Habitat dependent associations between parasitism and fluctuating asymmetry among endemic stickleback populations

C. A. BERGSTROM* & T. E. REIMCHEN†

*Bamfield Marine Sciences Centre, Bamfield, BC, Canada
†Department of Biology, University of Victoria, Victoria, BC, Canada

Abstract

Inconsistencies in the relationship between fluctuating asymmetry (FA) and fitness may be due to selection acting on the degree of trait asymmetry that differs among populations or among traits. We assessed relationships between parasite susceptibility and fluctuating asymmetry in the number of bony lateral plates among 83 populations of freshwater *Gasterosteus aculeatus* (three spined stickleback) and among lateral plate positions that vary in the selection they experience for symmetry. The correlation between FA and parasite infection was highly variable among samples. Excess of infected asymmetric *G. aculeatus* increased significantly as the robustness of structural predator defences decreased. This effect was found for one parasite species only (*Eustrongylides* sp.) and was slightly stronger in females. In addition, there was a trend for there to be an excess of infected females asymmetric in those lateral plates positions that did not experience selection for their symmetry, although the trend only approached significance. These results suggest that selection for trait symmetry can obscure relationships between fitness and individual-wide developmental stability, providing one possible explanation for some of the heterogeneity in FA/fitness relationships seen in the literature. These results are also consistent with previous reports showing that ecological segregation between symmetric and asymmetric *G. aculeatus* and between sexes can alter the FA/fitness relationship.

Introduction

Fluctuating asymmetry (FA), seen as small, random differences between sides of a bilateral character, is an indication of instability during an individual's development (Mather, 1953) and is generally thought to be negatively correlated with multiple components of fitness (Møller, 1997, but see Clarke, 1998). In most instances, the negative relationship between fitness and FA is indirect, in that both are symptoms of levels of developmental instability rather than being causally related with each other. Parasite infection in particular is a fitness measure that has been indirectly linked with elevated levels of FA in multiple taxa (Escos *et al.*, 1995; Møller, 1996; Agnew & Koella, 1997; Hunt & Allen, 1998; Sasal & Pampoulie, 2000), although many studies have failed

Correspondence: C. A. Bergstrom, Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada V0R 1B0.
Tel.: +1 250 7283301; fax: +1 250 7283452; e-mail: cbergstr@bms.bc.ca

to find this association (Ward *et al.*, 1998; Thomas *et al.*, 1998; Quek *et al.*, 1999).

Inconsistencies in the association between FA and parasite infection as well as other measures of fitness are prevalent in the literature and while the reasons for this are unclear, evidence suggests that the association is trait specific (Clarke, 2002). Most studies fail to find correlations in asymmetry among traits within individuals, resulting in fitness measures being correlated with asymmetry in some traits but not others (Lens *et al.*, 2002; but see Polak *et al.*, 2003). This suggests that the accuracy with which asymmetry indirectly reflects developmental instability varies among bilateral characters. One explanation for this is that the direct functional cost of asymmetry differs among traits (Bergstrom & Reimchen, 2000,2003). Consequently, the correlation between FA and other symptoms of developmental instability (i.e. parasite susceptibility) could be obscured by selection acting specifically against asymmetry in a bilateral trait (e.g. one whose functional efficacy

contributes directly to the individual's performance, such as wing length). Selection against asymmetry in a single trait would increase that trait's homeostasis, thus weakening the indirect association between its FA and fitness reductions resulting from organism-wide developmental instability.

As a result, patterns of FA/fitness relationships among multiple populations could emerge as either trait dependent (when selection against asymmetry varies among traits) and/or population dependent (when selection against asymmetry varies among habitats). Most previous studies that have looked at the relationship between FA and fitness as measured by parasite susceptibility have been restricted to single traits and/or single populations, making trait- and habitat-dependence difficult to detect. Meta-analyses have shown promise as a way to determine if broad patterns emerge among studies that vary in their methods by taxa, habitat or trait type (Leung & Forbes, 1996). However, it would be more effective to investigate trait and population dependence of the indirect relationship between fitness and FA by restricting the analysis to a large number of closely related populations that vary in the strength of selection acting on asymmetry both among traits and among habitats.

This study looks at the relationship between parasite infection and FA among 83 populations of *Gasterosteus aculeatus* (threespine stickleback) that occupy freshwater habitats on the Haida Gwaii archipelago (Queen Charlotte Islands) found off the north coast of British Columbia, Canada. A previous study of parasitism and composite multi-trait asymmetry in *G. aculeatus* found that most populations had greater infection rates in asymmetric than in symmetric fish (Bergstrom & Reimchen, 2002). Here we focus on a trait complex not included in the previous analysis: the number and position of heritable, bony lateral plates found along each side of the fish's body. These plates provide structural protection against predators (Reimchen, 1983,1992,2000) and demonstrate striking variation among populations on this archipelago in both total number and asymmetry (Moodie & Reimchen, 1976; Bergstrom & Reimchen, 2000). Plate number asymmetry can compromise the functional efficacy of structural defences against predators (Reimchen, 1983; Bergstrom & Reimchen, 2000) and the relative symmetry of plate number among populations covaries geographically with predator reaction distance and robustness of the predator defence structures (Bergstrom & Reimchen, 2003), both of which are indirect measures of the strength of post-capture selection on *G. aculeatus* by predators (Vinyard & O'Brien, 1976; Reimchen, 1989,1992; Bell, 2001). The expression of defence robustness and other morphological traits is unique to each geographically isolated population on Haida Gwaii (Reimchen et al., 1985). In addition, asymmetry in some plate positions, particularly those that support the large dorsal and pelvic spines,

