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

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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. Consistent with substantial sexually concordant fitness effects of immune response, we find alignment of multivariate selection across the sexes despite sexual antagonism over morphological shape. 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.

KEY WORDS: Fibrosis, life history tradeoffs, Schistocephalus solidus, sexual antagonism.
Schistocephalus solidus 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 (Weber et al. 2017a; Weber et al. 2017b; Scharsack et al. 2007; Scharsack et al. 2016).

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 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). Specifically, fish with severe fibrosis were more likely to have successfully ensnared small cestodes in a collagen/fibronectin sac (a granuloma). These granulomas frequently contained moribund or dead cestodes, indicating that fibrosis itself is an adaptation to reduce cestode infections (rather than a mere side-effect of inflammation). 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; Vrtilek and Bolnick 2020). However, some stickleback populations have recently co-opted this ancient pathway to respond to perceived S. solidus infections (Hund et al. 2020). Several of the strongest targets of selection in the diverging genomes of a fibrotic versus non-fibrotic population include fibroblast-regulating genes such as PU1 and STAT6 (Weber et al., in prep), further confirming that the fibrosis response itself appears to be an adaptation. 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 limit on ovary expansion. However, male reproduction does not rely on gonad enlargement and so we predicted that the costs of fibrosis may be sex-specific.

Two important questions in this stickleback-cestode system remain unanswered: why do populations vary in fibrosis immune response, and why do the sexes vary in cestode infection rates (Reimchen and Nosil 2001) but not in their innate immune response (Hund et al. 2020)? We predict that variation in fibrosis immune response across populations and cestode infection rates across sexes may be the result of differences in the cost/benefit trade-offs that dictate the optimal immune strategy. To test this prediction, we surveyed two wild stickleback populations that naturally differ in parasite infection rates. In particular, our focus on male component fitness allowed us to assess previously unknown costs to males. Both populations exhibit moderate rates of both infection and fibrosis. Crucially, there is an imperfect association between infection and fibrosis in both populations: not all individuals initiate fibrosis when infected. Also, fibrosis...
presents at least 3 months after the parasite is eliminated, so we find individuals with fibrosis but no surviving infection. These facts allow us to statistically separate the costs of infection from the costs of fibrosis, in both populations. We find massive fitness effects of both parasite infection and of the fibrotic immune response, in both sexes and across both populations. We show that these concordant fitness effects result in an alignment of multivariate selection across the sexes despite signatures of sexual antagonism over body shape. These results leave us with a puzzle: there are persistent sex differences in infection rates, but no corresponding difference in the probability of fibrosis or its costs. To explain this apparent contradiction, we construct and analyze an optimality model of immune response evolution. Our analysis indicates that immune response optima may often be shared across sexes that differ in parasite encounter/infection rates when costs of infection and immune response are concordant and high.

**Methods**

**FISH CAPTURE AND MEASUREMENT**

We captured adult three-spined stickleback from two lakes (Boot Lake and Roselle Lake), on Vancouver Island, British Columbia, Canada, between June 2 and June 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 (Weber et al. 2017b; Stutz et al. 2014; 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 1984a, 1984b). Although our correlative design cannot completely separate shared environmental effects on traits and fitness, we note that months-long development times of cestodes makes it unlikely that any correlation between nesting success and infection could be driven by nesting microhabitat differences; nesting territories are established immediately prior to reproduction in the spring, while tapeworm infections can last months. We also note that there is little evidence (based on cross-year mark-recapture surveys of two Vancouver Island lake populations) to indicate adults survive to reproduce for a second year (Bolnick, unpublished). Thus all or nearly all adults captured in the spring are expected to have been born the previous spring and so are approximately the same age. 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 are unlikely to be nesting. Trapped fish were euthanized and frozen. We sampled both lakes until at least 50 nesting males had been captured. This number was chosen to avoid excessive impact on the populations studied while still presenting a reasonable sample size; we note however that this sampling design precludes accurate estimation of population mean mating rate because we have controlled the number of nesting males captured. We also retained a random sample of trapped females in each lake, using ovary mass as a metric of component fitness. Because ovary mass is related to the number and size of eggs, it is a measure of female reproductive success subject to similar caveats as male mating success. We note again that there is little evidence that adults of either sex regularly survive to reproduce a second season in wild populations on Vancouver island.

