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Sex differences in disease avoidance behavior vary across modes of pathogen exposure

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Abstract

Sex differences in disease susceptibility are widespread, and these disparities are often compounded in cases where sexual dimorphism increases exposure risk to parasites for one sex more than the other. Studies rarely link sex differences in disease susceptibility to sex differences in infection avoidance behavior. Yet, understanding the intersection of hosts' susceptibility to infection and infection avoidance behavior is essential to predicting infection risk variation. Here, we use the fruit fly *Drosophila melanogaster* and a generalist entomopathogenic fungus, *Metarhizium robertsii*, which can be transmitted directly, indirectly, and post-mortem as a model host–pathogen system. We test whether the relationship between susceptibility to infection and pathogen avoidance behavior covaries with host sex. We first measured differences in resistance between male and female flies after three different types of exposure—direct, sexual, and environmental—to infectious fungal conidiospores. Then, we tested whether male and female flies differed in the likelihood of mating with infected partners and their avoidance of food patches with increased infection risk. Females were more susceptible to infection under all three exposure techniques. When confronted with an infectious partner, females mated sooner than males. However, when given a choice between an exposed partner and an unexposed partner, females take longer to begin copulating compared with males, though neither sex was more likely to choose the unexposed partner than expected by chance. Neither male nor females flies avoided food patches containing infectious conidiospores, though only females show an aversion to food sites containing an infectious fly corpse. These experiments suggest that sex differences in disease susceptibility may be counteracted via differential pathogen avoidance behavior, though the strength of avoidance behavior appears to vary across different contexts of infection risk.

KEYWORDS

Drosophila melanogaster, entomopathogenic fungus, habitat choice, infection avoidance, mate choice, sexual dimorphism

1 | INTRODUCTION

Differences among hosts in their resistance to infection and behaviors influencing exposure risk can be important drivers of disease dynamics. For many infectious diseases, males and females differ in their infection risk and severity of disease symptoms (Duneau, Luijckx, Ruder, & Ebert, 2012; Gipson & Hall, 2016; Zuk & McKean, 1996). In many cases, males are more susceptible to diseases than females (Klein, 2000; Zuk, 2009), an effect that can be exacerbated by differential exposure to pathogens via sex

differences in behaviors like space use, aggressiveness, and sexual behavior (De Lisle, Rowe, & evolution, 2015; Tinsley, 1989; Zuk & McKean, 1996). In host–parasite systems with sex differences in disease susceptibility, selection should favor more pronounced defenses against infection in the "sicker sex" (Nunn, 2003; Stoehr & Kokko, 2006), and the front-line of defense against disease is inherently behavioral, that is, avoidance of environments or situations of increased infection risk (Behringer, Butler, & Shields, 2006; Parker, Barribeau, Laughton, de Roode, & Gerardo, 2011; Parker, Elderd, & Dwyer, 2010). Although we expect more pronounced avoidance strategies to emerge in the more susceptible sex if all other factors are equal, all other factors are rarely equal and context-dependent behavioral strategies are common. Thus, infection avoidance behaviors should also be tested across different contexts in which hosts can encounter infectious agents.

Three fundamental strategies for avoiding parasite exposure are as follows: (a) avoiding the parasites themselves, (b) avoiding infectious conspecifics, and (c) avoiding environments of increased infection risk (summarized by Curtis, 2014). Animals avoid parasite-rich environments when foraging (Hutchings, Kyriazakis, Papachristou, Gordon, & Jackson, 2000), choosing nesting sites (Oppliger, Richner, & Christe, 1994), and when assessing social partners and mates (Behringer et al., 2006; Kavaliers, Fudge, Colwell, & Choleris, 2003; Khan & Prasad, 2013), and these responses can be sex-specific (Hund, Aberle, & Safran, 2015). However, the efficacy of these avoidance strategies may vary depending on the parasite's mode(s) of transmission and the context under which different individuals encounter infectious agents. For example, parasites that can be transmitted both directly and indirectly may require multiple cues to be recognized and avoided (e.g., parasite chemical cues and conspecific sickness behaviors). Because males and females may differ in infection risk across different modes of transmission, studies should link sex differences in susceptibility to sex differences in avoidance behavior using a host–parasite system in which hosts encounter disease-causing agents across multiple behavioral contexts or via multiple modes of transmission. We use a model host–pathogen system to ask whether sex differences in infection avoidance behaviors are more pronounced in the more susceptible sex, and whether this differs across different contexts of pathogen exposure.

