

Geographical variation in asymmetry in *Gasterosteus aculeatus*

C. A. BERGSTROM* and T. E. REIMCHEN

Department of Biology, University of Victoria, Victoria, BC, Canada.

Received 20 November 2001; accepted for publication 10 April 2002

We tested whether fluctuating asymmetry (FA) in undisturbed populations is associated with several natural environmental factors and whether FA is negatively correlated with fitness in the wild. We compared the FA of multiple bony structural defences among 87 endemic populations of threespine stickleback (*Gasterosteus aculeatus* L.) inhabiting pristine freshwater habitats on the islands of Haida Gwaii, British Columbia, Canada. Multi-trait FA for adults and juvenile fish varied extensively among populations, but only in adults did it correlate with geography and two habitat characteristics (pH and water colour). Mean FA among individual traits varied concordantly among populations but was not correlated within individuals. While asymmetrical fish showed slightly higher levels of parasitism as predicted, selection differentials based on age class comparisons suggested that asymmetrical fish had the same or marginally higher survival than symmetrical fish. Selection differentials of FA varied significantly among traits and may reflect variability in their functional importance and in the strength of selection on their developmental stability. The data imply that FA/fitness associations are heterogeneous and character-specific. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2002, 77, 9–22.

ADDITIONAL KEYWORDS: developmental stability – environmental stress – fitness – fluctuating asymmetry – parasitism – structural defences – survivorship – threespine stickleback – Queen Charlotte Islands.

INTRODUCTION

Over the last three decades, fluctuating asymmetry (FA) has been subject to an expanding repertoire of investigations encompassing genetics, development, behaviour, evolution, conservation biology and parasitology (Møller & Swaddle, 1997 for review). FA is generally thought to be associated with developmental instability (Mather, 1953; Van Valen, 1962; Soulé, 1967) and to reflect the failure of an individual to correct subtle and random departures from perfect symmetry during ontogeny (Waddington, 1942; Zakharov, 1992). Ability to correct developmental errors can be compromised by internal factors such as elevated homozygosity (Soulé, 1979; Palmer, 1986; Mitton, 1995), as well as by the disruption of co-adapted gene complexes through hybridization (Vrijenhoek & Lerman, 1982; Graham, 1992; Zakharov, 1992; Clarke,

1993). Developmental errors can also stem from physiological stress caused by extreme environmental conditions (Beardmore, 1960; Gest *et al.*, 1986; Hosken, Blanckenhorn & Ward, 2000). Elevated levels of FA are potentially relevant for conservation as these can indicate demographic bottlenecks and environmental deterioration (Wayne *et al.*, 1986; Pankakoski *et al.*, 1992; Manning & Chamberlain, 1993; Clarke, 1995; Gomendio, Cassinello & Rolden, 2000; Lens *et al.*, 2000). However, such inferences can be misleading without knowledge of the range in FA among undisturbed populations (Palmer, 1996).

FA can also reflect relative fitness of individuals as it is associated with reduced immunocompetence (Rantala *et al.*, 2000), increased susceptibility to parasitism (Escos *et al.*, 1995; Bonn *et al.*, 1996; Reimchen, 1997; Hunt & Allen, 1998), reduced survivorship (Packer & Pusey, 1993; Ueno, 1994; Pelabon & van Breukelen, 1998; Nosil & Reimchen, 2001), and avoidance by potential mates (Møller & Pomiankowski, 1993; Watson & Thornhill, 1994; Simmons & Ritchie, 1996). Given the associations

*Corresponding author. E-mail: cbergstr@uvic.ca

between FA and fitness, there should be correlations in the levels of FA among different traits on an individual. However, diverse studies (VanValen, 1962; Mason *et al.*, 1967; Ames *et al.*, 1979; Dufour & Weatherhead, 1996; Evans & Marshall, 1996; Clarke, 1998a) have found little support for such a correlation. This suggests that FA/fitness associations are heterogeneous among traits, and this heterogeneity may reflect the degree to which asymmetry compromises the functional integrity of some traits but not others (Moodie, 1977; Balmford *et al.*, 1993; Gummer & Brigham, 1995; Allen & Simmons, 1996; Crespi & Vanderkist, 1997; Swaddle, 1997; Bergstrom & Reimchen, 2000). Combined indices of FA from multiple traits can provide a more reliable estimate of developmental stability (Soulé & Baker, 1968; Dufour & Weatherhead, 1996; Gangestad & Thornhill, 1999; Bryden & Heath, 2000).

