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Signals of predation-induced directional and disruptive selection in the threespine stickleback

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ABSTRACT

Background: Different predation regimes may exert divergent selection pressure on phenotypes and their associated genotypes. Threespine stickleback *Gasterosteus aculeatus* have a suite of bony structures, which have been shown to be an effective defence against predation and have a well-known genetic basis.

Question: Do different predator regimes induce different selective pressures on growth rates and defence phenotypes in threespine stickleback between different habitats across distinct age classes?

Hypothesis: In the presence of predation-induced selection, we expect diverging morphological responses between populations experiencing either low or high predation pressure.

Study system: Threespine stickleback were sampled from two natural but recently established populations in an invasive range. One site has a high density of fish and insect predators, while at the other site predation pressure is low.

Methods: We inferred predator-induced selection on defence traits by comparing the distribution of size classes, defence phenotypes, and an armour-related genotype between different age classes in a high and a low predation regime.

Results: Under high predation, there are indications of directional selection for faster growth, whereas lateral plate phenotypes and associated genotypes show indications for disruptive selection. Heterozygotes at the *Eda*-gene have a lower survival rate than either homozygote. Neither pattern is evident in the low predation regime.

Conclusion: Potential evolutionary responses to divergent predation pressures between sites are apparent in a recently established system.

Keywords: age classes, defence traits, disruptive selection, *Eda*, predation.

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INTRODUCTION

Differences in habitat structure, resources, competitors, parasites, and predators are the main drivers of divergent natural selection, which can cause phenotypic differentiation, potentially leading to ecological speciation (Rundle and Nosil, 2005) and even adaptive radiation (Schluter, 2000). Although predation-driven phenotypic differentiation and diversification have been documented for many taxa (Vermeij, 1977), including insects (Nosil and Crespi, 2006) and vertebrates (Reznick and Endler, 1982; Langerhans *et al.*, 2007; Calsbeek and Cox, 2010), empirical evidence for a direct role of predation leading to speciation is limited (Endler, 1980; Reznick and Endler, 1982; Reimchen, 1994; Vamosi, 2005; Langerhans *et al.*, 2007; Marchinko, 2009). However, predation pressure can shape the evolution of divergent life histories (Reznick and Endler, 1982; Johnson and Belk, 2001). For example, increased predation rates on old or large individuals should favour the evolution of early maturation and a high reproductive effort, whereas the opposite is expected under an increased predation rate on young age-classes or small individuals (Ernande and Dieckmann, 2004; Gårdmark and Dieckmann, 2006; Walsh and Reznick, 2009).

The threespine stickleback *Gasterosteus aculeatus* is a powerful system in which to study adaptation and ecological speciation due to its repeated diversification into distinct ecotypes throughout its distribution range (McKinnon and Rundle, 2002). Stickleback were introduced into Switzerland about 140 years ago and spread rapidly across large parts of the country. In the invasive range the species displays a level of phenotypic diversity that is uncommon in freshwater populations elsewhere in Europe and is reminiscent of the entire habitat range of stickleback, including freshwater systems and the sea (Lucek *et al.*, 2010). Stickleback have an array of phenotypic traits related to anti-predator defence, including bony skeletal armour consisting of lateral plates, pelvic and dorsal spines, and a pelvic girdle (Reimchen, 1994). In their ancestral marine habitat, stickleback are generally heavily armoured with a full set of lateral plates covering the whole flank of the body and with long dorsal and pelvic spines (Reimchen, 1994; McKinnon and Rundle, 2002). This extensive armour is thought to provide protection in the marine habitat, which is dominated by gape-limited and puncturing fish and bird predators (Gross, 1978). Since the last glacial retreat about 15,000 years ago, threespine stickleback have invaded numerous temperate freshwater systems throughout the Holarctic independently, where a reduction in defence traits has occurred repeatedly (Bell and Foster, 1994; McKinnon and Rundle, 2002). Hypotheses to explain the reduction in defence traits in freshwater include adaptations to different predator regimes (e.g. Reimchen, 1980, 1991; Vamosi, 2002; Marchinko, 2009), food availability (Bjaerke *et al.*, 2010), buoyancy (Myhre and Klepaker, 2009), lower calcium availability (Giles, 1983; Bell *et al.*, 1993), and enhanced growth rate (Barrett *et al.*, 2008).