All fish were kept frozen until later laboratory dissection, upon which fish were thawed, measured, and dissected. We measured seven external morphological traits: standard length, head length (measured from the snout to the distal end of the operculum), snout length (measured from the snout to the orbital), eye width, body depth, body width at the pelvic girdle, and middle spine length. These traits were measured to two decimal places with digital calipers. We then dissected the left gills and counted gill raker number, in addition to photographing the gill rakers under a dissection microscope at fixed magnification to measure length of the longest raker. Following these measurements fish were dissected and all *Schistocephalus* counted and weighed. We note that *Schistocephalus* was the only abundant macroparasite found in our sample; we also examined internal organs, eyes, and the digestive tract for other parasite taxa. Gonads were removed and weighed. Stomach contents were removed and identified to (at least) order for all individuals, with the exception of samples from nesting males from Roselle, whose stomachs were lost during shipping. Although prey taxa likely differ in their rate of digestion (and thus detection probability in stomach contents), most prey taxa remain identifiable in stomachs for at least 4 h after ingestion (Svanbäck and Bolnick 2007), and we have no reason to expect different detection rates across sexes.
We scored fibrosis on an ordinal scale, where a value of 0 corresponds to no apparent fibrosis (organs move freely), a value of 1 corresponds to fibrosis between organs, a value of 2 corresponds to fibrotic connection between organs and the peritoneal tissue, and a value of 3 corresponds to excessive fibrosis across the entire body cavity. This scale is modified from that developed to approximate the range of fibrotic variation seen in both laboratory studies and natural populations of stickleback (A. Hund & L. Fuess, personal communication), and has previously been shown to be highly repeatable between independent observers blind to experimental vaccination treatment (Goldzmid and Trinchieri 2012). A video example of these fibrosis levels is available (https://www.youtube.com/watch?v=yKvcRVCSpWI&feature=youtu.be). Because there is no straightforward way to treat an ordinal categorical predictor in a linear model, when fitting fibrosis as a predictor in models (see below) we took the approach of fitting separate models assuming fibrosis as a continuous predictor and as a categorical, and present results from both approaches. However, we note that treating this trait as continuous carries perhaps greater biological justification, as a categorical assumption ignores ranking of levels.

**STATISTICAL ANALYSIS: BENEFITS OF FIBROSIS**

To evaluate the benefits of fibrosis, we used a generalized linear model to test whether *Schistocephalus* mass depends on host fibrosis score (treated as a continuous fixed effect which accommodates the ordinal nature of the variable), assuming exponential error. We avoided including fish body mass in this model because *Schistocephalus* mass is a direct component of total fish body mass and fibrosis is unrelated to mass, although results were unchanged when correcting for mass. Because our sample size for this analysis was limited to the subset of fish that were infected, we pooled lakes and sexes and did not model higher order interactions. We used a separate linear model with infection probability as a binomial response (cestode present or absent) to host fibrosis score, lake, and host sex; interaction terms were not significant and were dropped. We also repeated our analysis of fibrosis by fitting an ordinal multinomial generalized linear model with a cumulative logit link function with fibrosis score as the response and sex, lake, and infection status as fixed effects. We fit such a model with and without interaction terms, as the main effect of sex indicates whether the sexes differ in expressed fibrosis levels, while the sex*infection interaction indicates whether the sexes differ in fibrosis response to a given infection. We also refit this model after binning fibrosis into a binary variable (absent; 0/present; 1–3). Thus, our analyses assessed whether fibrosis was more prevalent in one sex than another, whether the ordinal amount of fibrosis was higher in one sex than another, and whether either of these measures differed in their association with parasite infection across the sexes.

**STATISTICAL ANALYSIS: COSTS OF FIBROSIS AND INFECTION**

We considered the fitness costs of fibrosis and infection on male and female reproductive success (nesting or not; ovary mass). We assessed component fitness costs for females in Roselle 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 in a model of size-corrected ovary mass (residuals from a regression of ovary mass on body mass) and report results of both analyses. 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 linear discriminant function scores calculated in a linear 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 an individual’s 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 importance of fibrosis using a full multivariate model; we explore such a multivariate model below (section Statistical Analysis: Alignment of multivariate selection across the sexes).