Here, we use the fruit fly *Drosophila melanogaster* to explore sex differences in susceptibility to a generalist entomopathogenic fungus and test whether more pronounced infection avoidance behaviors are observed in the more susceptible sex. We tested for susceptibility to infection by a generalist entomopathogen across three contexts: (a) direct inoculation to remove the influence of avoidance behaviors, (b) exposure to spores via an infectious mating partner, and (c) environmental exposure via a contaminated substrate. Testing across multiple exposure regimes is important because individuals may acquire different spore loads, and recognition of increased infection risk may require different cues across different contexts. We then tested for individuals' avoidance of infected mating partners and their avoidance of food patches containing either infectious conidiospores or a sporulating conspecific corpse.

2 | METHODS

2.1 | Test systems and laboratory maintenance

In the fruit fly *D. melanogaster*, sex differences in disease susceptibility vary across parasite species and host life stages (Kraaijeveld, Barker, & Godfray, 2008; Polak & Markow, 1995). For some bacterial and fungal pathogens, female *D. melanogaster* are more susceptible to infection compared with males (Lu, Wang, Brown, Euerle, & Leger, 2015; Wang, Lu, & Leger, 2017). Male *Drosophila* often exhibit superior innate immune function against pathogens which can be horizontally transmitted (McKean & Nunney, 2005), and females may compensate for this via the anticipatory upregulation of immune-response genes during courtship by males (Zhong et al., 2013). Female flies may also exhibit more pronounced infection avoidance behaviors, as Vale and Jardine (2016) found that previously exposed females are less likely than males to land on food patches that contain live Drosophila C virus. However, females may exhibit more of these avoidance behaviors than males in contexts under which they have increased exposure risk compared with males.

In all experiments, we used three-day- to five-day-old heterozygous virgin F1 offspring of flies collected from inbred parental lines obtained from the *Drosophila* Genetics Reference Panel (DGRP2. gnets.ncsu.edu; Mackay et al., 2012). We mated virgin males and virgin females from DGRP inbred homozygous lines to generate heterozygous F1 offspring of replicated genotypes. Flies were maintained in vials on standard fly media (see supplementary information at Dtyad https://doi.org/10.5061/dryad.gtht76hgr).

We tested flies' susceptibility to and avoidance of the generalist entomopathogenic fungus *Metarhizium robertsii*, which infects a broad host range including *Drosophila* flies. We used *Metarhizium* because generalist fungal pathogens are thought to cause the majority of insect diseases and thus impose important selective pressures (Roberts & St Leger, 2004). Further, *Metarhizium* spp. can be acquired via multiple routes of infection (e.g., sexual acquisition and environmental acquisition) (Dimbi, Maniania, & Ekesi, 2013; Keiser, Rudolf, Sartain, Every, & Saltz, 2018; Zhong et al., 2013) unlike some viruses and bacteria that are *Drosophila* specialists and often infect hosts only via the oral route. This strain (ARSF# 2576) was originally obtained from the USDA-ARS Collection of Entomopathogenic Fungi Cultures in Ithaca, New York, USA. Fungal cultures were grown for 3–4 weeks on Potato Dextrose Agar before collecting infectious conidiospores by using a sterile inoculating loop to scrape conidiospores into a sterile vial. Spore vials were vortexed for 15s to homogenize the collected spores into a fine powder for experimental applications. Different host genotypes were used haphazardly within and across experiments. However, in experiments where fly behavior was tested in pairs or groups, individuals were only tested with others of the same genotype. Given that innate immune responses can vary with age in *D. melanogaster* (Felix, Hughes, Stone, Drnevich, & Leips, 2012; Zerofsky, Harel, Silverman, & Tatar, 2005), in a preliminary experiment we verified that one-day-old and three-day-old flies did not vary in susceptibility to *M. robertsii* infection

(Mantel-Cox log-rank test; $\chi^2 = 0.73$; $p = .39$), suggesting that 3-day-old and 5-day-old flies may not vary drastically.

