ASYMMETRY IN STRUCTURAL DEFENSES:
INSIGHTS INTO SELECTIVE PREDATION IN THE WILD

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Abstract.—Assessment of geographical patterns in fluctuating asymmetry (small, random differences between sides of bilateral characters) among populations shows promise as a tool to resolve the relative biomechanical importance of traits, in addition to being a possible indicator of habitat quality. We used 115 endemic freshwater populations of threespine stickleback (*Gasterosteus aculeatus*) from Haida Gwaii (Queen Charlotte Islands), British Columbia, Canada, to explore the degree of concordance between geographical variation of asymmetry in a predator defense structure (bony lateral plates) and geographical variation in several indirect measures of predation regime as well as several abiotic habitat variables. We found a geographical cline in the population frequency of lateral plate asymmetries, with reduced asymmetry in the southern clear-water regions of the archipelago characterized by long reaction distances and greater chance of capture by predators, and elevated asymmetry in the northern stained-water regions with poor visibility and low chances of capture. Lateral plate asymmetry was strongly correlated with expression of several defensive armor traits, including total plate numbers among populations, mean cross-sectional diameter of stickleback with the dorsal and pelvic spines erect, and mean degree of overlap between the plates and spine supports. There were no significant correlations between frequency of asymmetric fish and any of our abiotic habitat variables. Stickleback with structural plate asymmetries had fewer trout-induced scars than symmetric fish in the significant majority of populations, and there was a decrease in structural plate asymmetry with age in stained-water habitats, suggesting that trout predators may be selectively removing asymmetric fish in some lakes. This study provides evidence that geographical variation in developmental stability of threespine stickleback, as seen in the frequencies of asymmetry, reflects differences among populations in the importance of structural defenses to fitness rather than differences in habitat quality, and that asymmetry may be a target of selection by predators in wild populations.

Key words.—Armor, fitness, fluctuating asymmetry, functional morphology, selection.

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Predation is widely recognized as a mechanism of natural selection and for its capacity to promote the persistence of diversity among populations (Cain and Sheppard 1954; Kettlewell 1955; Palmer 1979; Endler 1986; Reimchen 1994a). Yet one of the key problems in evolutionary biology is the identification of traits in wild populations that affect fitness by being targets of selective predation. Functional morphology has lent great insight into this problem, because it gives biomechanical explanations for the relationship between traits and their function in ecological contexts (Galis 1996; Irschick and Garland 2001). The combined approach of functional morphology and an expanding area of research involving the evolutionary implications of bilateral fluctuating asymmetry have shown promise in further clarifying the relative biomechanical importance of morphological traits in prey.

Fluctuating asymmetry (FA) reflects small random errors made during the development of a trait (Waddington 1942; Zakharov 1992). There has been growing interest in the evolutionary implications of FA in the last three decades, because a large range of taxa exhibit negative correlations between FA and components of fitness (Packer and Pusey 1993; Watson and Thornhill 1994; Hunt and Allen 1998; Rantala et al. 2000; Nosil and Reimchen 2001). In some cases, FA is an indication of fitness reduction associated with developmental instability (Mather 1953; VanValen 1962; Soulé 1967). In these cases, developmental instability results in both elevated FA and reductions in fitness components such as reduced immunocompetence or reduced survival, resulting in indirect, noncausal correlations between FA and fitness. In other cases, FA can directly cause fitness loss by reducing the functional

effectiveness of traits (Allen and Simmons 1996), particularly in the context of locomotion (Moodie 1977; Møller and Hoglund 1991; Thomas 1993; Gummer and Brigham 1995; Swaddle et al. 1996; Swaddle 1997a; Martín and López 2001).

Given the ubiquity of selective predation in the wild, it is likely that there are functional costs of asymmetry in traits used by prey for predator defense, and several examples demonstrate that the outcome of predator-prey interactions can be dependent on asymmetry of the participants. Houseflies with elevated wing asymmetry are more susceptible to predation by barn swallows (Møller 1996), whereas asymmetric barn swallows are more susceptible to predation by European sparrow hawks (Møller and Nielsen 1997). Houseflies with elevated tibial asymmetry are less likely to escape capture by dung flies, and predatory dung flies with elevated tibial asymmetry are less likely to successfully capture their prey (Swaddle 1997b). However, it is unknown whether susceptibility to predation is a direct functional consequence of asymmetry or an indirect effect of reduction in other fitness components that are associated with developmental instability.

The genetic basis of FA has received considerable attention, yet a consolidated model describing the heritability (h^2) of FA remains elusive. Published estimates of h^2 for FA in a range of characters and taxa are extremely variable (Hagen 1973; Møller and Thornhill 1997; Bryden and Heath 2000) and typically quite low. There is little support for the presence of any quantitative trait loci for FA (Leamy et al. 1997), but significant epistasis for FA has been demonstrated (Leamy et al. 2002). Nonetheless, selection can reduce trait-specific asymmetry over time (Reeve 1960; Møller and Thornhill

1997) and consequently, the degree of character asymmetry may reflect the functional impact of asymmetry on character performance (Mather 1953; Palmer and Strobeck 1986; Balmford et al. 1993; Gummer and Brigham 1995; Clarke 1998). Given this relationship, one would predict that structures that are important to the survival of prey after capture by predators would have reduced asymmetry in those habitats where selective predation is relatively strong. This study presents support for this prediction in natural populations of threespine stickleback (*Gasterosteus aculeatus*).

Stickleback have structural predator defenses that include two large dorsal spines, a pair of ventral pelvic spines, and a series of bony lateral plates that each lie on top of a myomere (body segment) and are heritable (Hagen 1973; Hermida et al. 2002). Lateral plate numbers are variable within and among populations, and are under selection pressure by predators (Hagen and Gilbertson 1973; Moodie and Reimchen 1976; Gross 1977; Bell and Haglund 1978; Bell and Richkind 1981; Bañbura et al. 1989; see review in Bell 2001), as they provide protection against puncture during predator handling (Reimchen 1992) and interfere with the pharyngeal actions of gape-limited predators (Reimchen 2000). Each lateral plate and its associated myomere can be identified by a numbered position (P1, P2, P3, . . . , P30) that is consistent among individuals (Reimchen 1983). The anterior lateral plates that overlie myomeres four through seven are adjacent to the dorsal and pelvic spines (Fig. 1A), and provide structural resistance against deflection of these spines (Reimchen 1983), whereas the nonstructural plates that lie anterior and posterior to myomeres four through seven do not. The dorsal and pelvic spines are modified fin rays, and are associated with skeletal pterygiophores. However, the major structural support to these spines comes from the overlap between the structural lateral plates and the bony external structures the spines sit in (the basal plates dorsally, and the ascending process ventrally; Fig. 1B). The dorsal and pelvic spines are erect during predator handling, and the resulting increase in cross-sectional diameter interferes with the handling efficiency of large predatory fish (Hoogland et al. 1957; Reimchen 1991) and is greater in stickleback sympatric with them (Hagen and Gilbertson 1972; Gross 1978). Experimental removal of the structural plates four through seven results in easier spine deflection, an effect that would have serious repercussions to a stickleback trying to escape predator manipulation (Reimchen 1983).

