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Investigating the association between armour coverage and parasite infection in an estuarine population of stickleback

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ABSTRACT

Background: When threespine stickleback colonized fresh water, they repeatedly evolved reduced armour plating via changes in *Eda* allele frequency. This evolution is typically attributed to differential predation pressure between marine and freshwater environments. However, the chromosomal region containing *Eda* is associated with many other phenotypes, including schooling, antipredator behaviour, and immunity. Consequently, the evolution of armour plating may be driven by multiple selective pressures acting on *Eda* or linked genes.

Question: Is parasite infection associated with armour phenotype?

Hypothesis: Parasite load differs between stickleback armour plate morphs.

Organisms: An armour-polymorphic population of threespine stickleback (*Gasterosteus aculeatus*), and their parasites.

Field site: In June 2009 and 2012, we sampled stickleback from a single human-made salt-marsh pool in the Campbell River Estuary on Vancouver Island.

Methods: We counted macroparasites on approximately 100 fish per year and counted lateral armour plates. We used generalized linear models to test for correlations between armour morph and parasite load.

Results: Most parasite species were not associated with armour. The gill parasite *Thersitina* was more abundant on more fully armoured fish in both years. The nematode *Eustrongylides* also exhibited a marginally significant positive trend. If parasitic infections reduce stickleback fitness, this positive covariance between armour and infection would accelerate the loss of armour plating in stickleback colonizing fresh water.

Keywords: *Ectodysplasin (Eda), Gasterostereus aculeatus,* lateral plates, pleiotropy, threespine stickleback.

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INTRODUCTION

Trait correlations are often discussed in terms of constraints on adaptation. Evolutionary responses to natural selection follow 'lines of least resistance' dictated by genetic covariances. Unless the major axis of genetic covariance happens to 'point' in the direction that selection acts, covariances between traits can constrain evolution. The trait mean can evolve more slowly than would occur with uncorrelated traits and can proceed at an angle to the direction of selection (e.g. Arnold, 1992; Schluter, 1996). However, phenotypic and genetic covariances can also accelerate adaptation when correlated traits are subject to selection along the same axis of covariance (e.g. Agrawal and Stitchcombe, 2009). Here, we present a case study of such a synergy between parasite load and armour plating in stickleback.

Threespine stickleback (*Gasterostereus aculeatus*) have repeatedly evolved reduced armour plating when completely-plated marine fish colonized freshwater habitats (Bell and Foster, 1994). Lateral plate number varies from low-plated (~0–10 plates) to completely-plated (upwards of 30 plates) (Hagen and Gilbertson, 1973; Bell, 1977). Partially-plated stickleback fall between these two extremes (typically 11–30 plates; exact cut-offs between morph categories vary between studies). This variation in the number of lateral plates in stickleback has been linked to variation in the *Ectodysplasin* (*Eda*) gene (Colosimo *et al.*, 2005), specifically in an enhancer region (O'Brown *et al.*, 2015). The completely-plated *Eda* allele shows incomplete dominance, therefore two copies of this allele produce completely-plated stickleback, zero copies produce low-plated individuals (Colosimo *et al.*, 2004; Cresko *et al.*, 2004), and one copy produces approximately 60% partially-plated and 40% either completely- or low-plated individuals (Colosimo *et al.*, 2004).

Plate morph evolution has long been attributed to differences in predation pressure. In marine and select lake environments dominated by gape-limited predators such as piscivorous fishes, predation pressure selects for large-spined and highly armoured stickleback (Hoogland *et al.*, 1956; Reimchen, 1992) with complete pelvic girdles (Lescak and von Hippel, 2011). Conversely, in freshwater environments dominated by aquatic insect and avian predators, reduced armour and spine length are favoured (Marchinko, 2009; Zanella *et al.*, 2015). Differences in refuge availability between marine and freshwater environments may also contribute to the loss of lateral plates in the transition to fresh water (Leinonen *et al.*, 2011).