appears to come at a greater cost to the fish and may be selected against more strongly than in plates not adjacent to the spines (Reimchen, 1983; Bergstrom & Reimchen, 2000,2003). As such, asymmetry in lateral plates appears to be a target of selection that varies in strength both among habitats and among plate positions, making this system an ideal one with which to test for population- and trait-dependence of the relationship between parasite infection and FA in wild populations.

Extensive investigations in one lake on Haida Gwaii have shown that relationships between FA and parasite resistance in *G. aculeatus* can be confounded by ecological segregation between sexes and between symmetric and asymmetric forms that results in differential exposure to parasite hosts (Reimchen & Nosil, 2001a,b). In this study we expand the analysis to over 80 geographically isolated populations on this archipelago and look at the relationship between plate asymmetry and infection in both sexes and by three parasite species associated with different micro-habitats within and among these lakes. If ecological segregation between symmetric and asymmetric *G. aculeatus* and/or between sexes plays a role in the relationship between FA and parasite infection at this large scale, any associations we find may be sex or parasite species-specific (e.g. asymmetric forms or sexes found more often in pelagic habitats will be more susceptible to parasites transmitted by pelagic hosts).

Our objectives are to determine (1) if *G. aculeatus* asymmetric for lateral plate position have greater parasite infection rates than symmetric *G. aculeatus* across multiple natural populations, (2) if the association between parasitism and lateral plate FA is dependant on the strength of selection for plate symmetry among populations from different habitats, as measured by robustness of the predator defence apparatus, (3) if the association between parasitism and lateral plate FA is dependent on strength of selection for symmetry among plate positions and (4) if the relationship between FA and fitness is dependent on sex or parasite species.

Methods

Samples of *G. aculeatus* were collected from geographically isolated lakes and streams across the archipelago during multiple expeditions from 1969 to 1997. The majority of *G. aculeatus* samples were collected from April to July using standard-mesh minnow traps placed in the littoral zone. Fish were fixed in 10% formalin and stored in 95% ethanol. Of these original collections, 83 were used for this study (this represents 69% of the total number of natural *G. aculeatus* populations on the archipelago, excluding those from artificial basins, those that had either no parasites present or no variation in lateral plate asymmetry and those with negligible sample sizes). Studies of long-term shifts in *G. aculeatus* morphology in some lakes included in this analysis suggest that while seasonal shifts in morphology do occur,

annual shifts in the predator defence structures are negligible over the time that sampling occurred (Reimchen & Bergstrom, unpublished data; Reimchen, 1995; Reimchen & Nosil, 2002). While half of the 83 samples shared watersheds with one or more samples, divergence among populations within and among watersheds due to different ecology and habitat related selection regimes has overshadowed similarities between them due to phylogenetic relatedness (Reimchen et al., 1985; Deagle et al., 1996). Therefore, each sample was treated as ecologically independent.

From each sample of *G. aculeatus*, 15–50 adults (>45 mm standard length; Wootton, 1976) were measured for standard length (SL), sex, robustness of the predator defence structures and lateral plate number asymmetry (mean sample size = 43; total n = 3569). Robustness was measured by scoring the number and positions of the lateral plates per side and the degree of overlap between the plates and the spine supports. Each lateral plate overlays a single myomere and can be identified by a numbered position (P1, P2, P3, …, P30) that is consistent among individuals (Reimchen, 1983). Lateral plate number was calculated per fish by taking the average number of plates on each side of the body. Mean lateral plate number was calculated for each population and log-transformed. Mean lateral plate number variation among populations on this archipelago is immense; ranging from <1 plate per side to the full suite of 30–35 (Moodie & Reimchen, 1976; Bergstrom & Reimchen, 2000) and is strongly positively correlated with selection by predators for post-capture survival (Reimchen, 1992,1994,1995,2000), as well as with symmetry of plate presence (Bergstrom & Reimchen, 2003).

A second measure of population-specific predator defence robustness we used was the mean degree of overlap between the spine supports and the lateral plates. The degree of overlap provides lateral structural support to the spines during predator handling (Reimchen, 1983) and therefore contributes to the robustness of the structural defences. Evidence suggests that selection for lateral plate number symmetry is associated with *G. aculeatus* populations with greater degrees of overlap (Bergstrom & Reimchen, 2003). For each fish, we categorized the degree of overlap between the anterior lateral plates and the spine supports as not touching (0), touching (1), partial overlap (2) and major overlap (3) (see Reimchen, 1983). We then calculated the average overlap per plate lying adjacent to the dorsal or pelvic spines for each sample. Mean degree of overlap between the spine supports and the plates was strongly positively correlated with mean lateral plate number among samples (R^2 = 0.69; d.f. = 82; P < 0.01); we therefore used residuals of mean overlap regressed on mean plate number for further analysis in order to avoid collinearity between these two measures in multiple regressions.