Since presence of structural plates on both sides of the fish is crucial to the effectiveness of the spines, it is likely that asymmetry in the presence of structural plates would be detrimental to stickleback during predator handling. Indeed, in stickleback from Haida Gwaii (Queen Charlotte Islands), which lie off the coast of central British Columbia, Canada, the structural plates exhibit reduced levels of asymmetry relative to nonstructural plates (Bergstrom and Reimchen 2000), suggesting that structural plate asymmetries may have been reduced by selection over time. Of two published estimates of the heritability of lateral plate number asymmetry, one detected no heritability (Hermida et al. 2002), whereas the other estimate was quite high ($h^2 = 0.63$; Hagen 1973), indicating that, at least in some populations, plate asymmetry may have the potential to respond to selection.

Frequencies of stickleback asymmetric for lateral plate numbers and positioning (structural and nonstructural) vary from 1% to greater than 75% among freshwater populations on Haida Gwaii (Bergstrom and Reimchen 2000). Extreme environmental conditions can impair developmental stability and inflate FA in individuals and populations in a range of taxa (Beardmore 1960; Parsons 1990). Negative correlations between population level FA and habitat quality have been reported (Valentine et al. 1973; Manning and Chamberlain 1993; Lens et al. 2000), but there are inconsistencies among traits and species. Although population level FA in three other traits in *G. aculeatus* (ascending process height, amount of overlap between plate 5 and the ascending process, and amount of overlap between plate 6 and the basal plate), did not show correlations with several habitat parameters on this archipelago (Bergstrom and Reimchen 2002), habitat correlates with lateral plate asymmetry have not been addressed. Plate number asymmetry is reduced in populations residing with predatory trout on this archipelago (Moodie and Reimchen 1976), but we do not know whether the variation in plate asymmetry among populations reflects differences in the importance of the lateral plates to fitness, or differences in abiotic habitat parameters among the lakes.

Here we investigate variation in lateral plate asymmetry among 115 endemic freshwater populations of *G. aculeatus* from the islands of Haida Gwaii. The objectives of this investigation are twofold. First, we determine whether the variation in developmental stability, as seen by the variation in the population frequencies of asymmetry, reflects differences among populations in the importance of structural predator defenses to survival and fitness, or differences in abiotic habitat characteristics. If the former, we predict that the frequency of lateral plate asymmetry will be negatively correlated with the degree of expression of structural defenses among populations of stickleback, as measured by total plate number, degree of overlap between the plates and the bony external spine supports, and cross-sectional diameter. Because water clarity and reaction distance can affect the outcome of predation events in aquatic systems (Vinyard and O'Brien 1976; Reimchen 1989, 1992), we also predict that plate asymmetry will be greater in populations in which visibility, and therefore the chance of capture, is reduced. Second, we determine whether there is evidence of selection acting on lateral plate asymmetry. Within each population, we compare the frequency of lateral plate asymmetry between stickleback with predator injuries and those without, as well as between two age classes (juvenile and adult). This allows us to compare the asymmetry of fish that have successfully escaped from and survived capture by a predator to fish in the rest of the population, as well as to calculate selection differentials for plate asymmetry. Knowledge of the function and sources of selection on lateral plates allows for the determination of whether the geographical distribution of plate FA among populations reflects differences in habitat quality or differences in the biomechanical importance of structural defenses to survival and fitness. This is the first investigation to address the ecological and functional implications of geographical variation in structural defense asymmetry among wild populations.
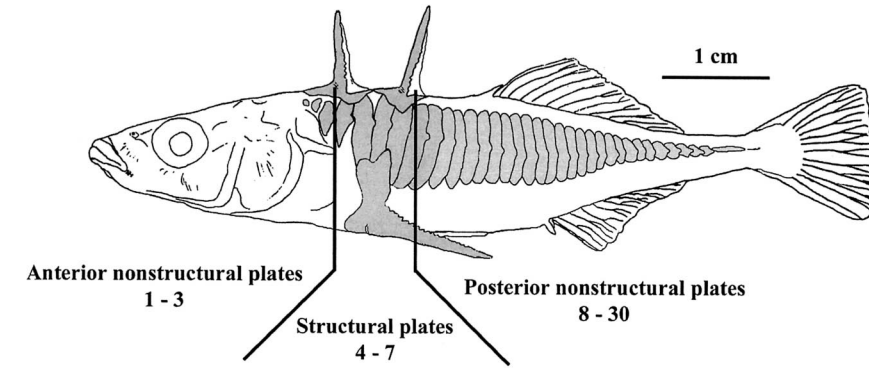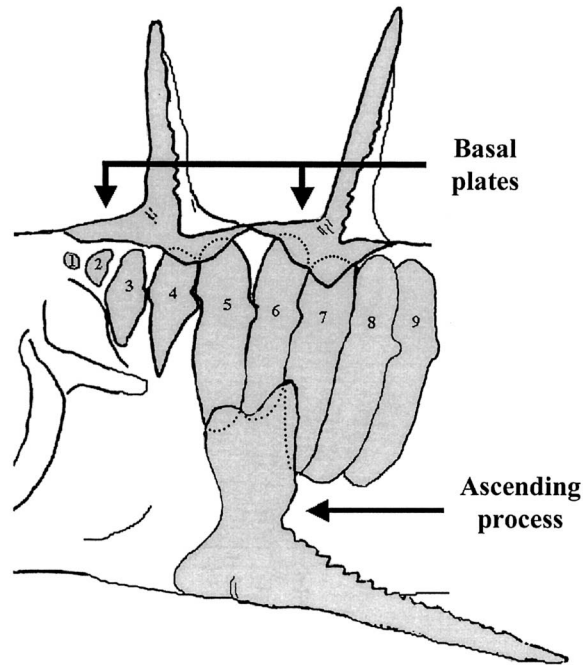
FIG. 1. (A) Camera lucida drawing of a completely plated threespine stickleback. The left pelvic fin has been removed for clarity. The two large dorsal spines, left pelvic spine, structural, and nonstructural lateral plates are highlighted in gray. (B) Close up of the anterior region of a stickleback showing lateral plates one through nine. Dashed lines show the regions of overlap between the lateral plates and the basal plates, as well as between the lateral plates and the ascending process.