The length of spines in stickleback is highly variable between populations and is predicted by the abundance of predatory fish in a given habitat (Hagen and Gilbertson, 1973). Erected spines increase the stickleback's effective diameter, making it more difficult for gape-limited predators to swallow, and increase the possibility of injury to predators. Lateral plates further increase the survival of stickleback after an attack by a fish or bird (Hoogland *et al.*, 1956; Reimchen, 1991, 2000).

Reimchen (1980, 1994) hypothesized that structures related to defence traits provide a means for grappling predators to grip their prey. Specifically, Reimchen (1980) suggested that spines might serve as levers that invertebrate predators such as dragonfly larvae could use to grasp the fish. Consequently, a reduction in spine-related phenotypes (i.e. their size and number) might confer a selective advantage in the face of strong dragonfly predation. He also

suggested that external structures that enhance frictional contact, such as lateral plates, ought to be disadvantageous in the presence of grappling predators.

The genetic basis of the lateral plate reduction (Colosimo *et al.*, 2004) is relatively well understood. The *Ectodysplasin* (*Eda*) gene has been reported to explain about 75% of the phenotypic variation between marine and freshwater stickleback with an almost Mendelian genetic basis (Colosimo *et al.*, 2004; Le Rouzic *et al.*, 2011). There are two classes of *Eda* alleles, referred to hereafter as the *Eda* L (low) and the *Eda* C (complete) allele. Most marine stickleback populations have mainly *Eda* C alleles, while most freshwater stickleback populations are fixed or nearly fixed for *Eda* L alleles (Cano *et al.*, 2006; Schluter and Conte, 2009). Individuals homozygous for the low-armour allele have few plates, whereas those homozygous for the high-armour allele have a complete array of lateral plates. Heterozygotes are either fully plated or have an intermediate number of plates. Furthermore, empirical evidence suggests selection acts on additional phenotypes related to the *Eda* gene (Barrett *et al.*, 2008; Le Rouzic *et al.*, 2011).

In this study, we compare the distribution of body size, defence phenotypes, and *Eda* allele-frequencies among different age classes in high and low predation regimes to test for signatures of predator-induced selection on defence traits and *Eda*. We predict that sub-adults in populations experiencing high fish and bird predation should grow faster and bigger to escape gape-limited predation. Similarly, relative spine lengths should be larger for fish experiencing gape-limited predation but counter-selected for under high invertebrate predation.

METHODS

Sample sites and data collection

We sampled stickleback from two sites within the Rhine-Aare drainage. Our first sample ($N = 130$) was from a pond (Jordeweiher) near Bern, Switzerland. This site has a moderate abundance of predatory insects, including large dragonfly larvae, and very few pike (we repeatedly observed one individual) (Table 1). The pond is approximately 70 m × 50 m and shallow (~2 m at its deepest point). The bottom substrate varies from highly structured along the edges, with rocks, dead branches and leaf litter, to soft and muddy, sometimes with vegetation, towards the middle. Reeds border one side of the pond. All parts of the pond were sampled during multiple sampling events. The second sample ($N = 160$) was from a stream (Gäbelbach) with a high abundance of insect and fish predators. This site is large and heterogeneous, consisting of a fast-flowing main stream, several side channels (sometimes slow-flowing, sometimes stagnant), seasonal ponds, reeds, and an estuary-like mouth into a lake (Wohlensee). Again, all parts of the habitat were sampled, although fish were never obtained from the small ephemeral ponds bordering the stream. The two sampling sites are hereafter referred to as the low and high predation regime respectively (abbreviated LPR and HPR hereafter). The two populations are spatially close (~800 m) and connected by a little stream interrupted by concrete pipes and sluices. Migration between the two sites might be possible downstream but unlikely to occur upstream due to an approximately 45 m difference in elevation. A genetic analysis using neutral microsatellites indicates a low but significant level of genetic divergence between the populations used in this study [F_{ST}: 0.015, $P = 0.002$ (K. Lucek, unpublished data; for technical details, see Sivasundar *et al.*, submitted)]. Fish were caught between 2007 and 2009 using minnow traps, euthanized in clove

