Gut cancer increases the risk for *Drosophila* to be preyed upon by hunting spiders in the laboratory

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Abstract

Predators are thought to prey on individuals that are in poor physical condition, although the evidence supporting this is ambiguous. We tested if sick individuals where more predated using *Drosophila melanogaster* flies as manipulable preys. We asked if hunting spiders, trapped from the wild, would selectively prey upon flies with compromised health (i.e. chronically infected or cancerous) versus healthy flies under laboratory conditions. Flies chronically infected with the bacterium *Providencia rettgeri*, a natural *Drosophila* pathogen, were not selectively preyed upon by neither jumping spiders, nor small wolf spiders. We suggest that this result support the hypothesis that chronic infection is associated with reduced symptoms to avoid the predation of their host. We then induced colon cancer in some of the flies and asked if the presence of cancer led to selective predation; there is little evidence for this, even in vertebrates. As the cancer developed, the incidence of predation by jumping spiders on the afflicted flies increased. We conclude that predation can impact disease lethality even in invertebrate species and that cancer is a factor in selective predation. Our results may explain why early tumors, but not metastasized cancers, are commonly detected in organisms in the wild, as cancer bearing individuals are rapidly eliminated due to the strong selective pressure against them.

Introduction

In vertebrates, individuals that are injured, or sick are often preyed upon, as are juveniles who have not reached adult speed or reflex (Mesa et al. 1994; Møller 2008; Genovart et al. 2010). This is especially true when a predator's preferred type of prey is difficult to catch (Temple 1987), however, there are exceptions (Penteriani et al. 2008). Although this theory of selective predation is largely accepted, only a few studies have tested it empirically. Selective predation can only be said to exist when the relative frequencies of the types of prey in a predator's diet differ from the frequencies of the prey in the environment (Chesson 1978). Ideally, to test the selective prey hypothesis in the wild, data on the types of prey sought by many of the predators and the health status of the whole prey population would be determined. This is obviously difficult, and although this information may be vitally important in the context of eco-evolutionary dynamics (Brunner et al. 2017, 2019), the topic has been subjected more to speculation than to empirical study.

Probably the most studied question regarding selective predation is whether predators prey more on sick over healthy individuals. Even if hosts predators may avoid diseased prey to avoid the risk of getting sick themselves (Goren and Ben-Ami 2017), it has been suggested, based on field observations, that infections could increase the vulnerability to predation (Møller and Erritzøe 2000; Duffy et al. 2005; Murray et al. 2006; Møller and Nielsen 2007; Miller et al. 2008; Møller et al. 2010; Adelman et al. 2017; Gooding et al. 2020) and there is some experimental support for this (Johnson et al. 2006; DeBlieux and Hoverman 2019; Gallagher et al. 2019). Consequently, by mediating selective predation, parasites can mediate the relationship between hosts and their prey (Hall et al. 2005; Møller 2008). For instance, removing predators can reduce vertebrate prey populations (Sih et al. 1985), which seems counterintuitive, as predators are expected to remove preys. However, mathematical models predict that virulent parasites may be selected against if predation disproportionately removes them before they can be transmitted to the host (Packer et al. 2003). Without this check on virulent parasites by the predator, more prey may die from lethal infections than from predation. Hence, if selective predation for infected individuals is a common pattern, it may also be a major driver of host and parasite evolution and cannot be ignored (Møller 2008).

It is also relevant to consider that not all sickness come from infections and that non-infectious diseases could also increase the risk of being predated. Hence, if it is accepted that parasites may evolve in response to the predator of their hosts, one can imagine that non-infectious diseases, when they have a genetic basis, could also evolve in response to the selective pressure imposed by predators. To our knowledge, few to nothing is known about the potential selective predation of prey that are sick due to a non-infectious disease.

Mutations can occur during the cell replication and division required to grow and maintain multicellular animals, and certain types of mutation can lead to the formation of tumors (National Cancer Institute 2020*a*). In most cases, tumors are benign (Bissell and Hines 2011), but they can be malignant, and even fatal (National Cancer Institute 2020*b*). Clinical categorization of tumors has been established in unchallenging, even 'optimal' environments (i.e. in the laboratory or clinic), usually to the point of death via organ failure. The impact of tumor progression on animal survival under more natural conditions has largely been neglected. However, depending on the relationship between tumor development and the health of the prey, predation could affect the selection of oncogenic mutations and thus the risk to develop cancer. To our knowledge, the possible interactions between a predator and a prey that is afflicted with cancer are poorly understood and have not been studied to date (Vittecoq et al. 2013).