MATERIALS AND METHODS

Samples of *G. aculeatus* from lakes and streams throughout the archipelago were obtained during multiple expeditions between 1969 and 1997. Of these original collections, 115 samples were available for this study (this represents 95% of the total number of natural stickleback populations on the archipelago, excluding those from artificial basins). Habitat descriptions and general collecting methods are published elsewhere (Reimchen et al. 1985; Reimchen 1989, 1994b). In summary, the majority of *G. aculeatus* samples was col-

lected 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. Water chemistry and lake data were collected from a subset of the localities at the time of collection. This included pH, percent light transmission at 400 nm (T400), specific conductance (μmhos cm^{-1}), water depth (m), and lake area (hectares). Specific conductance, water depth, and lake area were log transformed to normalize the skew in their distributions.

All localities were categorized as belonging in one of the

three recognized geographical areas on the archipelago: low-lands, plateaus, or mountains (Brown 1968). The lowlands lie in the northeastern region of the archipelago and are characterized by shallow, heavily stained, acidic lakes and ponds, the mountains lie in the southern region of the archipelago and are characterized by deep, clear, open-water lakes of neutral pH, and the plateaus lie in the northwestern and central region and are intermediate between the lowlands and the mountains in these characteristics (Reimchen 1994a,b).

From each sample of *G. aculeatus*, up to 100 individuals comprising approximately 50 juveniles (about 35–45 mm SL) and 50 adults (>45 mm) were measured for standard length (SL), sex, cross-sectional diameter with the spines erect, degree of overlap between the plates and the spine supports (the basal plates and the ascending processes), number of lateral plates per side, and the position of each lateral plate on the right and left. Degree of overlap between the lateral plates and the spine supports was scored as 0 (no contact), 1 (abutting), 2 (partial overlap), or 3 (major overlap; see Reimchen 1983). Lateral plate number for each individual stickleback was scored as the number of plate positions (P1, P2, P3, . . . , P30) with at least one plate present. This was used to determine the mean lateral plate number for each population. Lateral plate number was square-root transformed and cross-sectional diameter was log transformed. All measurements were made by CAB.

To determine whether the lateral plates exhibited fluctuating asymmetry, we calculated the plate number asymmetry for each individual (no. of plates on the right − no. of plates on the left), and then the signed mean plate number asymmetry for each sample of n individuals as follows:

$$[(R_1 - L_1) + (R_2 - L_2) + \cdots + (R_n - L_n)]/n. \quad (1)$$

We determined whether this mean differed significantly from zero by performing two-tailed t-tests for each sampled population. These tests for FA were run only on juveniles to minimize the possible effects of selection on the magnitude or sidedness of asymmetry, which may be evident in older fish. Stickleback with no plates and two samples that were comprised of entirely plateless stickleback (Slim and Sprint) were excluded from further analysis of plate asymmetry. Seventeen samples did not have sufficient numbers of juveniles to run the tests for FA. All of the remaining 96 samples had signed plate number asymmetry means that were not significantly different from zero after sequential Bonferroni corrections. Ten samples had signed means of exactly zero, and of the 86 samples with nonsignificant nonzero means, 43 were positive and 43 were negative. Kurtosis values for the signed plate asymmetry distributions of juveniles for each sample indicated no evidence of bimodality (antisymmetry). For all other analyses, juveniles were grouped with adults unless otherwise noted.

Measurement error is often disguised as FA, and thus can artificially inflate asymmetry estimates (Palmer and Strobeck 1986). Therefore, after initial measurements were complete, 12 localities were randomly chosen and 20 individuals from each were re-measured for number of lateral plates on both the left and right sides. A two-way mixed model ANOVA with plate number as the dependent and individual and side as the factors was used to determine whether the variance

between sides was significantly greater than the variance between replicate measurements. The side-by-individual interaction term was significant ($P < 0.001$), demonstrating that asymmetry variance was significantly greater than measurement error variance (Palmer and Strobeck 1986).

To determine whether one sex was significantly more asymmetric than the other, we compared absolute mean plate number asymmetry between sexes from each locality using two-tailed t-tests. None of the differences was significant at the $\alpha = 0.05$ level after sequential Bonferroni corrections, and there was no significant difference in the number of localities with greater absolute mean plate asymmetry in females than in males (55 vs. 50, respectively; $\chi^2 = 0.24$, df $= 1$; $P > 0.500$). The sexes were therefore pooled for further analysis.

Asymmetry in the presence of plates on the left and right sides at each plate position (P_1 through P_{30}) was also calculated ($R_{P1} - L_{P1}$, $R_{P2} - L_{P2}$, etc.). A fish was considered 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. Plate positions were subdivided into ''structural'' and ''nonstructural''. Structural plates are comprised of plates in positions four through seven, which lie directly beneath the two large dorsal spines and above the pelvic spines (Fig. 1A). Nonstructural plates are those plates anterior and posterior to the structural plates (1–3, and 8–30; Fig. 1A). Therefore, asymmetric stickleback could be classified as having either structural or nonstructural plate asymmetry. A stickleback was considered to have structural plate asymmetry if a structural plate was present on one side but absent on the corresponding myomere on the other side at one or more structural plate positions. Correspondingly, a stickleback was considered to have nonstructural plate asymmetry if a nonstructural plate was present on one side but absent on the corresponding myomere on the other side at one or more nonstructural plate positions.

