Cryptobiosis in Tardigrada

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Jonsson KI, Rebecchi L. 2002. Experimentally induced anhydrobiosis in the tardigrade *Richtersius coronifer*: phenotypic factors affecting survival. *Journal of Experimental Zoology* 293: 574-584.

Several freshwater invertebrate taxa are capable of producing resting (diapause) stages that are adaptive for survival in environmentally variable habitats. Common examples include gemmule production by freshwater sponges, statoblast production by bryozoans and rotifer resting eggs. The ability of tardigrades to enter cryptobiosis, defined as a state of life in which all metabolic processes have ceased, is well documented; tardigrades in anhydrobiosis (desiccation-induced cryptobiosis), have even been reported to survive the radiation and vacuum of low-orbit space! While considerable efforts have been made to understand the biochemical and physiological changes associated with sustaining this ametabolic state, little is known of how phenotypic characteristics may affect the probability of cryptobiotic survival. Such knowledge could provide insight into the selective forces and evolutionary constraints involved in the evolution of cryptobiosis.

Jonsson and Rebecchi (2002) experimentally induced anhydrobiosis in the arctic tardigrade *Richtersius coronifer* to study whether important phenotypic characteristics (body size, energetic condition, and reproductive condition) affected the probability of successful cryptobiosis. Body size was measured as a function of buccal tube length. The buccal tube is a rigid sclerified structure of the buccalpharyngeal apparatus; the length of this structure is strongly correlated with body size. Storage cell size was used as a proxy for energetic condition. Tardigrade storage cells act as fat storage and transport cells in the body cavity and the size of these cells is positively correlated with the amount of fat stored in each cell. Because *R. coronifer* are transparent, storage cells can be examined in live), individuals. Oocyte maturation stage was used to represent reproductive condition and was classified according to three categories. Only female specimens were included in this study. Buccal length, storage cell size and oocyte maturation stage were measured during the hydrated stage both before and after a 12-day period of experimentally induced anhydrobiosis. The predictive effects of phenotypic variables were modeled using a form of statistics known as logistic regression. This method of statistical analysis assesses the extent to which each independent variable (buccal tube length, storage cell size, oocyte maturation stage) and each combination of independent variables influences a predictive model that measures the probability of successful anhydrobiosis.

Results of the logistic regression analysis suggest that buccal tube length, storage cell size, and the interaction between buccal tube length and storage cell size significantly affected the probability that an individual tardigrade will survive anhydrobiosis. Oocyte maturation level was not a factor that affects anhydrobiosis survival. Overall, 84 of 139 tardigrades survived anhydrobiosis. Individuals with a small buccal tube length had the greatest likelihood of surviving anhydrobiosis. However, individuals with large buccal tube lengths showed a positive response in survival with larger storage cells, suggesting that energy constrained the ability of larger individuals to survive anhydrobiosis. These results support the hypothesis that that the ability to enter anhydrobiosis is subject to phenotypic selection and that some phenotypic characteristics, such as large body size, may be associated with anhydrobiotic failure.

Jonsson and Rebecchi observed a negative correlation between buccal tube length and energetic capacity; larger individuals generally had a smaller energetic capacity than medium sized individuals. These results suggest that the largest tardigrades were energetically constrained in their ability to enter anhydrobiosis. The authors suggest that this trend might be explained by a "terminal investment" life-history model. This model predicts that the largest individuals in a population are also the oldest individuals of the population. Furthermore, the terminal investment model predicts that these oldest/ largest individuals invest more energy in reproduction than do younger individuals, and therefore larger individuals would have less energy available for entering the anhydrobiotic state. More detailed studies that investigate whether tardigrades exhibit increased fecundity with increased size and age are necessary to further understand this phenomenon.

Nonetheless, the means by which these phenotypic traits act in shaping the evolution of cryptobiosis in tardigrades is still complex and mostly unknown. For example, Jonsson and Rebecchi found that storage cell size has a completely different effect on survival in large animals than on medium sized animals. The interaction between body size, storage size and anhydrobiosis survival is illustrated in Figure 1. This figure displays the tendency for the largest individuals (displayed as open circles) to have an increased probability of survival as storage cell size increases. However, medium sized individuals (displayed as 'x') with larger storage cells tend to experience a decline in survival probability. This disadvantage associated with larger energy reserves in smaller animals suggests an interesting evolutionary scenario, where the amount of energy reserves should be optimized rather than maximized. Future studies that examine the ratio between body size and energetic capacity in relation to cryptobiotic survival will help elucidate the role that energetic restrictions play in the evolution of cryptobiosis.

This work represents a starting point for further investigations into the phenotypic characteristics that may drive the evolution of cryptobiosis in tardigrades. Future research may focus on males, juveniles and eggs to create a more complete understanding of age-specific selection. Perhaps understanding the characteristics that drive the evolution of anhydrobiosis in tardigrades can illuminate some of the more general selective features that influence the evolution of cryptobiosis of other unrelated invertebrate taxa.

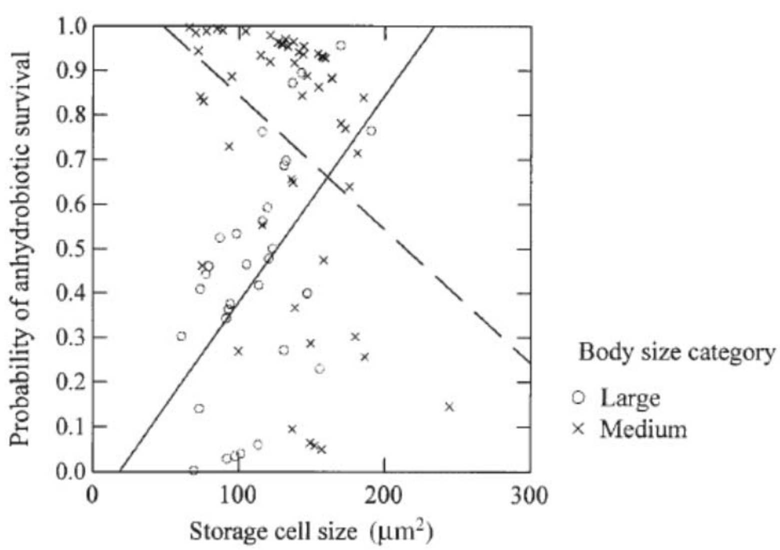

Figure 1. Probability of anhydrobiotic survival for large (o, solid line) and medium (x, broken line) tardigrades as a function of storage cell size. Larger individuals with larger storage cells are more likely to survive anhydrobiosis, while medium-sized individuals with smaller storage cells are more likely to survive anhydrobiosis. Adapted from Jonsson and Rebecchi, 2002.