

# Ecological opportunity and levels of morphological variance within freshwater stickleback populations

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The ‘ecological opportunity’ hypothesis predicts that when interspecific competition or predation is reduced, populations will exhibit increases in phenotypic variance as a result of colonization and adaptation to vacant or underutilized ecological niches (i.e. character release). We assessed this hypothesis by examining morphological diversity within stickleback populations in 40 undisturbed lakes from six islands off the mid-coast of British Columbia, Canada. Because larger lakes with well-developed littoral and limnetic zones will have greater trophic niche diversity than smaller lakes with only littoral zones, we predicted a positive association between lake size and variation in trophic morphology. Conversely, reduced vertebrate predation in small bog lakes allows increased variance in defensive structures without costs to fitness. Consistent with both predictions, we observed that phenotypic variance in two traits that are involved in feeding (gape width and pectoral fin length) increased with lake size while variability in defence structures (lateral plate number and dorsal spine length) was inversely related to lake size. Moreover, increased variance in defence morphology was accentuated in populations with severe armour reduction (spine loss, decreased plate overlap), another strong indicator of reduced vertebrate predation. In the majority of cases, these patterns were repeatable among islands, independent from the geographical distance between lakes, and arose from a combination of high variance within each of the sexes and increases in sexual dimorphism. These findings suggest that character release can be trait-specific and reflect the combined effects of competition, predation and habitat heterogeneity. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 86, 297–308.

**ADDITIONAL KEYWORDS:** character release – competition – defence morphology – *Gasterosteus* – natural selection – niche availability – niche width – predation – trait variance.

## INTRODUCTION

The ecological causes of adaptive radiation are a classical topic in evolutionary biology (Darwin, 1859; Lack, 1947; Simpson, 1953; Mayr, 1963), and have received renewed interest in recent years (Schluter, 2000). Numerous studies over the last century have shown that divergent natural selection, stemming from differences between environments or from competition between closely related species, can cause mean trait values to diverge between populations (Schluter, 2000 for review). Likewise, studies of intrapopulation variability have revealed that specialization on different resources is common among different age classes, sexes and individuals within

populations and that such specialization is often associated with specific morphological or behavioural adaptations (Reimchen, 1979, 1980, 1995; Schluter, 1993, 1995; Sinervo & Lively, 1996; Losos *et al.*, 1998; Reimchen & Nosil, 2001a, 2002, 2004; Grant & Grant, 2002; Bolnick *et al.*, 2003; for review). However, both the extent and ecological causes of geographical differences in the amount of intrapopulation variation are poorly documented.

Genetic variation within populations of many organisms is thought to correlate with environmental heterogeneity (for review see Hedrick, 1986) and thus ‘ecological opportunity’ is predicted to influence levels of intrapopulation variability. When interspecific competition or predation is reduced, competition within a population promotes diversification by favouring the utilization of unexploited resources (i.e. the coloniza-

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tion of vacant or under-utilized ecological niches; Simpson, 1953). Competition within a population leads to diversifying selection because individuals switching to new, under-exploited resources undergo weaker competition, and thus exhibit higher fitness (Slatkin, 1980; Wilson & Turelli, 1986; Doebeli, 1996; Bolnick, 2001). Such niche-width expansion is often associated with an increase in phenotypic variation (i.e. character release; Van Valen, 1965; Grant, 1972; Roughgarden, 1972; Lister, 1976; Taper & Case, 1985; Doebeli, 1996).

Direct tests of the 'ecological opportunity' hypothesis have generally taken two approaches. First, studies of natural populations have attempted to control for differences between study sites or time periods in niche availability and intraspecific competition and have revealed elevated trait variance in populations where and when competing species are known to be absent (Lister, 1976; Robinson & Wilson, 1994; Benton, 1996a, b; Robinson & Schluter, 2000; Simberloff *et al.*, 2000) or in geographical regions where interspecific competition is thought to be weak (e.g. islands vs. mainlands, birds, Van Valen, 1965; *Drosophila*, Carson & Kaneshiro, 1976; finches, Schluter, 1988b; Hawaiian silverswords and *Bidens*, Schluter, 2000; lake populations in northern latitudes, Robinson & Schluter, 2000). Second, laboratory experiments examining the factors facilitating diversification strictly within environments lacking interspecific competition have shown that habitat heterogeneity (Rainey & Travisano, 1998) and intraspecific competition (Rainey & Travisano, 1998; Bolnick, 2001; Buckling & Rainey, 2002) are factors promoting niche width expansion and subsequent character release. Conversely, phage-induced reductions in population density have been shown to result in decreased diversification within laboratory populations of bacteria (Buckling & Rainey, 2002).

There are few data on the effects of predator pressure or habitat heterogeneity on the opportunity for character release within natural populations. In this study, we provide a test of the ecological opportunity hypothesis by evaluating the influence of correlates of both these factors on levels of trait variation in natural populations of freshwater threespine stickleback (*Gasterosteus aculeatus*). Stickleback exhibit high levels of both within-population and between-population variation in morphology and the functional significance of defence and trophic morphology is well understood (Hoogland, Morris & Tinbergen, 1957; Reimchen, 1983, 1994; Schluter, 1993, 1995). Increased gape width is an adaptation to foraging on benthic macroinvertebrates while reduced gape is associated with foraging on zooplankton in the limnetic zone (Schluter & McPhail, 1992; Schluter, 1993, 1995). For defence morphology, predatory fish pro-

duce selection favouring increased size of spines and increased number of bony lateral plates (Moodie & Reimchen, 1976; Gross, 1978; Reimchen, 1983, 1994, 1995). Avian piscivores may generate selection in favour of lateral plate reduction (Reimchen, 1988, 1997; Bergstrom, 2002) while macroinvertebrate piscivores may cause selection favouring reduced number of plates and spines (Reimchen, 1980, 1994; Ziuganov & Zotin, 1995; Reimchen & Nosil, 2002, 2004; Vamosi, 2002).

