Article type : Articles

Allocation, not male resistance, increases male frequency during epidemics: A case study in facultatively sexual hosts

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Running Title: Infected hosts increase sex allocation

Abstract

Why do natural populations vary in the frequency of sexual reproduction? Virulent parasites may help explain why sex is favored during disease epidemics. To illustrate, we show a higher frequency of males and sexually produced offspring in natural populations of a facultative parthenogenetic host during fungal epidemics. In a multi-year survey of 32 lakes, the frequency of males (an index of sex) was higher in populations of zooplankton hosts with larger epidemics. A lake mesocosm experiment established causality: experimental epidemics produced a higher frequency of males relative to disease-free controls. One common explanation for such a pattern involves Red Queen (RQ) dynamics. However, this particular system lacks key genetic specificity mechanisms required for the RQ, so we evaluated two other hypotheses. First, individual females, when stressed by infection, could increase production of male offspring vs. female offspring (a tenant of 'Abandon Ship' theory). Data from a life table experiment supports this mechanism. Second, higher male frequency during epidemics could reflect a purely demographic process (illustrated with a demographic model): males could resist infection more than females (via size-based differences in resistance and mortality). However, we found no support for this resistance mechanism. A size-based model of resistance, parameterized with data, revealed why: higher male susceptibility negated the lower exposure (a size-based advantage) of males. These results suggest that parasite-mediated increases in allocation to sex by individual females, rather than male resistance, increased the frequency of sex during larger disease epidemics.

Keywords: *Daphnia*, parasite, parthenogenic, sex-specific infection, resistance, sex allocation

Introduction

Natural populations exhibit substantial variation in the frequency of sexual reproduction (Jokela et al. 2009; e.g., O'Connell and Eckert 2001; Tessier and Cáceres 2004). Given the myriad costs associated with sexual reproduction (Bell 1982; Maynard Smith 1978), it remains challenging to identify general mechanisms driving population-level variation in sex (reviewed by: Hartfield and Keightley 2012). The Red-Queen hypothesis poses that parasites can promote more sex (Decaestecker et al. 2003; Hamilton et al. 1990; Jokela et al. 2009). Here, parasites can increase the frequency of sexually reproducing hosts *via negative frequency-dependent selection*,

i.e., by sometimes granting sexual offspring fitness advantages over asexual ones (via host-parasite coevolution: Bell 1982). However, the Red-Queen does not apply to all host-parasite systems; mechanistically, it requires assumptions about specificity of infection genetics between the host and parasite. Numerous host-parasite systems lack these natural history ingredients (e.g., Clay and Kover 1996; Stelzer 2015, the focal system here). Hence, critics argue that the Red Queen remains too restrictive to generally drive population-level variation in sex (Otto 2009; Salathé et al. 2008; Stelzer 2015).

An alternative, 'Abandon Ship' hypothesis links stress to sex (Hadany and Otto 2009, and Mostowy and Engelstädter 2012). Stressors including drought, starvation, crowding, and predators can drive *increased allocation to sex* in a diverse array of organisms including facultative parthenogens (e.g., *Daphnia*: Cáceres and Tessier 2004; moths: Kumpulainen et al. 2004) and strictly sexual species (e.g., plants; Griffiths and Bonser 2013); (for more comprehensive lists see: Hadany and Otto 2009, and Mostowy and Engelstädter 2012). In the broadest sense, the Abandon Ship hypothesis posits that individual females in the poorest condition increase allocation to sex to escape eminent threats via dormancy or production of genetically diverse offspring (Hadany and Otto 2009). Hence, sex serves as a bet-hedging strategy governed by an individual's fitness prospects in the local environment. If variation in sex depends on the severity of stress, cahnges in environmental stressors could explain why natural populations vary in the frequency of sexual reproduction.

Here, we examine the ability of parasite-induced stress to drive allocation to sex in their hosts. While several studies have shown that parasites often increase outcrossing (e.g., Kovalchuk et al. 2003; Lucht et al. 2002), few studies have linked parasite-induced stress to sex-allocation decisions by individual hosts (but see Duncan et al. 2009; Duncan et al. 2006). This conceptual gap is quite surprising. Parasites are ubiquitous, often virulently exert physiological stress on hosts, and create spatio-temporal variation in infection-induced stress. Therefore, parasites likely serve as a key stressor catalyzing sex investment in non-Red Queen systems. We evaluate this allocation mechanism in this study.

However, a positive correlation between epidemics and sex could also emerge through an alternative, non-allocation mechanism: If males resist infection more than females, male frequency could increase during disease epidemics through demography, not via allocation decisions by individual females. In other words, male frequency could increase if females suffer

greater parasite-induced mortality. Such asymmetric mortality could leave behind more resistant males. To date, this hypothesis has remained mathematically and conceptually underdeveloped despite that males often resist infection [e.g., in Lyme disease (Jarefors et al. 2006), Schistosomiasis (Remoue et al. 2001), Toxoplasma (Walker et al. 1997), and Babesia (Aguilar-Delfin et al. 2001)]. Higher male resistance can arise through size-based, behavioral, or immunological traits that govern exposure or susceptibility to parasites (e.g., Cousineau and Alizon 2014; Moore and Wilson 2002; Perkins et al. 2003). Hence, male resistance, in its purely demographic form modeled here, could provide an underappreciated, yet important, alternative to more typical hypotheses attributing benefits of sex during epidemics.

We evaluate these two mechanisms (allocation vs. male resistance) using a facultatively sexual host (*Daphnia dentifera*; hereafter, 'hosts') and its virulent fungal parasite (*Metschnikowia bicuspidata*; hereafter, 'fungus' (Ebert 2005; Hall et al. 2009). In this system, the frequency of males provides an index of sexual reproduction and investment of hosts. We can use this system to link individual-level traits (e.g., allocation to male offspring by adult females, or male vs. female resistance) to population-level variation in sexual reproduction during natural and experimental epidemics. We combine multiple modes of inference to eventually confirm a parasite-driven allocation to sex mechanism. First, we illustrate a focal pattern: we see higher male frequency during larger fungal epidemics among natural lakes. We confirmed that parasites can cause higher male frequency using a mesocosm experiment deployed in a lake during the epidemic season (this experiment helps rule out other co-varying factors among lakes that could drive the field correlation). Then, a life table assay showed higher allocation to males by infected females. Finally, we ruled out a more complicated male resistance hypothesis, despite field and mesocosm data that seemed to support it (at first glance at least). We first used a demographic model to clarify conditions linking male resistance to higher male frequency during epidemics. However, a size-based resistance model revealed that males do not resist infection more than females (despite *a priori*, size-based expectations). Together, our results suggest that parasite-increased allocation to sex, rather than male resistance, drove this male frequency-epidemic size pattern.

