Male and female reproductive fitness costs of an immune response in natural populations

Stephen P. De Lisle*

Daniel I. Bolnick

Department of Ecology & Evolutionary Biology

University of Connecticut

Storrs, CT 06269

* email: stephen.de_lisle@uconn.edu

Keywords: life history tradeoffs, sexual antagonism, *Schistocephalus solidus*, fibrosis, indirect genetic effects

Abstract

Parasites can mediate host fitness both directly, via effects on survival and reproduction, or indirectly by inducing host immune defense with costly side-effects. The evolution of immune defense is determined by a complex interplay of costs and benefits of parasite infection and immune response, all of which may differ for male and female hosts in sexual lineages. Here, we examine fitness costs associated with an inducible immune defense in a fish-cestode host-parasite system. Cestode infection induces peritoneal fibrosis in threespine stickleback (*Gasterosteus aculeatus*), constraining cestode growth and sometimes encasing and killing the parasite. Surveying two wild populations of stickleback, we confirm that the presence of fibrosis

scar tissue is associated with reduced parasite burden in both male and female fish. However, fibrotic fish had lower foraging success and reproductive fitness (reduced female egg production and male nesting success), indicating strong costs of the lingering immunopathology. We show that these substantial sexually-concordant fitness effects of immune response act to align multivariate selection across the sexes, masking the signature of sexual antagonism that acted on morphology alone. Although both sexes experienced costs of fibrosis, the net impacts are unequal because in the two study populations females had higher cestode exposure. To evaluate whether this difference in risk should drive sex-specific immune strategies, we analyze a quantitative genetic model of host immune response to a trophically transmitted parasite. The model and empirical data illustrate how shared costs and benefits of immune response lead to shared evolutionary interests of male and female hosts, despite unequal infection risks across the sexes.

Introduction

Organisms' immune systems evolve to prevent or mitigate the fitness costs that parasites impose on their hosts. But, these immune systems can impose their own costs by consuming energy (Sheldon and Verhulst 1996) or attacking the host's own tissues (Billi et al. 2019). Consequently, parasite infection reduces host fitness both directly, via effects on survival and reproduction, and indirectly by inducing costly immune responses (Khan et al. 2017 , Armour et al. 2020). Host immunity is adaptive when the benefits of reducing direct effects of infection outweigh the indirect costs of the immune response itself. In this way, host immune response can be viewed and studied as a life history trait (Sheldon and Verhulst 1996, Schmid-Hempel 2003), whose expression may trade off with different fitness components and whose evolution depends upon these tradeoffs (Svensson et al. 1998, Lochmiller and Deerenberg 2000). Thus rather than simply being maximized, host immunity is expected to evolve to optimize these tradeoffs (Boots and Bowers 2004, Viney et al. 2005, Tschirren and Richner 2006, Houston et al. 2007, Graham 2013).

Evolution of immune defense becomes potentially more complicated when hosts reproduce sexually. Due to divergent gamete investment strategies and pervasive differences in the strength of sexual selection, males and females have sex-specific life history priorities (Rolff 2002, Stoehr and Kokko 2006). Shifts in the relative importance of longevity versus success in a single reproductive bout can alter the relative benefits of investing in immunological traits and mounting a response to a parasite or pathogen (Williams 1966, Zuk and Stoehr 2002, Gipson and Hall 2016, Hall and Mideo 2018). These strategic differences have been invoked to explain sex differences in the intensity and side-effects of male versus female immune response. There is some empirical evidence suggesting costs of immunity may be sex-specific: for example in

humans there are numerous examples of sex specific immunopathology, including heightened prevalence of autoimmune disease in females (Fish 2008, Billi et al. 2019) and greater severity and frequency of parasitic diseases in males (Klein 2000, Roberts et al. 2001). These differences in optimal immune investment can be further exaggerated because males and females often differ ecologically (Shine 1989). Differences in diet, habitat use, or activity levels can impart distinct risks of infection (Bolnick et al. 2020), and consequently sex differences in parasite infection rates in wild animal populations are commonplace but variable (Poulin 1996, Schalk and Forbs 1997, McCurdy et al. 1998, Sheridan et al. 2000, Zuk 2009). These differences in life history and exposure suggest that sexes might benefit from evolving sex specific intensities of immune response, or different means of mitigating costs.

To test this proposition, we need studies that quantify the fitness benefits and costs of immune responses in nature, for both sexes. Are males and females equally at risk of parasite exposure? Do they initiate similarly strong immune responses? Are those immune responses equally beneficial by reducing parasite load? And, do both sexes experience equivalent costs of initiating these immune responses? Or, would differences at any of these stages lead to the evolution of sex-specific immune optima? Determining the causes of variation in sex-specific immunity and parasite infection is a major challenge in evolutionary ecology.

Here we explore the component fitness costs and benefits of immune response to parasite infection in threespine stickleback (*Gasterosteus aculeatus*), where we had reason to believe parasite exposure, infection costs, and immune costs might all differ between sexes. Of the suite of macroparasites that infect stickleback in postglacial lakes of the Northern hemisphere, the cestode tapeworm *Schistocephalus solidus* is one of the most abundant and causes high host morbidity in wild populations (Reimchen and Nosil 2001, Barber and Scharsack 2010 , Stutz et

al. 2014). Stickleback are an obligate intermediate host of *Schistocephalus solidus,* which are transmitted trophically when a stickleback consumes an infected cyclopoid copepod; sex differences in *Schistocephalus* infection rates appear to be related to differences in male and female diet (Reimchen and Nosil 2001). *Schistocephalus* complete their lifecycle when the host fish is consumed by a bird, which is their definitive host where the cestode mates. *S.solidus* is well known for its capacity to manipulate host color, behavior, and buoyancy to increase sticklebacks' risk of avian predation. In addition to aiding host mortality, *Schistocephalus* infection is associated with reduced fecundity in females (Heins et al. 2010). Presumably to mitigate such fitness costs, some stickleback populations have evolved resistance to *Schistocephalus* infection, although other populations remain susceptible (Scharsack et al. 2007, Scharsack et al. 2016, Weber et al. 2017a, Weber et al. 2017b). In particular, fish in some populations develop peritoneal fibrosis in response to cestode infection, in which their body cavity and organs become engulfed in scar-like connective tissue (Weber et al in prep) similar to human fibrotic response during tissue repair (Mutsaers et al. 1997). In laboratory experimental infection trials, genotypes that initiated fibrosis were able to suppress cestode growth and occasionally trap and kill the parasite (Weber et al in prep). This fibrosis response is a deeply-conserved immune trait across ray-finned fishes, and can be induced by vaccination of a generic immune adjuvant (alum; Vrtlilek and Bolnick in preparation). However, some stickleback populations have recently co-opted this ancient pathway to respond to perceived *S.solidus* infections (Hund et al. 2020). Although both males and females respond to infection with similar levels of fibrosis, laboratory infection experiments reveal that fibrosis is associated with reduced female reproduction (Weber et al in prep). The web of fibrotic scar tissue might place an upper

115 limit on ovary expansion. However, male reproduction does not rely on gonad enlargement and
116 so we predicted that the costs of fibrosis may be sex-specific.

117 Two important questions in this stickleback-cestode system remain unanswered: why do
118 populations vary in fibrosis immune response, and why do the sexes vary in cestode infection
119 rates (Reimchen and Nosil 2001) but not in their innate immune response (Hund et al. 2020)?
120 We predict that variation in fibrosis immune response across populations and cestode infection
121 rates across sexes may be the result of differences in the cost/benefit trade-offs that dictate the
122 optimal immune strategy. To test this prediction, we surveyed two wild stickleback populations
123 that naturally differ in parasite infection rates. Both populations exhibit moderate rates of both
124 infection and fibrosis. Crucially, there is an imperfect association between infection and fibrosis
125 in both populations: not all individuals initiate fibrosis when infected. Also, fibrosis persists at
126 least 3 months after the parasite is eliminated, so we find individuals with fibrosis but no
127 surviving infection. These facts allow us to statistically separate the costs of infection from the
128 costs of fibrosis, in both populations. We find massive fitness effects of both parasite infection
129 and of the fibrotic immune response, in both sexes and across both populations. We show that
130 these concordant fitness effects act to align otherwise-antagonistic multivariate selection across
131 the sexes. These results leave us with a puzzle: there are persistent sex differences in infection
132 rates, but no corresponding difference in the probability of fibrosis or its costs. To explain this
133 apparent contradiction, we construct and analyze an optimality model of immune response
134 evolution. Our analysis indicates that immune response optima may often be shared across sexes
135 that differ in parasite encounter/infection rates when costs of infection and immune response are
136 concordant and high.
137

Methods

Fish capture and measurement

We captured adult threespine stickleback from two lakes (Boot Lake and Roselle Lake), on Vancouver Island, British Columbia, Canada, between June 2-8 2019. These lakes were chosen because *Schistocephalus* infection and fibrosis response are observed in both, although to substantially different degrees; fish in Roselle exhibit a strong fibrotic immune response yet relatively low cestode infection rates, while fish in Boot exhibit much higher infection rates and lower rates of fibrosis (Stutz et al. 2014, Weber et al. 2017b, Hund et al. 2020). As noted above, the presence of both fibrosis and infection, imperfectly correlated, allows us to statistically partition their effects on measures of male and female reproductive success.

