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Highlights

Vertically transmitted tumors influence the life history traits of hydras

Tumor-bearing hydras have a reduced survival rate

Tumorous hydras show increased early reproductive effort (asexual and sexual)

Changes in sexual reproduction pattern can be a compensatory response of the host

Boutry et al., iScience *25*, 105034
October 21, 2022
https://doi.org/10.1016/j.isci.2022.105034

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Tumors alter life history traits in the freshwater cnidarian, *Hydra oligactis*

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SUMMARY

Although tumors can occur during the lifetime of most multicellular organisms and have the potential to influence health, how they alter life-history traits in tumor-bearing individuals remains poorly documented. This question was explored using the freshwater cnidarian *Hydra oligactis*, a species sometimes affected by vertically transmitted tumors. We found that tumorous polyps have a reduced survival compared to healthy ones. However, they also displayed higher asexual reproductive effort, by producing more often multiple buds than healthy ones. A similar acceleration is observed for the sexual reproduction (estimated through gamete production). Because tumoral cells are not transmitted through this reproductive mode, this finding suggests that hosts may adaptively respond to tumors, compensating the expected fitness losses by increasing their immediate reproductive effort. This study supports the hypothesis that tumorigenesis has the potential to influence the biology, ecology, and evolution of multicellular species, and thus should be considered more by evolutionary ecologists.

INTRODUCTION

Since their evolution at the end of Precambrian, animals face the problem of the emergence and uncontrolled proliferation of constitutive abnormal cells (Aktipis and Nesse, 2013; Boutry et al., 2022b). The failure to remove abnormally proliferating cells may lead to the formation of neoplasms, also called tumors, which can be detrimental to the fitness of their bearers being, for instance, lethal at the metastatic stage (Dillekås et al., 2019; Lloyd et al., 2017). Although neoplasm formation is usually a slow process and can theoretically occur at any age in organisms of most multicellular species (Thomas et al., 2018), the importance of neoplastic cells for animal evolutionary ecology is largely unknown (Boutry et al., 2022a; Thomas et al., 2017). The ecological and evolutionary importance of cancer is an increasingly timely topic given the accumulating levels of carcinogenic pollutants in ecosystems because of anthropic activities (Giraudeau et al., 2018).

Host-tumor interactions are similar at several levels with host-parasite interactions (Ujvari et al., 2016b). For instance, tumoral cells depend on their hosts to survive and reproduce, and also induce fitness costs to their hosts (e.g., lower competitive ability, higher vulnerability to pathogens or predators (Vittecoq et al., 2013)). However, unlike parasites, only in a minority of instances tumoral cells are directly transmitted between host individuals of the same or different species, either horizontally (e.g., dogs and Tasmanian devil; (Bender et al., 2014; Ujvari et al., 2017)) or vertically (i.e., in hydra or bivalves; see (Domazet-Lošo et al., 2014; Ujvari et al., 2016b)). When tumoral cells can evolve the means to bypass the normal barriers that prevent mixing of cells from two genetically different organisms, they become subject to the evolutionary dynamics of an infectious agent, and act as a parasitic "species" (Dingli and Nowak 2006; Dujon et al., 2020; Tissot 2022; Burioli et al., 2021). Thus, although rare, transmissible cancers are fascinating models to study the ecology and evolution of host-tumor interactions based on a host-parasite interactions framework.

It is commonly accepted that changes in phenotypic (e.g., color, morphology, behavior, see for instance Bhattarai et al., 2021; Thomas et al., 2010) or life-history traits (Ewald, 1980; Thomas et al., 2010) in parasitized hosts can be classified into four major categories: (1) Host adaptations to eliminate the parasite and/or alleviate its fitness costs; (2) parasite adaptations to exploit the host and favor its reproduction and/or

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https://doi.org/10.1016/j.isci.2022.105034

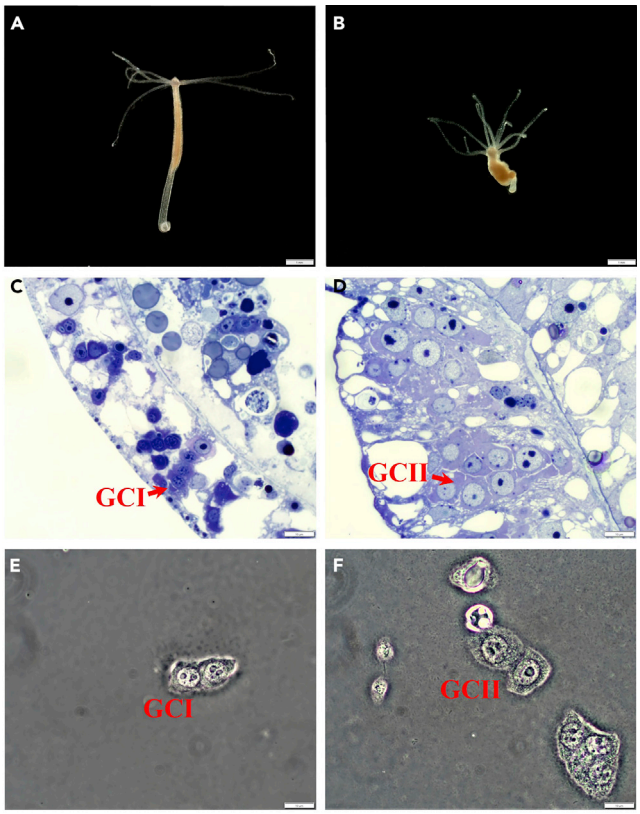

Figure 1. Germline stem cell tumors in *Hydra oligactis*

(A–F) Pictures of control (A) and tumorous (B) hydras; trinocular magnifier, scale bar: 1 mm. Photographs of histological sections showing germline stem cells in the ectoderm of a control hydra (C) at the stage I (GCI) and of a tumorous hydra (D) abnormally passed at the stage II (GCII); polarized optic microscope x100, scale bar: 10 μm. Photographs of germline cells, detached by maceration, from control (E) and tumorous hydras (F) showing differences in the size of germline cells and the development stage; polarized optic microscope x100, scale bar: 10 μm.

transmission; (3) a combination of the last two categories (Abbot and Dill, 2001); see also for synthesis (Lefèvre et al., 2008); and (4) a by-product of the infection without adaptive value for the host or the parasite. Although it is well known that tumor-bearing individuals also frequently display phenotypic changes (e.g., in sleep, libido, mood, food preferences (Davidson et al., 2002; Glaus et al., 1996), very few studies have until recently attempted to interpret these phenotypic changes using this host-parasite framework (Arnal et al., 2017; Dawson et al., 2018; Jones et al., 2008).