NATURAL HISTORY OF THE HOST-PARASITE SYSTEM

The focal hosts are key consumers in food webs in north temperate freshwater lakes. These

facultative parthenogenetic hosts produce asexual broods of females throughout most of the year (Fig. 1). However, environmental stress that signals winter's onset (e.g., decreasing water temperatures and daylight) induces some females to increase allocation to sex (these females can then alternate between sexual and asexual reproduction and can produce mixed broods with male and female offspring). Sexual reproduction involves: (1) the production of males and (2) eggs inside a durable case, called an ephippium. Males then, fertilize these eggs creating genetically diverse eggs that can survive winter inside the protective and durable ephippia (Ebert 2005; Fig. 1). This temporal sequence often produces a positive relationship between the frequency of males and ephippia-carrying females (Tessier and Cáceres 2004, this paper). Thus, the frequency of males indexes the host's investment in sexual reproduction.

The decline of conditions from fall to winter also coincides with peak infection by the fungal parasite. Before ultimately killing the host, the parasite fills the host's body cavity with spores; upon host death, spores are released into the environment where hosts inadvertently consume them while foraging. The potential for sex-based differences in infection arise because male *Daphnia* typically have a smaller body size than adult females and exposure to fungal propagules increases with size (Hall et al. 2007). Thus, size-based exposure advantages could allow males to resist infection more than females (assuming equal susceptibility to fungal spores consumed between sexes).

A Motivating Field Pattern and Experimental Confirmation

Methods: Field Survey

To investigate relationships between parasites and sexual reproduction, we sampled natural epidemics across a set of lakes in southwestern Indiana (Greene and Sullivan Counties, USA). We collected weekly or fortnightly samples throughout the epidemic season (mid-August through early-December) from 2009-2015. In total, we sampled 32 lakes, some only one year, others up to seven years. From each sampling date at each lake, we collected hosts with three vertical tows of a Wisconsin net (13 cm diameter, 153μm mesh; towed bottom to surface). From these samples, we estimated several key metrics. First, from ~ 400 *Daphnia* per sample, we visually diagnosed infection status, host stage, and ephippia production with a dissecting scope at 20 – 50X magnification (following Ebert 2005). The absence/presence of a brood chamber distinguishes juveniles and adult stages; males have a distinctive body shape and large first

antennules. For each lake-year combination, we calculated seasonal maxima for frequencies of males and ephippial females. We estimated integrated infection prevalence by calculating the area under the infection prevalence curve (Van der Plank 1963). For lakes sampled multiple years, we averaged these metrics among years (± 1 SE). We correlated maximum frequency of males and infection prevalence. (Results were similar with the mean frequency of males). All analyses used Matlab (Matlab v.9.0 R2016a; Mathworks, Natick, MA, USA).

Methods: Lake Mesocosms

To establish population-level causation between parasites and shifts in allocation to sex (indexed as male frequencies), we created experimental epidemics in large (6 m deep x 1 m wide) lake-deployed mesocosms. The experiment began during the typical autumnal epidemic season to ensure that hosts and parasites experienced natural changes in temperature, food, and daylight — the associated cues known to induce the sex response. In brief, we factorially manipulated epidemics and nutrients. (Nutrients conservatively reflect ranges from the field survey). We then tracked epidemics for 40 days post-inoculation (for detailed methods see Appendix S2). As in the field survey, we quantified stage-specific and overall infection prevalence. We analyzed differences in infection prevalence among males, juvenile females, and adult females with pair-wise randomization tests (10,000 iterations). To rule out crowding as a driver of male frequency (Hobaek and Larsson 1990), we estimated host density. We analyzed differences in the maximum male frequency (calculated as for the field survey) and density among treatments with generalized linear models (GLM) with binomial and log-normal errors, respectively. For both analyses, we ran saturated and reduced models and selected the best-fitting model with chi-squared or likelihood ratio tests. For all GLM models, we tested for overdispersion using $\hat{c}$, sum of the squared residuals from the fitted GLM/residual degrees of freedom (Burnham and Anderson 2002). We used the appropriate quasi-distribution if $\hat{c} > 1$.

Results: Field Survey

Maximum frequencies of males and ephippial females increased with epidemic size in the field survey (Fig. 2). Each point averages years (from 1 to 7 ± 1 SE). Male frequency is relative to adult females: male density / (male density + adult female density), excluding female juveniles (results were similar including juveniles). Male frequency (y-axis) was higher in lakes with

larger epidemics of the focal fungal parasite (*x*-axis, where each point is the integrated prevalence of infection in lake; r = 0.43, p = 0.017, Fig. 2*a*). Maximum frequency of males also positively correlated with maximal frequency of ephippium-carrying females among lakes (where each point is maximal frequency, averaged over years for a given lake; r = 0.55, p = 0.001, Fig. 2*b*). Together, these correlations suggest that larger epidemics led to more males, and more males led to increased sexual reproduction (indexed by frequency of ephippial females).

Time series from two lakes illustrate dynamics underlying these patterns. In these *examples*, we see a temporal cadence of increasing infection prevalence, then male frequency and the frequency of ephippial females (proportion of males and of ephippia-carrying females relative to non-ephippial adult females; Tessier and Cáceres 2004) through the seasonal epidemics. In the lake with a small epidemic, male production began on ordinal date 290 (1 October 2011, Fig 2*c*) but female hosts produced no detectable ephippia before the survey ended. In the lake with a large epidemic, male production began slightly earlier, ordinal date 278 (28 September 2011, Fig 2*d*), and male frequency was relative to the small epidemic lake; ephippial production began on ordinal date 299 (26 October 2011). This chronology shows that male and ephippial production are, in part, modulated by the onset of winter (since autumnal cooling triggers sex in this host). Yet, investment in sex was lower in the lake with a small fungal epidemic (Dogwood, 2011; Fig. 2*c*) relative to a lake with larger fungal epidemic (Midland, 2011; Fig. 2*d*). Among all lakes, similar dynamics produced the motivating correlation (Figs. *2a, b*).

Results: Lake mesocosms

In the field experiment (Fig. 3), host populations also shifted towards higher frequencies of males during fungal epidemics, as in the field survey. Maximum male frequency increased with the addition of fungal parasites (main parasite effect (P): $\chi^2 = 7.79$, p = 0.005, $\hat{c} = 0.07$, Fig. 3*a*). However, there was no effect of nutrients ($\chi^2 = 0.165$, p = 0.685) or their interaction ($\chi^2 = 1.52$, p = 0.218). In the high nutrient treatment, infection prevalence was slightly higher (Hite et al. 2016) and male production was of longer duration (Fig. 3*c,d*) relative to the low nutrient treatment (Appendix S2, Fig. S1). The key point, however: at two varying nutrient levels, parasites increased male frequency. As expected, disease decreased host density (P: $\chi^2 = 4.61$, p = 0.032, $\hat{c} = 0.1$, Fig. 3*b*). However, there were no main (N: $\chi^2 = 2.88$, p = 0.090) or interactive effects of nutrients on host density (P x N: $\chi^2 = 1.19$, p = 0.280). Thus, hosts did not produce

more males due to crowding (a common stressor). In other words, crowding did not explain the epidemic size-male frequency pattern in the mesocosms.