However, this intuitive story is complicated both by some negative results (Zeller *et al.*, 2012) and by some competing hypotheses. Numerous other ecologically relevant phenotypes have been mapped to *Eda*, genes linked to *Eda*, or are associated with lateral plate number. Therefore, these traits might be targets of selection instead of, or in addition to, armour plates, and cause correlated evolution of *Eda* and plate number. Transgenic manipulation of *Eda* has shown that this gene is pleiotropic, influencing both schooling behaviour (Greenwood *et al.*, 2016) and neuromast arrangement (Mills *et al.*, 2014) in addition to plate number (Colosimo *et al.*, 2005). Rennison *et al.* (2015) provide further evidence for pleiotropy, showing that selection acts similarly but not identically on *Eda* genotype (or genes in close linkage) and plate phenotype. *Eda* genotype is also associated with the tendency of fish to switch between fresh and saltwater environments (Barrett *et al.*, 2009a) and growth rate (Barrett, 2009b), potentially resulting from pleiotropic effects of *Eda.* Though not genetically linked to *Eda*, behavioural phenotypes such as aggression and boldness in juvenile stickleback (Lacasse and Aubin-Horth, 2012) are associated with plate morphology, and therefore could also result from pleiotropic effects of *Eda* or linked genes.

The co-localization of these numerous traits might be due to strong pleiotropic effects of *Eda*, or these findings may reflect a tight linkage between *Eda* and other genes on chromosome IV, potentially forming a co-adapted gene complex that could pre-adapt some marine stickleback genotypes towards colonizing fresh water. For example, a gene complex could play a role in the reduction in lateral plate number and dorsal spine length in the transition from marine to fresh water (Bell and Foster, 1994) due to two closely linked genes, *Eda* and *MSX2A*, which influence plate number (Colosimo *et al.*, 2005) and spine length (Howes *et al.*, 2017), respectively. Linkage group IV is a remarkably pleiotropic region, which raises questions about what the real sources of selection on *Eda* are. Is selection indeed acting directly on armour plating, or on a correlated phenotype, or on both? Are there multifarious selective forces acting synergistically to drive evolution of *Eda* and armour plating?

Data are emerging that suggest that the genetic region containing *Eda* also affects immune function. The expression of immune genes involved in the T-cell response and inflammation vary across *Eda* genotypes (Robertson *et al.*, 2017) and plate number is associated with parasite susceptibility (Simmonds, 2015; Robertson *et al.*, 2017). The *Eda* block is also in close linkage to immune genes such as the B-cell activating cytokine, *Tnfsf13b* (Colosimo *et al.*, 2005). Based on the repeated mapping of many traits to *Eda* and its neighbouring regions of linkage group IV, and the recent evidence of immune and parasitological effects of *Eda*, we sought to evaluate the impact of this armour phenotype on parasite infection in a wild population. In particular, if armour coverage is positively associated with parasite loads in a freshwater population, then parasitism might accelerate the evolutionary loss of armour plates. A couple of recent studies have addressed this same question, yielding inconsistent results. Østbye *et al.* (2018) surveyed stickleback from a brackish lake in Norway and found that the prevalence of trematodes increased with armour plating (from 69% to 99% from low- to completely-plated fish), whereas the gill louse *Thersitina gasterostei* affected all morphs at a similarly high rate. Scottish populations of stickleback, in contrast, exhibit higher infection loads in low-plated stickleback (Robertson *et al.*, 2017). Here, we contribute an additional test of this association from another region of sticklebacks' broad range, the Pacific Northwest.

METHODS

Collection and preservation of stickleback

We collected stickleback from a single pool in Campbell River Estuary, British Columbia, Canada (50°02′09.9″N, 125°15′08.5″W). Sampling a single population enabled us to focus on variation in parasite infections associated with armour plating within a population, minimizing confounding effects of population genetic structure and environmental effects.

The site is located in a salt marsh above high tide. The pool is human-made, built in 1999 as part of a habitat restoration project on formerly industrial land. It is a narrow (2 m wide, <1 m deep) channel in a figure-8 shape, 120 m long and 45 m wide (pictured at evolutionary-ecology.com/data/3183Appendix.pdf, Fig. A1). The pool contains fresh water; salinity is 20–25 μS/cm^3. There is a narrow connection to an outflow stream at its northern end, which drains into the mouth of the Campbell River. We sampled only the southern half of the figure-8-shaped pool.