To determine whether lateral plate asymmetry in these populations is a reflection of abiotic habitat characteristics that might disrupt developmental stability, we ran a multiple regression of the factors pH, percent light transmission at 400 nm (T400), specific conductance (μmhos cm^{-1}), water depth (m), and lake area (hectares) against the population frequency of asymmetric juveniles (structural and nonstructural combined). We used juveniles only, because asymmetry in adults may reflect selection on asymmetry as well as environmental factors that disrupt developmental stability. We combined structural and nonstructural asymmetrical juveniles under the assumption that environmental stress would disrupt developmental stability equally between these two plate regions.

Each stickleback was scored for the presence or absence of injuries left by avian and trout predators, as described in Reimchen (1988). Aquatic avian piscivores and predatory trout are the two major predators of stickleback on this archipelago (Moodie and Reimchen 1976; Reimchen 1994a). Although there are other fish species in these lakes, trout are the primary fish predators that forage on sub-adult and adult stickleback. Injuries were categorized as avian if there were parallel, compressionlike, dorso-ventral directed scars on both sides of the body, and as trout if there were punctures or long comblike antero-posteriad directed scars present.

TABLE 1. Multiple regression analysis showing correlations between population frequencies of asymmetric juveniles and pH, T400, specific conductivity (log-transformed), water depth (log-transformed), and lake area (log-transformed) with and without lateral plate number (square-root transformed) included as a factor. Multiple regression model results without plate number included: $R^2 = 0.25$; $F = 1.46$; df $= 27$; $P = 0.243$. Multiple regression model results with plate number included: $R^2 = 0.61$; $F = 5.49$; df $= 27$; $P = 0.001$.

	Without plate number		With plate number	
	Partial r	P	Partial r	P
Lateral plate number			−0.69	<0.001
pH	−0.45	0.026	−0.14	0.516
T400	0.26	0.217	0.16	0.480
Specific conductivity	0.08	0.729	0.13	0.613
Water depth	0.04	0.858	0.28	0.198
Lake area	−0.22	0.296	−0.36	0.089

RESULTS

Geographic Clines in Population Frequency of Asymmetric Stickleback

The population frequencies of stickleback with lateral plate asymmetries (structural and nonstructural combined) differed significantly among the three geographical areas (Kruskal-Wallis $\chi^2 = 19.78$; df $= 2$; $P < 0.001$), and increased from the mountain to the plateau to the lowland samples. Lateral plate asymmetry was comprised of asymmetry in both non-structural and structural plates, and there were geographical clines in the distributions of population frequencies of both types of asymmetry. The population frequencies of asymmetric fish differed significantly among the three geographical areas for both non-structural (Kruskal-Wallis $\chi^2 = 18.69$; df $= 2$; $P < 0.001$) and structural (Kruskal-Wallis $\chi^2 = 44.59$; df $= 2$; $P < 0.001$) plate asymmetries. Both types of asymmetry demonstrated an increase in population frequency from the mountain to the lowland samples, although the effect was strongest for structural asymmetries.

Habitat Correlates with Population Frequency of Asymmetric Stickleback

Multiple regression of the population frequency of asymmetric juveniles (structural and nonstructural combined) on several environmental variables was performed, and the only significant result was a negative correlation with pH (Table 1). However, lateral plate number may have confounded this effect, as pH and mean number of lateral plates are strongly positively correlated among population samples ($r = 0.66$; $P < 0.001$). When mean number of plates was included in the regression model, it became the only significant correlate with population frequency of asymmetric juveniles (Table 1). Not all samples were included in the multiple regression analysis since pH, specific conductivity, and T400 were only collected at a subset of localities. We had a record of water depth, lake area, and a categorical measure of water staining for all localities, and a three-way ANOVA of these variables on population frequency of asymmetric juveniles demonstrated no significant main effects or interaction terms (all $P > 0.250$), consistent with the multiple regression.
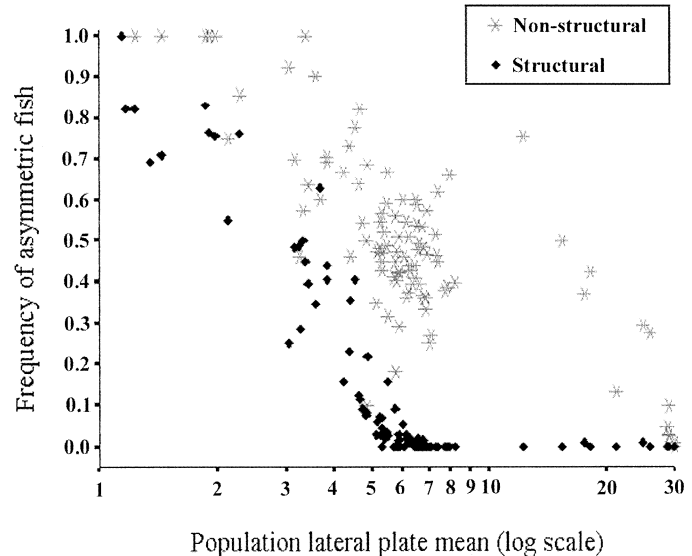

FIG. 2. Negative correlations between lateral plate mean and the frequency of specimens with nonstructural and structural lateral plate fluctuating asymmetry among population samples.

Expression of Structural Defenses and Population Frequency of Asymmetric Stickleback

There was a significant negative correlation between population lateral plate mean and the frequency of asymmetric individuals, including both juveniles and adults ($r = -0.80$; $P < 0.001$). The correlation was significant for both non-structural ($r = -0.70$; $P < 0.001$) and structural ($r = -0.68$; $P < 0.001$) plate asymmetries (Fig. 2). Frequency of non-structural asymmetry gradually decreased from close to 100% to less than 10% with increasing plate number. Frequency of structural plate asymmetry decreased sharply from close to 100% to about 2% as population plate means increased from zero to seven plates per side ($r = -0.95$; $P < 0.001$). In populations with plate means greater than seven, the frequency of structural plate asymmetries remained at or below 2% and no longer decreased significantly with plate number ($r = 0.17$; $P = 0.471$).

Mean cross-sectional diameter and mean lateral plate number were weakly but significantly positively correlated among populations ($r = 0.19$; $P = 0.044$). We removed the effect of plate number, and found that the residual cross-sectional diameter had a significant negative correlation with the frequency of structural plate asymmetry among populations (residual $r = -0.21$; $P = 0.025$) but not with the frequency of nonstructural plate asymmetry (residual $r = -0.01$; $P = 0.902$).