Table 1. Quantitative fish data from electrofishing for the HPR site in June 2009 and semi-quantitative observational data for the LPR site based on many hundred man-hours of snorkelling and diving (2007–2010)[a]

		HPR		LPR	
Fish species	Common name	Presence	Abundance	Presence	Abundance
Barbatula barbatula	Stone loach	+	1	–	–
Barbus barbus	Common barbel	+	2	–	–
Cyprinus carpio	Carp	–	0	+	A few
Esox lucius (P)	Pike	+	0*	+	1
Gasterosteus aculeatus	Stickleback	+	15	+	Many
Lampetra planeri	Brook lamprey	+	7	–	–
Perca fluviatilis (P)	Perch	+	15	–	–
Rutilus rutilus or *Scardinius erythrophthalmus***	Common roach or Common rudd	–	0	+	Many
Salmo trutta (P)	Brown trout	+	87	–	–

[a] In the HPR site, a 100 m stretch of the main stream was fished three times in succession using standard electrofishing protocols.
* Pike have been observed at the HPR site, but were not caught during the electrofishing effort reported here.
** Species identity could not be unambiguously identified in the field.
(P) = species generally known to prey on young stickleback.

Table 2. Sample sizes and age structure in the two sites

	Age	*N*	Year of sampling
Low predation regime (LPR)	YOY	30	2009
	1	45	2007, 2008, 2009
	2	52	2007, 2008, 2009
	3	3	2007, 2008, 2009
	Total	**130**	
High predation regime (HPR)	YOY	30	2009
	1	113	2008, 2009
	2	17	2008, 2009
	3	0	2008, 2009
	Total	**160**	

Note: YOY = young of the year.

oil, and stored in 96% ethanol. Sample sizes and other details are provided in Table 2. Four water samples (spanning four months, March to June 2009) were collected from each site to estimate the Ca^{2+} ion concentration using ion chromatography.

Standard length, first dorsal spine length, and pelvic spine length were measured for each fish using a digital calliper to the nearest 0.1 mm. Lateral plates were counted on the left side

after staining. Since stickleback lack scales, we used seasonal rings on otoliths to age fish following Jones and Hynes (1950).

We genotyped all individuals for the *Stn382* marker, which flanks a 60 bp indel in intron 1 of the *Eda* gene (Colosimo *et al.*, 2005). This locus has two alleles: the C-allele, associated with the fully plated phenotype, and the L-allele, associated with the low plated phenotype. In addition, the sex of each individual was determined using a molecular marker (*Idh*) yielding either one or two bands (separated by 30 bp) in females and males respectively (Peichel *et al.*, 2004). DNA was extracted using a 10% Chelex solution, following the manufacturer's protocol (Biorad, Hercules, CA). PCR conditions for the two markers multiplexed followed Peichel *et al*. (2004). PCR products were analysed on a 1.5% agarose gel and genotypes were scored by eye.

Statistical analyses

To test for a statistical difference in the overall age structure between sites, we used analysis of variance (ANOVA) to test for effects of site and sex on otolith readings from all individuals with sampling year as a random factor and treating it as a categorical variable. Because 3-year-old fish were only obtained in the LPR site at a very low frequency ($n = 3$; 2.3%), they were excluded from all statistical analyses. To estimate trait differences between sites and age classes, we used linear mixed models, testing each trait against site, age and sex with all potential interactions in the initial model. We used sampling year as random factor to account for potential differences between sampling events. We tested for a significant relationship between dorsal spine length and pelvic spine length with standard length using linear regressions. Because dorsal spine length and pelvic spine length were significantly correlated with standard length, we used standard length as a fixed factor in the linear mixed models to correct for size. To select the final model, we used a backward procedure based on a likelihood ratio test for models fitted using maximum likelihood, where all non-significant terms were removed sequentially ($\alpha = 0.05$). We fit the final models with restricted maximum likelihood to estimate differences between groups and their significance using *post hoc t*-tests.

