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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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.

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

87 However, whether the host or the tumor is responsible for the alteration of the
88 tentacles number is not clear yet. Furthermore, in one *H. oligactis* lineage, called the St.
89 Petersburg strain, tumor initiation and maintenance require the presence of a specific
90 microbiome (Rathje et al. 2020), suggesting that bacteria may also be involved in the
91 phenotypic alteration of the host to promote their own transmission. Here, through a series
92 of experiments using a set of healthy and tumor tissue grafts from tumors associated with
93 different phenotypes, we attempted to determine whether the host or the tumor is
94 responsible for the growth of additional tentacles in the tumorous hydra.

95

96 **Material and Methods**

97 *Biological material*

98 All hydras were maintained according to standard rearing protocols (Boutry et al.
99 2022a). Three different main lineages of *Hydra oligactis* were used in our study. The Saint
100 Petersburg (named thereafter SpB) and the Robusta (named thereafter Rob) strains were
101 obtained from Thomas Bosch's laboratory in Kiel (Domazet-Lošo et al. 2014). Rob are also
102 known under the denomination *Pelmatohydra robusta*, as they were described as a distinct
103 specie from Japan (Ito 1947). Current phylogenetic analyses suggest than they belong to *H.*
104 *oligactis* species. (Martínez et al. 2010), and it is currently unknown if populations related to
105 this strain still exist in nature. For each of these two lines, control lines were also isolated
106 from healthy polyps collected during the same sampling; these control groups do not harbor
107 any sign of transmitted tumors and are supposed to be genetically identical to their
108 respective tumorous lines (Boutry et al. 2022b). A third tumorous lineage has been recently
109 established in Frédéric Thomas's laboratory in France, and was isolated from a polyp

110 sampled in Montaud water pond (43.748211°N, 3.989871°E); this lineage, named Montaud
111 (thereafter Mt), was maintained in the lab since August 2021 (Boutry et al. 2023). To date,
112 we have been unable to establish a distinct control and vertically transmitted tumorous
113 lineage for this new strain, due to variable transmission rates of these tumors and the
114 sporadic occurrence of spontaneous tumors from initially healthy- looking polyps. In
115 addition, a group named "wild group" was used as recipient of the transplanted tissues; this
116 group was composed of randomly sampled *H. oligactis* and assumed to be genetically
117 diverse and distinct from the SpB and Rob lineage. These wild hydras were also sampled in
118 the Montaud pond, between 15/02/2022 and 15/04/2022.

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

127 *Grafting protocol*

128 The transplantation experiment was performed according to (Lenhoff 2013, Dujon et
129 al. 2022) using healthy and tumorous donors of the SpB, Rob, and Mt lineages. For the
130 recipients, we used healthy individuals of the SpB lineage and the wild group (see Figure 1
131 for an illustration of the experimental design). Since transplants on healthy Rob recipients
132 led to massive mortality, we excluded them from this analysis. The recipients from the “wild

133 group" (because they may be genetically diverse) were cut in half longitudinally one week
134 before transplantation and allowed to fully regenerate, in order to obtain two genetically
135 identical individuals on which to perform the control and tumor transplants. The
136 manipulations were performed under a binocular magnifying glass (Olympus© model SZ51,
137 magnification from 0.67X to 4.5X) and the tools were disinfected with 90% alcohol and
138 rinsed with Volvic© before and after each transplant. Donor and recipient hydras were
139 starved for 24 hours prior to transplantation to prevent digested food remaining in the
140 gastric cavity from being released during grafting.

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

156 of food for a week to avoid water pollution from feeding during regeneration, then returned
157 to a normal diet on day 8 post-transplant.

158 *Temporal follow-up and statistical analysis*

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

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

178 lines with spontaneous tumors and their controls. Random effects of transplantation date
179 and/or batch were evaluated and included in the models for which they were relevant.

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

188 The change in the number of tentacles was then analyzed using a delta variable: the
189 difference between the maximum number of tentacles developed during the study and the
190 number of tentacles of the recipient on the day of transplantation (see the analysis and
191 results in supplementary section 4). This variable was then modeled according to a random
192 generalized models with a Poisson distribution (we turned all values into positive values by
193 simply adding 2 to all values). In the models, several fixed effects were tested: tumor status
194 and lineage of the donor, lineage of the recipient, initial number of tentacles of the recipient
195 and donor. The impact of tumor development after transplantation on the number of
196 tentacles was excluded because of a confounding effect between donor status and tumor
197 development (see in supplementary). The effect of tumor size on the number of
198 supernumerary tentacles was evaluated using an intra-group linear model (donor lineage,
199 status, and recipient). This was done to assess how small, medium, and large tumors
200 influence the variation in tentacle numbers independently of donor and recipient lineage,

201 for both transmissible and spontaneous tumors (see the analysis and results in
202 Supplementary Section 4).