Because both trophic niche availability and predatory regime varies with lake size, we have analysed the association between lake size and intrapopulation variability in trophic and defence morphology in 40 stickleback populations on six islands from the west coast of Canada. Habitat heterogeneity, and thus trophic niche availability, is positively correlated with lake size (Barbour & Brown, 1974; Hildrew & Townsend, 1987). Therefore the ecological opportunity hypothesis predicts elevated levels of trait variation in trophic structures within large lakes. Conversely, populations in small lakes are generally subject to reduced levels of vertebrate predation, resulting in relaxed selection on lateral plates and spine morphology (Moodie & Reimchen, 1976; Reimchen, 1994, 2000). Reduced importance of vertebrate predation in small lakes results from an absolute decrease in levels of vertebrate predation and a decrease in the relative importance of vertebrate vs. invertebrate predation. This release from vertebrate predation will allow stickleback to exhibit morphologies that were previously selected against, resulting in an inverse association between lake size and variability in defence morphology. Moreover, this latter trend should be accentuated in lakes with spine loss and decreased plate overlap, both of which are strong indicators of reduced selection from vertebrate predators (Reimchen, 1980, 1983; Reimchen & Nosil, 2002, 2004). Finally, we assess whether increases in trait variance result from elevated sexual dimorphism, from increased trait variation within the each of the sexes, or from a combination of these processes.

Stickleback are particularly ideal for studies of character release because: (1) all the stringent criteria required for linking geographical variation in trait variability to character release are likely fulfilled (cf. Schluter & McPhail, 1992); (2) populations in postglacial lakes are species poor and recent in origin (10 000 years BP), minimizing the effects of interspecific competition and phylogenetic history on patterns of trait variation (Robinson & Schluter, 2000), and (3) studies of geographical differences in levels of within-population variability can be interpreted in light of detailed, long-term studies of variation within single populations (e.g. Reimchen, 1994, 1995; Reimchen & Nosil, 2002, 2004).

## STUDY AREA AND METHODS

Stickleback were sampled from small lakes located on the Dewdney-Banks archipelago off the mid-coast of British Columbia, Canada (52°59'N, 129°37'W). Lakes were sampled on Banks, Barnard, Dewdney, Trutch, Lotbiniere and Uni islands. The lakes on each of the islands are all low-elevation (less than 100 m) and found within several kilometres from marine waters. Fish were captured from 40 freshwater stickleback populations. Lake area was calculated from topographic maps and lake depth was estimated using bank profiles and lake cover (Reimchen, 1989). Lake volume was calculated as lake area  $\times$  average lake depth.

We assessed morphological differentiation within each population using linear measurements from 13 metric traits and counts from two meristic traits ( $N = 1754$  individuals, mean sample size per population = 44.0, SD = 10.9, min. = 14, max. = 54). Abbreviations are as follows: standard length of fish (LENGTH); maximum body depth (DEPTH), width of gape (GAPEW), length of gape (GAPEL), length of first dorsal spine (DORS1), length of left pelvic spine (LPELV), length of ventral plate (VPLATEL), width of ventral plate (VPLATEW), height of ascending process (APHGHT), maximum width of ascending process (APWDTH), pectoral length (PECTLNGT), eye diameter (EYEDIAM), length of 7th lateral plate (PLATEL7), number of lateral plates on left side of trunk (PLATENO) and number of gill rakers (RAKERS). Due to low levels of variability in raker number, only approximately half ( $N = 879$ ) of the fish were scored for RAKERS. The degree of overlap between plates and incidence of spine number reduction was also recorded.

All morphological characters were log-transformed prior to statistical analyses. For all the morphometric characters examined, trait size increased linearly with LENGTH (all  $P < 0.001$ , bivariate correlation). Consequently, morphometric data were size-standardized by taking the residual trait values from a regression of trait values on standard length. The slopes of the trait size vs. LENGTH regression differed among localities for all the metric characters (all  $P < 0.001$ , trait-LENGTH interaction, ANCOVA) and, consequently, all size standardizations were calculated using separate, within-group slopes (Sokal & Rohlf, 1981). Analyses using size-standardization with pooled, between-groups slope yielded congruent results (not shown). PLATENO did not increase with increasing SL ( $P > 0.05$ , bivariate correlation) and thus was not size-standardized.

### MORPHOLOGICAL VARIABILITY WITHIN POPULATIONS

We used several different measures of trait variability within populations, including: (1) the total sample

variance, (2) the sample variance within each of the sexes, and (3) the degree of sexual dimorphism (calculated as the absolute difference in mean trait values for males vs. females within each lake). To control for the potential effects of associations between sample variances and sample means, we also conducted analyses using residual values from a regression of sample variances against sample means (residual variance hereafter). Analyses using residual variances are analogous to using coefficients of variation (variance : mean ratios) but correct more precisely for associations between means and variances.

There was no correlation between levels of variance within a population and sample size ( $r < 0.20$ ,  $P > 0.15$  for all measures of trait variance; for 26 populations  $N = 50$ ) and levels of variance within a population did not tend to differ between the sexes (all  $t < 1.60$ , all  $P > 0.10$ ; paired  $t$ -tests using levels of trait variance in males vs. females for the 40 lakes).