Mean overlap between the plates and the bony external spine supports and mean lateral plate number was also significantly positively correlated among populations ($r = 0.70$; $P < 0.001$). We removed the effect of plate number on mean overlap, and found that the frequency of fish with structural plate asymmetry decreased significantly as residual mean overlap increased among populations (residual $r = -0.60$; $P < 0.001$), but found no significant change in the frequency of nonstructural plate asymmetry (residual $r = -0.06$; $P = 0.540$).
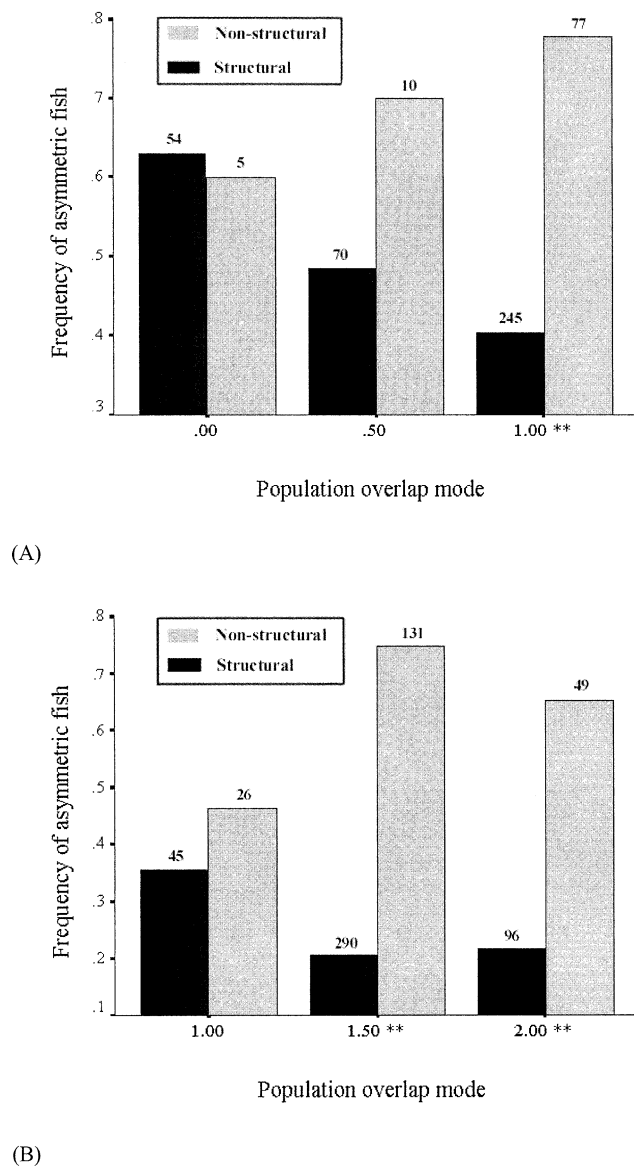
FIG. 3. Frequencies of structurally and nonstructurally asymmetric stickleback among groups of fish that differ in overlap modes from populations with lateral plate means of three (A) and four (B). Numbers above bars designate numbers of all individuals in those populations of particular overlap modes and lateral plate means. Double asterisks indicate $P < 0.001$.

TABLE 2. Means and standard errors of frequencies of asymmetric fish (nonstructural and structural combined) and lateral plate numbers of populations from localities of three different levels of water staining.

Level of staining	Frequency of asymmetric fish Mean ± SE	Lateral plate number Mean ± SE
Heavy	0.59 ± 0.03	3.70 ± 2.03
Moderate	0.49 ± 0.04	8.31 ± 7.65
Clear	0.37 ± 0.03	11.13 ± 8.99

Only populations with plate means of three and four provided suitable sample sizes of both types of asymmetry to make interpretable comparisons. We pooled individuals from populations with the same modal degree of overlap and the same lateral plate mean; these populations all represent the same end point in selection for structural predator defenses. As predicted, at the lowest levels of overlap, the frequency of structurally asymmetric fish was not significantly different from nonstructural asymmetry for either plate mean (overlap modes of 0.0 and 0.5 for plate means of 3, and overlap modes of 1.0 for plate means of 4; all $P > 0.05$; Fig. 3). However, as population overlap mode increased, structural asymmetries became significantly less frequent than nonstructural asymmetries in those individuals from populations of both plate means (overlap modes of 1.0 for plate means of 3, and overlap modes of 1.5 and 2.0 for plate means of 4; all $P < 0.001$).

Water Staining and Population Frequency of Asymmetric Stickleback

We compared population frequencies of plate asymmetry (including both juveniles and adults) among three different categorical levels of water staining that were assigned in the field at the time of collection: heavily stained (T400 < 70%), moderately stained (70% < T400 < 85%) and clear (T400 > 85%). Mean plate asymmetry frequency (structural and nonstructural combined) increased significantly as water staining increased (ANOVA: $F = 12.85$; df = 2; $P < 0.001$; Table 2), and multiple comparisons showed that heavily stained localities contained significantly more asymmetric fish than both moderately stained localities ($P < 0.025$) and clear localities ($P < 0.001$). The increase in plate asymmetry with water staining was evident in both nonstructural (Kruskal-Wallis: $\chi^2 = 21.42$; df = 2; $P < 0.001$) and structural (Kruskal-Wallis $\chi^2 = 43.19$; df = 2; $P < 0.001$) plate asymmetries.