Here, we assess the effect of chronic infection and cancer on the risk of predation. We have used the fruit fly *Drosophila melanogaster*, already a well-characterized model for infectious and non-infectious disease, as a novel model for sick prey. We assess the predation of flies by hunting spiders, using flies chronically infected by a wild-caught bacterial pathogen (*Providencia rettgeri*), or have cancer, under laboratory-controlled conditions. These conditions allow for the ideal experimental setting: to examine the prey choice of many individual predators while knowing the health status of the entire prey population.

Materials and methods

Preys and predators.

We used *Drosophila melanogaster* flies as prey in this study as they are tractable to genetic induction of tumours, are well-defined models for bacterial infection, and allow for control of environmental and genetic differences. Only one sex (males) were used, to avoid sex effects. We induced chronic infection by injecting 23 nL of a suspension of few thousand cells of the bacterium *Providencia rettgeri* (strain *Dmel*, isolated from wild-caught *Drosophila melanogaster;* Juneja and Lazzaro 2009) in phosphate-buffered saline (PBS) into the abdomen of flies (Canton S line). The same volume of sterile PBS without bacteria was injected into another group of flies that served as the control. Individuals surviving for three days or more after injection of the bacteria have an established chronic infection, as we have previously demonstrated (Duneau et al. 2017).

To induce tumors in the flies, we took advantage of signaling pathways that regulate cell growth in mammals, which have a conserved function in *Drosophila*, the EGFR and Wnt pathways (Rudrapatna et al. 2012; Millburn et al. 2016; Mirzoyan et al. 2019; Villegas 2019). We used targeted induction of these pathways (Rasv12 for EGFR, APC RNAi for the Wnt pathway) to induce two degrees of malignancy in the digestive tract of flies. This type of cancer is appropriate for this study, as it does not overtly affect physical performance including locomotion, and will thus allow for the study of the interaction of oncogenic phenomena and predation (Dawson et al. 2018). To generate tumors in the gut of *Drosophila*, flies esg^{TS} (*esgGal4*, $Gal80^{ts}$, *UAS-GFP*) were crossed to either background control flies (Trip stock 35786), flies carrying a $UAS\text{-}Ras^{v12}$ (Bloomington 64196) or flies carrying a combination of $UAS\text{-}Ras^{v12}$ (Bloomington 641926) and *UAS-APC-RNAi* (Bloomington Trip stock 28582) combined. The expression of Ras^{v12} in progenitor cells stimulates an accelerated turnover and a mild accumulation of epithelial cells in the gut, akin to a dysplastic tissue (Buchon et al. 2010; Houtz et al. 2019). The overexpression of Ras^{v12} combined with the knock-down of *APC* leads to increased proliferation, and accumulation of progenitor cells and epithelial cells in the gut, reminiscent of an intestinal disseminated tumor (Wang et al. 2013). F1 flies from these crosses were raised at 18°C (Gal4 off, transgene expression off, normal development and emergence), and 3 days after emergence were switched to 29°C (Gal4 on, transgene expression on) for 10 or 20 days before performing the experiment. As in (Wang et al. 2013), crosses with WT flies showed mild tissue renewal (restricted

GFP signal), flies with expressing *Ras*^{v12} showed disseminated GFP in their gut and flies expressing *Ras*^{v12} and *APC RNAi* showed gut enlargement and GFP accumulation.

We used two families of hunting spiders as predators: jumping spiders of several species from the family of Salticidae (see illustration in Fig. 1) and small wolf spiders from the family of Lycosidae (likely *Pardosa lugubris*). Both jumping and wolf spiders hunt by wandering rather than by using webs as traps.

Jumping spiders have the sharpest vision of any arthropod, even surpassing many vertebrates (Land and Nilsson 2012). They hunt during the day and rely on this astonishing vision when catching a prey. Similar to the way a cat stalks its prey, jumping spiders turn towards their prey, directed by a pair of lateral eyes that provide a nearly panoramic field of view with the ability to discern motion (Zurek and Nelson 2012). Then, the spiders track, approach, and jump on the prey. The vision accuracy is conferred by two pairs of forward-facing eyes that also provide a precise perception of depth (Zurek et al. 2010; Nagata et al. 2012).