To assess differences in diet, including potential costs of immune response and infection on resource acquisition, we used a
series of uni- and multivariate linear models to test for associations between diet and fibrosis or infection. Because of the sparse multivariate nature of diet data (i.e., many zeros for rare taxa), we constructed our models on three specific hypotheses, in all cases avoiding higher order interactions wherever appropriate. First, to evaluate variation in overall prey intake, we used a univariate linear model with total prey counts as the response and fibrosis, lake, and infection status as fixed effects. Prior studies have reported inconsistent sex-biased diet in stickleback (males often being more limnetic in most but not all lakes; Bolnick and Ballare 2020). This is relevant because cestodes are acquired by eating limnetic copepods. To assess differences across the sexes in diet composition we used a multivariate linear model where the response vector consisted of counts of each prey taxon in an individual’s diet. This model included sex, lake, and their interactions with prey taxon as fixed effects; in this model significant interactions with taxon type indicate differences in the taxonomic composition of diet across sexes or lakes. Finally, we used a similar multivariate model to test for diet differences between nesting and randomly sampled males in Boot Lake, although as a caveat we cannot control for variation in time spent in traps before euthanasia that may affect measured diet. Multivariate models with infection status as a predictor failed to converge, likely due to the relatively small number of infected fish. For all models of diet content, in which the data are counts of individual prey items in a stomach, we treated the response variable(s) as Poisson distributed. For multivariate models with a response vector of counts of each prey taxon, we modeled covariance in counts across individual fish as the Cholesky parameterization of an unstructured covariance matrix.

STATISTICAL ANALYSIS: ALIGNMENT OF MULTIVARIATE SELECTION ACROSS THE SEXES

Our analysis of fitness costs of fibrosis suggested shared reproductive costs of fibrosis across the sexes, and we were interested in quantifying effects of fibrosis on the geometry of multivariate selection. To assess the effects of fibrosis immune response on alignment between male and female selection, we compared the orientation of multivariate selection in males and females estimated in Roselle Lake. Small sample size of females in Boot Lake precluded such an analysis for those fish. For this analysis, we re-fit our model of male morphological sexual selection using a glm with nesting success as a response and morphological traits as a predictor, fit an equivalent model for females with ovary mass as a response, and compared the orientation of male and female selection (assuming a Gaussian error for both for comparison). We fit models including all morphological traits, and also models with reduced dimensionality where we used the first three principal components of the correlation matrix of morphological traits (both sexes pooled). We repeated this with and without including fibrosis score in as a predictor in our model in order to assess the effects of fibrosis on the alignment of selection across the sexes. Note that the non-binary fitness component for females precluded discriminant analysis as performed before for males. We compared orientation by calculating the vector correlation between the normalized selection gradients, \( \beta_m \beta_f^T \), which provides an estimate of sexual antagonism on a scale of \(-1\) to \(1\), where a value less than zero indicates selection acting on opposing directions across the sexes, while a value greater than zero indicates concordant 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 \( \beta_m \) and \( \beta_f \). We estimated the sampling distribution of this vector correlation by resampling \((100,000 \times)\) each vector from a multivariate normal distribution centered at the original REML estimates of \( \beta \) 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. Finally, we note that this approach may provide insight into how specific traits (e.g., fibrosis) contribute to the alignment of multivariate selection across the sexes, but cannot say anything about the causes of selection, as we have not experimentally manipulated phenotypic or ecological variables that might generate the variation in reproductive success.

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). Moreover, all of our selection analyses treat fibrosis as a continuous variable, as there is no straightforward way to incorporate an ordinal multinomial trait in a Lande-Arnold phenotypic selection analysis. However, we reached qualitatively equivalent conclusions when treating fibrosis as a binary categorical trait.

