Multi-generation selective landscapes and sub-lethal injuries in stickleback

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Abstract

The interaction between predation landscape and phenotypic variation within prey populations is of substantial significance in evolutionary biology. Extending from several decades of studies at a remote freshwater lake on Haida Gwaii, western Canada, we analyze the incidence of predator-induced sub-lethal injuries in 8,069 wild-captured threespine stickleback (Gasterosteus aculeatus) and using cohort analyses test whether the distribution of injuries informs the selective landscape influencing the bell-shaped frequency distribution of the traits. Our results indicate that (1) the incidence of injuries varies among phenotypes differing in the number and position of lateral plates, (2) these differences occur only among younger fish, (3) the incidence of injuries is inversely related to the estimated population frequencies of plate phenotypes, with the modal phenotype generally having the fewest injuries, (4) direct estimates of selective differentials and relative fitness based on analyses of 1,735 fish from 6 independent yearly cohorts indicates statistically informative elevated differentials in phenotypes with greater number of plates and elevated relative fitness of non-modal phenotypes, and (5) there are significant differences among yearly cohorts in strength and direction of selection, and an increased prevalence of diversifying versus stabilizing selection despite longer-term stasis (4 decades) in trait means. We conclude that the presence of multiple “optimal” phenotypes complements the renewed interests in quantifying short-term temporal or spatial variation in ecological processes in studies of fitness landscapes and intrapopulation variability.

Keywords: fitness, fluctuating selection, Gasterosteus, lateral plates, predator–prey, unsuccessful predation

Introduction

Escape from a predator following the initial stages of capture facilitates the evolution of behavioral or morphological defense adaptations in a prey species. The presence of predator-induced injuries such as wing damage in butterflies (Hale, 1942), shell fractures in gastropods (Vermeij, 1971), tail loss in lizards (Schoener, 1979) or scarring in cetaceans (McCordic et al., 2014) have been widely used as an ecological proxy for spatial and temporal differences in predation rate among prey species (Alexander, 1981; Bateman and Flemming, 2011; Dietl & Alexander, 2009; Edmunds, 1974; Ide, 2005; Lantry et al., 2015; Reimchen, 1982; Schoener & Schoener, 1980; Tyler et al., 2019; Vermeij et al., 1981, review in Vermeij, 1993). Yet there is ambiguity in this interpretation (Sih, 1985). Very young animals commonly have elevated risk of predation due to their small size and lack of defenses and as such, the absence of injuries in these animals is not indicative of low predation rate but rather high predator-induced mortality, sometimes referred to as “the invisible fraction” (Hadfield, 2008). Predators may develop improved capture techniques resulting in different incidence of injuries in the prey over time (Abrams, 1989). Injuries tend to be more common when there are prey defenses (i.e., large body size, armor, toxins) as these often prolong handling time by predators and increase post-capture escape (Vermeij, 1987).

If predation or mortality rate of the prey increases with phenotypic/genotypic distance from the mode, stabilizing selection is probable (Estes & Arnold, 2007; Falconer, 1981; Futuyma, 2009; Haller & Hendry, 2014). Broad recognition and generality of this adaptive peak scenario has led to the view that natural selection is primarily an “optimizing” or “purifying” process (Gould, 1984; Brunet et al., 2021). Under this scenario, and on the assumption that some of the prey escape after capture, the incidence of injuries within age classes should increase with phenotypic distance from the modal phenotype and parallel the probability of capture by the predator. In one of the few morphometric studies assessing these intrapopulation incidence of injuries, lizards with regenerated tails showed increased occurrence of extreme (non-modal) phenotypes indicating these had been attacked at a higher rate than modal phenotypes, although this varied among species (Seligmann et al., 2003). Yet alternatively, rather than injuries on peripheral phenotypes reflecting the presence of stabilizing selection, sub-lethal injuries may be a direct measure of positive fitness as these individuals have been “tested” by natural selection (Vermeij, 1982). If this is true, it could indicate evidence for either diversifying (disruptive) or fluctuating selection, each considered important additional processes facilitating intrapopulation variation (Nosil et al., 2018; Pfenninger et al., 2022, review in Bell, 2010).

The spine-plate complex of the threespine stickleback (Gasterosteus aculeatus) is an important defense adaptation (reviews in Reimchen, 1994; Wootton, 1976, 1984). While spines increase the probability of escape after capture...
by gape-limited piscivores (Hoogland et al., 1957), the bony lateral plates buttress the spines and provide epidermal protection from predator-induced punctures and tears (Reimchen, 1983, 1992a, 1994). Throughout its northern hemisphere freshwater distribution, there is great variability in this complex, both among and within populations (reviews in Bell, 1976; Bell & Foster, 1994; Oslund-Nilsson et al., 2007; Vamosi, 2005). Freshwater stickleback from Haida Gwaii, off the western coast of Canada, vary extensively among populations in the number of lateral plates. Early studies showed that those populations exposed to predatory fish have a mode near seven plates per side with low variance, possibly due to strong stabilizing selection, while those with few or no predatory fish have a reduced number of plates per side (modes near 4, range 0–5) and greater variance, possibly due to relaxed selection (Moodie & Reimchen, 1976). Subsequent studies with greater geographical breadth of sampling confirmed the lateral plate trends but found no evidence for reduction in predation among populations with fewer plates (Reimchen et al., 1985). Avian piscivores such as loons (Gaviiformes) and grebes (Podicephaliformes), less common relative to predatory fish and not previously recognized as important sources of predation, were consuming substantive numbers of adult and subadult stickleback (Reimchen, 1980; Reimchen & Douglas, 1984).