Hydras are freshwater cnidarians that can reproduce both asexually and sexually. During asexual reproduction, hydras produce buds that develop into different individuals (polyps) that can detach from the parental polyp and develop into independent individuals. Hydras can also reproduce sexually by producing eggs and sperm (on separate individuals). In the species *Hydra oligactis*, sexual reproduction is followed by a post-reproductive degeneration of the parental polyp resulting in increased mortality risk (Yoshida et al., 2006). Excluding this post-reproductive degeneration, hydras are considered "immortal" because of their longevity studied in detail in *Hydra vulgaris* (Dańko et al., 2015; Martinez, 1998; Tökölyi et al., 2017).

Some hydras can harbor tumors (e.g., *Hydra oligactis* (Domazet-Lošo et al., 2014)). These tumors, visible to the naked eye, are formed by an accumulation of germline stem cells, which proliferate in the ectoderm of the main body (see Figure 1). The tumorous polyps expressed differentially 44 genes that are involved in tumor development in mammals (Domazet-Lošo et al., 2014) for more detail). These tumors and the specific microbiome associated with them have the rare capacity to be vertically transmitted during asexual reproduction (Rathje et al., 2020). Specifically, the tumorous polyps transmit their neoplastic cells to their descendants during budding, which results in the development of tumors in the offspring hydra three or four

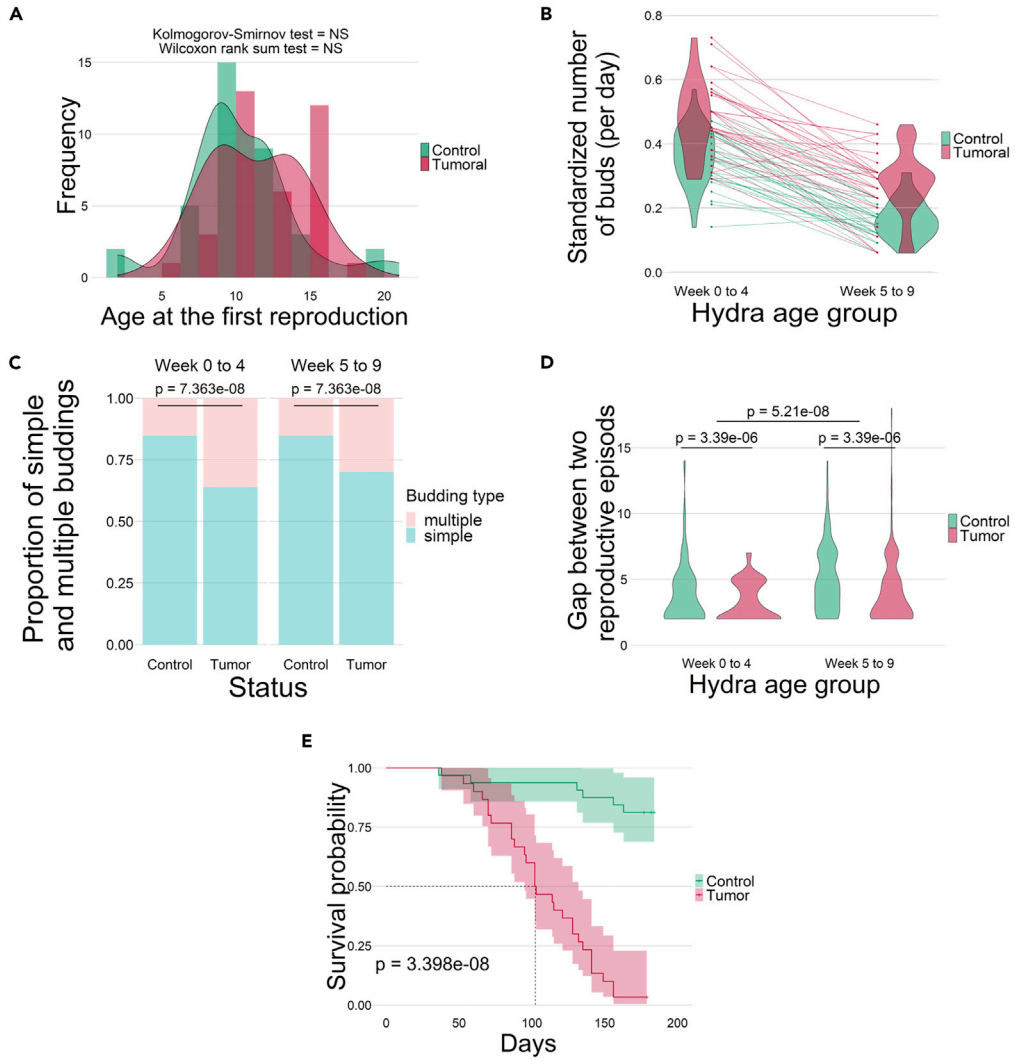

Figure 2. Asexual life-history traits of tumorous hydras

(A) The barplot and the slopes represent the distribution of the age at which hydras started to reproduce (in days). The control group is represented in green and with bars on the left and the tumorous hydras are in red with bars on the right. The Kolmogorov-Smirnov (N = 36, D = 0.22222, p value = 0.3364) and Wilcoxon tests (N = 36, W = 525, p value = 0.1543) are both non-significant.