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 are available on Dryad (https://doi.org/10.5061/dryad.5tb2rp3w) and complete script to reproduce all analyses and figures is provided in the Supporting Information.
Results

BENEFITS OF FIBROSIS

We sampled a total of 411 fish (Roselle, 156 M, 71 F; Boot, 154 M, 30 F), of which 102 were nesting males (50 Roselle, 52 Boot) and 81 (16 Roselle, 65 Boot) were infected with *Schistocephalus* (Table S1). Probability of cestode infection varied across sexes and lakes, with higher (odds ratio 4.79) infection rates in females across both Boot and Roselle lakes, and higher (odds ratio 10.5) overall infection rates in Boot than Roselle lake (Sex effect, $F_{1,407} = 21.06, P < 0.0001$; Lake effect, $F_{1,407} = 46.14, P < 0.0001$; Fig. 1). Across lakes and sexes, probability of cestode infection was reduced in fish with high levels of fibrosis (odds ratio of unit offset at the mean 0.649, $F_{1,407} = 6.15, P = 0.0135$; Fig. 1), consistent with prior observations that fibrosis contributes to elimination of the infection then lingers afterwards (Hund et al. 2020). This effect was also observed in a multinomial model with fibrosis as the response, where we observed a significant relationship with infection status and fibrosis score ($F_{1,405} = 6.56, P = 0.0108$) and no sex ($F_{1,405} = .29, P = 0.59$) or lake ($F_{1,405} = .45, P = 0.50$) differences in fibrosis levels. In a model with interaction effects, we found no significant interactions with sex (sex $\times$ infection $F_{1,401} = 1.95, P = 0.16$; sex $\times$ lake $F_{1,401} = 0.98, P = 0.32$; sex $\times$ lake $\times$ infection $F_{1,401} = 0.1, P = 0.74$). We also found no main or interacting effects of sex when fibrosis was binned intro a binary presence/absence trait (sex effect $F_{1,403} = 0.62, P = 0.43$; sex $\times$ infection $F_{1,403} = 1.5, P = 0.22$; sex $\times$ lake $F_{1,403} = 3.32, P = 0.07$; sex $\times$ lake $\times$ infection $F_{1,403} = 0.13, P = 0.72$). Thus we found no evidence that the sexes differ in prevalence of fibrosis, the severity of fibrosis, nor the association with fibrosis and infection. This is consistent with observations of fibrosis in laboratory infections (Weber et al., in prep) and artificial vaccinations (Hund et al. 2020), which both found no sex differences in infection response. Of those fish infected, host fibrosis was associated with reduced cestode mass ($F_{1,76} = 9.06, P = 0.0035$; Fig. 2; size-corrected: $F_{1,76} = 13.81, P = 0.0004$; categorical fibrosis $F_{2,75} = 8.34, P = 0.0005$). Thus, despite different parasite infection rates across sexes and lakes, we find no evidence of corresponding differences in fibrosis rates across the sexes, although we do find evidence that fibrosis

![Figure 1](image1)

**Figure 1.** Fibrosis phenotype is associated with reduced probability of cestode infection across lakes and sexes. Model fit is from a generalized linear model with binomial error and lake, sex, and fibrosis as fixed effects. Interaction terms were not significant and were dropped; all main effects were significant (see text). Blue solid, females; red dashed, males. Confidence limits are 95%.

![Figure 2](image2)

**Figure 2.** Fibrosis phenotype is associated with reduced cestode mass in infected individual hosts. *Schistocephalus* mass is the total mass in mg 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 $\times$ sex combinations make analysis of higher order interactions tenuous. Confidence limits are 95%.
REPRODUCTIVE COSTS OF IMMUNITY

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.

is associated with benefits in the form of both a reduction in cestode growth and a decrease in the probability of infection.

