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Extending the ecology of fear: Parasite-mediated sexual selection drives host response to parasites

Collin J. Horn*, Monika K. Mierzejewski, Maesha E. Elahi, Lien T. Luong

Department of Biological Sciences, University of Alberta, CW405 Biological Sciences Bldg., Edmonton, AB T6G 2E9 Canada

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ABSTRACT

The 'ecology of fear' describes the negative effects natural enemies have on potential victims even when those victims are not consumed or infected. Although recent work has demonstrated parasites have non-consumptive effects (NCE) on potential hosts, how these effects vary within host populations is not well understood. We investigated how NCE vary based on host risk of infection and relative cost of infection by measuring the metabolic rate (MR) of naive *Drosophila nigrospiracula* exposed to an ectoparasite, *Macrocheles subbadius*. We tested two mutually exclusive hypotheses: 1) asymmetrical costs of infection drive adaptions for stronger responses to parasite exposure; or 2) asymmetrical risks of infection drive adaptions for stronger responses to parasite exposure. In this system, male flies have higher costs of infection relative to female flies due to parasite-mediated sexual selection; similarly, virgin females experience higher costs of infection relative to mated females. Risk of infection also varies among flies because mites preferentially infect female flies over males, and mites preferentially infect mated females over virgin females. Our results were compatible with the hypothesis that costs of infection drive the strength of response to mite risk. Female flies responded to parasite exposure with a 15.1% increase in MR, while exposed males showed a stronger response with a 31.3% increase in MR. Mated females increased their MR by 34.8% during mite exposure whereas virgin females experienced an increase of 61.2%. Our findings suggest that NCE of parasites can vary based on state-dependent costs of infection.

1. Introduction

The ecology of fear describes the negative effects of predators on their potential prey outside of direct attack s, i.e. non-consumptive effects (NCE) [13,22,57,58]. Even if the individual prey is not consumed, it may still experience prolonged stress, decreased feeding success, depressed immunity, reduced growth, and ultimately lower fitness [53,47,35,17]. Males and females often differ in their predation risk [1,55]. Consequently, they may exhibit dissimilar responses to predation risk and resultant non-consumptive costs [5,39,71]. For example, male crickets experienced larger increases in metabolic rate (MR) when exposed to predator-derived fecal cues than female crickets [39]. These sex-biased outcomes show that the NCE of predators are not borne equally by members of the prey population [39].

A growing number of studies have attempted to apply the ecology of fear framework to describe host-parasite interactions outside of infection [9,16,49,63]. Tadpoles show comparable avoidance of parasite infectious stages and predator cues [65], albeit in a hierarchical manner [34]. Likewise, large tick populations can cause grazing small mammals and ungulates to leave foraging sites earlier than sites lacking ticks [3,4,21]. These studies suggest that ectoparasites can cause would-be hosts to forgo foraging at otherwise preferred sites and potentially cause trade-offs between nutrition and parasite avoidance [3,21].

Variation in the risks and costs of infection may influence the evolution of different parasite avoidance strategies, and it is reasonable to also expect intraspecific variation in NCE [9,76]. Individuals differ in their responses to infection risk by sex and reproductive status, and these differences could lead to variation in NCE [14,23, 31]. However, unlike studies examining predator-prey systems [39,71], little research has directly tested how parasite-mediated NCE vary within host species.

Here we measured intraspecific variation in NCE in terms of host metabolic changes (measured as rates of CO_2 production). Avoiding parasites and predators can be energetically costly [28,39], likely due to increases in activity, immunity, and/or stress [26,42]. Because of these energetic costs, MR measurements integrate the costs of behavioral and physiological responses into a shared currency [41].

We investigated the NCE an ectoparasitic mite, *Macrocheles subbadius*, has on *Drosophila nigrospiracula* [59]. Mites have consumptive effects on flies when they feed on host hemolymph [60]. Flies defend themselves primarily through bouts of intense grooming, kicking,

* Corresponding author.
E-mail address: chorn@ualberta.ca (C.J. Horn).

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jumping, or bursts of flight, and melanization is also observed at mite attachment sites [8,49,60]. Mite infection can more than halve fly longevity as well as reduce the fecundity of infected female flies by up to two-thirds [60,62]. Additionally, exposure to mites also elicits non-consumptive effects [7]. Flies have elavated MR when exposed to mites across a mesh screen and when induced to groom, [28,42]. Chronic parasite exposure also causes fitness losses among would-be hosts — females reared adjacent to mites had ~13% fewer adult offspring than unexposed flies and a ~23% reduction in lifespan [27]. Because parasites in this system are known to have short- and long-term NCE on potential hosts, it provides an opportunity to investigate variation in non-consumptive interactions. We anticipate variation between flies in the strength of NCE they experience based on the relative costs and risks of infection they experience.

Male and female *D. nigrospiracula* both experience intensity-dependent reductions in longevity and reproductive success due to infection with *M. subbadius* [60,61,62]. However, parasite-mediated sexual selection is significantly stronger for male flies than female flies [61]. Mite infection reduces the odds of copulating more for male than female flies, and fewer infections are required to completely exclude male flies from mating than female flies [61]. These differences may be partially due to mites physically obstructing mating attempts by infected males [43]. We, therefore, hypothesize that males are under selection to mount stronger anti-parasite responses than females due to asymmetrical costs of infection. Consequently, the cost of infection hypothesis predicts that male flies will experience larger increases in MR (a measure of energetic expenditure) upon exposure to mites than female flies.

If asymmetrical costs resulting from parasite-mediated sexual selection drive variation in responses to mites, then we also anticipate that virgin females will have stronger responses to mites than mated females. Mite infection reduces the odds of copulation for female flies, albeit less than males; however, sexual selection is tautologically reduced in already mated females [60,61]. As such, virgin females are likely adapted to respond more strongly to mites than mated females [61]. Therefore, the cost of infection hypothesis also predicts that virgin female flies will experience a larger increase in MR compared to mated females upon exposure to mites.

Sex-biased infections are commonly observed in vertebrate [12,50], invertebrate [11,67], and even dioceous-plant systems [51,78]. Differential risk of infection between sexes can occur because many ectoparasites actively seek out and selectively attack hosts based on host characteristics [11,12,73]. Currently, whether *M. subbadius* prefer to infect male or female *D. nigrospiracula* is not known, but it is known *M. subbadius* distinguish between potential hosts based on MR [29]. The congeneric mite *M. muscaedomesticae* preferentially infects female *D. hydei* over male flies in pair-wise choice tests [11]. Similarly, we predict *M. subbadius* will prefer to infect female *D. nigrospiracula* over males. In preliminary preference experiments, we found that *M. subbadius* selectively infects female *D. nigrospiracula* over males (Fig. 1). Since the preferred sex experiences a higher risk of infection, we hypothesize that the preferred sex will be under stronger selection to respond to parasites [15,33]. The asymmetrical risk of infection hypothesis predicts that female flies — the preferred host of the parasite — will exhibit a larger increase in MR upon parasite exposure than males. Further trials showed that mites have a weaker preference for mated females over virgin females (Fig 1). So, the asymmetrical risk hypothesis also predicts that mated females will have a stronger response to mite exposure than virgin females.