2.2 | Sex differences in susceptibility

2.2.1 | Direct inoculation

To test for sex differences in susceptibility across contexts (i.e., modes of pathogen exposure: via the sexual partners or via the environment), we exposed three-day-old male ($n = 48$) and female ($n = 35$) flies directly to infectious conidiospores and monitored their survival. To expose flies, we transferred each fly individually into a vial containing ~1 mg of freshly collected conidiospores and then using a fine paintbrush, transferred the fly back into its housing vial (similar to methods in Hunt et al., 2016; Keiser et al., 2018). We checked each fly daily for 20 days and recorded the number of days until death for each fly. To verify their infection status, we sterilized the cadaver's outer body surface and placed it on damp filter paper to allow fungal growth from inside the body (following protocols in Lacey, 1997). Any fly where we found no evidence for infection was removed from the analysis. Thus, we were comparing the disease-associated mortality among host sexes using a conservative estimate of infection status. No flies that died past the 20 day observation period were found to be infected, though our current methods cannot differentiate between individuals that were never infected versus individuals that cleared the infection. Control flies ($n = 30$ female and $n = 24$ male) were similarly exposed to autoclaved spores that are non-infectious.

2.2.2 | Exposure via mating partner

To test for differences in susceptibility to infection via sexual contact, we exposed male ($n = 26$) and female ($n = 27$) flies to conidiospores as before and placed them back in their solitary housing vial for 24 hr. After this time, the exposed fly was moved to another vial containing a single unexposed fly of the opposite sex (the "focal fly"). If a mating event occurred, we removed the focal fly and placed it back into its housing container and monitored its survival for 20 days. Any pairs that did not mate were excluded from the analysis; no unmated focal flies became infected. Further, we removed from analysis any pairing in which we found that the experimentally exposed stimulus fly did not become infected ($n = 2$ males and $n = 2$ females). Control pairs ($n = 14$) were set up where two unexposed flies were mated, and we monitored their survival for 20 days.

2.2.3 | Exposure via environment

We measured survival after exposure to infectious conidiospores in the environment by placing flies ($n = 29$ females and $n = 29$ males) individually into housing vials in which the food had been sprinkled with ~0.1 mg of spores and vortexed briefly to evenly apply the spores across the surface of the food. Flies were housed alone in these vials until death. We similarly prepared 20 vials with autoclaved, non-infectious conidiospores and added virgin flies ($n = 10$ females and $n = 10$ males) to test for non-disease related mortality but none of these individuals died before 20 days.

2.3 | Avoidance of infected mates

We tested for flies' avoidance of infected mating partners using both no-choice and two-choice experimental designs. Combining no-choice and two-choice experiments has previously been suggested for quantifying mating preferences (Dougherty & Shuker, 2014; Rutstein, Brazill-Boast, & Griffith, 2007; Shackleton, Jennions, & Hunt, 2005; Wagner, 1998). All mate-choice assays were conducted between the hours of 09:00hr and 12:00hr. None of the flies in behavioral assays were anesthetized to move between housing vials and experimental chambers to avoid effects on fly behavior. We first performed no-choice mating assays, where 3-day-old focal flies ($n = 21$ females and $n = 22$ males) were moved into the housing vial of a fly of the opposite sex, but of the same genotype, that had been exposed to *M. robertsii* conidiospores 24 hr prior. After pairing a focal fly and an infected fly, we observed the pair continuously and allowed them to interact undisturbed for 120 min, or until mating began. If the pair mated, we noted the latency until copulation began. After mating had ceased, we separated the pair back into their own housing vials and measured the time until death of focal flies. The no-choice assays were compared with control pairs ($n = 21$) where neither individual had been exposed to conidiospores.