(B) Each dot represents the average number of buds produced by a hydra per day during each period, in green for controls and in red for tumorous hydras. The lines connect the values of the same individual across the two stages. There is a significant effect of the health status depending on the phase (GLMM, IRR = 1.26, SE = 0.08, p value = 4.056e-04). N = 36 for controls in both phases and for asymptomatic tumorous hydras, N = 33 for tumorous symptomatic hydras.

(C) The barplots represent the proportion of the reproductive episodes that released simple (blue, below) versus multiple buds (pink, above) at the same time. On the left for hydras during their first month and on the right for hydras from the 5th to the 9th week. Tumorous individuals undergo a significant increase of the proportion of multiple budding episodes relative to control hydras, independently of their period of life (GLMM; effect of tumorous status, OR = 0.37, SE = 0.07, p value = 7.365e-08). N = 36 for controls in both phases and for asymptomatic tumorous hydras, N = 33 for tumorous symptomatic hydras.

(D) The violin plots represent the distribution of the delay of time (in days) between two detachments, depending on the tumorous status (control in green on the left, tumorous in red on the right) and the pathological stage (first month on the left, from the 5th to the 9th week on the right). There is a significant decrease of the time between two reproductive episodes for tumorous hydras (GLMM; IRR = 0.81, SE = 0.45, p value = 3.392e-06). In addition, hydras undergo a significant

Figure 2. *Continued*
increase of this delay after the 5th week (GLMM; IRR = 1.25, SE = 0.05, N = 678, p value = 5.208e-08). N = 36 for controls in both phases and for asymptomatic tumorous, N = 33 for symptomatic tumorous hydras.
(E) Probability of survival of control and tumorous hydras over time. The control hydras (in green) have a significant increase of the survival probability across time compared to the tumorous (in red; survival regression, estimate = 0.38, SE = 0.07, p value = 3.39e-08) with a half-life time superior to 200 for controls against to approximately 100 days for the tumorous. N = 36 in each group.

weeks later. With their slow development and the lack of impact on survival (Domazet-Lošo et al., 2014), these tumors provide a good model to study the effect of neoplastic cells on the host fitness before and after tumor development. Interestingly, Domazet-Lošo et al. (2014) reported a decrease of reproductive abilities – i.e., reduced budding rate and egg production, in hydras bearing well-developed tumors, which suggested that tumorigenesis is associated with fitness costs for hydras.

Although such selective pressures have the potential to favor the evolution of compensatory host strategies that alleviate these costs (see for instance (Arnal et al., 2017; Jones et al., 2008; Michalakis and Hochberg, 1994) for parasites, and also for cancer examples), this possibility has never been explored in the context of organisms harboring vertically transmitted tumors. In this study, we explored the link between tumor development and host life-history traits (e.g., survival, budding rate, health status of buds, sexual reproduction) in hydras. If compensatory responses exist in hydras to alleviate the fitness costs of tumors, we predict that offspring from tumoral hydra should enhance their reproductive effort early in their life, i.e., during their first four weeks, that is before developing detrimental tumors. Also, because during the first four weeks of their life, offspring from tumoral hydras look tumor-free, we also predicted that the buds produced by them during this time interval could potentially remain healthy thereafter.

RESULTS

Age at the first asexual reproduction

Figure 2A shows the age at first budding distributions of control and tumorous hydras. The Kolmogorov-Smirnov (D = 0.22, p value = 0.34) and Wilcoxon tests (W = 525, p value = 0.15) are both non-significant, which shows no difference in the distribution and the median of the values of age at first reproduction between control and tumor hydras. Even if we noted a tendency of a delayed reproduction for tumorous hydras, it remains non-significant.

Budding rate

The model selected to explain the budding rate takes into account the fixed effect of the interaction between the status and the phase, and the random effect of the individual. Tumorous hydras display a general increase of the budding rate, compared to control, then this budding rate is reduced beyond the fifth week for both hydra types (tumorous and control). However, the reduction is significantly more important for control individuals than for tumorous ones (Figure 2B. GLMM; effect of the interaction of status and phase, IRR = 1.26, SE = 0.08, p value = 4.056e-04).

Asexual reproductive episodes

Different components of reproduction were examined: the number of offspring produced in each budding episode and the gap between two of these episodes. To explain the budding type, the fixed effect of the status and the random effect of the individual were taken into account. Tumorous hydras show a significant increase in the proportion of multiple rather than single budding episodes, regardless of the phase considered (Figure 2C; GLMM; effect of tumor status, OR = 0.37, SE = 0.07, p value = 7.365e-08).

However, concerning the time between two detachments, the additional fixed effect of the phase and the status were selected with the random effect of the individual. The effect of the status (Figure 2D; GLMM; status effect, IRR = 0.81, SE = 0.45, p value = 3.392e-06) as well as the effects of phase are significant (Figure 2D; GLMM; phase effect, IRR = 1.25, SE = 0.05, p value = 5.208e-08). Then, this indicates that tumorous hydras show a reduction in the time required between two detachments: when the control ones need one day, the tumorous only need 0.81 days. However, independently of the status of the hydra, after the 5th week, the time between two reproductive episodes increases by 25%. Thus, during the four first weeks, tumorous hydras show (1) a reduction of the delay between two budding episodes and (2) an increase in