ASYMMETRY IN THREESPINE STICKLEBACK

Here we assess natural levels of FA in multiple traits among 87 endemic populations of freshwater threespine stickleback (*Gasterosteus aculeatus* L.) that colonized Haida Gwaii, an archipelago off the central coast of British Columbia, Canada, approximately 12 000 years ago (Moodie & Reimchen, 1976). Populations on this archipelago inhabit geographically isolated freshwater lakes, ponds, and streams, the majority of which are relatively pristine and undisturbed by human activity. The localities encompass a range of natural environmental variation making this system an ideal one with which to investigate baseline levels of FA in the wild as well as sensitivity of FA to natural levels of environmental variation. We investigate geographical variation in a multi-trait composite FA index in response to several environmental factors that vary among habitats from this archipelago. We also test for the predicted negative correlation

between FA and fitness among these natural populations by comparing mean FA between two age classes and by comparing FA among parasitized and unparasitized fish, as recent studies from one of these populations show broad temporal trends between FA and parasitism (Reimchen, 1997; Reimchen & Nosil, 2001). If fitness and FA are negatively associated, and if FA is an accurate indicator of organism-wide developmental stability, we should see a reduction in FA in adults relative to juveniles as well as a correlation in FA among traits within individuals. We investigate differences in asymmetry of the individual traits used in our multi-trait composite FA index to determine if there is evidence of differential levels of developmental stability among them. Consistent variation in the level of asymmetry among traits across a range of habitats implies that FA may have value as an indicator of the relative functional importance of the traits. Such an examination of geographical patterns of both composite and trait-specific asymmetry will provide context to the importance of environmental stress and natural selection in the expanding analyses of intra-individual variation among natural populations.

MATERIAL AND METHODS

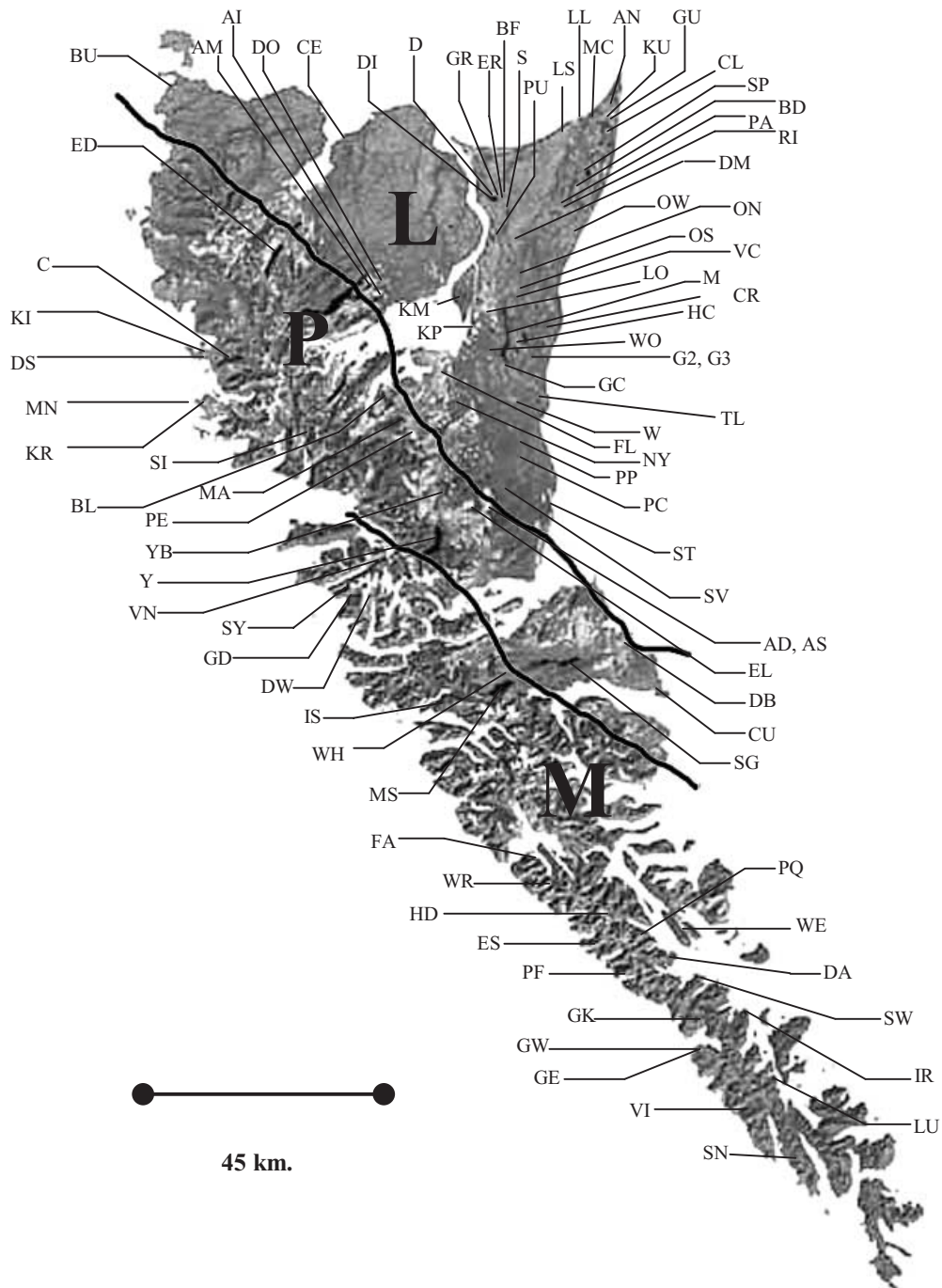
Samples of *G. aculeatus* from lakes and streams throughout the archipelago were obtained during multiple expeditions between 1969 and 1991. Of these original collections, 87 samples from the three major geographical areas of the archipelago (lowlands, plateaux and mountains) were used for this study (Fig. 1). Habitat descriptions and general collecting methods are published elsewhere (Reimchen, 1983, 1989, 1994; Reimchen *et al.*, 1985). In summary, the majority of *G. aculeatus* samples were collected from April to July using standard-mesh minnow traps placed in the littoral zones. Fish were fixed in 10% formalin and stored in 95% ethanol. Water chemistry and lake data

