Intraguild predation leads to genetically based character shifts in the threespine stickleback

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Intraguild predation is a common ecological interaction that occurs when a species preys upon another species with which it competes. The interaction is potentially a mechanism of divergence between intraguild prey (IG-prey) populations, but it is unknown if cases of character shifts in IG-prey are an environmental or evolutionary response. We investigated the genetic basis and inducibility of character shifts in threespine stickleback from lakes with and without prickly sculpin, a benthic intraguild predator (IG-predator). Wild populations of stickleback sympatric with sculpin repeatedly show greater defensive armor and water column height preference. We laboratory-raised stickleback from lakes with and without sculpin, as well as marine stickleback, and found that differences between populations in armor, body shape, and behavior persisted in a common garden. Within the common garden, we raised stickleback half-families from multiple populations in the presence and absence of sculpin. Although the presence of sculpin induced trait changes in the marine stickleback, we did not observe an induced response in the freshwater stickleback. Behavioral and morphological trait differences between freshwater populations thus have a genetic basis and suggest an evolutionary response to intraguild predation.

Interspecific resource competition can lead to increased phenotypic diversity as natural selection favors character shifts that decrease competition and promote the use of alternative resources (Schluter 2000a,b; Pfennig and Pfennig 2010; Stuart and Losos 2013). Other trophic interactions may also lead to divergence between closely related species (Schluter 2000b, 2003; MacColl 2011; Nosil 2012). Experimental studies have verified that divergence in traits in response to predation (Endler 1980; Vamosi 2002; Langerhans et al. 2007; Marchinko 2009) and parasitism (Hudson and Greenman 1998; Laine 2009) have a genetic basis. Intraguild predation has been predicted to increase phenotypic diversity between lineages (Schluter 2000b), but the evolution of character shifts in response to intraguild predation has not been tested.

Intraguild predation occurs when a predator is also a competitor of its prey species (Polis et al. 1989; Holt and Polis 1997; Hart 2002; Arim and Marquet 2004). Competition from intraguild predators (IG-predators) can shift the diet of intraguild prey (IG-prey) to include alternative food sources (Polis et al. 1989; Vance-Chalcraft et al. 2007; Ingram et al. 2012). Simultaneously, predation from IG-predators can result in increased antipredator behavior and defensive structures in IG-prey (Polis et al. 1989; Kratina et al. 2010; Vanak et al. 2013; Walzer and Schausberger 2013), and behavioral shifts to alternative habitats to reduce predation (Donadio and Buskirk 2006).

We investigated the evolution of character shifts in freshwater threespine stickleback (*Gasterosteus aculeatus*) in response to an IG-predator. Freshwater populations formed when marine or anadromous (hereafter, "marine") stickleback became isolated in numerous lakes at the end of the last ice age, approximately 12,000 years ago. These populations adapted rapidly to freshwater in isolation from each other and from the marine environment (Bell and Foster 1994). A subset of these lakes was also colonized by prickly sculpin (*Cottus asper*), a freshwater teleost fish and IG-predator

of the threespine stickleback (Dennenmoser et al. 2015). Sculpin grow to larger size than stickleback and consume juvenile and adult stickleback up to 60% of their body length (McPhail 2007; Reimchen 1994). Prickly sculpin are cryptic ambush predators of stickleback and they also eat benthic invertebrates (McPhail 2007).

Preliminary evidence indicates that intraguild predation has led to phenotypic changes in stickleback that decrease competition and/or predation from sculpin. In the wild, stickleback from lakes with sculpin show a shift to a limnetic-like body shape. In contrast, stickleback from lakes without sculpin are more benthic-like with a deeper body, a wider caudal peduncle, and a posterior shift in the first dorsal spine (Ingram et al. 2012). Differences in stickleback body shape correlate with diet (Willacker et al. 2010). In a mesocosm experiment, stickleback from a population sympatric with sculpin had more zooplankton in their diet than stickleback from a population without sculpin, whose diet consisted of more benthic prey. When sculpin were experimentally added to mesocosms, stickleback from both populations increased the proportion of zooplankton consumed (Ingram et al. 2012). The addition of sculpin also increased stickleback mortality and reduced growth rate, but to a lesser extent in the stickleback population sympatric with sculpin than stickleback from the sculpin-absent lake (Ingram et al. 2012), suggesting that they are less susceptible to predation. Differences between populations in other traits such as armor and behavior are likely, but have not been measured. The presence of predators is often associated with greater defensive armor in stickleback (Reimchen 1994; Vamosi and Schluter 2004; Willacker et al. 2010; Leinonen et al. 2011; Lescak and von Hippel 2011; Lacasse and Aubin-Horth 2012), as well as differences in sociality and shoaling (Vamosi 2002; Bell and Sih 2007; Dingemanse et al. 2007, 2009; Lacasse and Aubin-Horth 2012). Longer spines increase the body diameter of the stickleback, making them more difficult for gape-limited predators to ingest (Hoogland et al. 1956) and lateral plates provide structural support for spines (Reimchen 1983). Increased zooplankton in the diet suggests greater use of the water column by stickleback from lakes with sculpin, which may decrease the rate of encounter (Lima and Dill 1990).