Asymmetry in the presence of plates on the left-hand and right-hand sides at each plate position (P_1–P_{30}) was calculated (R_{P1}–L_{P1}, R_{P2}–L_{P2}, etc.). Overall, we considered a fish to be asymmetric if it had a plate present on one side but absent on the corresponding myomere on the other side at one or more plate positions. Fish that were asymmetric were further categorized as having the asymmetry in either one of the 'structural' plate positions, or one of the 'nonstructural' plate positions. Structural plates are those in the anterior region of the fish that lie directly adjacent to the dorsal and pelvic spines used for protection during handling by a predator. Experimental studies have shown that the absence of one of these plates from either side of the body significantly reduces the strength required to deflect the spines (Reimchen, 1983). Field studies have shown that the incidence of asymmetry in these plates is greatly reduced relative to the nonstructural plates (Bergstrom & Reimchen, 2000) especially in populations that have robust predator defence phenotypes indicative of strong selective predation for post-capture escape mechanisms (Bergstrom & Reimchen, 2003). We therefore looked for correlations between structural plate asymmetry and parasite infection, as well as nonstructural plate asymmetry and parasite infection, in order to compare the strength of this correlation between related traits that presumably vary in the selection they experience for symmetry.

Concurrent studies show that there is slight directional asymmetry (population-level bias in side of greater expression of a bilateral trait) in lateral plate number in very large samples (n > 10 000) from two lakes on the archipelago used in this study (Reimchen & Nosil, 2001b; Reimchen & Bergstrom, unpublished data). While it is possible that some of the 83 samples of 50 or fewer fish used here might demonstrate subtle directional asymmetry at larger n (which is currently unavailable), the significant majority of these at the available sample sizes exhibit true fluctuating asymmetry and negligible measurement error (Bergstrom & Reimchen, 2003).

Each individual fish was scored for the presence or absence of three parasites species: *Schistocephalus solidus*, *Eustrongylides* sp. and *Diplostomulum* sp. (Hoffman, 1967). *Schistocephalus solidus* is a segmented cestode that uses pelagic copepods as first intermediate hosts and *G. aculeatus* as second intermediate hosts. They grow into large plerocercoids in the body cavities of *G. aculeatus*. *Eustrongylides* sp. is a nematode that uses benthic oligochaetes as the first intermediate host and *G. aculeatus* as the second intermediate host. Larval forms of this parasite grow in the body cavities and occasionally the musculature of *G. aculeatus*. *Diplostomulum* sp. is a trematode that uses gastropods as the first intermediate host and *G. aculeatus* as the second intermediate host. The metacercariae becomes encysted in the musculature of *G. aculeatus* or in the lenses of the eye. Avian piscivores are the definitive hosts for each of these parasites species. Only adult *G. aculeatus* were used in this analysis since parasite infection in younger *G. aculeatus* is rare or

nonexistent. Less than 1% of all fish were infected with more than one parasite species ($n = 34$ of 3569).

In order to determine if asymmetric individuals were more likely to be parasitized than symmetric individuals, we compared the proportion of infected symmetric fish to the proportion of infected asymmetric fish within each population using G-tests and sequential Bonferroni corrections. We did this for all parasite species combined, as well as single parasite species infections and for combined sexes as well as separately for males and females. For all single parasite species comparisons in this study, G. aculeatus infected with multiple parasite species, although rare, were excluded to avoid confounding the results. Due to the large number of populations in this study, a probability of ≤0.0006 was required to denote significance in any single sample when using sequential Bonferroni corrections, which, especially at the sample sizes available, is likely to elevate the chances of making a Type-II error (Moran, 2003). Therefore, we also used Binomial tests to compare the total number of populations with elevated parasitism in asymmetric fish, regardless of magnitude, to the total number with elevated parasitism in symmetric fish.

Any relationship found between parasitism and FA in lateral plate number in G. aculeatus would not result from parasites causing asymmetry. Lateral plate development is complete by the time G. aculeatus are about 30 mm in standard length (Bell, 1981), but most parasite infections do not occur until the fish are older than this. In addition, once lateral plate development is complete it is a fixed trait that does not change throughout the fish's life.

To determine if parasitism/FA associations were dependent on the history of selective predation unique to the habitat of each population, we described populations by the mean degree of structural defence robustness in each sample, as measured by lateral plate number mean and residual overlap mean. We then calculated an index of the FA/parasitism association for each sex in each sample that was equivalent to the proportion of infected asymmetric fish minus the proportion of infected symmetric fish (values of 0 equate to no difference in infection rates between symmetric and asymmetric fish; + values equate to an excess of infected asymmetric fish, and − values equate to an excess of infected symmetric fish). We used this index as a parametric dependent variable in an ANCOVA with sex as the factor and with mean lateral plate number and residual mean overlap as the covariates. If slopes and elevations of the regression lines for the two sexes were not significantly different, they were pooled and a multiple regression was run with the parasitism/FA index as the dependent variable, and mean lateral plate number and residual mean overlap as the independent variables. This analysis was done for total parasite infection as well as for each parasite species separately. Due to nonnormality of indices for some parasite species and some sexes, we also used nonparametric Kendall's Rank Correlation tests to cross-validate the results.