We experimentally tested two mutually exclusive hypotheses by comparing the MR of male and female flies as well as mated versus virgin female flies at rest and during mite exposure. **H1: The asymmetrical costs hypothesis** predicts that male flies will experience larger increases in MR when exposed to mites than female flies, and virgin females will have larger increases in MR than mated females. **H2: The asymmetrical risks hypothesis** predicts that female flies will experience a larger mite-induced increase in MR than male flies, and that mated females will exhibit a larger increase in MR than virgin females. In order to elucidate the relative contributions of physiological and behavioral changes to inter-group differences in MR, we also measured fly activity simultaneously with respiration.

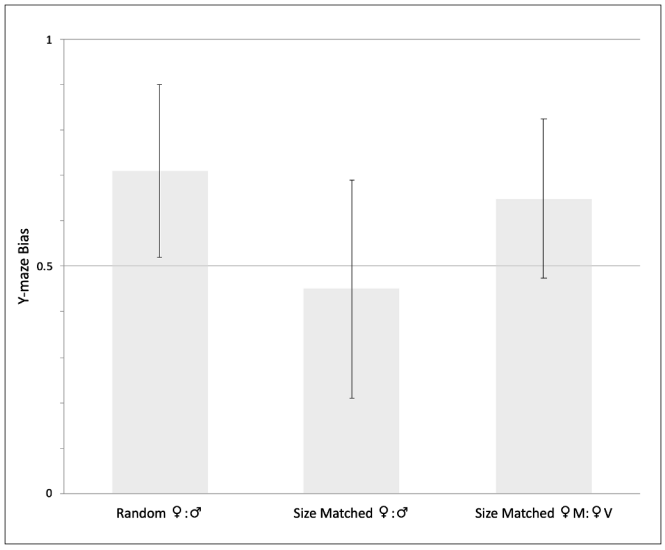

Fig. 1. Host preferences from y-maze experiments. Biases (A:B) represent the proportion of y-mazes in which the group A fly was infected. Flies were either selected at random or size-matched within 5% of mass. All flies were virgins, except when mites were given a choice between mated females (♀M) and virgin females (♀V). Error bars represent 95% CI (R, binom.test).

2. Methods

2.1. Mite and fly cultures

Drosophila nigrospiracula Patterson and Wheeler cultures were founded from approximately 150 flies of each sex collected from cactus (*Carnegiea gigantea*) rots located in the Sonoran Desert (Phoenix, Arizona). Flies were cultured on a 3:1 mix of instant potatoes to *Drosophila* formula (Formula 4–24 Instant *Drosophila* Medium, Carolina Biological Supply Company, Burlington, NC, USA). Because *D. nigrospiracula* larvae fail to pupate in the absence of host plant tissue, autoclaved cactus was added to culture bottles [49]. Nutritional yeast was added to the media as a supplement. Following emergence, adult flies were moved to sex-separate agar vials before reproductive maturity [45]. Flies were stored in incubators at 24 °C and 50% relative humidity until they were used in the experiments below (Percival Scientific, Perry, IA, USA).

Macrocheles subbadius (Berlese) cultures were founded from approximately 200 adult female mites collected from wild caught *D. nigrospiracula*. Mites were reared on a 2:1 wheat bran to wood chips mixture moistened with distilled water and co-cultured with bacteriophagic nematodes as a food source. Mite cultures were stored in incubators at 26 °C and 70% relative humidity (Percival Scientific, Perry, IA, USA). Adult female mites, the infectious stage, were collected from the stock culture using Berlese funnels and stored in specimen cups lined with moistened paper towel or Plaster-of-Paris until experiments. The number of mites used in each experiment was chosen based on the size of the lab population.

2.2. Host preferences of mites

2.2.1. Host sex

This experiment tested whether *M. subaddius* preferentially infect female or male *D. nigrospiracula* in choice-tests. Age-matched virgin

male and female flies were anesthetized with CO_2 and glued (Elmer's rubber cement) to cotton in order to eliminate behavioral resistance [11]. Following a recovery period, a fly was transferred to each arm of a y-shaped maze (Fisherbrand Tubing, Y Polypropylene Connectors). To control for idiosyncratic biases, the male and female flies alternated between the left and right arms. A single adult female mite was then placed in the free arm and the maze was sealed with cotton. We placed mazes under an opaque box to exclude light as mites are more likely to infect in the dark (pers. obs). After 1 h of mite exposure, the y-maze was inspected. A trial was considered successful if the mite infected either fly, then the sex of the infected fly was recorded. In a subset of successful trials (19 of 31) the masses of both flies were recorded before the assay. Between assays, the y-mazes were washed with detergent, sterilized with 70% ethanol, then rinsed with distilled water to remove chemical cues left behind by mites or flies.

A second experiment tested if the preference for female flies was driven by sexual dimorphism in size, since female *Drosophila* are on average larger than conspecific males ([11], 3.1.1). In this follow-up experiment male and female flies were size (mass) matched to within 5% and assigned as matched-pairs to the y-mazes. The choice tests were otherwise conducted as above. Binomial tests (binom.test) were used to test if flies disproportionately infected male or female flies (R Studio, Stats package).

2.2.2. Host reproductive status

Pair-wise choice tests, as described in 2.2.1, were used to determine if mites preferred to infect mated or virgin females. Flies were mated by placing them into agar vials at a 2:1 ratio of males to females. This biased ratio ensured nearly all female flies were mated following the 72-hour mating period (pers. obs). Pairwise choice tests were conducted 72 h post-mating. Mated and virgin females were size matched (mass difference within 5%) before each assay. Each matched-pair was then transferred to a y-maze. A single adult female mite was introduced to the maze and allowed 2 h to infect while the y-maze was covered with an opaque box. A binomial test (binom.test) was used to test if flies disproportionately infected mated or virgin females.

