Final report for National Research, Development and Innovation Office
Young investigator grant FK 124164

Project title: "The evolutionary trade-off between senescence and cancer"

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In this project, we aimed to understand how two physiological processes contributing to mortality (ageing on on hand and cancer on the other) relate to each other in an evolutionary ecology setting. We used the freshwater cnidarian *Hydra oligactis* as a model system, which is an emerging model species in the fields of both ageing and cancer research, it is amenable to laboratory experiments and, and exhibits substantial natural variation in ageing rates in nature.

Our premise was that protecting the organism against selfish cell lineages and tumor development requires mechanisms that limit uncontrolled cell proliferation. These mechanisms would sacrifice mutated / damaged cells to reduce the risk of developing cancerous cell lineages, but would also contribute to physiological decline due to removal of cells that would otherwise contribute to maintaining the organism. Accordingly, we hypothesized that a higher rate of phenotypic ageing at the organism level, or apoptosis and cellular senescence at the cell level would predict a better ability to protect the organism against development of cancerous cell lineages and thereby a lower rate of tumor prevalence and cancer development.

We aimed to test this hypothesis by establishing strains of *Hydra oligactis* with distinct genotypes from multiple populations, characterize variation in ageing rate in these strains, and test whether variation in the rate of ageing is associated with the frequency of somatic mutations, the risk of spontaneous tumor development or resistance to experimental tumor induction. This hypothesis was based on previous observations of tumor development in the strains maintained in our laboratory (Fig. 1.).

Two developments arose during the folding out of this project, both of them strongly influencing the direction of our research activity. We will discuss these developments in the two subsequent sub-chapters below.

1. Pervasive and marked phenotypic plasticity in the senescence rate of hydra genotypes

Senescence in *Hydra oligactis* occurs after sexual reproduction in lab strains, but no information was available about natural populations in field condition. Therefore, we performed a study to understand how variation in reproductive mode (sexual vs. asexual) predicts the rate of senescence in field-collected individuals. As proxies for senescence, we used regeneration loss and quantified the size of somatic stem cell pools and differentiated cells involved in food capture (nematocytes). We found that sexual individuals have significantly reduced somatic maintenance functions (reduced regeneration, diminished stem cell pools and nematocyte availability). The results of this study have been published in the journal Functional Ecology (Q1/D1; Sebestyén et al. 2018). Importantly, this study showed us that differences in reproductive mode can be taken as a proxy for variation in post-reproductive senescence to gain more insight into the the natural diversity of the latter.