To determine if parasitism/FA associations were trait dependent, we used a sub-set of samples that contained both fish with structural plate asymmetry and fish with nonstructural asymmetry within the same lake ($n = 23$ samples). These were primarily samples with plate number means between 2 and 6 per side. All other samples exhibited only one of the two types of asymmetry. Within each of these samples we calculated two indices: (1) the frequency of infected fish with structural asymmetries minus the frequency of infected symmetric fish and (2) the frequency of infected fish with nonstructural asymmetries minus the frequency of infected symmetric fish. Fish with both types of asymmetry were excluded. The values were compared within each sample using paired t-tests for combined and separate parasites and sexes, and confirmed with nonparametric Sign tests.

Results

Distribution of parasitized and asymmetric fish across archipelago

The population frequencies of parasitized fish varied greatly and were not randomly distributed with respect to geography or morphology. At least one parasite species was found in every sample and total incidence of infection ranged among samples from 2 to 89% with an average of 24% infected fish. *Schistocephalus solidus* was present in 41 of the 83 samples and ranged in infection frequency from 2 to 52% with a mean of 12%. *Diplostomulum* sp. was present in 43 samples and infection frequency ranged from 2 to 89% with a mean of 16%. *Eustrongylides sp.* was present in 59 samples and infection frequency ranged from 2 to 80% with a mean of 14%. *Schistocephalus solidus* and *Diplostomulum* sp. were found more often in lakes that contained G. aculeatus of higher plate counts, while *Eustrongylides* sp. tended to be found in lakes that contained G. aculeatus of lower plate counts (Table 1). Asymmetric G. aculeatus were also most common in populations with reduced plate number (Table 1; Moodie & Reimchen, 1976; Bergstrom & Reimchen, 2003). However, there were no significant correlations between the population frequency of total or single parasite species infection and frequency of asymmetric adult G. aculeatus across the archipelago (all $r < 0.15$; $40 < \text{d.f.} < 82$; all $P > 0.28$).

Relationships between parasite infection and FA

Overall effects
Among these 83 samples from isolated populations, there was no broad, consistent trend of asymmetric fish being more parasitized than symmetric fish. There were no significant differences between the proportion of infected asymmetric and infected symmetric individuals within

Table 1 Proportion of samples that contain parasite-infected adult *G. aculeatus* for each of the three parasite species.

| Plate number | Frequency of samples containing | | | Mean sample frequency of plate asymmetry ± SD |
	S. solidus	*Diplost*. sp.	*Eustr*. sp.	
Low (0.8–3.8)	8/20	2/20	17/20	0.73 ± 0.15
Medium–low (3.9–5.8)	7/21	9/21	18/21	0.52 ± 0.13
Medium–high (5.9–6.6)	11/21	19/21	17/21	0.45 ± 0.09
High (6.7–30.0)	15/21	13/21	7/21	0.42 ± 0.18
G	3.67	15.66	6.39	F = 19.65
P	0.30	<0.01	0.09	<0.01

Diplost.: *Diplostomulum*; *Eustr*.: *Eustrongylides*. Values in the numerator are number of samples containing fish infected with a particular parasite species and denominators are total number of samples in that plate number category. Mean sample frequency of plate asymmetry is compared among plate number categories with 1-way ANOVA and resulting F-ratio is given instead of G-values. For G-tests and ANOVA all d.f. = 3.

Table 2 Results of ANCOVA showing no significant differences between sexes in slope or elevation of regression lines of the FA/parasitism index of a sample and its mean lateral plate number and mean residual overlap between the plates and spine supports.

Parasite	Interaction	F	P
Combined	Plates × sex	1.67	0.20
	Overlap × sex	0.34	0.56
	EMM	0.17	0.68
S. solidus	Plates × sex	3.27	0.07
	Overlap × sex	0.17	0.68
	EMM	1.13	0.29
Eustrongylides sp.	Plates × sex	3.31	0.07
	Overlap × sex	0.22	0.64
	EMM	2.06	0.15
Diplostomulum sp.	Plates × sex	0.01	0.92
	Overlap × sex	<0.01	0.98
	EMM	0.29	0.59

EMM: Estimated marginal means.
All d.f. = 1.

any sample for total or single parasite species, or for pooled or separate sexes (*G*-tests: all $P > 0.21$ after sequential Bonferroni corrections). The number of significant cases before Bonferroni corrections was the same or less than what was expected due to chance in multiple comparisons. The number of samples that had a greater frequency of infected asymmetric fish than infected symmetric fish, regardless of magnitude, was not significantly different than the number of samples that showed the reverse for total and separate parasite species and combined and separate sexes (binomial tests: all $P > 0.22$).