Stickleback must have colonized the site after the restoration programme. The present-day population in this pool is polymorphic for armour plating. The pool is not connected to any other (e.g. upstream) freshwater populations. The stickleback in the main estuary are

completely-plated, so we infer that the polymorphic population in the focal pool constitutes a permanent resident population. This is supported by the observation that stickleback are present at this site all year, and breed and nest within the site, whereas anadramous populations elsewhere in this estuary, and in neighbouring estuaries, typically migrate into fresh water in late spring.

We present data from two samples from this population. Each sample was obtained by placing 30 unbaited minnow traps in the pool for 2 hours. We sampled the population on a single day in June 2009 ($N = 105$) and another day in June 2012 ($N = 105$). For the 2009 collection, we retained the first ~100 fish captured, providing a random sample estimate of plate phenotypes. For the 2012 sample, we collected approximately 30 fish of each plate morph (low, partial, complete) to get a balanced representation.

Collected fish were placed in individually labelled tea bags and stored in 20% neutral formalin (2009) or 70% ethanol (2012) for subsequent analysis. The collections were conducted with Scientific Fish Collecting permits from the British Columbia Ministry of the Environment (NA07-32612, NA12-84189), with approval from the University of Texas IACUC Committee (Protocols #07-032201, #07-N100201, and AUP-2012-00065).

We stained fish with Alizarin Red to count left and right armour plates and averaged these to obtain an armour score. Plate categories were defined using this mean armour score. The distribution of mean plate number was divided into three approximately equal groups: low-plated fish were categorized as fish with fewer than 10 lateral plates, completely-plated fish were those with more than 20 plates, and partially-plated fish were intermediate (10–20 plates) (Fig. 1). Photographs of examples are provided in the Appendix (Fig. A2).

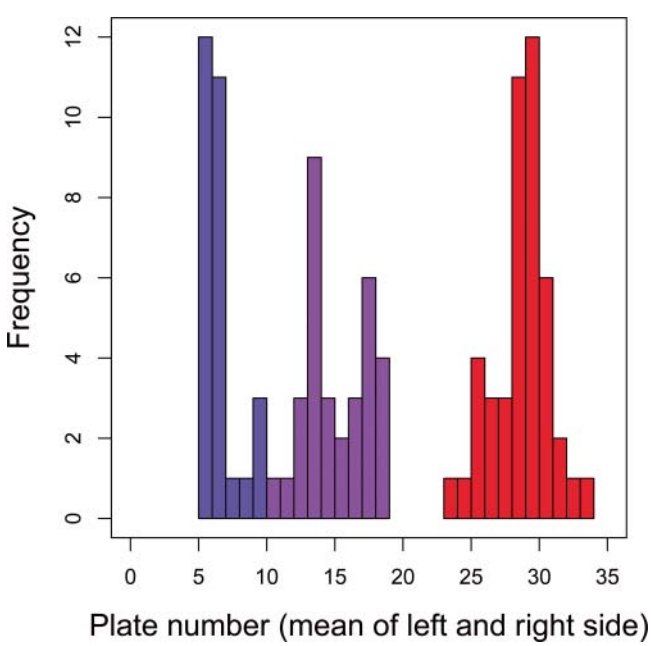

Fig. 1. Frequency distribution of average lateral plate counts in the random (2009) sample from Campbell River Estuary. For our analysis, fish with <10 plates were considered low-plated morphs (blue bars), fish with >20 plates were considered completely-plated morphs (red bars), and fish with an intermediate number were considered partially-plated morphs (purple bars).

This categorization was based on the breakpoints of the observed distribution within this estuary, recognizing that our completely-plated group has fewer plates (20–30) than seen in most marine populations [usually >30 plates (Hagen and Gilbertson, 1973; Bell, 1977; Colosimo *et al.*, 2005)]. Nevertheless, the 'completely-plated fish' in this sample had complete coverage of armour to the caudal peduncle, including the typical caudal keel. Their lower total number of plates might reflect smaller overall body size compared with typical anadramous marine fish. We also weighed each fish to an accuracy of 0.1 g and inspected the gonads to determine individuals' sex.

Quantification of parasites

We scanned each fish under a dissection stereomicroscope to count macroparasites (helminths, crustaceans, molluscs, and microsporidia). We started by scanning the exterior (skin, fins, and armour plates), then the buccal cavity and gills. We then dissected each fish to examine the body cavity and organs (liver, swim bladder, gonads, eyes) before opening the digestive tract. All macroparasites were identified to the lowest feasible taxonomic unit (typically genus) and counted, as described in greater detail in Stutz and Bolnick (2017).