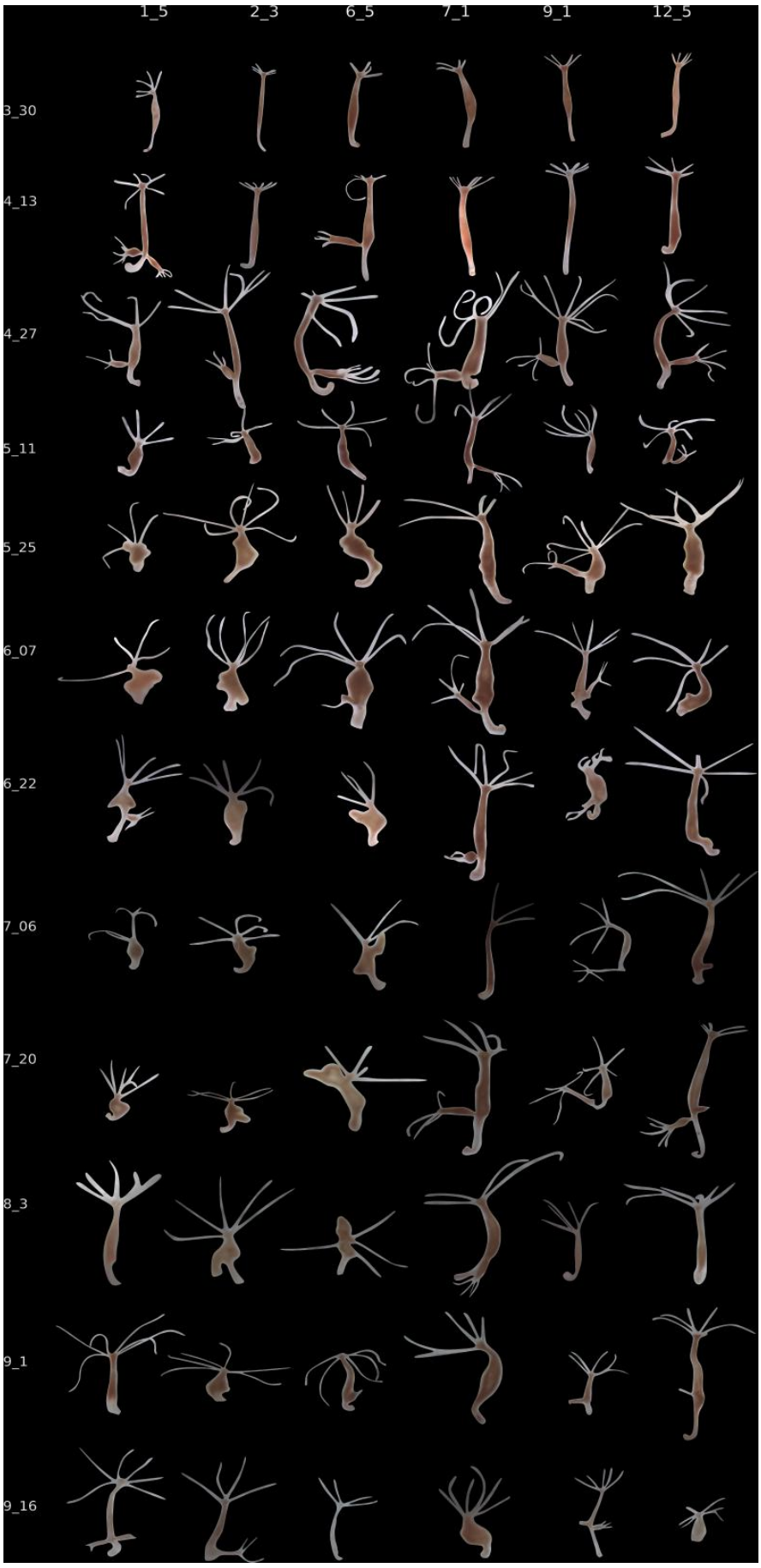

Fig. 1. Development of tumors in six hydra individuals that were followed over a period of ~6 months (top to bottom).

Building on this study, we sampled natural *Hydra oligactis* populations (13 populations in total, all in Eastern Hungary), during their autumn sexual period. We recorded their reproductive mode and selected N=120 individuals (both sexual and asexual) for genotyping using Restriction-site Associated DNA-Sequencing (RAD-Seq). This method relies on sequencing a random subset of the genome of the target organism and extracting SNPs from these sequences, which can then be used for downstream population genetic, phylogeographic or phylogenetic analysis. RAD-Seq works quite well for non-model organisms even when other methods fail. Indeed, no previous population genetic information was available for the *Hydra* genus (partly because our previous attempts using conventional methods, like microsatellites, failed). Our results revealed a remarkable pattern: the genetic differentiation of our *Hydra oligactis* populations was very low, and populations that were about a hundred km. apart could contain related individuals. More importantly, we were able to show that sexual and asexual individuals within populations do not form distinct genetic clusters and often polyps belonging to the same genotype can have contrasting reproductive modes. The results have been published in the journal Molecular Ecology (Q1/D1, Miklós et al. 2021). Importantly, this study told us that there is very little genetic differenciation and much higher plasticity than we suspected when we designed this project.

What could be the drivers of this plasticity? Age and size are important life history traits in hydra polyps. Therefore, we first investigated how age might affect senescence in hydra. We took two laboratory strains, isolated adult (at least 3 weeks old) individuals and freshly detached buds (around 3 days old), induced sexual reproduction in them by cooling and compared their reproductive investment and post-reproductive senescence. We found that young individuals responded by having a reduced sexual investment (lower chance of initiating sexual reproduction and fewer gonads if they did initiate gonad development), and meanwhile a higher somatic maintenance level (higher regeneration capacity, more stem cells retained during reproduction and a higher overall survival rate) compared to adults exposed to the same environmental cue (cooling). The results have been published in the Journal of Animal Ecology (Q1/D1, Sebestyén et al. 2020).

Secondly, we performed a correlative analysis to understand the role of body size in generating variation in post-reproductive senescence. We found that larger polyps were more likely to initiate sexual reproduction in response to cooling, had a higher fecundity and were more likely to die after reproduction. To causally ascertain this relationship, we next exploited the ability of hydra polyps to incorporate foreign tissue that is introduced into their body column through surgical manipulation and experimentally increased or decreased the size of the animals. The results were concordant with the correlative data: polyps with a reduced body size had lower sexual investment but experienced a higher post-reproductive survival rate, suggesting that body size *per se*, rather than any of the factors correlating with it determine senescence in hydra. To our knowledge, this is the first experimental study to show that body size directly regulates senescence in animals. The results have been published in the journal Ecology Letters (Q1/D1; Ngo et al. 2021).

The previous two studies ascertained that senescence in hydra is a highly plastic trait, where the same genotype can be both senescing or non-senescing based on its age or size. Next, we wanted to find out whether the sexual, senescing phenotype shows a consistent pattern at the population level. We performed serial samplings from a single population at four time points (spring and autumn for two years), established laboratory strains from the sampled individuals and phenotyped them for sexual reproduction and senescence. We found that the frequency of the sexual, senescing phenotype varies with the season, such that we are more likely to detect them in strains established from individuals collected in the autumn. An accompanying experiment showed that propensity to initiate sex in response to cooling changes with warm-exposure in cold-accomodated individuals, suggesting that temperature fluctuations drive seasonal variation in reproductive readiness in this species. The results have been published in the journal Ecosphere (Q1, Tökölyi et al. 2021).

