Summary

Having a cellular body is a universal feature of organisms on Earth, but we do not understand the origin of dramatic differences between organisms in the size and number of cells in their body. Cell size diverges between species and populations as a result of phenotypic plasticity and evolution, and this variance is likely to have an adaptive nature, at least in part. Organisms' life history strategies involve biochemical pathways that regulate cellular processes during development, and thus determine whether an adult body is built from many small cells or fewer large cells. Following the theory of optimal cell size (TOCS), the cellular architecture of the body, including cell size, is not a neutral trait but is a subject of optimising selection that leads to a compromise between the costs of the cell membrane maintenance and benefits resulting from capacity to perform physiological functions. Small cells are more costly than large cells due to relatively more area of plasma membrane and thus more energy to maintain ionic gradients at cell surface and membrane structure. However, at the same time, because of short distances within the cell and the more exchange area created by the cell surface, the capacity of small cells to transport resources (nutrients and oxygen) exceeds that of large cells.

My thesis explores the role of cell size in insect functioning, addressing the TOCS framework and physiological limitations caused by oxygen demand and supply. I use genetic lines of *Drosophila melanogaster* flies to focus on the coupling of cell size with body size and ageing, two important elements of the life history strategy, and the effects of cell size on physiological performance (here flight) in gradients of temperature and oxygen conditions. To obtain adult flies with changes in cell size for all my studies (Study I-III), I reared larvae in standard food or food with rapamycin (control and rapamycin flies). Rapamycin, a bacterial antibiotic used in pharmacology, is useful for my research, as it affects the activity of the co-called target of rapamycin (TOR), a crucial protein kinase in cell size control pathways. Rapamycin also prolongs the lifespan of model organisms and thus is considered a potential drug in anti-ageing interventions.

My first study (Study I) investigates whether (i) manipulation of TOR activity during fly development leads to orchestrated changes in cell size in different organs and whether (ii) changes in cell size in different organs contribute in a consistent way to differences in adult body size. To address these questions, I measured body size and the size of five cell types, namely wing and leg epidermal cells, ommatidial cells, flight muscle cells, and epithelial cells in Malpighian tubules in two cell-size phenotypes (rapamycin and control flies). My next study (Study II) explores the performance of flies during oxygen-demanding activities (here tethered

flight), under different environmental conditions with reference to cell size. I measured wing flapping under warm and hot conditions, combined with normal and reduced oxygen concentrations in two cell-size phenotypes (rapamycin and control flies), and measured cell size in the same flies. Due to such an experimental design, the flies experienced different balances between oxygen demand and supply. My third study (Study III) examines whether cell size differences correspond to changes in ageing via an allocation trade-off between the costs of anti-ageing mechanisms and the costs of plasma membrane maintenance. In this study, I measured survival patterns in flies that emerge with small vs. large cells in the adult body (rapamycin and control flies).

Study I demonstrated that rapamycin supplementation of *D. melanogaster* larvae produced a distinct phenotype of adult flies, with a small body and reduced cell sizes in different cell types. This provides strong support for earlier hypotheses that organisms evolved developmental mechanisms that coordinate cell changes in different organs. This, for the first time, demonstrates the role of the TOR pathway in driving such coordination, leading to organism-wide changes in the cellular structure of organs. Study II showed that flies slowed wing beat rates under cooler and less oxygenated conditions, but as expected, small-celled flies were less oxygen limited than large-celled flies. This is the first published evidence showing that poor oxygen conditions promote small-cell life strategies in ectotherms involved in demanding activities. It is also a valuable demonstration that the performance of tiny terrestrial insects can become limited by oxygen conditions in the environment. Study III revealed that small-celled adult flies survived worse than large-celled flies, at least at the beginning of adult life. This study provides pioneering evidence suggesting links between cell size and organismal survival.

My findings on *D. melanogaster* permit some generalisations: (i) cell size changes within a body occur in a systemically coordinated manner in different tissue and organs; (ii) one of the mechanisms involved in this coordination is the TOR signalling pathway; (iii) body size differences among organisms involve organism-wide changes in cell size; (iv) organismal performance depends on cell sizes that build the body and environmental conditions that dictate the demand and supply of oxygen; (v) variation in cell size between organisms, and thus in the costs and benefits associated with the amount of plasma membranes relative to the amount of cytoplasm, may be an overlooked factor shaping mortality (or even ageing) patterns in nature. These conclusions provide an important contribution to scientific discussions on the adaptive value of cell size and cell size changes as an element of life history strategies.