One approach to testing evolutionary character shifts in IG-prey is to ask whether putative cases fulfill criteria analogous to those routinely used to test for ecological character displacement (modified from Schluter and McPhail 1992): (1) phenotypic differences have a genetic basis, (2) differences are not due to chance, (3) divergence should be the outcome of evolution rather than species sorting, (4) shifts in phenotype reflect differences in resource use and/or predation risk, (5) shifts are not the result of other environmental differences between sites with and without IG-predators, and (6) there is independent evidence that preshift IG-prey phenotypes compete with and suffer predation from the IG-predator.

Here, we evaluate the first criterion. Character shifts in response to intraguild predation might be the result of either phenotypic plasticity or genetic change (West-Eberhard 2003). Plasticity can lead to rapid character shifts because the match of phenotype to environment occurs without waiting for the spread of adaptive alleles (Schlichting and Pigliucci 1998; West-Eberhard 2003). Adaptive phenotypic plasticity in IG-prey behavior (Heithaus 2001; Janssen et al. 2007; Amarasekare 2008) or inducible antipredator defenses (Kratina et al. 2010; Nakazawa et al. 2010; Urbani and Ramos-Jiliberto 2010) have been shown to increase survival of IG-prey in theoretical models. Alternatively, genetic mapping studies based on crosses between marine and freshwater stickleback populations have found different quantitative trait loci (QTL) between populations inhabiting lakes with and without sculpin associated with body shape differences and armor components (Rogers et al. 2012), suggesting that many trait differences between the population types have at least a partial genetic basis. Distinguishing between phenotypic plasticity and genetic evolution is also important for predicting community dynamics (Cortez 2011; Yamamichi et al. 2011). However, experimental studies are required to test whether character shifts have a genetic basis (Scheiner 1993).

In this study, we describe character shifts in body armor, body shape, and behavior among natural populations of stickleback that occur with and without prickly sculpin. Stickleback were raised in a common garden to determine the relative role of genetics and phenotypic plasticity in these shifts. We assessed the inducibility of these traits by rearing split families in the presence and absence of sculpin. We included marine stickleback in the experiment to determine if phenotypic plasticity was present in the form representing the ancestral state. If prickly sculpin have led to the evolution of character shifts in stickleback, individuals raised in a common garden will replicate the phenotypes of the parental populations and the presence of sculpin will not induced trait shifts.

Materials and Methods
STUDY POPULATIONS AND SAMPLE COLLECTION

Lake characteristics and information on fish community composition were obtained from Habitat Wizard (www.env.gov.bc.ca/habwiz) maintained by the British Columbia Ministry of Environment. We identified eight lakes (8.0–58.7 ha) in southwestern British Columbia with a simple fish community of threespine stickleback, cutthroat trout (*Oncorhynchus clarkii*), and prickly sculpin (*C. asper*), and contrasted these populations with eight lakes (3.7–44.6 ha) containing only threespine stickleback and cutthroat trout (Fig. S1). Cutthroat trout are ubiquitous in lakes in this region. All lakes are in separate watersheds, ensuring no gene flow between populations. Lakes with and without sculpin did not differ in mean area (Mann–Whitney U-test $= 18$,

$P = 0.16$), perimeter ($U = 16$, $P = 0.10$), mean depth ($U = 25$, $P = 0.77$), elevation ($U = 34$, $P = 0.88$), or distance to the ocean ($U = 28$, $P = 0.72$). The study populations also included "marine" stickleback from two geographically distinct populations. Modern marine (including anadromous) stickleback are thought to be phenotypically similar to the ancestral populations that initially colonized the freshwater lakes following the last ice age (Bell and Foster 1994). Marine stickleback have a diverse and largely uncharacterized predator community including several species of marine sculpin (McPhail 2007).

Adult stickleback were collected in May–June 2011 and 2012 using 10–15 baited minnow traps placed overnight along the shoreline of each lake and at the marine sites. Specimens collected for morphological analysis from all populations (Table S1; $n = 7$–26/population) were euthanized using buffered MS-222 (Argent Chemical Laboratories, Redmond, WA) and preserved in 95% ethanol. Some sites were sampled in subsequent years to increase sample size. Additional adult stickleback were collected for behavioral experiments in 2011, but a sufficient number of specimens was only available for seven lakes without sculpin, three lakes with sculpin, and one marine population ($n = 12$–27/population). Stickleback were transported to the aquatics facility at the University of British Columbia and allowed to acclimatize for one week prior to behavioral trials. In 2012, adult stickleback in reproductive condition were collected from three lakes with sculpin (Ambrose, Paq, and Rosseau), three lakes without sculpin (Trout, Cranby, and Kirk), and from two marine populations (Oyster Bay and Little Campbell) for a common garden and plasticity experiment (Fig. S2). Sculpin were collected from Paq Lake at this time and were transferred to the aquatics facility.