We tested for a difference in lateral plate number between age classes within sites using an ANOVA with sampling year as a random factor and a Tukey's HSD *post hoc* test. Because heterozygote individuals for the *Stn382* C allele can either have an intermediate or full set of lateral plates, whereas homozygote individuals for the *Stn382* L allele are generally fixed for the low plated phenotype, we compared the distribution of plates only for individuals with more than 10 plates on the left side (Lucek *et al.*, 2012). We further compared the *Stn382* genotype frequencies between consecutive years within sites using χ^2 tests. All statistical analyses were performed using R v.2.14.1 (R Development Core Team, 2011).

RESULTS

Age structure and morphology

Age structure differed significantly between the two habitats, with a higher average age in the LPR site (LPR mean = 1.323; HPR mean = 1.013; $F_{1,286} = 11.24$, $P < 0.001$). Age, however, did not differ between the sexes, either within a site (LPR: $F_{1,126} = 0.39$, $P = 0.535$; HPR: $F_{1,157} = 0.55$, $P = 0.461$) or overall ($F_{1,285} = 0.75$, $P = 0.386$).

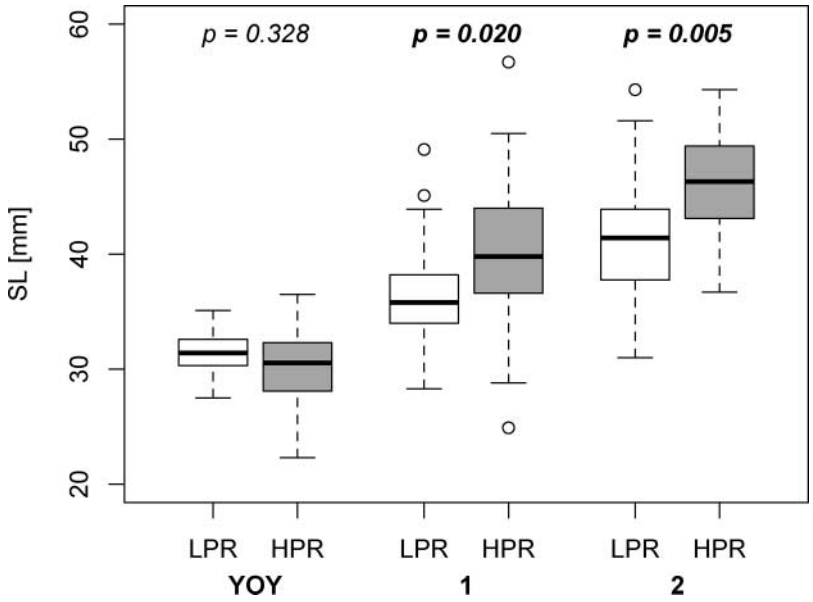


Fig. 1. Boxplots of size (standard length, SL) for each age class at each site (YOY = young of the year individuals; LPR = low predation regime; HPR = high predation regime). *P*-values associated with significant differences between sites for a given age class, based on *post hoc* estimates from a linear mixed model (see text for details). The sexes were pooled because they did not differ statistically.

The model that best explained the variation in standard length included sampling site and age, with a significant interaction between the two factors ($F_{2,278} = 4.101$, $P = 0.017$). Young of the year from the two sites did not differ in size ($t_{1,278} = 0.981$, $P = 0.328$), whereas adult individuals from the HPR site were significantly larger than those from the LPR site at both age 1 ($t_{1,278} = 2.332$, $P = 0.020$) and age 2 ($t_{1,278} = 2.838$, $P = 0.005$; Fig. 1).

In both systems, dorsal spine length and pelvic spine length were significantly correlated with standard length in each age class (Table 3, Fig. 2). Sites differed for pelvic spine length at ages 1 and 2; dorsal spine length, however, differed only at age 1. For dorsal spine length, size-corrected residuals differed significantly only between sites ($F_{1,281} = 33.634$, $P < 0.001$; Fig. 3). Individuals from the HPR site generally had smaller dorsal spines at similar size. For pelvic spine length, both site and age were retained in the best model, where individuals in the HPR site had generally smaller spines when corrected for size ($F_{1,279} = 15.927$, $P < 0.001$). For both sites, spine length increased significantly in the first year ($t_{2,279} = 3.881$, $P = 0.001$) but did not differ between the two adult age classes ($t_{2,279} = 1.637$, $P = 0.103$).