Statistical analysis

The core question of this study is whether armour plate morphs differ with respect to parasite infection load, or prevalence. Due to low prevalence of many parasites, we only analysed parasite taxa that occurred in at least five individual hosts. To compare infection loads between plate morphs, we used a generalized linear model (GLM) with Poisson distribution and log-link function. We used body size (mass) and sex as covariates in the GLMs. We tested for overdispersion using the *dispersiontest* function in R (package *AER*). For parasite taxa showing significant overdispersion (*Thersitina* and *Dermocystidium*), we re-analysed the data using a GLM with negative-binomial distribution and log-link function (*glm.nb* function in R package *MASS*). To correct for multiple comparisons, a Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) with a false discovery rate of 5% was applied to all *P*-values from the GLM. To test for differences in parasite prevalence, we categorized individuals as infected or uninfected, and used a binomial GLM, again with sex and mass as covariates. Statistics were done with R software v.3.5.1 (R Core Team, 2018).

RESULTS

In the 2009 sample, most parasite species were rare. We observed a number of parasite taxa that were each present in only a single fish (*Acanthocephalus*, Black-spot, *Capillaria*, *Crepidostomum*), or three fish (*Eustrongylides*). Only four taxa were widespread enough to warrant further investigation for association with armour plating.

One parasite exhibited a significant association with armour in the 2009 sample. The crustacean *Thersitina gasterostei* (commonly known as the gill louse) was abundant on sticklebacks' gills (averaging 6.4 *Thersitina* per fish). A Poisson GLM supported effects of plate category, sex, and mass on *Thersitina* infection intensity ($P = 0.005$, $P < 0.0001$, and $P < 0.0001$, respectively), but was significantly overdispersed ($P = 0.0001$). The louse was more abundant in males, and in larger fish. Re-analysing the trend with a negative binomial GLM, sex and size remained significant, and the main effect of armour was again sig-

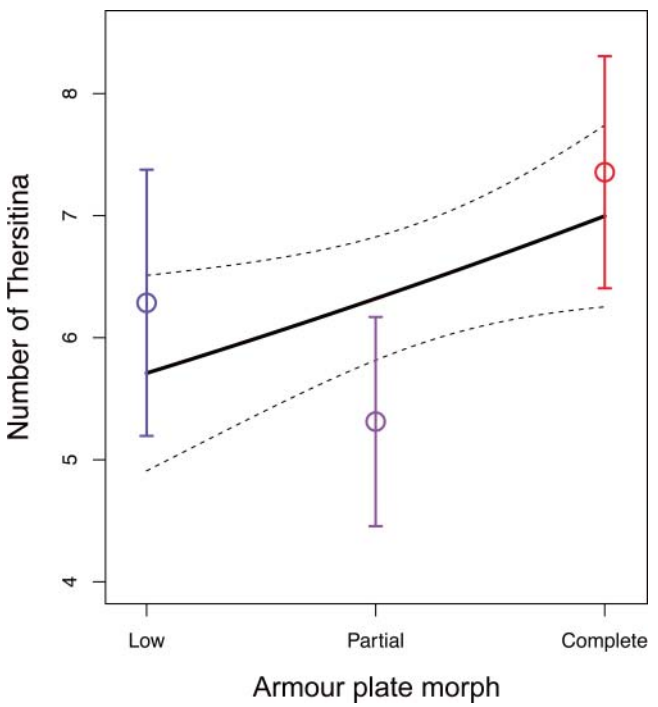

Fig. 2. Mean parasite count of *Thersitina gasterostei* (2009) in low- (blue), partially- (purple), and completely-plated (red) stickleback. Error bars represent standard error of the mean. Linear relationship predicted by the GLM is graphed (black line) with standard error (dashed line).

nificant ($P = 0.021$) once we accounted for armour interactions with fish mass ($P = 0.042$). We conclude that more armour plating coincides with increasing prevalence of *Thersitina* (Fig. 2). However, *Thersitina* infection prevalence (the proportion of fish infected) was unrelated to armour phenotype, whether categorized into the three morphs (binomial GLM $P = 0.8779$) or treated as a continuous trait ($P = 0.613$). This is because prevalence was uniformly high (92.8% of low-, 93.8% of partial-, and 95.6% of completely-plated fish), so infection intensity is more informative.

