (*, n = 12).

SCP was not dependent on water content (both W and WQ). It was correlated positively with body weights (FW, DW, and LW, all three ***, n = 108), but might have been caused by the difference between sexes. If compared separately, SCP was size-dependent only in males (**, n = 54).

There were low values of SCP during the diapause (diapause development considered to be the same as described in Wolda & Denlinger, 1984) and rised at the end of diapause in April. After one further month, active beetles deprived of food again lowered SCP to an intermediate value (Fig. 4). This pattern occurred in both 'untreated' and 'JHA' samples. This increase and consecutive decrease in freezing characteristics were accompanied by increase (onset) and decrease in motile activity, and no mating was observed.

Our results on supercooling changes in *S. rotundus* were supported by partial measurements on an undescribed species of *Stenotarsus*. We found an aggregation of this species in late February on the trunk of *Tetragastris panamensis* on Gigante Peninsula, several kilometres from the aggregation of *S. rotundus*. The SCP was low just after collecting, and there was a considerable increase in SCP value in April in a sample taken to Czech Republic. The only difference mentioned was that, on the contrary to the moving *S. rotundus*, the 'Gigante' species was still found in large aggregations on bark in late February.

DISCUSSION

Metabolic level in the 'insectary' sample was twice as high as in beetles on the palm, which may represent the role of high humidity in accelerating diapause termination (Tanaka et al., 1987a,b). These results also suggest that *S. rotundus*, used in some experiments in Ohio (Tanaka et al., 1988; Yoder et al., 1992), may have undergone diapause development more rapidly than those on the palm. Beetles treated with acetone displayed some activation, but not to the same extent as those treated with JHA. The recorded metabolic rates (oxygen consumption calculated from rate of fat utilization) were comparable to those of previous studies (30 vs. 22 μl O_2/g/h in Tanaka et al., 1988).

Accumulation of water, probably of metabolic origin from the fat, compensated for the loss of mass (FW) caused by fat utilization (Fig. 3). Fat content (F and FQ) was correlated positively to the lean dry weight as found previously (Wolda & Denlinger, 1984). Water content (W and WQ), however, was correlated negatively to lean dry weight, contrary to the earlier study. Apparently, metabolic water serves as a compensation of transpirational water losses. If the beetles are kept in moist conditions, water accumulates in the body. The process is more rapid in smaller individuals, as a rule of size in metabolic rate.

Changes in water quotient, after mass movement of beetles to positions lower on the tree, can mean that those beetles with low water content in the upper part of the aggregation eventually moved to a more humid place at the trunk base. Thus, the beetles possess both physiological and behavioural adaptations against desiccation.

The low mean value of SCP during diapause (–13°C) as well as the increase of SCP after termination of dormancy (–7°C) in the two *Stenotarsus* species are in agreement with values in many diapausing insects from temperate regions (e.g., in a sister group of endomychids, Coccinellidae; Lee, 1980; Nedvěd, 1993). Thus a decrease in SCP appears to be a widespread (though not unique) trait of the diapause syndrome (De Wilde, 1970), independent of cold acclimation and true cold tolerance, at least in a part of the Insecta with imaginal diapause. This study extended such a knowledge to tropical species. The described response may be a preadaptation for cold hardiness in the evolution in some insects in temperate regions (the freezing-avoidance strategy).

Flesh fly pupae from BCI have a SCP as low as that of temperate-zone flies (–20°C), yet they are quite intolerant of temperatures much higher than the SCP (Denlinger, personal communication). Whether the supercooling ability in *S. rotundus* corresponds with an ability to survive frosts or not (as in flesh flies) remains debatable. There is a long list of insects species, which are able to supercool, but are unable to survive even a short time at low temperatures above SCP [pupae of *Delia radicum* (Košťál, 1993), pupae of *Sarcophaga crassipalpis* (Lee & Denlinger, 1985) and some aphids (Knight et al., 1986)] or even above 0°C.

