

First evidence for the evolution of host manipulation by tumors during the long-term vertical transmission of tumor cells in *Hydra oligactis*

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eLife Assessment

This interesting study explores whether tumor cells can manipulate their Hydra hosts, and includes **important** findings on the consequences for the fitness of the host Hydra. The evidence supporting these findings is **convincing**. The work will be of broad interest to many fields including development biology, evolutionary biology and tumor biology.

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Abstract

While host phenotypic manipulation by parasites is a widespread phenomenon, whether tumors – which can be likened to parasite entities, can also manipulate their hosts is not known. Theory predicts that this should nevertheless be the case, especially when tumors (neoplasms) are transmissible. We explored this hypothesis in a cnidarian *Hydra* model system, in which spontaneous tumors can occur in the lab, and lineages in which such neoplastic cells are vertically transmitted (through host budding) have been maintained for over 15 years. Remarkably, the hydras with long-term transmissible tumors show an unexpected increase in the number of their tentacles, allowing for the possibility that these neoplastic cells can manipulate the host. By experimentally transplanting healthy as well as neoplastic tissues derived from both recent and long-term transmissible tumors, we found that only the long-term transmissible tumors were able to trigger the growth of additional tentacles. Also, supernumerary tentacles, by permitting higher foraging efficiency for the host, were associated with an increased budding rate, thereby favoring the vertical

transmission of tumors. To our knowledge, this is the first evidence that, like true parasites, transmissible tumors can evolve strategies to manipulate the phenotype of their host.

Introduction

It is now widely established that animals (metazoans) are not autonomous entities, but rather holobionts composed of the host cell community, various commensal and mutualistic microorganisms, a diversity of parasitic taxa, and in many cases, tumor cells (Ujvari et al. 2016a [↗](#), Dheilly et al. 2019 [↗](#)). While it has been shown that parasites and mutualistic organisms have frequently evolved the ability to manipulate the host phenotype [e.g., reproduction, behavior, morphology, physiology, body odor (2012, Johnson and Foster 2018 [↗](#), Naundrup et al. 2022 [↗](#))] to favor their transmission and/or survival, sometimes inducing dramatic alterations (Doherty 2020 [↗](#)), this phenomenon has been poorly investigated in the context of host-tumor interactions. The only unambiguous evidences of such manipulation is found in the tumoral microenvironment, when, for instance, malignant cells manipulate the surrounding healthy cells to induce the growth of blood vessels that bring oxygen and nutrients to the tumor, a process called neoangiogenesis (Hicklin and Ellis 2005 [↗](#)). Evidence regarding the ability of tumor cells to manipulate their host phenotype at other levels is scarce [see (Tissot et al. 2016 [↗](#)) for a review] and this probably reflects a real rarity of this phenomenon. Indeed, host manipulation capabilities often rely on sophisticated and complex adaptations that evolve during long coevolutionary processes, whereas tumors (neoplasms) most often evolve for only a few years and then die with their host without being able to transmit any acquired traits (Arnal et al. 2015 [↗](#)). However, like “true parasites”, transmissible tumor cells [vertically – such as in *Hydra oligactis* (Domazet-Lošo et al. 2014 [↗](#), Boutry et al. 2022b [↗](#)); or horizontally – such as in Tasmanian devils (Jones et al. 2008 [↗](#))] could more easily evolve manipulative abilities (Tissot et al. 2016 [↗](#), Ujvari et al. 2016b [↗](#)). Nevertheless, to date, we lack evidence that transmissible cancers can manipulate the phenotype of their hosts.

Hydra oligactis are cnidarians that frequently develop spontaneous tumors in the lab, noticeable as individuals with significant increases in body size and visible deformities. These tumors correspond to abnormal proliferation of germline cells in the hydra’s ectoderm (Boutry et al. 2023 [↗](#)). In 2014, Domazet-Lošo and colleagues (Domazet-Lošo et al. 2014 [↗](#)) described a fascinating case of two hydra lineages harboring neoplasms capable of vertical transmission through buds, during the asexual reproduction of hydras. Thanks to this transmission, these two tumorous hydra lines have been maintained under laboratory conditions for more than 15 years now (Lange 2010 [↗](#)). Beyond the presence of large tumor masses, a curious feature of these hydras is their increased number of tentacles. Specifically, while healthy polyps typically have 6-7 tentacles, tumorous polyps have between 8 and 13 tentacles, even up to 20 (Domazet-Lošo et al. 2014 [↗](#)). This phenotype is particularly impressive given that tentacle numbers in hydras usually vary in a very small range [from 5 to 8 tentacles, depending on temperature and body size (Shostak 2012 [↗](#))]. Recently, Boutry and colleagues (Boutry et al. 2022a [↗](#)) demonstrated that tumorous hydras featuring these supernumerary tentacles capture more prey than healthy ones, regardless of whether prey is rare or abundant in the environment. In contrast, wild specimens of *H. oligactis* developing spontaneous tumors when placed in laboratory conditions do not seem to develop additional tentacles (Domazet-Lošo et al. 2014 [↗](#)). Since food intake, survival, as well as budding rate are usually positively correlated in hydras (Tököllyi et al. 2016 [↗](#)), the presence of additional tentacles in certain tumorous hydras is likely to have an adaptive value, favoring host survival despite the cost of bearing tumors, and this can ultimately enhance the hydra’s reproductive potential and/or the tumor cells’ transmission [see also (Boutry et al. 2022b [↗](#))].

However, whether the host or the tumor is responsible for the alteration of the tentacles number is not clear yet. Furthermore, in one *H. oligactis* lineage, called the St. Petersburg strain, tumor initiation and maintenance require the presence of a specific microbiome (Rathje et al. 2020 [↗](#)),

suggesting that bacteria may also be involved in the phenotypic alteration of the host to promote their own transmission. Here, through a series of experiments using a set of healthy and tumor tissue grafts from tumors associated with different phenotypes, we attempted to determine whether the host or the tumor is responsible for the growth of additional tentacles in the tumorous hydra.

Material and methods

Biological material

All hydras were maintained according to standard rearing protocols (Boutry et al. 2022a [↗](#)). Three different main lineages of *Hydra oligactis* were used in our study. The Saint Petersburg (named thereafter SpB) and the Robusta (named thereafter Rob) strains were obtained from Thomas Bosch's laboratory in Kiel (Domazet-Lošo et al. 2014 [↗](#)). Rob are also known under the denomination *Pelmatohydra robusta*, as they were described as a distinct specie from Japan (Ito 1947 [↗](#)). Current phylogenetic analyses suggest that they belong to *H. oligactis* species. (Martínez et al. 2010 [↗](#)), and it is currently unknown if populations related to this strain still exist in nature. For each of these two lines, control lines were also isolated from healthy polyps collected during the same sampling; these control groups do not harbor any sign of transmitted tumors and are supposed to be genetically identical to their respective tumorous lines (Boutry et al. 2022b [↗](#)). A third tumorous lineage has been recently established in Frédéric Thomas's laboratory in France, and was isolated from a polyp sampled in Montaud water pond (43.748211°N, 3.989871°E); this lineage, named Montaud (thereafter Mt), was maintained in the lab since August 2021 (Boutry et al. 2023 [↗](#)). To date, we have been unable to establish a distinct control and vertically transmitted tumorous lineage for this new strain, due to variable transmission rates of these tumors and the sporadic occurrence of spontaneous tumors from initially healthy-looking polyps. In addition, a group named “wild group” was used as recipient of the transplanted tissues; this group was composed of randomly sampled *H. oligactis* and assumed to be genetically diverse and distinct from the SpB and Rob lineage. These wild hydras were also sampled in the Montaud pond, between 15/02/2022 and 15/04/2022.