203 The relationship between the number of buds produced and the number of tentacles
204 developed was subsequently examined on the complete dataset (transmissible and
205 spontaneous tumors together) utilizing the same delta variable (refer to the analysis and
206 results in Supplementary Section 5). The number of buds produced during the 10-week
207 observation period was then subjected to modeling through random linear models. In these
208 models, various fixed effects were assessed, including the tumor status of the grafted
209 individuals, the delta variable representing the number of additional tentacles, and the
210 group consisting of the lineage and cancerous status of both the donor and recipient. The
211 latter was treated as random effects to emphasize our focus on intra-group variations in
212 drawing conclusions. Figures and tables were made with the packages GGplot2 (Wickham
213 2016), Sjplot (Lüdecke 2023), ggstatsplot (Patil 2021) and the software Keynotes©.

214

215 **Results**

216 *1. Tentacle numbers in hydras bearing transmitted versus spontaneous tumors*

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

228 *2. Tumor development following tissue transplantation*

229 On average, healthy hydras grafted with tumorous tissue, either from the transmissible or
230 the spontaneous tumors, have an increased risk to subsequently develop tumors compared
231 to those grafted with healthy tissue (Figure 3 pie charts). Hydras grafted with transmissible
232 tumors developed on average 8 times more tumors (IRR (incidence rate ratio) = 8.00 [2.73 -
233 23.49], $p<0.001$) than those transplanted with healthy tissue, whereas for spontaneous
234 tumor transplants the risk only increased 2.61 times (IRR = 2.61 [1.09 - 6.23], $p=0.031$).
235 Nevertheless, for the transplantation of transmissible tumors from Rob donors on SpB
236 healthy recipients, the effect appears weaker (see Figure 3.A pie charts), although it is not
237 detected as significantly different from the other transplant combinations (see in

238 supplementary section 3). However, an intriguing result is that hydra grafted with healthy
239 tissues also developed tumors but at lower, yet non negligible, rate compared to hydra
240 grafted with tumorous tissues (Figure 3 pie charts; 48.75%, 48.12% and 28.92%, on average,
241 for healthy tissues from Mt, Spb, and Rob, respectively).

242 *3. Development of new tentacles following tissue transplantation*

243 The number of tentacles (Figure 3A, dot plots) showed a significant increase in individuals
244 transplanted with tissue from transmitted tumors (even if the effect remains low when the
245 donor is Rob and the recipient is SpB), whereas there was no significant effect in individuals
246 transplanted with tissues from spontaneous tumors (Figure 3B&C, dot plots), relative to
247 individuals transplanted with healthy tissue. On average, an individual transplanted with
248 transmitted tumor tissues developed 1.45 [1.21 - 1.73] additional tentacles while individuals
249 transplanted with healthy tissue developed only one additional tentacle (IRR= 1.45 [1.21 -
250 1.73], $p<0.001$), with no significant effect of the donor lineage. Furthermore, we also
251 detected a negative effect of the initial number of tentacles of the recipient (Figure 3, dot
252 plots): when the recipient already had a relatively high number of tentacles, the increase in
253 the number of tentacles after transplantation was reduced (IRR=0.82 [0.74 - 0.92], $p=0.001$),
254 highlighting a possible threshold effect. In contrast, we do not detect any significant effect of
255 the number of tentacles of the recipient on the number of tentacles developed after the
256 graft of tissues from spontaneous tumors (see model selection in supplementary section 4).

257 Considering the effect of tumor size on the number of supplementary tentacles
258 independently of their donor and recipient lineage, large tumors developed after the graft of
259 transmissible tumors demonstrated a statistically significant increase in the number of
260 supernumerary tentacles compared to small tumors (Estimate = 1.40, 95% CI [0.14, 2.65], p =

261 0.03; see figure 5). For spontaneous tumorous donors, the results indicated no significant
262 effect of the size of the tumors developed after grafting on the number of tentacles (see
263 Figure 5, model selection and complete results in supplementary section 4).