COMMON GARDEN AND PLASTICITY EXPERIMENT

We raised stickleback in a common garden laboratory environment in the presence and absence of sculpin. We created four families from each population by artificially crossing pairs of wild-caught fish at the lakeside. Eggs were obtained by gently pressing on the sides of females and placed into lake water. Males were euthanized with an overdose of MS-222 and testes were dissected, minced, and added to the eggs. We made reciprocal F1 crosses between stickleback from a lake with sculpin (Paq) and a lake without sculpin (Trout) to test for maternal effects on phenotypes. Paq and Trout lake populations have divergent body shape (Ingram et al. 2012), but are less differentiated in armor (Table S1). Four crosses used females from Paq Lake and males from Trout Lake and four crosses used females from Trout Lake and males from Paq Lake.

Fertilized eggs were transferred to the University of British Columbia within 24 h. At that time, each clutch was split. Half the eggs were assigned to a sculpin treatment and half to a control treatment. Each 100 L experimental tank was divided in the center with window screen and contained 3 kg of coarse limestone gravel and 1 ppt sodium chloride. Each half clutch was added to one side, while the other side was left empty. A low concentration of methylene blue was added to inhibit fungal growth. Tanks were kept at 16-h light:8-h dark photoperiod. One Ambrose clutch, one Rosseau, and two F1 clutches did not hatch (Fig. S2).

The development of induced defenses may depend upon the timing of exposure to the stimulus (Harvell 1990). Limited evidence suggests that even stickleback embryos can change behavior in response to cues from trout predation (Golub 2013). Because we were uncertain of the stage at which exposure to sculpin might lead to induced defenses, we provided sculpin cues for the duration of the experiment, from fertilized eggs until nine months of age. To provide possible olfactory cues, daily we added a 50 mL aliquot of water from a tank containing four adult sculpin to unhatched eggs in each sculpin treatment tank. This continued until stickleback hatched and fry were four weeks of age. Dechlorinated water was added to the control treatment during this time. At four weeks posthatching, stickleback fry were too large to pass through the window screen dividing each tank and were gathered and moved to a random side of the tank. At that time, we reduced the number of fry to 20 per tank. In the case of half clutches with fewer than 20 fry, we reduced the number of fry to an equal density in the control and sculpin treatment tanks. In the sculpin treatment, a single adult sculpin was added to the other side of the tank. In the control treatment, an equal biomass (four fish) of adult stickleback was added. Adult stickleback were laboratory reared F2 hybrids between Paxton Lake benthic and limnetic stickleback that had been raised for an unrelated study. The window screen dividing each tank allowed experimental stickleback to receive constant visual and chemical cues from the sculpin or the adult stickleback.

Stickleback were fed hatched brine shrimp nauplii for the first four months, and then a mixture of brine shrimp and bloodworms for the remainder of the experiment. Adult stickleback in the control treatment were fed a 3:1 mixture of bloodworms and mysis shrimp to satiation daily. Sculpin do not eat mysis shrimp and were fed only bloodworms.

The experiment was stopped at 36 weeks posthatching. Several adult control stickleback died during the experiment and were immediately replaced upon discovery. There were no sculpin mortalities. A Rosseau family was excluded from analysis after a sculpin jumped to the other side of the tank and consumed the experimental stickleback. The final sample size was 35 families in 70 tanks.

MORPHOLOGY

Samples stored in 95% ethanol were rehydrated, fixed in 10% formalin, and stained with alizarin red to highlight bony

characteristics following standard procedures (Peichel et al. 2001). We measured standard length, gape width, first and second dorsal spine length, pelvic spine length, pelvic girdle length, and lateral plate number on both wild-caught and experimental stickleback (Fig. S3). Spine measurements were made on the left side of the fish using digital calipers. Lateral plates were counted under a dissecting microscope. All armor traits were $\log(x + 1)$ transformed to homogenize variance. Experimental stickleback smaller than 28 mm were excluded from analysis because the development of lateral plates may be incomplete in smaller stickleback (Hagen 1973; Bell 2001; Rennison et al. 2015). All wild-caught stickleback were >28 mm.

To compare traits among stickleback of different sizes, all traits except lateral plates were size-adjusted to the mean standard length of the wild-caught samples (46.3 mm). For each trait, we fit a linear model with standard length as a covariate and population as a factor. All measurements were adjusted using the residuals from each regression (Vamosi 2002). The wild-caught samples were size-corrected separately from the common garden stickleback.