Temporal dynamics in the experiment (Fig. 3*c*, *d*) largely mirror those from the field (Fig. 2*c*,*d*). They also underlie the summary patterns from the experiment (Fig. 3*a*). Across all high nutrient replicates, the onset of male production occurred on ordinal date 278 (5 October 2011; Fig. 3*c*-*d*). (See Appendix S2 for similar patterns in the low nutrient treatments, Fig. S1). In the absence of parasites, peak male frequency reached c. 52% (dashed line, both figures) on ordinal date 292 (19 October); then, it declined on ordinal date 295 (22 October). With parasites, male frequency peaked later and was higher (Fig. 3*d*). Note that, unlike in the field survey, the experiment ended before ephippium-carrying females appeared.

TEST OF THE ALLOCATION TO SEX MECHANISM

Methods: Life-table Assay

We used a life-table experiment to test for increased allocation to sex (male frequency) by individual, infected females. In short, we first created six environments (flasks) that contained the requisite cues to catalyze a transition to the sexual stage (higher density, end of epidemic season temperature and light conditions: 15°C, 8:16 light: dark cycle (Tessier and Cáceres 2004). We added parasites to three flasks and kept the other three flasks parasite-free. After epidemics began, we collected 15 individual females from each flask and tracked their allocation to sex (# males/total offspring produced) over three clutches while keeping them exposed to environmental cues from their natal flask (for expanded details see Appendix S2). To test for increased allocation to sex (frequency of males) and fecundity declines due to infection, we fit a mixed-effects generalized linear model (GLMM) with binomial errors (male frequency) or Poisson errors (fecundity). We checked for overdispersion with visual diagnostics and the scale parameter (Pinheiro and Bates 2000). This model also accounted for potential differences among flasks.

Results: Life-table Assay

Data from the life table assay supported the 'allocation to sex' mechanism. Infected female hosts in the life table assay significantly increased allocation to sex compared to uninfected females. These females came from and received cues from flasks where final infection

prevalence ($\hat{c}$ = 0.04, p = 0.530) and final host density were similar across all treatments (Flask: p = 0.768; Spore level: p = 0.433). All females originally exposed in those flasks, then used for the life table assay, became infected. These infected females in the life table produced higher frequencies of males (GLMM, parasite treatment: $0.75 > \hat{c} < 1.4$, $\chi^2 = 5.46$, p = 0.019, Fig. 4*a*) and produced smaller clutches (p = 0.018, Fig. 4*b*). Thus, infected females incurred a parasite-mediated reduction in fecundity but allocated that reduced reproduction towards males. Hence, the epidemic size-male frequency pattern seen in these lakes could have arisen because infection-stressed females increased allocation to sex (male production).

DEVELOPMENT OF THE MALE RESISTANCE MECHANISM: A DEMOGRAPHIC MODEL

The alternative male resistance mechanism poses that correlations between male frequency and epidemic size in the field could reflect demography. Do males resist infection and increase in frequency due to parasite-driven mortality of less resistant females? We evaluate this possibility using a demographic model of disease, reproduction, and sexual allocation. This model separated feedbacks and identified key metrics from field and mesocosm data to evaluate the hypothesis. The details of this model appear in Appendix S1. In brief: we highly simplify reproduction, assuming that changes in male and female density reflect allocation (s) to each sex from a constant reproductive flux (R). Then, we assume a constant force of infection. These two assumptions removed some density-dependent feedbacks on reproduction and disease, but enabled analytical tractability. We derive conditions under which male frequency increases with larger epidemics, like in the field pattern, and over a disease-free baseline, as in the experiment. Importantly, differential mortality of infected males vs. females placed some important demographic bounds on this male-resistance mechanism. We then compared and contrasted infection prevalence of females vs. males. How does male resistance influence patterns of infection prevalence between females and males?

Here in the main text, we summarize key results from the demographic model. (For analytical and graphical details, see Appendix S1). First, the model predicts that *complete* male resistance (an extreme example) almost certainly leads to increasing male frequency with epidemic size (version A) and higher male frequency over a disease-free baseline (version B). However, if males become infected, *moderate* male resistance can: (1) produce higher male frequency with epidemics and over a disease-free baseline and (2) lead to higher infection

frequency in females and males. However, both infection prevalence and male frequency results depend on stage-specific mortality: males cannot suffer severe mortality from infection. This result puts some demographic bounds on the male resistance mechanism. The model readily captures the increase above the disease-free baseline version (like in the mesocosm experiment: Fig. 3). Thus, male resistance provides a mathematically viable alternative mechanism for the epidemic size-male frequency pattern — as long as males do not suffer extreme virulence.

QUANTITATIVE EVALUATION OF THE ALTERNATIVE MECHANISM: MALE RESISTANCE

Methods: Field Survey and Mesocosms vs. Lab Assay

We empirically tested the hypothesis that smaller males have higher resistance due to less (slower) contact with spores. First, we perform an 'indirect test': we evaluated infection prevalence in the field. This test is indirect because field prevalence does not just mirror resistance. Any epidemiological model, like the one above, shows how prevalence during an epidemic combines additional traits besides resistance and various dynamical feedbacks. Therefore, prevalence can reflect resistance— assuming all else equal. Thus, for this indirect text, we established that smaller size of males with measurements of c. 40 individuals of each host stage in 23 lakes on each sampling date during epidemic season of 2015. Then, we estimated mean stage-specific infection prevalence (e.g., # infected males/total # males; see Appendix S2 for extended details) in each lake and mesocosm population for each sampling date.

Second, we performed a more direct test of male resistance. Specifically, we estimated resistance of each stage directly from a highly controlled lab assay, essentially eliminating the influence of other traits that also shape prevalence during field and mesocosm epidemics. In this lab experiment, we measured exposure (feeding) rate and infection prevalence (and then used those data to estimate per-spore susceptibility and resistance in the model below). In brief, we measured food/spore consumption by males, juvenile females, and adult females exposed to one of three parasites doses (0, 150, or 350 sp/mL) for 48 hours. We then measured hosts and maintained them for subsequent visual diagnosis for 19 days post exposure. (For details, see Appendix S2). We analyzed differences in infection prevalence from this controlled assay across stages and spore doses with logistic regression.

Results: Field Survey and Mesocosms vs. Lab Assay

In the indirect test, the field survey and mesocosm experiment produced, at first glance, some support for the resistance mechanism. However, the controlled assay undermined this support. Together, these results highlight important distinctions between prevalence and resistance. In the field survey, male and juvenile female hosts were similarly sized ($p = 0.175$), but both males ($p < 0.001$) and juvenile females ($p < 0.001$) were smaller than adult females (Fig. 5*a*). Hence, males likely have lower exposure than adult females, all else equal (i.e., the exposure part of the hypothesis might apply). Then, in the field survey, infection prevalence was similar among males and juvenile females (squares Fig. 5*b;* $p = 0.409$) but lower than adult females (all p-values< 0.001). The mesocosm experiment mirrored these results, except that males had lower infection prevalence relative to both female stages (high nutrient treatments: triangles Fig. 5*b,* all p-values < 0.0001); low nutrient treatments (not shown) showed similar results. However, in the controlled, lab-based assay, logistic regression quantified no difference in infection prevalence between stages, suggesting similar resistance levels among stages (for full results, with dose effects, see Appendix S2). (We discuss possible reconciliation between the indirect test [Fig. 5*b*] vs. the direct test estimates of infection prevalence and resistance [Figs. 5*c* and 6*c*] below).