Reimchen (1994, 2000) proposed that puncturing predators (fish) should favor development of post-capture adaptations (i.e., more lateral plates) while pursuit and compression predators (birds) should favor increased burst acceleration (i.e., fewer plates). Extended investigations at one of the isolated Haida Gwaii stickleback populations (Drizzle Lake) was undertaken to identify sources of mortality throughout the life history curve (Reimchen, 1990, 1994) and assess whether they influenced morphological variability in predator defense structures. These studies showed that mean lateral plate number among sub-adult age classes was greater during seasons with mortality primarily from predatory fish, but was lower during seasons with greater importance of predatory birds, consistent with the proposal and suggestive that fluctuating selection had a diversifying effect and was a contributing mechanism to the large variance in the population (Reimchen, 1995). During initial sampling of stickleback in this population, healed predator-induced injuries were identified in 15–30% of the adult age classes (Reimchen, 1988, 2021) and it seemed possible that these injuries might provide insight into relative capture rate or post-capture survival of lateral plate phenotypes. Here, we assume that injuries are a direct result of attacks and we initially test the data against the neutral model that injuries are independent of plate phenotype. However, if injuries are associated with phenotype, we predict that peripheral phenotypes will be subject to greater predation and have higher injury rates than the mode if stabilizing selection is occurring. Alternatively, if injuries reflect positive values of fitness because such phenotypes have survived a predator encounter and have been “tested” by natural selection (Vermeij, 1982), diversifying selection would be implicated. We differentiate these alternatives using direct measures of selection and relative fitness. Non-modal phenotypes should decrease in frequency over ontogeny if stabilizing selection is occurring but increase if diversifying selection is occurring.

Methods

Study area and sampling protocol

The Drizzle Lake watershed is on an expanse of raised bog on the north-eastern region of Haida Gwaii and is protected as an Ecological Reserve (details in Reimchen & Douglas, 1980, 1984). The dystrophic lake (110 ha) has four resident fish species: Threespine Stickleback, Cutthroat Trout (Oncorhynchus clarkii), Dolly Varden (Salvelinus malma) and juvenile Coho Salmon (O. kisutch). The cutthroat trout is the major predator on juvenile and sub-adult stickleback (Reimchen, 1990). There are also multiple species of avian piscivores, predominantly Common Loon (Gavia immer) and Red-necked Grebe (Podiceps grisegena). The former is the major avian predator on adult and sub-adult stickleback (Reimchen & Douglas, 1984). Population size of adult and sub-adult stickleback (60–90 mm standard length [SL]) is estimated at 580,000 (Reimchen, 1990). The general natural history and predator assemblage of this locality is broadly representative of temperate lakes although actual taxonomic assemblages vary geographically (Reimchen, 1994, 2021). Stickleback were sampled (1980–1983, 1985–1990, 2006, 2011–2013, 2015–2016) with minnow traps baited with 1cc old cheddar cheese (total N = 8,069). For each collection prior to 2006, all fish with any evidence of injury as well as an equivalent sub-sample of uninjured fish were retained (details in Reimchen, 1995) while from 2006 onwards, sampling effort was greatly reduced but all fish (injured and uninjured) were retained. Fish were anesthetized with MS-222 or Clove Oil and preserved in 10% formalin and later transferred to isopropanol alcohol or ethanol. Collections were made under the Ministry of Environment (Gov’t of British Columbia) permits (SM09-51584 and SM10-62059) and University of Victoria Aquatic Unit facility Standard Operating Procedure OA2003.

Measurements

We measured standard length (anterior edge of the jaw to the posterior edge of the caudal peduncle ±1mm), sex (examination of gonads), number and position of left lateral plates, length of the ventral plate of the pelvic girdle, and skeletal Measurements


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Cohort analyses

Although SL is usually sufficient to determine general age on stickleback with a 1 to 3 year life span (Baker, 1994), this becomes more difficult in the current study population in which adult fish can live to 8 years and growth rates are largely asymptotic after 4 years (Reimchen, 1999). In a subset of adult fish in the samples (N = 5,611), the pelvic spines were removed, decalcified, embedded in plastic, sectioned, and stained (ibid) from which age in years was determined. As fish were collected in sequential years, this allowed identification to hatching year and therefore assignment to respective yearly cohorts. Twenty-two cohorts were identified from 1978 to 2014, six of which (1978–1983) had much larger sample sizes that allowed partitioning among lateral plate phenotypes, sexes and age classes. Incidence of predator-induced injuries increases with body size (SL) in this population (Reimchen, 1988) that may confound analyses of any defense trait that also covaries with SL. As such, for most analyses, the samples were split into two age classes: younger (≤3 years) and older (>4 years) to give, where possible, similar numbers in each age group. The two size classes approximately separated fish above or below 75 mm SL, and this size corresponds to the position on the curve that approaches the size by age asymptote (Reimchen, 1999).

Estimates of selection were evaluated using phenotype frequencies within the two age classes in these six cohorts (1978–1983). We used multiple methods to evaluate the cohort data. Initially, we used the complete dataset that comprised all uninjured and injured fish in the samples. As all injured fish had been explicitly retained in the field samples, their relative frequency was inflated relative to uninjured fish in the samples. In order to reduce bias that this might entail, second, we re-analyzed all uninjured fish and a random sample of injured fish, thus approximating the percentage of injured fish in the lake population (Reimchen, 1988, 2021). We chose to do a 25% random sample as this was representative of the frequencies of injured adult fish randomly collected from the population (Reimchen, 1988, 2021). We refer to this as the primary dataset as this is the major data used in the analyses. Third, to further exclude the potential bias from the use of any injured fish, we analyzed the data only for uninjured fish. In each of these three separate data partitions, selection estimates were made for sexes combined (to maximize sample size) and for sexes separate. We first treated lateral plate number (LPNUM) as a continuous metric and tested for directional selection (i) as well as variance selection (j). Second, we binned the 18 lateral plate position (LPPOS) phenotypes into five categories: LPPOS 5-7 (includes also small numbers of fish with 2 or fewer plates-2le), LPPOS 3-7 (includes 4-8), LPPOS 3-8 and LPPOS X (includes all rare phenotypes with four or more plates including P4odd, P5odd, P6odd, P7odd, each with a plate missing in the normally continuous series, and 8ge, which is fish with eight or more plates). We determined fitness of each of these four phenotypes relative to the modal phenotype (LPPOS 4-7).

Directional selection (i) and significance levels (t) were measured based on Endler (1986, eq. 6.1, 6.3):

\[ i = \frac{(X_a - X_b)}{\sqrt{t}} \]

where \( X_a \) is the mean frequency after selection (older fish), \( X_b \), mean frequency before selection (younger fish) and \( V_a \), the variance before selection.