Habitat-specific selection

In order to determine if the relationship between FA and parasite infection was dependent on the strength of selection for plate symmetry among populations, we compared the difference in infection frequency between symmetric and asymmetric fish among populations of different levels of armour robustness. This was measured by both mean total plate number and the degree of overlap between the spine supports and the plates, both of which are indirect measures of the strength of selective predation for plate symmetry (Bergstrom & Reimchen, 2003). There were no significant differences between sexes in the relationship between the FA/parasitism index and mean plate number or mean residual overlap (Table 2), although the sex by plate number interaction terms approached significance for *S. solidus* and *Eustrongylides* sp. infections. Therefore sexes were pooled in the multiple regressions.

The FA/parasitism index was correlated with predator defence robustness in several instances. For combined parasites there was a significant shift from symmetric infection excess to asymmetric infection excess among

samples as the residual degree of overlap decreased (Table 3; Fig. 1). For *Eustrongylides* sp. infections there was a significant shift from symmetric infection excess to asymmetric infection excess as both lateral plate number and residual degree of overlap decreased (Table 3; Fig. 2a and b). In all three cases the effect was stronger for females than males, although in no case was the difference in slope between the sexes significant (Table 2).

The nonparametric Kendall's rank correlation tests were consistent with the regressions in all cases (significance vs. nonsignificance for separate and combined sexes and parasites) except two. For combined parasites, females had a significant negative Kendall's rank correlation between plate number and the FA/parasitism index (Kendall's $\tau = -0.18$; $n = 79$; $P = 0.02$) but not males (Kendall's $\tau = -0.09$; $n = 83$; $P = 0.22$) or combined sexes (Kendall's $\tau = -0.12$; $n = 83$; $P = 0.11$). In addition, the association between residual mean overlap and the relative *Eustrongylides* sp. infection rates between symmetric and asymmetric fish for both sexes

Table 3 Results of multiple regressions testing the effects of mean plate number and mean residual overlap between the plates and spine supports on the FA/parasitism relationship.

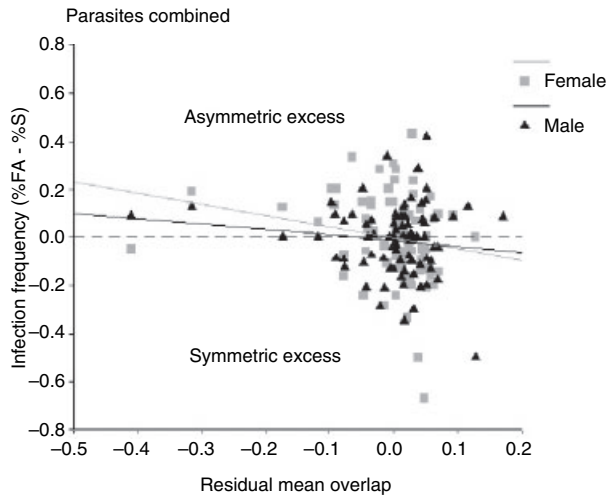| Parasite	Factor	Partial r	t	d.f.	P
Combined	Plates	−0.11	−1.43	82	0.15
	Overlap	−0.16	−2.03	82	0.04
S. solidus	Plates	−0.05	−0.47	40	0.64
	Overlap	−0.02	−0.20	40	0.84
Eustrongylides sp.	Plates	−0.22	−2.38	58	0.02
	Overlap	−0.20	−2.13	58	0.04
Diplostomulum sp.	Plates	−0.15	−1.35	42	0.18
	Overlap	−0.13	−1.17	42	0.24

Fig. 1 Difference in infection frequency between asymmetric (FA) and symmetric (S) fish (%FA−%S) for each sample as a function of mean residual overlap between the plates and spine supports (with the effects of mean lateral plate number removed). Results are shown for combined parasites (all species) and for both sexes. Dashed line represents equal proportions of infected asymmetric and symmetric fish. Solid lines represent least squares linear regression fits for each sex.

became nonsignificant (Kendall's $\tau = -0.14$; $n = 83$; $P = 0.07$).

Trait effects

We did not find strong significant differences in the relationship between FA and parasitism between the two trait types in this study: nonstructural plates vs. structural plates (Table 4). The nonparametric Sign tests were consistent with the paired *t*-tests. However, while there were no consistent trends in the difference between the two trait types for combined sexes or for males, non-structurally asymmetric females had relatively greater infection rates than structurally asymmetric females for all separate and combined parasites (Fig. 3). The difference approached significance for combined parasites (paired $t = -1.75$; d.f. = 18; $P = 0.10$; Sign test: $P = 0.09$) and was strongest, although nonsignificant, for *Eustrongylides* sp.