In two-choice assays, a focal fly ($n = 25$ females and $n = 15$ males) was moved into a vial with two "stimulus" flies of the opposite sex: One exposed fly and one unexposed fly. To differentiate the stimulus flies, the exposed fly was marked with a small dot of acrylic green paint atop its dorsal thorax, and the unexposed fly was marked with a pink dot. With a separate experiment, we found no evidence that paint color influences fly mate choice (unpublished data). Control groups ($n = 10$ male and 10 female focal flies) were formed similarly where no fly was exposed to conidiospores, but were marked in the same way. In two replicates (5% total) with male focal flies, we found that the unexposed stimulus female became infected.

2.4 | Avoidance of contaminated environments

2.4.1 | Conidiospores present

We tested for flies' avoidance of food patches that have been exposed to *M. robertsii* conidiospores following methods similar to Vale and Jardine (2016). We placed groups of four 5-day-old flies of the same sex and genotype into a 100 mm petri dish containing two smaller food patches (diameter = 35 mm) containing grapefruit juice agar (see supplementary information at Dtyad https://doi.org/10.5061/dryad.gtht76hgr). One of the food patches was inoculated with 100 µl of a *M. robertsii* conidiospore suspension (7.26×10^7 conidiospores/ml) in 0.05% sterile Triton X-100 (Sigma-Aldrich). The solution was spread evenly across the surface of the food patch using a sterile inoculating loop and allowed to dry overnight. The uncontaminated

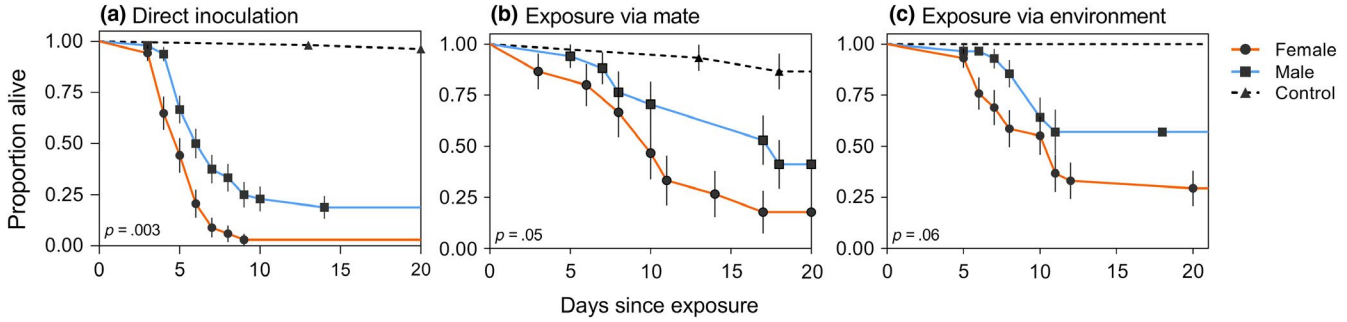

FIGURE 1 Sex differences in susceptibility to infection. (a) Females die more rapidly when exposed directly to *M. robertsii* conidiospores. (b) After mating with an exposed partner, females died more rapidly than males. (c) When housed in a vial with infectious conidiospores previously deposited by an exposed conspecific, we found no evidence for sex differences in mortality. *p*-Values in each panel represent the male–female comparison for that exposure type [Colour figure can be viewed at wileyonlinelibrary.com]

patch was treated only with sterile Triton X-100. We then allowed four flies of the same genotype and sex to enter the arena through a micropipette tip (see supplementary information at Dtyad https://doi.org/10.5061/dryad.gtht76hgr) at 09:30hr. We made 10 observations in total, 30 min apart, each time recording the number of flies that were on either of the food patches.

2.4.2 | Corpse present

We also tested for flies' avoidance of food patches containing an infectious conspecific cadaver using the same experimental design described above (four flies of the same sex), but the contaminated food patch contained a single infected fly cadaver that had begun sporulating 24 hr prior while the other patch contained a recently frozen and thawed uninfected corpse of the same sex. We manipulated focal fly sex (n = 19 females and n = 20 males) and the sex of the fly corpse in a fully factorial design.