Since the concentration of cryoprotective compounds in haemolymph is too low to promote a great decrease in the SCP (Pullin & Wolda, 1993) the main factor causing this decrease might be the loss of ice nucleating agents (INAs) from the body fluids. The increase of SCP after diapause termination was not connected with food intake, thus INAs seem to be reactivated in the body (within cells, according to Zachariassen, 1985).

Insects treated with juvenile hormone analogue showed a rapid increase in metabolic rate (decrease in fat content) but, even in these activated beetles, the increase in SCP occurred after several weeks. This could be analogous to the cold hardiness in some temperate insects, maintained for some time after termination of diapause (during postdiapause quiescence) (Lee, 1980; Nedvěd, 1993).

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REFERENCES

Brunnhofer V., Nedvěd O. & Hodková M. 1991: Methodical improvement of the determination of supercooling point in insects. *Acta Entomol. Bohemoslov.* **88**: 349–350.

Denlinger D.L. 1991: Relationship between cold hardiness and diapause. In Lee R.E.Jr. & Denlinger D.L. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, London. pp. 174–198.

De Wilde J. 1970: Hormones and insect diapause. *Mem. Soc. Endocrinol.* **18**: 487–514.

Hodek I. 1973: *Biology of Coccinellidae.* Academia, Prague, 260 pp.

Hodková M. & Hodek I. 1994: Control of diapause and supercooling by the retrocerebral complex in Pyrrhocoris apterus. *Entomol. Exp. Appl.* **70**: 237–245.

Knight J.D., Bale J.S., Franks F., Mathias S.F. & Baust J.G. 1986: Insect cold hardiness: supercooling points and pre-freeze mortality. *Cryo-Letters* **7**: 194–203.

Košťál V. 1993: Cold-hardiness and supercooling capacity in diapausing and nondiapausing stages of the cabbage root fly Delia radicum. *Cryobiology* **30**: 524–531.

Lee R.E.Jr. 1980: Physiological adaptations of Coccinellidae to supranivean and subnivean hibernacula. *J. Insect Physiol.* **26**: 135–138.

Lee R.E. & Denlinger D.L. 1985: Cold tolerance in diapausing and nondiapausing stages of the flesh fly, Sarcophaga crassipalpis. *Physiol. Entomol.* **10**: 309–315.

Nedvěd O. 1993: Comparison of cold hardiness in two ladybird beetles (Coleoptera: Coccinellidae) with contrasting hibernation behaviour. *Eur. J. Entomol.* **90**: 465–470.

Pullin A. & Wolda H. 1993: Glycerol and glucose accumulation during diapause in a tropical beetle. *Physiol. Entomol.* **18**: 75–78.

Tanaka S., Denlinger D.L. & Wolda H. 1987a: Daylength and humidity as environmental cues for diapause termination in a tropical beetle. *Physiol. Entomol.* **12**: 231–224.

Tanaka S., Wolda H. & Denlinger D.L. 1987b: Seasonality and its physiological regulation in three neotropical insect taxa from Barro Colorado Island, Panama. *Insect Sci. Appl.* **8**: 507–514.

Tanaka S., Wolda H. & Denlinger D.L. 1988: Group size affects the metabolic rate of a tropical beetle. *Physiol. Entomol.* **13**: 293–241.

Tauber M.J., Tauber C.A. & Masaki S. 1986: *Seasonal Adaptations of Insects.* Oxford University Press, New York, 412 pp.

Wolda H. & Denlinger D.L. 1984: Diapause in a large aggregation of a tropical beetle. *Ecol. Entomol.* **9**: 217–230.

Yoder J.A., Denlinger D.L. & Wolda H. 1992: Aggregation promotes water conservation during diapause in the tropical fungus beetle, Stenotarsus rotundus. *Entomol. Exp. Appl.* **63**: 203–205.

Zachariassen K.E. 1985: Physiology of cold-tolerance in insects. *Physiol. Rev.* **65**: 799–832.

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