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

265 Considering only individuals with a stable number of tentacles (-2, +2), we observed that
266 tumorous individuals produced significantly fewer buds during the ten weeks of the study
267 (Wilcoxon test, W = 959, p-value = 0.0094, n=171). Regardless of the type of donor and
268 recipient, the number of buds produced per individual was found to be significantly
269 correlated with the number of supernumerary tentacles developed by the individual during
270 the same period (Figure 4). However, the slope of this correlation was notably reduced for
271 hydras with tumors (Figure 4, n=92, estimate = -2.75 [-5.21 – 0.29], p= 0.029). Consequently,
272 all other factors being equal, an individual developing a tumor will require more
273 supplementary tentacles than a healthy individual to achieve the same number of buds
274 produced. Noticeably, we observed that the impact of the number of tentacles and tumor
275 development on the number of buds produced became statistically detectable only after the
276 7th week of the experiment.

277
278 **Discussion**

279 Many parasites from different taxa (e.g., viruses, bacteria, protozoa, helminths, fungi,
280 arthropods) have evolved the ability to manipulate the phenotype of their host to favor their
281 own transmission and/or survival (2012). A wide variety of host traits can be modified by
282 parasites, including reproduction, behavior, color, morphology and/or physiology(Barnett
283 1983, Dawkins 1999, Agnew et al. 2000). Although theory predicts that transmissible

284 cancers should evolve a true parasitic lifestyle over time (Dujon et al. 2020, Burioli et al.
285 2021), to date there has been no evidence of host phenotypic manipulation by transmissible
286 tumor cell lines (Tissot et al. 2016).

287 In their pioneering work on transmissible tumors in hydra, Domazet-Lošo and
288 colleagues (Domazet-Lošo et al. 2014) mentioned the existence of an increased number of
289 tentacles in the tumor-bearing lineages that have been propagated in the laboratory, but the
290 precise cause of this phenotypic change was not understood. Here, we first highlight that
291 this is not a general attribute of tumor-developing hydras, since both Mt and SpB polyps
292 developing spontaneous neoplasms do not show a similar large increase in the number of
293 tentacles (Figure 2A). These results support the hypothesis that spontaneous tumors, which
294 have no evolutionary history with their host, do not possess (or just to a small extent) the
295 ability to influence its phenotype. Conversely, our results showed that transmissible tumors
296 propagated in the laboratory for more than 10 years can induce the growth of additional
297 tentacles even when they are transplanted in new hosts (even if this effect seems low with
298 SpB grafted with tumors from Rob), as they seem to do in their original host lineage. In
299 addition, while transplantation of spontaneous tumors never promoted an increase in
300 tentacle numbers, transmissible tumors did, regardless of the origin of the recipient hydra
301 (although one may suspect a different pattern between recipient strains that may not be
302 detected due to a lack of power). These results are not consistent with the hypothesis that
303 the development of additional tentacles could indicate a compensatory response of tumor-
304 bearing polyps, for example, to better cope with resources redirected to growing tumors.
305 Similarly, in agreement with previous work showing that the tumor phenotype can be
306 observed in tumor-bearing hydras that do not consistently have an altered microbiome [this
307 is the case for SpB, but not Rob (Domazet-Lošo et al. 2014)], our transplantation

308 experiments do not support the hypothesis that the alteration in tentacles' number could be
309 caused entirely by bacteria. Indeed, both the SpB and Rob tumors were able to trigger
310 supernumerary tentacles in their new hosts, even if Rob microbiome is the same between
311 tumorous and non-tumorous hydras (Domazet-Lošo et al. 2014). Considering a potential size
312 effect of the donor, individuals with spontaneous tumors grafted also display an enlarged
313 body similarly to transmissible ones, as the grafted tissues were of similar size compared to
314 transmissible tumor grafts. Therefore, this is not likely to explain the effect measured here,
315 which is also consistent with the literature (Ngo et al. 2021).