In absence of detectable effect on selective predation of infected preys, we decided to test our hypothesis with another type of hunting spider species with different hunting behavior. Wolf spiders are nocturnal hunters and their eyes function mainly as low-light movement detectors. Their vision is not as acute as that of jumping spiders (Land and Nilsson 2012). The use of visual cues in prey detection and orientation have only been well-studied in jumping spiders, and empirical evidence suggests that wolf spiders rely more on vibrations to capture their prey than on vision (Lizotte and Rovner 1988). Hence, the differences between these two types of hunting spiders primarily lie in their ability to prey on flies that are at rest. In an environment where there is no place to hide, jumping spiders would likely prey equally well on moving and non-moving prey, and selective predation in this case should depend largely on the prey's capacity to escape.

All spiders in the study were obtained from the wild in the Cornell University campus (Ithaca, NY, USA) or in the campus of the University of Toulouse 3 (Toulouse, France).

Predation trials

Predation trials were performed by incubating five healthy and five sick flies at 20°C with a spider in a round plastic box (11.5cm x 8.5cm) containing a wet piece of cotton or foam. The flies were first added to the box to settle before the introduction of the predators. After about 30 min, spiders were put in the box. The trials generally lasted for about 4 hours or were stopped when 50% of the flies had been preyed upon. This goal was sometimes exceeded as some spiders ate faster than expected. Individual spiders have never been used twice as predators within an experiment.

To determine the number of surviving flies that were infected, we ground the remaining flies at the end of the predation period and suspended the mixture in 250 µL of Luria-Bertani (LB) medium. A droplet of five microliters of the resulting suspension was spread on a petri dish filled with agar containing LB medium, and the plates were incubated overnight at 37°C to assess for the presence of *P. rettgeri*. We calculated the proportion of infected flies at the end of predation period by determining the number of droplets in which *P. rettgeri* were present. For a control comparison, we performed the same process with infected and healthy flies, but did not expose them to predatory spiders. The number of surviving flies that had cancer were counted by dissecting all surviving flies and observing their guts under a fluorescent microscope, which reveals the presence/absence of cancer.

Statistical analysis

The use of the Fisher exact test or a chi-squared test would have permitted us to test for every trial regardless of whether the number of flies from a particular category was more or less preyed upon than those from another category. However, we used a hypergeometric distribution instead of a Chi-squared distribution because, as flies were preyed upon, the population size decreased. The *p*-value for each experimental trial was added up and compared to the summed *p*-value obtained by simulating random trials. We assessed the overall *p*-value by determining the number of simulated trials required to have an observed result (summed *p*-value observed) as probable as the simulation (summed *p*-value simulated). Hence, a *p*-value of 0.05 indicated that 20 trials would be sufficient to have the same result as the empirical data by chance, whereas a *p*-value of 0.0001 meant that 10,000 trials would be required to achieve the same result as the empirical data by chance. We used the Manly Alpha Index as the preference index to illustrate the selective predation on sick individuals (index 0: preference for

healthy individuals, index 0.5: no preference, index 1: preference for sick individuals). It was calculated as log (total flies sick / flies sick but not eaten) / [(log(total flies sick / flies sick but not eaten) + log(total flies healthy / flies healthy but not eaten)] as established by Manly (1972).

Results

Predation of chronically infected flies by hunting spiders. We tested whether chronic infection of the flies by *P. rettgeri* affected the risk of being preyed upon by the spiders. The flies had been infected for several days before exposure to the spiders, and their immune response was expected to be activated (Chambers et al. 2019). We did not detect selective predation by either of the hunting spider types (Fig. 2A and B for jumping and wolf spiders, respectively).

Predation of WT flies with cancer by hunting spiders. We asked if cancer of the gut, which we induced, affected the risk for predation of flies by the hunting spiders. Hyperplasia is the enlargement of an organ or tissue caused by increased cell division and is often an initial stage in the development of cancer. We observed that flies in this early hyperplastic stage (esg^{ts} > $UAS\text{-}Ras^{v12}$) did not show an increased likelihood of getting preyed upon by the jumping spiders, which are active diurnal hunters (Fig. 3A). However, jumping spiders did catch more successfully flies with an advanced stage of cancer (esg^{ts} > $UAS\text{-}Ras^{v12}$, *UAS-APC-IR*) (compared with healthy flies, and the preference seemed to increase with the length of time after the tumor had been induced (Fig. 3B).