Lateral plate phenotypes and *Stn382* genotypes

The distribution of *Stn382* genotype frequencies changed significantly between young of the year and one-year-old individuals in both sites (LPR: $\chi^2_2 = 6.55$, $P = 0.038$; HPR: $\chi^2_2 = 7.56$, $P = 0.023$). Frequencies between one- and two-year-old individuals changed significantly only in the HPR site (LPR: $\chi^2_2 = 3.81$, $P = 0.149$; HPR: $\chi^2_2 = 11.98$, $P = 0.003$). In the LPR the young of the year-to-age 1 transition was accompanied by an increase in CC

Table 3. Linear regression coefficients for each trait and site per age class and comparison of the regression slopes between sites (see text for details)

		LPR			HPR			LPR vs. HPR
Trait	Age	F	d.f.	R^2	F	d.f.	R^2	P
Dorsal spine length	YOY	7.82	1,28	**0.218****	20.61	1,28	**0.424*****	0.689
	1	18.72	1,43	**0.303*****	84.85	1,111	**0.433*****	**0.046**
	2	4.89	1,49	**0.091***	4.92	1,15	**0.247***	0.276
Pelvic spine length	YOY	11.29	1,28	**0.287****	68.06	1,28	**0.709*****	0.861
	1	11.09	1,43	**0.205****	178.00	1,111	**0.616*****	>**0.001**
	2	9.74	1,49	**0.166****	94.30	1,15	**0.863*****	>**0.001**

Note: Statistically significant values are indicated in **bold** font. YOY = young of the year.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

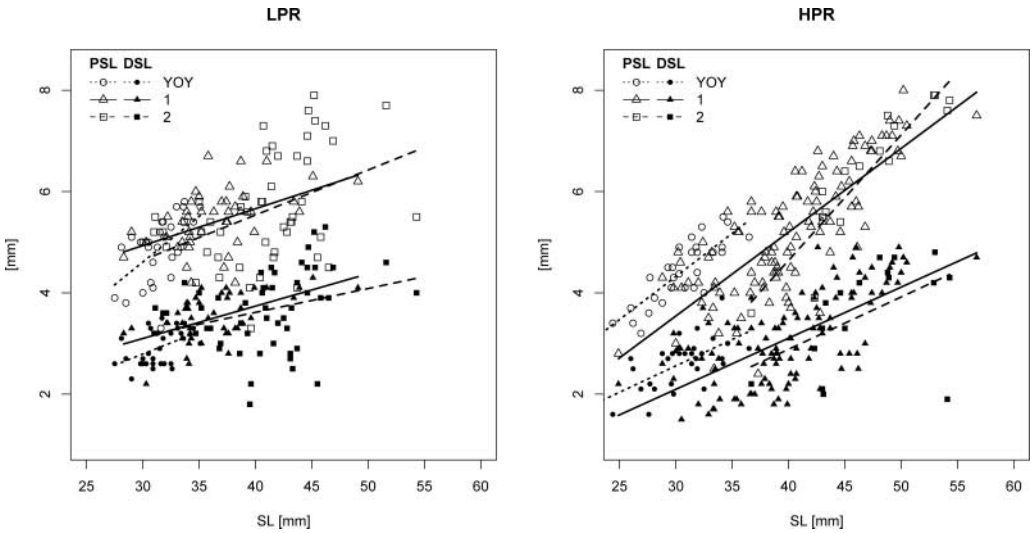


Fig. 2. Linear relationship between pelvic spine length (PSL, open symbols) and dorsal spine length (DSL, solid symbols) with size (standard length, SL) for both the LPR (left) and HPR (right) site. Symbols further indicate different age classes (YOY = circles, age 1 = triangles, age 2 = squares). The regression line for each age class is shown (YOY = dotted, age 1 = solid, age 2 = dashed); see Table 3 for details. YOY = young of the year.

and LC genotypes, whereas in the HPR there was a decrease in the CC genotype and almost no change in the LC genotype. In the age 1-to-age 2 transition there was an increase in both homozygote genotypes and a very marked decrease in the heterozygote genotype in the HPR site. In the LPR site, the pattern was similar but the frequency change was not significant (Fig. 3). Reflecting the changes in genotype frequencies, intermediate plate morphs became less frequent in successive age classes in the HPR site but not in the LPR