2.5 | Ethics statement

These experiments were conducted on invertebrate animals and thus are not under the governance of the US National Research Council, though we adhered to the guidelines of the ASAB/ABS Guidelines for the care and research of animals wherever possible.

2.6 | Statistical analyses

2.6.1 | Sex differences in susceptibility

Female *D. melanogaster* typically live longer than males (Linford, Bilgir, Ro, & Pletcher, 2013; Nuzhdin, Pasyukova, Dilda, Zeng, & Mackay, 1997), so natural sex differences in life span may influence the interpretation of infection-induced mortality. However, only four control flies died within our 20 day observation period across all three experiments, so we instead focused our analyses on the direct comparison between male and female infected flies. Female *D. melanogaster* also experience reduced life span after mating (Fowler & Partridge, 1989), though this phenomenon occurs on a timeframe that surpasses our window of observation (20 days). Sex differences in susceptibility across the three exposure types were analyzed using Mantel-Cox log-rank tests with host sex as an independent variable.

2.6.2 | Avoidance of infected mates

Sex differences in mate choice in no-choice and two-choice assays were analyzed by log-transforming latency-to-copulate values and then analyzing them with general linear models (GLM) with treatment as an independent variable (male focal fly, female focal fly, or control). This GLM was based on Type III Sum of Squares. In two-choice tests, we analyzed whether males and females differed in their likelihood of choosing the infected or uninfected partner with a nominal logistic regression.

2.6.3 | Avoidance of contaminated environments

To assess avoidance of food patches contaminated with conidiospores, we analyzed the average number of flies observed on the contaminated and control food patches with a repeated measures MANOVA using an unstructured covariance matrix to account for non-independence between the response variables. We included host sex, patch type (contaminated vs. control), and their interaction term as independent variables. To assess avoidance of food patches containing an infectious corpse, we used another repeated measures MANOVA as above, including host sex, corpse sex, patch type, and interaction terms between patch type, host sex, and corpse sex as independent variables. For all GLMs and MANOVAs, we confirmed normality of model residuals using Shapiro–Wilk tests and assessed homogeneity of residual variance using visual inspection. All statistical analyses were performed in JMP Pro version 14.1.

3 | RESULTS

3.1 | Sex differences in susceptibility

Females were more susceptible to infection compared with males when directly exposed to conidiospores, with a median time to death 2.5 days

sooner than males (χ^2 = 8.75, *df* = 1, *p* = .003; Figure 1a). This trend is especially notable given that female *D. melanogaster* typically live longer than males (Linford et al., 2013; Nuzhdin et al., 1997). About 20% of directly exposed males never became infected whereas only 3% of directly exposed females remained uninfected. Females also died sooner when infection was acquired via sexual contact with an infectious mate, with a median time to death of 10 days compared with 18 days for males (χ^2 = 3.75, *df* = 1, *p* = .05; Figure 1b). We found less statistical support for sex-difference in survival when flies were exposed to spores via a contaminated environment (*p* = .06; Figure 1c). However, the curves appear similar across all infection contexts, where females die more rapidly or a larger proportion of females died during 20 days of observation.

3.2 | Avoidance of infected mates

When confronted with an infected partner in no-choice tests, focal females mated in 71% of cases and males mated in 60% of cases (82% of control pairings resulted in copulation), though the sex of the focal fly did not predict whether mating would occur (χ^2 = 2.79, *df* = 2, *p* = .25). In no-choice tests, females began mating more quickly when confronted with an infected partner compared to males and compared to control pairs where no one was exposed ($F_{2,44}$ = 3.4, *p* = .04; Figure 2a).

