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Some Features of Water Saving in *Aporia crataegi* L. Caterpillars Inhabiting Areas of Dry Climate in Eastern Siberia (Yakutia, Russia)

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Abstract

Climate in Yakutia is characterized by low winter and hot summer temperatures that cause extreme low air humidity reaching up to 30% in varied periods. Therefore, insects in Yakutia are seasonally faced to extremely dry air that forced them to create special adaptation mechanisms during evolution.

Estimation of water balance for *Aporia crataegi* L. caterpillars was based on measurements of rates of water loss, respiration and total water content. Our research shows that *Aporia crataegi* exposed to dry environmental conditions have low cuticular water permeability that was close to that of African beetles. This mechanism allows the insect to keep significant amount of water: up to 70% inside their bodies despite extreme dry conditions that surround them. The study shows that another mechanism making up insect water balance during winter associated with production of ice nucleating proteins transforming water into ice in an insect's hemolymph is not sufficient for the given species. Additionally, a cocoon they are hidden inside since early autumn, the whole winter and spring also protect the caterpillars from dissociation.

Keywords: *Aporia crataegi*; Resistance to drought; Cuticle water permeability; Rates of water loss; Metabolic rates; Cocoon; Eastern Siberia

Introduction

One of the most important environmental factors determining the performance of insects in dry areas is the level of humidity. There are few general survival strategies used by insects to be drought resistant. They either may evolve ability to tolerate extensive loss of body water by means of anhydrobiosis [1] or they may develop drought tolerance by reducing transcuticular permeability of their body wall [2]. Finally, they may use behavioral implications for avoiding excessive water loss such as migration to protected sites [3].

Climate in Yakutia is characterized by low winter and hot summer temperatures that cause extreme low air humidity reaching up to 30% in varied periods [4,5]. Therefore, insects in Yakutia seasonally face extremely dry air conditions. Investigation of adaptation mechanisms to extreme conditions is a matter of significant interest but the problem has not been yet systematically studied.

As broadly known, insects inhabiting temperate and cold regions evolve two general strategies to be cold hardy. They may seek to avoid freezing, in many cases by allowing their body fluids to stay supercooled at low temperatures, or they may develop tolerance to freezing [6,7]. Usually, freeze-avoiding insects avoid freezing by removing or inactivating all particles that potentially may cause freezing. Insects surviving freezing establish a protective extracellular freezing at a high subzero temperature by the aid of potent extracellular ice nucleating agents (INAs), produced for this function [8].

In their studies, Lundheim and Zachariassen stated that because the vapour pressure of ice is lower than that of supercooled water at the same temperature the hibernating freeze avoiding (supercooled) insects may lose substantial amounts of body water during winter [2]. Therefore, freeze avoiding insects have exceptionally low water permeability of the body wall. In contrast, body fluids of freeze tolerant insects stay in vapour pressure equilibrium with ice in frozen state. Frozen insects will neither lose nor gain water during winter diapause and many appear to have leaky body walls [2]. This is why freezing tolerance seems to offer advantages with regard to water balance.

In our early studies of water balance of the Siberian freeze tolerant *Acanthocinus aedilis* larvae was shown that they have a low cuticular water permeability preventing it from excessive water loss [9]. Interestingly, that this species is not only freeze tolerant, but has also developed ability to supercooling. The study was based on measurements of rates of water loss and metabolism of the larvae and data were then related to water balance curve of the African desert beetles obtained by Zachariassen et al. [10] (Figure 1). According to Figure 1, the regression line presents the rates of water loss of various

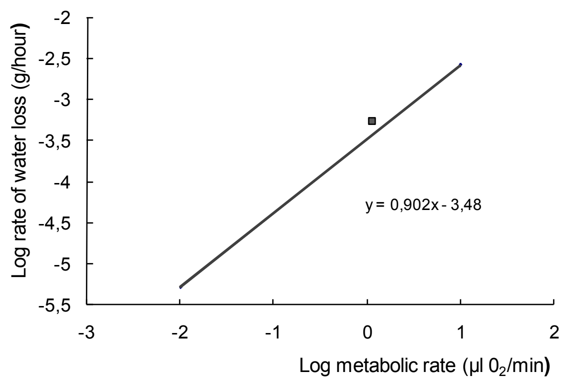

Figure 1: Rate of water loss and metabolic rate of *Acanthocinus aedilis* larvae (redrawn after ref. 5).

