Proasellus thrive in the dark – plasticity enabling cave colonisation?

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Abstract

Darkness is the hallmark of all subterranean habitats and, to some degree, of many surface habitats. Many surface animals that live in semi-dark habitats, such as freshwater benthos, are photophobic. Yet, few of them can colonize caves and form subterranean populations. We hypothesize that phenotypic plasticity induced by darkness enables surface crustaceans to colonize caves, as has been previously shown for the cavefish *Astyanax mexicanus*.

Our target organisms were surface species that form distinct subterranean populations with different adaptations to caves and have closely related cave species. *Proasellus* proved to be a good model system, and we selected the surface species *Proasellus coxalis* and its cave relative *P. anophtalmus* to learn what adaptations evolve in subterranean forms. We exposed a randomly selected group of both species to complete darkness (DD) where we could establish breeding colonies. Control breeding colonies are exposed to a 12:12 h light-dark photoperiod (LD). Other conditions are kept as constant and equal as possible, including conditioned *Alnus glutinosa* leaves used for shelter and food.

We examined the molecular phenotypes of both *Proasellus* species, animals originally collected from the wild and preserved after four months under the experimental conditions DD and LD. The number of observed differentially expressed genes (DEGs) was significantly higher between cohorts DD and LD of *P. coxalis* compared to *P. anophtalmus*. The direction of regulation of DEGs also showed the opposite effect, with the cohort of *P. coxalis* DD showing predominant upregulation and the cohort of *P. anophtalmus* DD showing predominant downregulation relative to their cohorts LD. A greater number of DEGs in both species were associated with various metabolic processes such as lipid regulation. In contrast, DEGs related to growth, cell division, rhythmic processes, sensory perception of taste, and sexual reproduction were observed only in *P. coxalis*.

We examined the changes at the phenotypic level in the first generation born in the experiment. Surprisingly, *P. coxalis* showed better fitness under DD conditions (growth rate, fecundity, survival). Other phenotypes were altered in both adaptive and non-adaptive

directions i. e. towards vs. opposite of cave adaptations. We conclude that darkness is not a limiting factor in cave colonization and suggest that it may even be a key factor in the evolution of troglomorphy via phenotypic plasticity.

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Funding program

Tenure Track Pilot Programme with funds of the Croatian-Swiss Research Programme

Grant title

TTP-2018-07-9675 EvoDark

Hosting institution

Ruđer Bošković Institute, Zagreb

Conflicts of interest

No