To minimize trait redundancy, stickleback armor variation was summarized with the first principal component (PC1) based on the correlation matrix between size-corrected spine traits and lateral plates, separately for wild-caught and experimental stickleback. All armor traits had significant positive loadings on PC1, which accounted for 74.6 and 79.7% of the variance in wild stickleback and laboratory-raised stickleback (Table S2). PC1 was the only PC with an eigenvalue greater than 1, therefore PC2-5 were not examined further.

We examined body shape in the experimental stickleback. The left side of each stickleback was photographed using a Nikon D300 camera (Nikon, Melville, NY). We placed 20 landmarks outlining the shape of the fish as well as the insertion points of spines and fins (Fig. S3; Walker 1997; Ingram et al. 2012). Landmarks were digitized using tpsDig 2.16 software (Rohlf 2008) and were centered, scaled, and rotated using the *shapes* package (Dryden 2012) in the R 3.0 environment (R Core Team 2014). We performed a linear discriminant analysis (LDA) with the *MASS* package (Venables and Ripley 2010) to visualize shape differences among lakes. We used the tank (half-family) as our classification variable, and thus the LDA did not a priori differentiate between treatment or population type. An LDA was preferable to other types of multivariate methods such as a PCA because it ignores trait combinations that vary only within populations (Tabachnick and Fidell 2013) and those resulting from measurement error or specimen bending. The first and second linear discriminant axes (LD1 and LD2) accounted for 34.3 and 15.9% of the observed variation in shape among half-families.

STICKLEBACK BEHAVIOR

We used a behavioral assay to measure position in the water column preferred by stickleback. Vertical position in the water column is a proxy for habitat use in guppies (Torres-Dowdall et al. 2012) and correlates with anxiety behavior in zebrafish (Egan et al. 2009; Cachat et al. 2010; Stewart et al. 2012). Limnetic stickleback from Paxton Lake prefer to be higher in the water column than benthic stickleback (Larson 1976).

Wild-caught stickleback in nonreproductive condition were transferred from their home tank to a holding basket next to the assay. Although Cachat et al. (2010) recommends a 1-h recovery period, preliminary trials showed that a 15-min acclimation period was sufficient. At the start of each trial, a focal fish was gently introduced to the top of an unfamiliar tank and was allowed to move freely for 330 sec (Fig. S4). The first 30 sec of each trial were not analyzed, because the introduction of the stickleback into the tank often resulted in erratic movement. Trials were recorded and videos were subsampled to 0.5 frames/sec using VirtualDub (www.virtualdub.org). The *x* and *y* coordinate position of the focal fish was measured every 2 sec using MtrackJ (Meijering et al. 2012) in ImageJ (Schneider et al. 2012). For each trial, we calculated the mean vertical position and the total movement of the stickleback in pixels (distance traveled).

The water column height preference of the experimental stickleback was assayed at 28–31 weeks of age using 10 stickleback chosen at random from each experimental tank. Tanks containing the same family were tested in the sculpin and control treatments sequentially in random order.

We further characterized the behavior of experimental stickleback using a shoaling assay (Vamosi 2002; Kozak and Boughman 2008; Wark et al. 2011). A 100 L tank was divided into two end compartments and one center arena using window screen (Fig. S4). An experimental shoal of 10 unfamiliar stickleback was added to one end and two stickleback were added to the other end (Wark et al. 2011). A focal fish was introduced into the center arena and its distance to the stimulus shoal arena was used as a measure of shoaling preference. Shoaling assays were conducted two days after the water column preference assay using 10 randomly chosen stickleback from each experimental tank. Stimulus stickleback were chosen from a stock of laboratory-reared Priest Lake benthic stickleback. The stimulus population was selected because stickleback were similar in age and size to the experimental fish and were unrelated to all of the experimental populations. Experimental stickleback were moved to holding baskets near the shoaling assay for a 15-min acclimation period. At the start of each trial, the focal stickleback was introduced into the center arena. Trials were recorded for 630 sec and the first 30 sec of each trial were not analyzed. The *x* and *y* coordinate position of the

focal fish was calculated as described above. For each trial, we calculated the time spent within one body length of the stimulus shoal as well as the distance traveled. Four hundred sixty-three shoaling videos were scored (Table S3).

STATISTICAL ANALYSIS

We tested for differences in mean trait values between wild-caught stickleback from lakes with sculpin and lakes without sculpin using linear models. Tests involving freshwater fish from the common garden used the tank mean as the unit of replication because each half-family was raised in the same tank. The experiment was analyzed using a linear mixed effects model with treatment (sculpin or control), population type (from a lake with or without sculpin), and their interaction as fixed factors and lake and family as random factors. Inducibility in the marine population was assessed in a separate analysis using a linear mixed effects model with treatment (sculpin or control) as a fixed factor and lake and family as random factors. Maternal effects were tested by comparing F1 crosses raised without sculpin using direction of cross as a fixed factor and family as a random factor.