Methods: A Size-Based Model of Resistance

In the indirect test of male resistance, field and mesocosm data suggested that males were more resistant than adult females (based on infection prevalence, which again is an indirect measure of resistance). Yet, the controlled lab experiment indicated similar infection prevalence among smaller males and larger females. Why did the size-based hypothesis for male resistance fail? To answer this question, we fit data from the lab assay to a size-based model of resistance (modified from Bertram et al. 2013). For details of this model see Appendix S2. Briefly: the model assumes that exposure, $E(L,Z)$, scales with surface area (L^2) and with size-corrected rate $\hat{E}$ but declines with exposure to spores, Z (via sensitivity α). Susceptible hosts which contact spores are then infected with per spore susceptibility u, resistance is $\beta(L,Z) = u\,E(L,Z)$. Estimation of $\hat{E}_j$ and u_j for each stage j and two other common parameters uses maximum likelihood. We also calculated size-corrected resistance as $\hat{\beta}_j = \mu\,\hat{E}_j$. We then bootstrapped 95% confidence intervals around each parameter and compared estimates among stages using randomizations. Finally, we bootstrapped confidence envelopes on feeding rate, $E_j(L,Z)$, and resistance, $\beta_j(L,Z)$,

as functions of length and spore dose.

Results: A Size-Based Model of Resistance

The size-based model of resistance explains why males are not more resistant despite being smaller than adult females. Indeed, the size-based exposure part of the resistance model works well. In fact, compared to both juvenile ($p = 0.0004$) and adult females ($p = 0.0004$), males had much lower size-corrected exposure ($\hat{E}$) rates, i.e., lower foraging/exposure — even after accounting for their small size (Fig. 6*a*). After controlling for size, juvenile and adult females had similar exposure rates (size-corrected $\hat{E}$; $p = 0.0684$, adults trending higher). All else equal, then, males should have been more resistant. However, males had similar per-spore susceptibility (u) compared to both juveniles ($p = 0.5838$) and adult females ($p = 0.1112$, Fig. 6*b*; adults trending lower than males) and adult females had lower per-spore susceptibility (u) relative to juveniles ($p = 0.0344$). Combined, tension between exposure, $\hat{E}$, and susceptibility led to no significant differences between males and adult females in size-corrected resistance,

$$\widehat{\beta_J}$$

, (all p-values of pair-wise comparisons > 0.05, Fig. 6*c*). Additionally, adding in variation in size among stages, both exposure rate, $E_j(L,Z)$ (Fig. 6*d*), and resistance, $\beta_j(L,Z)$, increased with host size (but flattened and then decreased as large adult females depressed their feeding at high dose; Fig. 6d; see also Appendix S2 for results at lower doses which show less foraging depression). Hence, larger adult females and smaller males had similar levels of resistance (i.e., point estimates with confidence envelopes overlapped considerably, Fig. 6*e*). Taken together, these results do not support the hypothesis that smaller males resist infection through lower exposure. Thus, through rigorous evaluation of male resistance, we conclude that the male resistance mechanism likely did not drive the epidemic size-male frequency pattern in the field.

DISCUSSION

We evaluated two mechanisms which could link disease epidemics to the frequency of sex. In a multi-year, multi-lake field survey, the frequency of males (an index of sex) was higher in lake populations of zooplankton hosts with larger fungal epidemics. A mesocosm experiment confirmed causality: the frequency of males increased with parasites relative to disease-free controls. (Since it directly manipulated parasites in the field, this experiment obviates worry

about spurious correlation). Following Abandon Ship theory (Hadany and Otto 2009), these epidemic size-male frequency patterns could arise if infection-stressed females increased allocation to sex (males) (Duncan and Little 2007; Griffiths and Bonser 2013; Mostowy and Engelstaedter 2012). However, it could have emerged due to population-level consequences of male resistance (a typically overlooked but important possibility that could also drive a positive relationship between epidemics and sex).

We found that individual, infected females allocated more to male offspring. Stress from infection manifested (at least in part) as virulence on fecundity; infected hosts produced clutches with fewer offspring relative to uninfected hosts. Those infection-stressed females then produced a higher proportion of males per clutch. This 'Abandon ship' stress response resembles that of other facultatively pathenogenic and strictly sexual organisms which plastically alter investment in sex when stressed (e.g., by drought, low resources, and crowding; for comprehensive lists: Hadany and Otto 2009; Mostowy and Engelstaedter 2012). Here, plastic allocation choices by infection-stressed females most likely produced the sex-epidemic size pattern seen in the field.

We arrive at that conclusion because the alternative, 'male resistance' mechanism failed. Males were indeed smaller, in the field and lab experiment, than adult females. Furthermore, they had slower foraging (and thus, exposure) rates. Such size and exposure differences should have yielded male resistance. Yet, even after accounting for exposure, smaller males and larger females resisted infection similarly. The mechanistic model of resistance explained why: males were equally susceptible to infection as juvenile females and tended to be more susceptible than adult females. Furthermore, higher spore doses depressed exposure of larger adult females but not males. Both factors negated the size-based exposure advantage of males. Hence, we find no support for the male-resistance mechanism. Still, sex-based differences in resistance arise frequently in other systems (e.g., Aguilar-Delfin et al. 2001, Jarefor et al. 2006, Remoue et al. 2001, Walker et al. 1997) and could drive population-level differences in the frequency of sex more broadly.

The failure of the male resistance mechanism seemed surprising given differences in infection prevalence between males and females in the indirect test, i.e., using prevalence data from the survey and field experiment. If males resisted infection more than females, the demographic model predicted that female infection prevalence should (usually) exceed male prevalence, as seen here in the survey and experiment in the field. Yet, the resistance model and

experiment ruled out male resistance. One must remember, however, that infection prevalence in the field (and fully dynamical models) does not simply mirror resistance. Hence, the contrast between the prevalence-based indirect test vs. the actual resistance metric highlights key differences between prevalence and resistance.

This allocation response by infected females did not arise due to a Red Queen mechanism. The epidemic size-male frequency correlation detected here superficially resembled predictions from the Red-Queen hypothesis (RQH). In the RQH, parasites can increase frequency of sexually reproducing hosts by sometimes granting them fitness advantages over asexual ones (via host-parasite coevolution). The RQ selection mechanism can produce positive correlations between epidemic size and frequency of sex, often indexed as percent males (Decaestecker et al. 2007; Hamilton et al. 1990; Jokela et al. 2009). However, the *Daphnia*-fungus system here clearly lacks essential components required for the RQH (summarized in Appendix S2). Thus, while the Red Queen provides a powerful model for parasite-induced sex, the focal system lacks most of the requisite natural history ingredients.