To compare reproductive success for males with versus without fibrosis (or, cestode infection), we compared the rates of fibrosis (infection) in randomly caught males, versus males that had successfully nested. We snorkeled in the littoral zone to search for nesting males; we identified nesting males based on their behavior (territory defense) and presence of a nest (e.g. arrangement of vegetative debris). Males exhibiting these qualities were observed until egg fanning behavior, or hatched fry, were observed, upon which the male was deemed to be a successful nester and was captured, immediately euthanized, and placed on ice and frozen. During this period we also placed minnow traps nearby to capture a random sample of males and females; this allowed us to obtain a random sample of unmated males for comparison to those that were successfully defending nests. Thus we obtained data required to measure total sexual selection acting via variance in male mating success (defining sexual selection sunsu Arnold and Wade 1984b, Arnold and Wade 1984a). Traps were placed at varying depths and distance from shore, and we avoided placing traps directly in areas of high nest density so most trapped males

161 are unlikely to be nesting. Trapped fish were euthanized and frozen. We sampled both lakes
162 until at least 50 nesting males had been captured. This number was chosen to avoid excessive
163 impact on the populations studied while still presenting a reasonable sample size; we note
164 however that this sampling design precludes accurate estimation of population mean mating rate
165 because we have controlled the number of nesting males captured. We also retained a random
166 sample of trapped females in each lake, using ovary mass as a metric of reproductive stage.
167 All fish were kept frozen until later laboratory dissection, upon which fish were thawed,
168 measured, and dissected. We measured seven external morphological traits: standard length,
169 head length (measured from the snout to the distal end of the operculum), snout length (measured
170 from the snout to the orbital), eye width, body depth, body width at the pelvic girdle, and middle
171 spine length. These traits were measured to two decimal places with digital calipers. We then
172 dissected the left gills and counted gill raker number, in addition to photographing the gill rakers
173 under a dissection microscope at fixed magnification to measure length of the longest raker.
174 Following these measurements fish were dissected and all *Schistocephalus* counted and weighed.
175 We note that *Schistocephalus* was the only abundant macroparasite found in our sample; we also
176 examined internal organs, eyes, and the digestive tract for other parasite taxa. Gonads were
177 removed and weighed. Stomach contents were removed and identified to (at least) order for all
178 individuals, with the exception of samples from nesting males from Roselle, whose stomachs
179 were lost during shipping.
180 We scored fibrosis on an ordinal scale, where a value of 0 corresponds to no apparent
181 fibrosis (organs move freely), a value of 1 corresponds to fibrosis between organs, a value of 2
182 corresponds to fibrotic connection between organs and the peritoneal tissue, and a value of 3
183 corresponds to excessive fibrosis across the entire body cavity. This scale is modified from that

184 developed to approximate the range of fibrotic variation seen in both laboratory studies and
185 natural populations of stickleback (A. Hund & L. Fuess, personal communication), and has
186 previously been shown to be highly repeatable between independent observers blind to
187 experimental vaccination treatment (Goldzmid and Trinchieri 2012). A video example of these
188 fibrosis levels is available
189 (https://www.youtube.com/watch?v=yKvcRVCSpWI&feature=youtu.be).
190
191 *Statistical Analysis- Benefits of fibrosis*
192 To evaluate the benefits of fibrosis, we used a generalized linear model to test whether
193 *Schistocephalus* mass depends on host fibrosis score (treated as a continuous fixed effect which
194 accommodates the ordinal nature of the variable), assuming exponential error. We avoided
195 including fish body mass in this model because *Schistocephalus* mass is a direct component of
196 total fish body mass and fibrosis is unrelated to mass, although results were unchanged when
197 correcting for mass. Because our sample size for this analysis was limited to the subset of fish
198 that were infected, we pooled lakes and sexes and did not model higher order interactions. We
199 used a separate linear model with infection probability as a binomial response (cestode present or
200 absent) to host fibrosis score, lake, and host sex; interaction terms were not significant and were
201 dropped.
202
203 *Statistical Analysis- Costs of fibrosis and infection*
204 We considered the fitness costs of fibrosis and infection on male and female reproductive
205 success (nesting or not; ovary mass). We assessed component fitness costs for females in Roselle
206 lake using a linear model with ovary mass as a response, fibrosis score and infection status as

fixed effects, and exponential error. Small sample size of gravid females precluded such an analysis for Boot fish. We obtained qualitatively equivalent conclusions including body mass in the model, although present the results without size correction. A caveat with our analysis of female ovary mass is that we cannot control for females that may have already laid a clutch and are in the early stages of developing a second. We repeated this analysis for males from Boot and Roselle lakes, using binomial nesting success as the response variable and fitting separate models for each lake. Male testes mass is less clearly related to reproductive success than is female ovary mass and so we focus on male nesting success.

The above analysis of male nesting success indicated strong sexual selection against fibrosis, and we were interested in if and how this selective force acts in conjunction with sexual selection on morphology. To do this, we estimated the bivariate fitness surface for morphology and fibrosis for males separately for Boot and Roselle lakes using binomial linear models with nesting success as the response variable and fibrosis score and score on the multivariate morphological selection gradient as fixed effects. Score on the morphological selection gradient was obtained as discriminant function scores calculated in discriminant function analysis of nesting success and morphology, performed separately for each lake; this is equivalent to calculating individual scores on the vector of multivariate directional sexual selection on morphology (Mitteroecker and Bookstein 2011). This approach essentially estimates individual score on the morphological sexual selection gradient and then uses this score as a predictor in a linear model with fibrosis as an additional predictor of fitness. This approach allowed us to 1) estimate the effects of fibrosis on fitness accounting for effects of morphological traits, where morphology has reduced dimensionality yielding increased power and 2) allowed us to plot the corresponding bivariate fitness surface. We obtained qualitatively equivalent conclusions on the

230 importance of fibrosis using a full multivariate model; we explore such a multivariate model
231 below (*Statistical Analysis - Alignment of multivariate selection across the sexes*).
232 To assess differences in diet, including potential costs of immune response and infection
233 on resource acquisition, we used a series of uni- and multivariate linear models to test for
234 associations between diet and fibrosis or infection. Because of the sparse multivariate nature of
235 diet data (i.e., many zeros for rare taxa), we constructed our models to test three specific
236 hypotheses, in all cases avoiding higher order interactions wherever appropriate. First, to
237 evaluate variation in overall prey intake, we used a univariate linear model with total prey counts
238 as the response and fibrosis, lake, and infection status as fixed effects. Prior studies have reported
239 inconsistent sex-biased diet in stickleback (males often being more limnetic in most but not all
240 lakes; Bolnick and Ballare 2020). This is relevant because cestodes are acquired by eating
241 limnetic copepods. To assess differences across the sexes in diet composition we used a
242 multivariate linear model where the response vector consisted of counts of each prey taxon in an
243 individual's diet. This model included sex, lake, and their interactions with prey taxon as fixed
244 effects; in this model significant interactions with taxon type indicate differences in the
245 taxonomic composition of diet across sexes or lakes. Finally, we used a similar multivariate
246 model to test for diet differences between nesting and randomly sampled males in Boot lake,
247 although as a caveat we cannot control for variation in time spent in traps before euthanasia that
248 may affect measured diet. Multivariate models with infection status as a predictor failed to
249 converge, likely due to the relatively small number of infected fish. For all models of diet
250 content, in which the data are counts of individual prey items in a stomach, we treated the
251 response variable(s) as Poisson distributed. For multivariate models with a response vector of

252 counts of each prey taxa, we modeled covariance in counts across individual fish as the Cholesky
253 parameterization of an unstructured covariance matrix.
254
255 *Statistical Analysis - Alignment of multivariate selection across the sexes*
256 Our analysis of fitness costs of fibrosis suggested shared reproductive costs of fibrosis
257 across the sexes, and we were interested in quantifying effects of fibrosis on the geometry of
258 multivariate selection. To assess the effects of fibrosis immune response on alignment between
259 male and female selection, we compared the orientation of multivariate selection in males and
260 females estimated in Roselle lake. Small sample size of females in Boot lake precluded such an
261 analysis for those fish. For this analysis, we re-fit our model of male morphological sexual
262 selection using a glm with nesting success as a response and morphological traits as a predictor,
263 fit an equivalent model for females with ovary mass as a response, and compared the orientation
264 of male and female selection (assuming gaussian error for both for comparison). We fit models
265 including all morphological traits, and also models with reduced dimensionality where we used
266 the first three principal components of the correlation matrix of morphological traits (both sexes
267 pooled). We repeated this with and without including fibrosis score in as a predictor in our
268 model in order to assess the effects of fibrosis on the alignment of selection across the sexes.
269 Note that the non-binary fitness component for females precluded discriminant analysis as
270 performed before for males. We compared orientation by calculating the vector correlation
271 between the normalized selection gradients, $\boldsymbol{\beta}_{\mathbf{m}}\boldsymbol{\beta}_{\mathbf{f}}^{\mathrm{T}}$, which provides an estimate of sexual
272 antagonism on a scale of -1 to 1, where a value less than zero indicates selection acting on
273 opposing directions across the sexes, while a value greater than zero indicates concordant
274 selection. Note that this approach compares orientation only, and not magnitude of selection,

and so avoids likely issues arising from use of different fitness components in estimation of β_m and β_f. We estimated the sampling distribution of this vector correlation by resampling (100,000x) each vector from a multivariate normal distribution centered at the original REML estimates of β with covariance equal to the covariance matrix of the fixed effects from the fitted linear model, calculating the vector correlation for each sample. This approach is a modification, focusing on fixed effects, of a similar resampling approach (Houle and Meyer 2015) used to estimate sampling distributions of arbitrary functions of parameter estimates of random effects.

We focus our selection analyses on absolute component fitness, rather than relative fitness, because our fitness component estimates precluded estimation of population mean component fitness. This is because, by targeting a minimum number of nesting males, we lack a meaningful estimate of population-wide mean nesting rate. In such a situation, use of an arbitrary estimate of mean fitness to relativize fitness can be misleading when comparing selection among groups (De Lisle and Svensson 2017). Moreover, a focus on absolute component fitness is also appropriate because our interest is in performance costs associated with immune traits, rather than potential evolutionary response per se (De Lisle and Svensson 2017).