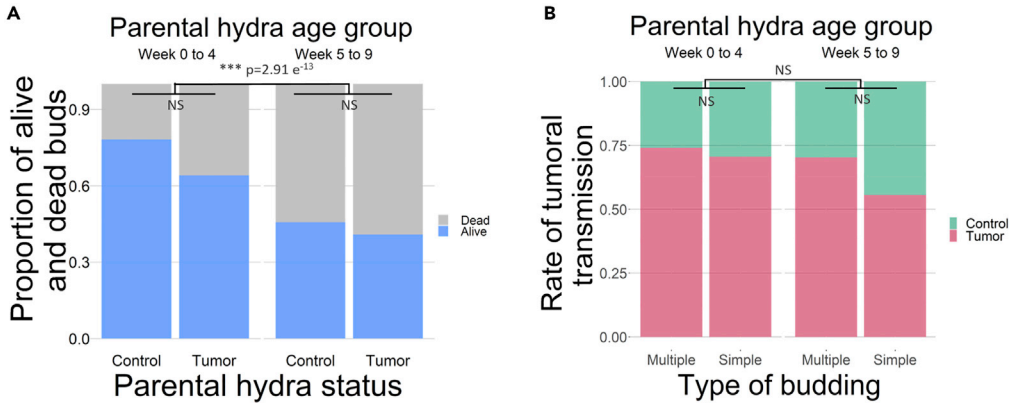

Figure 3. Survival and transmission of the tumorous phenotype to the buds

(A) The barplot represents proportions of alive (in blue, below) and dead (in grey, above) buds one month after their detachment, depending on the status (control or tumorous) and the age group (first or second month) of the parental hydra. There is a significant decrease in the proportion of survival of the buds after the 5th week (GLMM; OR = 5.22, SE = 2.89, p value = 2.907e-03). During the first phase; N = 87 for control and N = 117 for tumorous and during the second phase; N = 81 for control and N = 88 for tumorous.

(B) The barplot represents the rate of tumoral transmission by the proportion of buds produced by tumorous parents that developed tumors (in red, below) or remain asymptomatic (in green, above) after 5 weeks, according to the type of budding episode from which they are originated (multiple or simple) and the age of their parent. The tumoral transmission rate does not vary significantly among the different groups (GLMM, the best-fitted model does not include the effect of any parameter, see appendix Table S3). N = 75 for the first phase and N = 36 for the second.

the number of offspring produced at each of these episodes. These two effects explain how tumorous hydras increase their budding rate.

Survival during asexual reproduction

Concerning the survival time of the parental hydras, only the fixed effect of the status has been selected. Figure 2E represents the probability of survival over time of control and tumorous hydras as well as the results of the survival regression. Tumorous hydras have a significantly increased risk of mortality compared to control ones (Figure 2E; survival regression; estimate = 0.38, SE = 0.07, p-value = 3.39e-08) with a half-life time equal to approximately 100 days against at least 200 for controls (their half-life time couldn't be estimated during the experiment's time).

Bud survival and tumor transmission

The model selected to explain the proportion of alive and dead buds only takes into account the fixed effect of the age of the parental polyp and the random nested effect of the survey date and the batch. The effect of the phase of bud's production is significant (Figure 3A; GLMM; phase effect, OR = 5.22, SE = 2.89, p value = 2.907e-03). Specifically, this indicates that the buds produced when the parental polyps are five weeks aged are less likely to survive than the ones produced earlier no matter the parental status.

Then, within the surviving buds coming from tumorous parental hydras we found no significant variations in the proportion of individuals developing tumors after five weeks. The selected model explains the transmission of the tumorous phenotype with no impact from either of the pathological phase of the parental hydra or the type of budding episode from which the bud detached. The model takes into account the random effect of the batch. Thus, the tumoral transmission does not seem to be influenced by the age of the parental polyp and by the type of budding episode from which they are generated (Figure 3B).

Age at the first sexual reproduction

The age at the first eggs produced is explained by a model taking into account the effect of the interaction between the status and the phase with the random effect of the date. Figure 4A represents the average age

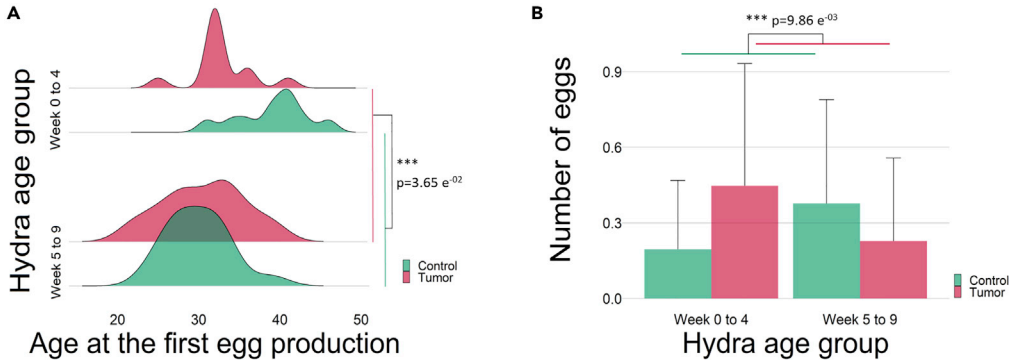

Figure 4. Sexual life-history traits of tumorous hydras

(A) Age distribution at the first egg production according to the hydra status and their age at the induction of sexual reproduction. The tumorous hydras (in red, above) induced during their first month display a significantly reduced time to produce their first egg compared to the control (in green, below), whereas the hydras sexually induced during their second month need a similar time to produce their first egg independently of their group (LMM; estimate = 5.11, SE = 2.44, p value = 3.65e-02). For hydras induced during the first phase; N = 10 for control and N = 12 for tumorous, for hydras induced during the second phase; N = 17 for control and N = 10 for tumorous.

(B) The barplot represents the average number of eggs per individual produced by control (in green) and tumorous (in red) hydras depending on their age at sexual induction. The control hydras (in green) produced more eggs when they were induced during their second month whereas it is the opposite for the tumorous hydras (in red) that produced more eggs in average when they were sexually induced during their first month (GLMM; IRR = 0.23, SE = 0.13, p value = 9.86e-03). For hydras induced during the first phase; N = 72 for control and N = 56 for tumorous, for hydras induced during the second phase; N = 69 for control and N = 79 for tumorous hydras.

at the first egg production, taking into account the phase and the status as well as the results of the linear regression. The age at which the first eggs are produced decreases after the fifth week, but this slowdown is stronger for the control compared to the tumorous hydras (Figure 4A; LMM; effect of the interaction of the status and the phase, estimate = 5.11, SE = 2.44, p value = 3.65e-02).