### ASSOCIATIONS BETWEEN TRAIT VARIANCE AND LAKE VOLUME

We tested for linear relationships between trait variance and log-transformed lake volume (log-transformation normalized the distribution of lake volumes,  $Z = 0.68$ ,  $P = 0.74$ , Kolmogorov-Smirnov test). Analyses using raw variances and log-transformed variances yielded similar results. Consequently, we report results using log-transformed variances because they are conservative with respect to the potential effects of outliers and resulted in normally distributed residuals from all subsequent regression analyses (Kolmogorov-Smirnov tests on residuals, all  $P > 0.25$ ). Analyses using lake area or lake depth alone yielded results similar to analyses using lake volume (results not shown).

Because of substantive departures from linearity, we also used quadratic and cubic regression. For all analyses, we assess the significance of  $r^2$  using the  $F$ -ratio. We assessed the overall importance of quadratic and cubic components using two methods. First, we tested the significance of quadratic and cubic regression coefficients, in regression models including both linear and quadratic terms or linear, quadratic and cubic terms, respectively (cf. Lande & Arnold, 1983). Second, we used partial  $F$ -tests to compare the fit of models including only a linear term to the fit of models including higher-order terms (i.e. we assessed the significance of increases in  $r^2$  when quadratic or cubic terms were added to the regression model; Norusis, 1993).

We examined eight trophic traits and seven defensive traits and thus applied sequential Bonferroni corrections to significance levels within each type of trait

(for the significance of  $r^2$  in overall regression analyses; Rice, 1989). This approach is highly conservative because levels of variance for different traits were often not correlated with each other (trophic traits: 11 of 55 pairwise comparisons where  $P < 0.05$ ; defensive traits: 13 of 28 pairwise comparisons where  $P < 0.05$ ; bivariate correlations).

Departures from linearity were also examined by plotting associations between trait variance and lake size using the non-parametric cubic spline (Schluter, 1988a). Finally, we used multiple regression analyses to test for associations between trait variance and lake size when partial associations among traits were controlled for statistically.

#### REPLICATION OF TRENDS AMONG AND WITHIN ISLANDS

Replication of associations between trait variance and lake volume was assessed by testing whether such associations were dependent upon island of origin (i.e. testing for significant interactions between 'island' and linear, quadratic and cubic lake volume terms in ANCOVA analyses, island treated as a factor). Second, we used Mantel randomization tests (10 000 randomizations; Manly, 1997) to test whether

interpopulation differences in levels of trait variance were correlated with the geographical distance between lakes. This analysis was conducted using all the study populations and for each island separately (for the four islands with more than two lakes per island).

## RESULTS

### ASSOCIATIONS BETWEEN TRAIT VARIABILITY AND LAKE VOLUME

We detected significant associations between overall trait variance and lake volume for standard length and four additional traits (Table 1, Fig. 1). The relationship was positive for GAPEW and PECTLNGT but negative for DORS1 and PLATENO, with substantial departures from linearity in each case (Table 1; all other traits  $P > 0.05$  in linear regression analyses). When variance within each of the sexes was considered separately, within-population variance increased with lake size for the two trophic traits in males but not females and decreased with lake size for the two defensive traits within each of the sexes (Table 2).

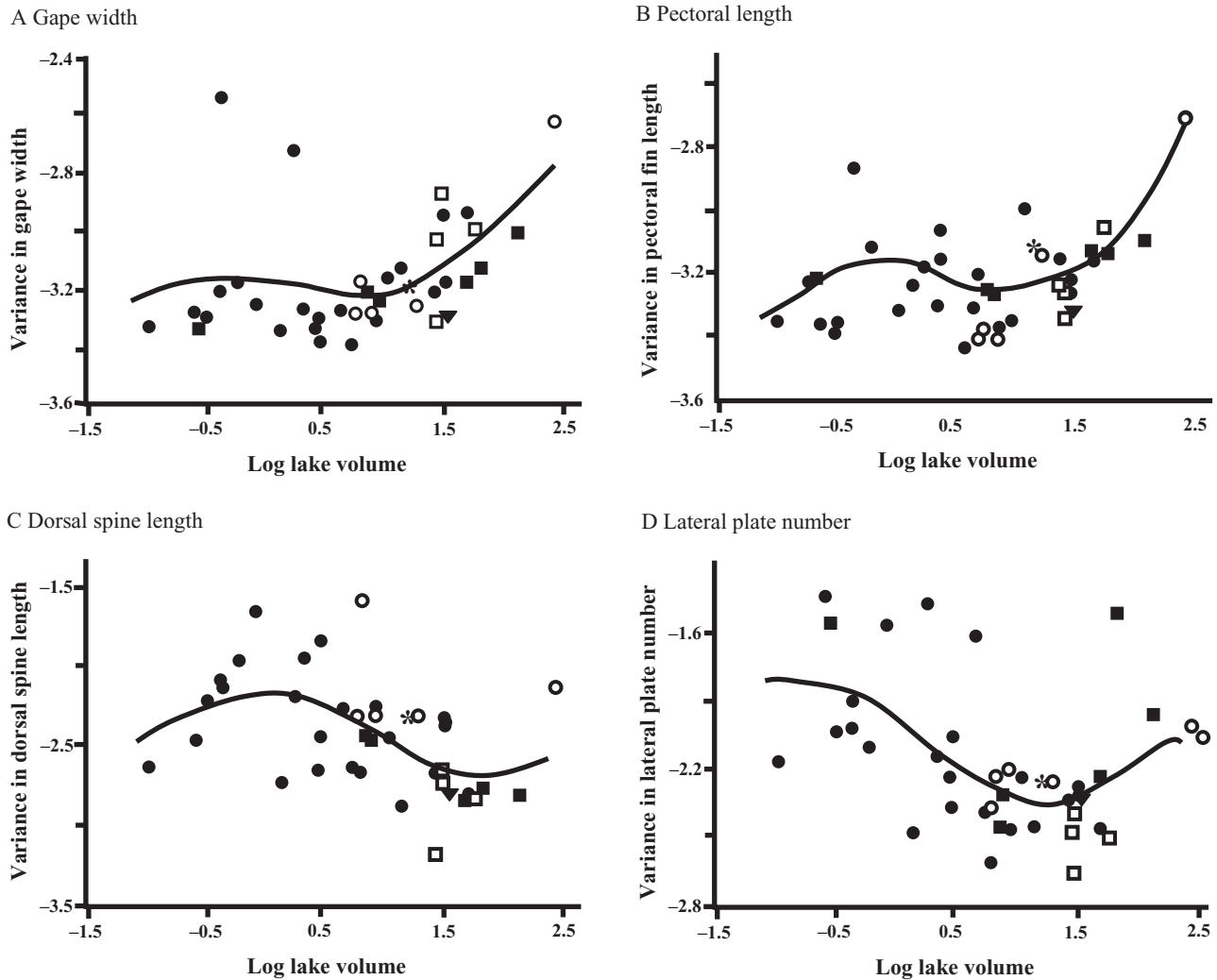
A substantial proportion of the elevated variance in