For two-choice tests, the latency to copulate did not differ between focal males or females in control groups (*p* = .35), so they were combined for analysis. When confronted with two potential mates, neither male nor female focal flies were more likely to mate with the uninfected partner over the infected partner (47% of females and 60% of males mated with the infected partner; χ^2 = 0.06, *df* = 1, *p* = .81). However, when given the choice between an infected and uninfected partner, focal males mated faster than focal females: males in under 300s on average, while female focal flies mated at 650s on average, and control groups mated after 1200s on average ($F_{2,53}$ = 13.02, *p* < .0001; Figure 2b). Although male and female focal flies in the two-choice control trials did not differ in latency to mate, we performed an additional analysis to control for potential inherent sex differences in mating behavior. We standardized the latency-to-copulate values by dividing each replicate by the average latency value for to their respective controls (male vs. female focal flies), log-transformed the standardized values, and ran another GLM. This additional analysis corroborated the previous result: Focal males mated

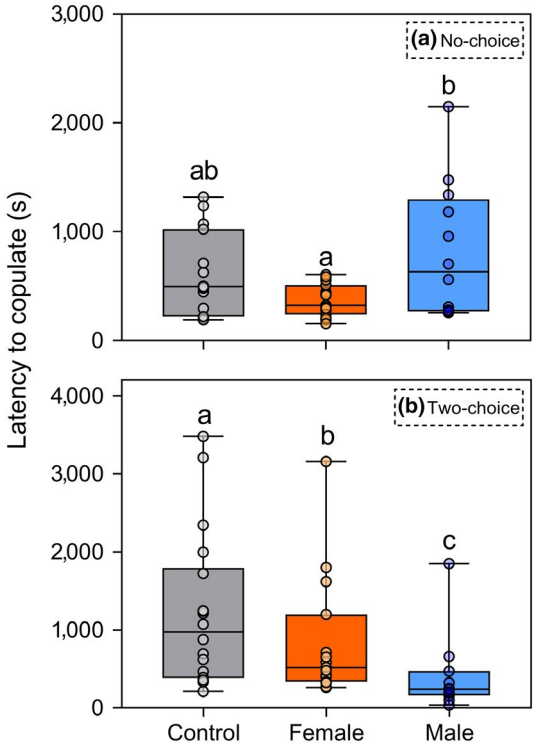

FIGURE 2 Sex differences in mate choice. (a) In no-choice tests, females mated more rapidly when confronted with an infected mating partner compared with males. Control pairs of unexposed males and females exhibited intermediate latency values. (b) In two-choice tests, when confronted with an exposed and unexposed individual of the opposite sex, focal females took longer to begin mating compared with focal males, regardless of with which fly they mated. We found no evidence for a difference in latency to mate when males and females were confronted with two unexposed partners, so those data were compiled in the control group. The boxes extend from the 25th to the 75th percentiles, the horizontal line represents the median, and the bars extend to the smallest and largest values. Circles represent individual data points [Colour figure can be viewed at wileyonlinelibrary.com]

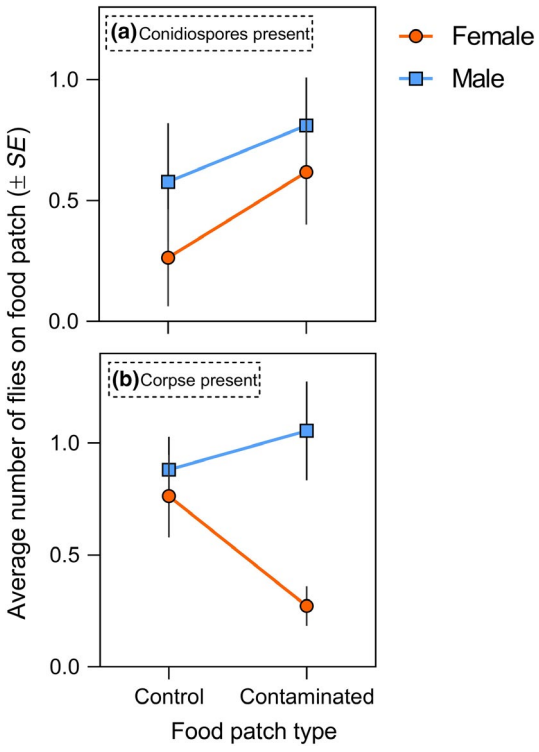

FIGURE 3 Sex differences in avoidance behavior. (a) When given the choice between an unexposed food patch and a patch that had been contaminated with conidiospores 24 hr earlier, males and females were equally likely to be found on both patches. (b) When given the choice between a food patch with an infectious corpse present and a patch with a non-infectious corpse, we observed fewer females on the contaminated patch compared with males, but males and females were equally likely to be found on the control patch [Colour figure can be viewed at wileyonlinelibrary.com]