The reduction in frequency of asymmetric stickleback in clear water habitats may have been confounded by population lateral plate mean, as plate number increases significantly from stained to clear water systems (Kruskal-Wallis $\chi^2 = 47.76$; df = 2; $P < 0.001$; Table 2). Populations with plate means of five through seven were present at all three staining levels, allowing us to compare asymmetry among staining levels while minimizing variance in mean plate number. Mean population frequencies of nonstructural plate asymmetry did not change significantly at different degrees of water staining for this lateral plate number range (Kruskal-Wallis: $\chi^2 = 3.98$; df = 2; $P = 0.137$), but population frequencies of structural plate asymmetries did show a signif-

On first principles, we expect the support that structural plates provide to the spines to decrease as the amount of overlap between these plates and the spine supports decreases (Reimchen 1983). Therefore, in populations with low overlap means, we expected to find structural asymmetry frequencies comparable to nonstructural asymmetries, because the biomechanical difference between these two types of plates would be minimized. We calculated the modal degree of overlap for each population, and compared the frequency of all structurally and nonstructurally asymmetric individuals among different population overlap modes using chi-squared tests. We controlled for lateral plate number by doing separate comparisons for each population lateral plate number mean.
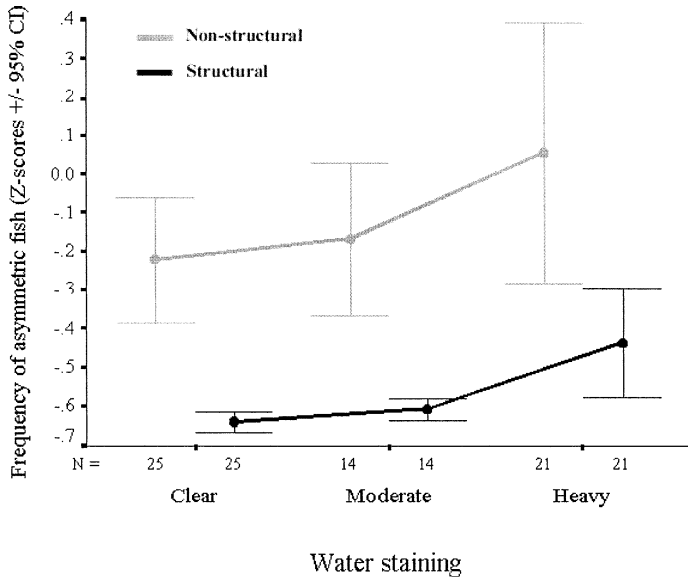
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FIG. 4. Mean population frequencies of nonstructurally and structurally asymmetric fish among different levels of water staining. Includes populations with plate means of five through seven only. Numbers above staining categories represent number of populations. Mean frequency of structural plate asymmetry varies significantly among water staining levels (Kruskal-Wallis $\chi^2 = 15.21$; df $= 2$; $P < 0.001$), whereas mean frequency of nonstructural plate asymmetry does not (Kruskal-Wallis: $\chi^2 = 3.98$; df $= 2$; $P = 0.137$). Nonstructural and structural frequencies are converted to Z-scores in order to place both on a single y-axis.

icant increase from clear to moderate to heavily stained localities (Kruskal-Wallis $\chi^2 = 15.21$; df $= 2$; $P < 0.001$; Fig. 4).

Selection on Lateral Plate Asymmetry

We found no significant differences between the numbers of asymmetric stickleback (nonstructural or structural) with predator-induced scars (avian or trout) and the numbers of symmetric stickleback with predator-induced scars within any population after sequential Bonferroni corrections (all $P > 0.150$). However, significantly more populations had a greater incidence of trout-induced injuries in symmetric rather than structurally asymmetric stickleback than the number of populations that did not (Table 3).

We calculated frequency shifts of symmetric fish between juvenile and adult age classes within each locality. There were no significant differences in the proportion of either structurally or nonstructurally asymmetric stickleback between age classes in any population after sequential Bonfer-

roni corrections (χ^2 tests: all $P > 0.200$). The number of populations that had increases in asymmetry with age was not significantly different from the number that had decreases for either structural asymmetries (32 vs. 28, respectively; binomial: $P = 0.699$) or nonstructural asymmetries (47 vs. 36, respectively; binomial: $P = 0.272$). For those populations that had a greater incidence of trout-induced injuries in symmetric stickleback than structurally asymmetric stickleback, there was no significant difference between the numbers of populations with positive or negative shifts in structural asymmetry with age. There were no significant differences among the three levels of water staining in the mean shift in asymmetry between sub-adult and adult stickleback for structural asymmetries (Kruskal-Wallis: $\chi^2 = 0.08$; df $= 2$; $P = 0.961$) or non-structural asymmetries (Kruskal-Wallis: $\chi^2 = 0.73$; df $= 2$; $P = 0.695$). Among populations within clear and moderate levels of staining, neither structural or nonstructural asymmetry frequencies shifts with age were significantly correlated with plate mean, cross-sectional diameter or mean level of plate overlap (all $P > 0.250$). Among heavily stained sites, there were suggestive but nonsignificant negative correlations between mean shifts in structural asymmetry frequency with age and both cross-sectional diameter and mean level of plate overlap ($P = 0.090$ and $P = 0.067$, respectively). However, as mean lateral plate number increased, there was a significantly greater reduction in the frequency of structural plate asymmetry with age among populations ($r = -0.32$; $P = 0.050$), but no significant change in frequency of nonstructural asymmetry ($r = -0.01$; $P = 0.944$).

DISCUSSION

These results demonstrate that there is a strong cline in the distribution of asymmetry in structural defenses among populations of stickleback from Haida Gwaii, and this cline is correlated with two measures of predation regime: the expression of defensive armor and inferred reaction distance. The northeastern lowlands of the archipelago contain the most asymmetric populations, and are characterized by muskeg bog swamps with low conductivity, heavy water staining, and acidic, shallow waters (Reimchen 1994b). However, the distribution of this cline in asymmetry was not a function of geographical variation in several abiotic factors (T400, conductivity, lake area, or water depth), although there was greater asymmetry in lakes with low pH. This last correlation appeared to be a function of total lateral plate number, because plate numbers are lower in the acidic lowlands and the inclusion of plate number into our multiple regression rendered the association between FA and pH nonsignificant (Ta-

TABLE 3. Numbers of populations that have more symmetric fish with scars versus the numbers that have more asymmetric fish with scars. Includes both avian and trout scars and is segregated into nonstructural and structural symmetries.

	No. of populations with more avian scars on fish that are:			No. of populations with more trout scars on fish that are:		
	Symmetric	Asymmetric	χ^2	Symmetric	Asymmetric	χ^2
Nonstructural	16	19	0.26	33	34	0.02
Structural	16	11	0.93	37	19	5.79*

* $P < 0.025$.

ble 1). This is consistent with another study that also found no correlations between asymmetry in other traits in juvenile stickleback from this archipelago and the same habitat characteristics (Bergstrom and Reimchen 2002). It is possible that there are other abiotic factors in these lakes of which we are unaware that are capable of disrupting developmental stability of stickleback, as studies using other taxa have found evidence that poor environmental conditions can elevate FA (Beardmore 1960; Valentine et al. 1973; Parsons 1990; Manning and Chamberlain 1993; Lens et al. 2000). However, unless environmental predictors of FA in stickleback are identified in future studies, we should use caution when drawing inference of habitat quality based on stickleback FA.