**Table 1.** Linear (lin.), quadratic (quad.) and cubic (cub.) regression analyses of the relationship between measures of trait variance [Overall Variance (OV) and sexual dimorphism (SexDi)] and lake volume for 40 populations of threespine stickleback.  $r^2$  refers to the fit of the overall regression model, F-change to the F-ratio from a partial  $F$ -test assessing whether adding a quadratic term to a linear model improves the overall fit of the model,  $\beta_1$  to the linear regression coefficient,  $\beta_2$  to the quadratic regression coefficient and  $\beta_3$  to the cubic regression. In general, adding a quadratic term improved the fit of the regression model and quadratic regression coefficients were often statistically significant. Sequential Bonferroni corrections were also applied to the significance of overall regression analyses (i.e.  $r^2$  values), with separate adjustments for trophic (GAPEW, PECTLENG) vs. defensive traits (DORS1, PLATENO), see methods for details. LENGTH, standard length of fish. Analyses where  $r^2$  retains significance at  $P < 0.05$  after this correction are in bold

Trait	$r^2$	OV				SexDi				
		F-change	$\beta_1$	$\beta_2$	$\beta_3$	$r^2$	F-change	$\beta_1$	$\beta_2$	$\beta_3$
LENGTH (lin.)	0.29***		0.23***	–	–	0.11*		0.08*	–	–
LENGTH (quad.)	0.34**	2.32	0.10	0.09	–	0.14	1.48	0.002	0.005	–
LENGTH (cub.)	0.35**	0.78	0.11	–0.03	0.06	0.16	0.58	0.002	–0.002	0.003
GAPEW (lin.)	0.10*		0.08*	–	–	0.03		0.002	–	–
GAPEW (quad.)	0.22**	6.52*	–0.06	0.10*	–	<b>0.30**</b>	14.16**	–0.007*	0.007**	–
GAPEW (cub.)	<b>0.30**</b>	3.95*	–0.05	–0.07	0.08*	<b>0.34**</b>	2.36	–0.007	0.001	0.003
PECTLNGT (lin.)	0.12*		0.06*	–	–	0.11*		0.004*	–	–
PECTLNGT (quad.)	<b>0.26**</b>	6.92*	–0.04	0.08*	–	0.17*	2.70	–0.003	0.003	–
PECTLNGT (cub.)	<b>0.41***</b>	9.33**	–0.03	–0.11	0.08**	<b>0.38**</b>	12.06**	0.001	–0.010*	0.007**
DORS1 (lin.)	0.15*		–0.17*	–	–	0.01		–0.003	–	–
DORS1 (quad.)	0.17*	0.93	–0.08	–0.07	–	0.03	0.78	–0.010	–0.005	–
DORS1 (cub.)	<b>0.37**</b>	11.40**	–0.06	–0.56**	0.22**	<b>0.31**</b>	14.27**	–0.008	–0.002**	0.002**
PLATENO (lin.)	0.15*		–0.16*	–	–	<b>0.17**</b>		–0.015**	–	–
PLATENO (quad.)	<b>0.26**</b>	5.37*	–0.35	0.14*	–	0.18*	0.40	–0.020*	0.004	–
PLATENO (cub.)	<b>0.35**</b>	4.80*	–0.33**	–0.16	0.14*	0.22*	1.60	–0.020*	–0.010	0.008

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .





**Figure 1.** The relationship between total trait variance within populations of *Gasterosteus* and lake volume. The association was positive for two traits involved in feeding (A, B, gape width; pectoral fin length) and negative for two defensive traits (C, D, dorsal spine length; number of lateral plates). Substantial departures from linearity were detected in all cases (Table 1 for statistics). The form of the relationship is depicted by solid lines derived using nonparametric cubic spline analysis (Schluter, 1988a). Symbols denote the island a population originates from: ▼, Uni; \*, Trutch; ■, Lotbiniere; ●, Dewdney; □, Banks; ○, Barnard.

trophic and defensive traits in large and small lakes, respectively, could be attributed to increases in the degree of sexual dimorphism. Thus the relationship between sexual dimorphism and lake size was positive for GAPEW and PECTLNGT and negative for DORS1 and PLATENO (Table 1).