Sexual reproduction effort

The model selected explains the number of eggs produced by the fixed effect of the phase in interaction with the status and the random effect of the date. Figure 4 represents the results of the model used and the average number of eggs produced for each phase and status. The pattern of egg production is opposite between control and tumorous hydras (Figure 4B; GLMM; effect of the interaction of status and phase, IRR = 0.23, SE = 0.13, p value = 9.86e-03). The production of eggs by the tumorous hydras decreases after the fifth week whereas it increases for the control hydras. When the control hydras produce on average one more egg in their second phase than in their first, the tumorous hydras produce only 0.23 eggs.

DISCUSSION

Despite the ubiquity of tumorigenesis processes among metazoans (Aktipis et al., 2015; Boutry et al., 2022b; Vincze et al., 2021), changes in life-history traits in tumor-bearing individuals remains a poorly investigated topic (Ujvari et al., 2016a). This is unfortunate because phenotypic variations arising from tumorigenesis are likely to contribute to inter-individual variability, and thus have the potential to influence evolutionary ecology processes (Thomas et al., 2017). Here, using as a model system a species harboring vertically transmitted tumors (Domazet-Lošo et al., 2014), we found that tumorous individuals, compared to healthy ones, have a reduced survival but also display enhanced asexual reproductive efforts in their early life and an earlier development of the sexual trait (Figure 5). These findings are discussed in the sections below.

Age at first reproduction

The age at which hydra, once detached from their parental polyp, produce themselves a first bud was not significantly different between tumorous and healthy individuals. Thus, young hydras during the pre-pathological period apparently do not advance their first reproduction. This result also suggests that the health status of the parental hydra does not influence, at least for this criterion, offspring reproductive strategies through

Figure 5. Graphical abstract of the main results for asexual reproduction, bud outcome, and sexual reproduction in the two phases studied.

trans-generational effects. Whether an earlier age at first reproduction would be useless in terms of fitness gains, and hence not favored by selection, or not possible because of physiological constraints remains to be answered.

Asexual and sexual reproduction

Once asexual reproduction has begun, tumorous hydras in the pre-pathological period produce a greater number of buds, i.e., multiple polyps simultaneously and more frequently, than during the pathological period, beyond 5 weeks. Healthy hydra budding rate displayed the same temporal trends, but it remained lower than the one of tumorous individuals. The precise mechanisms behind the enhanced asexual reproductive effort in tumorous polyps remain to be determined. Symptomatic individuals possess increased number of cells because of the increased proliferative abilities of tumoral cells (Domazet-Lošo et al., 2014), and this could mechanically increase the number of cells available to produce buds. However, this is undoubtedly not the sole possibly acting mechanism because the higher budding rate of tumorous hydras was already observed during the pre-pathological stage, whereas tumorous and healthy polyps have the same morphological aspects. It is also established that hydras have two ways to reduce their number of cells, namely bud detachment or tentacle erosion (Shostak, 2017). In this context, the increased budding rate in tumorous hydras, coupled with their increased number of tentacles (Domazet-Lošo et al., 2014), might correspond to adaptive mechanisms to maintain cellular homeostasis. Because those adaptations would remain insufficient through time in front of tumor cells' proliferation, tumorous hydras would keep

this altered phenotype without being able to prevent all tumor cells from accumulating and forming neoplasms. It would also be interesting to explore if budding in tumorous hydras yield a reduction in tumor cell load.

The higher budding rate of tumorous hydras compared to healthy ones, during their two first months, contrasts with findings from other studies. Domazet-Lošo et al. (2014) reported that tumorous *H. oligactis* produce almost no buds. In the same lab, but 6 years later, Rathje et al., (2020) conversely observed that tumorous hydras displayed a significant budding rate (around 30 buds in 25 days), even if the number of buds consistently remained lower than that produced by healthy polyps. The lack of consistency through time and/or between labs in the budding rate of tumorous hydras could have different non-mutually exclusive explanations. First, we cannot exclude that differences in the rearing protocol (e.g., nutritional levels, experimenters) could differentially affect tumorous and healthy hydras, and would mostly influence the budding rate of the former. Second, it is possible that the tumoral lineage used, although considered as the same, has evolved during the 6 years of clonal reproduction separating the two studies mentioned before, favoring mutants able to better tolerate tumors, thereby budding despite the presence of tumors. What remains interesting with the present study is however the fact that, in contrast to the two previous studies, the budding rate of tumorous hydras was constantly higher than the one of healthy ones. Last but not least, another explanation to this discrepancy could be the existence of a non-voluntary bias introduced on hydras' age in the previous studies. Indeed, if previous experimenters, in their need to collect typical tumorous specimens for their experiment, selected in mass culture those individuals displaying large tumors, they were likely to preferentially collect relatively older specimens. Because the sampling of healthy individuals did not rely on visual clues correlated to age, individuals were collected randomly and hence would have been on average younger than tumorous ones. Given that these studies did not control for the age of polyps, this explanation could be relevant: If, for instance, in our study we consider healthy polyps freshly detached and tumorous hydras of 5 weeks old (see Figure 3), we could conclude that healthy hydras bud more than tumorous ones, as in Domazet-Lošo et al. (2014). In addition, the fact that reproductive investment varies through lifetime in *H. oligactis* has already been described in the literature (Sebestyén et al., 2020). To clarify our findings and explore deeper the possibility of such an age bias in the previous studies, one would need to monitor the budding rate of hydras beyond nine weeks, to verify if older tumorous hydras display a budding rate reduction strong enough to be on the long term constantly below the rate of healthy ones of the same age. In any case, the current results are not in contradiction with those obtained previously but offer us a more detailed picture of the kinetics of the impact of tumors on host fitness. This monitoring over time also allows us to highlight a dynamic adjustment of the life history traits before the deleterious impact of the tumors on host fitness.

