Supplement Discussion

To ensure comparability of the *Hydra* mutation rate determined in this study with the human and mouse mutation rates measured by Milholland et al, 2021, we applied almost the same methodology regarding wet-lab steps and bioinformatics (see Supplement Table S6). Unlike Milholland et al., however, who examined fibroblasts, we sought to sample stem cells or early progenitors, called herein 'stem-like'. Nevertheless, we argue that regardless of the exact differentiation status of the actual cells studied, we measured the mutation rate of stem-like cells. This follows from the circumstance that we averaged the mutation rate per cell division over thousands of cell generations since 1973 (approximate time of last sexual reproduction, see Fig. 1, Methods). Whereas, even if we had sampled a fully differentiated cell, the number of their non-stem like cell ancestors would only comprise a few cell generations, i.e. a vanishingly small fraction of the total ancestors since 1973. For this fraction to have any appreciable effect in the overall calculation of mutation rate, the mutation rate in differentiated cells, would have to be many orders of magnitude higher than in stem cells. This seems to be highly unlikely given that in other species these rates differ "just" by a factor of two (Brazhnik, et al., 2020). Nevertheless, it would be interesting to determine in the future whether this factor is lower or higher in *Hydra* by comparing single-cells of defined differentiation states. Of note, our approach of estimating the mutation rate is based on the assumption that the impact of back mutations and negative selection is neglectable. If these assumptions do not hold, the mutation rate of the *Hydra* would be even higher than reported here.

We found a U-shaped bipartite pattern with most single-cell SNVs being either present in all nine examined individuals or in none (Fig 3A). Given the relationship structure (Fig. 1), the somatic mutations generating single-cell SNVs that were observed also in one to all nine individuals must have happened somewhere between the zygote and LCA2. Those single-cell SNVs that were not detected at the level of *Hydra* individuals, if not representing amplification artifacts, likely occurred after LCA2 on the branch to the donor of the single cells. The single-cell SNVs detected in one to eight individuals, due to limitations of our sequencing depth, in fact may also be present in more if not all individuals examined. Alternatively, genetic drift and bottleneck effects at budding may have eliminated or diminished those SNVs in some individuals.

By construction, variants found at the single-cell level distinguish stem cell lineages from each other. Therefore, these SNVs were likely absent in the zygote (Fig.1). If such a somatic variant is found in a significant proportion of the cell population of a *Hydra* individual, the respective cell clone might have expanded by somatic positive selection. The high observed vs. expected ratios for the different classes of sharing single-cell SNVs by *Hydra* individuals (Fig. 3A) can be interpreted in this sense.

Another potential indicator of positive selection is the high fraction of homozygous variants detected in the single-cell experiment and (Fig. 3A). Given the background of lacking regular sister chromosome pairing and recombination during somatic evolution of I- and E-cells, it has to be considered extremely unlikely that by chance so many somatic SNV alleles become fixed on both sister chromosomes. On the other hand, the observation of apparent homozygosity could also be explained by allelic imbalance in early steps of the amplification. Three reasons suggest that these are more than mere amplification artifacts: (1) the majority of these homozygous somatic variants found initially at the single-cell level could be confirmed at the whole-animal level, (2) those variants were found in individuals with a significantly higher MAF and (3) in more individuals than heterozygous ones. Two explanations appear to be plausible for this loss of heterozygosity: unidirectional "copy and paste" transfer of the mutated allele to its sister chromosome, e.g., as a consequence of the repair of a double strand break; or deletion of one non-mutated allele before or after the mutation event. Loss of heterozygosity was frequently observed in the context of continued mitotic cell divisions, such as many cancers as well as asexually unicellular organisms such as various yeast and funghi (Ryland, et al. 2015; James, et al. 2019; Dutta, et al. 2021). Our finding indicates that this phenomenon can also occur at a high frequency in a multicellular organism in the context of continued high

physiological mitotic activity. On the other hand, it may further underline the perspective on *Hydra* as an active stem-cell community sharing features of both multi- and unicellular organisms. It is tempting to speculate that this phenotype and the respective underlying molecular mechanisms have evolved *Hydra* specifically to accelerate *Hydra's* adaptation to environmental changes.

Given the importance of the processes found to be under strong negative selection in the wild for *Hydra's* fitness, it is unsurprising that the corresponding genes tend to be more frequently expressed than genes in general in our samples. Particularly noteworthy, however, is that the same is true also for positively selected processes during the time in the laboratory, especially since the mutation-bearing genes in general do not show an elevated proportion of expressed genes. This underlines that these adjusting these processes is likely to be relevant for adapting to the laboratory environment.

Since mutation rate seems to play no or only a minor role in the non-senescense of the *Hydra* strain studied, it would be interesting to identify genomic differences to more rapidly aging species in the future. An obvious comparison would be that with *Hydra oligactis*, in which rapid aging can be induced when held at a water temperature of less than 10 ° C. It would be exciting to verify whether genomic differences would be enriched in pathways similar to those highlighted by recent gene expression comparisons between aging and non-aging *Hydra* such as cellular senescence, apoptosis and autophagy, which did not appear in our analyses (Tomczyk et al, 2020; Sun et al. 2021).

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