Aim & Hypothesis: Watching a single-cell protist, a pine tree or an extinct Tyrannosaurus, it becomes immediately evident that millions of years of evolution caused gigantic differences in the size of organisms. Differences in body size simply arise from changes in the number and size of cells in a body, but the effect of such changes in a cellular architecture on the functioning of organisms in their environments is one of the greatest unsolved mysteries of life. Does an organism that consists of many small cells copes with environmental challenges equally well to an organism that consists of few large cells? This project explores the role of cell size in the ecology and evolution of organisms. According to the theory of optimal cell size, which is being developed by our research group, the size of cells results from costs and benefits that vary with the environment. On one hand, building a body from small cells can be beneficial, because small cells have relatively larger area of cell membranes. Acting as busy transport hubs, the semi-permeable cell membranes allow vital substances to enter cells, stop harmful substances from entering cells and remove wastes from cells. A body that consists of many small cells gains increased capacity to deliver oxygen and fuel to power generators inside cells. But, small cells are also costly to an organism which has to spend more energy for the maintenance of large area of cell membranes - keeping the transport hubs ready to work requires a lot of energy. According to our hypothesis, small cells help ectotherms (organisms that change their body temperatures with ambient temperatures) in warm, food-rich but oxygen-poor environments, but they harm ectotherms in cold, food-poor and oxygen-rich environments. The Earth is a mosaic of such conditions, and these conditions are constantly changing through time with global processes. A warm ectotherm, e.g. a fly heated by sunrays, has speeded metabolism and it is ready for intense flight and reproduction. Nevertheless, this potential will be lost, if not enough oxygen and fuel reaches cells in a fly's body on time. We propose that a fly with small cells would perform better in this situation that a fly with large cells. In cold and oxygen-rich environment, where organisms do not need super-fast cellular transport but should save energy, large and cheap cells would be the best option for ectotherms.

Methods: Our laboratory experiments on the fruit fly, *Drosophila melanogaster*, examine how cell size differences between flies affect their performance at different thermal and oxygen conditions. Our study involves flies that differ in the size of cells that build their body. One group of such flies (i) have mutations in genes that control a cell cycle, and these mutations were produced in laboratories by genetic engineering of flies. It means that cell size differences among these flies have well known genetic basis. Other flies (ii) come from an evolutionary experiment, in which flies evolved differences in cell size by natural mutations and natural selection. So, cell size differences among these flies have also genetic basis, but these differences occurred through natural processes. Another flies (iii) will have non-genetic differences in cell size, induced environmentally. To create these flies, we will catch wild flies and develop their eggs at different thermal and oxygen conditions. We predict that the warm and low-oxygen environment will cause small cells in a fly's body, whereas the cold and high-oxygen environment will cause large cells. So, differences in cell size among these flies will not be genetic, but they will resemble plastic changes in cell size, that are a part of life of flies, which live in various environments on Earth. In all studied flies (i-iii), we will measure cell size in five different cell types and determine an architecture of the respiratory (tracheal) system, which delivers oxygen to cells. We will learn whether cell size undergoes concerted changes in different cell types, whether changes in cell size play a role in the differentiation of body size, and whether the tracheal system changes its architecture together with cell size. In the first experiment (1), we will allow flies to fly in the air with normal and low amount of oxygen. By raising the temperature, we will test whether small cells help flies to fly in warm and low-oxygen conditions. In the next two experiments (2, 3), we will measure wing-beat frequency and egg production at different temperatures and oxygen concentrations. We will test whether flies with small cells are superior to other flies in warm and oxygen-poor conditions (the ability to fly and lay eggs decides about the capacity of a fly to spread its genes). In another experiment (4), we will compare oxygen consumption in flies with different cell sizes (the amount of oxygen which enters cells decides about the capacity to fly and reproduce). We will see whether small cells help flies to acquire oxygen in the warm and low-oxygen environments, where demand for oxygen is high but the supply of oxygen is low.

Significance: If we find that cell size affects the capacity of flies to acquire oxygen, then this discovery will change current views on the efficiency of a respiratory system in insects. Understanding the role of cell size in the performance of ectotherms is crucial for testing the theory of optimal cells size. No earlier study has ever examined this role, which is the great weakness of the theory. Addressing the role of cells is organismal performance is important for better understanding of fundamental ecological and evolutionary phenomena. We can gain a deeper insight into previously unexplored mechanisms which shape environmental sensitivity of organisms, and which have impact on the history of life on Earth, geographic distribution of organisms, and the ongoing transitions of life driven by global climatic changes.