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宇宙生物学の実験材料としてのネムリユスリカ

An anhydrobiotic insect, *Polypedilum vanderplanki* as a tool for astrobiology

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Abstract: Larvae of African chironomid *Polypedilum vanderplanki* represent highly developed mechanism of anhydrobiotic conservation of metabolism which allowing them to stay alive for many years in the absence of water and be utilized as a model organisms in long-term projects, including the spaceflight. In this paper we shortly overview the main properties of anhydrobiosis of the larvae and describe the main space experiments where the insect was used as a model organism for investigation of influence of spaceflight environment of latent stages of invertebrates.

Key words: International Space Station, outer space, anhydrobiosis, chironomid larvae, stress, Biorisk

The progress achieved in a few last decades in interplanetary spaceflights allows planning and realizing long-duration spaceflights and exploration of perspective planets such as Mars and Moon with a direct human participation. Intensive investigations of influence of simulated and real space experiments on living organisms revealed that microgravity itself is not a limitation factor for normal metabolism and full-cycle reproduction of plants and invertebrates (Sychev et. *al.*, 2007). Instead, some organisms were found to be much stronger than it was suggested before and recent studies showed that, at least, microorganisms are able to resist a harsh combination of abiotic stresses of outer space environment for a long period of time without losing potency for reviving from resting stages and reproduction (Hornek, 2003; Baranov *et. al.*, 2006). Considering prolonged duration of spaceflights (including interplanetary missions), cargo limitation of spacecrafts, biohazard and interplanetary quarantine issues and perspectives of continued storage of alive higher organisms, we propose utilization of larvae of an African chironomid as a tool for astrobiological studies.

Water usage limitation is a one of the most serious limitation factor for planning space mission and conducting scientific experiments in manned spaceflight. Water is the major component of living organisms. The average content of water in invertebrates is around 70% (from 17% to 90%) and water makes up 95–99% of the total number of molecules (for review see Watanabe, 2006). Most organisms have only limited ability to survive water loss. At the same time, some organisms posses ability to stay alive for a extended period even after they are almost completely dehydrated. This biological state is termed "anhydrobiosis" and the taxonomic range of desiccation tolerance in animals covers rotifers, tardigrades, nematodes, rotifers, and a arthropods (Alpert, 2006). The common feature of anhydrobiotic species is their relatively small size, which places limitations for implying them as model animals for molecular and cellular studies.

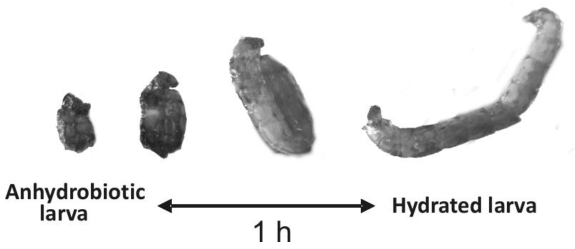

Fig. 1 Rehydration of the larvae of *P. vanderplanki*

Larvae of African chironomid *Polypedilum vanderplanki*, is the largest and one of the the most complex anhydrobiotic organisms known to date. Originally described by Hilton (1960), larvae of this species experience cycles of drying and rehydration in their habitats in the temporary pools on the rocks in Nigeria. Metabolism in the larva of *P. vanderplanki* can be brought reversibly (in a less than an hour for rehydrating larvae) to a standstill by dehydration and exposure to very low or very high temperatures: 102–104°C for 1 min when dry, and recovers temporarily after exposure to 106°C for 3 hr or 200°C for 5 min. (Hinton, 1960, Watanabe, 2006). Larvae recovered after immersion in absolute ethanol or pure glycerol when dry. The larvae recovered temporarily after being stored dry for at least 10 years.

During last few years, impressive progress was achieved in understanding of molecular mechanism of anhydrobiosis in *P. vanderplanki*. Constructing EST databases of dehydrating larvae and annotating and analysis of gene expressions allowed isolating and characterizing of a number of active molecules directly involved in formation of anhydrobiotic stage of a larva (Kikawada, *et. al.*, 2007) and creation of the databases of stress-related gene transcripts for further analysis.