Links between parasite-induced stress and allocation to sex are particularly intriguing for facultative parthenogens. For these organisms, sex intricately links to dormancy and dispersal (Bell 1982; Bonner 1958). Hence, ecological conditions that induce allocation to sex can also modulate population genetic variance, rates of evolution (Balloux et al. 2003; Wright 1931), and inbreeding depression (Cáceres et al. 2009). Therefore, connections between parasites and allocation to sex in these (and other) organisms may help clarify how and when parasites drive/maintain variation in their host populations. How generally, then, do parasites stress hosts enough to alter allocation to sex at the individual and population levels? What genetic components (e.g., modifier genes, Hadany and Otto 2009) regulate the switch to sexual reproduction? Future studies that address these physiological and genetic questions will advance our understanding of the factors driving variation in the frequency of sexual reproduction.

ACKNOWLEDGMENTS

We are grateful to K. Boatman, A. Bowling, and Z. Brown for field assistance. S. Siscoe, R. Ronk, B. Feaster, and T. Stoelting at the Indiana DNR facilitated the field survey. Discussions with A. de Roos (UvA) and the Bever and Lively labs (IU) improved this manuscript. An EPA STAR Fellowship supported JLH. NSF GRFs supported RMP, MSS, and ATS. NSF funded this

work: (DEB-0841679, 0841817, 1120316, 1120804, 1353749, 1354407, 1353806). Parameter estimates utilized Karst, funded through the Lilly Endowment, Inc. and the Indiana METACyt Initiative.

LITERATURE CITED

Aguilar-Delfin, I., M. J. Homer, P. J. Wettstein, and D. H. Persing. 2001. Innate resistance to Babesia infection is influenced by genetic background and gender. Infection and Immunity 69:7955-7958.

Balloux, F., L. Lehmann, and T. de Meeus. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164:1635-1644.

Bell, G. 1982, The Masterpiece of Nature: The evolution of genetics of sexuality. Berkeley, CA, USA, University of California Press.

Bertram, C. R., M. Pinkowski, S. R. Hall, M. A. Duffy, and C. E. Cáceres. 2013. Trait-mediated indirect effects, predators, and disease: test of a size-based model. Oecologia 173:1023-1032.

Bonner, J. T. 1958. The relation of spore formation to recombination. American Naturalist 92:193-200.

Burnham, K. P., and D. R. Anderson. 2002, Model selection and multimodel inference: a practical information-theoretic approach New York., Springer-Verlag.

Cáceres, C. E., C. Hartway, and K. A. Paczolt. 2009. Inbreeding depression varies with investment in sex in a facultative parthenogen. Evolution 63:2474-2480.

Clay, K., and P. X. Kover. 1996. The Red Queen Hypothesis and plant/pathogen interactions. Annual Review of Phytopathology 34:29-50.

Cousineau, S. V., and S. Alizon. 2014. Parasite evolution in response to sex-based host heterogeneity in resistance and tolerance. Journal of Evolutionary Biology 27:2753-2766.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host-parasite 'Red Queen' dynamics archived in pond sediment. Nature 450:870-873.

Decaestecker, E., A. Vergote, D. Ebert, and L. d. Meester. 2003. Evidence for strong host clone-parasite species interactions in the *Daphnia* microparasite system. Evolution 57:784-792.

462 Duncan, A. B., S. A. Hall, and T. J. Little. 2009. Parasitism and environmental sex determination
463 in *Daphnia*. Evolutionary Ecology Research 11:965-973.
464 Duncan, A. B., and T. J. Little. 2007. Parasite-driven genetic change in a natural population of
465 *Daphnia*. Evolution 61:796-803.
466 Duncan, A. B., S. E. Mitchell, and T. J. Little. 2006. Parasite-mediated selection and the role of
467 sex and diapause in *Daphnia*. Journal of Evolutionary Biology 19:1183-1189.
468 Ebert, D. 2005, Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia*. Bethesda, MA,
469 USA, National Library of Medicine, National Center for Biotechnology Information.
470 Ebert, D. 2008. Host-parasite coevolution: Insights from the *Daphnia*-parasite model system.
471 Current Opinion in Microbiology 11:290-301.
472 Griffiths, J. G., and S. P. Bonser. 2013. Is sex advantageous in adverse environments? A test of
473 the Abandon-Ship hypothesis. American Naturalist 182:718-725.
474 Hadany, L., and S. P. Otto. 2009. Condition-dependent sex and the rate of adaption. American
475 Naturalist 174:S71-S78.
476 Hall, S. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009.
477 Friendly competition: evidence for a dilution effect among competitors in a planktonic
478 host-parasite system. Ecology 90:791-801.
479 Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist
480 parasites (a review). Proceedings of the National Academy of Sciences 87:3566-3573.
481 Hartfield, M., and P. D. Keightley. 2012. Current hypotheses for the evolution of sex and
482 recombination. Integrative Zoology 7:192-209.
483 Hite, J. L., R. M. Penczykowski, M. S. Shocket, A. T. Strauss, P. A. Orlando, M. A. Duffy, C. E.
484 Cáceres et al. 2016. Parasites destabilize host populations by shifting stage-structured
485 interactions. Ecology 97:439-449.
486 Hobaek, A., and P. Larsson. 1990. Sex determination in *Daphnia magna*. Ecology 71:2255-2268.
487 Jarefors, S., L. Bennet, E. You, P. Forsberg, C. Ekerfelt, J. Berglund, and J. Ernerudh. 2006.
488 Lyme borreliosis reinfection: might it be explained by a gender difference in immune
489 response? Immunology 118:224-232.
490 Jokela, J., M. F. Dybdahl, and C. M. Lively. 2009. The maintenance of sex, clonal dynamics, and
491 host-parasite coevolution in a mixed population of sexual and asexual snails. American
492 Naturalist 174:S43-S53.