All statistical analyses were performed in SAS/IML version 9.3 (Cary Institute, NC). Generalized linear models were fit by REML using the glimmix procedure. Raw data and complete script to reproduce all analyses and figures is provided in the supplemental material.

Results

Benefits of fibrosis

We sampled a total of 411 fish (Roselle, 156 M, 71 F; Boot, 154 M, 30F), of which 102 were nesting males (50 Roselle, 52 Boot) and 81 (16 Roselle, 65 Boot) were infected with

298 *Schistocephalus* (Table S1). Probability of cestode infection varied across sexes and lakes, with
299 higher (odds ratio 4.79) infection rates in females across both Boot and Roselle lakes, and higher
300 (odds ratio 10.5) overall infection rates in Boot than Roselle lake (Sex effect, $F_{1,407} = 21.06$, $P <$
301 0.0001; Lake effect, $F_{1,407} = 46.14$, $P < 0.0001$; Figure 1). Across lakes and sexes, probability of
302 cestode infection was reduced in fish with high levels of fibrosis (odds ratio of unit offset at the
303 mean 0.649, $F_{1,407} = 6.15$, $P = 0.0135$; Figure 1), consistent with prior observations that fibrosis
304 contributes to elimination of the infection then lingers afterwards (Weber et al. in prep, Hund et
305 al. 2020). Of those fish infected, host fibrosis was associated with reduced cestode mass ($F_{1,76} =$
306 9.06, $P = 0.0035$; Figure 2), consistent with laboratory infection experiments (Weber et al. in
307 prep). Thus, across sexes and lakes, fibrosis confers benefits in the form of both a reduction in
308 cestode growth and a decrease in the probability of infection.
309
310 *Costs of fibrosis and infection*
311 Countering these benefits, we also see evidence for costs of fibrosis. In both Boot and
312 Roselle lakes, males defending active nests exhibited reduced fibrosis levels compared to
313 randomly sampled males (Boot, odds ratio of unit offset at the mean 0.56, $F_{1,151} = 6.87$, $P =$
314 0.0097; Roselle, odds ratio of unit offset at the mean 0.41, $F_{1,153} = 12.57$, $P = 0.0005$) and
315 reduced cestode infection in Boot lake (odds ratio 2.45, $F_{1,151} = 4.3$, $P = 0.039$; Roselle lake
316 odds ratio 1.89, $F_{1,153} = .57$, $P = 0.45$). This is reflected in significant (Roselle, $F_{1,152} = 6.24$, P
317 $= 0.0135$) or nearly significant (Boot, $F_{1,147} = 3.52$, $P = 0.062$) negative effects of fibrosis on
318 mating probability in models including morphological discriminant function score, that is the
319 vector of sexual selection on morphology, as a predictor of mating success ($P < 0.0001$ for both

lakes). Thus, for both lakes, nesting success was determined by the independent effects of ecomorphology and fibrosis (Figure 3).

Although we lack data on mating success for females, we did obtain data on ovary mass, which is expected to be directly related to fecundity. For females in Roselle lake, we found that fibrosis was associated with a significant reduction in ovary mass ($F_{1,67} = 10.65$, $P = 0.0017$; Figure 4) while controlling for infection; ovary mass was also significantly reduced in cestode-infected females ($F_{1,67} = 4.92$, $P = 0.03$). Notably, the fibrosis response explained more variance in female ovary mass than the cestode infection itself explained. These results remained qualitatively equivalent when including body length as a covariate in the model. From Boot Lake we trapped too few gravid females to warrant analysis. Effect sizes for costs and benefits of fibrosis in both sexes are also presented below (see 'A model of immune response evolution').

In our analysis of diet content, we found individuals with high fibrosis had fewer total prey items in their stomach ($F_{1,341} = 7.75$, $P = 0.0057$; Figure 5A) controlling for lake effects ($F_{1,341} = 43.11$, $P < 0.0001$); this model indicated no difference in total prey items in infected versus uninfected individuals ($F_{1,341} = .79$, $P = 0.37$). In a multivariate model, we find no evidence of sex differences in the total number of prey items in an individual's diet (sex effect, $F_{1,343} = 0.06$, $P = 0.81$), although the taxonomic content of individual diet varied with sex (sex*prey taxon effect, $F_{7,2393} = 2.39$, $P = 0.019$) while controlling for lake effects (lake*prey taxon effect, $F_{7,2393} = 4.04$, $P = 0.0002$). This sex effect was primarily driven by differences in the abundance of dipteran larvae, fish eggs, and zooplankton (Figure 5B), with females tending to have more limnetic prey. For the subsample of males from Boot lake, for which we had diet data for both nesting and non-nesting males, we found a main effect of nesting status in a multivariate model ($F_{1,145} = 7.15$, $P = 0.0084$) with nesting males having more prey items in their

343 stomachs than a random sample of males in the population (Figure 5C). We found no evidence
344 of a difference in the relative amounts of prey taxa in the diet of nesting and non-nesting males
345 (mate status*prey taxa effect, $F_{3,431} = 7.15$, $P = 0.35$).
346
347 *Alignment of multivariate selection*
348 Given that fibrosis appears to confer similar component-fitness costs in males and
349 females, we can examine the effects of fibrosis on geometric alignment of multivariate selection
350 across the sexes. We find weak or even antagonistic alignment between multivariate selection
351 on morphological traits in males and females (Figure 6), with evidence of sexually antagonistic
352 selection on body shape independent of body size. This is reflected in a negative estimate of the
353 correlation between male and female multivariate selection gradients (Figure 6B). However,
354 including fibrosis as a predictor of component fitness results in a geometric alignment between
355 male and female multivariate selection (Figure 6, A-C). Note that we avoid formal statistical
356 comparison of male and female selection gradients because they were estimated in separate
357 models using different fitness components.
358
359 **A model of immune response evolution**
360 Our empirical results indicate substantial sexually-concordant fitness effects of fibrosis. We also
361 find sex differences in cestode infection rates in the two populations we surveyed, and sex-
362 specific infection rates are commonplace but variable across populations in Western Canada
363 (Reimchen and Nosil 2001; Bolnick unpublished data). Yet, males and females do not appear to
364 differ in fibrosis response (Hund et al. 2020, Weber et al in prep). These results are somewhat
365 paradoxical because intuition suggests substantial sex differences in infection rates may be

366 expected to lead to the evolution of sex-specific immune response. Here we construct and
367 analyze a simple model of immune response evolution, in order to reconcile these results and
368 illuminate how shared fitness costs of immunity may in part determine the optimum immune
369 response in males and females.

370 We consider how selection acts on an underlying latent trait, the sensitivity of immune
371 response to parasite exposure. Proximately, such a latent trait reflects mobilization of the
372 immune system in response to a parasite infection/exposure. We seek to identify the rules that
373 would determine the optimum sensitivity of immune response to parasite exposure and infection.
374 We define individual fitness as a function of expressed immune response and infection status,

$$\omega_{i,j} = \mathrm{a}_{w,i} - \beta_{N,i} z_{F,i,j} + \gamma_i z_{F,i} z_{I,i,j} - \beta_{S,i} z_{I,i,j} + \varepsilon \qquad 1$$

375 where $\omega_{i,j}$ is the fitness of individual j of sex i. In this linear equation a_w is an intercept (keeping
376 in mind mean male and female fitness will be equal in sexual populations with a Fisherian sex
377 ratio; Kokko and Jennions 2003), β_N is the natural selection cost of an immune response trait,
378 fibrosis z_F, and β_S is the cost associated with being infected, a trait termed z_I. Definitions of all
379 parameters in our model are presented in Table 1. The coefficient γ_i represents the fitness
380 benefit of an immune response, which manifests depending on both infection status and immune
381 response phenotypes. Alternatively, we could define an alternative but equivalent algebraic
382 expression for γ_i where it is defined as a multiplicative function of β_S; we retain the formulation
383 in equation 1 because it is more readily estimable from data. The traits z_F and z_I can be
384 quantitative on a latent scale (i.e., describing probability of immune expression or parasite
385 infection), continuous, or binary.

386 Importantly, the two traits in this model z_F and z_I are not expected to be expressed
387 independently, and in fact are measurable outcomes of underling latent trait(s). Fibrosis is

388 induced by parasite exposure, but in turn it acts to reduce the probability of successful infection,
389 z_I. Thus fibrosis, z_F, is a trait whose expression is determined not only by host genotype but also
390 by parasite exposure, an environmental effect. Infection, z_I, is a trait determined both by parasite
391 encounter rates as well as the mitigating effects of fibrosis response. We define trait expression
392 as

$$z_F = a_F + \varepsilon + \psi_{F,I} z_I$$
$$z_I = a_I + \varepsilon + \psi_{I,F} z_F \qquad 2$$

393 where a_F and a_I are intercepts that describe the additive breeding value for the trait. For
394 fibrosis, this represents fibrotic expression in the absence of exposure to parasites, and may
395 typically be close to zero. For parasite infection, a_I represents exposure rate in the absence of
396 immune response, or the probability of exposure to parasites, and thus is an ecological variable
397 determined by diet and habitat choice.