2.3. Fly responses to mite exposure

2.3.1. Sex differences

To test which fly sex has a stronger response to parasites we measured the rate of CO_2 production, a proxy for metabolic rate, of male and female flies either unexposed or exposed to mites using flow-through respirometry (illustrated in [28]). Unexposed flies were in otherwise empty respirometry chambers, whereas flies in the exposed condition were in chambers with 3 mites. During the exposed trials an extra chamber containing only 3 mites was also measured. A Li-7000 infrared analyzer was used to measure the concentration of CO_2 produced by individual flies (Li-COR Biosciences, Lincoln, NE). A MAVen-FT system (Sable Systems, Las Vegas, NV) was used to direct inflow air, at 30 mL/min, through either a respirometry chamber or the baseline. The real-time flow rate was recorded by the MAVen-FT and used in the calculation of CO_2 production rate (calculation described in [29]). In order to improve sensitivity, incoming air was purged of CO_2 and water vapor using a purge gas generator (FT-IR Purge Gas Generator 75–45, Parker Canada Division, Milton Ontario). Excurrent air was scrubbed of water vapor by passing the excurrent air through a magnesium perchlorate column before analysis with the Li-7000. A reference cell of bone-dry CO_2-free air also produced via the purge generator was used to enable the Li-7000 to measure the excurrent gas. The Li-7000 was spanned periodically with dry 20 ppm CO_2 at 30 mL/min (Praxair, Danbury, CT) and zeroed before each assay. Female and male flies were placed in alternating experimental chambers and the CO_2 production of each fly was recorded sequentially for 300 s (1 observation / second). The 300 observations were averaged to calculate a mean respiration rate for each fly using the Expedata software. Following respirometry measurements the flies were frozen so that mass could be measured afterwards.

The MAVen-FT system infrared monitor recorded the activity (arbitrary Voltage, V) of the fly in the chamber simultaneously with respiratory measurements to test if activity drove sex differences in MR (Sable Systems, Las Vegas, NV). Activity measurements for flies within the same replicate block were conducted concurrently. The activity monitor primarily detects translocation and large movements, which increase during parasite exposure [28].

Following each assay, we visually inspected the flies for mites. Infection occurred at negligible rates and at sub-pathological intensities (2 flies across all experiments acquired 1 mite) [42]. Thus, the MR changes we detected are primarily due to fear, not infection. This low rate of infection is likely due to ample space permitting fly defenses, the presence of light that inhibits mite infection, and/or relatively short exposure times precluding infection.

We analyzed MR using the lmer and glmer (Gamma) functions (lme.4 package). Backwards model selection was performed by sequentially removing fixed effects from the model. Model comparison was carried out with the anova function (test = χ^2) for lmer models and the Wald t-statistic for glmer models. If there was a significant difference between models with and without the explanatory variable the variable was retained. CO_2 production was modeled with the fixed predictors: fly sex, mass, activity, and chamber. Replicate block was included as a random effect within experiment date. Linear rescaling of CO_2 production was necessary in some glmer models. We examined the residuals of models for normality (shapiro.test, R Stats package). We considered the potential of non-singularity in mixed effect models by examining models that only use replicate block or date, but models based on single random effects always lead to the inclusion of the same fixed effects as models based on both.

2.3.2. Mating status

We tested if mated or virgin females had larger changes in MR during mite exposure by measuring CO_2 production of flies either exposed to mites or left undisturbed using flow-through respirometry. Flies were mated as in 2.2.2. Respiratory and activity measurements were conducted as in the sex-difference experiments (2.3.1), except for this mite exposure condition using 5 mites. Backwards model selection was performed as in 2.3.1

3. Results

3.1. Host preferences

3.1.1. Mites prefer to infect female flies over males in a size-mediated manner

This experiment tested if *M. subbadius* prefer to infect male or female flies in pairwise choice-tests. Female flies were infected in 22 of 31 successful trials (71%), significantly more often than the male flies (29%) (binom.test, $P = 0.029$, Fig. 1). Males on average weighed 2.35 ± 0.09 mg and females weighed 2.86 ± 0.15 mg, a 19.6% difference.

A follow-up experiment was conducted to test if the observed preference for female flies was driven by sexual dimorphism in mass by size-matching male and female flies. Size matching essentially eliminated the observed difference in mass between the sexes: females in this experiment on averaged weighed 2.22 ± 0.09 mg ($N = 29$) and males on average weighed 2.20 ± 0.09 mg ($N = 29$). The largest difference in size between a paired male and female fly was 3.1% and the mean percent difference was less than 1%. The female fly was infected in 13 of 29 successful trials (45%) and the male was infected in 16 trials (55%) (Fig. 1). When flies were size-matched, there was no significant preference for either sex (binom.test, $P = 0.55$). Taken together our results show mites selectively infect female flies because females are larger than males.

3.1.2. Mites prefer to infect mated female flies over virgin females

We tested if flies preferentially infected mated or virgin female flies in size-matched preference experiments. In 24 of 37 (65%) trials, the mite infected the mated fly whereas only 35% of mites preferentially infected the virgin fly (binom.test, $P = 0.099$) (Fig. 1). The confidence interval (0.47–0.80) of this weak preference slightly overlapped 0.5 (95% CI, Fig. 1). Following size-matching, the average mass of the mated flies was 2.56 ± 0.04 mg and the average mass of the virgin female was 2.53 ± 0.04 mg. On average there was a 1.3% difference in mass between the mated and virgin fly in a y-maze and the largest difference was 4.2%.

3.2. Fly responses to mite risk

3.2.1. Male flies have stronger responses to mite risk than female flies

The MR of unexposed flies was best described with a normal distribution upon visual inspection, thus the lmer function was used for modeling. Unexposed females ($N = 36$) had substantially higher CO_2 production rates than males ($N = 36$), 0.063 ± 0.003 μL/min versus 0.053 ± 0.003 μL/min respectively (16.3% higher), and sex was a significant predictor of MR among unexposed flies ($\chi^2 = 6.34$, $P = 0.012$) (Fig. 2a). Because of sexual dimorphism, we performed backwards model selection on initial models of MR with mass and sex separately to avoid co-linearity. There was a 9.5% difference in mass between male and female flies in this experiment. Males had an average mass of 2.20 ± 0.08 mg and females had an average mass of 2.43 ± 0.09 mg, but mass was not a significant predictor of MR ($\chi^{2<0.001}$, $P = 0.99$). Nor was mass a significant predictor in models without sex ($\chi^2 = 0.43$, $P = 0.51$). Respirometry chamber was a significant predictor of MR ($\chi^2 = 12.3$, $P = 0.0004$).