Relative fitness (w) and variance were calculated based on Manly (1985, eqs 2.18, 2.21):

\[ w = \frac{(\text{var} - \text{var}_b)}{\text{var}_b} \]

where var is the variance of the trait after selection, \( \text{var}_b \) the variance before selection, \( N_a \) the sample size after selection, and \( N_b \) the sample size before selection.

All statistical analyses (Generalized Linear Model, ANOVA, Contingency Tests, Log-Linear, Spearman’s Rank test, Binomial tests, t-tests, F-ratio tests) were carried out using SPSS version 27/28 and unless noted otherwise, the sexes were separated for analyses and split between younger and older age classes. Incidence of injuries were assessed for associations with (1) total number of left lateral plates (LPNUM), (2) cumulative plate position phenotypes (LPPOS), and (3) presence/absence of a plate for each sequential myomere 1 through 10. Due to the low sample size of uncommon phenotypes, these were binned with adjacent LPNUM or LPPOS phenotypes. We did not adjust p-values for multiple comparisons, but rather considered each analysis as a separate inference, and focused on
Results

Lateral plates and incidence of injuries

Lateral plate number (LPNUM)

Frequency distributions of LPNUM indicates that 86% of the fish have from four to six plates with a mode at 5 and with a weak left skew (−0.06; Figure 1). Mean number of plates were similar between the sexes (male: $\bar{x} = 4.78$, SD = 1.08; female: $\bar{x} = 4.75$, SD = 1.08, $F_{1,4030} = 1.9$, $p = .17$). Incidence of injuries varied among LPNUM phenotypes (Figure 2). Younger males with intermediate number of plates (4) had lowest incidence of injuries, which progressively increased in phenotypes with fewer or greater number plates (GLM, Walds Chi = 12.3, df = 5, $p < .04$). For older female, those with the fewest plates had lowest injuries, which increased with greater number of plates (GLM, Walds Chi = 11.9, df = 5, $p < .04$). For older fish in both sexes, injuries were about twice as abundant compared with younger fish but there were no statistical associations between LPNUM and injuries (Male, Walds Chi = 3.2, df = 5, $p = .67$; Female: Walds Chi = 0.64, df = 5, $p = .99$). Partitioning the data to include only fish injured with aviscars also showed higher incidence of injuries with greater number of plates and strong similarity between the sexes for younger age classes. There was no effect for older age classes.

The cumulative data were partitioned among 6 yearly sampling periods (1980–1981, 1982, 1983–1984, 1985, 1986–1990, 2006–2016) to determine whether there was any temporal consistency in these relationships. For younger males, the interaction between injury and LPNUM persisted (Walds Chi = 10.5, df = 3, $p < .02$), but only approached significance in females (Walds Chi = 6.9, df = 3, $p < .08$), and there were no significant differences among years ($p > .3$ in both sexes). Among older fish, there was no evidence for any association between injury and LPNUM ($p > .35$ in all cases).

Since the relationship between plate number and injury was significant for the younger age classes, and since body size (SL) is positively correlated with incidence of injuries (Reimchen, 1988), we re-analyzed the data using SL as a co-variante within each of the two age classes. The results removed any statistical support for the injury/LPNUM association (younger males: Walds Chi = 0.79, df = 3, $p = .85$; younger females: Walds Chi = 1.38, df = 3, $p = .71$; older males: Walds Chi = 3.93, df = 3, $p = .27$; older females: Walds Chi = 0.32, df = 3, $p = .96$) but yielded a highly significant contribution to SL ($p < .001$ in all cases) indicating co-variation between LPNUM and SL. Consequently, we examined whether uninjured LPNUM phenotypes differed in SL within individual year classes. For 1-year-old individuals in both sexes, phenotypes with more plates had a marginally larger SL than those with fewer plates (males, 2% increase, $F_{1.340} = 2.5$, $p < .06$; females, 5% increase, $F_{1.31} = 6.5$, $p < .001$), but there were no trends in any of the remaining age classes (ages 2–5, ANOVA, all $p > .5$). We also examined whether the size of the pelvic plate (size-corrected by using regression residuals against SL) was associated with lateral plate phenotype for each year class. There was no effect of LPNUM * AGE interaction for males ($F_{5.798} = 0.89$, $p = .53$) or for females ($F_{5.792} = 0.78$, $p = .62$). All multiple range comparisons were not significant ($p > .1$).

Lateral plate position (LPPOS)

Using the pooled samples, fish were categorized into 18 phenotypes according to the position and number of left lateral plates and analyzed for relative frequencies of injured individuals. Of the 18 phenotypes, four (P4–P7, P3–P7, P4–P8, P3–P8) accounted for the majority of fish (76%), of which P4–P7 was the modal phenotype that has the four major buttressing plates (Reimchen, 1983), and with a rank abundance that was largely identical for the two sexes (Figure 3). For younger males (Figure 3A), all nonmodal phenotypes had a higher incidence of injuries than the modal phenotype; 7 of the 17 differences reached statistical significance ($p < .05$). Differences in injuries occurred even among phenotypes that had the same or similar number of plates. For example, P4odd has 4 plates that are not in sequential positions and this phenotype had significantly more injuries than the modal phenotype with four plates in sequential positions.
The second most common phenotype in the population (P3–P7), differing from the modal phenotype by the presence of the third plate above the cleithrum, had significantly more injuries than the modal phenotype. The highest incidence of injuries (>60%) occurred in the least common phenotypes, which usually had six or more plates. For younger females (Figure 3B), trends were generally similar to those of younger males in that 13 phenotypes (rarest phenotypes grouped) had higher incidence of injuries than the modal phenotype, four of which reached statistical significance (p ≤ 0.05). As well, P3–P7, the second most common phenotype, had significantly more injuries than the mode, while the highest frequencies of injuries (>70%) occurred in phenotypes with six or more plates that have non-continuous (odd) sequences. In females, the only two phenotypes (2le, 4–6) that exhibited lower injuries than the mode were each missing one or more of the 4 buttressing plates. Overall, among the younger fish, the frequency of injuries was inversely proportional to the population frequency of the phenotypes (Spearman’s ρ = −0.50, p = .002), this effect being much larger in males (Spearman’s ρ = −0.76, p < .001) than in females (Spearman’s ρ = −0.28, p = .27). Among older males (Figure 3C), there was little evidence for associations between injury frequencies and plate phenotypes. Eight phenotypes had marginally higher and six had marginally lower incidence of injuries relative to the modal phenotype, one of which (P5–P8) reached statistical significance with a deficiency of injuries. Among older females (Figure 3D), 6 phenotypes had a marginal excess of injuries and 10 had a marginal deficiency relative to the mode, of which the single phenotype (P5odd) reached significant differences but with a deficiency relative to the mode. Among the older fish in both sexes, there was no overall relationship between injuries and phenotype (Spearman’s ρ = −0.1, p = .6), with only a weak negative association in males (Spearman’s ρ = −0.34, p = .18) and a weak positive association in females (Spearman’s ρ = 0.26, p = .32).