Solid line (calculated linear regression line by Zachariassen) presents rate of transpiratory water loss of East African habitat carabids and tenebrionids, plotted as a function of their metabolic rate.

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species of African beetles that linearly relate to their metabolic rate implying that the line provides an approximate measure of the rate of respiratory water loss. It was concluded that due to low air humidity in the frozen pupal chambers under the bark in winter and poor water diet in the combination with low humidity in summer, the Siberian timberman is likely to have the similar water balance problems as African insects and therefore their cuticular water permeability was close to that of African beetles. The study [9] apparently testifies that supercooled state of overwintering insects seems to be the most critical factor determining their low transcuticular permeability. More data is required for understanding extent, to which water balance depends from cold adaptation strategy. To fill a gap in this field, freeze tolerant *Aporia crataegi* L. caterpillars (Lepidoptera: Pieridae) were used in this research to learn the mechanism they use to adapt to dry air conditions in central Yakutia, as compared to other insects inhabiting this region.

Materials and Methods

Insects

Leaf nests, each containing a few caterpillars, were collected in the vicinity of the Russian city Yakutsk (62°N, 130°W) in different seasonal periods when experiments were planned to be conducted. Before the experiments the caterpillars were removed from their nests and were kept at +4°C during 2 hours for cleaning their gut.

Only overwintering specimens were used without pre-incubation at +4°C because their guts were empty. The mean initial body weight of the caterpillars was 8.3 mg.

Determination of seasonal variations in water content

To determine water content the caterpillars were dried to constant weight at 60⁰C. Weight loss was determines by weighing the animals before and after desiccation on a Mettler AC88 balance.

Preparation of hemolymph samples

Samples of hemolymph were obtained by careful pressing the caterpillar's body, the exuding hemolymph being drawn into a capillary tube by means of capillary forces.

Water balance

Estimating of water balance was made by method described by Lundheim et al. for desert beetles [2]. According to this method, the total water loss was determined as loss of body mass while the caterpillars were kept inside a desiccators with silica gel (relative air humidity was approximately 4%) at +22°C. The body mass was measured by means of a Mettler analytical balance. Because the caterpillars are very small, the loss of body mass was determined on the group consisting of 8 caterpillars at intervals of approximately 10 hours. Cuticular water permeability of the caterpillars was expressed as fraction of cuticullar water loss**.** Metabolic rates were measured on the smaller group (up to 3 specimens) at interval of 30 min by means of Engelmann constant pressure respirometer at +22°C [11]. All values of metabolic rate were calculated to standard conditions and in relation to the initial fresh body mass. For comparison, in this study a few freeze tolerant species such as *Upis ceramboides*, *Pieris rapae*, *Delia floralis*, larvae belonging to Diptera overwintering of which occur in well-buffered places (under bark of trees in case of beetles; or inside soil in case of *Delia floralis*) were also measured to estimate their water balance. The data on metabolic rates and cuticular water permeability of the insects was then related to a curve obtained for tropical desert beetles to evaluate how degree of exposition to dry air influence the water balance of the insects (regression line by Zachariassen (Figure 1).

Test for ice nucleating agents in the hemolymph

The method of determination of ice nucleating activity in the hemolymph of the insects is based on measurements of the supercooling point of 5 samples of a 0.9% solution of NaCl, containing 5 vol% hemolymph. Undiluted samples of NaCL solution is supercooled to the temperature range from -15 to 20⁰C whereas the addition of hemolymph containing nucleating agents raise the supercooling points to temperatures above -10⁰C. The method is described in detail by Zachariassen and Hammel [12].