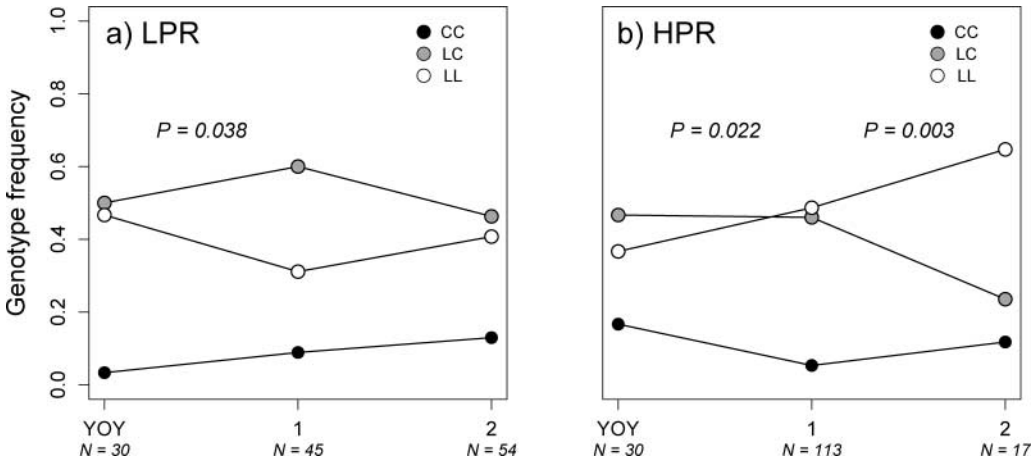


Fig. 3. Genotype and allele frequencies at *Stn382* by age class at the (a) LPR and (b) HPR site. Black circles = CC; grey circles = LC; open circles = LL. *P*-values associated with significant differences between successive age classes are shown. Missing values represent statistically non-significant differences. Total sample sizes for each age class at each site are given below the x-axis.

site (Fig. 4). The lateral plate numbers for individuals with >10 lateral plates changed significantly in the HPR site ($F_{2,79} = 5.57$, $P = 0.006$), differing between young of the year and age 1 ($P = 0.017$) and young of the year and age 2 ($P = 0.017$). Age classes did not differ at the LPR site ($F_{2,74} = 0.68$, $P = 0.510$; Fig. 5).

Calcium concentration did not differ statistically between sites (mean ± S.D.): LPR 896 ± 269 μmol·L^{-1}, HPR 881 ± 301 μmol·L^{-1} (Mann-Whitney *U*-test, $P = 0.889$).

DISCUSSION

We observed different growth rates for stickleback from the two habitats, which differ in predation pressure. Juvenile individuals from both sites were similar in size but, with increasing age, size increased more rapidly under high predation, suggesting a faster growth rate in the HPR site. Faster growth might confer a selective advantage under high predation on juveniles or sub-adults by gape-limited predators (Reimchen, 1992, 1994, 2000). Even olfactory cues of predatory fish can lead to an increased growth rate with a concordant increase of spine lengths (Frommen *et al.*, 2011). Consistent with this, age structure differed among the sites with lower average survival in the high predation site. This may lead to adaptive divergence in life-history strategies, where high predation on juveniles and sub-adults would favour investment in fast growth but further experiments are needed.

Young of the year fish have significantly longer pelvic spines (when corrected for size) compared with age class 1 and 2 in both sites. The relatively long pelvic spine length might compensate for small size at younger age. Interestingly, individuals from the HPR site have generally smaller spines at a given size compared with fish from the LPR site. Thus, we do not find selection for longer spines in the high predation site. This could reflect costs associated with the development of larger spines (Frommen *et al.*, 2011), where fish in the HPR would invest in size rather than spine length.