Because SL differed among uninjured LPNUM phenotypes at least for AGE 1 fish, we also examined whether this

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**Figure 3.** Frequency distribution of lateral plate position phenotypes (LPPOS) in the population separated for sex and age class, (a) younger males ≤3 years age, (b) younger females ≤3 years age, (c) older males ≥4 years age, (d) older females ≥4 years. Phenotypes ranked inverse to their population frequency. Solid symbols show percentage of predator-induced injuries for each phenotype. Horizontal dashed line indicates frequency of injury for shaded modal phenotype (4–7). Value adjacent to each symbol is the probability from a 2 × 2 Pearson’s Chi-square test or Fisher’s Exact test comparing incidence of injuries in each phenotype relative to 4–7. Rare position phenotypes: 2le- less than or equal than two plates, 4odd-usually 3–7 with one plate missing within the series, 5odd- usually 3–8 with one plate missing in the series, 3odd- usually 4–7 with one plate missing in the series, 6odd- usually 2–8 with one plate missing in the series, 7odd- usually 2–9 with one plate missing in the series, ge8- usually 2–9 or greater number of plates. The three least common phenotypes (3–6, ge8, 1–7) were pooled to increase minimum cell size (>10) and probability based on the pooled values. Plate positions can be derived from Figure 1 (inset).
association occurred for LPPOS phenotypes. There were no significant associations in any year classes for either sex (1 year old: males \( F_{1,135} = 1.3, p = .25 \), females \( F_{1,212} = 1.1, p = .39 \); 2 years old: males \( F_{1,152} = 1.6, p < .1 \), females \( F_{1,379} = 1.5, p = .13 \)); 3 years and older, all \( p > .5 \) for both sexes). As well, size-corrected pelvic length did not differ among LPPOS phenotypes (male: \( F_{1,272} = 1.28, p = .22 \), LLPOS*AGE: \( F_{1,272} = 0.93, p = .36 \); females, \( F_{1,604} = 0.46, p = .95 \), LLPOS*AGE: \( F_{1,604} = 0.53, p = .98 \)). Although even further partitioning of the data might yield associations, the current analyses implies that the differential injury rates on LPPOS phenotypes cannot be ascribed to correlated effects with SL or size-corrected pelvis.

### Presence/absence of plates

Presence or absence of a plate at each position (#1 thru #10) provides an additional comparison of injured and uninjured fish. In younger males (Figure 4A) and younger females (Figure 4B), injuries were associated with presence of anterior (#1, #3) and posterior (#9, #10) lateral plates. In younger males, positions #3 and #6, which are two of the spine-buttressing positions (see inset), also showed significant differences in incidence of injuries but in these positions, elevated injuries occurred when the plates are absent. Younger females did not show this pattern but had a significant reduction in injuries where plates were absent at #7. Older males (Figure 4C) and older females (Figure 4D) showed no trends and no significant associations between injuries and positions for individual positions except for the most posterior position (#10), rarely occupied by a plate, which exhibited significantly fewer injuries when the plate was present.

### Selection differentials and relative fitness

Lateral plate phenotypes in six yearly cohorts (1978–1983) were assessed for evidence of directional and variance selection between younger and older age classes. We present results for the primary dataset: to check for consistency, we also show the results for the complete dataset and for the subset of uninjured fish. In the primary dataset, three of the six cohorts (1979, 1981, 1982) had statistically significant positive directional (i) differentials, with an average 4.4% increase in mean plate count (Figure 5A, Table 1). When partitioned by sex, both males and females showed the same trends although with some deviation with respect to significance (Figure 5B, Table 2). With all cohorts pooled, there was a significant positive directional differential for sexes combined (Figure 5A, Table 2) and when separated for sexes, this remained significant for females and weakly positive for males (Figure 5B, Table 2).

These results were largely consistent for the complete data set and for the subset of uninjured fishes. For the complete dataset (all injured and uninjured with sexes combined), two of six cohorts (1978, 1981) had statistically significant positive directional selection differentials (1978, 1981), both showing an approximate 3% increase in mean plate count over ontogeny while a single year (1980) showed a weak non-significant negative directional differential (Supplementary Figure 1, Supplementary Table 1). Separating the sexes (Supplementary Figure 2, Supplementary Table 2) indicated relative consistency with these associations for both sexes, with four significant positive directional differentials (1979, 1981, 1982) and with each sex showing a weak but non-significant negative differential in 1980. For the subset of data containing only uninjured fish, one of the six directional differentials (1982) was significant and positive, with similar trends for both sexes in 1982, although 1978 had significant positive differentials for females only (Supplementary Figure 3, Supplementary Tables 3 and 4).

### Variance selection

Variance selection (j) in the primary dataset (Table 1) indicated significant stabilizing selection in 1978 and diversifying selection in the remaining cohorts, of which 1980 reached statistical significance. The combined cohorts showed significant diversifying selection. When partitioned by sex (Table 2), the directionality of trends were similar but significant levels occurred only for females in 1978 (stabilizing) and 1980 (diversifying). With all cohorts combined, there was overall diversifying selection for each sex but neither was significant. These variance selection differentials were largely consistent with the complete dataset with sexes combined and separated (Supplementary Tables 1 and 2). In the subset containing only uninjured fish with sexes combined (Supplementary Table 3), 1979 and 1980 both showed significant diversifying selection while with sexes separated, all cohorts of both sexes showed diversifying in direction apart from 1978 (Supplementary Table 4).