COSTS OF FIBROSIS AND INFECTION

Countering these benefits, we also see evidence for costs of fibrosis. In both Boot and Roselle lakes, males defending active nests exhibited reduced fibrosis levels compared to randomly sampled males (Boot, odds ratio of unit offset at the mean 0.56, $F_{1,151} = 6.87, P = 0.0097$; although sensitive to scale; categorical fibrosis $F_{3,149} = 1.49, P = 0.21$; Roselle, odds ratio of unit offset at the mean 0.41, $F_{1,153} = 12.57, P = 0.0005$, categorical fibrosis $F_{3,151} = 2.91, P = 0.036$). Nesting males also exhibited reduced cestode infection in Boot lake (odds ratio 2.45, $F_{1,151} = 4.3, P = 0.039$; Roselle lake odds ratio 1.89, $F_{1,153} = .57, P = 0.45$). This is reflected in significant (Roselle, $F_{1,152} = 6.24, P = 0.0135$) or nearly significant (Boot, $F_{1,147} = 3.52, P = 0.062$) negative effects of fibrosis on mating probability in models including morphological discriminant function score, that is the 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 (Fig. 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 (justification provided in detail in Bolnick 2004). For females in Roselle lake, we found that fibrosis was associated with a significant reduction in ovary mass ($F_{1,67} = 10.65, P = 0.0017$; Fig. 4; size-corrected: $F_{1,67} = 8.33, P = 0.005$; categorical fibrosis $F_{3,66} = 3.41, P = 0.023$) while controlling for infection; ovary mass was also significantly reduced in cestode-infected females ($F_{1,67} = 4.92, P = 0.03$; size-corrected: $F_{1,67} = 3.9, P = 0.052$). Notably, the fibrosis response explained more variance in female ovary mass than the cestode infection itself explained. 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 ($F_{1,341} = 7.75, P = 0.0057$; Fig. 5A, categorical fibrosis:...
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.

ALIGNMENT OF MULTIVARIATE SELECTION

Given that fibrosis appears to confer component-fitness costs in males and females, we can examine the effects of fibrosis on geometric alignment of multivariate selection across the sexes. We find weak or even antagonistic alignment between multivariate selection on morphological traits in males and females (Fig. 6), with evidence of sexually antagonistic selection on body shape independent of body size. This is reflected in a negative estimate of the correlation between male and female multivariate selection gradients (Fig. 6B). However, including fibrosis as a predictor of component fitness increases geometric alignment between male and female multivariate selection (Figure 6A–C), although sampling error is substantial in some cases. Note that we avoid formal statistical comparison of male and female selection gradients because they were estimated in separate models using different fitness components.

A MODEL OF IMMUNE RESPONSE EVOLUTION

Our empirical results indicate substantial fitness effects of fibrosis in both sexes. We also find sex differences in cestode infection rates in the two populations we surveyed, and sex-specific infection rates are commonplace but variable across populations in Western Canada (Reimchen and Nosil 2001; Stutz et al. 2014). Populations differ in whether a fibrosis response is mounted upon exposure to cestode protein homogenate, and how quickly fibrosis develops after vaccine adjuvant exposure (Hund et al. 2020). By scoring fibrosis in >700 cestode-exposed F2 hybrid fish,
Figure 6. Sexually concordant effects of fibrosis on reproductive fitness result in alignment of multivariate selection across the sexes in Roselle Lake, particularly selection on multivariate shape. Histograms represent the empirically constructed sampling distributions of the vector correlation between male and female multivariate selection gradients estimated in separate linear models, when gradients are estimated using morphological traits alone (red) or including fibrosis (blue). Panel (A) shows this contrast in an analysis using the first three morphological principle components (i.e., size and two dimensions of shape that in total capture 85% of the phenotypic variation). Panel (B) shows the contrast in an analysis using PC2 and PC3, showing sexually antagonistic selection on shape traits alone. Panel (C) shows the contrast when all raw morphological traits are modelled. Dashed lines represent the correlation estimated from the original REML estimates. Histograms are correlations calculated from gradient vectors obtained from resampling (100,000 ×) from a multivariate normal distribution centered on the original estimates and covariance from the estimated covariance matrices of the fixed effects.

Weber et al. (in prep) mapped quantitative trait loci for fibrosis, containing fibrosis-associated candidate genes exhibiting strong signatures of selection. These results confirm that the fibrosis response pathway is evolvable. However, in both studies males and females do not differ significantly in fibrosis response (Hund et al. 2020, Weber et al., in prep), nor does fibrosis map to the sex chromosomes. These results are somewhat paradoxical because intuition suggests substantial sex differences in infection rates may be expected to lead to the evolution of sex-specific immune response. Here, we construct and analyze a simple model of immune response evolution, in order to reconcile these results and illuminate how shared fitness costs of immunity may in part determine the optimum immune response in males and females.