None of the other parasite taxa in the 2009 sample exhibited associations with armour plating. The cestode *Cyanthocephalus truncatus* infected five sampled stickleback; the parasite loads were not significantly associated with host mass, sex, or armour phenotype (Table 1). The nematode *Raphidascaris* infected four fish. It was significantly more common in males (2/9) than in females (2/92), but parasite loads were unrelated to fish mass or armour plating (Table 1). The eye fluke *Diplostomum spathaceum* was widespread, and was more abundant in larger fish, but prevalence was unrelated to sex or armour phenotype (Table 1).

In the 2012 sample, we observed a greater diversity of parasites, but again most had very low prevalence. Many parasites occurred only in one (*Actheres*, *Anisakis*, *Crepidostomum*) or two sampled fish (*Bunodera*, *Glugea*, *Schistocephalus*, and two unidentified nematodes). Several species were abundant enough for further analysis: the eye fluke *Dermocystidium*, the nematode *Eustrongylides*, the cestode *Cyanthocephalus truncates*, *Echinocephalus*, and *Thersitina*. Of these, infection loads of both *Thersitina* (Fig. 3) and *Eustrongylides* (Fig. 4)

Table 1. Results of a GLM testing the effect of fish mass, sex, and plate morph (low, partial, complete) on the number of parasites in stickleback collected in either 2009 ($N = 105$) or 2012 ($N = 105$)

Parasite genus	Year	Factor	$\beta \pm$ S.E.	Z	P
Cyanthocephalus	2009	Mass	0.08 ± 0.90	0.09	0.93
		Sex	−16.48 ± 2811.68	−0.01	1.00
		Plate morph	−0.23 ± 0.50	−0.45	0.65
Diplostomum spathaceum	2009	Mass	0.65 ± 0.24	2.73	**0.01**
		Sex	−0.98 ± 0.72	−1.35	0.18
		Plate morph	−0.09 ± 0.16	−0.53	0.60
Thersitina gasterostei (NB)	2009	Mass	1.45 ± 0.35	4.20	**<0.01**
		Sex	1.20 ± 0.42	2.88	**<0.01**
		Plate morph	0.88 ± 0.38	2.31	**0.02**
		Sex × Plate morph	−0.39 ± 0.27	−1.43	0.15
		Mass × Plate morph	−0.48 ± 0.23	−2.03	0.04
Cyanthocephalus	2012	Mass	0.44 ± 0.53	0.84	0.40
		Sex	0.22 ± 0.84	0.26	0.80
		Plate morph	0.72 ± 0.54	1.34	0.18
Dermocystidium (NB)	2012	Mass	008 ± 0.22	0.35	0.72
		Sex	−1.29 ± 0.35	−3.64	**<0.01**
		Plate morph	0.02 ± 0.15	0.11	0.91
Eustrongylides	2012	Mass	0.43 ± 0.44	0.97	0.33
		Sex	−18.04 ± 2897.50	−0.01	1.00
		Plate morph	1.40 ± 0.67	2.09	0.04
Nematode sp.	2012	Mass	1.27 ± 0.44	2.99	**<0.01**
		Sex	2.23 ± 0.76	2.93	**<0.01**
		Plate morph	0.31 ± 0.48	0.65	0.51
Neoechinorhyncus	2012	Mass	1.10 ± 0.26	4.24	**<0.01**
		Sex	−17.12 ± 1665.12	−0.01	0.99
		Plate morph	−0.06 ± 0.26	−0.22	0.82
Thersitina gasterostei	2012	Mass	0.99 ± 0.07	14.77	**<0.01**
		Sex	0.81 ± 0.12	6.70	**<0.01**
		Plate morph	0.25 ± 0.07	3.60	**<0.01**

Note: Significant *P*-values following Benjamini-Hochberg correction are shown in **bold** font. Negative binomial models are marked with (NB) next to the parasite and were used when overdispersion was significant in a regular Poisson GLM. We included interaction effects in the *Thersitina* negative binomial model from 2009 after visual inspection of the data suggested a strong interaction might exist.