The fitness of the lateral plate position phenotypes relative to the modal phenotype (LPPOS 4-7) varied in magnitude and direction, varied among cohorts, and to a lesser degree varied between sexes. In the primary dataset with sexes combined, the 1982 cohort showed the most consistent trend where each of the non-modal phenotypes exhibited higher fitness than the modal phenotype, although only LPPOS 3-7 and LPPOS X reached significance (Figure 6A, Table 3). LPPOS X also showed a significant positive fitness in 1981. Cohorts 1978 and 1980 tended to showed minimal fitness differences from the modal phenotype apart from LPPOS 3-7 which showed a significant fitness reduction in 1980. With cohorts combined, each of the non-modal phenotypes showed positive fitness, and LPPOS 3-8 and LPPOS X reached significance. Separating the sexes (Figure 6B, Table 4) yielded a relatively consistent trend between the them with occasional (e.g., LPPOS X, 1983) nonsignificant opposite trends relative to the modal phenotype. With cohorts combined, females exhibited significantly elevated fitness relative to the mode in three of the four phenotypes and in all cases exhibited higher fitness differences than do males.

These trends in relative fitness were largely consistent with the two other datasets, both of which tended to show elevated fitness in non-modal phenotypes in most cohorts but with occasional (1980) reversals with higher fitness of modal phenotypes (Supplementary Figures 1–3, Supplementary Tables 5–8).

Overall positive directional differentials of higher plated phenotypes relative to the mode (LPPOS4-7) among multiple cohorts (1978–1983) implies directional selection and a presumed gradual increase in mean number of lateral plates over time. To test this, we examined mean LPNUM for 22 yearly cohorts (1975–2014), and while there were significant differences among cohorts \( F_{21,651} = 2.6, p < .001 \), including a reduction in the 1981, 1988, and 2013 relative to adjacent years (LSD; \( p < .05 \)), there was no overall directionality in mean LPNUM over the four decades (Figure 7). Modal LPPOS phenotype (P4–P7) over this time period shows general stability in frequencies as well.

In sum, our data demonstrate that (1) incidence of injuries differs among lateral plate phenotypes, (2) these differences occur...
among younger rather than older age classes, (3) the incidence of injuries is inversely related to the population frequencies of the phenotypes, with the modal phenotype generally having the lowest injuries, (4) analyses of ontogenetic changes in phenotype frequencies within each of six yearly cohorts demonstrate multiple instances of directional selection favoring higher number of plates, (5) relative fitness of non-modal phenotypes is on average higher than modal phenotypes, although this can switch to lower fitness within individual cohorts, (6) the strength of selection and relative fitness tends to be accentuated in females relative to males, and (7) the intrapopulation variability is influenced by a complex selective landscape of directional, diversifying and stabilizing selection but overall, relative stasis in population means is evident between the earliest and latest of the 22 cohorts.

**Discussion**

The rationale for this study was to assess the extent to which predator-induced injuries impact intrapopulation variability in defense traits of prey, specifically the lateral plates of threespine stickleback. These plates have a typical bell-shaped frequency distribution, comparable to the variability in most quantitative traits in most species. Natural selection acts on and shapes genetic and phenotypic variability within and among populations across taxa (Endler, 1986). Our understanding of the mechanics of this process in populations has improved considerably since the explicit models of ecological genetics were formalized in the mid-1900s (Ford, 1964), and then interpreted in the context of niche space partitioning among population subsets (Levene, 1953) and the maintenance of intrapopulation morphological variation (Van Valen, 1965). After establishment of some widely applied methods to estimate selection (e.g., Endler, 1986; Lande & Arnold, 1983), there have been a plethora of examples of selection in wild populations (Kingsolver et al., 2001; Stinchcombe et al., 2008). Despite this, there is still not a comprehensive understanding of one of the classic questions in evolutionary biology: how genetic variability is maintained in
the face of selection (Johnson & Barton, 2005). One potential answer to this question is that population selection landscapes are not static, but instead are fluctuating moving targets over space and time, generating changes in type of and direction of phenotypic selection (Fisher & Ford, 1947; Slatkin and Lande, 1976; Pelabon et al., 2010; Reimchen et al., 2019). However, quality datasets demonstrating how selection can fluctuate within a single population remain scarce, especially those that link selection to a causal ecological factor, although there has been a recent resurgence of efforts to do so across spatial (Marrot et al., 2021; Taverne et al., 2021; Van Buskirk & Smith, 2021) and temporal scales (Bonnet & Postma, 2018; Reimchen, 1995; Reimchen & Nosil, 2002; Siepielski et al., 2009). Our data on predator-induced injuries identify a complex interplay between phenotype and fitness that varies over time and is more consistent with diversifying selection in its broad context.

Total number of plates has been the predominant classification method of these heritable defense structures (reviews in Bell & Foster, 1994; Wootton, 1984). There are reasons to suspect that this common classification obscures functionally relevant differences among phenotypes that have the same number of plates in atypical positions (Reimchen, 1983). We considered three classifications of the lateral plate defense system: (1) total number of plates (LPNUM), (2) plate positions (LPPOS), and (3) presence/absence of a plate at individual myomeres. These are not independent of each other but provide slightly different perspectives on lateral plate function and variability. We initially tested our injury data against the neutral model, that injuries would be independent of number or positions of lateral plates. Among older fish in both sexes, we observed little evidence for differential injuries among phenotypes, consistent with the neutral model and suggestive that predators are capturing the different plate number phenotypes randomly. In contrast, among younger fish, which as a group have fewer injuries, there were significant differences in injuries among plate phenotypes for both sexes, trends that were relatively consistent among cohorts and among the three partitions of the dataset. As such, we reject the neutral model for younger age classes and focus our discussion on these fish.