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

\[
\omega_{i,j} = a_{w,i} - \beta_{F,i}z_{F,i,j} + \gamma_{I,z_{I,i,j}}z_{I,i,j} - \beta_{I,z_{I,i,j}} + \epsilon_{i,j}
\]
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 |
| $a_o$ | Fitness intercept |
| $\beta_F$ | Fitness cost of expressing immune trait $z_F$ |
| $\beta_I$ | Direct fitness cost of parasite infection $z_I$ |
| $\gamma$ | 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 |
| $\theta_o$ | Optimum immune sensitivity $\psi_{F,I}$ |

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

Importantly, the two traits in this model $z_F$ and $z_I$ are not expected to be expressed independently, and in fact are measurable outcomes of underlying latent trait(s). Fibrosis is induced by parasite exposure, but in turn it acts to reduce the probability of successful infection, $z_I$. Thus fibrosis, $z_F$, is a trait whose expression is determined not only by host genotype but also by parasite exposure, an environmental effect. Infection, $z_I$, is a trait determined both by parasite encounter rates as well as the mitigating effects of fibrosis response. We define trait expression as

$$z_F = a_F + \epsilon + \psi_{F,I}z_I$$
$$z_I = a_I + \epsilon + \psi_{F,F}z_F$$

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

The terms $\psi$ represent the degree to which trait expression is influenced by another trait, in this case infection status or fibrosis. Thus, $\psi_{F,I}$ is a latent trait describing the degree to which fibrosis is induced in response to a parasite exposure, and is thus the key variable of interest in our model, whose evolution we seek to predict. We note mathematical similarity with the literature on indirect genetic effects (Moore et al. 1997; McGlothlin et al. 2010), in particular because effects of immune response on infection repression and infection induction of immune response are analogous (but not equivalent) to indirect genetic effects. We can now define each trait explicitly in terms of the other,

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

with a similar equation for $z_I$. We note that in our model of antagonistic interactions between immune response and parasite infection, we expect $\psi_{F,I}$ to range from 0 to 1 and for $\psi_{I,F}$ to range from 0 to $-1$.

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

$$\frac{d\omega}{d\psi_{F,I}} = -\beta_F a_I + \beta_F \psi_{F,I}a_I + \frac{\gamma a_I^2}{(1 + \psi_{F,I})^2} + \frac{-2\gamma a_F a_I a_I}{(1 + \psi_{F,I})^2} + \frac{\beta_I a_I}{(1 + \psi_{F,I})^2}$$

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

$$\theta_o = \frac{a_I \gamma - \beta_F + \beta_I}{a_I \gamma + \beta_F - \beta_I}$$

which holds for $a_I \gamma + \beta_F \neq \beta_I$ and $a_I \gamma \neq 0$, and is a local optimum (see Appendix).

Equation 5 describes the location of the optimum immune response sensitivity as a function of the costs and benefits of immune response and parasite infection. Immediately notable is that increases in parasite prevalence, captured by the intercept $a_I$, lead...
Figure 7. A model of immune response evolution. Panels (A) and (B) show fitness as a function of immune response sensitivity $\varphi_{FI}$ under two parasite encounter rates, $a_I = 0.5$ (blue optimum, $\theta_B$) and $a_I = 0.9$ (red optimum, $\theta_B$), where costs of fibrosis exceed costs of infection (A) and where costs of fibrosis equal costs of infection (B). Panel (C) illustrates the optimum as a function of costs of fibrosis and costs of infection, holding $\gamma$ and $a_I$ constant; values where $\beta_F < \beta_I$ all exceed unity. When $\beta_F > \beta_I$, the optimum is determined by the mitigating effects ($\gamma$) of fibrosis on fitness costs of parasite infection, as well as parasite encounter rate $a_I$ (Panel D). When $\beta_F \leq \beta_I$, these mitigating effects and encounter rates play little role in the position of the optimum (Panel E).

to an increase in the optimum sensitivity (Fig. 7A). However, regardless of the magnitude of $a_I$, when the natural selection cost of fibrosis, $\beta_F$, is less than or equal to the cost of infection $\beta_I$, the optimum is for high sensitivity (Figure 7B, C, and E). Intriguingly, we can also see that immune response can evolve even when the natural selection costs of fibrosis outweigh the costs of carrying an infection (Fig. 7D), if the prevalence of parasites is high enough and the costs of infection are in part mitigated by the interaction term $\gamma$.