One of the most attractive features for implication of the chironomid in space-related studies is tolerance of the larvae to very high doses of ionizing radiation

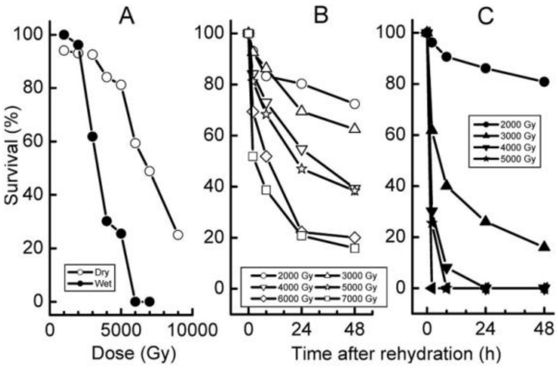

Fig. 2 Viability of the larvae after gamma-irradiation (from Watanabe, et. *al* 2006)

Watanabe *et. al.* (2006) demonstrated that wet *P. vanderplanki* larvae survive for at least 48 h after irradiation with 2000 Gy of gamma-rays. The radiation-tolerance even increased after entry into an anhydrobiotic state: a number of dehydrated larvae recovered and moved after extremely high doses (up to 9000 Gy) of gamma-rays (Fig. 2).

In the frame of joint-study supported by JSPS (Japan) and Russian Academy of Science, larvae of *P. vanderplanki* were selected as a model object for investigation of influence of space radiation and other factors of a manned spaceflight on the resting stages of invertebrates. The exposition of the packed dried larvae (Fig. 3A) to the spaceflight environment was conducted for two periods: 30 days and 210 days onboard Russian segment of International Space Station (ISS) in ISS-11 and ISS-12 missions in 2005/2006. The samples were returned to the Earth by members of the astronauts' crew by "Souz" spacecraft in October 2005 and May 2006. After transferring the larvae to Japan, their viability and life cycle patterns were confirmed to be similar to ones of the larvae from the ground control group. No significant differences were observed in the larvae exposed to real space flight for 30 and 210 days and *P. vanderplanki* was agreed to be stable and safe biological object accepted as an object for further studies onboard of manned spacecrafts.

In 2005, after discovering unexpectedly high resistance of some prokaryotes and fungi to continued (more than 1.5 years) outer space environment exposure in "Biorisk" project, new studies were suggested to be conducted to elucidate a possibility of long-term survival of higher eukaryotes in outer space and re-evaluation of the present regulations of space biohazard and space contamination issues to prevent possible contamination of other planets with Earth life for, as well as contamination of spacecrafts and astronauts with potential alien life forms.

A

B

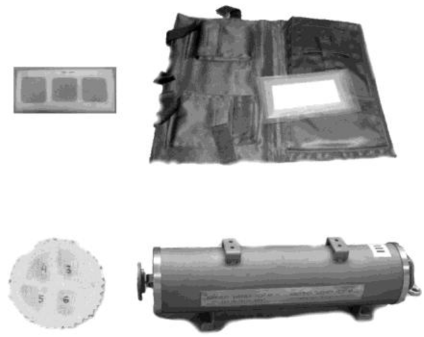

Fig. 3 Packing of the dried larvae of *P. vanderplanki* for exposition inside ISS (A) and "Biorisk" outer space experiment (B)

By possessing the abilities to stand complete desiccation, temperature fluctuation, and high level of radiation, anhydrobiotic larvae of *P. vanderplanki*, as well as a number of other anhydrobiotic animals and plant seeds, fulfilling the key criteria for tolerating exposure to outer space conditions, were selected for a 12 months exposure to the natural space conditions in the "Biorisk" canisters to analyze higher organisms tolerance to outer space (Fig. 3B). The canisters were fixed on the platform of the outer side of "PIRS" docking compartment of ISS by Russian astronauts on June 6, 2007 and to be retrieved in July 2008.

Confirmed biosafety and simple storage without losing viability, made the chironomid larvae an attractive candidate to be a test living organism for the spaceflight to Phobos in the frame of "Phobos-Grunt" space mission. The unique mission aims to retrieve soil samples from the

Mars satellite and several biological objects are suggested to be included in the mission, as a test for transportation of resting stages of organisms in interplanetary spaceflight.

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