The reason why tumorous hydras displayed an enhanced asexual reproductive effort early in life may have several causes. As mentioned before, we cannot exclude that artificial lab conditions continued to select for hydra that better tolerate tumors (e.g., (Thomas et al., 2020; Yamamura, 1993)). This hypothesis, however, seems unlikely to yield a situation in which tumorous hydras would bud more than healthy conspecifics. In parallel, it is also theoretically predicted that animals whose future reproduction expectancy is compromised because of pathological disorders that are detrimental for survival and/or fecundity, are favored by selection if they compensate the expected losses by increasing their immediate reproductive effort (see, for instance, (Michalakis and Hochberg, 1994; Vézilier et al., 2015) for examples involving parasitic diseases). Although the same prediction could apply in regard to hosts suffering from detrimental tumoral pathologies, evidence of early reproductive effort in tumorous animals is still scarce at the moment. Among them, Arnal et al. (2017) showed that *Drosophila* females harboring early stage of gut tumors alleviate the associated fitness costs (i.e., a reduced survival) by reaching their oviposition peak earlier than healthy females. (Dawson et al., 2018) studied the social behavior of tumorous flies and found that, when given the choice between groups of tumorous or healthy flies, a tumorous fly prefers to join other cancerous individuals, with which interactions are less stressful. This preference significantly decreases the progression of their own tumor, and potentially provides them with more opportunities to reproduce before dying (Dawson et al., 2018). Finally, in Tasmanian devils (*Sarcophilus harrisii*) harboring horizontally transmissible tumors called DFTD (devil facial tumor disease), individuals in populations severely impacted by the disease reach their sexual maturity earlier than elsewhere, which allows them to reproduce despite an early death caused by almost unavoidable DFTD infections (Jones et al., 2008; Lachish et al., 2009). In our study, we have not been able to demonstrate that tumors induce castration in the host within their two first months of life. However, tumorous hydras display a considerably reduced survival compared to healthy ones (see below). In addition to this intrinsic reduced life-expectancy, Boutry et al. (2022b) recently demonstrated that tumorous hydras,

because of their altered morphology when symptomatic, have a significantly increased predation rate by fish (*Betta splendens*) that use visual clues to hunt. Because symptomatic tumorous hydras experience a higher risk of predation, selection on the host should presumably favor any combinations in which the budding rate is maximized early, even if it directly compromises the host survival thereafter. Also, tumorous hydras, which have supernumerary tentacles (Rathje et al., 2020) capture more prey (Boutry et al., 2022b). Even if a part of the higher level of resources acquired is probably diverted to the tumoral cells, one can hypothesize that the higher budding of tumorous hydra is the direct consequence of their better food intake. In accordance with this hypothesis, it is known in hydra that the budding rate is linked to food availability (Tökölyi et al., 2016). We do not favor this hypothesis, at least to be the only explanation, because during the early life of tumorous hydras, the number of tentacles is equivalent to the one observed in healthy polyps. Thus, it could not explain *per se* the enhanced reproductive effort observed in the asymptomatic phase of tumorous hydras. However, because the parents of asymptomatic tumorous hydras harbor supernumerary tentacles and thus consume more prey, we cannot exclude possible trans-generational effects, resulting in the production of high-quality buds almost immediately able of enhanced reproductive performance. Trans-generational effects of parasitism and predation exposure over generations have been described in numerous vertebrates and invertebrates (Gallizzi et al., 2008; Hasselquist and Nilsson, 2009; Sternberg et al., 2015; Tariel et al., 2020). The existence of trans-generational effects of tumors in hydras remains an interesting perspective for further research.

Although the conditions mentioned above could select in tumorous hydras for precocious reproductive investments as a compensatory response, we cannot exclude that this could also result from a host manipulation by tumor cells to favor their transmission before the host dies (Hughes et al., 2012). A potential way to determine whether the enhanced budding rate in tumorous hydra is controlled by the host and/or by the tumor is to look at the tumor cell transmission rate in the buds produced during the asymptomatic and the symptomatic periods (e.g., Locke et al., 2012; Simon et al., 2011; Werren et al., 2008). The health status change through time of the polyps produced by single or multiple budding episodes is informative as well. For instance, if the polyps produced by asymptomatic tumorous hydras, or those coming from multiple budding episodes, remain thereafter tumor-free (e.g., because of a reduced number of tumor cells transmitted to the bud), it would be difficult to argue that the enhanced reproductive effort of hydra is under the control of tumor cells to favor their transmission. Conversely, it would support the idea that asymptomatic tumorous hydras, or those that produce multiple buds, maximize their fitness not only by optimizing their descendants' number before dying (Polak and Starmer, 1998), but also by improving their phenotypic quality because the latter would be tumor-free (Singh et al., 2015). Our data show that polyps produced by asymptomatic hydras, develop tumors at the same rate as the symptomatic polyps. In addition, the transmission rate is also stable during simple or multiple budding episodes. Thus, the potential alleviation of the fitness cost in tumorous hydras, by increasing budding rate, is here superimposed with the opportunity for tumor cells to be transmitted. Therefore, without additional experiments, it is difficult to determine which of the two partners is responsible for this alteration in the host reproductive schedule.

Sexual reproduction, in contrast to asexual one, is not associated with the transmission of tumor cells to the descendants because it involves gametes. Therefore, a maximization of the early sexual reproduction would support the hypothesis of a host adaptive response rather than a tumor manipulation aimed at increasing tumor cell transmission. Of interest, our data show that tumorous hydras display, all else being equal, an earlier and enhanced reproductive effort when they are sexually induced in the asymptomatic phase. This is characterized by the reduced time of gamete formation and the increased number of eggs produced when tumorous hydras started their sexual reproduction in the asymptomatic phase. Conversely, older symptomatic hydras produced fewer eggs than healthy individuals of the same age. Given the germline origin of the tumors, one might have expected that these cells could be reinvested in an increased egg production. However, because the increase in egg production was only observed in polyps that did not yet have produced visible tumors, this hypothesis could be ruled out. This pattern is however consistent with a final investment in reproduction before tumors become too impactful and partially castrate their host. This type of final investment is potentially comparable to the one classically observed in the context of host-parasite interactions. For instance, (Minchella and Loverde, 1981) found that molluscs infected by castrating worms of the trematode group lay massive numbers of eggs before being castrated (see also Agnew et al. (2000) for synthesis).