The main conclusion from this model is that although optimum immune sensitivity can indeed be governed by encounter rate, when costs and benefits of infection and immune response are high these encounter rates play less of a role in determining the optimum immune response. We find evidence of sex and population differences in parasite encounter rates, $a_I$, based on differences in worm infection rates across populations and sexes (Fig. 1) and sex and population differences in diet (Figure 5; see also Bolnick and Ballare 2020). Yet we find no evidence of
Table 2. Estimates of parameters in Equation 1, describing costs and benefits of fibrosis and parasite infection on male nesting success and female ovary mass. $\beta_F$ indicates the fitness cost of fibrosis, $\beta_I$ the fitness cost of parasite infection, and $\gamma$ the benefit of fibrosis immune response. ± 1 Standard Error.

| Population | $\beta_F$ | $\beta_I$ | $\gamma$ |
|------------|-----------|-----------|-----------|
| Roselle Males | 0.24 ± 0.07 | 0.22 ± 0.23 | 0.19 ± 0.31 |
| Boot Males | 0.24 ± 0.08 | 0.27 ± 0.13 | 0.18 ± 0.17 |
| Roselle Females | 64 ± 16 | 88 ± 35 | 62 ± 46 |

sex differences in fibrotic immune response in the wild, and past work in the lab confirms this for Roselle lake and other Vancouver Island populations (Hund et al. 2020). Moreover, QTL mapping of fibrosis response to experimental cestode infection in the lab revealed no sex effect on fibrosis (Weber et al., in prep). At present, we lack data on lifetime reproductive success (in addition to a way to estimate $a_I$ directly), and so cannot confront our model directly with the data at hand. But, this model provides guidance for key parameters that should be measured in the future. And, the data we do have shows that in both populations and sexes there are measurable fitness costs of infection and fibrosis. We can get a better understanding of these costs by re-fitting linear models with component fitness as a predictor and binary infection status, fibrosis status, and their interaction as fixed effects. From these regression coefficient estimates, we can take the linear terms and multiply them by $-1$ and use the raw interaction term to obtain estimates of the parameters in Equation 1. We assume normality for the sake of parameter estimation and comparison. Corresponding estimates of $\gamma, \beta_F,$ and $\beta_I$ for each subpopulation sampled are provided in Table 2.

We can reach two key conclusions from these parameter estimates. First, costs of fibrosis and infection ($\beta_F$, and $\beta_I$) are always higher than the mitigating effects of $\gamma$. Second, $\beta_F$ and $\beta_I$ are either similar in magnitude (males in Boot and Roselle) and/or $\beta_F < \beta_I$ (Roselle females, Roselle males). We note that actual costs of infection are likely much higher than estimated here, as our dataset lacked information on mortality induced by infection (particularly because the parasite facilitates bird predation to complete its life cycle). Thus, these values are consistent with a high optimum for $q_{F,I}$ 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 balance the costs and benefits of immunity (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Zuk and Stoehr 2002; Graham 2013). The costs of immunity are well documented and include increased energetic expenditure and decreased resource acquisition (Houston et al. 2007), increased vulnerability to predators (Navarro et al. 2004), loss of social status (Stockmaier et al. 2020), and the increased risk of immune pathology (Goldzmid and Trinchieri 2012). These costs combine with the benefits of immune response to costly infections to determine the optimum immune response. Thus, quantification of the costs and benefits of immune response is key to understanding the evolutionary origins of variation in parasite infection rates and host immune evolution observed across populations and sexes in the wild.

Here, we have presented a rare instance of an immune response with measurable benefits and costs in the wild. In two lakes on Vancouver Island, we found that peritoneal fibrosis is associated with reduced cestode infection, both in terms of infection probability and parasite growth when infected. However, we find that this immune response comes at substantial component fitness costs for both sexes. Fibrosis is associated with a reduction in prey consumption. In both lakes, fibrotic males are less likely to be reproductively successful, indicating consistent costs of this immune response trait. Similar reproductive costs are observed in females, with substantial reduction in ovary mass in individuals expressing fibrosis (in the one lake with enough infected females to test this trend). We show that these shared fitness costs result in alignment of multivariate selection across males and females, despite evidence of sexually-antagonistic selection on trophic morphology.