The MR of the flies exposed to mites was best described using a gamma distribution (glmer, family = "Gamma"). In the exposed treatment, the MR of male (0.073 ± 0.008 μL/min) and female (0.073 ± 0.007 μL/min) flies were nearly identical, and sex was not a significant predictor of MR (Wald $t = 0.24$, $P = 0.81$, Fig. 2a). However, since control females started off with a higher MR, the relative increase in MR upon exposure was higher among males than females. When exposed to mites, the rate of CO_2 production increased by 15.1% among females and 31.3% in males. Flies exposed to mites had higher MRs than unexposed flies overall, but, as predicted in the cost of infection hypothesis, male flies showed a stronger response compared to female flies upon exposure to mites.

Female flies in the exposed experiment on average had 24% higher masses than male flies, 2.48 ± 0.06 mg and 1.95 ± 0.03 mg respectively. Due to this sexual dimorphism, we modeled MR with either mass or sex. In models with mass as a predictor, but not sex, mass significantly predicted MR in the exposed condition (Wald $t = 2.27$, $P = 0.023$). Chamber was a significant predictor in the exposed condition model of MR (Wald $t = 5.84$, $P < 0.0001$). On average the chamber with three mites ($N = 4$) produced 0.00052 ± 0.00006 μL/min of CO_2, and as such the respiration of the mites was a negligible contributor to the difference in metabolic rates between the exposed and unexposed conditions.

We recorded the activity of flies using an infrared monitor simultaneously with MR in order to test if changes in activity accounted for differences between male and female MR. In the control condition (no mites), the level of activity among females (0.037 ± 0.007 V, $N = 36$) did not differ substantially from males (0.040 ± 0.008 V, $N = 36$, Fig. 2b), and activity was not a significant predictor of MR ($\chi^2 = 0.11$, $P = 0.74$). In the *exposed* treatment, activity was a significant predictor of MR (Wald $t = 4.47$, $P < 0.0001$). This result was sensitive to the inclusion of a single male fly, the removal of which led

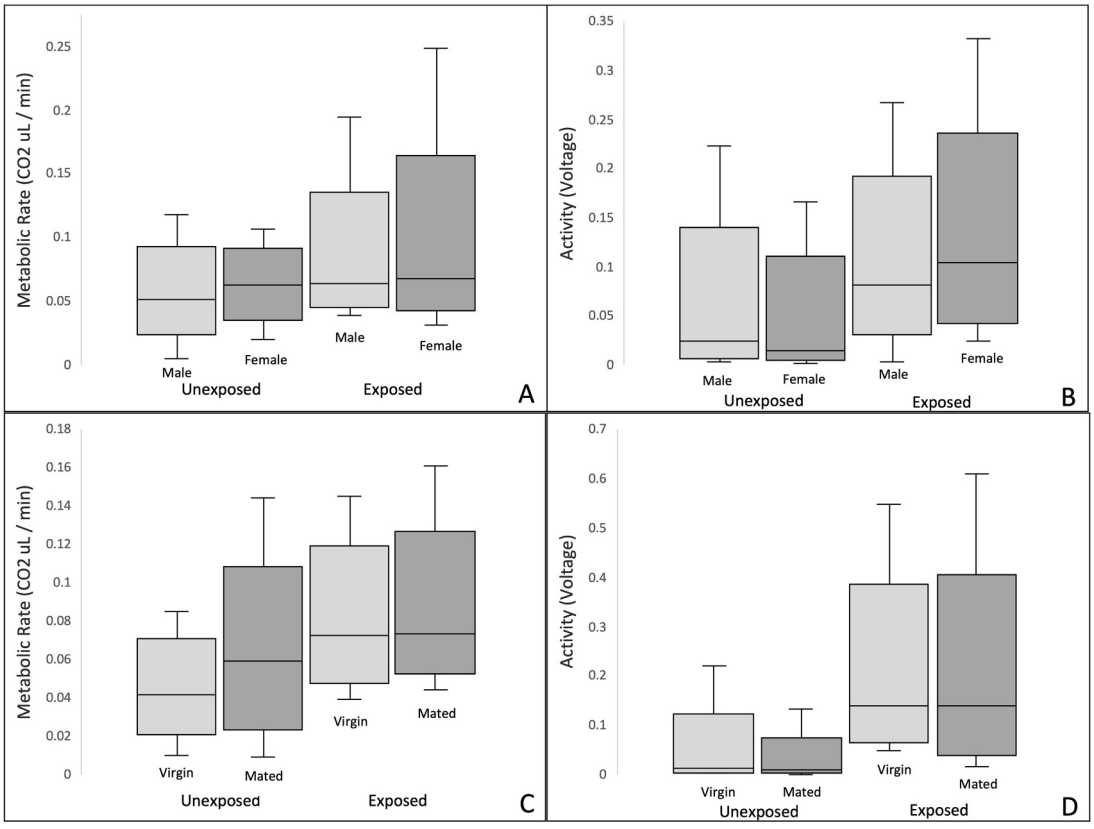

Fig. 2. Fly responses to mite exposure. A) Metabolic rates of male and female flies at rest or exposed to mites. B) Activity of male and female flies at rest or exposed to mites. One male fly was removed due to high activity. C) Metabolic rates of virgin and mated females at rest or exposed to mites. D) Activity of virgin and mated females at rest or exposed to mites. Boxplots represent minimum, 25th percentile, median, 75th percentile and maximum,.

to activity not being a significant predictor of exposed MR (Wald $t = -1.301$, $P = 0.19$). There was no substantial difference in activity between male (0.13 ± 0.04 V, $N = 28$) and female (0.11 ± 0.01 V, $N = 28$) flies, even if the male with the highest activity is removed (male: 0.10 ± 0.01 V, $N = 27$) (Fig. 2b). Unsurprisingly, the presence of mites increased fly activity 3–4 times compared with unexposed flies. However, male and female flies had comparable activity at rest or when exposed to mites.

3.2.1. Virgin females have stronger responses to mite risk than mated females

The MR of unexposed flies was best described with a normal distribution upon visual inspection, thus the lmer function was used for modeling. Mating status was a moderate predictor of MR when flies were at rest ($\chi^2 = 3.23$, $P = 0.07$) (Fig. 2c). In the unexposed experiment, the mated group (0.057 ± 0.004 μL/min, $N = 27$) produced 24.8% more CO_2 than the virgin group (0.042 ± 0.004 μL/min, $N = 28$). Mass did not differ substantially between the virgin and mated group (2.54 ± 0.06 mg and 2.52 ± 0.07 mg respectively), or significantly predict unexposed MR ($\chi^2 = 0.72$, $P = 0.40$).