Discussion

This study has demonstrated that correlations between elevated asymmetry and reduced fitness (in this case parasite susceptibility) are geographically variable among 83 closely related wild populations of *G. aculeatus* on the Queen Charlotte Island archipelago. This is congruent with the lack of strong, general trends in this association among a wide range of trait types and taxa (Leung & Forbes, 1996) and is a powerful reinforcement of meta-analyses that have demonstrated heterogeneity in
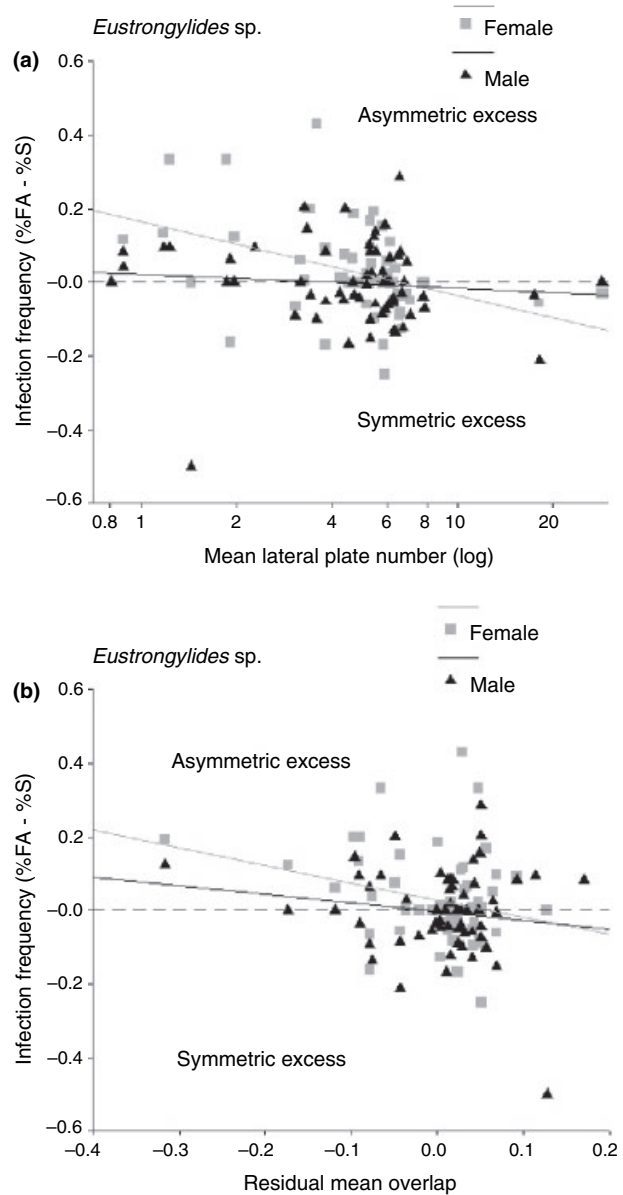

Fig. 2 Difference in infection frequency between asymmetric (FA) and symmetric (S) fish (%FA−%S) for each sample as a function of (a) mean lateral plate number and (b) mean residual overlap between the plates and spine supports (with the effects of mean lateral plate number removed). Results are shown for *Eustrongylides* sp. infections only and for both sexes. Dashed line represents equal proportions of infected asymmetric and symmetric fish. Solid lines represent least-squares linear regression fits for each sex.

FA/fitness associations among studies. Other studies with very large sample sizes ($n > 10\ 000$) from a single population on this archipelago have demonstrated temporal and demographic variability in this association (Reimchen & Nosil, 2001a,b), while the data presented here reveals previously un-addressed geographical vari-

Table 4 Paired *t*-tests of the difference between the frequency of infected fish with nonstructural vs. structural lateral plate asymmetries.

Sex	Parasite	Paired-*t*	d.f.	*P*
Both	Combined	0.62	22	0.54
	S. solidus	0.86	6	0.42
	Eustrongylides sp.	0.59	21	0.56
	Diplostomulum sp.	0.80	5	0.46
Males	Combined	0.70	17	0.49
	S. solidus	0.93	4	0.41
	Eustrongylides sp.	0.59	16	0.56
	Diplostomulum sp.	0.22	4	0.84
Females	Combined	1.75	18	0.10
	S. solidus	1.00	4	0.37
	Eustrongylides sp.	1.53	17	0.14
	Diplostomulum sp.	1.18	4	0.30

Degrees of freedom vary because not all samples contain all three parasite species or have them in both sexes.

ability in the association by using smaller samples from a large number of populations. That the inconsistency is prevalent in a system of closely related populations residing in a relatively small area emphasizes that the relationship between fitness and FA is complex and likely to be confounded by other factors.

Although the correlation between FA and parasite infection was variable among populations, it was not random. Intriguingly, there was a significant shift towards greater asymmetric excess of fish parasitized by *Eustrongylides* sp. as the robustness of predator defences decreased. These results suggest that increased susceptibility to parasites in individuals asymmetric for lateral plate number is accentuated in populations residing in habitats where the functional importance of lateral plates and selection against their asymmetry is reduced. Relaxed selection for lateral plate symmetry may allow FA of this trait to more accurately reflect the developmental stability of the whole fish and other fitness measures symptomatic of that stability, such as parasite resistance. Other studies have found increased parasite infection rates for multiple years in large samples of asymmetric adult *G. aculeatus* in Boulton Lake, one of the lakes included in the present study (Reimchen, 1997; Reimchen & Nosil, 2001b). This lake has a population with a lateral plate mode of three and virtually no overlap between the plates and the spine supports, which