493 Kovalchuk, I., O. Kovalchuk, V. Kalck, V. Boyko, J. Filkowski, M. Heinlein, and B. Hohn.
494 2003. Pathogen-induced systemic plant signal triggers DNA rearrangements. Nature
495 423:760-762.
496 Lively, C. M., M. F. Dybdahl, J. Jokela, E. E. Osnas, and L. F. Delph. 2004. Host sex and local
497 adaptation by parasites in a snail-trematode interaction. The American Naturalist 164:S6-
498 S18.
499 Lucht, J. M., B. Mauch-Mani, H. Y. Steiner, J. P. Metraux, J. Ryals, and B. Hohn. 2002.
500 Pathogen stress increases somatic recombination frequency in *Arabidopsis*. Nature
501 Genetics 30:311-314.
502 Matlab. R2016a. Mathworks, Natick, MA, USA.
503 Maynard Smith, J. 1978, The evolution of sex. Cambridge, England, Cambride Univ. Press.
504 Moore, S. L., and K. Wilson. 2002. Parasites as a viability cost of sexual selection in natural
505 populations of mammals. Science 297:2015-2018.
506 Mostowy, R., and J. Engelstaedter. 2012. Host-parasite coevolution induces selection for
507 condition-dependent sex. Journal of Evolutionary Biology 25:2033-2046.
508 O'Connell, L. M., and C. G. Eckert. 2001. Differentiation in reproductive strategy between
509 sexual and asexual populations of *Antennaria parlinii* (Asteraceae). Evolutionary
510 Ecology Research 3:311-330.
511 Otto, S. P. 2009. The evolutionary enigma of sex. The American Naturalist 174:S1-S14.
512 Perkins, S. E., I. M. Cattadori, V. Tagliapietra, A. P. Rizzoli, and P. J. Hudson. 2003. Empirical
513 evidence for key hosts in persistence of a tick-borne disease. International Journal for
514 Parasitology 33:909-917.
515 Pinheiro, J. C., and D. M. Bates. 2000, Mixed Effects Models in S and S-Plus. New York, NY
516 USA, Springer New York
517 Remoue, F., D. T. Van, A. M. Schacht, M. Picquet, O. Garraud, J. Vercruysse, and A. Ly. 2001.
518 Gender-dependent specific immune response during chronic human *Schistosomiasis*
519 *haematobia*. Clinical and Experimental Immunology 124:62-68.
520 Salathé, M., R. D. Kouyos, R. R. Regoes, and S. Bonhoeffer. 2008. Rapid parasite adaptation
521 drives selection for high recombination rates. Evolution 62:295-300.
522 Stelzer, C. P. 2015. Does the avoidance of sexual costs increase fitness in asexual invaders?
523 Proceedings of the National Academy of Sciences 112:8851-8858.

Tessier, A. J., and C. E. Cáceres. 2004. Differentiation in sex investment by clones and populations of *Daphnia*. Ecology Letters 7:695-703.

Van der Plank, J. E. 1963, Plant disease: Epidemics and Control. N.Y., N.Y. USA, Academic Press.

Walker, W., C. W. Roberts, D. J. P. Ferguson, H. Jebbari, and J. Alexander. 1997. Innate immunity to *Toxoplasma gondii* is influenced by gender and is associated with differences in interleukin-12 and gamma interferon production. Infection and Immunity 65:1119-1121.

Wright, S. 1931. Evolution in mendelian populations. Genetics 16:0097-0159.

FIGURE CAPTIONS

Figure 1. Life cycle of the host, *Daphnia dentifera*. Solid lines depict the asexual parthenogenetic phase. Dashed lines depict the sexual phase. Numbers in parentheses reflect the ploidy of the gametes produced by different stages. Note the smaller size of adult males relative to adult females. Illustration by Julia Ferguson.

Figure 2. *The epidemic size-male frequency pattern-field survey*: Variation in the maximum frequency of males, relative to adult females, in populations of a zooplankton host with varying epidemic sizes (indexed as integrated prevalence [proportion days]). Each point is one of 32 lakes, with the maximum frequency or integrated epidemic prevalence averaged across 1-7 years (2009 – 2015) ± SE. Regression best-fit line (black line) and lower/upper 95% confidence envelopes (grey lines). (*A*) Males became more frequent during larger epidemics of the fungal parasite. (*B*) Populations with higher maximal frequency of males had higher maximal frequency of ephippial females. *Examples*: (*C, D*) Mean frequency of infection (grey), males (black), and ephippial females (white) through the autumnal epidemic season in a lake with (*C*) a small fungal epidemic (Dogwood, 2011) and (D) a larger one (Midland, 2011).

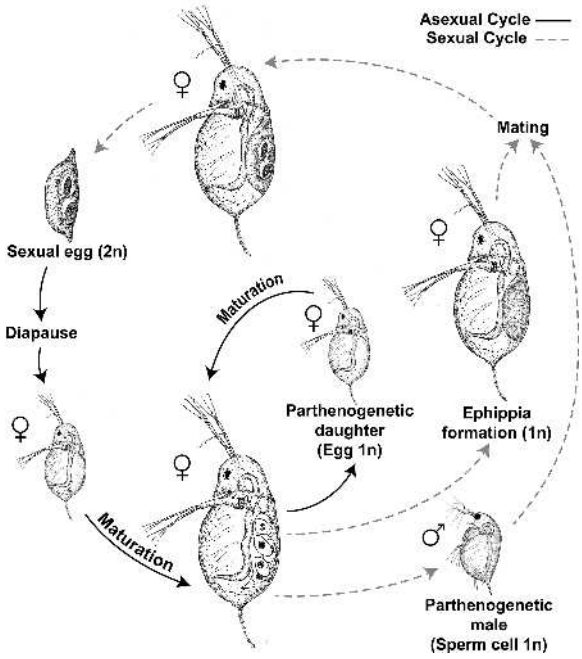
Figure 3. *Experimental confirmation of the epidemic size-male frequency pattern: (A)* Lake-deployed mesocosms confirmed that fungal epidemics caused host populations to shift toward higher mean frequency of males (accounting for a nutrient effect and interaction: see text). (*B*) Disease significantly decreased host density. Thus, stressful overcrowding did not explain higher male frequency in parasite treatments. For GLM-produced *P*-values, ‘F’, ‘N’, and ‘F´N’ indicate fungal parasite, nutrient, and interactive effects, respectively. (*C, D*) Seasonal dynamics from the

high nutrient treatment illustrate mean frequency of males (black) without (—, panel C) and with (+, panel D) parasites. Grey squares denote parasite prevalence. The dashed line marks maximum frequency of males in the parasite-free treatment. Points are means ± SE.

Figure 4. *Experimental test of the 'allocation to sex' mechanism: life-table assay:* Individual, infected female hosts increase production of males in a life-table assay. (*A)* Infected female hosts, from '+ parasite' flasks, significantly increased the frequency of males produced per clutch. (*B*) Virulence on fecundity: infected hosts produced fewer offspring relative to uninfected hosts. P-values come from a generalized linear mixed effects model. Filled and unfilled symbols are '— parasite' and ' + parasite' treatments, respectively. Data are means ±SE.

Figure 5. *Quantifying 'male resistance': field survey and lake mesocosms vs. lab experiments:* (*A*) In the field survey (2015), males and juvenile females were significantly smaller than adult female hosts, confirming the size component of the hypothesis. (*B*) In the field survey (squares) and mesocosm experiment (triangles), males and juveniles had lower infection prevalence (means ± SE) relative to adult females. Lower case letters indicate significant differences between stages; survey and mesocosm data analyzed separately. (*C*) In the lab experiment, males also tended to have lower infection prevalence relative to juvenile and adult females (means ± bootstrapped 95% CI). However, infection prevalence did not differ significantly across stages. P-values are from a logistic regression model with "D" representing parasite-dose effects, "St" representing stage effects, and "D x St" representing their interaction.

Figure 6. *Quantifying 'male resistance' with a size based model:* A size-based model of resistance shows that smaller males do not resist infection more than adult females. (*A*-*C*) Parameter estimates (± 95% CI) from the model (equs. 1,2) fit to a joint foraging-infection assay. (*A*) Size-corrected exposure rate, $\hat{E}$ (equ. 1), (*B*) per-spore susceptibility, *u* (i.e., susceptibility of hosts to infection to consumed hosts), and (*C*) size-corrected resistance, β (i.e., the product of $\hat{E}$ and *u*; low β means high resistance). (*D*, *E*) Best-fit model predictions of (D) exposure rate, $Ej(L,Z)$, and (E) resistance $\beta j(L,Z)$, for each host stage (means ± 95% confidence envelopes).