Although trait means and trait variances were correlated for the two armour traits (DORS1, PLATENO; Table 3), associations between trait variance and lake size were not driven entirely by this correlation. Specifically, the relationship between lake size and trait variability persisted using residual variances (i.e. controlling for differences among populations in trait

means, PECTLNGT, DORS1, PLATENO,  $P < 0.05$ , GAPEW,  $P = 0.08$ ; Table 3). Second, associations among variances for the different traits were weak (DORS1 by PLATENO,  $r = 0.37$ ,  $P < 0.05$ , all other bivariate correlations,  $r < 0.35$ ,  $P > 0.05$ ) and accounting for them in a multiple regression analysis resulted in pronounced partial associations between trait variance and lake size (overall  $r^2 = 0.41$ ,  $P < 0.01$ ; Table 3 for partial associations).

Graphical analyses using the non-parametric cubic spline were consistent with the statistical analyses; the relationship between trait variance and lake volume increased with lake size for GAPEW and PECT-

**Table 2.** Linear (lin.), quadratic (quad.), and cubic (cub.) regression analyses of the relationship between measures of trait variance and lake volume for each sex for 40 populations of threespine stickleback.  $r^2$  refers to the fit of the overall regression model, F-change to the F-ratio from a partial  $F$ -test assessing whether adding a quadratic term to a linear model improves the overall fit of the model,  $\beta_1$  to the linear regression coefficient,  $\beta_2$  to the quadratic regression coefficient and  $\beta_3$  to the cubic regression. In general, adding a quadratic term improved the fit of the regression model and quadratic regression coefficients were often statistically significant. Sequential Bonferroni corrections were also applied to the significance of overall regression analyses (i.e.  $r^2$  values), with separate adjustments for trophic (GAPEW, PECTLENG) vs. defensive traits (DORS1, PLATENO), see methods for details. LENGTH, standard length of fish. Analyses where  $r^2$  retains significance at  $P < 0.05$  after this correction are in bold

Trait	$r^2$	males				$r^2$	females			
		F-change	$\beta_1$	$\beta_2$	$\beta_3$		F-change	$\beta_1$	$\beta_2$	$\beta_3$
LENGTH (lin.)	0.13*	–	0.18*	–	–	0.27**	–	0.28**	–	–
LENGTH (quad.)	0.15	0.56	0.10	0.06	–	0.37***	6.06*	0.03	0.19*	–
LENGTH (cub.)	0.15	0.20	0.09	0.15	–0.04	0.45***	4.81*	0.04	–0.18	0.17*
GAPEW (lin.)	0.07	–	0.07	–	–	0.00	–	–0.02	–	–
GAPEW (quad.)	0.16*	4.03*	–0.05	0.09*	–	0.02	0.51	0.06	–0.06	–
GAPEW (cub.)	0.22*	2.52	–0.04	–0.07	–0.08	0.03	0.39	0.06	–0.18	0.06
PECTLNGT (lin.)	0.07	–	0.06	–	–	0.03	–	0.03	–	–
PECTLNGT (quad.)	0.17*	4.80*	–0.05	0.08*	–	0.11	3.35	–0.05	0.07	–
PECTLNGT (cub.)	0.22*	1.98	–0.05	–0.04	–0.06	0.13	0.99	–0.05	–0.02	0.04
DORS1 (lin.)	0.13*	–	–0.16*	–	–	<b>0.19**</b>	–	–0.20**	–	–
DORS1 (quad.)	0.13	0.18	–0.12	–0.03	–	<b>0.23**</b>	2.02	–0.06	–0.11	–
DORS1 (cub.)	0.24*	5.08*	–0.10	–0.41*	0.17*	<b>0.37***</b>	7.36*	–0.04	–0.54**	0.20*
PLATENO (lin.)	0.11*	–	–0.13*	–	–	0.10*	–	–0.14*	–	–
PLATENO (quad.)	<b>0.28**</b>	8.48**	–0.38**	0.18**	–	0.16*	2.06	–0.28	0.11	–
PLATENO (cub.)	<b>0.34**</b>	3.37	–0.36**	–0.07	0.12	0.21*	2.70	–0.27*	–0.17	0.12

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

LNGT but decreased with lake size for DORS1 and PlateNO (Fig. 1).

( $r^2 = 0.02$ ,  $0.49$ ,  $\beta = 0.03$ ,  $0.05$ ,  $P = 0.49$ ,  $0.12$ , respectively).

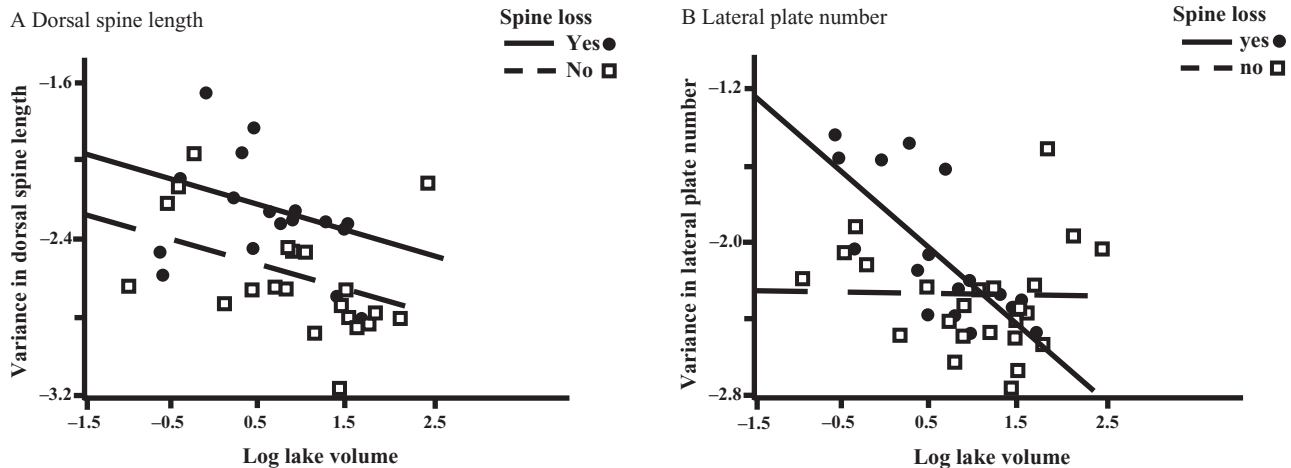
#### REPLICATION OF TRENDS AMONG AND WITHIN ISLANDS