The MR of the flies exposed to mites was best described using a gamma distribution (glmer, family = "Gamma"). Upon exposure to mites, mated flies (0.081 ± 0.005 μL/m, $N = 31$) produced CO_2 at nearly the same rate (Fig. 2c) as virgin flies (0.079 ± 0.004 μL/min, $N = 33$), and mating status was not a significant predictor of MR (Wald $t = -1.08$, $P = 0.28$). Among flies that were mated, exposure to mites resulted in a 34.8% rise in CO_2 production compared to the unexposed experiment. By comparison, virgin flies responded to mite exposure by increasing CO_2 production by 61.2% over the unexposed group.

In the exposed condition, virgin flies were 9% heavier than mated flies, 3.19 ± 0.06 mg ($N = 35$) and 2.91 ± 0.05 mg ($N = 35$), respectively; however, mass was not a significant predictor of exposed MR (Wald $t = 1.06$, $P = 0.29$). On average the chamber with five mites ($N = 5$) produced 0.000084 ± 0.000013 μL/min of CO_2, and as such the respiration of the mites was a negligible contributor to the difference in metabolic rates between the exposed or unexposed experiments.

The activity of mated and virgin females was recorded simultaneously with MR when exposed or unexposed to mites. When flies were not exposed to mites, activity did not substantially differ between virgin and mated groups: 0.025 ± 0.008 V and 0.017 ± 0.003 V respectively (Fig. 2d). Nor did activity significantly predict MR when flies were not exposed ($\chi^2 = 0.03$, $P = 0.87$). By contrast, mated and virgin female flies exposed to mites were 8–9 times more active than flies not exposed to mites, and activity was a significant predictor of MR (Wald $t = 4.45$, $P < 0.0001$, Fig. 2d). However, the activity levels of the virgin (0.16 ± 0.02 V, $N = 35$) and mated (0.16 ± 0.02 V, $N = 35$) groups were indistinguishable during mite exposure (Fig. 2d).

4. Discussion and conclusion

4.1. Discussion

We set out to test two mutually exclusive hypotheses: 1) asymmetrical costs primarily drive selection for stronger responses to parasite exposure; or 2) asymmetrical risks primarily drive selection for stronger responses to parasite exposure. The latter hypothesis predicted a stronger response (i.e. increase in MR upon exposure to mites) among females compared to males, and that mated females would have stronger responses than virgin females. This prediction was not borne out, even though mites have a preference for female flies over male flies, and a moderate preference for mated females over virgin females independent of size. In other words, although the risk of infection is greater for female flies than males, and more for mated females than virgins, it did not determine the relative magnitude of the host response to parasitic threat. By contrast, the asymmetrical cost hypothesis predicted a stronger response among male flies than female flies, and a stronger response among virgin females relative to mated females. Our results support the asymmetrical costs hypothesis: male flies had a larger increase in metabolic rate than female flies when exposed to mites — 31.3% versus 15.1% respectively. Furthermore, virgin females had larger increases in MR, 61.2%, when exposed to mites than mated females, 34.8%. Hence unequal costs of infection, arising from parasite-mediated sexual selection, are likely driving the relative strength of responses of potential fly hosts to mites.

Fly activity was a significant predictor of MR in the exposed treatments, and activity was 3–9 times higher on average among flies exposed to mites than flies at rest. Perhaps unsurprisingly, activity was not a significant predictor of MR among unexposed flies. Intraspecific differences in MR may be present under conditions of high activity or recovery but absent during routine conditions [10,75,77]. Our results are consistent with the general finding that energetically demanding activities correlate more strongly with overall MR than low energy activities [44]. The increase in activity observed here may be adaptive as activity can aid in parasite avoidance, particularly those that attack via the integument [6,35,28]. However, there was no substantial difference in activity levels between male and female or mated and virgin flies under any condition we tested. While activity may explain why flies exposed to mites generally have higher MR than unexposed flies, it does not fully account for the sex and mating status differences observed here.

Differences in NCE experiencd by potential hosts may instead be due to physiological (e.g. stress) or immunological mechanisms [69,24,70,71]. In *Drosophila,* the stress hormone octopamine appears to increase MR since octopamine knock-out flies have significantly reduced CO_2 production [40]. Similarly, mammalian stress hormones are positively correlated with MR [25]. Short-term stress, e.g. during initial exposure, can be adaptive and assists crickets and tadpoles in evading predation [2,37]. Comparably, stress may also help hosts avoid infection by mobile parasites, particularly ectoparasites [9,36].

Predation risk can also alter immune responses in insects [20,52]. Dragonfly larvae exposed to cannibalistic conspecifics had increased melanization upon sham infection with microfilaments – potentially because melanization promotes both wound healing and immune function [52]. Predator presence can increase melanization responses in potential prey even when those predators are caged [19]. In our study, flies may respond to the presence of mites with preemptive increases in immunity which could impose an energetic cost. Potential changes could occur in components of the phenoloxidase (PO) system which governs melanin production by PO via activation of PO zymogens (see mini-review [66]). Future research should test how the mere presence of mites impacts the stress and immune systems of flies [7].

Although hosts may benefit from stress responses and/or preemptive priming of immune systems (e.g. melanization) to parasite and predator exposure in the short term, these responses likely decrease with time since long-term stress can negatively impact fitness [72,37,17]. For example, exposure to predator cues increases tadpole oxygen consumption initially, but with repeated exposures oxygen consumption is suppressed [72]. A meta-analysis of vertebrate species enduring parasite infection found that stress hormones are highest early in infection, and, although still generally higher than uninfected conspecifics, this difference decreased over time [56]. Additionally, short-term evasion strategies, e.g. bolting, may differ from longer-term strategies, e.g. hiding, and require different energy investment [72]. Thus, it is important for future studies on parasite-mediated NCE to consider a range of non-consumptive effects across different time scales.

Our results suggest that, at least in the short term, mated and virgin female flies experience unequal non-consumptive effects from parasite exposure. However, previous research did not find a significant difference in longevity between mated female flies and virgin female flies chronically exposed to mites [27]. This relationship may be obscured by the complex and not well-understood link between mating and survival in *Drosophila* [46]. Alternatively, NCE, and potentially intraspecific

variation in NCE, can be increased or decreased depending on the environment [18,32,49,68]. Differential fitness impacts of NCE may only manifest under poor conditions where organisms cannot easily compensate, e.g. with increased feeding. As a result, short-term NCE on MR may not always predict relative long-term NCE on longevity and may be environment dependent.

Organisms vary intra- and inter-specifically in how they compensate for long-term risk. Compensatory physiological changes can help damselfly larvae cope with reduced feeding during predator exposure [74]. Male and female prey can differ in how much they compensate risk. For example, female lizards, *Podarcis hispanicus*, habituate to the presence of predators more than conspecific males [64]. Variation in compensation has implications for the ecology of prey species as interspecific differences in habituation to predation risk may drive competitive advantages among amphipod prey [30]. Future research should consider intra- and inter-specific variation in habituation to the presence of parasites and consequent implications for unequal long-term NCE.