398 The terms ψ represent the degree to which trait expression is influenced by another trait,
399 in this case infection status or fibrosis. Thus $\psi_{F,I}$ is a latent trait describing the degree to which
400 fibrosis is induced in response to a parasite exposure, and is thus the key variable of interest in
401 our model, whose evolution we seek to predict. We borrow our notation here from the literature
402 on indirect genetic effects (Moore et al. 1997, McGlothlin et al. 2010), because these effects of
403 immune response on infection repression and infection induction of immune response can be
404 considered a special case of an indirect genetic effect (we develop IGE models of host-parasite
405 coevolution more extensively in another paper). We can now define each trait explicitly in terms
406 of the other,

$$z_F = \frac{a_F + \psi_{F,I} a_I}{1 - \psi_{F,I}\psi_{I,F}} \qquad 3$$

407 with a similar equation for z_I. We note that in our model of antagonistic interactions between

408 immune response and parasite infection, we expect $\psi_{F,I}$ to range from 0 to 1 and for $\psi_{I,F}$ to

409 range from 0 to -1.

Table 1. Definition of model parameters

parameter	definition
z_F, z_I	Expressed individual trait values of immunity (fibrosis) and parasite infection status
a_F, a_I	Individual breeding values of fibrosis response (in absence of infection) and parasite infection; the latter reflects the encounter rate with parasites
a_w	fitness intercept
β_N	Fitness cost of expressing immune trait z_F
β_S	Direct fitness cost of parasite infection z_I
γ	Fitness benefit of immunity
$\psi_{F,I}$	Sensitivity of expressed immunity to parasite infection; the degree to which expressed fibrosis depends on upon parasite infection
$\psi_{I,F}$	Sensitivity of parasite infection to expressed immunity; the degree to which parasite infection depends on upon fibrosis
θ_ψ	Optimum immune sensitivity $\psi_{F,I}$

410

411 We can now analyze this model to determine how selection acts on the latent immune
412 response trait $\psi_{F,I}$. Substituting equations 2 and 3 into 1 and differentiating with respect to $\psi_{F,I}$
413 yields a selection gradient:
414

$$\frac{d\omega}{d\psi_{F,I}} = \frac{-\beta_N a_I}{1+\psi_{F,I}} + \frac{\beta_N \psi_{F,I} a_I}{(1+\psi_{F,I})^2} + \frac{\gamma a_I^2}{(1+\psi_{F,I})^2} + \frac{-2\gamma\psi_{F,I} a_I^2}{(1+\psi_{F,I})^3} + \frac{\beta_S a_I}{(1+\psi_{F,I})^2} \quad 4$$

415 assuming for simplicity that $a_F = 0$ and $\psi_{I,F} = -1$. Equation 4 has three roots representing
416 evolutionary equilibria. One is at $a_I = 0$, an ecological scenario where parasites are absent or
417 never encountered. Another is at $\gamma = 0$ and $\beta_N = \beta_S$, where there is no benefit of fibrosis and
418 costs of immune response equal costs of parasite infection. Finally, the relevant root for
419 evolution of ψ is
420

$$\theta_\psi = \frac{a_I\gamma - \beta_N + \beta_S}{a_I\gamma + \beta_N - \beta_S} \quad 5$$

421 which holds for $a_I\gamma + \beta_N \neq \beta_S$ and $a_I\gamma \neq 0$, and is a local optimum (see Appendix).
422 Equation 5 describes the location of the optimum immune response sensitivity as a
423 function of the costs and benefits of immune response and parasite infection. Immediately
424 notable is that increases in parasite prevalence, captured by the intercept a_I, lead to an increase in
425 the optimum sensitivity (Figure 7A). However, regardless of the magnitude of a_I, when the
426 natural selection cost of fibrosis, β_N, is less than or equal to the cost of infection β_S, the optimum
427 is for high sensitivity (Figure 7B, C, E). Intriguingly, we can also see that immune response can
428 evolve even when the natural selection costs of fibrosis outweigh the costs of carrying an

429 infection (Figure 7D), if the prevalence of parasites is high enough and the costs of infection are
430 in part mitigated by the interaction term γ.

431 The main conclusion from this model is that although optimum immune sensitivity can
432 indeed be governed by encounter rate, when costs and benefits of infection and immune response
433 are high these encounter rates play less of a role in determining the optimum immune response.
434 We find evidence of sex and population differences in parasite encounter rates, a_I, based on
435 differences in worm infection rates across populations and sexes (Figure 1) and sex and
436 population differences in diet (Figure 5; see also Bolnick and Ballare 2020). Yet we find no
437 evidence of sex differences in fibrotic immune response in the wild, and past work in the lab
438 confirms this for Roselle lake and other Vancouver Island populations (Hund et al. 2020).
439 Moreover, QTL mapping of fibrosis response to experimental cestode infection in the lab
440 revealed no sex effect on fibrosis (Weber et al. in prep). At present, we lack data on lifetime
441 reproductive success (in addition to a way to estimate a_I directly), and so cannot confront our
442 model directly with the data at hand. But, this model provides guidance for key parameters that
443 should be measured in the future. And, the data we do have shows that in both populations and
444 sexes there are measurable fitness costs of infection and fibrosis. We can get a better
445 understanding of these costs by re-fitting linear models with component fitness as a predictor and
446 binary infection status, fibrosis status, and their interaction as fixed effects. From these
447 regression coefficient estimates, we can take the linear terms and multiply them by -1 and use the
448 raw interaction term to obtain estimates of the parameters in Equation 1. We assume normality
449 for the sake of parameter estimation and comparison. Corresponding estimates of γ, β_N, and β_S
450 for each subpopulation sampled are provided in Table 2.
451

Table 2. Estimates of parameters in Equation 1. Bold indicates statistical significance, with the caveat that response variables (component fitness) were not normally distributed. ± 1 Standard Error

Population	β_N	β_S	γ
Roselle Males	**.24 ± .07**	.22 ± .23	.19 ± .31
Boot Males	**.24 ± .08**	**.27 ± .13**	.18 ± .17
Roselle Females	**64 ± 16**	**88 ± 35**	**62 ± 46**

We can reach two key conclusions from these parameter estimates. First, costs of fibrosis and infection (β_N, and β_S) are always higher than the mitigating effects of γ. Second, β_N, and β_S are either similar in magnitude (males in Boot and Roselle) and/or $\beta_N < \beta_S$ (Roselle females, Roselle males). Thus, these values are consistent with a high optimum for $\psi_{F,I}$ and relatively low sensitivity of that optimum to parasite encounter rates, a_I. Thus, our model and data suggest the sexes can be expected to exhibit similar optimum immune responses when the costs and benefits of immune response are high and sexually concordant, even if exposure rates to the parasites are sexually dimorphic due to sex differences in diet or habitat use.

Discussion

Numerous evolutionary models suggest that the strength or sensitivity of an immune response should be subject to net-stabilizing selection, rather than persistent directional selection, to

471 balance the costs and benefits of immunity (Sheldon and Verhulst 1996, Lochmiller and
472 Deerenberg 2000, Zuk and Stoehr 2002, Graham 2013). The costs of immunity are well
473 documented and include increased energetic expenditure and decreased resource acquisition
474 (Houston et al. 2007), increased vulnerability to predators (Navarro et al. 2004), loss of social
475 status (Stockmaier et al. 2020), and the increased risk of immune pathology (Goldzmid and
476 Trinchieri 2012). These costs combine with the benefits of immune response to costly infections
477 to determine the optimum immune response. Thus, quantification of the costs and benefits of
478 immune response is key to understanding the evolutionary origins of variation in parasite
479 infection rates and host immune evolution observed across populations and sexes in the wild.
480 Here, we have presented a rare instance of an immune response with measurable benefits
481 and costs in the wild. In two lakes on Vancouver Island, we found that peritoneal fibrosis is
482 associated with reduced cestode infection, both in terms of infection probability and parasite
483 growth when infected. However, we find that this immune response comes at substantial
484 component fitness costs for both sexes. Fibrosis is associated with a reduction in prey
485 consumption. In both lakes, fibrotic males are less likely to be reproductively successful,
486 indicating consistent selection against this immune response trait. Similar reproductive costs are
487 observed in females, with substantial reduction in ovary mass in individuals expressing fibrosis
488 (in the one lake with enough infected females to test this trend). We show that these shared
489 fitness costs act to align multivariate selection across males and females, which would otherwise
490 entail sexually-antagonistic selection on trophic morphology.
491 Consistent with our results, recent laboratory experimental infection studies demonstrate
492 that exposure to cestode antigens induces fibrosis (Hund et al. 2020) which in turn suppresses
493 cestode growth and viability (Weber et al. in prep). In these laboratory assays, the sexes were

494 equally prone to fibrosis following an immune challenge. Although males and females initiate
495 similar levels of fibrosis following a controlled immune challenge, their risk of cestode infection
496 should be unequal. In many lake populations of stickleback females feed on more benthic prey
497 than males do, though in some lakes this diet dimorphism is absent or reversed. *S.solidus* is
498 acquired by eating limnetic prey (cyclopoid copepods), so diet dimorphism should, and does,
499 generate sex differences in cestode encounter and infection rates (Reimchen and Nosil 2001,
500 Stutz et al. 2014). At face value, this presents a conundrum because substantial costs of fibrosis,
501 coupled with sex differences in parasite encounter rates, might be intuited to result in evolution
502 of sex differences in immune sensitivity to cestode infection. Our analysis of a simple model of
503 immune response evolution, however, indicates that optimum immune response can be
504 surprisingly insensitive to parasite encounter rates when costs of both immune response and
505 parasite infection are high. This finding can reconcile our results, and also indicates that
506 interpreting infection rates alone as a measure of immune activity (as is often done in the case of
507 sex differences in parasite loads) may be problematic (Poulin 1996). Our model also provides a
508 glimpse into the complex forms of selection that may inherently act on indirect genetic effects,
509 which are typically (Moore et al. 1997, McGlothlin et al. 2010) assumed to be fixed population
510 parameters in theoretical models.