Levels of trait variance did not vary among islands (all  $F_{1,35} < 1.50$ , all  $P > 0.25$ , ANOVA analyses on population variance, with island as the factor; Fig. 1) and interpopulation differences in levels of phenotypic variance were positively correlated with the geographical distance between lakes only in one case (variance in PLATENO within Lotbiniere island,  $r = 0.80$ ,  $P < 0.01$ ; all other positive correlations among islands, within islands and within watersheds on Dewdney,  $r < 0.27$ ,  $P > 0.05$ , Mantel tests).

Associations between trait variance and lake size were comparable among islands as the slope of the relationship between trait variance and lake size differed among islands for only a single trait (PECTLNGT,  $F_{3,40} = 6.87$ ,  $P < 0.01$ ; other traits, all  $F_{3,40} < 2.10$ , all  $P > 0.10$ , island–lake size interactions, ANCOVA). For PECTLNGT, the relationship between trait variance and lake size was strong and positive for Barnard and Banks island ( $r^2 = 0.98$ ,  $0.80$ ,  $\beta = 0.43$ ,  $0.68$ ,  $P = 0.001$ ,  $0.10$ , respectively) but much weaker for Dewdney and Lotbiniere island

#### ASSOCIATIONS BETWEEN VARIANCE AND MEANS FOR FUNCTIONAL TRAITS

For the four traits where variance was correlated with lake size, we examined their relationship with defensive traits that have known functional significance. Spine loss is a proxy for reduced vertebrate predation and did not affect the relationship between variance in the two trophic traits and lake size (GAPEW,  $F_{1,40} = 0.00$ ,  $0.33$ ,  $P = 0.98$ ,  $0.57$  for interaction term and main effects, respectively; PECTLNGT,  $F_{1,40} = 1.69$ ,  $0.31$ ,  $P = 0.21$ ,  $0.58$  for interaction term and main effects, respectively). Conversely, the incidence of spine loss was associated with levels of trait variability in defensive structures. For DORS1, populations with spine loss exhibited greater trait variance for a given lake size than populations without spine loss ( $F_{1,40} = 0.00$ ,  $P = 0.98$ , spine loss–lake size interaction term;  $F_{1,40} = 8.63$ ,  $P < 0.01$ , main effects; Fig. 2). Conversely, the negative association between trait variance in PLATENO and lake size was driven almost entirely by populations with spine loss ( $F_{1,40} = 11.65$ ,  $P < 0.01$ , spine loss–lake size interac-



**Figure 2.** The effects of spine loss on the relationship between trait variance in defensive traits within *Gasterosteus* populations and lake volume. A, variance in dorsal spine length. B, variance in lateral plate number.

**Table 3.** Results of regression analyses testing for associations between residual variance and lake size (residual variance), between trait variances and trait means (correlation with trait means) and between trait variance and lake size once partial association among traits were statistically accounted for (variance in four traits) for 40 *Gasterosteus* populations from the mid-coast of British Columbia, Canada

Trait	Residual variance			Correlation with trait means			Variance in four traits		
	$r^2$	$\beta$	$P$	$r^2$	$\beta$	$P$	partial $r^2$	$\beta$	$P$
GAPEW	0.08	0.07	0.08	0.01	-1.05	0.49	0.10	1.12	0.05
PECTLNGT	0.11	0.06	0.04	0.03	-1.69	0.33	0.10	1.47	0.05
DORS1	0.12	-0.12	0.03	0.41	-2.02	<0.001	0.10	-0.65	0.05
PLATENO	0.11	-0.06	0.04	0.81	-2.51	<0.001	0.14	-0.84	0.02

tion;  $r^2 = 0.01$ ,  $0.56$ ,  $\beta = -0.01$ ,  $0.40$ ,  $P = 0.88$ ,  $0.001$ , relationship between trait variance and lake size for populations without and with spine loss, respectively).