4.2. Conclusions

Community ecology and parasitology have increasingly recognized that parasites have important impacts on host populations and their communities outside of infection [38,48,54]. Infection does not impact all hosts equally, and our results suggest parasites also do not have equal NCE on all potential hosts. Male flies and virgin flies had stronger bioenergetic responses to parasites than female and mated flies respectively. These unequal reactions were not fully explained by intergroup differences in activity. Our results are compatible with the hypothesis that costs of infection primarily determine the relative strength of response upon exposure to parasites. Further research should investigate the specific mechanisms that drive this intraspecific variation in NCE. Organisms face a complex landscape of fear, and how they navigate that landscape will depend on optimal investment in defense based on their relative costs of infection.

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References

[1] L. Acharya, Sex-biased predation on moths by insectivorous bats, Anim. Behav. 49 (1995) 1461–1468, https://doi.org/10.1016/0003-3472(95)90067-5.
[2] S.A. Adamo, I. Kovalko, B. Mosher, The behavioural effects of predator-induced stress responses in the cricket (Gryllus texensis): the upside of the stress response, J. Exp. Biol. 216 (2013) 4608–4614, https://doi.org/10.1242/jeb.094482.
[3] B.F. Allan, T.S. Varns, J.M. Chase, Fear of parasites: lone star ticks increase giving-up densities in white-tailed deer, Isr. J. Ecol. Evol. 56 (2010) 313–324, https://doi.org/10.1560/ijee.56.3-4.313.
[4] S.B.S. Baleba, B. Torto, D. Masiga, M.N. Getahun, C.W. Weldon, Stable Flies, *Stomoxys calcitrans* L. (Diptera: muscidae), improve offspring fitness by avoiding oviposition substrates with competitors or parasites, Front. Ecol. Evol. 8 (2020) 1–5, https://doi.org/10.3389/fevo.2020.00005.
[5] S.L. Ball, R.L. Baker, Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? Ecology 77 (1996) 1116–1124, https://doi.org/10.2307/2265580.
[6] R.L. Baker, B.P. Smith, Conflict between antipredator and antiparasite behaviour in larval damselflies, Oecologia 109 (1997) 622–628, https://doi.org/10.1007/s004420050125.
[7] J.B. Benoit, J. Bose, S. Bailey, M. Polak, Interactions with ectoparasitic mites induce host metabolic and immune responses in flies at the expense of reproduction-associated factors, Parasitology (2020) 1–10, https://doi.org/10.1017/S0031182020000918 In press.
[8] D.V. Beresford, J. Sutcliffe, The effect of *Macrocheles muscaedomesticae* and *M. subbadius* (Acarina: macrochelidae) phoresy on the dispersal of *Stomoxys calcitrans* (Diptera: muscidae), Syst. Appl. Aracol. 23 (2009) 1–30, https://doi.org/10.11158/saasp.23.1.1 Special Publications.
[9] J.C. Buck, S.B. Weinstein, H.S. Young, Ecological and evolutionary consequences of parasite avoidance, Trends Ecol. Evol. 33 (2018) 619–632, https://doi.org/10.1016/j.tree.2018.05.001.
[10] I.C. Caballero, A.J. Sakla, J.T. Detwiler, M. Le Gall, S.T. Behmer, C.D. Criscione, Physiological status drives metabolic rate in Mediterranean geckos infected with Pentastomes, Plos One 10 (2015) e0144477, , https://doi.org/10.1371/journal.pone.0144477.
[11] E.O. Campbell, L.T. Luong, Mite choice generates sex- and size-biased infection in Drosophila hydei, Parasitology 143 (2016) 787–793, https://doi.org/10.1017/s0031182016000305.
[12] P. Christe, O. Glaizot, G. Evanno, N. Bruyndonckx, G. Devevey, G. Yannic, P. Patthey, A. Maeder, P. Vogel, R. Arlettaz, Host sex and ectoparasites choice: preference for, and higher survival on female hosts, J. Anim. Ecol. 76 (2007) 703–710, https://doi.org/10.1111/j.1365-2656.2007.01255.x.
[13] M. Clinchy, M.J. Sheriff, L.Y. Zanette, Predator-induced stress and the ecology of fear, Funct. Ecol. 27 (2013) 56–65, https://doi.org/10.1111/1365-2435.12007.
[14] C.J. Cloutier, M. Kavaliers, K.P. Ossenkopp, Rodent sex differences in disgust behaviors (anticipatory nausea) conditioned to a context associated with the effects of the toxin LiCl: inhibition of conditioning following immune stimulation with lipopolysaccharide, Pharmacol. Biochem. Behav. 152 (2017) 4–12, https://doi.org/10.1016/j.bb.2016.08.006.
[15] E.W. Daly, P.T.J. Johnson, Beyond immunity: quantifying the effects of host anti-parasite behavior on parasite transmission, Oecologia 165 (2011) 1043–1050, https://doi.org/10.1007/s00442-010-1778-y.
[16] Daversa D.R., Hechinger R.F., Madin E., Fenton A., Dell A.I., Ritchie E., Rohr J., Rudolf V.H.W., Preprint2019. Beyond the ecology of fear: non-lethal effects of predators are strong whereas those of parasites are diverse. Preprint at bioRxiv. doi: doi.org/10.1101/766477.
[17] DeWitt P.D., Visscher D.R., Schuler M.S., Thiel R.P. Predation risks suppress lifetime fitness in a wild mammal. Oikos128: 1–8. 2019, doi: 10.1111/oik.05935.
[18] S.C. Donelan, J.H. Grabowski, G.C. Trussell, Refuge quality impacts the strength of consumptive effects on prey, Ecology 98 (2016) 403–411, https://doi.org/10.1002/ecy.1647.
[19] Tammy M Doug, Shannon J McCauley, Predation risk increases immune response in a larval dragonfly (Leucorrhinia intacta), Ecology 97 (6) (2016) 1605–1610, https://doi.org/10.1890/15-1964.1.
[20] T.M. Duong, S.J. McCauley, Predation risk increases immune responses in a larval dragonfly (Leucorrhinia intacta), Ecologu 97 (2016) 1605–1610, https://doi.org/10.1890/15-1964.1.
[21] A. Fritzsche, B.F. Allan, The ecology of fear: host foraging behavior varies with the spatio-temporal abundance of a dominant ectoparasite, Ecohealth 9 (2012) 70–74, https://doi.org/10.1007/s10393-012-0744-z.
[22] A.J. Gallagher, S. Creel, R.P. Wilson, S.J. Cooke, Energy landscapes and the landscape of fear, Trends Ecol. Evol. 32 (2017) 88–96, https://doi.org/10.1016/j.tree.2016.10.010.
[23] D Gálvez, M Chapuisat, Immune priming and pathogen resistance in ant queens, Ecology and Evolution 4 (10) (2014) 1761–1767, https://doi.org/10.1002/ece3.1070.
[24] D.F. Garcia-Diaz, J. Campion, F.I. Milagro, A. Lomba, F. Marzo, J.A. Martinez, Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats, J. Physiol. Biochem. 63 (2007) 337–346, https://doi.org/10.1007/bf03165765.
[25] C.G. Haase, A.K. Long, J.F. Gillooly, Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals, Biol. Lett. 12 (2016), https://doi.org/10.1098/rsbl.2015.0867.
[26] D. Hawlena, O.J. Schmitz, Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 15503–15507.
[27] C.J. Horn, L.T. Luong, Proximity to parasites reduces host fitness independent of infection in a Drosophila-Macrocheles system, Parasitology 145 (2018) 1564–1569, https://doi.org/10.1017/s0031182018000379.
[28] C.J. Horn, L.T. Luong, Current parasite resistance trades off with future defenses and flight performance, Behav. Ecol. Sociobiol. 73 (2019), https://doi.org/10.1007/s00265-019-2697-5.
[29] C.J. Horn, M.K. Mierzejewski, L.T. Luong, Host respiration rate and injury-derived cues drive host preference by an ectoparasite of fruit flies, Physiol. Biochem. Zool. 91 (2018) 896–903, https://doi.org/10.1086/697466.
[30] L. Jermacz, J. Kobak, Keep calm and don't stop growing: non-consumptive effects of a sympatric predator on two invasive Ponto-Caspian gammarids Dikerogammarus villosus and Pontogammarus robustoides, PLoS One 12 (2017), https://doi.org/10.1371/journal.pone.0182481.
[31] C.N. Keiser, V.H.W. Rudolf, M.C. Luksi, J.B. Saltz, Sex differences in disease avoidance behavior vary across modes of pathogen exposure, Ethology 126 (2019) 304–312, https://doi.org/10.1111/eth.12969.
[32] M.F. Kersh-Becker, J.S. Thaler, Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids, J. Anim. Ecol. 84 (2015) 1222–1232, https://doi.org/10.1111/1365-2656.12371.
[33] J. Koprivnikar, M.R. Forbes, R.L. Baker, On the efficacy of anti-parasite behaviour: a case study of tadpole susceptibility to cercariae of Echinostoma trivolvis, Can. J. Zool. Rev. Can. Zool. 84 (2006) 1623–1629, https://doi.org/10.1139/z06-158.