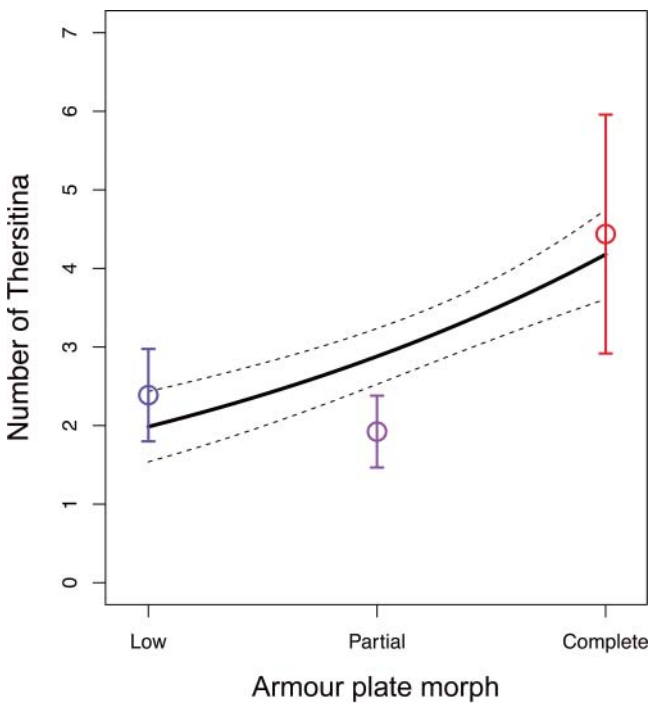
Number of Thersitina
0
1
2
3
4
5
6
7
Low
Partial
Complete
Armour plate morph

Fig. 3

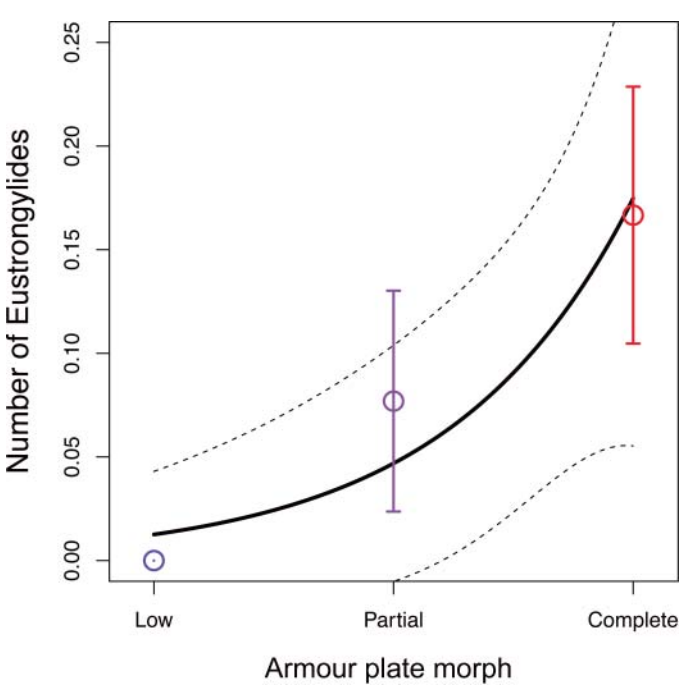
Number of Eustrongylides
0.00
0.05
0.10
0.15
0.20
0.25
Low
Partial
Complete
Armour plate morph

Fig. 4

were positively related to the number of armour plates in the sampled fish (Table 1), although this relationship was only significant for *Thersitina* ($P = 0.0003$). The relationship between plate number and *Eustrongylides* was significant but did not survive corrections for multiple comparisons. Plate phenotype did not significantly affect the counts of any other parasites tested. Fish mass significantly affected the counts of *Neoechinorhyncus* and *Thersitina*. Sex had a significant influence on the occurrence of *Dermocystidium* (lower in males) and a nematode (more common in males).

When we instead focus on infection prevalence (proportion of infected fish), plate number had a marginally significant positive effect on *Thersitina*, which infected 64% of low-plated fish, 71.4% of partially-plated fish, and 82.1% of completely-plated fish (binomial GLM $P = 0.059$). The effect was significant for *Eustrongylides*, which infected 0%, 8.6%, and 15.4% of the low-, partially-, and completely-plated fish ($P = 0.015$).