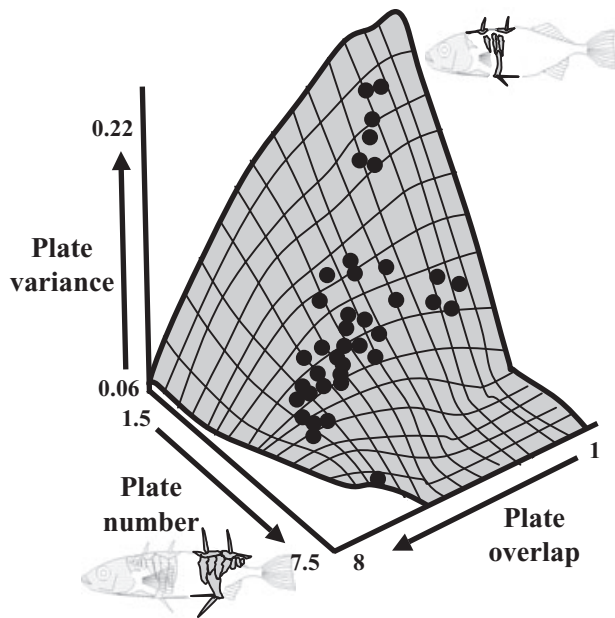
We also examined the relationship between PLATENO variance and lateral plate overlap (which is inversely related to vertebrate predation). For the association between PLATENO variance and lake size (for populations without spine loss), populations with positive residuals (i.e. greater trait variances than predicted by the least-squares regression line) exhibited significantly lower mean plate overlap than populations with negative residuals (mean overlap = 4.34, 6.16, respectively,  $t_{1,21} = 3.90$ ,  $P < 0.01$ ; Fig. 2), resulting in an inverse relation between residual variance and plate overlap ( $r = -0.76$ ,  $P < 0.001$ ). Moreover, the three populations without spine loss that exhibited much greater PLATENO variance than expected also exhibited exceptionally low plate overlap (2.54, 3.56, and 3.88, respectively; Fig. 2B). Specifically, mean overlap for these three populations (3.32) was significantly lower than mean overlap for all pop-

ulations without spine loss ( $t_{1,22} = 6.58$ ,  $P < 0.001$ , one-sample  $t$ -test against 3.32).

We also examined associations between PLATENO variance, mean PLATENO, and plate overlap. Trait variance in PLATENO was inversely related to both mean PLATENO (Table 3) and to plate overlap ( $r^2 = 0.42$ ,  $\beta = -0.13$ ,  $P < 0.001$ ). Thus the greatest PLATENO variances were observed when both mean PLATENO and plate overlap were low (Fig. 3). However, the negative relationship between PLATENO variance and lake size persisted once these associations among PLATENO characteristics were statistically accounted for (partial  $r^2 = 0.13$ ,  $\beta = -1.89$ ,  $P < 0.05$ ; multiple regression of plate variance, number and overlap against lake size).

## DISCUSSION

The ecological opportunity hypothesis predicts that when interspecific competition or predation is reduced, populations will exhibit increases in pheno-



**Figure 3.** The relationship between lateral plate number, lateral plate overlap and lateral plate trait variance among 40 freshwater *Gasterosteus* populations. Low plate number and low plate overlap are indicators of reduced vertebrate predation and was associated with high levels of trait variability for lateral plate number.

typic variance as the result of the invasion and adaptation to vacant niches (Simpson, 1953; Van Valen, 1965; Roughgarden, 1972; Schluter, 2000; for review). Under this hypothesis high niche availability facilitates diversification. We suggest that the positive association between variance in gape width and pectoral fin length and lake size detected in the current study reflects the effects of greater trophic niche availability in large lakes (Barbour & Brown, 1974; Hildrew & Townsend, 1987), and is consistent with the ecological opportunity hypothesis.

Conversely, stickleback populations in small bog lakes that experience low levels of vertebrate predation are predicted to experience reduced selection for well-developed body armature (Reimchen, 1994). This release from strong vertebrate predation will allow stickleback to exhibit morphologies that were previously selected against, resulting in elevated variability in defence morphology within small lakes. Consistent with the prediction, variability in dorsal spine length and lateral plate number within the study populations was inversely related to lake size. These increases in trait variance were associated with armour reduction (spine loss and reduced plate overlap), another reliable proxy for reduced exposure to vertebrate predation (Reimchen, 1994, 1995). Furthermore, trait variance decreased with lake size even

when the effects of armour reduction were statistically accounted for.

Collectively, our results are consistent with studies of laboratory populations that have documented elevated levels of phenotypic variance when vacant niches are available (Rainey & Travisano, 1998; Buckling & Rainey, 2002). However, Robinson, Wilson & Margosian (2000) report no association between lake size and ecological diversification within sunfish populations. Notably, we also did not detect associations between trait variance and lake size for numerous characters. These findings suggest that predation regime and niche availability do not always vary with lake size, or that they are sometimes unrelated to diversification.

#### CRITERIA FOR CHARACTER RELEASE

Previous authors have outlined the criteria required to implicate character release or displacement as an explanation for patterns of trait variation (Schluter & McPhail, 1992; Schluter, 2000). Freshwater stickleback populations likely fulfil all six criteria. Most of the evidence that these criteria are fulfilled is presented elsewhere (Schluter & McPhail, 1992; Schluter, 1994, 2000). As such, we provide only a brief summary of the previous evidence and focus on evidence that applies specifically to the populations examined in the current study.

First, phenotypic differences among populations should reflect differences in resource use such that functional relationships among diet, habitat use and morphology have been clearly demonstrated. In threespine stickleback, variation in trophic morphology has been linked to differences in resource use via both observation (Schluter & McPhail, 1992; Walker, 1997; Walker & Bell, 2000) and experiment (Schluter, 1993, 1994, 1995). Similarly, the functional significance of defence morphology in stickleback is well established (Hoogland *et al.*, 1957; Reimchen, 1983, 1994) and intrapopulation variation in defence morphology has been linked to differences in both habitat use (Reimchen, 1980, 1994) and diet (Reimchen & Nosil, 2001a, b, c). Moreover, evolutionary shifts in defence morphology can be driven by temporal and spatial shifts in divergent predation regimes (Reimchen, 1980, 1994, 1995; Reimchen & Nosil, 2002, 2004).