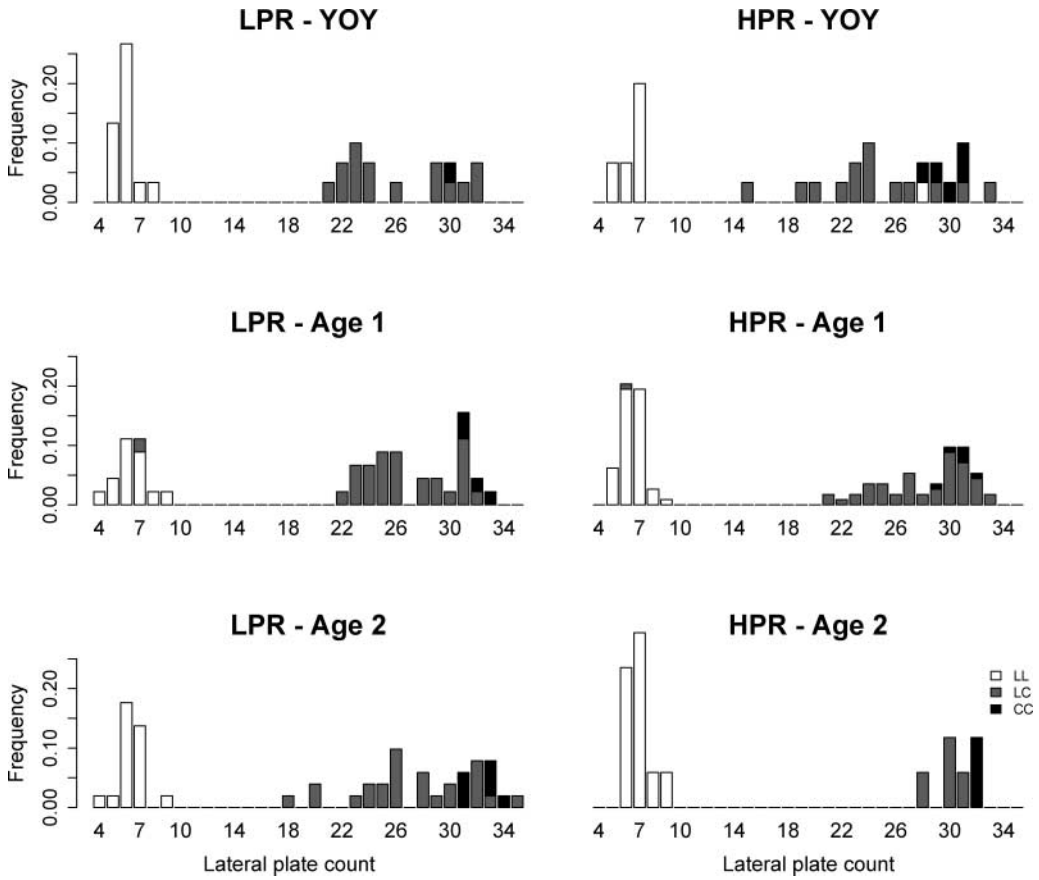


Fig. 4. Lateral plate frequency distributions within each age class for the LPR and HPR sites according to *Stn382* genotype (black = CC; grey = LC; white = LL). YOY = young of the year.

Lateral plate phenotypes and their associated genotypes showed an indication for disruptive selection. In the high predation system, intermediate plate phenotypes appear to have reduced survival. The average lateral plate number for individuals with >10 lateral plates increased significantly with age in the HPR site because intermediate plate phenotypes disappear successively with increasing age. In contrast, the plate phenotype distribution in the low predation system remains similar across age classes. The pattern of decreased intermediate plate phenotypes in the HPR site is also reflected in the *Eda*-genotype frequencies. A significant change in the transition from age 1 to age 2 was only detectable in the HPR site. Heterozygous fish decrease while both homozygote genotypes increase with age. In contrast, heterozygous fish are constant in frequency over all age classes in the LPR site. Where both gape-limited and insect predators are abundant, fully and low plated fish tend to survive longer. These two phenotypes might provide defence against the two major classes of predator types. Heterozygotes and intermediate phenotypes appear to be at a selective disadvantage, as they do not have effective protection against either predator type. Alternatively, there might be divergence in predator avoidance behaviour between plate morphs, where the right combination of trait and behaviour would