Although the cline in asymmetry on this archipelago did not reflect differences in abiotic conditions, it did reflect differences among populations in the importance of structural predator defenses to survival. The highly asymmetric populations found in the northeastern lowlands of the archipelago also tend to have reduced expression of body armor, a result either of relaxed selection for post-capture defenses (Moodie and Reimchen 1976), direct selection for improved hydrodynamic performance (Reimchen 1992; Bergstrom 2002), or low levels of dissolved calcium (Giles 1983; Francis et al. 1986; Bell et al. 1993). Across the archipelago, frequency of asymmetric fish was positively correlated with three measures of post-capture defenses: mean lateral plate number, mean cross-sectional diameter, and mean overlap between the plates and the spine supports. Populations with greater expression of lateral plate numbers are associated with predation regimes in which the functional importance of structural defenses is high (Reimchen 1994a). Asymmetric fish, especially those with structural plate asymmetries, may be at a functional disadvantage in these predation regimes and therefore may have been selected out of the gene pool during the last 12,000 years. In addition, nonstructural asymmetries are much more common than structural asymmetries within and among populations (Bergstrom and Reimchen 2000). The disparity in frequency between nonstructural and structural asymmetry supports this interpretation since it indicates that, during predator handling, the functional consequences of structural plate asymmetry may be more serious than nonstructural asymmetry (Bergstrom and Reimchen 2000). Presence of structural plates on both sides of the fish is crucial to the predator-deterring effectiveness of the adjoining spines (Reimchen 1983), whereas symmetry at the nonstructural plate positions would presumably have little direct functional impact on post-capture survival.

It is possible that the low asymmetry of structural plates is a correlated effect of selection for high plate counts. During evolution of reduced plate number, plates are lost from the front and rear progressively toward the structural plates near the pelvis. These structural plates are the last to be lost in the evolution of plate reduction (Reimchen 1983). In almost all cases, it is the plates on the anterior and posterior ends of a series that are asymmetric and rarely is a plate missing that is positioned between two others. Therefore, structural plate asymmetry would be uncommon until the evolution of plate loss had progressed to a point at which only the structural plates were still present.

Although this is possible, it does not explain the negative

correlations between structural plate asymmetry and cross-sectional diameter or between structural plate asymmetry and overlap with the spine supports. Both of these measures of structural defenses are important to defensive robustness during predator handling (Hoogland et al. 1957; Reimchen 1991). These correlations were significant and independent of mean lateral plate number, and provide support for our hypothesis that lateral plate asymmetry reflects the degree of expression of structural defenses in stickleback, and potentially the importance of structural defenses to survival and fitness. That we found these effects in structural plate asymmetry but not nonstructural plate asymmetry further supports this hypothesis, because functional morphology has demonstrated that the presence of structural plates on both sides is crucial to the post-capture effectiveness of the dorsal and pelvic spines (Reimchen 1983), whereas the presence of nonstructural plates on both sides is not. In addition, if the low frequency of structural plate asymmetry was the result of the fact that they are rarely on the anterior or posterior ends of a plate sequence, and thus are only a correlated effect of high plate counts, one would expect to find no correlation between the frequency of nonstructural plate asymmetry and plate number since nonstructural plates are always on the ends of a sequence. However, our data demonstrate a significant negative correlation between the frequency of nonstructural asymmetry and mean plate number (Fig. 2)

Directional selection has the capacity to disrupt developmental stability and elevate FA in other taxa (Leamy and Atchley 1985), so it is possible that directional selection for armor reduction in freshwater stickleback could be causing the negative association between plate asymmetry and plate number. However, a multi-trait index of FA in three other characters (ascending process height, amount of overlap between plate 5 and the ascending process, and amount of overlap between plate 6 and the basal plate) in these populations did not show a negative correlation with mean plate number ($r = 0.11$, $P = 0.334$; Bergstrom and Reimchen, unpubl. data), which would be expected if directional selection for armor reduction were generating developmental instability. In addition, if directional selection for plate reduction were disrupting developmental stability and inflating FA, we would expect to see similar levels of asymmetry in both structural and nonstructural asymmetries rather than the strong dissimilarity found in the data. Although unlikely, it is possible that directional selection is responsible for a portion of the asymmetry we find in these populations, and a detailed experimental analysis of the effects of directional selection on FA in this species would help to clarify this issue.

The likelihood of prey capture increases with reaction distance in aquatic systems (Vinyard and O'Brien 1976) and reaction distance can therefore be used as an indirect measure of the importance of post-capture defenses that is independent of prey morphology (Reimchen 1989, 1992). We have provided evidence that structural lateral plate asymmetry is reduced in lakes where the chances of capture by predators is greater. Our results showed an increase in structural plate asymmetry as water staining increased (Fig. 4). Again, this effect persisted even when lateral plate number was controlled for, and was not present for nonstructural plate asym-

metries. The correlation was weak but significant, and it suggests that structural plate asymmetries may be functionally disadvantageous in habitats where the chances of capture by visual predators are high. In heavily stained water with poor visibility, reaction distances are reduced, therefore increasing the chances of escape during the pursuit phase of predation (Reimchen 1989, 1992). The most heavily stained water bodies in this archipelago are in the lowland muskeg bog regions of the northeast, which also contain the most asymmetric stickleback and relatively high avian piscivore activity. In these heavily stained habitats, morphological attributes that enhance fast-start performance may be more important to survival than post-capture structural defenses, and thus selection against asymmetry that compromises the integrity of structural defenses would be relaxed. In clear water with good visibility, the reaction distance is increased as well as the chance of capture, which may result in stronger selection for both the strength and symmetry of post-capture defenses.