Fig. 3 Mean difference in infection frequency between asymmetric (FA) and symmetric (S) fish (%FA−%S) as a function of trait type (nonstructural vs. structural plate positions). Only those populations with asymmetry in both types of plate positions were included. Results are shown for (a) combined sexes, (b) males and (c) females. *n* is the number of samples with that parasite species present. Dashed line represents equal proportions of infected asymmetric and symmetric fish.
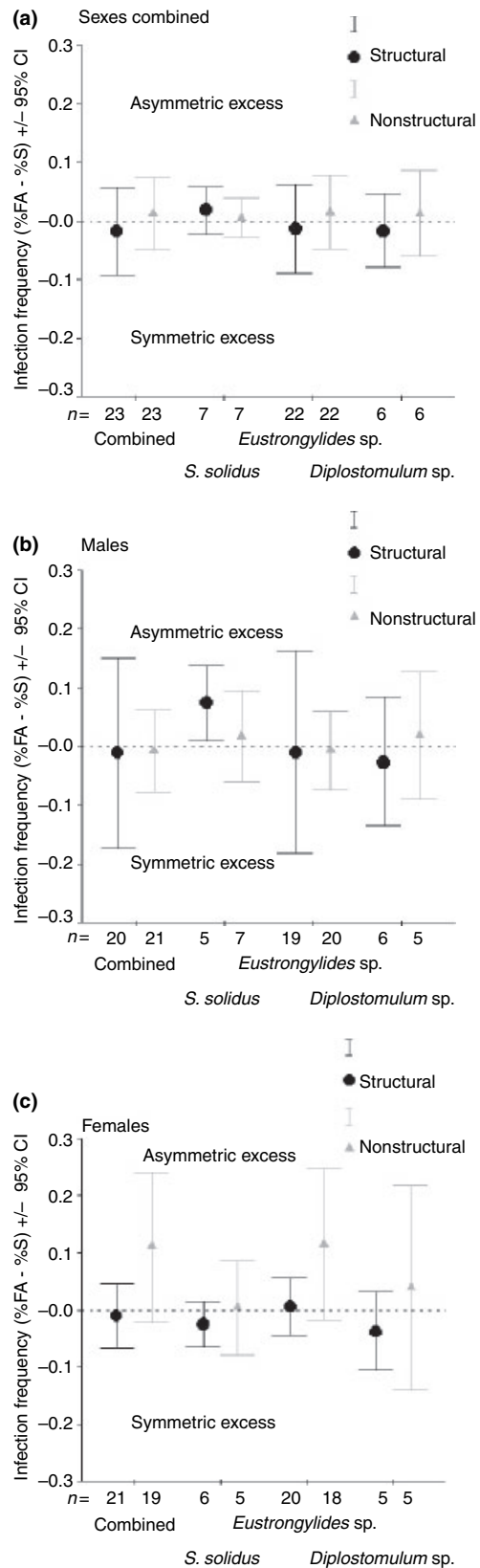
implies that there is little selection occurring to maintain the rigidity, and therefore the symmetry, of the lateral plate/spine complex. Reduction in the functional importance of lateral plates in this and other low-plated populations may have removed the effects of selection from the relationship between developmental stability, asymmetry and resistance to parasite infection. The inconsistency of the association between FA and parasitism in other taxa could be due to undetected biomechanical selection against asymmetry that is obscuring the sensitivity of the trait-specific asymmetry to the developmental stability of the individual and indirectly to other components of fitness. This implies that there may be two levels of developmental stability operating: at the level of the trait and at the level of the whole organism.

Most studies suggest that FA is a subtle signal of developmental instability and correlations between FA and fitness usually have small effect sizes, especially when sample sizes are small (Polak *et al.*, 2003). Heterogeneity in sampling effort among the 83 samples in this study may therefore be responsible for some of the heterogeneity we found between FA and parasite infection. However, despite the heterogeneity in sampling effort, the heterogeneity in the relationship between FA and parasite infection was not random, as there was a significant shift in the relationship as defence robustness decreased among samples. This effect was probably swamped somewhat by variable sampling effort, making these results conservative.

There may be variance in genetic stress in these populations that is interacting with the relationship between FA and parasite resistance. Genetic stress such as homozygosity, for example, has been correlated with both elevated FA and reduced fitness in other taxa (Soulé, 1979; Mitton, 1997). Therefore, if some *G. aculeatus* populations have experienced bottlenecks or selection that results in elevated homozygosity for lateral plate loci, this may have confounded FA/fitness associations. Low plated *G. aculeatus* on this archipelago tend to reside in habitats having diving bird activity but with few or no predatory trout, while high plated *G. aculeatus* reside in habitats containing both types of predators (Reimchen, 1994). These predators can place opposing selection forces on morphology of *G. aculeatus* within a single population (Reimchen, 1995), thus maintaining relatively high genetic variance and heterozygosity compared to *G. aculeatus* that reside with at most one major predator type. Therefore, it is possible that elevated homozygosity for the loci that determine plate number is occurring in low-plated populations (Ziuganov, 1983), thus magnifying the relationship between FA and fitness.