Our results show higher incidence of injuries in non-modal phenotypes; this is accentuated in peripheral and uncommon phenotypes. We predicted that predator-induced injuries should be lowest on fish with the fewest plates due to several processes, (1) that these fish would have the best burst acceleration (i.e., low capture rates) and (2) that if captured would have the poorest defense apparatus (i.e., increased consumption rate by the predator). The corollary of this was that injuries would be highest in fish with the greatest number of plates due to poorest acceleration (i.e., high capture rates) and higher escape and survival rates if captured. Our data indicate mixed support for these predictions. For younger age classes for both males and females, there were significantly more injuries among LPNUM phenotypes with greater numbers of plates, consistent with the prediction. As well, including only injuries of clear avian origin indicates the lowest capture rate of phenotypes with the fewest plates and highest capture rate of those with the most plates, also consistent with the prediction. However, younger males showed a bimodal distribution of injuries, in which phenotypes with

![Figure 5](https://academic.oup.com/evolut/article/77/10/1101/7049771)

**Figure 5.** Selective differentials (i) for LPNUM over six cohorts (1978-1983). A: sexes combined. B: sexes separate. *- statistically significant differentials (t-test). Horizontal bar on y-axis shows mean value of i based on cumulative cohorts. Positive i indicates selection for greater LPNUM. Cohorts represent year of hatching.

### Table 1

Mean number (x̄) of lateral plates separated for cohorts (1978–1983), age class (younger/older) and sexes combined with directional selection (i) and variance selection (j) (see Methods) for Primary data.

| Cohort | Younger | | Older | | | i | | | j |
|--------|---------|----------------|--------|----------------|--------|--------|--------|--------|
|        | x̄      | N   |   | x̄      | N   |   |   |   |   |
| 1978   | 4.76    | 88  |   | 4.68    | 111 |   | -.06 | | -.42 |
| 1979   | 4.67    | 213 |   | 4.84    | 149 |   | .17  | | .29  |
| 1980   | 4.73    | 558 |   | 4.67    | 120 |   | -.06 | | .35  |
| 1981   | 4.55    | 436 |   | 4.78    | 203 |   | .23  | | .10  |
| 1982   | 4.74    | 277 |   | 4.95    | 162 |   | .21  | | .24  |
| 1983   | 4.79    | 137 |   | 4.79    | 136 |   | .00  | | .17  |
| All    | 4.69    | 1735|   | 4.79    | 855 |   | .10  | | 1.1  |

*Note. N = sample size. Significant values (p < .05) are shaded. Cohorts represent year of hatching.*
the lowest and highest plate number had higher incidence of injuries than intermediate phenotypes. Splitting the pooled data into 6-year class groups indicated no significant differences among years.

These results appear to support opposite interpretations: both stabilizing and diversifying selection are demonstrated.

If injuries reflect negative effects on fitness (fishes have been captured at a higher rate), then stabilizing selection is indicated, a major process facilitating the removal of sub-optimal phenotypes in natural population (Bell, 2015; Endler, 1986; Futuyma, 2009). If injuries reflect positive fitness effects (fishes have been “tested” by natural selection as proposed by Vermeij, 1982), then diversifying selection is indicated with a broader fitness curve and maintenance of variability. Our direct measures of selection differentials (\(i\)), variance selection (\(j\)), and relative fitness (\(\bar{w}\)) from cohort analyses largely support diversifying selection as the major process in this population although there is some temporal variability. Three cohorts (1979, 1981, 1982) each showed significant positive differentials for increased number of plates, while the remaining three (1978, 1980, 1983) showed weak negative differentials or no effect. These differentials averaged 0.1 (max = 0.32) and were comparable to the most common differentials (\(<0.2\)) but were much lower than those for the average differentials (0.59) observed in natural populations of diverse taxa (summary in Endler, 1986). Such directional selection should be associated with reduction in variance (Falconer, 1981); however, while we observed significant stabilizing selection in 1978, diversifying selection was observed in the five remaining cohorts, of which 1980 reached significance. The results for relative fitness estimates are consistent with differentials as each of the four non-modal phenotypes usually had elevated fitness relative to the mode in each cohort, with the exception of reduced fitness in one of the phenotypes in 1978 and 1980. These trends were also present in the complete dataset, which had over-representation of injured fish, as well as the subset of data with only uninjured fish (see Methods). Our data add to the broader base of studies that variance-increase (diversifying selection) is much more common than previously assumed, can be as prevalent as variance-reduction

### Table 2. Mean number (\(\bar{x}\)) of lateral plates separated for cohorts (1978–1983), age class (younger/older) and sex (1-male, 2-female) with directional selection (\(i\)) and variance selection (\(j\))(see Methods) for Primary data.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Sex</th>
<th>Younger</th>
<th>Older</th>
<th>(i)</th>
<th>(j)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\bar{x})</td>
<td>N</td>
<td>(\bar{x})</td>
<td>N</td>
</tr>
<tr>
<td>1978</td>
<td>1</td>
<td>4.74</td>
<td>47</td>
<td>4.80</td>
<td>35</td>
</tr>
<tr>
<td>1979</td>
<td>1</td>
<td>4.75</td>
<td>128</td>
<td>4.82</td>
<td>67</td>
</tr>
<tr>
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<td>1981</td>
<td>1</td>
<td>4.57</td>
<td>224</td>
<td>4.81</td>
<td>110</td>
</tr>
<tr>
<td>1982</td>
<td>1</td>
<td>4.78</td>
<td>155</td>
<td>5.01</td>
<td>68</td>
</tr>
<tr>
<td>1983</td>
<td>1</td>
<td>4.81</td>
<td>96</td>
<td>4.59</td>
<td>46</td>
</tr>
<tr>
<td>1978</td>
<td>2</td>
<td>4.78</td>
<td>41</td>
<td>4.62</td>
<td>76</td>
</tr>
<tr>
<td>1979</td>
<td>2</td>
<td>4.55</td>
<td>85</td>
<td>4.85</td>
<td>82</td>
</tr>
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<td>1980</td>
<td>2</td>
<td>4.70</td>
<td>213</td>
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<td>56</td>
</tr>
<tr>
<td>1981</td>
<td>2</td>
<td>4.53</td>
<td>212</td>
<td>4.75</td>
<td>93</td>
</tr>
<tr>
<td>1982</td>
<td>2</td>
<td>4.69</td>
<td>122</td>
<td>4.90</td>
<td>94</td>
</tr>
<tr>
<td>1983</td>
<td>2</td>
<td>4.87</td>
<td>67</td>
<td>4.81</td>
<td>64</td>
</tr>
<tr>
<td>All</td>
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<td>4.72</td>
<td>995</td>
<td>4.81</td>
<td>390</td>
</tr>
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<td>All</td>
<td>2</td>
<td>4.65</td>
<td>740</td>
<td>4.77</td>
<td>465</td>
</tr>
</tbody>
</table>

Note. \(N\) = sample size. Significant values \((p < .05)\) are shaded. Cohorts represent year of hatching.