316 Thus, although the exact mechanisms promoting the growth of additional tentacles
317 remain to be identified, this is the first evidence of transmissible tumor cells that have
318 evolved the ability to manipulate an external phenotypic trait in their host. Interestingly, we
319 have shown that individuals with large tumors developed after a graft with transmissible
320 tumors exhibited more supernumerary tentacles, whereas individuals with large tumors
321 developed after spontaneous tumor grafts did not show the same pattern. This suggests a
322 proportional effect depending on the number of transmissible tumor cells developing in the
323 host. Further experiments focusing on transcriptional and proteomic changes (Takahashi et al.
324 2005) within grafted individuals, with a quantitative assessment of tumors size, will certainly
325 improve our knowledge of these phenotypic manipulations.

326 While it seems clear that the growth of supernumerary tentacles is induced only by
327 the presence of transmissible tumor cells, to argue for host manipulation in the strict sense
328 [as observed in the case of manipulative parasites, see (Ewald 1980, Poulin 2010, 2012)], it is
329 necessary to demonstrate that this phenotypic change is indeed associated with an increase
330 in tumor transmission. One possibility is that the supernumerary growth of tentacles, by

331 increasing the rate of prey capture, merely compensates for the pathological costs
332 associated with the presence of the tumor, the growth of which requires additional
333 resources. Under this hypothesis, tumorous hydra with supernumerary tentacles would not
334 necessarily exhibit an increased budding rate, as it would be more indicative of a host
335 manipulation aimed at better tolerating the tumor [i.e., compensating for vital resources
336 diverted toward the tumor (Thomas et al. 2020)]. It is also possible than the growth of
337 supernumerary tentacles, through a higher feeding rate, enhances the host's budding rate
338 and, consequently, the vertical transmission of the tumor. Our results do support the
339 hypothesis that tumors do indeed impose a cost on the reproductive potential of the host in
340 terms of the weaker budding of tumoral individuals, and this cost can be offset by the
341 growth of supernumerary tentacles (see Figure 4). Beyond this first conclusion, our data also
342 support the hypothesis that the supernumerary growth of tentacles can sometimes not only
343 compensate for the cost of the tumor, but also leads for some cancerous individuals to a
344 significant increase in the number of buds, beyond the values presented by healthy
345 individuals with a normal number of tentacles (c.f., overcompensation area in Figure 4).
346 Given that the transmission rate of tumors remains constant regardless of the budding rate
347 (Boutry et al. 2022b), all our results thus suggest that tumor-induced supernumerary
348 tentacle growth has likely evolved as a tumoral adaptation that reduces the cost for the host
349 in order to maintain and enhance tumor transmission. Further research should focus on
350 underlying the bio-physiological pathways and energetical costs of this manipulated
351 phenotype (Takahashi et al. 2005, Sebens et al. 2017).

352 An intriguing finding from the present study is that in *H. oligactis*, new tumors can
353 develop following injury and/or transplantation of healthy tissue alone. Additional
354 experiments are required to formally describe these outgrowths as tumors [although there

355 are strong phenotypic similarities with spontaneous tumors; see (Domazet-Lošo et al. 2014)]
356 and to understand the cause and mechanisms underlying their development. We can
357 speculate that the act of grafting is likely to weaken the hydra's ability to control abnormal
358 cell proliferation and/or make them more susceptible to dysbiosis known to promote
359 tumorigenesis (Rathje et al. 2020). In any case, despite the appearance of spontaneous
360 tumors even after transplantation of healthy tissue, it is striking that only transmissible
361 tumors are able to consistently alter and increase the number of tentacles in the
362 transplanted individuals, which ultimately reinforces our conclusions.

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

379 Transmissible tumors in hydras have significant implications for their ecological (Boutry et al.
380 2022a) and reproductive dynamics (Boutry et al. 2022b). For instance, these tumors have
381 been associated with changes in life history traits, notably increased early budding rates and
382 reduced sexual reproduction. Under experimental conditions, transmissible tumors have
383 also been shown to influence biotic interactions with other species (Boutry et al. 2021).
384 While our study focused on the modification of tentacle number, it is also promising to
385 investigate behavioral traits and the life cycle of grafted individuals in future research. This
386 would provide a more comprehensive understanding of the phenotypic changes in hydras
387 bearing transmissible tumors.

388

389 **Supplementary material:** This repository contains complete dataset, details of the
390 performed analysis and supplementary analysis at
391 **https://justineboutry.github.io/Supplementary.io/**

392 **Funding:** This work was supported by the Hoffmann Family and by the ANR EVOSEXCAN
393 (ANR-23-CE13-0007) to FT. JB salary was funded by the university of Montpellier.