511 Cestode prevalence varies widely across lakes in Western Canada (Stutz et al. 2014,
512 Weber et al. 2017b), and some stickleback populations do not exhibit fibrotic response to cestode
513 infection (Weber et al. in prep, Hund et al. 2020). Our results indicate that variation in the costs
514 and benefits of fibrosis across populations could explain this variation in stickleback immune
515 response. When the fitness cost of fibrosis exceeds the costs associated with cestode infection,
516 the parameter space where fibrosis response to parasite exposure is expected to evolve in our

model is limited. Our data from two populations indicate that the magnitude of these fitness costs are very similar, and even slightly higher for fibrosis in males from Roselle lake. Thus, even slight variation in the relative costs of fibrosis across populations could result in selection against fibrosis response in some lakes. Variation in the cost of fibrosis could arise from variation in the strength of competition for mating opportunities in males and/or variation in the strength of selection on female reproductive output, both of which (Boughman 2007, Baker et al. 2008, Heins and Baker 2014) are likely to vary across stickleback populations.

Sexual conflict theory indicates that sexually concordant fitness effects can mask the signature of sexual antagonism. This occurs if concordant effects are strong enough to override otherwise-conflicting selection acting upon the sexes, thus acting to align net selection across males and females. One way these effects are often envisaged is as a displacement from sex-specific optima, where maladaptation pushes both sexes away from their respective fitness peaks, aligning selection on the sexes transiently until the population adapts to its new environment and conflict again ensues (Long et al. 2012, Connallon 2015, Connallon and Hall 2016). Our analysis demonstrates a slightly different manifestation of how concordant fitness effects can change the nature of fitness variance across the sexes. In our study, we show that concordant fitness effects of a shared trait – fibrosis – act to align otherwise antagonistic selection acting through morphology when both suites of traits are included in a multivariate analysis. Selection is antagonistic and concordant in different dimensions of multivariate trait space, with the direction of net multivariate selection largely aligned due to the strong concordant fitness effects of fibrosis that swamp out antagonistic effects of morphological shape. Thus, our work illustrates how specific traits can contribute to or mask sexually antagonistic fitness variance. To the extent that immune response optima are shared across the sexes, such effects could lead to

the appearance of pervasive sexual conflict when they are not measured, creating a 'hidden-trait' problem (Morrissey et al. 2010) that could contribute to the appearance (Cox and Calsbeek 2009) of widespread unresolved sexual conflict in the wild.

By surveying component-fitness costs and benefits of parasite infection and immune response, we show that relative costs of infection and immune response can be similar across males and females despite acting through different fitness components across the sexes. Our simple analytical model of immune response evolution indicates that similar male and female immune optima may be a common feature across a range of ecological scenarios, especially when fitness costs of immune response and parasite infection are high. Thus, our work illustrates how shared fitness costs in host-pathogen interactions can lead to shared evolutionary optima for male and female host immunity.

Acknowledgements We are grateful to Will Shim and Jacqueline Salguero for their help in sampling fish, as well as Nathalie Steinel and Jesse Weber for logistical support in the field. Lauren Fuess and Amanda Hund provided help on scoring fibrosis. We thank Katherine Lewkowicz and Megan Braat for assistance with the dissections. A. Hund, Milan Vrtilek, and Foen Peng provided valuable feedback on the manuscript. Funding was provided by the University of Connecticut to DIB, and NIAID Grant 1R01AI123659-01A1 to DIB.

Appendix

We can confirm that the root of equation 4 given in equation 5 is a local optimum by solving for the second derivative at the root,

$$\frac{d^2\omega}{d{\psi_{F,I}}^2}(\psi_{F,I} = \theta_\psi) = -\frac{(a_I\gamma + \beta_N - \beta_S)^4}{8a_I^2\gamma^3} \quad 6$$

Which will be negative for all $\gamma > 0$.

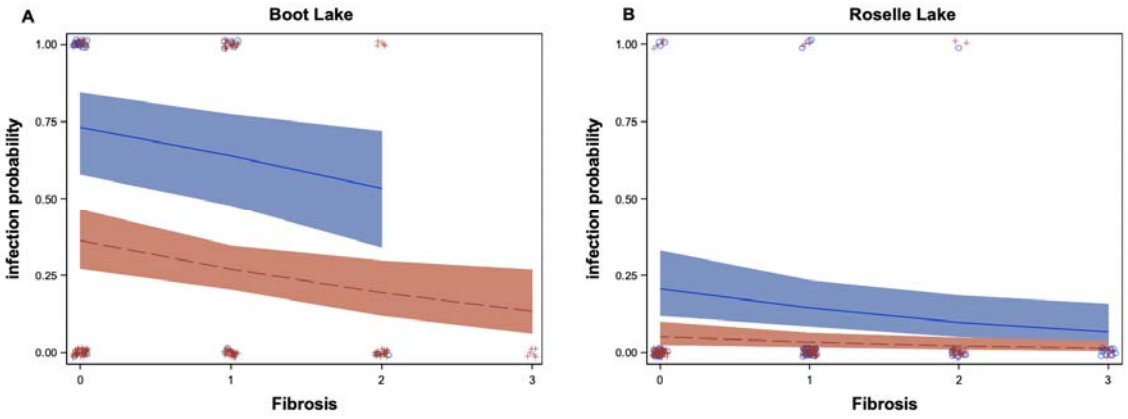
Table S1 Fish numbers sampled

Boot Lake

	Infected	Uninfected
Nesting Male	9	43
Trapped Male	32	70
Trapped Female	24	6

Roselle Lake

	Infected	Uninfected
Nesting Male	2	48
Trapped Male	7	99
Trapped Female	7	64



576

577

578 **Figure 1. Fibrosis phenotype is associated with reduced probability of cestode infection**

579 **across lakes and sexes.** Model fit is from a generalized linear model with binomial error and

580 lake, sex, and fibrosis as fixed effects. Interaction terms were not significant and were dropped;

581 all main effects were significant (see text). Blue = females, red = males.

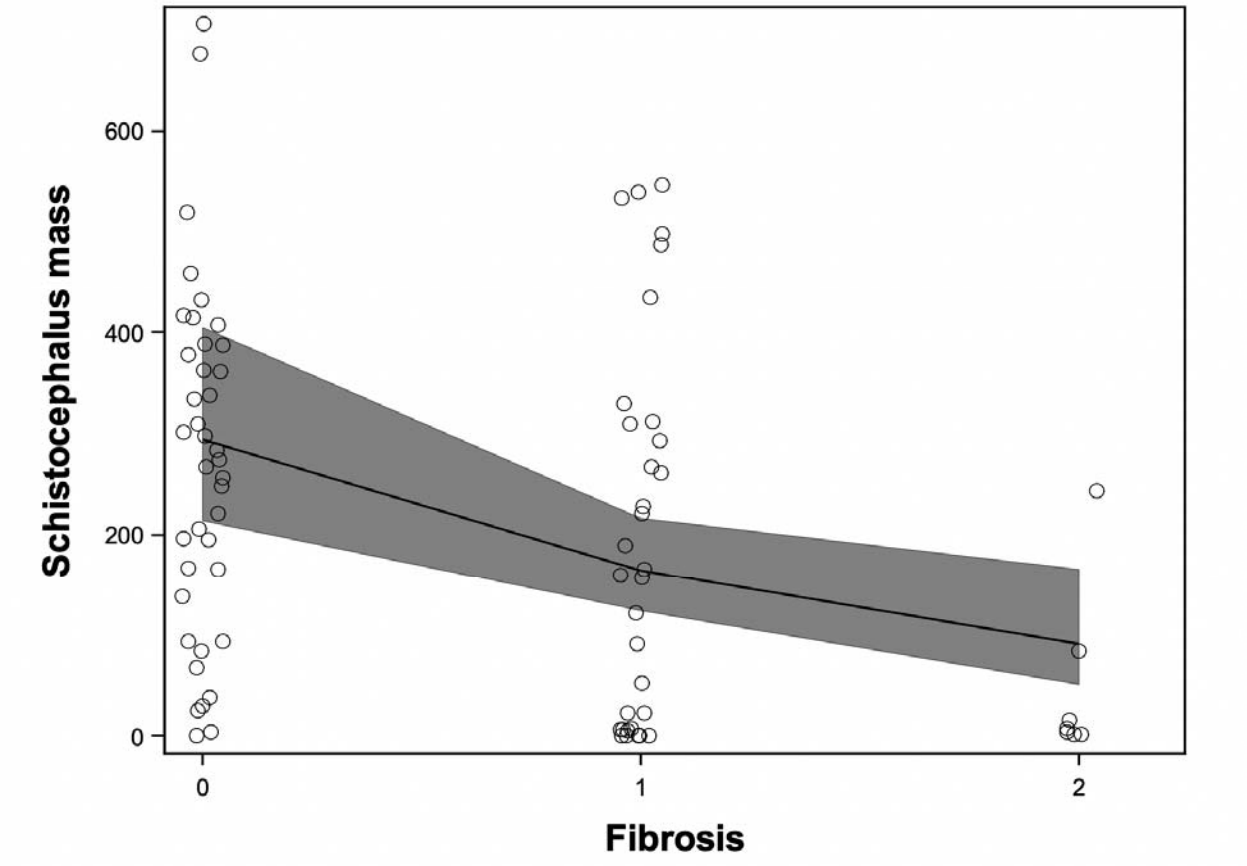


Figure 2. Fibrosis phenotype is associated with reduced cestode mass in infected individual hosts. *Schistocephalus* mass is the total mass of all worms found in the individual host. Model fit is from a generalized linear model with exponential error. Data are pooled across lakes and sexes; small sample sizes of infected individuals in lake x sex combinations make analysis of higher order interactions tenuous.