DISCUSSION

In this study, we found a tendency for stickleback with more lateral plates to be more heavily infected by a few parasite taxa. In 2009, this trend was seen for gill lice (*Thersitina gasterostei*), which infects the sticklebacks' gills, and was most pronounced in smaller fish. This same parasite was present three years later (2012), and we observed an identical trend. In addition, the nematode *Eustrongylides* (inhabiting cysts in the body cavity) was more common on more heavily armoured fish, although this was not significant after correction for multiple comparisons. Like *Eustrongylides*, the other parasites that occurred at sufficient numbers to warrant further analysis were endoparasites. Perhaps endoparasites in general are not significantly associated with plate morph.

Other studies have found an opposite association between plate number and parasite infection. For example, two recent studies found that low-plate morphs had increased susceptibility to *Schistocephalus solidus* (Simmonds, 2015) and *Gyrodactylus arcuatus* (Robertson *et al.*, 2017). Simmonds (2015) experimentally exposed various plate morphs to *S. solidus* and found that the low-plate morph was more susceptible to infection. That result is in contrast to another study showing that freshwater fish, which have reduced plates (Bell and Foster, 1994), are generally more resistant to *S. solidus*, to which they are more frequently exposed (Weber *et al.*, 2017). In our dataset, *Schistocephalus* was rare (only one infected fish of over 200 sampled). In yet another study, Østbye *et al.* (2018) found no relationship between plate morph and *Thersitina* infection (contrary to our results), but instead found a positive relationship with trematode infections that were rare in our sample.

From these inconsistent results, we infer that plate morph is associated with parasite infections in the wild, but these are apparently population- or region-specific. Such heterogeneous effects might be explained by regional differences in the parasite genotypes.

Fig. 3. (opposite top) Mean parasite count of *Thersitina gasterostei* (2012) in low- (blue), partially- (purple), and completely-plated (red) stickleback. Error bars represent standard error of the mean. Linear relationship predicted by GLM is graphed (black line) with standard error (dashed line).

Fig. 4. (opposite bottom) Mean parasite count of *Eustrongylides* (2012) in low- (blue), partially- (purple), and completely-plated (red) stickleback. Error bars represent standard error of the mean. Linear relationship predicted by GLM is graphed (black line) with standard error (dashed line).

A number of phenomena could explain the higher parasite load in completely-plated morphs that we document. In principle, some of the completely-plated fish might be anadromous fish that transiently entered this pool in the estuary. Marine fish are generally less resistant to freshwater parasites (MacColl and Chapman, 2010), such as *Eustrongylides*, which requires freshwater oligochaetes to develop (Measures, 1988), or *Schistocephalus*, which does not hatch in brackish water (Weber *et al.*, 2017). This explanation appears to be unlikely, however, because even the 'completely-plated' fish in our sample had fewer plates, and were smaller, than typical anadromous fish. We are therefore sceptical that the completely-plated individuals are anadromous migrants. Also, we note that *Thersitina gasterostei* infects both freshwater (De Roij and MacColl, 2012) and marine (Marcogliese, 1995) threespine stickleback, and therefore marine stickleback are not necessarily more susceptible than freshwater fish to this parasite.

Energetic trade-offs may also contribute to a dampened immune response to parasitic infection in completely-plated fish, as immune responses to parasites (reviewed in Lochmiller and Deerenberg, 2000) and the production of bony armoured plating (Giles, 1983) are metabolically expensive tasks. Perhaps low-plated morphs can allocate more energy to immune system functions, like fighting off parasitic infections, because they require less energy for the mineralization of bony plates. However, this explanation might be expected to affect many parasite taxa equally, rather than a subset of the extant common parasites. Also, the energetic costs of armour production should affect fish growth, causing low-plated fish to show elevated growth rates (Marchinko and Schluter, 2007; Barrett *et al.*, 2008), and hence larger adult body size. Our data do not support this possibility: we observed no significant association between the armour phenotype and fish mass, in either year (2009: $t = 0.663$, $P = 0.509$; 2012: $t = 0.915$, $P = 0.362$).