Our results suggested that population-dependence of the relationship between FA and parasitism may be stronger in females than males, although comparisons using larger sample sizes are needed to confirm this since our relatively small sample sizes only approached significance for sex differences. Studies of asymmetry and

parasitism using large samples from one of the lakes included in the present analysis (Boulton L.) show that micro-spatial habitat differences between symmetric and asymmetric individuals can affect FA/parasitism associations due to differences in diet and differential exposure to primary and intermediate parasite hosts (Reimchen & Nosil, 2001a,b). There is also ecological segregation between the sexes in this and other lakes on the archipelago that expose them to different diets (Reimchen & Nosil, 2001c). This raises the possibility that asymmetric fish in low-plated/low-overlap populations are occupying micro-habitats within the lakes that result in elevated consumption of benthic oligochaetes (the primary host of *Eustrongylides* sp.) relative to that of symmetric fish, and that this effect could be amplified in females relative to males. In addition, populations of *G. aculeatus* with low lateral plate means tend to be associated with small, shallow, acidic lake habitats on this archipelago, which generally have fewer species of fishes present in them than the large, deep, neutral lakes. *Gasterosteus aculeatus* are frequently the only fish species present in these habitats, and the resulting decrease in fish richness allows for greater micro-niche segregation within *G. aculeatus* populations.

The shift towards asymmetric excess of parasitized fish as predator defence robustness decreases is seen with *Eustrongylides* sp.; the only parasite species of the three that moves through a benthic primary host before secondary infection of *G. aculeatus* occurs. Populations with low plate number means are typically found in habitats with a greater area of shallow, benthic habitat available for utilization by both primary hosts and *G. aculeatus* and indeed, *Eustrongylides* sp. were present more frequently in *G. aculeatus* from these habitats (see Table 1). Ecological segregation in these lakes that is resulting in asymmetric fish becoming more benthic would result in their elevated consumption of these parasites.

The significant majority of these samples of ≤50 fish exhibit true fluctuating asymmetry, yet two of these from lakes where much larger sample sizes have been acquired (*n* > 10 000), demonstrate subtle but significant left-side biased directional asymmetry across multiple years (Reimchen & Nosil, 2001b; Reimchen & Bergstrom, unpublished data). It is possible that more of the samples from this archipelago would demonstrate subtle directional asymmetry if larger sample sizes were acquired. The reliability of directional asymmetry as a measure of developmental instability is questionable since it presumably has a genetic basis that would confound any nonheritable variance resulting from ontogenetic errors (Palmer & Strobeck, 1992). However, evidence suggests that in some cases subtle directional asymmetry and fluctuating asymmetry may both be reflections of developmental instability (Graham *et al.*, 1998; Leamy, 1999; Reimchen & Nosil, 2001b) and that the exclusion of the former based on statistical definitions may result in the loss of information. It would be interesting to assess

the association between asymmetry and susceptibility to parasite infection in additional, larger samples of *G. aculeatus* from this archipelago to see if this association in low plated populations persists even if directional asymmetry is detected.

We found no consistent evidence of differences in the strength of the relationship between FA and parasite infection between structural and non-structural plates, which differ in the functional costs of their asymmetry. In females, there was a weak but non-significant trend showing that fish with nonstructural plate asymmetries were relatively more parasitized than fish with structural plate asymmetries; consistent with the hypothesis that selection against asymmetry in some traits obscures their sensitivity to genome-wide developmental instability. However, this effect (trait type) was not as strong as the effect of population-specific defence robustness. This suggests that the strength of selection against asymmetry in lateral plates varies more among geographically isolated populations than it does among lateral plate positions within populations. This could be due to genetic and developmental linkage between these two trait types, in that the degree of homeostasis they express may not be able to evolve independently of each other to the degree where they would reflect developmental instability of the whole organism at different resolutions.

In summary, we have demonstrated that correlations between parasite susceptibility and FA are geographically variable among 83 natural populations of *G. aculeatus*, contributing to the growing body of evidence against the generality of FA/fitness associations. We found that elevated *Eustrongylides* sp. infection rates in asymmetric fish increased as the robustness of predator defences and thus history of selection for plate symmetry, decreased among populations. This effect was slightly but non-significantly stronger in females than males, consistent with previous studies that have found ecological segregation between the sexes and between symmetric and asymmetric *G. aculeatus*. Therefore, it appears that the heterogeneity in the relationship between asymmetry and parasite resistance among these populations is due to a combination of factors: differences in selection for trait symmetry among populations and differential exposure of *G. aculeatus* to parasite hosts, as the effect was found for only one parasite species. This supports the hypothesis that selection for symmetry in specific traits can weaken the relationship between fitness, FA and developmental stability; offering one possible explanation for the perplexing heterogeneity of fitness/FA associations in the literature. While the exact mechanism by which selection could affect the relationship between FA and parasite susceptibility is not clear, the identification of whether selection acts on the asymmetry of a trait, as well as whether ecological segregation occurs between symmetric and asymmetric forms, may be crucial in resolving how asymmetry and components of fitness interact in different habitats.

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