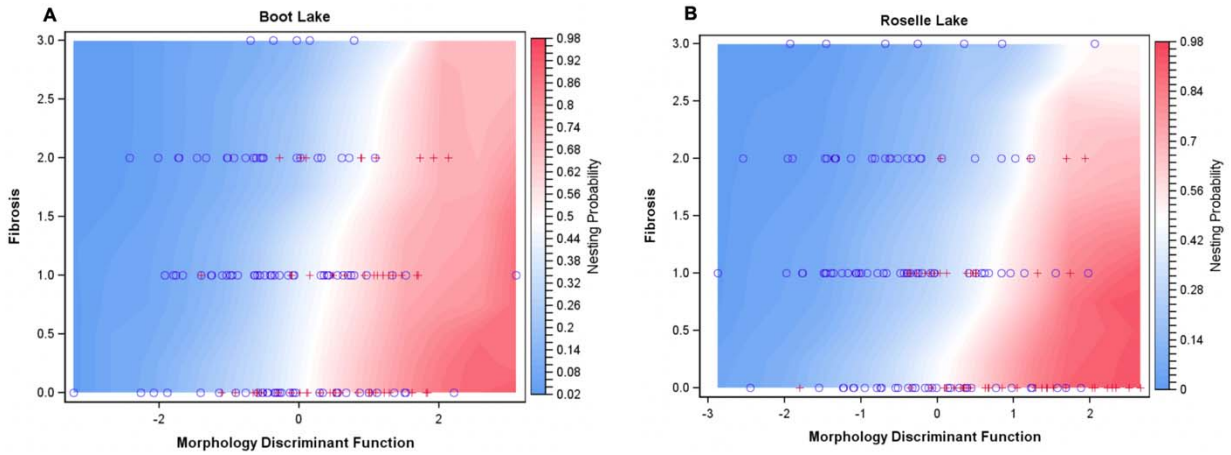
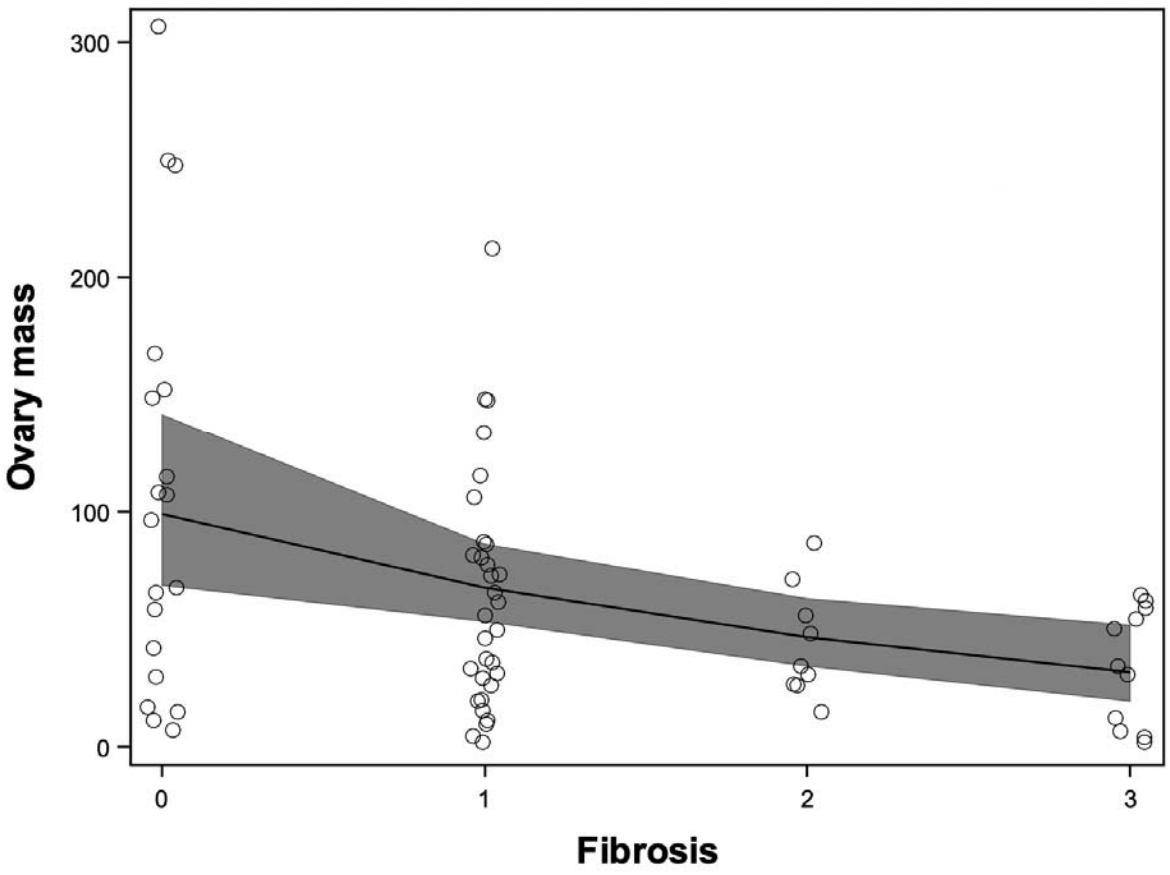


Figure 3. Sexual selection on fibrosis and morphology. Heat maps show male nesting probability as a function of fibrosis and sexually-selected multivariate morphology. The x axis is the discriminant function vector of morphometric traits that defines the direction of multivariate directional sexual selection on morphology alone, estimated separately for each lake. In both lakes, sexual selection acts against fibrosis independently of selection on size and shape. See text for details.



609

610 **Figure 4. Fibrosis is associated with reduced female ovary mass in Roselle lake.** Model fit is

611 from a generalized linear model with exponential error. The small sample size of gravid females

612 in Boot precluded analysis of female fitness effects of fibrosis.

613

614

615

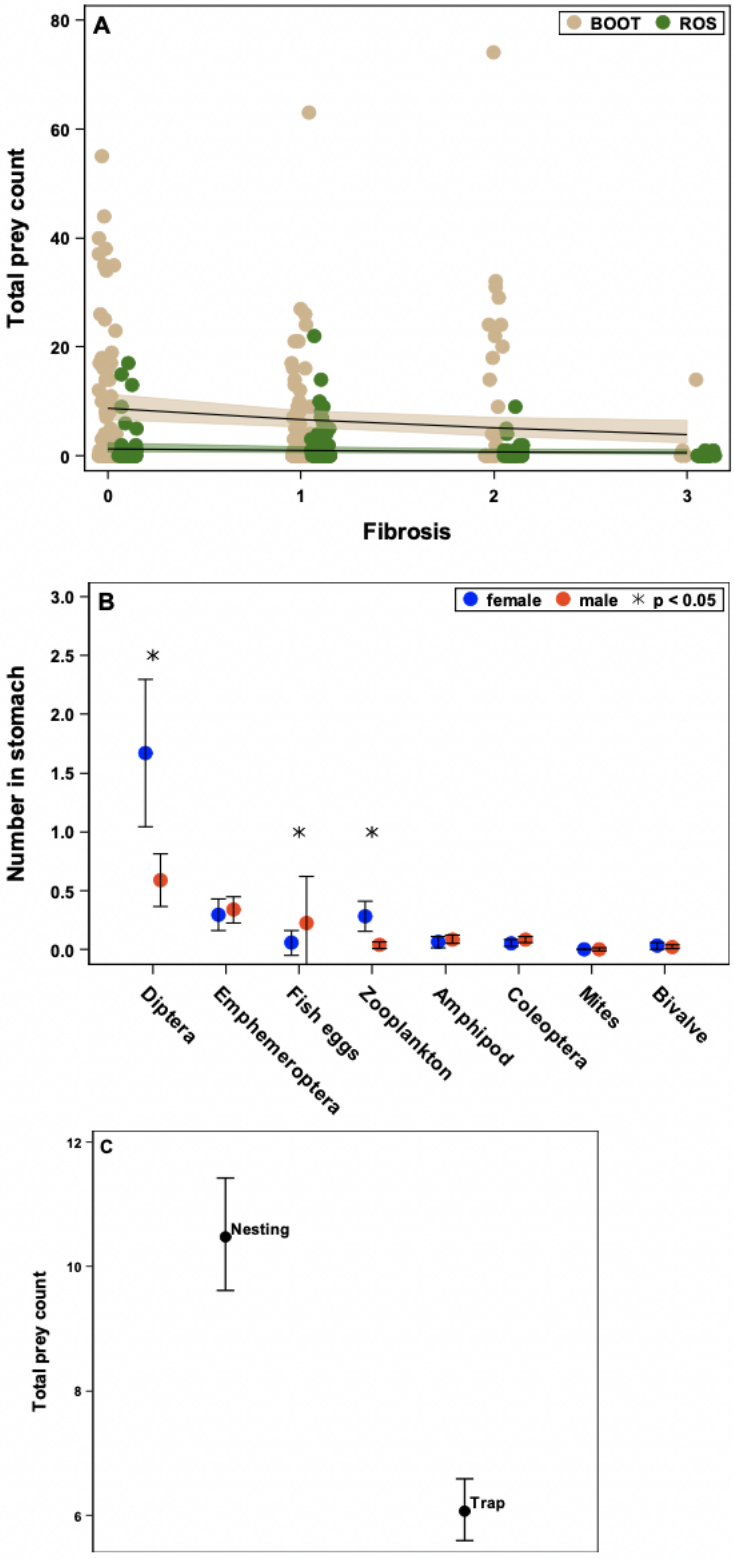
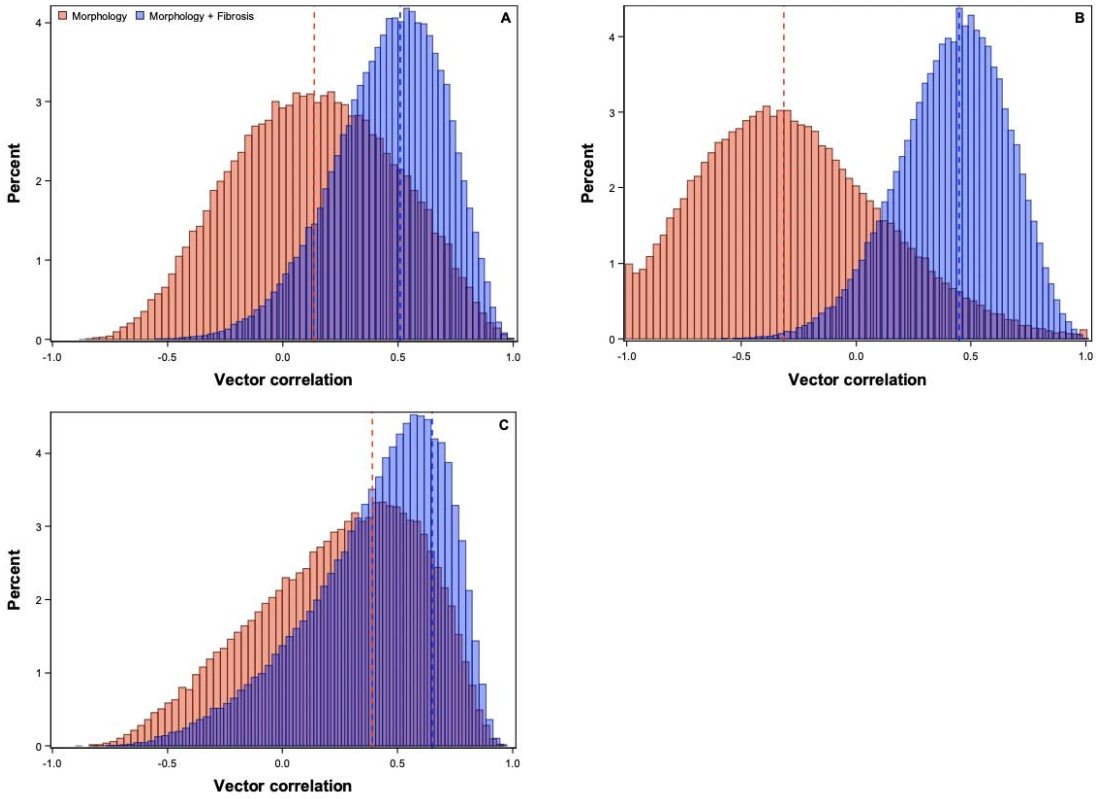