Discussion

Many diseases do not kill their hosts and are considered benign in optimal conditions, such as in a clinic or laboratory. However, it is very likely that non-lethal illnesses still increase the risk of mortality through indirect interactions with other environmental factors. Indeed, being sick may reduce foraging and mating abilities, increase the risk for lethal superinfections, or of being preyed upon. Even though many biological questions are presently being addressed using an integrative approach, the question of whether a non-lethal disease is truly benign in natural conditions seems to

have been overlooked in many cases. We hope to generate interest in evaluating the lethality of diseases when their interaction with other risks, such as predation, is considered. Chronic infection by a wild-caught *Drosophila* pathogen did not significantly affect the risk of being preyed upon in the laboratory. In contrast, we observed that while the presence of tumors in the early stage of development did not change predation risk, the presence of advanced cancer did.

Little is known about selective predation in individuals with non-infectious diseases. The prevailing opinion that general health, which can be affected by non-infectious sickness, correlates with the risk of being preyed upon, is largely observational (Hoey and McCormick 2004; Genovart et al. 2010). Nonetheless, although we should not be jumping to hasty conclusions, our results on the effects of non-infectious disease on selective predation suggests a stronger selective pressure than natural chronic infections.

Cancers are common in multicellular organisms and can occur whenever cells evade the normal cell checkpoints that control cell division, proliferation, and apoptosis, thus leading to uncontrolled cell division (Aktipis et al. 2015). Individuals always have some tumors, most of which never become lethal (Bissell and Hines 2011; Abu-Helil and van der Weyden 2019). Our understanding of oncogenic phenomena in wild populations of animals is limited, in particular whether tumors that are clinically non-lethal affect the well-being of organisms in the wild (McAloose and Newton 2009; Vittecoq et al. 2013; Pesavento et al. 2018). If tumor formation is inevitable, it is likely that multicellular organisms have evolved ways to control and tolerate them, perhaps resulting in a trade-off between surviving the tumor and surviving other perils, such as infections or predation (Pavard and Metcalf 2019). In some cases, selection for tumor tolerance may have arisen from selection for tolerance to other factors, perhaps explaining why early stages of cancer, such as the colon hyperplasia that we induced in *Drosophila*, do not change the likelihood of being preyed upon. In our study, when the tumor developed into a cancer, predation of the flies increased, suggesting that uncontrolled tumors strongly increase the likelihood of being preyed upon. This conclusion supports the idea that individuals with uncontrolled tumors will suffer the risk of increased predation compared with healthy individuals. Predation is a critical force shaping natural selection (Wade and Kalisz

1990). By removing prey genotypes susceptible to tumors, predators are likely to have selected for the tumor tolerance largely observed in nature (Bissell and Hines 2011). Furthermore, by removing individuals with advanced tumors, predation may explain why cancerous animals are rarely recorded in autopsies of wild animal populations (Vittecoq et al. 2013).

The progression of cancer can, in many ways, be compared with infectious diseases. However, if both reduce viability and survival, infectious diseases are, unlike oncogenic processes, under selective pressure to ensure transmission of the pathogen to another host. For both non-infectious diseases and cancer, selective predation could increase the selection against the sickness, as diseases that are moderate and non-lethal could lead to a higher risk of the host being preyed upon, resulting in mortality rates that are similar to those of infectious diseases. With infectious diseases, there may be additional consequences. First, selective predation could affect the transmission of pathogens, spreading them through predator feces over long distances. Second, it might affect epidemics, either preventing them by removing parasite spreaders from the host population (Duffy et al. 2005) or by increasing their likelihood of occurrence by dispersing parasites instead of containing them in dead hosts (Strauss et al. 2016). An additional consideration is that predation may affect the evolution of parasite virulence. It is generally assumed that predation can reduce virulence because hosts with rapidly proliferating parasites would suffer more from the infection and may be eaten before transmitting the parasite to another host, whereas the less rapidly proliferating parasite would impact less host health and would be transmitted before the host is eaten (Møller et al. 2010). However, lower virulence could evolve also through lower pathogenicity, that is by inducing less damage to the host, and not only through reduced proliferation. The most important factor being that bacteria can proliferate within their host as long as the symptoms do not increase the risk of predation. The bacteria studied here, *P. rettgeri*, was obtained from the wild and is prevalent in populations of *D. melanogaster* (Juneja and Lazzaro 2009). Despite being chronic and the fact that the pathogen can trigger an immune response through the course of the infection (Duneau et al. 2017; Chambers et al. 2019), the chronic infection did not increase the predation in our study. This result supports the

hypothesis that chronic infections are selected for such that the symptoms that enhance predation are reduced.