Results

CHARACTER SHIFTS IN WILD-CAUGHT STICKLEBACK

The presence of sculpin was associated with character shifts in armor and behavior in wild populations of stickleback. Stickleback from lakes with sculpin had higher mean armor PC1 scores than stickleback from lakes without sculpin (Fig. 1A; $F_{1,14} = 33.9$, $P < 0.001$). All individual armor traits were greatest in stickleback from lakes with sculpin (Table S3). There was no difference in gape width ($F_{1,14} = 3.01$, $P = 0.11$) or standard length ($F_{1,14} = 1.86$, $P = 0.19$) between lakes with and without sculpin.

Stickleback from lakes with sculpin also preferred a higher mean vertical position in the water column than stickleback from lakes without sculpin (Fig. 1B; $F_{1,8} = 8.0$, $P = 0.02$). Distance traveled was not different between stickleback from the two types of lakes ($F_{1,8} = 0.12$, $P = 0.73$).

CHARACTER SHIFTS PERSISTED IN A COMMON GARDEN

Common garden stickleback from lakes with sculpin had a higher mean armor PC1 score than populations from lakes without sculpin (Fig. 2, filled circles; $F_{1,4} = 12.5$, $P = 0.047$). Individual armor traits were similar between stickleback raised in the control treatment of the common garden and values of wild caught stickleback from the same lake (Table S4). Exposure to sculpin did not induce a detectable change in PC1 score (Fig. 2, open circles; treatment: $F_{1,19} = 0.17$, $P = 0.38$; treatment × population-type: $F_{1,19} = 0.41$, $P = 0.53$).

Stickleback from lakes with and without sculpin differed in mean body shape in the common garden (Fig. S5, filled circles).

Lakes with sculpin were significantly differentiated in both LD1 ($F_{1,4} = 13.2$, $P = 0.022$) and LD2 ($F_{1,4} = 31.1$, $P = 0.005$). Stickleback from lakes with sculpin had an anterior shift in first dorsal spine, decreased body depth, a narrower caudal peduncle, larger eye diameter, and a longer jaw. Exposure to sculpin did not induce a detectable difference in mean shape (LD1: $F_{1,19} = 0.0$, $P = 0.995$; LD2: $F_{1,19} = 0.26$, $P = 0.62$; all treatment × population-type interactions were nonsignificant, $P > 0.1$).
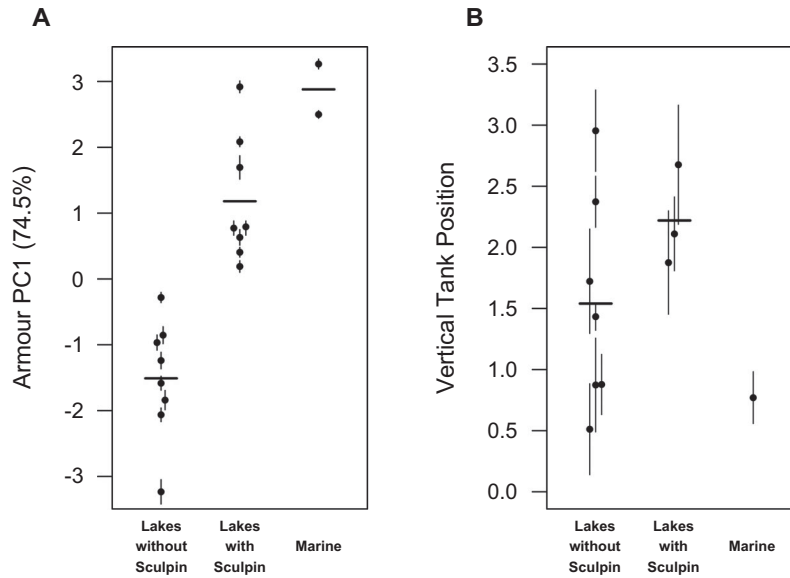
Common garden stickleback from lakes with and without sculpin also differed in behavior. As we saw in wild-caught stickleback, laboratory-raised stickleback from lakes with sculpin preferred a higher mean position in the water column (Fig. 3, filled circles; type: $F_{1,4} = 16.1$, $P = 0.016$). Stickleback from lakes with and without sculpin traveled a similar distance during the trials (type: $F_{1,4} = 0.8$, $P = 0.41$). In the shoaling assay, stickleback from lakes with sculpin spent less time near the stimulus shoal (decreased shoaling preference) than stickleback from lakes without sculpin (Fig. 4, filled circles; $F_{1,4} = 18.1$, $P = 0.013$). Population types traveled a similar distance during the shoaling assay ($F_{1,4} = 0.9$, $P = 0.39$). Exposure to sculpin did not detectably alter any behavior (water column position: $F_{1,17} = 0.1$, $P = 0.76$; water column distance: $F_{1,17} = 0.4$, $P = 0.54$; shoaling preference: $F_{1,16} = 2.6$, $P = 0.13$; shoaling distance: $F_{1,16} = 1.4$, $P = 0.25$; all treatment × population-type interactions were nonsignificant, all $P > 0.1$).