Given that the frequency of plate asymmetries in these populations reflected importance of the structural defenses to post-capture survival, we predicted that there may be present-day selection acting against plate asymmetry, and indeed this is supported by the data. Structurally asymmetric fish had fewer trout injuries than symmetric fish in a significantly greater number of populations than those that did not, but there was no effect with nonstructural asymmetries or with avian scars (Table 3). One interpretation of this is that symmetric stickleback are more likely to escape after capture from predatory fish than are stickleback with asymmetry in one or more of their structural plates. This is consistent with experimental work that found a significant decrease in the integrity of the spines when one or more of these structural plates were absent (Reimchen 1983). If the spines are collapsed more easily in stickleback with missing structural plates, gape-limited trout will be more likely to successfully swallow the individual. Our results are also consistent with a previous study of lateral plate number asymmetry among a subset of these populations that found that plate number asymmetry was significantly reduced in localities in which trout predators were present (Moodie and Reimchen, 1976). Avian piscivores are less successful at capturing stickleback than are predatory fish (Bergstrom and Reimchen, unpubl. data), but enjoy relatively high post-capture success rates (Reimchen 1994a), which may result in little to no opportunity for generating selection on post-capture defenses (Vermeij 1982; Reimchen 1992). Therefore, we would expect to see elevated structural plate asymmetry in the lowland northeastern habitats where avian piscivores predominate. This was in fact the case, but at this stage is only a correlational effect. Although the relationship between predator efficiency and strength of selection remains largely unexplored experimentally, it may explain the lack of an association between avian injuries and structural plate asymmetry, especially if spine deflection is largely unrelated to the sticklebacks' chances of escaping handling by a large bird predator.

An additional explanation of the association between trout injuries and structurally asymmetric fish is that the asymmetric fish may be captured less frequently, resulting in lower injury rates. One way to resolve this is to look at asymmetry frequency shifts between juvenile and adult stickleback. If asymmetric fish are being captured less frequently, there should be a general increase in their frequency with age. However, we found no evidence of increase in frequency of asymmetric fish with age overall. To the contrary, in lakes with high levels of water staining, there was a significantly greater *reduction* in the frequency of structural plate asymmetry with age among populations as mean lateral plate number increased; an effect not seen in nonstructural asymmetry. This suggests that fish with structural asymmetries were more likely to be selected out of those populations with greater plate numbers in stained sites. Lateral plate means range from zero to seven in stained sites, with greater population plate means being generally associated with the presence of large predatory fish (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1977; Bell and Richkind 1981). This, combined with the association between trout-induced injuries and structural asymmetries, implicate large predatory fish as potential agents of selection against structural asymmetries in some localities on this archipelago. This appears to be a result of biomechanical weakness in structurally asymmetric fish, because there are no associations between nonstructural plate asymmetries and trout injuries or survivorship.

The lack of correlation between changes in the frequency of asymmetrical structural plates with age and lateral plate mean in moderately stained and clear localities may be a result of the lack of structural asymmetry variance among these sites. There could be two explanations for this. First, reduced visibility in heavily stained sites may reduce reaction distances enough to minimize the magnitude of selection acting on structural defenses. This relaxed selection may have allowed FA in plates to persist even though it is still selected against in the trout-dominated sites with greater plate numbers. Secondly, selection against structural plate asymmetry in relatively clear-water habitats may have been strong enough over the last 12,000 years to reduce asymmetry levels to a state of equilibrium, resulting in shifts in asymmetry frequency between generations that are negligible and undetectable with current methods.

We found no general trend for reduced survival in either structurally or nonstructurally asymmetric stickleback within populations across the archipelago; the overall number of populations with greater incidence of asymmetry with age did not differ significantly from the number of populations with reduced incidence of asymmetry with age. This conflicts with the broad generalization that FA and fitness are negatively correlated (Møller 1997) but is consistent with some other studies of FA in stickleback that have failed to find evidence of reduced survivorship and other fitness measures in asymmetric individuals (Moodie and Reimchen 1976; Bergstrom and Reimchen 2000, 2002; but see Reimchen 1997; Reimchen and Nosil 2001a,b). This may be due in part to variability in the heritability of asymmetry among populations. Concurrent studies show that in very large samples ($n > 10,000$) from two lakes on this archipelago, there is slight directional asymmetry (DA) in lateral plate number for multiple years, with more plates on the left side (Reimchen and Nosil 2001b; Reimchen and Bergstrom, unpubl. data). At smaller sample sizes, the DA is undetectable. This raises the possibility that weak directional asymmetry may be more common among these populations than the sample sizes of

this study allow us to detect, resulting in a mixture of populations ranging from true FA to weak DA. If this were the case, the detection of geographical patterns in selection differentials among populations at one point in time may be nearly impossible, since these two types of asymmetry presumably have different genetic backgrounds (Palmer and Strobeck 1986) and possibly different mechanisms of inheritance. Regardless, the strong correlations between plate asymmetry and the importance of structural defenses to fitness that are found consistently across this archipelago suggest that the overwhelming majority of these populations have responded to determining factors concordantly over the last 12,000 years, despite the lack of large present-day selection differentials.

This study provides evidence that the geographical distribution of asymmetry in structural defenses among natural populations reflects the relative importance of these defenses to survival in different habitats, underscoring the utility of bilateral asymmetry to infer the impact traits have on individual fitness. This demonstrates that fitness reduction in asymmetric individuals can be a direct functional consequence of the asymmetry, in addition to being a reflection of underlying developmental instability. Moodie (1977) found reduced pectoral fin ray asymmetry in stream populations of brook stickleback relative to lake populations, suggesting that pectoral fin ray asymmetry may have more serious hydrodynamic costs in habitats with higher currents, and thus may be subject to selection for increased maneuverability during pursuit by predators. Studies in other taxa have found correlations between symmetry and success for both prey and predator (Møller 1996; Møller and Nielsen 1997; Swaddle 1997b), all of which potentially have biomechanical explanations. It would be interesting to experimentally determine whether trait asymmetry in these studies has direct biomechanical consequences that affect evasiveness of prey or the pursuit and manipulation capabilities of predators. Consistent with the hypothesis that geographical distributions of asymmetry reflect variation in the biomechanical importance of the trait to fitness, we found evidence of selection acting directly against structural plate asymmetry in some habitats. The strong and predictable degree of concordance between geographical variation of asymmetry in structural defenses among more than 100 natural populations and geographical variation in the importance of these defenses to survival is particularly surprising given the ambiguity of previously reported studies of the evolutionary implications of FA. This suggests that subtle asymmetries may be sensitive to selective pressures in the wild, and as such, the distributions of asymmetry in natural populations has potential for use in identifying traits whose biomechanical efficacy are important to fitness.

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