**Figure 6.** Fitness \((\bar{w}-1)\) of lateral plate position phenotypes (LPPOS) relative to modal phenotype (P4–P7). A: sexes combined. B: sexes separate. * statistically significant relative fitness (Chi-square). Horizontal bars on y-axis show mean value of \(\bar{w}\) for males and females based on cumulative cohorts. Positive \(\bar{w}-1\) indicates higher fitness relative to mode (P4–P7). Cohorts represent year of hatching.
in natural populations (Kingsolver & Diamond, 2011) and can be driven by predation pressure (Caldwell, 1982; Martin & Pfennig, 2009; Moredo-Rueda, 2009).

Frequency-dependent selection by visual predators can lend fitness advantage to rarer prey phenotypes (Allen, 1988; Olendorf et al., 2006) and thus be a diversifying agent. However, this is due to the development of search images by predators for common phenotypes. If this were taking place in this study we would expect to find fewer injuries in peripheral phenotypes and more injuries in the most common modal phenotypes, opposite to what our data demonstrates.

We were able to quantify number of plates and frequencies of the modal phenotype over 22 cohorts (1978–2014). Despite the presence of directional selection favoring greater plate number, the prevalence of diversifying rather than stabilizing selection, as well as temporal variability in these metrics, there was no overall evidence of directional evolutionary change over this time frame. Time scale can affect the detection of selection-driven phenotypic changes in natural populations; changes on shorter time scales are easily overlooked, only to be smoothed out over longer ones (Bonnet & Postma, 2018). Galapagos Finch hybrids demonstrated rapid changes in their fitness relative to a non-hybrid species in response to an El Nino event in 1982, yet the years before and after that event were marked by little change (Grant & Grant, 2002). Anolis hindlimb length changed rapidly within one or two generations after the experimental introduction of a predator on multiple small islands, but that change was followed by years of stasis (Losos et al., 2006). The multi-decade time scale of lateral plate evolution demonstrated here in Drizzle Lake stickleback (Figure 7) suggests stasis, yet it obscures the large changes occurring within it at annual (Figures 5 and 6) and intra-annual scales (Reimchen, 1995). Drizzle Lake had experienced significant changes in predation regime between 1981 and 1982, which is linked to changes in lateral plate number in sub-adult stickleback (Reimchen, 1995). Similar rapid shifts in lateral plate number are also evidence in transplant experiments of giant stickleback into ecologically opposite habitats (Leaver & Reimchen, 2012) as well as in recent colonization of marine stickleback to freshwater habitats in Alaska (Bell et al., 2004). These regime transitions at fine timescales, associated with large shifts in relative fitness and genomic structure (Bell & Windsor, 2013; Kingman et al., 2021; Marchinko et al., 2014; Marques et al., 2018; Schluter et al., 2021) exemplify the magnitude of change that rapid environmental transitions can induce, and can potentially be relevant to the maintenance of standing genetic variation.

Incidence of injuries was also evaluated with respect to biomechanics and functionality of lateral plate positions rather than as a quantitative trait such as number of plates. Stickleback populations in western Canada typically have seven plates per side, usually at positions 2 through 8 (Moodie, 1972; Hagen & Gilbertson, 1973; Moodie & Reimchen, 1976, reviews in Wootton, 1984), which are known to provide epidermal defense and spine supports when punctured by predators such as fish (Reimchen, 1992a). The modal LPPOS phenotype (P4–P7) in the current study population only has four plates. These are the major buttressing plates for the dorsal and pelvic spines (see Figure 1) and are the most conserved of all plates throughout the circumboreal distribution of Gasterosteus (Reimchen, 1983). Because bony armor can compromise swimming speed (Aleyev, 1977; Andraso, 1997; Bergstrom, 2002; Taylor & McPhail,
Table 4. Frequency (N) of plate position phenotypes separated for cohorts (1978–1983), age class (younger/older) and sex (1-male, 2-female) with estimates fitness (\(\hat{w}; SE\)) relative to the modal phenotype for primary data.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Sx</th>
<th>LPPOS 4-7 (mode)</th>
<th>LPPOS 5-7\textsuperscript{a}</th>
<th>LPPOS 3-7\textsuperscript{b}</th>
<th>LPPOS 3-8</th>
<th>LPPOS X\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>yng</td>
<td>old</td>
<td>yng</td>
<td>old</td>
<td>w</td>
</tr>
<tr>
<td>1978</td>
<td>1</td>
<td>14</td>
<td>11</td>
<td>7</td>
<td>3</td>
<td>.59</td>
</tr>
<tr>
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<td>36</td>
<td>17</td>
<td>11</td>
<td>8</td>
<td>1.54</td>
</tr>
<tr>
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<td>.81</td>
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<td>11</td>
<td>5</td>
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<td>13</td>
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<td>1.88</td>
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<td>5</td>
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</tr>
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<td>207</td>
<td>103</td>
<td>73</td>
<td>56</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Note. Phenotype classes: \(\textsuperscript{a}\) also includes all rare phenotypes with \(\leq3\) plates, \(\textsuperscript{b}\) also includes P4–P8, \(\textsuperscript{c}\) includes all rare phenotypes with \(\geq4\) plates (see Figure 3). Significant values \((p < .05)\) are shaded. Cohorts represent year of hatching.
1986) and is costly to produce (Barrett, 2010; Marchinko & Schluter, 2007), this modal phenotype retains the minimal number of plates without compromising swimming ability or the defense integrity of the spine complex. Predatory fish are also found in this population and might be expected to produce selection favoring a higher plate mode as is found in other coastal populations with predatory fish (Reimchen et al., 2013). This does occur (Reimchen, 1995), but 69% of the total predation on stickleback greater than 40 mm SL in this population (the size classes used in the current study) is due to avian piscivores (Reimchen, 1994). Although these are compression rather than puncturing predators (Reimchen, 1988), stickleback still benefit from the spine buttressing plates but there is reduced importance of the non-buttressing plates. That this phenotype is the most frequent in the population and has the lowest overall injury rate strongly suggests that its high fitness is related to having the lowest capture rate by avian piscivores.