Water loss of caterpillars inside and outside of the cocoon

Overwintering *A. crataegi* caterpillars are covered by transparent cocoons and located in leaf nests. To study the role of cocoon in protection of the caterpillars from evaporative water loss they were divided in two groups: one group consisted of caterpillars inside the cocoon, another one of caterpillars on the outside. Each cocoon contained 1-2 caterpillars. The caterpillar´s evaporative water loss was determined in the model experiment when the caterpillars from both groups were kept at +37°C to shorten the period during which they lose most of the water. Thus, the choice of such temperature was connected with exceptionally low rate of water loss at standard laboratory conditions (it could be a period the caterpillars potentially died). The rates of water loss were established by daily weighing of the cocoons.

Statistical methods

Comparison of means between samples was made with ANOVA/ Tukey's test using the statistical package Statistica v6.0.

Results and Discussion

Life's cycle of *A. crataegi* is linked with branches of bushes that make it to be exposed to dry air seasonally. However, *A. crataegi* caterpillars contain significant amount of water up to 70% during long period since September until May (Figure 2). As broadly known, insects use to be dehydrated in some degree before they will enter diapause during winter to decrease a risk of formation too large ice crystals inside body. Ability of *A. crataegi* to keep such amount of water inside body during harsh winter seems to be strange and perform rather unique feature.

To estimate the water balance of *A. crataegi* the method of determination of rates of water loss and metabolic rates was used in this study. The parameters of water balance were measured and the

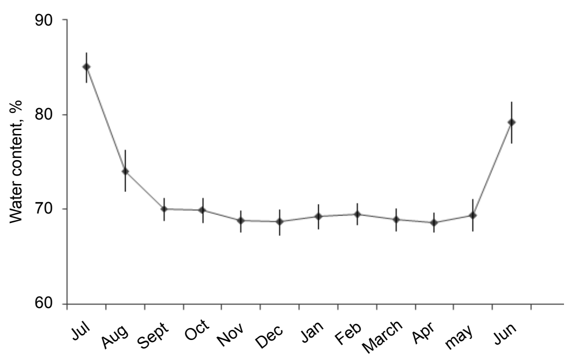

Figure 2: Seasonal variations in water content (%) of *Aporia crataegi* caterpillars

values were related to a curve obtained for desert beetles (Figure 3). According to Figure 3, the values for water loss and metabolic rate related to *A. crataegi* are situated along the line that testifies reduced cuticular permeability of this species implying that their water loss is close to African beetles. Such water balance of *A. caretegi* is obviously to be an adaptation to the extreme dry conditions they are exposed to on the different life stages. In winter they are located in leaf nests, fixed to bush branches (*Crataegus dahurica*) above the snow; therefore they are exposed both to as low ambient temperatures as -47°C…-55°C and low air humidity reaching 30% during May. In July the young caterpillars get out of eggs laid by butterflies. They live in a large group and feed on leaves. The caterpillars 2-3 weeks of age begin to make a cocoon inside of which they hide and glue leaves, turning them into the nest. In this period they are faced to drying risk because they stop to feed, their cuticle is not properly developed and ambient temperatures are still warm (in average: +15…+25°C) [13]. The way to save water inside body during this period is apparently associated with ability of caterpillars

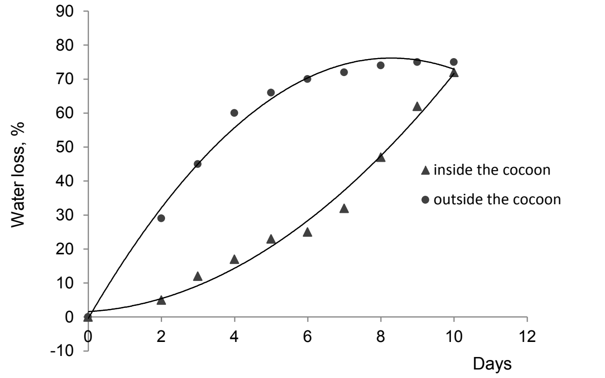

Figure 4: Comparative dynamics of water loss (%) by *Aporia crataegi* caterpillars being inside and outside the cocoon (each point is an average of 8 caterpillars measured)