In this study, two types of tumors were distinguished. “Transmitted tumors” correspond to those from the tumorous hydra lineages (SpB and Rob) obtained from Thomas Bosch's laboratory in Kiel; hydras bearing these tumors possess an enhanced number of tentacles and these tumors have been vertically transmitted through budding for 15 years (Lange 2010 [↗](#)). “Spontaneous tumors” correspond to tumors that sporadically appear in Mt lineage, but also among healthy polyps of the SpB control lineage (which were isolated from the common culture as soon as they appeared). Noticeably, tumors were never observed to appear spontaneously in the Rob control lineage.

Grafting protocol

The transplantation experiment was performed according to (Lenhoff 2013 [↗](#), Dujon et al. 2022 [↗](#)) using healthy and tumorous donors of the SpB, Rob, and Mt lineages. For the recipients, we used healthy individuals of the SpB lineage and the wild group (see **Figure 1** [↗](#) for an illustration of the experimental design). Since transplants on healthy Rob recipients led to massive mortality, we excluded them from this analysis. The recipients from the “wild group” (because they may be genetically diverse) were cut in half longitudinally one week before transplantation and allowed to fully regenerate, in order to obtain two genetically identical individuals on which to perform the control and tumor transplants. The manipulations were performed under a binocular magnifying glass (Olympus® model SZ51, magnification from 0.67X to 4.5X) and the tools were disinfected with 90% alcohol and rinsed with Volvic® before and after each transplant. Donor and recipient hydras were starved for 24 hours prior to transplantation to prevent digested food remaining in the gastric cavity from being released during grafting.

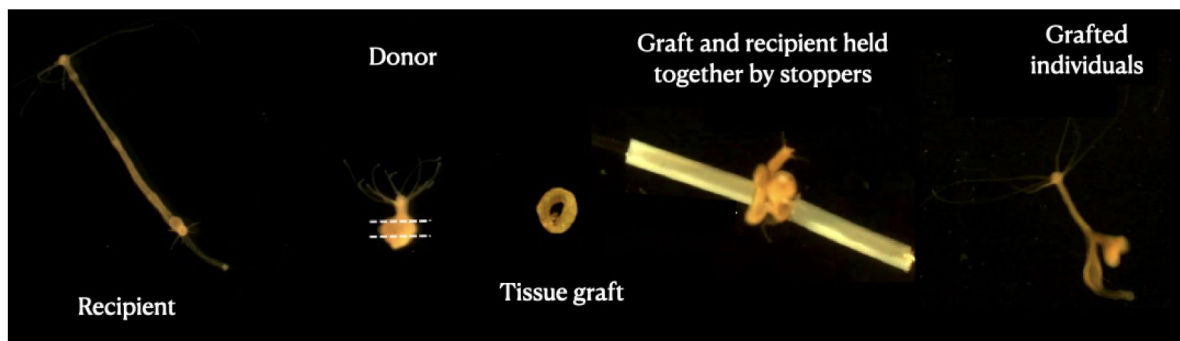


Figure 1.

Grafting protocol.

Images represent each step of the transplantation process, starting with the recipient polyp (left); the donor is then cut into a ring-shaped tissue graft that is inserted on a needle, put in contact with the recipient tissue and blocked by stopping tubes. At the end (right) a transplanted individual can be seen 3 days after the transplant.

Transplantations were realized in a glass Petri dish containing 150 mL of Volvic®. Using a flat scalpel, the head of the donor hydra was cut and removed from the beaker. Two cuts were made in the body of the hydra, resulting in a ring-shaped graft (**Figure 1**). Using fine forceps, a piece of fishing line (nylon, diameter 0.28 mm) was inserted into the center of the graft. A small incision was made halfway up the recipient hydra, and the fishing line that has been previously inserted into the graft was then passed through this incision and exited through the mouth of the recipient hydra to limit tissue damage. The graft was held to the recipient by sections of polyethylene tubing (**Figure 1**, internal diameter 0.30 mm, Sensa®). The fishing line and the tubes were removed three hours after the graft, and the experimenter could then check the acceptance of the graft. The hydras whose graft was not accepted were eliminated and the grafting was repeated [see (Dujon et al. 2022) for a video of the grafting steps]. Three days later, the protrusion created by the graft was excised using a scalpel blade, allowing the individual to return to a normal morphology. The period of three days was assumed to be sufficient time to allow tumor cells to migrate into the tissue before graft removal (Domazet-Lošo et al. 2014). Freshly transplanted hydras were deprived of food for a week to avoid water pollution from feeding during regeneration, then returned to a normal diet on day 8 post-transplant.

Temporal follow-up and statistical analysis

The number of tentacles in donor and recipient individuals was assessed before the transplantations. The individuals with transplants were then followed weekly for 70 days to record the number of tentacles, the date of tumor onset, the asexual reproduction through bud release and the date of eventual death. Detached buds from the grafted individuals were removed after each feeding and the size of the tumors was qualitatively assessed at the death of the individual (following the method of Tissot et al. 2023).

Generalized linear mixed-effects models were used to quantify the impact of donor and recipient characteristics on the different traits observed in grafted individuals. For each analysis, the most parsimonious model was selected following the method used by Zuur (Zuur et al. 2009). The use of Akaike's conditional criterion (AICc), therefore, allows us to exclude some irrelevant effects and to present here only the results of the most relevant models (see supplementary). All the models, their effects (fixed and random), and their respective AICc are presented in the appendix. The absence of overdispersion and the balance of the residuals were also checked using the DHARMA package (Team et al. 2021). Analyses were performed using R software (version 4.1.3) (R Core Team 2022) and various packages (see the exhaustive list in Appendix). Because of the unbalanced distribution of tumor type modalities within the different lines (transmissible tumors in SpB and Rob, spontaneous tumors in Mt and SpB), we had to separate all analyses into two parts, one including all lines with transmissible tumors as well as their controls, and one including all lines with spontaneous tumors and their controls. Random effects of transplantation date and/or batch were evaluated and included in the models for which they were relevant.

The median number of donor tentacles was compared using Wilcoxon tests between the tumorous and the control groups. Mortality rates during the experiment were compared using a random generalized models with a binomial distribution (see the analysis and results in supplementary section 2). The rate of tumor development was modeled using a random generalized model with a binomial distribution (see the analysis and results in supplementary section 3). The different models constructed evaluated the fixed effects of donor lineage (SpB, Rob, or Mt), donor status (tumor or healthy) and recipient lineage (SpB or Wild group).

The change in the number of tentacles was then analyzed using a delta variable: the difference between the maximum number of tentacles developed during the study and the number of tentacles of the recipient on the day of transplantation (see the analysis and results in supplementary section 4). This variable was then modeled according to a random generalized models with a Poisson distribution (we turned all values into positive values by simply adding 2 to all values). In the models, several fixed effects were tested: tumor status and lineage of the donor,

lineage of the recipient, initial number of tentacles of the recipient and donor. The impact of tumor development after transplantation on the number of tentacles was excluded because of a confounding effect between donor status and tumor development (see in supplementary). The effect of tumor size on the number of supernumerary tentacles was evaluated using an intra-group linear model (donor lineage, status, and recipient). This was done to assess how small, medium, and large tumors influence the variation in tentacle numbers independently of donor and recipient lineage, for both transmissible and spontaneous tumors (see the analysis and results in Supplementary section 4).