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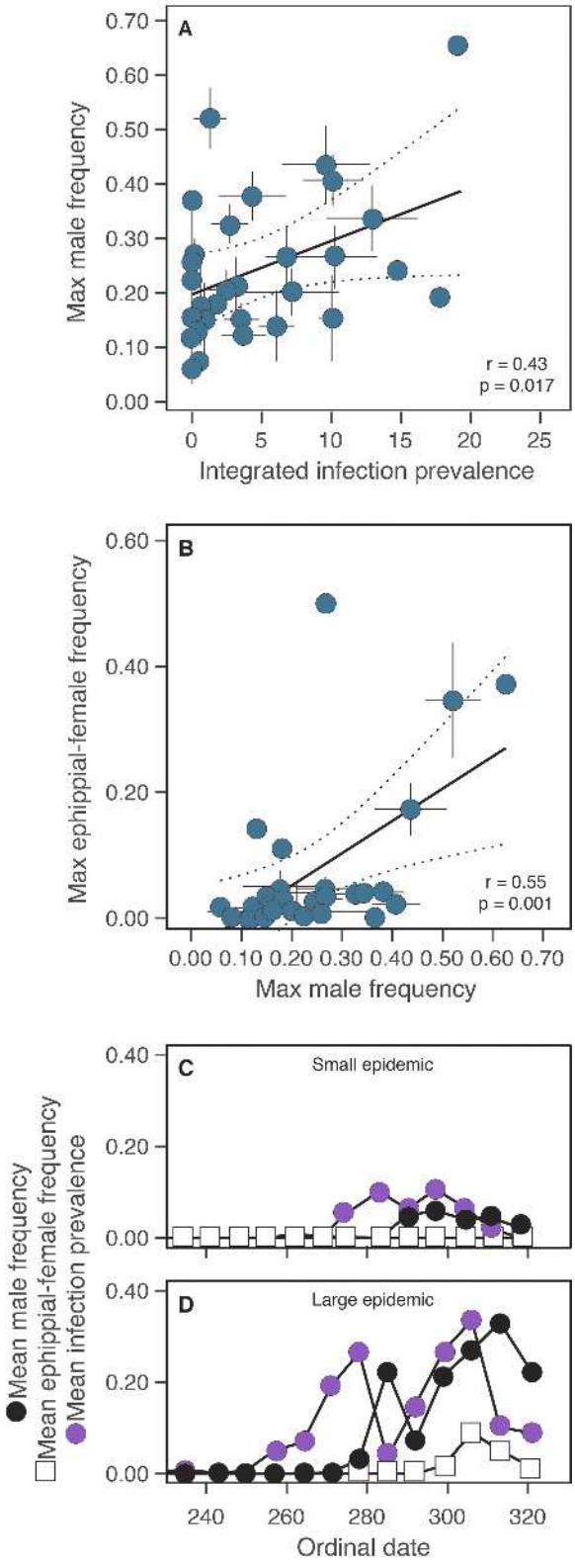

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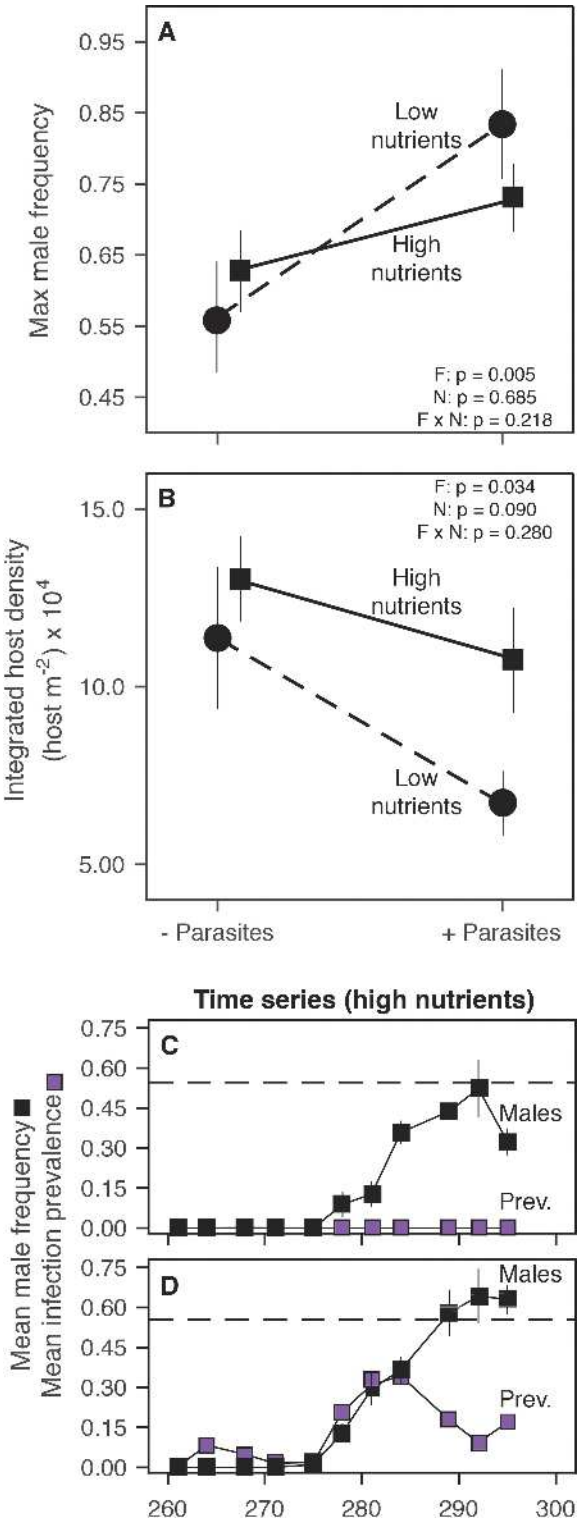

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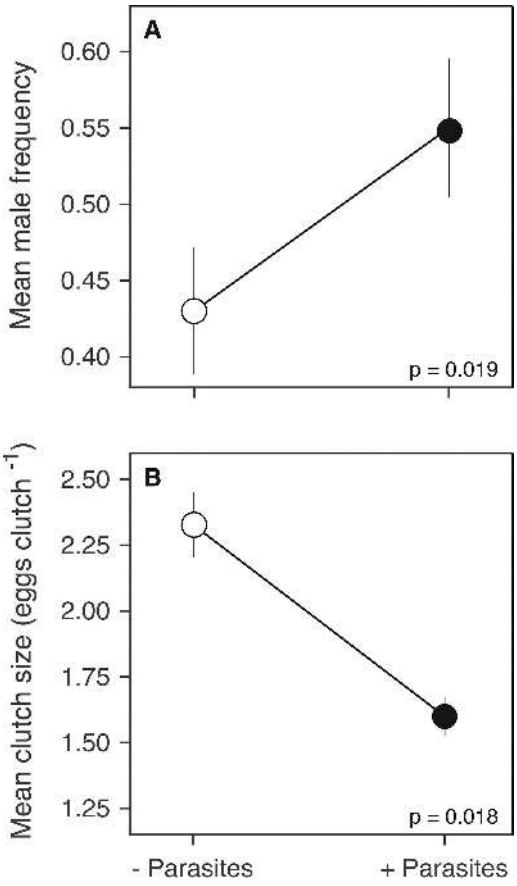