Differential exposure risk of different plate morphs to these parasites could also contribute to this positive association between plate number and parasite load. Lacasse and Aubin-Horth (2012) found a negative correlation between behaviours like aggressiveness and boldness and defensive morphology, including plate number. Therefore, the low-plated fish may be bolder, which could reduce their interactions with conspecifics and their exposure to parasites. A similar mechanism is seen in guppies; bolder individuals tend to have weaker social networks (Croft *et al.*, 2009), reducing their risk of exposure to parasites that can be socially transmitted during shoaling (Richards *et al.*, 2010). The lower incidence of *Thersitina* infection in low-plate morphs could therefore be a result of reduced exposure to this horizontally transferrable parasite. In addition to being less bold, which may positively influence the closeness of social networks and therefore increase exposure risk, completely-plated fish are also more effective at schooling behaviours (Greenwood *et al.*, 2016), potentially making them more susceptible to horizontally transmitted ectoparasites.

Although we did not directly measure genotype in our study, genetic mapping and transgenic experimentation have shown that plate number is controlled by *Eda* genotype (Colosimo *et al.*, 2005); this genotype explains ~75% of the variance in plate phenotype with *Eda* modifier loci likely contributing to the unexplained variance (Colosimo *et al.*, 2004). Assuming a close correlation between plate number and *Eda* genotype, the association we find between plate number and the occurrence of *Thersitina* may indicate a connection between *Eda* or closely linked genes and immune phenotype. Although *Thersitina* does not penetrate the skin, ectoparasites still induce measurable immune responses in fishes (e.g. Lindenstrøm *et al.*, 2004; Gonzalez *et al.*, 2007; Forlenza *et al.*, 2008). The positive association between parasite load and armour plating could result if *Eda* pleiotropically affects immune performance or is

linked to polymorphic immune genes. Colosimo *et al.* (2005) found that the majority of low-plate morphs shared both the *Eda* gene and a closely flanking gene that encodes a cytokine, *Tnfsf13b* (also known as BAFF). This cytokine is associated with adaptive immunity; it promotes B-cell survival and maturation and is involved in T-cell regulation (reviewed in Mackay and Browning, 2002). In fish, specifically, *Tnfsf13b* influences B-cell activation and maturation (Liu *et al.*, 2016) and in humans, this gene is involved in the immune response to nematode infection (Acevedo *et al.*, 2009), indicating that it could play a role in parasite immunity. An adaptive immune effect of *Eda*-linked genes may explain why we observed significant effects for one parasite, but not others, if the response is pathogen-specific.

Another possibility is that armour phenotype might affect individuals' foraging behaviour. Diet is known to affect parasite susceptibility in stickleback (Stutz *et al.*, 2014). The *Eda* region, although not directly linked to feeding morphology or behaviour, is associated with schooling behaviour (Greenwood *et al.*, 2016), which can influence foraging (Lachlan *et al.*, 1998). Alterations in feeding behaviour may affect exposure to trophically transmitted parasites, such as *Eustrongylides*, and therefore a linkage of *Eda* to such behaviours can explain the correlation of armour plating and parasite load.

Our results indicate a positive correlation between infection by two parasite species and armour plating within a geographically restricted population of threespine stickleback. Macroparasites like *Eustrongylides* (Paperna, 1974; Kaur *et al.*, 2013) are known to impose fitness costs on fishes. *Thersitina* infection negatively affects respiration (Rokicki, 1994) in stickleback, and crustacean ectoparasites like *Thersitina* are well known to reduce fitness in salmonids (e.g. Bui *et al.*, 2016; Herron *et al.*, 2018; Susdorf *et al.*, 2018). The correlations that we observe between plate morph and parasite load will likely impose additional selection on the genomic region containing *Eda* and its neighbouring genes. Both parasitism and osmotic stress in fresh water should act jointly to drive the evolution of reduced armour, which changes rapidly following the introduction of marine stickleback to fresh water (Bell *et al.*, 2004). Our results add to the growing evidence that the region of chromosome IV containing *Eda* may be subject to multifarious selective pressures that act in concert on the same gene or linked genes. Such simultaneous selective pressures, acting on the same locus (or, tightly linked loci), will tend to accelerate the rate of adaptive evolution.

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