SCULPIN EXPOSURE INDUCED CHARACTER SHIFTS IN MARINE STICKLEBACK
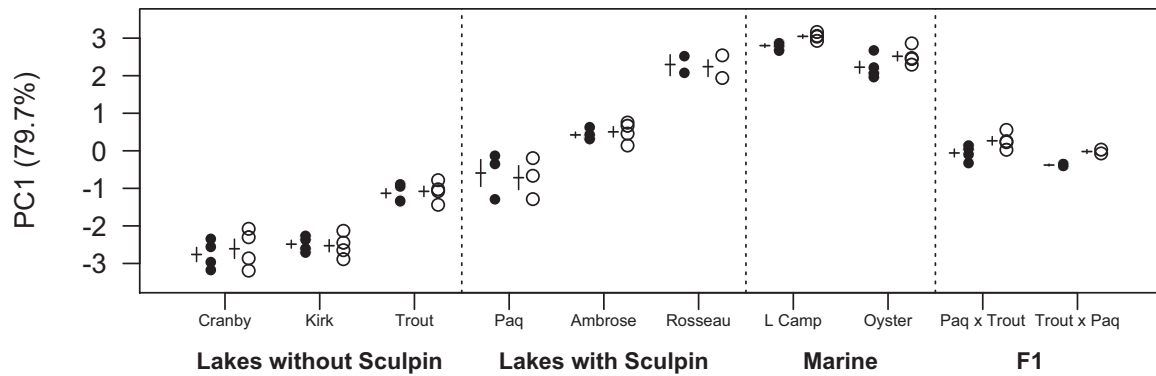
Phenotypic plasticity was observed in several traits in marine stickleback. Marine stickleback raised in the sculpin treatment had higher armor PC1 scores than those raised in the control treatment (Fig. 2; $F_{1,7} = 6.7$, $P = 0.016$). Adding the category "marine" as a population type to our previous analysis of experimental populations from lakes resulted in a significant treatment × population-type interaction (PC1: $F_{1,27} = 5.65$, $P = 0.025$), hinting that the marines are more plastic than the freshwater populations. Body shape did not differ between treatments (LD1: $F_{1,7} = 0.1$, $P = 0.81$; LD2: $F_{1,7} = 0.1$, $P = 0.76$). In the water column preference assay, marine stickleback from the sculpin treatment showed a marginal but nonsignificant increase in mean water column position (Fig. 3; $F_{1,7} = 4.5$, $P = 0.07$; treatment × population type: $F_{1,25} = 3.39$, $P = 0.08$), and in the shoaling assay, marines in the sculpin treatment had a decrease in shoaling tendency (Fig. 4; $F_{1,5} = 8.0$, $P = 0.037$) and a significant treatment × population-type interaction ($F_{1,22} = 6.77$, $P = 0.016$).
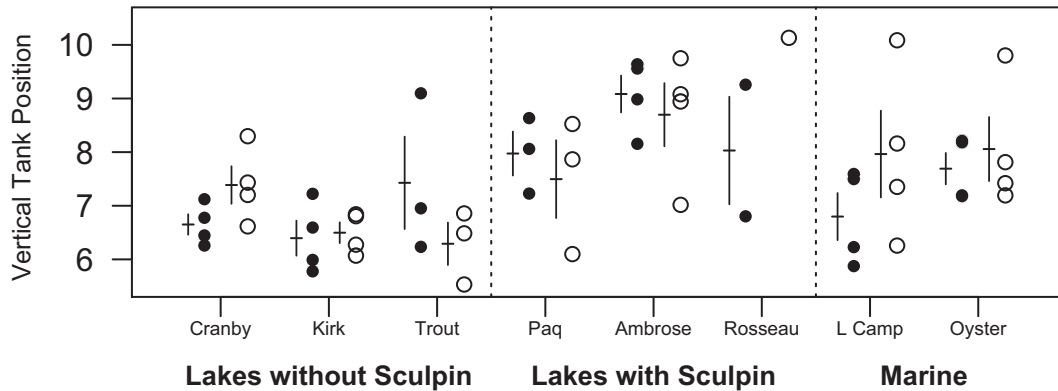
MATERNAL EFFECTS