The relationship between the number of buds produced and the number of tentacles developed was subsequently examined on the complete dataset (transmissible and spontaneous tumors together) utilizing the same delta variable (refer to the analysis and results in Supplementary Section 5). The number of buds produced during the 10-week observation period was then subjected to modeling through random linear models. In these models, various fixed effects were assessed, including the tumor status of the grafted individuals, the delta variable representing the number of additional tentacles, and the group consisting of the lineage and cancerous status of both the donor and recipient. The latter was treated as random effects to emphasize our focus on intra-group variations in drawing conclusions. Figures and tables were made with the packages GGplot2 (Wickham 2016 [\[4\]](#)), Sjplot (Lüdecke 2023 [\[5\]](#)), ggstatsplot (Patil 2021 [\[6\]](#)) and the software Keynotes©.

Results

1. Tentacle numbers in hydras bearing transmitted versus spontaneous tumors

As expected (see Introduction), hydras from both strains harboring transmissible tumors had an enhanced number of tentacles, with typically more than 4 additional tentacles in both the Rob (**Figure 2A** [\[4\]](#), control, $\bar{x} = 6[6-6]$ (median, first and third quartiles); transmissible tumors $\bar{x} = 10[9-12]$, $p < 0.001$) and the SpB (**Figure 2A** [\[4\]](#), control $\bar{x} = 6[5.5-6]$; transmissible tumors $\bar{x} = 10[8-10]$, $p < 0.001$) strains. In contrast, SpB hydras bearing spontaneous tumors did not show an increased number of tentacles compared to healthy controls (**Figure 2A** [\[4\]](#), control $\bar{x} = 6[5.5-6]$; spontaneous tumors $\bar{x} = 6[6-6.5]$, $p = 0.371$). However, Mt hydras bearing spontaneous tumors showed an increase in the number of tentacles compared with controls, but of lower magnitude than for Rob and SpB strains with transmissible tumors, with typically one additional tentacle (**Figure 2A** [\[4\]](#), control $\bar{x} = 6[5-6]$; spontaneous tumors $\bar{x} = 7[6-7.5]$, $p < 0.001$).

2. Tumor development following tissue transplantation

On average, healthy hydras grafted with tumorous tissue, either from the transmissible or the spontaneous tumors, have an increased risk to subsequently develop tumors compared to those grafted with healthy tissue (**Figure 3** [\[4\]](#) pie charts). Hydras grafted with transmissible tumors developed on average 8 times more tumors (IRR (incidence rate ratio) = 8.00 [2.73 – 23.49], $p < 0.001$) than those transplanted with healthy tissue, whereas for spontaneous tumor transplants the risk only increased 2.61 times (IRR = 2.61 [1.09 – 6.23], $p = 0.031$). Nevertheless, for the transplantation of transmissible tumors from Rob donors on SpB healthy recipients, the effect appears weaker (see **Figure 3** [\[4\]](#).A pie charts), although it is not detected as significantly different from the other transplant combinations (see in supplementary section 3). However, an intriguing result is that hydra grafted with healthy tissues also developed tumors but at lower, yet non negligible, rate compared to hydra grafted with tumorous tissues (**Figure 3** [\[4\]](#) pie charts; 48.75%, 48.12% and 28.92%, on average, for healthy tissues from Mt, Spb, and Rob, respectively).

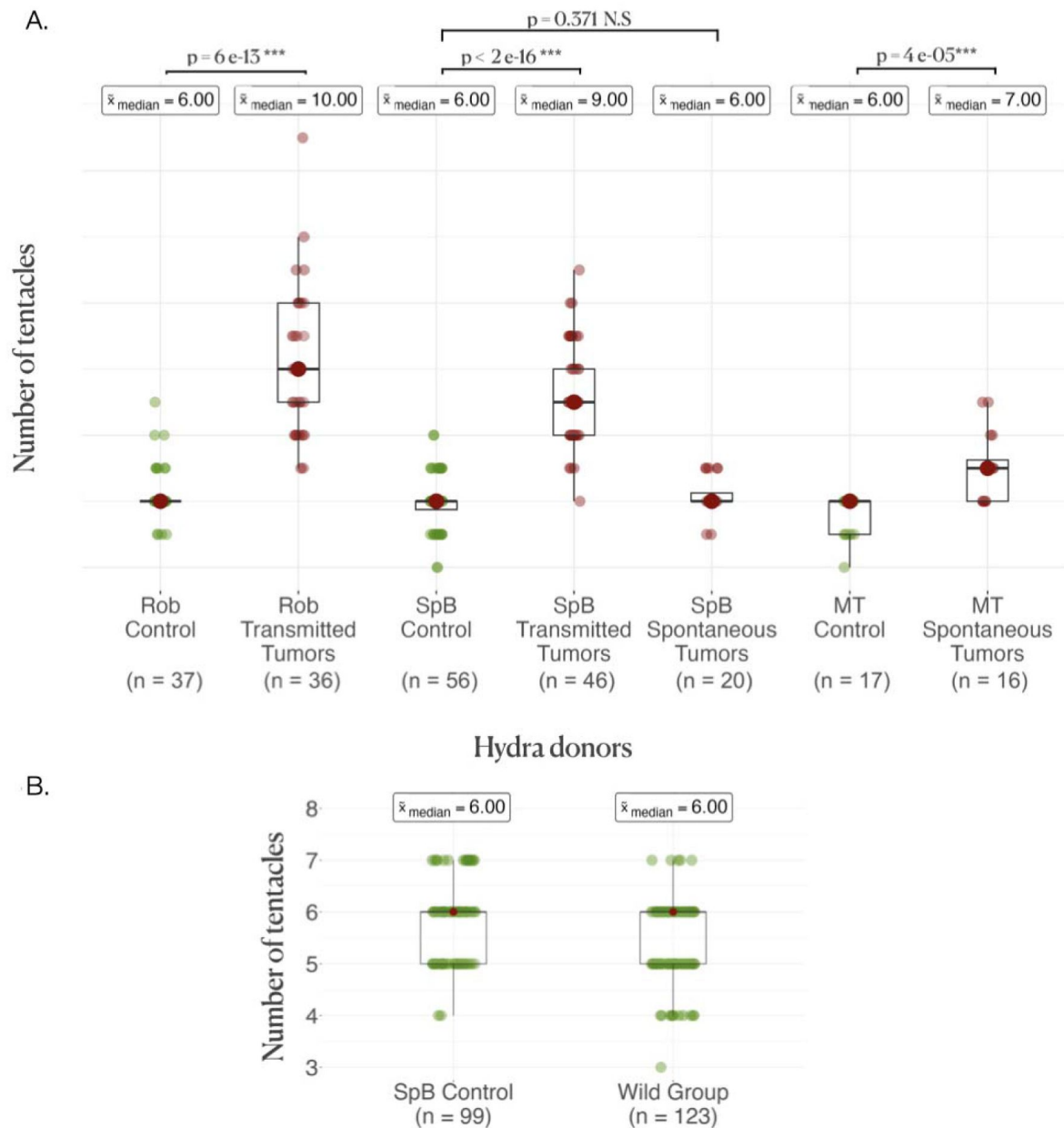


Figure 2.

Donor and recipient tentacle phenotype.

(A) Boxplots represent the number of tentacles in each donor line; the average number of tentacles in each group is written above in the boxes, and the rows represent the comparison between the control and the tumor medians in each line, with the p-values of the corresponding Wilcoxon-Mann-Whitney tests. (B) Boxplots represent the number of tentacles in different groups of recipients.