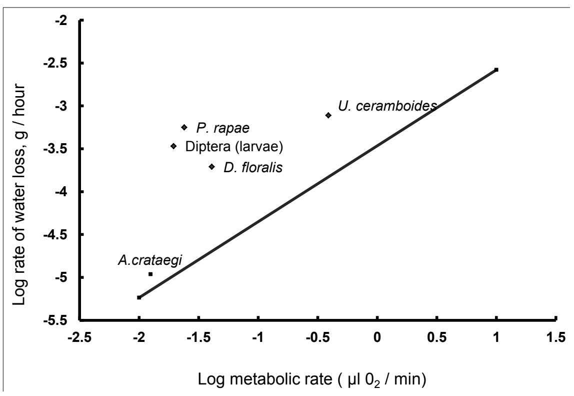

Figure 3: Logarithmic values of rates of water loss of a few Siberian insects as a function of the logarithm of their metabolic rate. The solid line represents corresponding data for East African desert beetles obtained by Zachariassen and Maloiy [18].

Photo 1: Discovered leaf's nests containing *Aporia crataegi* caterpillars and fragments of the cocoons.

to make a cocoon they are hidden inside since early autumn, a whole winter and spring (Photo 1).

As it was established in previous studies, the cocoon is built from protein consisting of a few aminoacids: serine, asparagin, threonin with a prevalence of proline [14]. According to this research, the caterpillars inside the cocoon are characterized by significantly more efficient water saving mechanism than those outside of the cocoon (Figure 4). As seen at Figure 4, they lost approximately 75% of water during 10 days even at temperatures such as +37°C.

Thus, the main principal of water saving mechanism in *A. crataegi* is apparently associated with exceptionally low trans cuticle permeability that ensure prolonged stability in water content inside body. This feature makes *A. crataegi* to be close to African desert beetles that as it well known have completely water proof cuticle [15-17]. These data indicates that climate in Yakutia is actually dry like in African savannas and therefore species the living cycle of which goes on exposed sites acquired during evolution the cuticle with exceptionally low permeability. It is probably the most important adaptation for some species inhabiting such dry conditions.

According to this study, other freeze tolerant insects (*Upis ceramboides, Pieris rapae, Delia floralis,* and larvae belonging to Dipera) have high permeability of body wall (Figure 4 and Table 1) that is apparently associated with low degree of their exposition to dry air. For these insects, freezing of body liquid initiated by ice nucleating proteins [16,17] seems to be a sufficient part of their water saving mechanism during winter (Table 1). As *Aporia crataegi,* ice nucleation seems not to be a significant component of water saving in caterpillars because according to the Figure 2 they keep significant amount of water not only during winter but also during another seasons. It indicates that

Insect	Water content,%	SCP, °C	Ice nucleating activity (0.9% NaCl + hemolymph	Metabolic rate, µl O_2/min	Water loss, g/h	Winter habitat
A. crataegi	70.0	-11.5 ±1.2	-10.5 ± 0.78	0.012 ± 0.08	$1.12 \cdot 10^{-5}$ (± 0.95)	Inside leaf nests, above the snow line
U. ceramboides	59.3	-7.5 ± 2.6	-7.3 ± 0.28	0.354 ± 0.05	$7.94 \cdot 10^{-4}$ (± 0.61)	Under the bark of birch, sometimes above the snow
D. floralis	48.7	-5.8 ± 0.8	-5.5 ± 0.71	0.039 ± 0.07	$1.99 \cdot 10^{-4}$ (± 0.87)	In the soil, under the snow
P. rapae	65.5	-8.5 ± 1.6	-8.3 ± 1.1	0.022 ± 0.06	$6.3 \cdot 10^{-4}$ (± 0.56)	In the soil, under the snow

*Measurements were made in January, 2012-2013

Table 1: Comparative data of water balance, SCP, hemolymph ice nucleating activity and winter habitat obtained for freeze tolerant insects inhabiting central Yakutia*.

water saving mechanism of *A. crataegi* is more linked with low cuticle permeability than with ice nucleation.

At last, cocoon is also important part of water saving during early autumn when caterpillars stop feeding but still exposed to hot and dry conditions without proper cuticle protection. In sum, all these mechanisms not only allow to *A. crataegi* avoid excessive water loss but also favor to keep significant amount of water even during the most dry air conditions in central Yakutia.

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