Figure 5. Individual diet varies with fibrosis, sex, and mating status. Across both lakes, fibrosis was associated with a reduction in the total number of prey items found in an individual's stomach (panel A). Controlling for differences among lakes, males and females differed in their multivariate diet content, and this effect was driven by females consuming more dipteran larvae and zooplankton and males consuming more fish eggs (panel B; least square means and standard errors). In males from Boot lake, from which stomach contents of nesting males were available, we find that successfully nesting males had consumed more total prey items than a random sample of males captured in minnow traps (panel C; least square means and 95% CIs). From univariate (A and C) and multivariate (B) linear models with Poisson error.



640

641

642 **Figure 6. Sexually-concordant effects of fibrosis on reproductive fitness act to align**

643 **multivariate selection across the sexes in Roselle lake.** Histograms represent the empirically

644 constructed sampling distributions of the vector correlation between male and female

645 multivariate selection gradients estimated in separate linear models, when gradients are estimated

646 using morphological traits alone (red) or including fibrosis (blue). Panel A shows this contrast in

647 an analysis using the first three morphological principle components (i.e., size and two

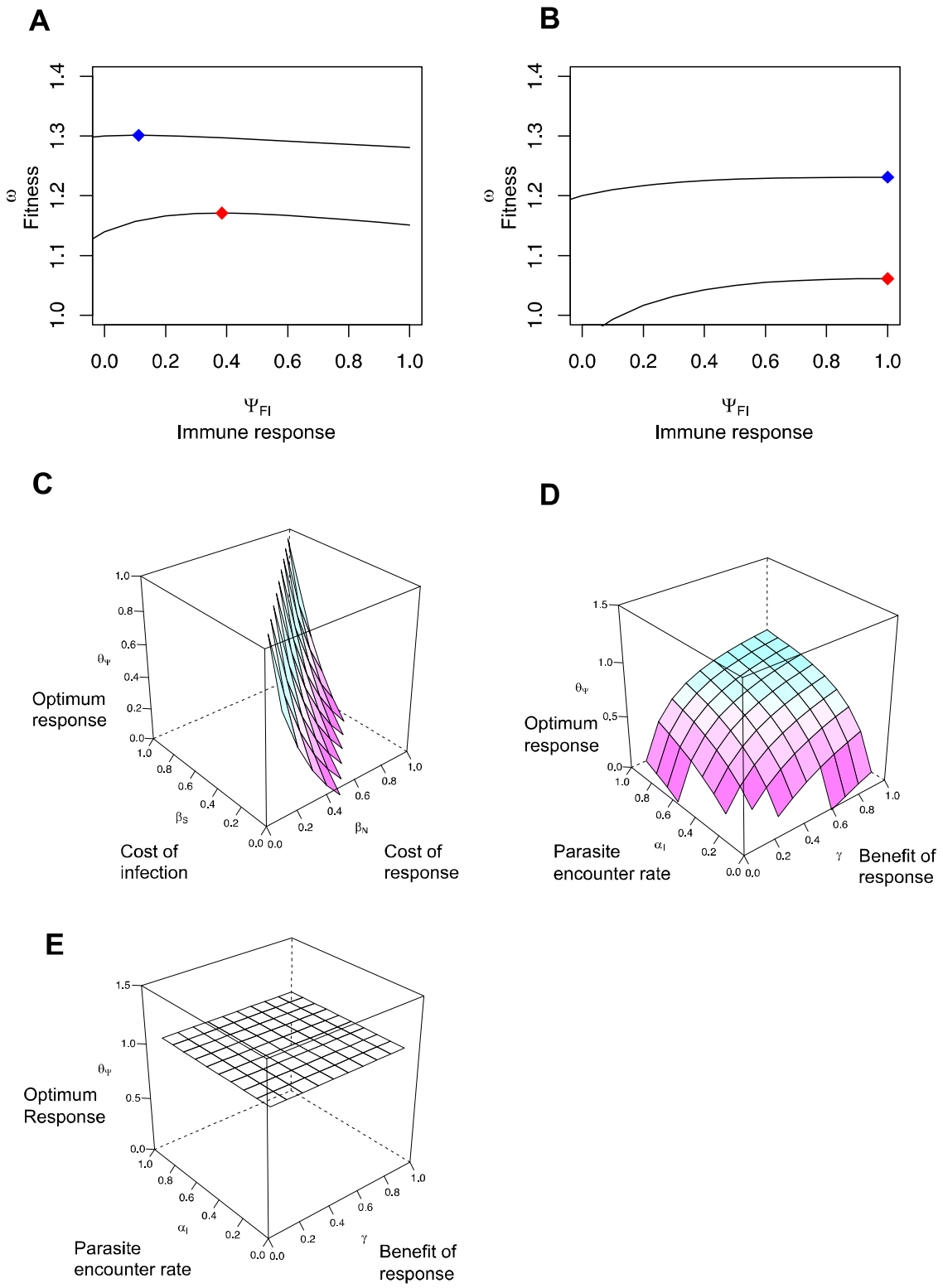
648 dimensions of shape that in total capture 85% of the phenotypic variation). Panel B shows the

649 contrast in an analysis using PC2 and PC3, showing sexually-antagonistic selection on shape

650 traits alone. Panel C shows the contrast when all raw morphological traits are modelled. Dashed

651 lines represent the correlation estimated from the original REML estimates. Histograms are

652 correlations calculated from gradient vectors obtained from resampling (100,000x) from a
653 multivariate normal distribution centered on the original estimates and covariance from the
654 estimated covariance matrices of the fixed effects.
655
656
657
658



660 **Figure 7. A model of immune response evolution**. Panels A and B show fitness as a function
661 of immune response sensitivity $\psi_{F,I}$ under two parasite encounter rates, $a_I = 0.5$ (blue optimum,
662 θ_ψ) and $a_I = 0.9$ (red optimum, θ_ψ), where costs of fibrosis exceed costs of infection (A) and
663 where costs of fibrosis equal costs of infection (B). Panel C illustrates the optimum as a function
664 of costs of fibrosis and costs of infection, holding γ and a_I constant; values where $\beta_N < \beta_S$ all
665 exceed unity. When $\beta_N > \beta_S$, the optimum is determined by the mitigating effects (γ) of fibrosis
666 on fitness costs of parasite infection, as well as parasite encounter rate a_I (Panel D). When
667 $\beta_N \leq \beta_S$, these mitigating effects and encounter rates play little role in the position of the
668 optimum (Panel E).
669
670
671
672
673
674
675
676
677
678
679
680
681
682