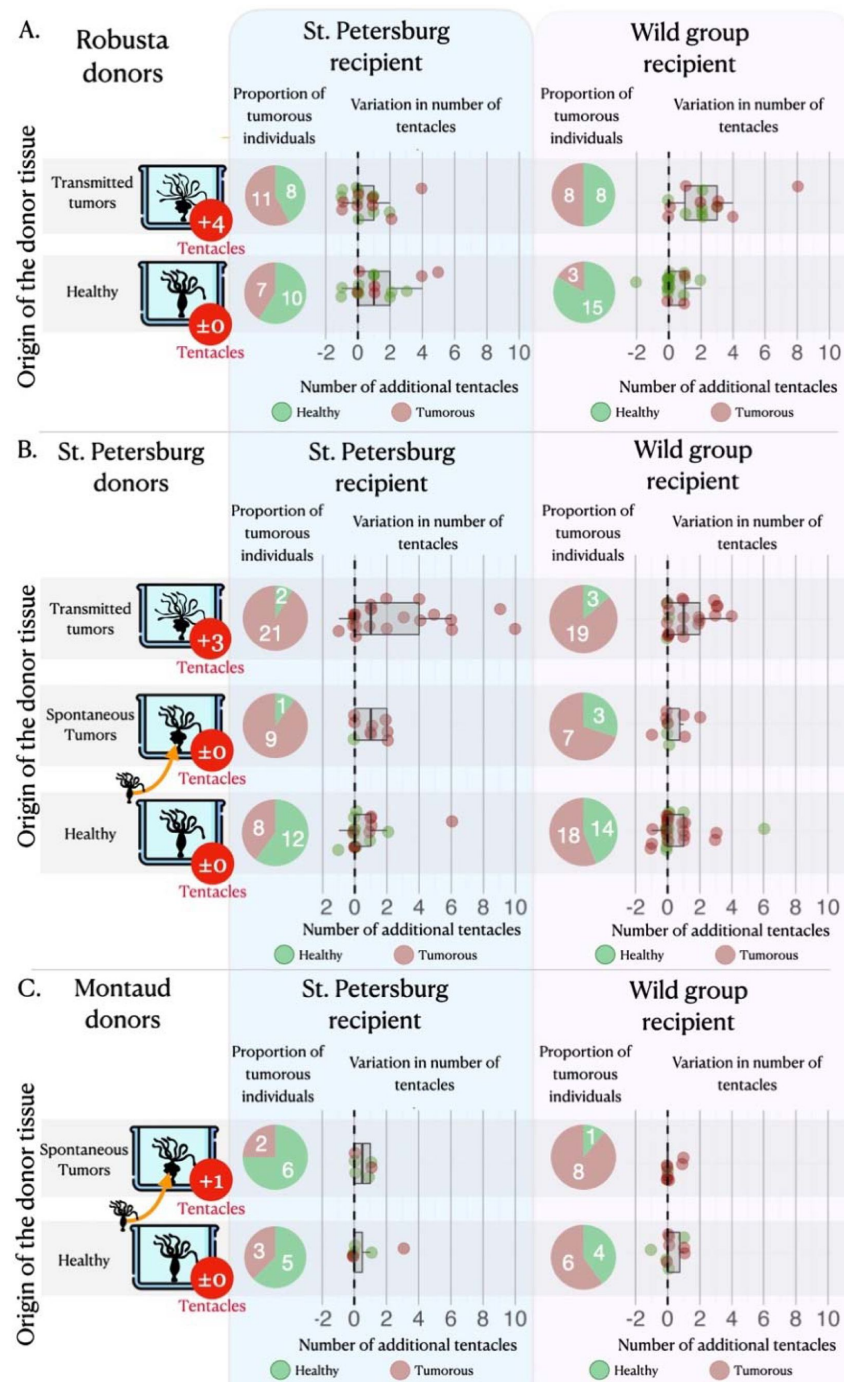


Figure 3.

Tumor appearance and changes in tentacle number after the grafts.

Three sets of matrices showing (i) the proportion of individuals (pie charts) that have developed tumors (in red) or lacked tumors (in green) and (ii) the variation in tentacle numbers (graphs) among non-tumorous individuals (green dots) and tumorous individuals (red dots), following the grafting of healthy tissue (from healthy tissue (green bars) or tumorous tissue (from spontaneous – orange bars, and transmitted – red bars, tumors) from Robusta, St. Petersburg or Montaud donor lineages (left panel) onto healthy recipients from the St. Petersburg or wild groups (top panels). For the donor lineages, the arrows indicate that the individuals with spontaneous tumors were isolated from the same cultures as the healthy individuals, whereas the individuals with transmitted tumors represent a distinct lineage; the median number of supernumerary tentacles in the tumorous donor individuals are also shown (in red circles).

3. Development of new tentacles following tissue transplantation

The number of tentacles (**Figure 3A** [↗](#), dot plots) showed a significant increase in individuals transplanted with tissue from transmitted tumors (even if the effect remains low when the donor is Rob and the recipient is SpB), whereas there was no significant effect in individuals transplanted with tissues from spontaneous tumors (**Figure 3B** [↗](#) & **C**, dot plots), relative to individuals transplanted with healthy tissue. On average, an individual transplanted with transmitted tumor tissues developed 1.45 [1.21 – 1.73] additional tentacles while individuals transplanted with healthy tissue developed only one additional tentacle (IRR= 1.45 [1.21 – 1.73], $p < 0.001$), with no significant effect of the donor lineage. Furthermore, we also detected a negative effect of the initial number of tentacles of the recipient (**Figure 3** [↗](#), dot plots): when the recipient already had a relatively high number of tentacles, the increase in the number of tentacles after transplantation was reduced (IRR=0.82 [0.74 – 0.92], $p = 0.001$), highlighting a possible threshold effect. In contrast, we do not detect any significant effect of the number of tentacles of the recipient on the number of tentacles developed after the graft of tissues from spontaneous tumors (see model selection in supplementary section 4).

Considering the effect of tumor size on the number of supplementary tentacles independently of their donor and recipient lineage, large tumors developed after the graft of transmissible tumors demonstrated a statistically significant increase in the number of supernumerary tentacles compared to small tumors (Estimate = 1.40, 95% CI [0.14, 2.65], $p = 0.03$; see **figure 5** [↗](#)). For spontaneous tumorous donors, the results indicated no significant effect of the size of the tumors developed after grafting on the number of tentacles (see **Figure 5** [↗](#), model selection and complete results in supplementary section 4).

4. The reproductive costs and benefits of tumors and supernumerary tentacles

Considering only individuals with a stable number of tentacles (−2, +2), we observed that tumorous individuals produced significantly fewer buds during the ten weeks of the study (Wilcoxon test, $W = 959$, p -value = 0.0094, $n = 171$). Regardless of the type of donor and recipient, the number of buds produced per individual was found to be significantly correlated with the number of supernumerary tentacles developed by the individual during the same period (**Figure 4** [↗](#)). However, the slope of this correlation was notably reduced for hydras with tumors (**Figure 4** [↗](#), $n = 92$, estimate = −2.75 [−5.21 – 0.29], $p = 0.029$). Consequently, all other factors being equal, an individual developing a tumor will require more supplementary tentacles than a healthy individual to achieve the same number of buds produced. Noticeably, we observed that the impact of the number of tentacles and tumor development on the number of buds produced became statistically detectable only after the 7th week of the experiment.