A limitation of our observations is the impossibility to access the viability of the eggs produced, because fertilization in the lab is difficult to achieve for this strain. In addition, we noticed that the global rate of sexual reproduction is much lower than what is commonly reported for this species (Tökölyi et al., 2021)

independently of tumor presence, which might suggest a possible loss of sexual qualities in this strain. To go further, it would be interesting to repeat this protocol on other strains of hydras harboring tumors and displaying stronger sexual abilities.

Survivorship

Tumorous hydras, in our experimental conditions, have a significantly decreased survivorship compared to control ones, a result that may indicate, as hypothesized before, that tumoral progression directly reduces the host survival, and/or that tumorous hydras invest in an early reproduction directly at the cost of their later survival. This mortality rate in tumorous hydras, as well as the one in healthy ones, albeit much more reduced, contrasts with the findings from other studies, and with the general belief that *Hydra vulgaris* do not senesce (Dańko et al., 2015; Martinez, 1998). A possible explanation is that our lab maintenance conditions are, for reasons that are not clear at the moment, not optimal for hydras, and that tumorous individuals are more fragile to adverse environmental conditions than healthy ones. The observed increasing mortality rate of buds (from both tumorous and healthy hydras) with the age of the parental hydra may support the hypothesis that our conditions are not optimal for hydra; i.e., older individuals would be weakened and produce buds of a lower quality, with a low survival rate. Alternatively, other explanations could exist if, again, we consider the supernumerary tentacles present in symptomatic tumorous polyps (Domazet-Lošo et al., 2014). Although this enhanced capacity is likely to be advantageous where prey is scarce, it could result in a detrimental overfeeding in the *ad libitum* conditions in which we performed our experiment. Rather than being directly detrimental, an overfeeding could also mimic a transient period that is favorable in the wild, yielding hydra to a fatal asexual reproductive exhaustion (see above).

Conclusion

This study shows that symptomatic tumorous hydras, at least in our experimental conditions, suffered from a reduced lifespan compared to healthy ones, but also an increased asexual budding rate in their early life. In addition, sexual reproduction efforts are increased and initiated earlier for asymptomatic tumorous hydras, whereas the egg production is reduced when tumors reach a visible stage. Because with asexual reproduction this enhanced effort also favors tumor cells' transmission, it is difficult to determine if this phenotypic alteration is governed by the host and/or the tumor cells. However, because the same tendency is observed with parameters linked to sexual reproduction, and because tumor cells are not transmitted this way, our findings support the hypothesis that, at least certain responses in the altered phenotype of tumorous hydras are adaptive only for the host, through a maximization of the reproduction before death and/or the end of the reproductive capacity. The extent to which the enhanced early reproductive effort in tumorous hydras is a cause or a consequence of their low survival remains to be precisely elucidated. Also, at present, we do not have enough information to establish whether this alteration is inherited or constitutive. One way to explore this question would be to monitor the reproductive schedule of the few tumorous hydra polyps that remain asymptomatic. If the higher early budding rate is a phenotypic plastic response, these polyps should exhibit the same reproductive patterns as healthy/control hydras in general. Conversely, if the trait is now a constitutive response, these asymptomatic polyps should display the same reproductive patterns as the tumorous polyps. This study is among the first ones that experimentally demonstrated host life-history traits alterations in relationships with tumorigenesis. Overall, these results currently support the hypothesis that within the hydra strain harboring tumors, selection has favored traits (genetic adaptation and/or phenotypic plastic response) that allow reproductive compensation despite restricted lifespan.

Limitations of the study

This study was conducted in an experimental setting, not directly on wild specimens. Therefore, these results may vary in the wild.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105034.

ACKNOWLEDGMENTS

We are grateful to Alexander Klimovich for providing us with hydras and his valuable advice that helped us during the follow-up of this study. We want to thank the two anonymous referees for their constructive comments that contributed to improving this article. This work was funded by the MAVA Foundation and by the following grants: ANR TRANSCAN (ANR-18-CE35-0009), ARC Linkage (LP170101105), and a CNRS International Associated Laboratory Grant. The APC was funded by the ANR TRANSCAN (ANR-18-CE35-0009). This work was supported by the international research project CANECEV.

AUTHOR CONTRIBUTION

The original idea for the article came from discussions between R.H., B.U., A.N., and F.T. J.B., S.T., and F.T. designed the study. J.B., S.T., and N.M. collected the data whereas J.M. took care of the hydras rearing. S.T., J.B., J.T., and A.M.D. performed the analyses. J.B. and S.T. were supported by F.T. and B.R. S.T. and J.B. wrote the manuscript, and all authors significantly contributed to revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: May 9, 2022
Revised: July 13, 2022
Accepted: August 24, 2022
Published: October 21, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Data analysed	This paper	Not applicable
Experimental models: Organisms/strains		
Hydra oligactis, St Petersburg lineage	Domazet-Lošo et al. (2014)	Not applicable
Software and algorithms		
RStudio	(RStudio Team, 2020). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.	Not applicable

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Justine BOUTRY (Justine.boutry@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data used are available in supplementary information (see Tables S5–S9).
- The codes are available in the appendix in the explained Markdown version (see Datas S1, and S2).
- Any additional information required to reanalyze the data reported in this article is available from the lead contact on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Control and tumorous *H. oligactis* used in this study originated from clonal strains (St. Petersburg lineages from Domazet-Lošo et al., 2014). Hydras were maintained at 18°C in Volvic© water, with a photoperiod of 12 h, and fed three times per week with *Artemia salina* hatched following standard protocols. We obtained Artemia nauplii by adding 0.5 g of egg microcysts (*Artemia salina*, Planktovie S.A.S., Marseille, France) in 400 mL of seawater (36 g/L of sea salt, Reef Crystals, Aquarium systems, Sarrebourg, France) oxygenated with an air pump and illuminated continuously inside a 500 mL glass bottle. After 24 h of incubation at 30°C or 72 h at 18°C (over the weekend), nauplii were collected with a pipette, rinsed on a filter, and suspended in a 200 mL beaker of Volvic© water. Each feeding was followed by two water changes, the first 30 min after the feeding and the second 8 h later. For this experiment, control and tumorous hydras were first taken randomly from the collective cultures and maintained separately. Buds from these hydras were isolated to be monitored during this experiment. They were placed individually in wells of standard cell culture plates (12-well plates, 1.5 mL/well, Thermos Scientific), containing Volvic© and maintained as previously described above.