more quickly when provided the choice between and infected and uninfected partner compared with focal females and disease-free controls ($F_{2,53}$ = 13.02, p < .0001).

3.3 | Avoidance of contaminated environments

In trials where flies could choose between an infectious food patch (containing infectious conidiospores) and a non-infectious control patch, we found no evidence that males and females differed in patch choice (repeated measures MANOVA: $F_{1,18}$ = 2.21, p = .15; Figure 3a) and neither sex was more likely to be found on the control patch (i.e., no evidence for avoidance: repeated measures MANOVA: $F_{1,19}$ = 1.34, p = .26; Figure 3a). When food patches contained either an infectious conspecific corpse or an uninfected corpse, we found no evidence that males avoided the patch containing the infectious corpse, whereas we observed 22% fewer females on the infectious patch compared with males (sex × patch interaction term: $F_{1,37}$ = 4.21, p = .04; Figure 3a). Female corpses also attracted more flies onto the food patch compared with male corpses, regardless of infection status ($F_{1,37}$ = 5.48, p = .02). Thus, we found no evidence that males and females preferred the control food patch over a food patch containing infectious conidiospores, but females were less likely than males to be found on a food patch containing an infectious conspecific corpse.

4 | DISCUSSION

In host–parasite systems with sex differences in susceptibility, selection should favor more pronounced disease avoidance behaviors in the more susceptible sex. Here, we tested for sex differences in disease avoidance behavior and susceptibility to a generalist entomopathogenic fungus in the fruit fly *D. melanogaster*. We found that females were more susceptible to direct and sexually acquired infections compared with males, and females also exhibited stronger avoidance behaviors when confronted with infectious conspecifics (living and dead) but not infectious environments.

4.1 | Avoidance of infected mates

The risk of acquiring parasites via conspecifics is a key selective pressure on social behavior (Altizer et al., 2003; Côté & Poulin, 1995). The *Contagion Indicator Hypothesis* posits that females choose males based on traits that indicate a male's infection status to reduce the risk that females will acquire these parasites (Able, 1996). We found no evidence that males or females avoided mating with infected partners in both no-choice and two-choice assays. Female *D. melanogaster* are larger than males and thus have more surface area on which spores may attach, and they experience reduced life span after mating even in the absence of sexually transmitted infections (Fowler & Partridge, 1989), which may compound the sex differences in disease susceptibility, suggesting that selection should favor females that discern the infection status of potential mates. It may be that flies are unable to recognize the infection status of potential mates at this stage of infection, especially if infection-induced changes to behavior have not set in. Previous reports have noted male aversion to mating with bacteria-infected females (Wittman & Fedorka, 2015), so this may be a pathogen-specific effect. Future studies would benefit from observing mate choice across a time course of infection to observe the onset of potential sickness behaviors and how this coincides with the period of infectiousness.

We found that the latency to begin mating, considered to reflect a component of mate-preference (Shackleton et al., 2005), differed between sexes in both no-choice and two-choice tests: Females mated faster when presented with only an infected partner while males mated faster when presented with an infected and uninfected partner simultaneously. Reproductive patterns can be influenced by multiple non-independent factors, including male courtship effort (e.g., Wignall & Herberstein, 2013) and female receptivity to mating (e.g., Roberts, Cushing, & Carter, 1998). Thus, several non-exclusive mechanisms could have caused the outcomes we observed. For example, perhaps infected males increase their courtship effort to compensate for decreased life span and to capitalize on immediate reproductive opportunities ('host compensation hypothesis'; Polak & Starmer, 1998), leading to a decreased latency to mate with uninfected focal females. Conversely, females may reduce their sexual receptivity after exposure, thus producing an increased latency to mate with focal males.