Consistent with our results, recent laboratory experimental infection studies demonstrate that exposure to cestode antigens induces fibrosis (Hund et al. 2020) that in turn suppresses cestode growth and viability (Weber et al., in prep). In these laboratory assays, the sexes were equally prone to fibrosis following an immune challenge. Although males and females initiate similar levels of fibrosis following a controlled immune challenge, their risk of cestode infection should be unequal. In many lake populations of stickleback females feed on more benthic prey than males do, although in some lakes this diet dimorphism is absent or reversed. *S. solidus* is acquired by eating limnetic prey (cyclopoid copepods), so diet dimorphism should, and does, generate sex differences in cestode encounter and infection rates (Reimchen and Nosil 2001; Stutz et al. 2014). At face value, this presents a conundrum because substantial costs of fibrosis, coupled with sex differences in parasite encounter rates, might be intuit to result
in evolution of sex differences in immune sensitivity to cestode infection. Our analysis of a simple model of immune response evolution, however, indicates that optimum immune response can be surprisingly insensitive to parasite encounter rates when costs of both immune response and parasite infection are high. This finding can reconcile our results, and also indicates that interpreting infection rates alone as a measure of immune activity (as is often done in the case of sex differences in parasite loads) may be problematic (Poulin 1996).

Cestode prevalence varies widely across lakes in Western Canada (Weber et al. 2017b; Stutz et al. 2014), and some stickleback populations do not exhibit fibrotic response to cestode infection (Hund et al. 2020). Our results indicate that variation in the costs and benefits of fibrosis across populations could explain this variation in stickleback immune response. When the fitness cost of fibrosis exceeds the costs associated with cestode infection, 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.

Three major caveats exist in interpreting our results. First, it is likely that other immune pathways beyond the peritoneal fibrosis we measured play a role in suppressing cestode infections. QTL mapping of cestode survival and growth in F2 hybrids between resistant and susceptible populations finds that fibrosis is the strongest predictor of cestode growth, and maps to some of the same loci (Weber et al., in prep), but there remains as-yet-unexplained host genetic control of cestode growth. Our focus on fibrosis is justified in that it is a common phenotype in both populations surveyed, yet alternative immune pathways may explain residual variation in tapeworm infection rates in our data and that from other populations (Stutz et al. 2014). A second caveat is that our selection analysis is correlative and cannot separate shared environmental effects on traits and component fitness, although we note that trapped fish were sampled from as close as possible to nesting males. Our sample of infected fish is also likely incomplete due to selection bias owing to mortality induced by parasite infections prior to our sampling. Manipulative experiments assessing immune response effects on fitness are an important next step in understanding immune evolution in stickleback. Finally, a third caveat, particularly in interpretation of our model, is that we have ignored coevolution of the parasite and the possibility that parasites may themselves evolve strategies specific to host sex (Duneau and Ebert 2012).

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 contribute to the nature of fitness variance across the sexes. In our study, we show that concordant fitness effects of a shared trait — fibrosis — result in alignment of multivariate selection despite sexually-antagonistic selection on a suite of morphological traits. Although there is substantial sampling error, inclusion of fibrosis in a multivariate selection analysis consistently results in increased inferred alignment between male and female selection gradients in comparison to analysis of just morphology. This suggests 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. We emphasize that our analysis is limited to understanding how specific traits contribute to the geometry of selection, and cannot speak to the causes of selection on specific traits. 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.
AUTHOR CONTRIBUTIONS
Both authors designed the study. S.D. performed the study and drafted the manuscript. Both authors revised the manuscript.

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DATA ARCHIVING
All data are provided as supplemental material and archived on DRYAD https://doi.org/10.5061/dryad.5tb2rbp3w

CONFLICT OF INTEREST
The authors have no conflict of interest to declare

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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,

\[ \frac{d^2\omega}{d\phi_{F,I}^2} (\phi_{F,I} = 0) = -\frac{(a_I\gamma + \beta_F - \beta_I)^4}{8a_I^2\gamma^3} \]

which will be negative for all \( \gamma > 0 \).

Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Fish numbers sampled. Total (unfibrosed / fibrosed).