Discussion

Many parasites from different taxa (e.g., viruses, bacteria, protozoa, helminths, fungi, arthropods) have evolved the ability to manipulate the phenotype of their host to favor their own transmission and/or survival (2012). A wide variety of host traits can be modified by parasites, including reproduction, behavior, color, morphology and/or physiology (Barnett 1983 [↗](#), Dawkins 1999 [↗](#), Agnew et al. 2000 [↗](#)). Although theory predicts that transmissible cancers should evolve a true parasitic lifestyle over time (Dujon et al. 2020 [↗](#), Burioli et al. 2021 [↗](#)), to date there has been no evidence of host phenotypic manipulation by transmissible tumor cell lines (Tissot et al. 2016 [↗](#)).

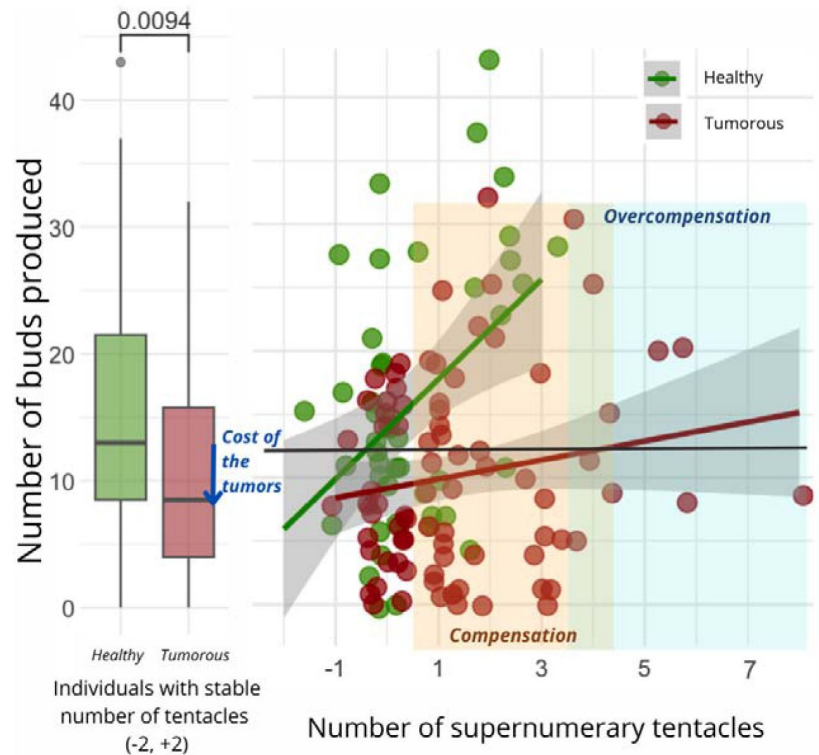


Figure 4.

Number of buds produced depending on the development of supernumerary tentacles.

On the left, the box plot exhibits the bud production of healthy ($n=47$, green) and tumorous individuals ($n=28$, red) with a constant number of tentacles $(-2, +2)$ during the 10-week monitoring post-grafting (Wilcoxon test, $W=959$, $p=0.009$, $n_{\text{healthy}}=79$, $n_{\text{tumorous}}=92$). The arrow signifies the reduction in bud production attributed to tumor costs. On the right, each point represents bud production for an individual (healthy in green, $n=83$, and tumorous in red, $n=116$) based on the number of tentacles developed in the 10 weeks following the transplantation. The orange zone represents tentacle-induced compensation for tumor costs compared to healthy individuals with a stable number of tentacles, while the blue zone indicates overcompensation beyond expected values for a healthy individual. The interaction between tentacle number and tumor occurrence significantly explains bud production variation (Random linear model, estimate = -2.75 , CI: $-5.21 - -0.29$, $p=0.029$).

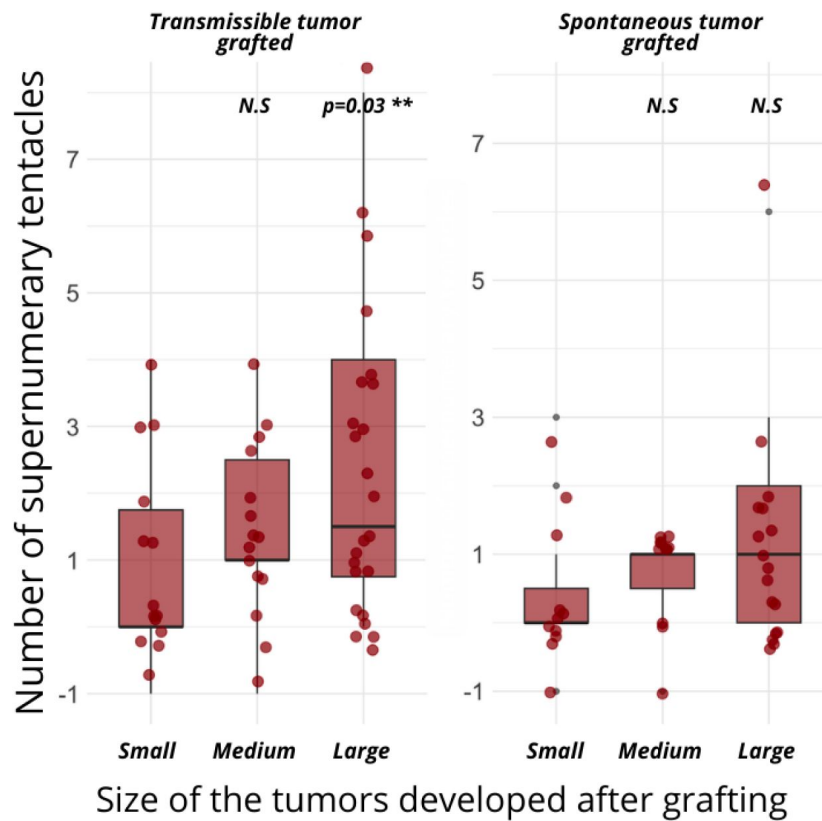


Figure 5.

Effect of tumor size on the number of supernumerary tentacles developed after grafting of transmissible tumors (left panel, n=53) versus spontaneous tumors (right panel, n=39).

For transmissible tumors, there was a significant increase in supernumerary tentacles with large tumors (linear model, estimate=1.40 [0.14-2.65], $p=0.03$) compared to small ones, while medium tumors show no significant difference (N.S.). In contrast, spontaneous tumors do not show a significant effect on the number of supernumerary tentacles across all tumor sizes (N.S.).

In their pioneering work on transmissible tumors in hydra, Domazet-Lošo and colleagues (Domazet-Lošo et al. 2014 [DOI](#)) mentioned the existence of an increased number of tentacles in the tumor-bearing lineages that have been propagated in the laboratory, but the precise cause of this phenotypic change was not understood. Here, we first highlight that this is not a general attribute of tumor-developing hydras, since both Mt and SpB polyps developing spontaneous neoplasms do not show a similar large increase in the number of tentacles (**Figure 2A** [DOI](#)). These results support the hypothesis that spontaneous tumors, which have no evolutionary history with their host, do not possess (or just to a small extent) the ability to influence its phenotype. Conversely, our results showed that transmissible tumors propagated in the laboratory for more than 10 years can induce the growth of additional tentacles even when they are transplanted in new hosts (even if this effect seems low with SpB grafted with tumors from Rob), as they seem to do in their original host lineage. In addition, while transplantation of spontaneous tumors never promoted an increase in tentacle numbers, transmissible tumors did, regardless of the origin of the recipient hydra (although one may suspect a different pattern between recipient strains that may not be detected due to a lack of power). These results are not consistent with the hypothesis that the development of additional tentacles could indicate a compensatory response of tumor-bearing polyps, for example, to better cope with resources redirected to growing tumors. Similarly, in agreement with previous work showing that the tumor phenotype can be observed in tumor-bearing hydras that do not consistently have an altered microbiome [this is the case for SpB, but not Rob (Domazet-Lošo et al. 2014 [DOI](#))], our transplantation experiments do not support the hypothesis that the alteration in tentacles' number could be caused entirely by bacteria. Indeed, both the SpB and Rob tumors were able to trigger supernumerary tentacles in their new hosts, even if Rob microbiome is the same between tumorous and non-tumorous hydras (Domazet-Lošo et al. 2014 [DOI](#)). Considering a potential size effect of the donor, individuals with spontaneous tumors grafted also display an enlarged body similarly to transmissible ones, as the grafted tissues were of similar size compared to transmissible tumor grafts. Therefore, this is not likely to explain the effect measured here, which is also consistent with the literature (Ngo et al. 2021 [DOI](#)).