[34] J. Koprivnikar, L. Penalva, Lesser of two evils? Foraging choices in response to threats of predation and parasitism, PLoS One 10 (2015), https://doi.org/10.1371/journal.pone.0116569.

[35] J. Koprivnikar, T.M.Y. Urichuk, Time-lagged effect of predators on tadpole behaviour and parasite infection, Biol. Lett. 13 (2017), https://doi.org/10.1098/rsbl.2017.0440.

[36] E.A. Krusemark, W. Li, Do all threats work the same way? Divergent effects of fear and disgust on sensory perception and attention, J. Neurosci. 31 (2011) 3429–3434, https://doi.org/10.1523/jneurosci.4394-10.2011.

[37] P.S. Kulkarni, N.P. Gramapurohit, Effect of corticosterone on larval growth, anti-predator behaviour and metamorphosis of Hylarana indica, Gen. Comp. Endocrinol. 251 (2017) 21–29, https://doi.org/10.1016/j.ygcen.2016.09.001.

[38] K.D. Lafferty, A.P. Dobson, A.M. Kuris, Parasites dominate food web links, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 11211–11216, https://doi.org/10.1073/pnas.0604755103.

[39] P.A. Lagos, M.E. Herberstein, Are males more scared of predators? Differential change in metabolic rate between males and females under predation risk, Physiol. Behav. 173 (2017) 110–115, https://doi.org/10.1016/j.physbeh.2017.02.002.

[40] Y. Li, J. Hoffmann, F. Stephano, I. Bruchhaus, C. Fink, T. Roeder, Octopamine controls starvation resistance, life span and metabolic traits in Drosophila, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep35359.

[41] Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, Oxford New York.

[42] L.T. Luong, C.J. Horn, T. Brophy, Mitey costly: energetic costs of parasite avoidance and infection, Physiol. Biochem. Zool. 90 (2017) 471–477, https://doi.org/10.1086/691704.

[43] Michal Polak, Lien T Luong, William T Starmer, Parasites physically block host copulation: a potent mechanism of parasite-mediated sexual selection, Behavioral Ecology 18 (15) (2007) 952–957, https://doi.org/10.1093/beheco/arm066.

[44] K.J. Mathot, N.J. Dingemanse, S. Nakagawa, The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights, Biol. Rev. 94 (2019) 1056–1074.

[45] T.A. Markow, Reproductive behavior of Drosophila melanogaster and Drosophila nigrospiracula in the field and in the laboratory, J. Comp. Psychol. 102 (1988) 169–173.

[46] T.A. Markow, “Cost” of virginity in wild *Drosophila melanogaster* females, Ecol. Evol. 1 (2011) 596–600, https://doi.org/10.1002/ece3.54.

[47] S.J. McCauley, L. Rowe, M.J. Fortin, The deadly effects of "nonlethal" predators, Ecology 92 (2011) 2043–2048, https://doi.org/10.1890/11-0455.1.

[48] K.M. McKee, J. Koprivnikar, P.T.J. Johnson, M.T. Arts, Parasite infectious stages provide essential fatty acids and lipid-rich resources to freshwater consumers, Oecologia 192 (2020) 477–488, https://doi.org/10.1007/s00442-019-04572-0.

[49] M.K. Mierzejewski, C.J. Horn, L.T. Luong, Ecology of fear: environment-dependent parasite avoidance among ovipositing Drosophila, Parasitology 146 (2019) 1564–1570, https://doi.org/10.1017/s0031182019000854.

[50] S. Morand, J.G. De Bellocq, M. Stanko, D. Miklisova, Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe? Parasitology 129 (2004) 505–510, https://doi.org/10.1016/ensam.inra.fr.

[51] K.K. Moritz, C. Bjorkman, A.L. Parachnowitsch, J.A. Stenberg, Female Salix viminalis are more severely infected by Melampsora spp. but neither sex experiences associational effects, Ecol. Evol. 6 (2016) 1154–1162, https://doi.org/10.1002/ece3.1923.