Armor traits in F1 hybrids between Trout Lake and Paq Lake stickleback were intermediate between the parental populations (Fig. 2) and direction of cross did not affect trait value (PC1: $F_{1,4} = 8.0$, $P = 0.11$). Similarly, overall body shape was intermediate
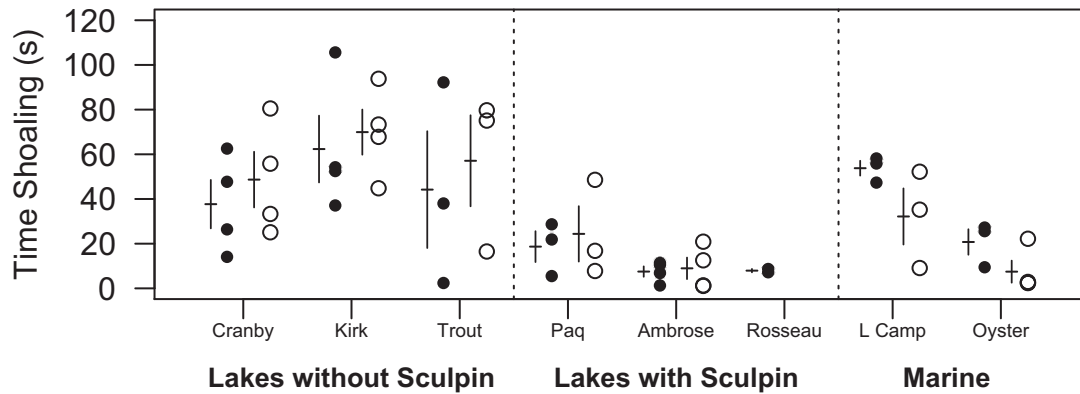
A

B

Figure 1. Character shifts in wild populations of stickleback. (A) Mean and SE of armor PC1. Each point represents a single population. The solid horizontal lines give the means for each type of population. (B) Mean and SE of vertical position in the water column (y-axis position) of wild caught stickleback measured in the laboratory in an unfamiliar tank. Each point is a single population. Horizontal lines give the means of each population type.

Figure 2. Mean armor PC1 for experimental stickleback from each family raised in the control common garden (filled) and the sculpin treatment (open). The F1 is a cross between fish from Trout (sculpin absent) and Paq (sculpin present) Lakes. The father is first and the mother is second for F1 crosses. The mean and SE of each lake and treatment is given on the left.

Figure 3. Mean vertical position in the water column in an unfamiliar tank (y-axis position) of stickleback raised in a common garden. The control treatment is represented by closed symbols and the sculpin treatment is represented by open symbols. The mean and SE of each lake and treatment is given on the left.

Figure 4. Mean time spent near the shoal. The control treatment is represented by closed symbols and the sculpin treatment is represented by open symbols. The mean and SE of each lake and treatment is given on the left.

between the parental populations, but F1 families with Trout Lake mothers (without sculpin) had a larger mean LD1 score (LD1: $F_{1,4} = 9.5$, $P = 0.037$) than F1 families with Paq Lake mothers (with sculpin), indicating that maternal effects may impact body shape in these populations. There was no difference in LD2 ($F_{1,4} = 1.4$, $P = 0.3$).

Discussion

TRAIT SHIFTS IN RESPONSE TO INTRAGUILD PREDATION

The presence of an IG-predator, prickly sculpin, is associated with character shifts in multiple traits in the threespine stickleback, and the results herein indicate that these trait shifts have a genetic basis. Wild populations of stickleback sympatric with sculpin show parallel increases in armor morphology, prefer to be higher in the water column, and have been previously shown to differ in body shape (Ingram et al. 2012). These differences in armor, shape, and behavior persisted in a common garden. To our knowledge, this system is the first confirmed case of genetically based character divergence associated with intraguild predation.

Competition, predation, or both might produce character shifts in response to intraguild predation and disentangling these interactions will be challenging. Piscivorous predators have previously been associated with longer spines and an increased number of lateral plates (Hagen and Gilbertson 1972; Moodie 1972; Bell et al. 1993; Reimchen 1994; Reimchen and Nosil 2002; Baker et al. 2010; Leinonen et al. 2011, Lescak and von Hippel 2011). Increased armor in lakes with sculpin might be a response to increased predation, though the number of lateral plates might also affect buoyancy (Myhre and Klepaker 2009) and drag (Walker 1997). Alternatively, it is possible that shifts in armor are the indirect outcome of a habitat shift between sculpin and stickleback. Prickly sculpin prefer the littoral zone of lakes where there is easy

access to cover and benthic invertebrates (McPhail 2007). Sculpin may displace stickleback into the pelagic environment either by decreasing benthic resources, increasing the threat of predation, or both. Because cutthroat trout are more prevalent in the open water, longer spines might be an adaptation to increased predation from trout, rather than a direct response to predation by sculpin. A third, less plausible, hypothesis is that sculpin predation on benthic invertebrates indirectly relaxes selection for reduced spines. Juvenile stickleback are eaten by large aquatic insects and studies suggest that some insects capture stickleback by grabbing the spines (Reimchen 1980; Reist 1980; Marchinko 2009; although see Lescak et al. 2012 and Mobley et al. 2013). Spine length might represent a balance between selection for longer spines by gape-limited predators and selection for shorter spines via predation by aquatic insects upon juvenile stickleback (Reimchen 1980).