Thus, although the exact mechanisms promoting the growth of additional tentacles remain to be identified, this is the first evidence of transmissible tumor cells that have evolved the ability to manipulate an external phenotypic trait in their host. Interestingly, we have shown that individuals with large tumors developed after a graft with transmissible tumors exhibited more supernumerary tentacles, whereas individuals with large tumors developed after spontaneous tumor grafts did not show the same pattern. This suggests a proportional effect depending on the number of transmissible tumor cells developing in the host. Further experiments focusing on transcriptional and proteomic changes (Takahashi et al. 2005 [DOI](#)) within grafted individuals, with a quantitative assessment of tumors size, will certainly improve our knowledge of these phenotypic manipulations.

While it seems clear that the growth of supernumerary tentacles is induced only by the presence of transmissible tumor cells, to argue for host manipulation in the strict sense [as observed in the case of manipulative parasites, see (Ewald 1980 [DOI](#), Poulin 2010 [DOI](#), 2012)], it is necessary to demonstrate that this phenotypic change is indeed associated with an increase in tumor transmission. One possibility is that the supernumerary growth of tentacles, by increasing the rate of prey capture, merely compensates for the pathological costs associated with the presence of the tumor, the growth of which requires additional resources. Under this hypothesis, tumorous hydra with supernumerary tentacles would not necessarily exhibit an increased budding rate, as it would be more indicative of a host manipulation aimed at better tolerating the tumor [i.e., compensating for vital resources diverted toward the tumor (Thomas et al. 2020 [DOI](#))]. It is also possible that the growth of supernumerary tentacles, through a higher feeding rate, enhances the host's budding rate and, consequently, the vertical transmission of the tumor. Our results do support the hypothesis that tumors do indeed impose a cost on the reproductive potential of the host in terms of the weaker budding of tumoral individuals, and this cost can be offset by the growth of supernumerary tentacles (see **Figure 4** [DOI](#)). Beyond this first conclusion, our data also

support the hypothesis that the supernumerary growth of tentacles can sometimes not only compensate for the cost of the tumor, but also leads for some cancerous individuals to a significant increase in the number of buds, beyond the values presented by healthy individuals with a normal number of tentacles (c.f., overcompensation area in **Figure 4**). Given that the transmission rate of tumors remains constant regardless of the budding rate (Boutry et al. 2022b), all our results thus suggest that tumor-induced supernumerary tentacle growth has likely evolved as a tumoral adaptation that reduces the cost for the host in order to maintain and enhance tumor transmission. Further research should focus on underlying the bio-physiological pathways and energetical costs of this manipulated phenotype (Takahashi et al. 2005, Sebens et al. 2017).

An intriguing finding from the present study is that in *H. oligactis*, new tumors can develop following injury and/or transplantation of healthy tissue alone. Additional experiments are required to formally describe these outgrowths as tumors [although there are strong phenotypic similarities with spontaneous tumors; see (Domazet-Lošo et al. 2014)] and to understand the cause and mechanisms underlying their development. We can speculate that the act of grafting is likely to weaken the hydra's ability to control abnormal cell proliferation and/or make them more susceptible to dysbiosis known to promote tumorigenesis (Rathje et al. 2020). In any case, despite the appearance of spontaneous tumors even after transplantation of healthy tissue, it is striking that only transmissible tumors are able to consistently alter and increase the number of tentacles in the transplanted individuals, which ultimately reinforces our conclusions.

An alternative explanation for our results would be that the increase in tentacle number following transplantation is a response of the hydra when non-self-tissues are introduced into the polyps. This would imply that the tumors, owing to their own evolution over years of vertical transmission, have diverged so significantly that they are perceived as non-self by the recipient hydras. However, we do not favor this hypothesis since when *Hydra vulgaris* are grafted with tissue from *Hydra oligactis*, we simply observe a destruction of the non-self-tissue (Bosch and David 1986). On the opposite, there is evidence of a lack of intra-specific non-self-recognition in *H. vulgaris* (Kuznetsov and Bosch 2003), without any reporting of growth of additional tentacles. Also, since healthy and spontaneously tumorous hydras have been shown to sometimes grow a few additional tentacles, it would necessary to explore in the future the extent to which the responsible environmental factors are similar to those potentially produced/exploited by transmissible tumors [see, for instance (Lefèvre et al. 2008)]. Further work, including experimental evolution, would be needed to assess the extent to which tentacle growth promotion is a capability that could be acquired over time from spontaneous tumor cells, at least for those that also acquired the capacity to be transmitted.

Transmissible tumors in hydras have significant implications for their ecological (Boutry et al. 2022a) and reproductive dynamics (Boutry et al. 2022b). For instance, these tumors have been associated with changes in life history traits, notably increased early budding rates and reduced sexual reproduction. Under experimental conditions, transmissible tumors have also been shown to influence biotic interactions with other species (Boutry et al. 2021). While our study focused on the modification of tentacle number, it is also promising to investigate behavioral traits and the life cycle of grafted individuals in future research. This would provide a more comprehensive understanding of the phenotypic changes in hydras bearing transmissible tumors.

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We are grateful to Thomas Bosch's laboratory, and in particular to Alexander Klimovich, for providing the first hydras, methods and ideas on which the authors first met and built their research. We would like to thank the two referees who reviewed the previous version of this manuscript. Their comments have significantly improved the manuscript.

Supplementary material

This repository contains complete dataset, details of the performed analysis and supplementary analysis at <https://justineboutry.github.io/Supplementary.io/> 

Additional information

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Author contributions

Conceptualization: JB, FT; Methodology and data collection: JB, OR, LG, JM, ST; Analysis and visualization: JB, OR, LG; Supervision: JB and FT; Writing of the original draft: JB, FT, OR, Review & editing: JB, JT, AMN, ST, NS, BU, RH, AD

Competing interests

All other authors declare they have no competing interests.

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Reviewer #1 (Public review):

Summary:

In this manuscript, Boutry et al examined a cnidarian Hydra model system where spontaneous tumors manifest in laboratory settings, and lineages featuring vertically transmitted neoplastic cells (via host budding) have been sustained for over 15 years. They observed that hydras harboring long-term transmissible tumors exhibit an unexpected augmentation in tentacle count. In addition, the presence of extra tentacles, enhancing the host's foraging efficiency, correlated with an elevated budding rate, thereby promoting tumor transmission vertically. This study provided the evidence that tumors, akin to parasitic entities, can also exert control over their hosts.

Strengths:

The manuscript is well-written, and the phenotype is intriguing.