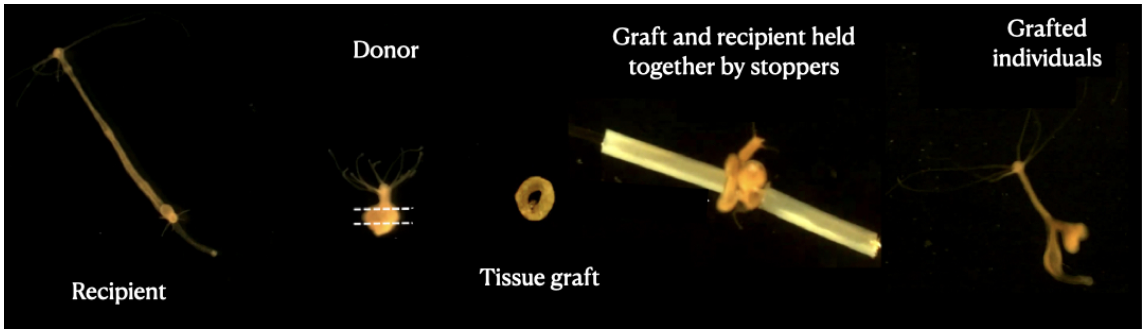
394 **Acknowledgment:** We are grateful to Thomas Bosch's laboratory, and in particular to
395 Alexander Klimovich, for providing the first hydras, methods and ideas on which the authors
396 first met and built their research. We would like to thank the two referees who reviewed the
397 previous version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

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

402 **Competing interests:** All other authors declare they have no competing interests.