683 **Literature cited**

684 Armour, E., T. L. Bruner, J. K. Hines, and M. W. Butler. 2020. Low-dose immune challenges
685 result in detectable levels of oxidative damage. Journal of Experimenal Biology **223(Pt**
686 **6):jeb220095**.
687 Arnold, S. J., and M. J. Wade. 1984a. On the measurement of natural and sexual selection:
688 Applications. Evolution **38**:720-734.
689 Arnold, S. J., and M. J. Wade. 1984b. On the measurement of natural and sexual selection:
690 theory. Evolution **38**:709-719.
691 Baker, J. A., D. C. Heins, S. A. Foster, and R. W. King. 2008. An overview of life-history
692 variation in female threespine stickleback Behavior **145**:579-602.
693 Barber, I., and J. P. Scharsack. 2010 The three-spined stickleback-Schistocephalus solidus
694 system: An experimental model for investigating host-parasite interactions in fish.
695 Parisitology **137**:411-424.
696 Billi, A., J. M. Kahlenberg, and J. E. Gudjonsson. 2019. Sex bias in autoimmunity. Current
697 Opinion in Rheumatology **31**:53-61.
698 Bolnick, D. I., and K. M. Ballare. 2020. Resource diversity promotes among-individual diet
699 variation, but not genomic diversity, in lake stickleback Ecology Letters **23**:495-505.
700 Bolnick, D. I., E. J. Resetarits, K. Ballare, Y. E. Stuart, and W. E. Stutz. 2020. Host patch traits
701 have scale-dependent effects on diversity in a stickleback parasite metacommunity
702 Ecography **43**:990-1002.
703 Boots, M., and R. G. Bowers. 2004. The evolution of resistance through cotsly acquired
704 immunity Proceedings of the Royal Society of London Series B. **271**:715-723.
705 Boughman, J. W. 2007. Condition-dependent expression of red colour differs between
706 stickleback species. Journal of Evolutionary Biology **20**:1577-1590.
707 Connallon, T. 2015. The geography of sex-specific selection, local adaptation, and sexual
708 dimorphism. Evolution **69**:2333-2344.
709 Connallon, T., and M. D. Hall. 2016. Genetic correlations and sex- specific adaptation in
710 changing environments. Evolution **70**:13.
711 Cox, R. M., and R. Calsbeek. 2009. Sexually antagonistic selection, sexual dimorphism, and the
712 resolution of intralocus sexual conflict. American Naturalist **173**:176-187.
713 De Lisle, S. P., and E. I. Svensson. 2017. On the standardization of fitness and traits in
714 comparative studies of phenotypic selection. Evolution **71**:2313-2326.
715 Fish, E. N. 2008. The X-files in immunity: sex-biased differences predispose immune responses
716 Nature Reviews Immunology **8**:737-744.
717 Gipson, S. A. Y., and M. D. Hall. 2016. The evolution of sexual dimorphism and its potential
718 impact on host-pathogen coevolution Evolution **70**:959-968.
719 Goldzmid, R. S., and G. Trinchieri. 2012. The price of immunity Nature Immunology **13**:932-
720 938.
721 Graham, A. L. 2013. Optimal immunity meets natural variation: the evolutionary biology of host
722 defence Parasite Immunology **35**:315-317.
723 Hall, M. D., and N. Mideo. 2018. Linking sex differences to the evolution of infectios disease
724 life-histories Philosophical Transactions of The Royal Society B. **373**:20170431.
725 Heins, D. C., and J. A. Baker. 2014. Fecundity compensation and fecundity reduction among
726 populations of the three-spined stickleback infected by Shistocephalus solidus in Alaska.
727 Parisitology **141**:1088-1096.

Heins, D. C., J. A. Baker, M. A. Toups, and E. L. Birden. 2010. Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyloobothriidean cestode Schistocepalus solidus Biological Journal of the Linnean Society **100**:845-846.

Houle, D., and K. Meyer. 2015. Estimating sampling error of evolutionary statistics based on genetic covariance matrices using maximum likelihood. Journal of Evolutionary Biology **28**:1542-1549.

Houston, A. I., J. M. McNamara, Z. Barta, and K. C. Klasing. 2007. The effect of energy reserved and food availability on optimal immune defence Proceedings of the Royal Society of London Series B. **274**:2835-2842.

Hund, A. K., L. Fuess, M. L. Kenney, M. F. Maciejewski, J. M. Marinin, K. C. Shim, and D. I. Bolnick. 2020. Rapid Evolution of parasite resistance via improved recognition and accelerated immune activcation and deactivation BioRxiv.

Khan, I., D. Agashe, and J. Rolff. 2017 Early-life inflammation, immune response and ageing. Proceedings of the Royal Society of London Series B. **284**:1850.

Klein, S. L. 2000. The effects of hormones on sex differences in infection: from genes to behavior Neuroscience and Biobehavioral Reviews **24**:627-638.

Kokko, H., and M. Jennions. 2003. It takes two to tango. Trends in Ecology & Evolution **18**:103-104.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? . Oikos **88**:87-98.

Long, T. A. F., A. F. Agrawal, and L. Rowe. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. Current Biology **22**:204-208.

McCurdy, D. G., D. Shutler, A. Mullie, and M. R. Forbes. 1998. Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. Oikos **82**:3030-3312.

McGlothlin, J. W., A. J. Moore, J. B. Wolf, and E. D. I. Brodie. 2010. Interacting phenotypes and the evolutionary process. III. Social evolution. Evolution **64**:2558-2574.

Mitteroecker, P., and F. Bookstein. 2011. Linear discimination, ordination, and the visualization of selection gradients in modern morphometrics. Evolutionary Biology **38**:100-114.

Moore, A. J., E. D. I. Brodie, and J. B. Wolf. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution **51**:1352-1362.

Morrissey, M. B., L. E. B. Kruuk, and A. J. WIlson. 2010. The danger of applying the breeder's equation in observational studies of natural populations. Journal of Evolutionary Biology **23**:2277-2288.

Mutsaers, S. E., J. E. Bishop, G. McGrouther, and G. J. Laurent. 1997. Mechanisms of tissue repair: from wound healing to fibrosis The International Journal of Biochemistry and Cell Biology **29**:5-17.

Navarro, C., F. de Lope, A. Marzal, and A. P. Møller. 2004. Predation risk, host immune response, and parasitism. Behavioral Ecology **15**:629-635.

Poulin, R. 1996. Sexual inequalities in helminth infections: a cost of being a male? American Naturalist **147**:287-295.

Reimchen, T. E., and P. Nosil. 2001. Ecological causes of sex-biased parasitism in three-spined stickleback. Biological Journal of the Linnean Society **73**.

Roberts, C. W., W. Walker, and J. Alexander. 2001. Sex-associated hormones and immunity to protozoan parasites. Clinical Microbiology reviews **14**:476-488.

773 Rolff, J. 2002. Bateman's principle and immunity. Proceedings of the Royal Society B:
774 Biological Sciences **269**:867-872.
775 Schalk, G., and M. R. Forbs. 1997. Male biases in parasitism of mammals: effects of study type,
776 host age, and parasite taxon. Oikos **78**:67-74.
777 Scharsack, J. P., F. A. Franke, N. I. Erin, A. Kuske, J. Büscher, H. Stolz, I. E. Samonte, J. Kurtz,
778 and M. Kalbe. 2016. Effects of environmental variation on host-parasite interaction in
779 three-spined stickelbacks (Gasterosteus aculeatus). Zoology **119**:375-383.
780 Scharsack, J. P., M. Kalbe, C. Harrod, and G. Rauch. 2007. Habitat-specific adaptation of
781 immune responses of stickleback (Gasterosteus aculeatus) lake and river ecotypes.
782 Proceedings of the Royal Society of London Series B. **274**:1523-1532.
783 Schmid-Hempel, P. 2003. Variation in immune defenxe as a question of evolutionary ecology
784 Proceedings of the Royal Society of London Series B. **270**:357-366.
785 Sheldon, B. C., and S. Verhulst. 1996. Ecological Immunology: costly parasite defences and
786 trade-offs in evolutionary ecology Trends in Ecology and Evolution **11**:317-321.
787 Sheridan, L. A. D., R. Poulin, D. F. Ward, and M. Zuk. 2000. Differences in parasitic infections
788 among arthropod hosts: is there a male bias? Oikos **88**:327-334.
789 Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the
790 evidence. Quarterly Review of Biology **64**:419-461.
791 Stockmaier, S., D. I. Bolnick, R. A. Page, and G. G. Carter. 2020. Sixkness effects on social
792 interactions depend on the type of behaviour and relationship Journal of Animal Ecology
793 **89**:1387-1394.
794 Stoehr, A. M., and H. Kokko. 2006. Sex differences in immunocompetence: what does life
795 history theory predict? . Behavioral Ecology **17**:751-756.
796 Stutz, W. E., O. L. Lau, and D. I. Bolnick. 2014. Contrasting patterns of phenotype-dependent
797 parasitism within and among populations of threespine stickleback The American
798 Naturalist **183**:810-825.
799 Svensson, E. I., L. Råberg, C. Koch, and D. Hasselquest. 1998. Energetic stress, immuno
800 suppression and the costs of an antibody response Functional Ecology **12**:912-919.
801 Tschirren, B., and H. Richner. 2006. Parasites shape the optimal investment in immunity
802 Proceedings of the Royal Society of London Series B. **273**:1773-1777.
803 Viney, M. E., E. M. Riley, and K. L. Buchanan. 2005. Optimal immune responses:
804 immunocompetence revisited. Trends in Ecology and Evolution **20**:665-669.
805 Weber, J. N., M. Kalbe, K. C. Shim, N. I. Erin, N. C. Steinel, L. Ma, and D. I. Bolnick. 2017a.
806 Resist globally, infect locally: a transcontinental test of adaptation by stickleback and
807 their tapeworm parasite The American Naturalist **189**:43-57.
808 Weber, J. N., N. C. Steinel, K. C. Shim, and D. I. Bolnick. 2017b. Recent evolution of extreme
809 cestode growth suppression by a vertebrate host Proceedings of the National Academy of
810 Sciences **114**:6575-6580.
811 Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's
812 principle. The American Naturalist **100**:687-690.
813 Zuk, M. 2009. The sicker sex. PloS Pathogens **5**:e1000267.
814 Zuk, M., and A. Stoehr. 2002. Immune defense and host life history The American Naturalist
815 **160** S9-S22.
816