<https://doi.org/10.7554/eLife.97271.2.sa2>

Reviewer #2 (Public review):

Background and Summary:

This study addresses the intriguing question of whether and how tumors can develop in the freshwater polyp hydra and how they influence the fitness of the animals. Hydra is notable for its significant morphogenetic plasticity and nearly unlimited capacity for regeneration. While its growth through asexual reproduction (budding) and the associated processes of pattern formation have been extensively studied at the cellular level, the occurrence of tumors was only recently described in two strains of Hydra oligactis (Domazet-Lošo et al, 2014). In that research, an arrest in the differentiation of female germ cells led to an accumulation of germline cells that failed to develop into eggs. In hydra, fertile egg cells typically incorporate nurse cells, which originate from large interstitial stem cells (ISCs) restricted to the germline, through apoptosis. However, this increase in apoptosis activity is absent in "germline tumors," and germline ISCs instead form slowly growing patches that do not compromise tissue integrity. Despite the upregulation of certain genes associated with mammalian neoplasms (such as *tpt1* and *p23*) in this tissue, determining whether this differentiation arrest and the resulting egg patches truly constitute neoplasms remains a challenge.

The authors have recently published two papers on the ecological and evolutionary aspects of hydra tumor formation (Boutry et al 2022, 2023), which is also the focus of this manuscript. They transplanted tissues derived from animals with germline tumors to wildtype animals and analyzed their growth patterns, specifically the number of tentacles in the host tissue. They observed that such tissues induced the growth of additional tentacles compared to tissues without germline tumors. The authors conclude that this growth pattern (increased number of tentacles) is correlated with "reducing the burden on the host by (over-)compensating for the reproductive costs of tumors" and claim that "transmissible tumors in hydra have evolved strategies to manipulate the phenotype of their host". While it might be stimulating to add a fresh view from other disciplines (here, ecological and evolutionary aspects), the authors completely ignore the current knowledge of the underlying cell biology of the processes they analyze. Major criticisms are:

Strengths:

intriguing question of whether and how tumors can develop in the freshwater polyp hydra and how they influence the fitness of the animals.

Reviewing Editor note on revisions: Most of the reviewer's comments have been addressed.

<https://doi.org/10.7554/eLife.97271.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

In this manuscript, Boutry et al examined a cnidarian Hydra model system where spontaneous tumors manifest in laboratory settings, and lineages featuring vertically transmitted neoplastic cells (via host budding) have been sustained for over 15 years. They observed that hydras harboring long-term transmissible tumors exhibit an unexpected augmentation in tentacle count. In addition, the presence of extra tentacles, enhancing the host's foraging efficiency, correlated with an elevated budding rate, thereby promoting tumor transmission vertically. This study provided evidence that tumors, akin to parasitic entities, can also exert control over their hosts.

Strengths:

The manuscript is well-written, and the phenotype is intriguing.

Weaknesses:

The quality of this manuscript could be improved if more evidence were to be provided regarding the beneficial versus detrimental effects of the tumors.

We thank the reviewer for taking the time to examine our work carefully and for their highly relevant comments and precise suggestions. We have incorporated these suggestions, which greatly improved the clarity of our manuscript concerning the beneficial and detrimental effects of tumors. Specifically, we have added a new analysis and rephrased the results section, as well as the corresponding sentences in the discussion, to enhance clarity.

Additionally, regarding the impact of tumor size on the development of supernumerary tentacles, we have included as suggested a new analysis that was previously only available in the supplementary materials of the earlier version. This addresses the reviewer's question and significantly enhances the quality of our paper.

We have thanked the two referees in the Acknowledgements section of our article.

Reviewer #2 (Public Review):

Background and Summary:

This study addresses the intriguing question of whether and how tumors can develop in the freshwater polyp hydra and how they influence the fitness of the animals. Hydra is notable for its significant morphogenetic plasticity and nearly unlimited capacity for

regeneration. While its growth through asexual reproduction (budding) and the associated processes of pattern formation have been extensively studied at the cellular level, the occurrence of tumors was only recently described in two strains of *Hydra oligactis* (Domazet-Lošo et al, 2014). In that research, an arrest in the differentiation of female germ cells led to an accumulation of germline cells that failed to develop into eggs. In *hydra*, fertile egg cells typically incorporate nurse cells, which originate from large interstitial stem cells (ISCs) restricted to the germline, through apoptosis. However, this increase in apoptosis activity is absent in "germline tumors," and germline ISCs instead form slowly growing patches that do not compromise tissue integrity. Despite the upregulation of certain genes associated with mammalian neoplasms (such as *tpt1* and *p23*) in this tissue, determining whether this differentiation arrest and the resulting egg patches truly constitute neoplasms remains a challenge.

The authors have recently published two papers on the ecological and evolutionary aspects of *hydra* tumor formation (Boutry et al 2022, 2023), which is also the focus of this manuscript. They transplanted tissues derived from animals with germline tumors to wildtype animals and analyzed their growth patterns, specifically the number of tentacles in the host tissue. They observed that such tissues induced the growth of additional tentacles compared to tissues without germline tumors. The authors conclude that this growth pattern (increased number of tentacles) is correlated with "reducing the burden on the host by (over-)compensating for the reproductive costs of tumors" and claim that "transmissible tumors in *hydra* have evolved strategies to manipulate the phenotype of their host". While it might be stimulating to add a fresh view from other disciplines (here, ecological and evolutionary aspects), the authors completely ignore the current knowledge of the underlying cell biology of the processes they analyze.

Strengths:

The study focuses on intriguing questions. Whether and how tumors can develop in the freshwater polyp *hydra*, and how they influence the fitness of the animals?

Weaknesses:

Concept of germline tumors.

The conceptual foundation of their experiments on germline tumors was the study of Domazet-Lošo et al (2014) introducing the concept of germline tumors in *hydra* (see above). While this is an intriguing hypothesis, there has been little advancement in comprehending the molecular mechanisms underlying tumor formation in *hydra* beyond this initial investigation. Germline tumors in *hydra* do not fully meet the typical criteria for neoplasms observed in mammalian tissues. More importantly, a similar phenotype was already reported by the work of Paul Brien and described as "crise gamétique" (Brien, 1966, *Biologie de la reproduction animale - Blastogenèse, Gamétogenèse, Sexualisation*, ed. Masson & Cie, Paris). This phenomenon of gametic crisis is unique to *Hydra oligactis*, a stenotherm, cold-adapted cosmopolitan species. In this species, gametogenesis severely impacts the vitality of the polyps, often leading to complete exhaustion and death (Tardent, 1974). Animals can only be rescued during the initial phase of the cold-induced sexual period (see also the research of Littlefield (1984, 1985, 1986, 1991). The observed arrest in differentiation arrest in germline tumors might represent an epigenetically established consequence of surviving gametogenesis. Regrettably, this important work was not mentioned by the authors or by Domazet-Lošo et al. (2014), highlighting a notable gap in the recognition of basic research in this area that might challenge the *hydra* tumor hypothesis.

"Super-nummary" tentacles in graft experiments.

*The authors describe that after grafting tissue from animals with germline tumors to wild-type animals, the number of tentacles in the host tissue increased when the donor tissue had germline tumors. A maximum effect of four additional tentacles was found with donor strain *H. oligactis robusta* and three additional tentacles with donor strain *H. oligactis* St Petersburg. In general, *H. oligactis* wild-type host strains had fewer tentacles than *H. oligactis* St Petersburg strains. This is consistent with the results of Domazet-Lošo et al (2014) who showed that the number of tentacles increased in the strains with germline tumors. What conclusions can be drawn from these experiments?*

The authors might want to conclude that transmissible tumors in Hydra have developed strategies to manipulate the phenotype of their host. But there is no evidence for this, as essential controls are missing. It is known that the size of hydra polyps is proportion-regulated, i.e. the number of tentacles varies with the size and number of (epithelial) cells. Such controls are missing in the experiments. There is also a lack of controls from wild-type animals in gametogenesis: it is very likely that grafts with wild-type animals with egg spots of comparable size as the germline tumors (see above) will result in similar numbers of tentacles in host tissue.