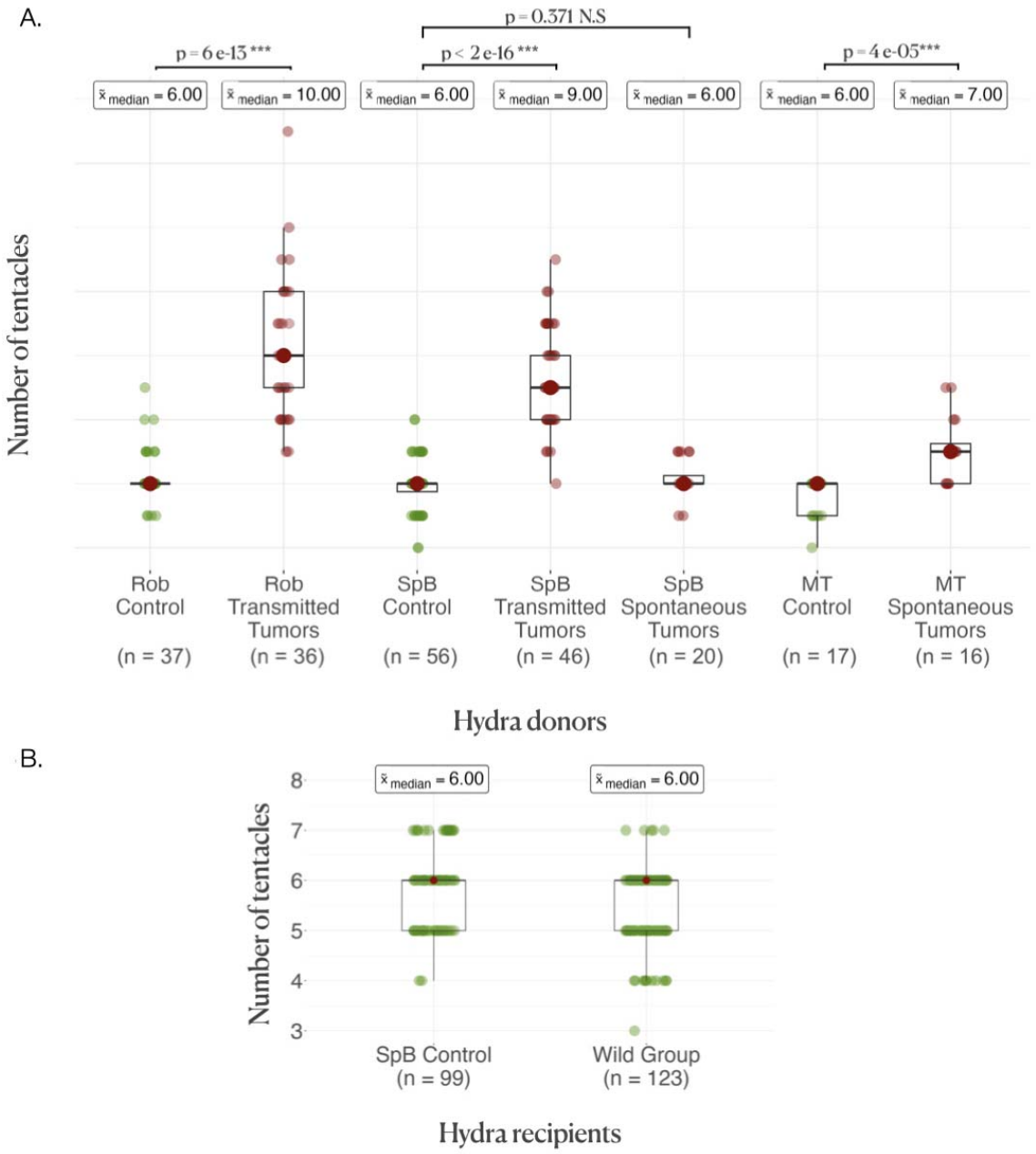
403

404 **Figures**



405

406 **Figure 1.** Grafting protocol. Images represent each step of the transplantation process,
407 starting with the recipient polyp (left); the donor is then cut into a ring-shaped tissue graft
408 that is inserted on a needle, put in contact with the recipient tissue and blocked by stopping
409 tubes. At the end (right) a transplanted individual can be seen 3 days after the transplant.



410

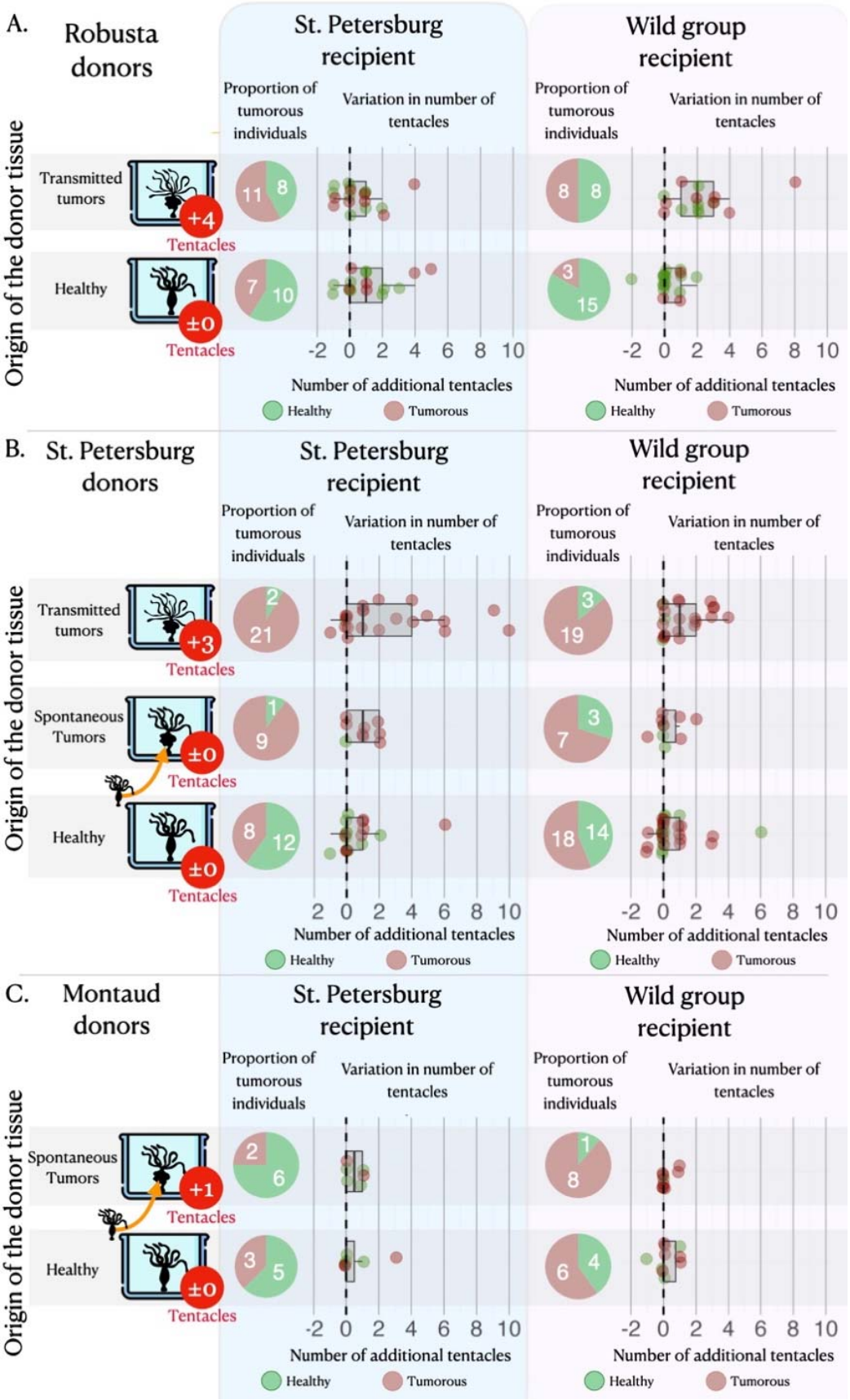
411 **Figure 2.** Donor and recipient tentacle phenotype. (A) Boxplots represent the number of