In two-choice tests, focal males mated more quickly when presented with an infected and uninfected partner compared with focal females confronted with an infected and uninfected male. In control trials where focal flies were confronted with two uninfected flies, we found no sex-difference in mating latency. This may have been driven by courtship interference between the two "stimulus" males, extending the latency until any copulation could have occurred (Gabor, Krenz, & Jaeger, 2000; Savalli & Fox, 1999; Wong & Candolin, 2005), which may be exacerbated when one male is infected. We know of no current evidence for courtship interference between females. Recent data suggest that *D. melanogaster* males decrease their aggregation behavior after viral infection (Siva-Jothy & Vale, 2019), though how infection alters courtship and courtship interference remains unknown. These potential explanations are non-exhaustive, and further experiments measuring courtship effort, mating interference, and female receptivity will shed more light on how individuals avoid disease in a sexual context.

Overall, we found little evidence that either sex avoids mating with infected partners, even when an uninfected partner is available. This suggests ample opportunities for both sexes to become infected via sex, contrary to our predictions. Sex differences in mating behavior when flies encounter an infectious mate, therefore, may be driven by the behavioral responses of the focal individual to an infected potential mate, by the behavior of the infected fly, or both.

4.2 | Avoidance of infectious environments

Identifying and avoiding environmental sources of infection are important components of disease risk. For insects, avoidance of infectious

cadavers even on small scales can alter individuals' infection risk and potentially their role in epizootics (Eakin, Wang, & Dwyer, 2015). We found no evidence that males avoided food patches that contained infectious conidiospores or sporulating cadavers, though females appeared to avoid food patches that contained infectious corpses. Males may activate immune defenses in the presence of infectious corpses, thus reducing the need for avoidance behaviors (see Klemme & Karvonen, 2016 for evidence in sea trout), though this remains to be tested in our system and others. We also observed three cases where males were courting and attempting to copulate with female corpses, one of which was with an infectious corpse. Thus, the trend where 35% of males were found on the infectious patch when the corpse was female compared with 20% when the corpse was male suggests that the benefits of potential reproduction may override infection avoidance behaviors in males but not females.

4.3 | Conclusions and future directions

In cases where males and females differ in susceptibility to disease, we expect more pronounced avoidance strategies to emerge in the more susceptible sex, all other factors being equal. Of course, all other factors are rarely equal, and context-dependent behavioral strategies are common. Here, we found that female *D. melanogaster* were more susceptible to infection by a generalist entomopathogenic fungus, though females only exhibited a greater degree of infection avoidance than males under some contexts but not others. We addressed three parasite-avoidance strategies described by Curtis (2014): avoidance of parasites, avoidance of infectious conspecifics, and avoidance of infectious environments. Surprisingly, we found little evidence that flies of either sex were adept at avoiding situations where they may become infected with this deadly fungus. An interesting possibility is that high-quality mates (i.e., the most attractive individuals) may be the most likely to acquire *and* pass along sexually transmitted infections, generating contrasting selective pressures in the absence of cues to suggest partner infection status (Kokko, Ranta, Ruxton, & Lundberg, 2002).

Identifying which cues are used by male and female fruit flies to determine infection risk under each of these contexts may explain the observed patterns. Given that we tested for avoidance of infectious environments using flies in groups, the role of sex differences in socially acquired information in the avoidance of pathogens should also be addressed (Kavaliers & Choleris, 2018). The mere presence of parasites in the environment can alter host behavior before infection takes place, with important consequences for ecology and evolution (Buck, Weinstein, & Young, 2018; Weinstein, Buck, & Young, 2018). Thus, incorporating sex differences in parasite-avoidance strategies across different modes of parasite infection will aid in our understanding of how parasite distribution across environments alters patterns of disease prevalence in nature.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data associated with this manuscript are available at Dryad Digital Repository (https://doi.org/10.5061/dryad.gtht76hgr).

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