It is reasonable to assume that the success of predators is not simply due to favorable luck. In fact, selective predation may be more the rule than the exception, although it is not understood what makes a prey susceptible. The value of an integrative approach is recognized by most biologists and dictates that we attempt to understand the role of predation in the evolution of diseases, infectious or not. On the one hand, selective predation on sick individuals is likely to counter-select against parasites or genetic diseases more strongly than if selection occurred upon disease-mediated host death. On the other hand, in the same way that some parasites evolved to manipulate their host to increase transmission to intermediate hosts, parasites may have evolved to make their hosts less conspicuous to predation, for example, by interfering with the search for mates or by lessening the effects of the disease on the host; for example, by reducing symptoms. Because cancer progression generally results in the death of the organism, we cannot expect the incidence of lethal cancer to have evolved in the same way as parasites. We argue that selective predation on preys with cancer may be one of the reasons why tumors are commonly tolerated in animals, where advanced cancer is only rarely detected.

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Figure legends

Figure 1. Photograph of a jumping spider consuming *Drosophila melanogaster* flies in the laboratory.

Figure 2. Predation by hunting spiders on a population of *Drosophila melanogaster* Canton S, of either healthy flies or flies chronically infected with *P. rettgeri*. A. Predation by jumping spiders (Hypergeometric test: n= 27 trials, *p*-value > 0.05, Median pref. index = 0.64) B. Predation by wolf spiders (Hypergeometric test: n= 30 trials, *p*-value > 0.05, Median pref. index = 0.43). Each dot represents the proportion of individuals eaten over five flies and the lines connect the groups of individuals from a same trial. Each trial represents ten flies (five from each type) presented to one spider at once. Preference index is calculated as in Manly (1972) and ranges from 0 to 1, from a preference to healthy flies to a preference for sick flies, respectively.

Figure 3. Predation by hunting spiders in a population of *Drosophila melanogaster* that were either healthy or in which colon tumours (hyperplasia) had been induced. Tumour size increased with induction time, during which uncontrolled cell division occurred. A. Uncontrolled cell division was triggered for 10 or 20 days in $esg^{TS} > Ras^{v12}$ flies to lead to hyperplasia (respectively with n= 17 and 15 trials). In both cases, individuals with hyperplasia were not differently predated upon compared with the healthy individuals (Hypergeometric test *p*-value: 10 days > 0.05; 20 days > 0.05, Median pref. index = 0.5). B. Fast uncontrolled cell division was triggered for 10 or 20 days in $esg^{TS} > Ras^{v12}$, *APC-RNAi* flies to lead to cancer (respectively with n= 16 and 20 trials). In both cases, individuals with cancer were more likely to be predated upon than individuals who did not have cancer (Hypergeometric test *p*-value: 10 days = 0.025; 20 days = 0.002). This pattern tended to be stronger as the tumour grew larger (Median pref. index: 10 days = 0.64; 20 days = 0.8). Each dot represents the proportion of individuals eaten over five flies and the lines connect the groups of individuals from a same trial. Each trial represents ten flies (five from each type) presented to one spider at once. Preference index is calculated as in Manly (1972) and ranges from 0 to 1, from a preference to healthy flies to a preference for sick flies, respectively.

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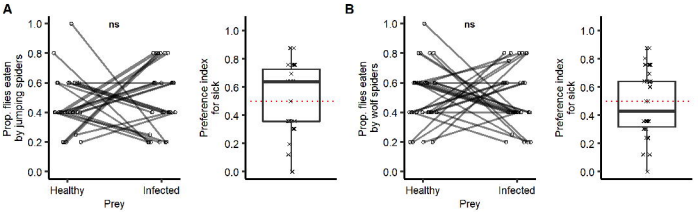

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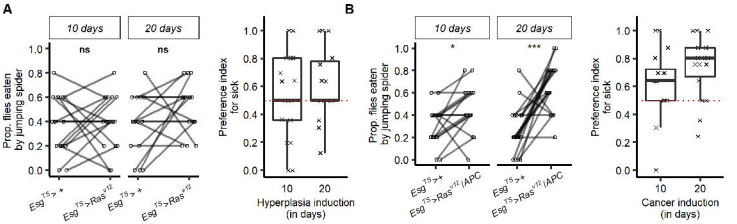

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