412 tentacles in each donor line; the average number of tentacles in each group is written above

413 in the boxes, and the rows represent the comparison between the control and the tumor

414 medians in each line, with the p-values of the corresponding Wilcoxon-Mann-Whitney tests.

415 (B) Boxplots represent the number of tentacles in different groups of recipients.


A.
Robusta donors
St. Petersburg recipient
Wild group recipient
Proportion of tumorous individuals
Variation in number of tentacles
Origin of the donor tissue
Transmitted tumors
+4
Tentacles
Healthy
±0
Tentacles
11
8
7
10
8
8
3
15
-2 0 2 4 6 8 10
Number of additional tentacles
Healthy
Tumorous
B.
St. Petersburg donors
St. Petersburg recipient
Wild group recipient
Proportion of tumorous individuals
Variation in number of tentacles
Origin of the donor tissue
Transmitted tumors
+3
Tentacles
Spontaneous Tumors
±0
Tentacles
Healthy
±0
Tentacles
2
21
1
9
8
12
3
19
3
7
18
14
2 0 2 4 6 8 10
-2 0 2 4 6 8 10
Number of additional tentacles
Healthy
Tumorous
C.
Montaud donors
St. Petersburg recipient
Wild group recipient
Proportion of tumorous individuals
Variation in number of tentacles
Origin of the donor tissue
Spontaneous Tumors
+1
Tentacles
Healthy
±0
Tentacles
2
6
3
5
1
8
6
4
-2 0 2 4 6 8 10
Number of additional tentacles
Healthy
Tumorous

416

417 **Figure 3.** Tumor appearance and changes in tentacle number after the grafts.
418 Three sets of matrices showing (i) the proportion of individuals (pie charts) that have
419 developed tumors (in red) or lacked tumors (in green) and (ii) the variation in tentacle
420 numbers (graphs) among non-tumorous individuals (green dots) and tumorous individuals
421 (red dots), following the grafting of healthy tissue (green bars) or tumorous tissue (from
422 spontaneous – orange bars, and transmitted – red bars, tumors) from Robusta, St.
423 Petersburg or Montaud donor lineages (left panel) onto healthy recipients from the St.
424 Petersburg or wild groups (top panels). For the donor lineages, the arrows indicate that the
425 individuals with spontaneous tumors were isolated from the same cultures as the healthy
426 individuals, whereas the individuals with transmitted tumors represent a distinct lineage; the
427 median number of supernumerary tentacles in the tumorous donor individuals are also
428 shown (in red circles).