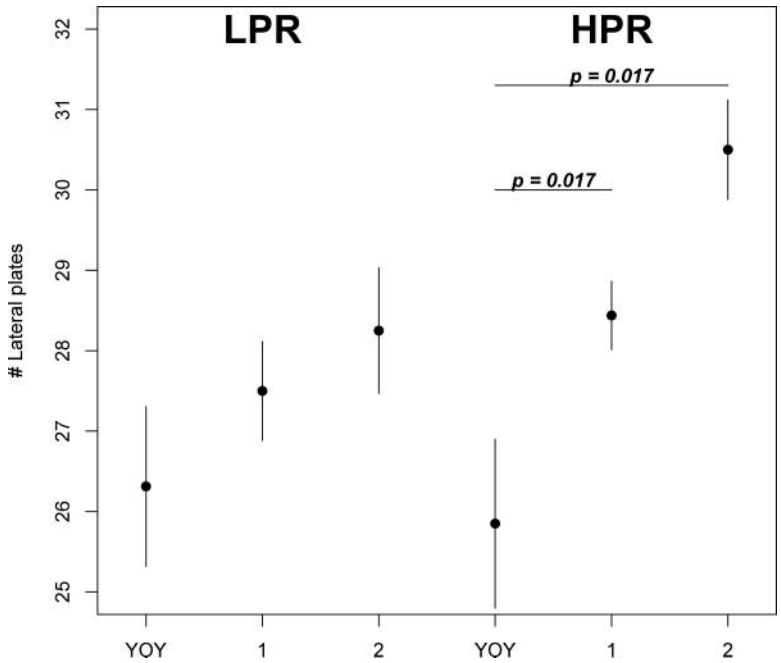


Fig. 5. Mean number of lateral plates (±1 S.E.) for individuals with more than 10 plates for each age class for the LPR and HPR sites. *P*-values associated with significant differences between successive age classes, based on *post hoc* ANOVA.

protect against predation. Heterozygotes, however, would fail to be effectively defended because their behaviour/morphology defence strategy would not match.

In the long term, disruptive selection with heterozygous disadvantage would mean selection against the rare allele, which could only be maintained by frequency-dependent selection (Lewontin, 1958; Wilson and Turelli, 1986). Perhaps there is differentiation in feeding niche between the two phenotypes, which can result in frequency-dependent selection (Wilson and Turelli, 1986). However, examination of the stomach contents provides no support for a relationship between plate phenotype and feeding niche both within and among populations (K. Lucek, unpublished data). It is also possible that this is a transient polymorphism, and over time the population will become fixed for the more common allele and phenotype, either via selection or drift.

One aspect of predation that has not been considered here is the role of avian piscivores. Given the much larger area and heterogeneity of habitats at the HPR site, the abundance and diversity of fish-eating birds is likely to differ compared with the LPR site, although we did not quantify this. In general, however, piscivorous birds are not very common, having been observed on very few occasions at either site.

We cannot, of course, unequivocally attribute all of the differences in size and defence traits to predation – the two sites also differ in physical and environmental characteristics, and perhaps food availability and strength of intra- and inter-specific competition, all of which may play some role in phenotypic divergence. One such factor is water clarity, which may be a relevant parameter for visual predators. The LPR site is a small pond, with very high visibility throughout the year. The HPR site is a much more heterogeneous

environment and, consequently, there is a great deal of both spatial and temporal variation in water clarity there. Another factor that has been shown to play a limiting role in the formation of defence traits is the availability of environmental calcium (Giles, 1983; Bell *et al.*, 1993). However, the calcium concentrations at our sites exceeded those suggested to be limiting by a factor of at least four. It is thus highly unlikely that environmental calcium availability plays any limiting role in armour formation at these sites.

Thus, despite a number of other biotic and abiotic differences between the sites, given the demonstrated utility of lateral plates and spines as defence against predation, at least a significant proportion of the differences between the sites is likely explained by the marked difference in their predator composition. Phenotypic differentiation as a consequence of the presence of a predator has been attributed to phenotypic plasticity on body size and spine lengths before (Frommen *et al.*, 2011), In contrast, we observed a pattern consistent with disruptive selection acting on genotypes. This suggests that there might be directional selection on body and spine size under high predation; simultaneously, there could be disruptive selection on lateral plate number, with intermediates (and heterozygotes for *Eda*) being at a disadvantage compared with either extreme phenotype. Under low predation, neither of these patterns is apparent. Taken together, predator-driven evolution has the potential to manifest itself even over very short evolutionary time scales.

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