Second, ecological release in the classic sense implies that phenotypic differences have a genetic basis. However, an environmentally induced or plastic response to elevated ecological opportunity can also generate increased morphological variation that has a functional role (Meyer, 1987). In the case of stickleback, morphological differences among populations likely involve a partial genetic component because



they persist when individuals from different populations are raised in the laboratory on the same diet (McPhail, 1984; Lavin & McPhail, 1987; Hendry, Taylor & McPhail, 2002). Additionally, within-population variation in morphology has a strong genetic component (Lavin & McPhail, 1987; Peichel *et al.*, 2001). While differences in levels of trait variance among the lakes examined in this study thus likely reflect, at least in part, genetic differences among them it is possible that some of the variance reflects environmental differences. For example, both genetics and plasticity are known to contribute to morphological differences among stickleback species (Day, Pritchard & Schluter, 1994) and genetic data are unavailable for the populations examined in this study. Potentially, small ponds might exhibit more abiotic sources of variance (e.g. temperature fluctuations) or lower variance in food variability, thereby influencing observed levels of trait variability.

Third, when addressing the effects of niche availability on levels of trait variability the populations being compared should experience similar amounts of interspecific competition. Other species of fish were rarely captured while sampling our study populations (Reimchen, unpubl. data) but would be expected to occur primarily in large lakes. The resulting increase in interspecific competition in large lakes would produce reduced, rather than elevated, variance in trophic traits.

Fourth, stochastic processes such as founder effects, random population fluctuations or genetic drift in allopatry, which most likely occur in small populations, need to be excluded as explanations for the patterns observed. Such processes are exceedingly unlikely to account for our particular results as the patterns detected represent parallel divergence across evolutionary independent populations as (1) they were replicated over multiple populations found on several islands, and (2) interpopulation differences in levels of phenotypic variance were independent of the geographical distance between lakes. Moreover, stochastic processes are not likely to account for the results with defensive traits as greater variance was detected in smaller lakes where bottlenecks are more likely.

Fifth, differences among populations should reflect evolutionary shifts and not some other process, such as a biased extinction or colonization process. This criterion applies more readily to comparisons involving multiple, highly differentiated lineages than to studies of intrapopulation variation (Schluter & McPhail, 1992). Moreover, there is no reason to suspect that more divergent lineages would come into contact in small vs. large sized lakes.

Finally, to infer that intraspecific competition is the mechanism facilitating the use of vacant niches, inde-

pendent evidence of resource competition between similar phenotypes is required. This final criterion is likely to be met for our study populations because previous work on stickleback has revealed disproportionately severe competition between similar phenotypes (Schluter, 1994, 2003; Pritchard & Schluter, 2001; Gray & Robinson, 2002) and stronger disruptive selection when intraspecific competition was increased (Bolnick, 2004).

Although some of the evidence that the criteria for character release are fulfilled is indirect, the collective results strongly suggest that all the criteria are fulfilled. Notably, very few other studies of character displacement or release have fulfilled all these criteria (Schluter, 2000).

#### PARALLELS WITH PREVIOUS TESTS OF ECOLOGICAL OPPORTUNITY

Previous studies on natural populations of birds, lizards, fish and mammals have provided support for the ecological opportunity hypothesis by documenting increased levels of trait variance or greater niche width when populations are released from interspecific competition (see Introduction for examples). In freshwater fishes in particular, a comprehensive review of the evidence for character displacement and release (Robinson & Wilson, 1994) revealed that phenotypic variation within populations is often elevated in the absence vs. presence of competitors. More recently, Robinson & Schluter (2000) showed that fish populations in depauperate environments (few competitors and predators) tend to exhibit elevated levels of niche-based phenotypic variation, often in the form of trophic or resource polymorphisms (see also Robinson *et al.*, 2000). Our results complement and expand previous work on character release by showing that once interspecific competitors are removed, niche availability and predatory regime are both factors influencing diversification.

Previous work on sticklebacks has revealed evidence of niche partitioning between the sexes in terms of habitat, diet and parasite load (Reimchen, 1980; Reimchen & Nosil, 2001a); ecological correlates of sexual dimorphism are common in nature (Selander, 1966; Slatkin, 1984; Shine, 1989; for review; Reimchen & Nosil, 2004). In the current study, increased sexual dimorphism was associated with variation in lake size, providing evidence that site-specific ecological pressures can affect the evolution of sexual dimorphism. Differences in sexual dimorphism in context of ecological release are also reported by Simberloff *et al.* (2000), who found greater dimorphism in a mongoose species where congeners were absent. In contrast, Van Valen's (1965) classic study of character release found no evidence for greater difference between the sexes in

mainland vs. island birds. The role of ecology in driving geographical variation in sexual dimorphism remains unclear, but offers a promising avenue of further research.

Finally, numerous studies have detected no evidence for character release in the absence of interspecific competition (e.g. Soule & Stewart, 1970; Patterson, 1983; Rising, 1987; Werner & Sherry, 1987). Conceivably, these may represent cases where the availability of vacant niches (i.e. habitat heterogeneity) was low, where intraspecific competition was weak (see also Roughgarden, 1972; Slatkin, 1980 for theoretical treatments) or where genetic variation was lacking and thus niche width expansion was not favoured or possible.

### CONCLUSIONS

Our results provide correlative evidence that increased niche availability and reduced predation pressure can promote evolutionary diversification, but also indicate that these effects are trait-specific. This finding is consistent with the ecological opportunity hypothesis and complements field studies demonstrating that variation within populations is linked to specialization on different resources or habitats (e.g. Reimchen, 1980, 1995; Grant & Grant, 2002; Reimchen & Nosal, 2002, 2004; Bolnick *et al.*, 2003; for review). Field experiments testing the effects of shifts in predation pressure and niche availability on patterns of trait variability are now required to provide further evidence that these factors facilitate diversification within natural populations.

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