Greater incidence of injuries also occurred in phenotypes that were only marginally less common than the modal phenotype. Relative to the mode, the three most common phenotypes in the population each had elevated injuries differing from the mode (P4–P7) by addition of a single plate at position 3 or position 8 (Figure 3). There is no obvious reason why these phenotypes would be biomechanically sub-optimal. Rather, they have marginally more epidermal protection and more structural support for the second dorsal spine and pelvic spine (Reimchen, 1983). However, it remains possible that due to the additional armor, specifically the addition of P8 which is positioned immediately behind the center of body mass, there could be a marginal reduction in trunk flexibility and burst acceleration. That these phenotypes with more plates have a slightly higher age-specific SL than the mode might also contribute to the elevated escape after capture (Reimchen, 1991). If so, this would be consistent with diversifying selection and the persistence of non-modal phenotypes due to post-capture advantages.

The incidence of injuries among non-modal phenotypes is inversely related to the frequencies of the different phenotypes in the population. To what extent is this associated with functionality of specific lateral plate positions or combinations? Some 95% of the stickleback have plates in a contiguous series (Figure 1). Five of the uncommon phenotypes in this population had one or more plates missing from the contiguous series and each of these phenotypes have particularly greater incidence of injuries. An additional five uncommon phenotypes had a contiguous series but were missing one or more of the major buttressing plates; these also exhibit elevated incidence of injuries, probably as the spines are more readily fractured after capture. This could reflect the tendency for predators to target sub-optimal or peripheral phenotypes (Curio, 1976; Genovart et al., 2010; Mesa et al., 1994; Temple, 1987; Rutz, 2012) possibly due to atypical swimming efficiency or poor physiological condition. We suspect that the higher incidence of injuries on these infrequent and atypical plate phenotypes do not reflect fitness advantages but rather are more consistent with fitness reduction and stabilizing selection.

The fact that the injury differences among phenotypes for the younger fish are no longer detectable in the older age classes was not expected and we have no clear understanding of this counter-intuitive pattern. We speculate that it could be associated with the increased frequencies of post-capture predator failures in progressively larger fish. In predation experiments with stickleback and trout from this population, trout had about a 90% post-capture failure rate on larger stickleback (70–90 mm SL), a consequence of their reduced ability to manipulate them (Reimchen, 1991). These size-dependent manipulation failures also parallel the incidence of injuries in the population (Reimchen, 1988, 2021). Furthermore, young age classes of prey that survive a capture event by a predator can reduce chances of secondary capture through behavioral avoidance of the predator (Kelley & Magurran, 2003). If applicable to the current study population, in which the majority of older fish are eventually captured and escape, this would result in gradual increased similarity in the incidence of injuries among all plate phenotypes over ontogeny.
We partitioned the sexes in much of our analyses because previous studies in stickleback from throughout the Haida Gwaii archipelago have shown relatively consistent morphological and niche differentiation between the sexes (Reimchen, 1980; Reimchen & Nelson, 1987, Reimchen & Nosil, 2004; Reimchen et al., 2009, 2016). While the sexes did not differ significantly in lateral plate number, the relative fitness (w) for non-modal plate phenotypes was usually higher in females than in males in multiple cohorts. Female and male stickleback differ in a range of additional morphological traits including eye diameter, jaw size, head shape, and stable isotope signatures (Reimchen et al., 2016), potentially indicative of niche segregation not directly related to sexual selection. Females are more abundant in limnetic zones and males in littoral, resulting in divergent predator exposure between sexes given that avian predators are also more common in limnetic zones and piscivorous predators in littoral zones (Reimchen, 1994). This spatial sex partitioning is consistent with widespread habitat segregation between sexes across vertebrate lineages (Wearmouth & Sims, 2008), and could indicate subtle differences between sexes in selection landscape that are driving differences in fitness estimates in the Drizzle Lake population. Greater fitness differences in females than males in lateral plate phenotypes is a relatively unusual finding, given that a recent meta-analysis across diverse taxa found stronger phenotypic selection on average in males, and mostly in traits related to reproduction and not predator evasion (Singh & Punzalan, 2018).

We believe this study is the first to integrate predator-induced injuries and fitness as a means of investigating ecological and selective processes influencing intrapopulation variation. In broad context, the large dataset, combined with long-term assessment of ecological interactions between predator and prey indicates a complex fitness landscape. While we have differentiated the two major predator groups (fish and birds), this is a conservative evaluation of the different selective pressures as these two groups are composed of 21 species of predators that use stickleback in this lake, each of which differ in their foraging behavior (Reimchen, 1994). The importance of diversifying, directional, and stabilizing selection changes over time and in combination, facilitates the bell-shaped frequency distribution of defense structures and overall relative stasis in population means. If these processes are representative of other natural systems, the almost universal occurrence of the bell-shaped frequency distribution of metric and meristic traits may be rooted more in ecological diversity and reflect a flatter fitness curve among phenotypes rather than a single fitness peak bounded by sub-optimality, a theme consistent with the renewed interests in fluctuating and balancing selection in natural populations (Nosil et al., 2018).

Supplementary material

Supplementary material is available online at Evolution (https://academic.oup.com/evolut/qpad032)

Data availability

All data associated with this article will be archived with Dryad. https://doi.org/10.5061/dryad.5hqbzkhh9t

Author contributions

T.E.R. designed the research, made the collections and measurements on the fish and developed the first draft of the manuscript. C.A.B. contributed to the analyses and manuscript preparation.

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Conflict of interest: The authors declare no conflicts of interest.

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