We thank the reviewer for their thoughtful comments. While we appreciate the concerns raised, we maintain that the evidence provided by Domazet-Lošo et al. (2014, Nature Communications) supports the relevance of this model, including the suggested comparisons with the expression profiles of individuals undergoing induced sexual reproduction. Our study focuses primarily on the impact of these tumors on the host phenotype rather than their origin. Tumors are defined as accumulations of abnormally proliferating cells. This includes the definition provided by the referee, which describes “apoptosis activity as absent in ‘germline tumors,’ with germline ISCs forming slowly growing patches.” Compromise of tissue integrity is not a criterion for defining neoplasms, and many benign neoplasms do not meet this criterion. We are interested in continuing this discussion with the referee to better understand the expected evidence and agree that histological nomenclature could be improved. While further investigation into the cell biology of these tumors would be valuable, this is currently beyond the scope of our article but is being pursued in separate research.

We also appreciate the points raised regarding the definition of germline tumors and the reference to the pioneering work of Paul Brien. However, in that publication, the concept of gametic crisis in *H. oligactis* describes reproductive exhaustion leading to death, rather than abnormal cell proliferation indicative of a tumor-like phenotype. This distinction likely explains why this specific paper was not cited previously.

Our study builds on prior research using the same model (e.g., Domazet-Lošo et al. 2014; Boutry et al. 2023) and describes observations across different hydra strains from various locations worldwide (not just two), all conducted under stable warm temperatures that are not conducive to sexual development. These investigations reveal a phenomenon distinct from the senescence observed post-reproduction in *H. oligactis*. The phenotype we describe, characterized by an accumulation of cells in the ectoderm, aligns with studies referenced by the reviewer from leading groups in hydra research, known for their expertise in hydra cellular biology. We have relied on these studies after carefully reviewing their results and receiving training from these experts. Furthermore, our team is focused on eco-evolutionary topics and does not aim to specialize in cellular biology, as other teams are already dedicated to that field.

We also thank the reviewer for their comments on the relevance of our findings and the missing controls. However, we have noted that the reviewer may have misunderstood our experimental design and results.

Firstly, it appears that the reviewer based their critique mainly on the initial sentences of our Results section (illustrated in Figure 2), which outline the donor groups used in our study rather than presenting the results of the grafting experiments. This description alone is insufficient for drawing conclusions, which is why we conducted further analyses using these donor groups grafted onto different recipients. The maximum effects mentioned by the reviewer (+10 tentacles with St. Petersburg tumoral tissue and +8 tentacles with Robusta tumoral tissue, Results Section 2) represent only a part of our study. We encourage the reviewer to focus on the model analyses presented in Results Section 2, which directly relate to the grafting experiments and provide a more comprehensive evaluation of our results and conclusions. These analyses include comparisons between transmissible tumors and spontaneous tumors, offering deeper insights into their effects on tentacle development.

In our methods (as depicted in Figure 3), we explicitly compared different types of tumorous tissue from various donors, distinguishing between spontaneous and transmissible tumors. Although we avoid labeling spontaneous tumors as "controls" to prevent confusion with healthy tissue controls, they serve as controls to the "treatment" that involves transmissible tumors, and thus are appropriate comparisons for assessing the size effect suggested by the reviewer. Spontaneous and transmissible tumors share similar size and cellular characteristics but differ significantly in the number of tentacles their hosts possess. Furthermore, we refer the reviewer to a relevant study (Ngo et al. 2021) that found no increase in tentacle numbers with larger polyps of healthy tissue. This reference has been included in the revised discussion (line 309 to 312), which now also addresses the potential effect of body size with additional explanations.

Regarding the suggestion to include controls from animals undergoing gametogenesis, we did not find evidence in the literature indicating an increase in tentacle numbers during this process in hydra. If such studies exist, we kindly request the complete references so we can include them in our discussion. Additionally, as noted in Brien's work, *Hydra oligactis* undergoing gametogenesis are known to either die or experience significant degeneration afterward. Transplanting tissue from dead or dying (and reproducing) hydras poses technical challenges and raises questions about whether any observed effects result from incomplete gametogenesis, the onset of senescence, or both. While these questions are intriguing, they fall outside the scope of our article.

In conclusion, we appreciate the opportunity to address these points and reaffirm that our study offers valuable insights into the evolutionary dynamics of interactions between transmissible tumor tissues and host phenotypes in hydra. We remain open to further discussion and welcome any additional feedback to enhance the clarity and robustness of our manuscript.

Recommendations for the authors:

Reviewer #1 (Recommendations For The Authors):

(1) *If the fitness of hydra is altered in those with spontaneous tumors is the increased number of tentacles associated with those with transmitted tumors able to rescue this phenotype?*

We thank the reviewer for reformulating our results. Indeed, fitness can be restored and even improved in tumorous polyps harboring supernumerary tentacles. This phenomenon, which we referred to as compensation and over-compensation in Section 3 and Figure 4, was initially discussed only in the discussion section. To improve the clarity of our manuscript, we have now specified this in the Conclusion (lines 345 to 347 and some minor rewording in the same paragraph) in the Results section (lines 284 to 286).

(2) Does the size of the tumor predict the number of tentacles formed?

We agree that this would be a valuable complementary analysis. We have conducted an analysis considering the qualitative size of the tumors (based on visual categories) and the number of tentacles, which is now included in our paper (lines 160-161; lines 193 to 198; lines 253 to 259; lines 314 - 322).

(3) Considering the mentioned association of body size with tentacle numbers for hydra, is a change in size a phenotype associated with transmitted tumors, and is such a phenotype transmittable.

All tumorous individuals, regardless of their tumor type, exhibit a swollen body. We have added a sentence in the introduction to clarify this point (line 62).

(4) Is there anything unique about the Rob population that would explain their mass mortality following transplantation? For instance, their resistance to spontaneous tumor formation? Similarly, is there a difference in transplantation success based on the type of tissue transplanted? The authors could address this point in the discussion.

It is a very old lineage described nearly 80 years ago. It is unknown whether natural populations of Robusta exist, and no reports of any male individuals have been documented. We have added a sentence in the Materials and Methods section to clarify this information (lines 98 to 102).

(5) What downsides are known about the transmittable tumors in hydra and how present are they in the grafted individuals? Are other physiological aspects such as mobility, regeneration, or sexual reproduction hindered?

Transmissible tumors have been associated with increased vulnerability to predation and alterations in life history traits, including a higher budding rate and decreased sexual reproduction. While we were unable to measure behavioral traits in this study of our grafted individuals, this is an intriguing avenue for further research. We have included this perspective in the discussion section as a concluding remark (lines 375 to 382). Thanks a lot for the suggestion of this conclusion.

(6) It is important to explore the mechanisms behind the phenotypic variation conferred by the types of tumors, whether of different lineage or transmissibility. For this purpose, RNA-Seq on the recipients seems like a good starting point.

Thanks for this suggestion, we've reworded the sentence about this perspective in our discussion to be more precise (line 320).

Boutry, Justine, Marie Buysse, Sophie Tissot, Chantal Cazevielle, Rodrigo Hamede, Antoine M. Dujon, Beata Ujvari, et al. 2023. « Spontaneously Occurring Tumors in Different Wild-Derived Strains of Hydra ». *Scientific Reports* 13 (1): 7449. <https://doi.org/10.1038/s41598-023-34656-0>.

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