We also wanted to find out if there is changing population genetic structure underlying these seasonal differences. Therefore, we genotyped the strains in the previous study and compared the distribution of genotypes in the two seasons. Our results revealed no clear seasonal pattern: broadly the same genotypes are present in spring and autumn (some genotypes even persisted for the complete study period spanning two years). These results were submitted for publication in the journal Freshwater Biology.

The overall conclusion from the above studies is that senescence in *Hydra oligactis* is decisively a plastic trait. The same genotype can show a sexual, senescing phenotype or an asexual, non-senescing phenotype depending on age, size or environmental conditions. This suggests that - at the geographic scale of the planned samplings - most genotypes should have a similar ability to prevent the development of cancerous cell lineage, contrary to our hypothesis.

2. Tumors in hydra are caused by components of the microbiome

A second development crucial for the implementation of this project was a finding made by a German group studying tumorous hydra in the laboratory. They were able to conclusively show that tumor development in hydra is induced by components of the microbiome, such that experimental removal of these components (e.g. through administering specific antibiotics) results in tumor regression (Rathje et al. 2020, Plos Pathogens, 16(3), 1008375). This result suggests that tumor development in hydra is not due to somatic mutations and the subsequent proliferation of mutated stem cells as we premised, but is instead the consequence of specific association with components of the microbiome.

To obtain more insight into the role of the microbiome in tumor development of our model system, we initiated scientific collaborations with two research groups: the group of prof. Sebastian Fraune (Heinrich-Heine University, Düsseldorf, Germany) and the group of prof. Frédéric Thomas (IRD/MIVEGEC Montpellier, France). In collaboration with the Fraune group, we have sampled a total of 21 Central European hydra populations to identify their microbiome composition and describe the factors that drive variation in microbiome composition. The results have been written up and will be soon submitted for publication. It still remains to be shown how this variation in microbiome composition correlates with tumor development.

We have also performed a detailed analysis of several Hungarian tumorous hydra lineages, together with a number of French ones, identified by the Thomas group. These results showed that, remarkably, the physiological background of tumor development appears to be very similar in both the Hungarian and the French strains, since we found an overproliferation of germline stem cells that are closely located to the mesoglea in these animals. We also started to identify the microbial species that might potentially involved in driving tumorous development, which suggest that distinct taxa are causing the same phenotype. These results are in the process of being written up and will be soon submitted for publication.

While working with these tumorous strains, we noticed clear phenotypic changes between tumorous and non-tumorous individuals (e.g. in size or number of tentacles). These phenotypic differences affected prey capture ability, exposure to predators, as well as the coexistence of hydra polyps with commensal ciliates. To our knowledge, this is among the first studies to show how tumors alter the ecological interactions of the affected individuals. The results have been published in the journal Science of the Total Environment (Q1/D1; Boutry et al. 2022).

Conclusions

Together, our observations clearly suggest that variation in senescence in hydra is a strongly plastic trait, such that a given phenotype can show both a sexual, senescent phenotype and an asexual, non-senescent phenotype depending on its internal state or environmental conditions. Therefore, we cannot really expect variation in senescence to be associated with variation in tumor development in this system. Moreover, these tumors appear to be caused by components of the microbiome, again suggesting that distinct drivers are behind the two processes. The two processes might still converge, but not in the way we hypothesized. Specifically, the mechanism through which microbes cause tumors in hydra (inducing the overproliferation of germline stem cells), could also result in increased sexual reproduction. Analyzing our multi-strain dataset for which we have data on both sexual reproduction, senescence and tumor development, we found that tumors are more likely to occur in individuals that show the sexual, senescing phenotype (i.e. exactly the opposite of what we predicted). However, it is still unclear why microbes would cause tumor development that results in increased sexual investment and senescence.

As planned, we also performed quantification of somatic mutation rate on our strains from the RAD-Seq data, quantified gene expression and did microscopic observations but found no clear patterns. Furthermore, we attempted to induce tumors experimentally in this species (through repeated exposure to UV light), without results: hydra polyps were remarkably resistant to sublethal irradiation. This is a very exciting finding, and identifying the mechanisms through which hydra resist tumorigenesis so exceptionally promises to be an interesting research topic for the future.

During the course of the project, we attempted to engage a large number of students in research activities. A total of 10 BSc students, 6 MSc students and 2 Phd students worked actively in our lab for their BSc, MSc or Phd theses. Of these, 2 BSc students and 3 MSc students were co-authors of the scientific papers resulting from the project, while the two Phd students finalized their theses and are in the process of defending them. Finally, we also wrote two popular science articles together with these students.

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