429

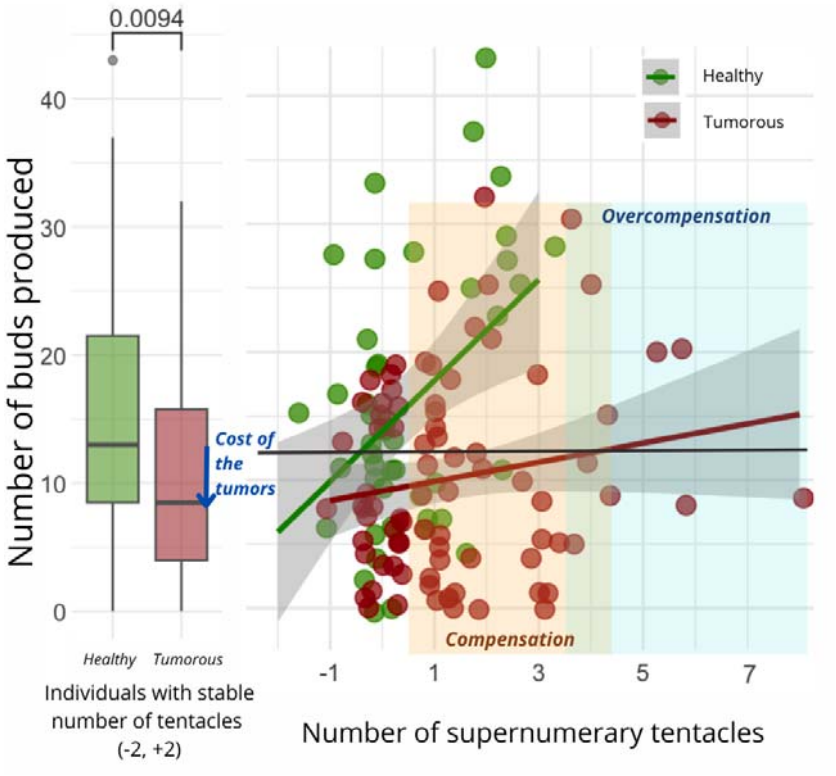


Figure 4. Number of buds produced depending on the development of supernumerary tentacles. On the left, the box plot exhibits the bud production of healthy (n=47, green) and tumorous individuals (n=28, red) with a constant number of tentacles (-2, +2) during the 10-week monitoring post-grafting (Wilcoxon test, W=959, p=0.009, $n_{healthy}$=79, $n_{tumorous}$ = 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 mode, estimate = -2.75, CI: -5.21 – -0.29, p=0.029).

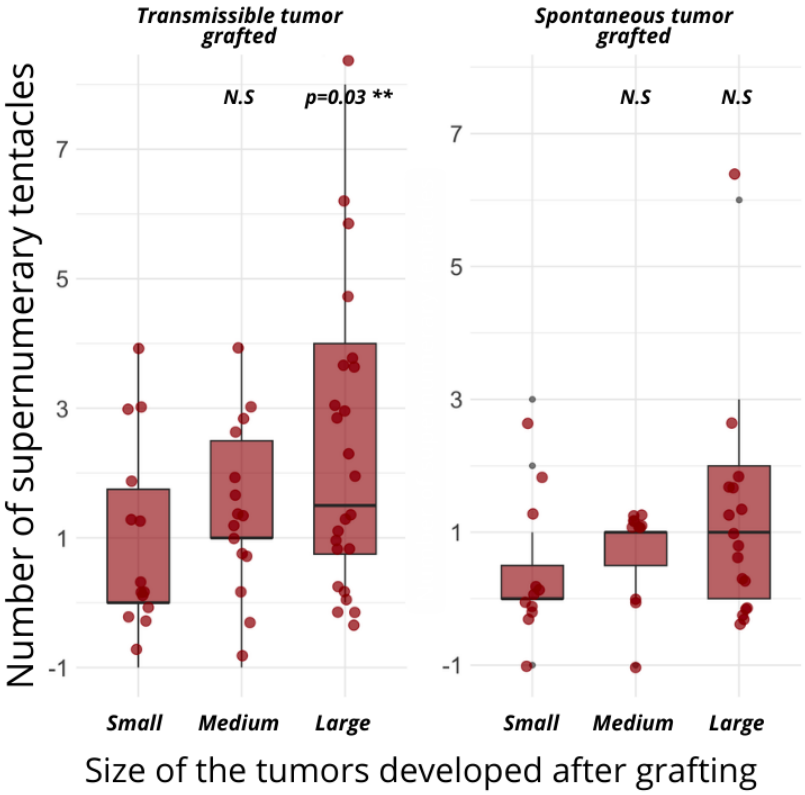


Figure 5. Effect of tumor size on the number of supernumerary tentacles developed after grafting of transmissible tumors (left panel, n=53) versus spontaneous tumors (right panel, n=39). For transmissible tumors, there was a significant increase in supernumerary tentacles with large tumors (linear model, 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).

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