Similarly, trait shifts in behavior could also be attributed to either competition or predation. We found that in the wild and in the laboratory, stickleback from lakes with sculpin preferred to be higher in the water column. A position higher in the water column might lessen risk of predation from sculpin. We also observed a decreased shoaling preference in stickleback from lakes with sculpin and in marine stickleback reared in the sculpin treatment. Shoaling may not be an effective method for escaping sculpin predation. Alternatively, differences in water column and shoaling preference may be a response to changes in foraging behavior caused by resource depletion by sculpin. The presence of sculpin has been demonstrated to induce a higher proportion of zooplankton in the stickleback diet (Ingram et al. 2012), and zooplankton is most abundant in the open water. Trait shifts in behavior could also interact with shifts in morphological traits. For example, diet preference and body shape vary with lateral plate number (Bjaerke et al. 2010). Intraguild predation may independently select for trait shifts in behavior and morphology, or changes in behavior may have led to selection for changes in morphology (or vice versa).

These alternatives underscore the challenge of elucidating the relative impacts of competition, predation, and their interaction in character shifts via intraguild predation.

TRAIT INDUCIBILITY HAS BEEN LOST IN FRESHWATER POPULATIONS

Phenotypic plasticity has been proposed as a possible explanation for trait shifts in IG-prey (Kratina et al. 2010; Nakazawa et al. 2010; Urbani and Ramos-Jiliberto 2010). Although adaptive plasticity has been reported in stickleback feeding morphology (Day et al. 1994; Day and McPhail 1996; Wund et al. 2008; Svanbäck and Schluter 2012) and body shape (Garduño-Paz et al. 2010; Svanbäck and Schluter 2012), we found no evidence for sculpin-induced plasticity in freshwater populations. However, marine stickleback reared in the presence of sculpin exhibited slightly increased armor, an increase in preferred water column height, and a decrease in shoaling behavior compared to the controls. To our knowledge, the increased armor in marine stickleback in the presence of sculpin is the first observation of induced structural defenses in stickleback. Importantly, induced trait changes in the presence of sculpin were in the same direction as the trait shifts among freshwater stickleback populations with and without sculpin. Phenotypic plasticity in the ancestral colonizing population may have aided in the initial divergence between freshwater populations (Wund et al. 2008).

It should be noted that while stickleback in the sculpin treatment received lifelong visual and olfactory cues from sculpin, they were not exposed to predation. Stickleback in this treatment might not have recognized sculpin as a threat or constant exposure to sculpin may have resulted in habituation (Kelley and Magurran 2003). All behavioral assays were conducted without sculpin, and including sculpin cues during these assays might induce a change in behavior.

This study provides evidence that intraguild predation leads to evolutionary divergence among stickleback populations (Schluter and McPhail 1992). Phenotypic differences between lakes with and without sculpin have a clear genetic basis. Character shifts have occurred in parallel across replicated populations; therefore, these differences are not due to chance. Preliminary comparisons found no evidence of consistent environmental differences among lakes. However, the biotic and abiotic environment can influence species interactions and affect the structure of piscivorous communities (Jackson et al. 2001). To fully rule out the role of the environment in generating these evolutionary shifts will require further investigation of abiotic characteristics (e.g., pH, vegetation), and the biotic community (e.g., aquatic insects, avian predators). Phenotypic differences between lakes with and without sculpin suggest that stickleback have evolved in response to competition and/or predation with sculpin.

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Supporting Information

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Supporting Figures
Figure S1. Map of sampling locations.
Figure S2. Schematic of crosses used in the common garden experiment.
Figure S3. Landmarks coordinates used for morphometrics and armor traits (Ingram et al. 2012).
Figure S4. The setup for behavioral assays.
Figure S5. Mean value of shape axis 1 from stickleback families reared in a common garden in a control treatment (filled) and a sculpin treatment (open).
Supporting Tables
Table S1. Mean and SE of traits in wild caught stickleback.
Table S2. PC loadings for armor traits.
Table S3. Sample sizes for behavioral assays.
Table S4. Mean and SE of traits in experimental stickleback from the "control" treatment.