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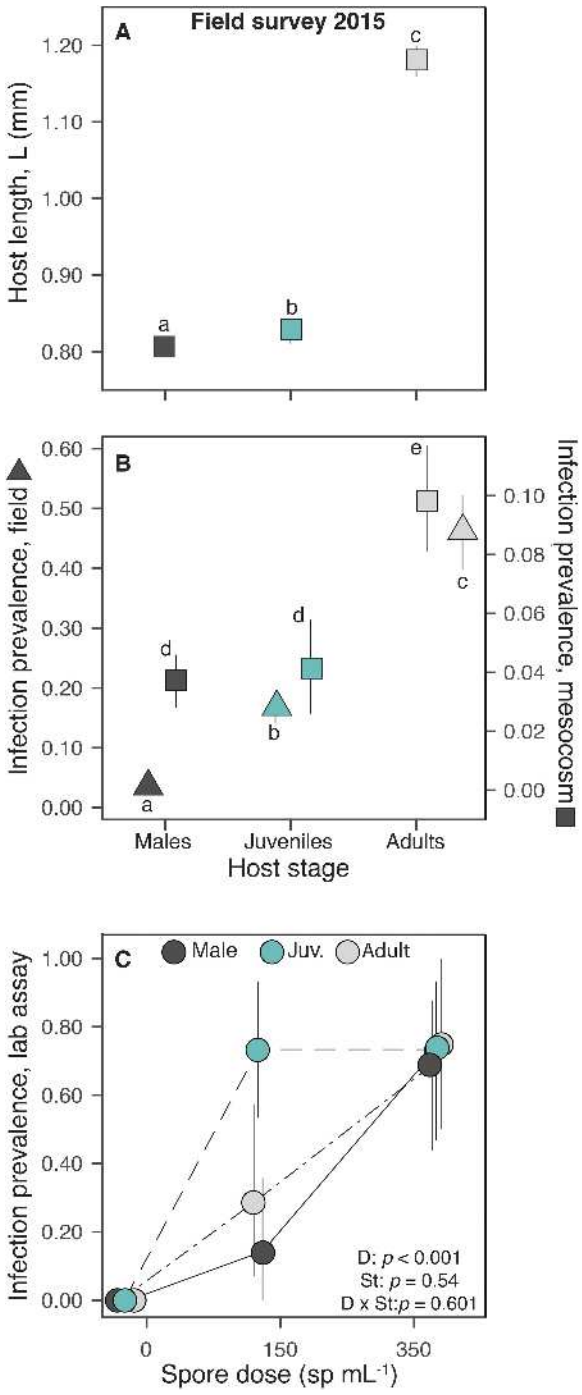

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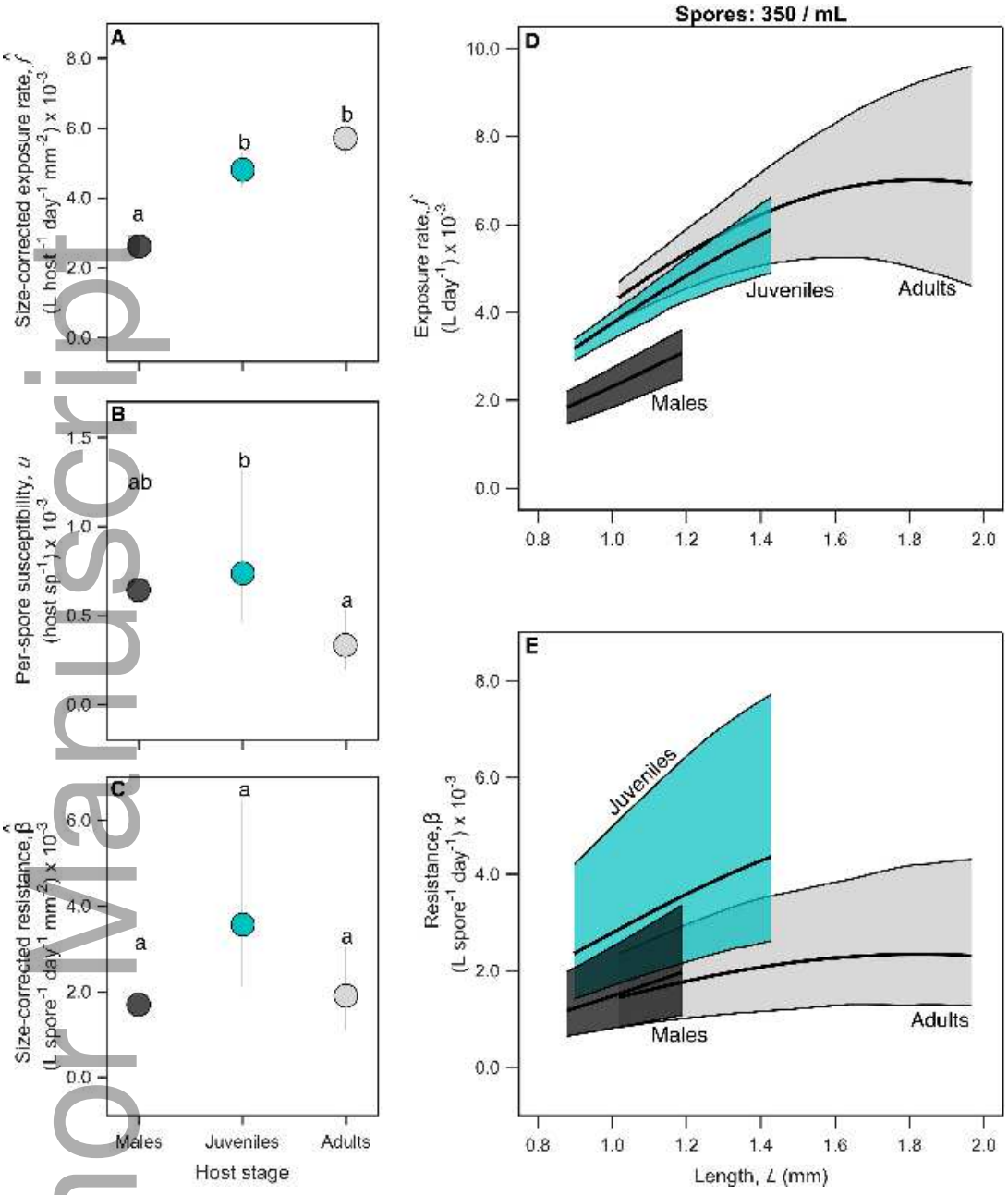

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