[52] R.L. Murray, S. Tah, J. Koprivnikar, L. Rowe, S.J. McCauley, Exposure to potentially cannibalistic conspecifics induces an increased immune response, Ecol. Entomol. 45 (2020) 355–363, https://doi.org/10.1111/een.12806.

[53] C. Navarro, F. de Lope, A. Marzal, A.P. Moller, Predation, host immune response, and parasitism, Behav. Ecol. 15 (2004) 629–635, https://doi.org/10.1093/beheco/arh054.

[54] V. Nehring, H. Teubner, S. König, Dose-independent virulence in phoretic mites that parasitize buring beetles, Int. J. Parasitol. 49 (2019) 759–767, https://doi.org/10.1016/j.ijpara.2019.05.011.

[55] K. Norrdahl, E. Korpimaki, Does mobility or sex of voles affect risk of predation by mammalian predators? Ecology 79 (1998) 226–232.

[56] K. O'Dwyer, F. Dargent, M.R. Forbes, J. Koprivnikar, Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: a meta-analysis, J. Anim. Ecol. 89 (2019), https://doi.org/10.1111/1365-2656.13123.

[57] S.D. Peacor, E.E. Werner, Nonconsumptive effects of predators and trait-mediated indirect effects, Encyclopedia of Life Sciences (ELS), John Wiley & Sons, Ltd., Chichester, United Kingdom, 2008, pp. 1–8.

[58] B. Peckarsky, C. Cowan, M. Penton, C. Anderson, Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity, Ecology 74 (1993) 1836–1846, https://doi.org/10.2307/1939941.

[59] A. Perez-Leanos, M.R. Loustalot-Laclette, N. Nazario-Yepiz, T.A. Markow, Ectoparasitic mites and their Drosophila hosts, Fly 11 (2017) 10–18, https://doi.org/10.1080/19336934.2016.1222998.

[60] M. Polak, Ectoparasitic effects on host survival and reproduction: the Drosophila-Macrocheles association, Ecology 77 (1996) 1379–1389, https://doi.org/10.2307/2265535.

[61] M. Polak, T.A. Markow, Effect of ectoparasitic mites on sexual selection in a sonoran desert fruit-fly, Evolution 49 (1995) 660–669, https://doi.org/10.2307/2410319.

[62] M. Polak, W.T. Starmer, Parasite-induced risk of mortality elevates reproductive effort in male Drosophila, Proc. R. Soc. B Biol. Sci. 265 (1998) 2197–2201.

[63] T.R. Raffel, L.B. Martin, J.R. Rohr, Parasites as predators: unifying natural enemy ecology, Trends Ecol. Evol. 23 (2008) 610–618, https://doi.org/10.1016/j.tree.2008.06.015.

[64] I. Rodriguez-Prieto, J. Martin, E. Fernandez-Juricic, Individual variation in behavioural plasticity: direct and indirect effects of boldness, exploration and sociability on habituation to predators in lizards, Proc. R. Soc. B Biol. Sci. 278 (2011) 266–273, https://doi.org/10.1098/rspb.2010.1194.

[65] J.R. Rohr, A. Swan, T.R. Raffel, P.J. Hudson, Parasites, info-disruption, and the ecology of fear, Oecologia 159 (2009) 447–454, https://doi.org/10.1007/s00442-008-1208-6.

[66] I. González-Santoyo, A. Córdoba-Aguilar, Phenoloxidase: a key component of the insect immune system, Entomol. Exp. Appl. 142 (2011) 1–16, https://doi.org/10.1111/j.1570-7458.2011.01187.x.

[67] L.A.D. Sheridan, R. Poulin, D.F. Ward, M. Zuk, Sex differences in parasitic infections among arthropod hosts: is there a male bias? Oikos 88 (2000) 327–334, https://doi.org/10.1034/j.1600-0706.2000.880211.x.

[68] O.J. Schmitz, A.E. Rosenblatt, The temperature dependence of predation stress and prey nutritional stoichiometry, Front. Ecol. Evol. 14 (2017) 1–8, https://doi.org/10.3389/fevo.2017.00073.

[69] K.A. Sloman, G. Motherwell, K.I. O'Connor, A.C. Taylor, The effect of social stress on the standard metabolic rate (SMR) of brown trout, Salmo trutta, Fish Physiol. Biochem. 23 (2000) 49–53, https://doi.org/10.1023/a:1007855100185.

[70] S. Slos, R. Stoks, Predation risk induces stress proteins and reduces antioxidant defense, Funct. Ecol. 22 (2008) 637–642, https://doi.org/10.1111/j.1365-2435.2008.01424.x.

[71] S. Slos, L. De Meester, R. Stoks, Food level and sex shape predator-induced physiological stress: immune defence and antioxidant defence, Oecologia 161 (2009) 461–467, https://doi.org/10.1007/s00442-009-1401-2.

[72] U.K. Steiner, J. Van Buskirk, Predator-induced changes in metabolism cannot explain the growth/predation risk tradeoff, PLoS ONE 4 (2009), https://doi.org/10.1371/journal.pone.0006160.

[73] T. Szentivanyi, O. Vincze, P. Estok, Density-dependent sex ratio and sex-specific preference for host traits in parasitic bat flies, Parasit. Vectors 10 (2017), https://doi.org/10.1186/s13071-017-2340-0.

[74] M. Van Dievel, L. Janssens, R. Stoks, Short- and long-term behavioural, physiological and stoichiometric responses to predation risk indicate chronic stress and compensatory mechanisms, Oecologia 181 (2016) 347–357, https://doi.org/10.1007/s00442-015-3440-1.

[75] M. Walkey, R.H. Meakins, An attempt to balance the energy budget of a host-parasite system, J. Fish Biol. 2 (1970) 361–372.

[76] S.B. Weinstein, J.C. Buck, H.S. Young, A landscape of disgust, Science 359 (2018) 1213–1214, https://doi.org/10.1126/science.aas8694.

[77] B.W.M. Wone, P. Madsen, E.R. Donovan, M.K. Labocha, M.W. Sears, C.J. Downs, D.A. Sorensen, J.P. Hayes, A strong response to selection of mass-independent maximal metabolic rate without a correlated response in basal metabolic rate, Heredity 114 (2015) 419–427, https://doi.org/10.1038/hdy.2014.122.

[78] K.J. Yule, K.C. Burns, Parasite - offspring competition for female resources can explain male-biased parasitism in plants, Biol. Lett. 15 (2019), https://doi.org/10.1098/rsbl.2018.0761.