METHOD DETAILS

Temporal monitoring of asexual and sexual reproduction

For asexual reproduction, control (n = 36) and tumorous hydras (n = 36) were collected at the time of their detachment from the parental polyp and monitored individually for 9 weeks. Previous observations (e.g., (Domazet-Lošo et al., 2014; Rathje et al., 2020) have shown that when a bud inherits tumor cells from its parent, weeks 0 to 4 of the detached polyp correspond to a pre-pathological phase (i.e., the hydra looks tumor-free) and it is only from weeks 5 to 9 that a tumoral growth can be visually detected. Three times per week, the number of buds produced was recorded for each polyp. We distinguished simple from

multiple budding episodes by the number of buds produced by the hydra and detached at the same sampling day (simple: one bud released, multiple: more than one bud released at the same time). The time between two budding episodes was calculated as the number of days between each episode. After the counting, the buds produced were removed from the parental well. The survival of the monitored polyps was also noted before each feeding, for 5 months.

Sexual reproduction in hydras can be activated by changing environmental parameters, namely temperature and photoperiod. Control and tumorous female hydras were thus placed at 10°C with a photoperiod of 8 h/16h day/night cycle to observe the development of their gonads. The hydras were divided into two subgroups depending on their age when we triggered their sexual development, during their second week of life (control, n = 72; tumorous, n = 56) or in their 5th week (control, n = 69; tumorous, n = 79). Three weeks after the starting of sexual differentiation, the number of detached eggs per female was counted three times a week, and the survival was monitored once a week until the death of all individuals.

Tumoral cell transmission to the offspring

Forty-eight control and forty-eight tumorous hydras were isolated with the goal of sampling the buds they would produce at different ages and assessing the transmission rate of the tumoral phenotype. When the isolated hydras reached their second week (i.e., within the pre-pathological phase for tumorous ones), we sampled the buds produced by half of the isolated polyps. We also noted the type of budding episode from which they were originated (simple or multiple). The same measurement was re-iterated during the 5th week (coinciding with the pathological phase for the tumorous hydra) for the rest of the hydras.

After five weeks, we assessed the presence or absence of visible tumors on the buds detached, to estimate the rate of transmission of the tumorous cells depending on the age and the pathological status of the parental hydra, but also depending on the type of budding event (simple or multiple) from which the bud originated.

QUANTIFICATION AND STATISTICAL ANALYSIS

Life history traits related to asexual reproduction

All analyses were performed using RStudio software (version 3.6.1 (2019-07-05)). Comparisons of the distributions of the age at the first reproduction between tumorous and control groups were performed by a two-sample Kolmogorov-Smirnov test. A Wilcoxon test was also performed to compare the medians of these two groups.

To compare the traits related to asexual reproduction – (1) Budding rate, (2) the gap between two reproductive episodes, and (3) the number of buds produced per reproductive episode according to hydras status (control or tumorous) and the pathological status, generalized linear mixed-effects models were used (taking into account the non-normality of the variables). Random effects of the individual and bud detachment's date were added to correct the part of variability explained by the measurement of the same individual over time. The fixed effects were the status of the hydras (control or tumorous) and their age. Model selection was based on the weight of the Akaike information criterion (AICc) and following the procedure described by (Zuur et al., 2009). The formulas of all different models constructed as well as the value of AICc's weight were obtained with the "MuMIn" package (Bartoń, 2022) are summarized in Table S1 and see Data S1 and Tables S5 and S6 in for the details. All model residuals' distribution was checked with the "DHARMa" package (Hartig, 2021) which applies an over-dispersion, outlier, and Kolmogorov-Smirnov test.

A Poisson distribution with a logarithmic link function was used in the models explaining the budding rate and the gap between two reproductive episodes because they correspond to count data. The type of reproductive episode (single or multiple) was analyzed by a binomial distribution with a logit link function. The analysis of survival over time, depending on the presence of tumors, was carried out by a survival regression to take into account the censored data. The Weibull distribution was used to model the evolution of the instantaneous risk over time with version 3.2–11 of the survival package of (Therneau, 2022).

Tumor transmission rate and bud survival

To explore the transmission of tumor cells to the offspring during the asexual reproduction and bud survival, generalized linear mixed-effects models with a binomial distribution and a logit link function were

used to explain the bud's status (healthy or tumorous) according to the fixed effects of budding type and the production phase. Similar model types were used to explain the survival according to the fixed effects of the budding type, the pathological status, and the production phase. Random effects of the date of the statement and the batch's number were added to correct the part of variability existing between these times and batches. The formulas of all different models constructed as well as the value of AICc's weight are summarized in Table S2 (for tumor transmission rate) and S3 for the details (for survival), see Data S1 and Tables S8 and S7 for the details.

Temporal monitoring of sexual reproduction

To compare the traits related to sexual reproduction, namely (1) ability to produce eggs and (2) age at the production of the first egg, according to hydra status and the pathological status, generalized linear mixed-effects models were used. For the number of detached eggs, also according to hydras status and the pathological status, linear mixed-effects models were used because the residuals followed a normal distribution. The random effect of the date was added in these models to correct the part of variability explained by the difference of time. The formulas of the different models constructed and the value of the weight of AICc were obtained are summarized in Table S4 and see Data S2 and Table S9 for the details.

Graphic representation

All figures and tables of results were formatted with, respectively, version 3.3.3 of the package "ggplot2" (Wickham, 2021) and version 2.8.7 of the package "sjPlot" (Lüdecke, 2021).