Figure 1. Map of Haida Gwaii showing sampled localities used for this analysis. Three major geographical zones of the islands defined as L (lowlands), P (plateaux) and M (mountains) as defined in Brown (1968). $N=87$. AI=Ain L., AM=Amber L., AD=Anderson North L., AS=Anderson South L., AN=Anser L., BF=Bigfish L., BL=Blackwater Cr., BD=Blue Danube L., BU=Bruin L., CR=Capeball R., CE=Cedar L., CL=Clearwater L., C=Coates L., CU=Cumshewa L., DM=Dam L., DA=Darwin L., DW=Dawson L., DB=Debris L., DS=Desolate L., DO=Downtree L., D=Drizzle L., DI=Drizzle Inlet, ED=Eden L., EL=Elk Survey Cr., ER=Eriophorum L., ES=Escarment L., FA=Fairfax L., FL=Florence Cr., G2=Geikie 2 Cr., G3=Geikie 3 Cr., GC=Gold Cr., GK=Goski L., GE=Gowgaia L. (east), GW=Gowgaia L. (west), GR=Gross L., GU=Grus L., GD=Gudal L., HC=Hickey L., HD=Hidden L., IS=Inskip Lagoon, IR=Irridens L., KI=Kiokathli L., KR=Krajina L., KU=Kumara L., KM=Kumdis L., KP=Kumdis P., LO=Loon L., LL=Lumme L., LS=Lumme Swamp, LU=Lutea L., MA=Marie L., M=Mayer L., MN=Menyanthes L., MC=Mica L., MS=Mosquito L., NY=New Years L., ON=Otter North L., OS=Otter South L., OW=Oeanda R., PA=Parkes L., PE=Peter L., PC=Pontoon Center L., PP=Pontoon Tlell P., PQ=Poque L., PF=Puffin L., PU=Pure L., RI=Richter L., SI=Seal Inlet L., SG=Skidegate L., S=Skonun L., SN=Snub L., SP=Spence L., ST=Stellata L., SY=Stiu L., SW=Sundew L., SV=Survey Cr., TL=Tlell Estuary, VC=Vaccinium L., VN=Van Inlet L., VI=Victoria L., WE=Wegner L., WH=White Swan P., WI=Wiggins L., WO=Woodpile L., WR=Right L., Y=Yakoun L., YB=Yakoun Backwater R.

were collected from a subset of the localities at the time of collection. This included pH, percentage light transmission at 400nm (T400), specific conductance ($\mu\text{mhos cm}^{-1}$), maximum water depth (m), and lake area (ha). Specific conductance, maximum water depth and lake area were log transformed to normalize the skew in their distributions.

MORPHOMETRICS

From each sample of *G. aculeatus*, up to 97 individuals were measured for standard length (SL), sex, parasite infection (*Schistocephalus solidus*, *Eustrongylides* sp., *Diplostomum* sp.), and asymmetry. Each sample was comprised of up to 50 juveniles (c. 35–45 mm SL) and



Downloaded from https://academic.oup.com/biolinnean/article/77/1/9/2639736 by guest on 23 April 2024

50 adults (>45mm). We also measured asymmetry in 10 bilateral traits on the predator defence apparatus (Fig.2). These traits included: number of forks on the dorsal edge of the ascending process (FORKS), amount of overlap between lateral plate(s) 4,5,6,7 (if present) and the basal plates (BP4, BP5, BP6 and BP7), amount of overlap between lateral plate(s) 5,6,7 (if present) and the ascending process (AP5, AP6 and AP7), ascending process height (APH), and ascending process width (APW). Overlap values ranged from 0 (not touching) to 3 (strong overlap). The overlap in this defensive apparatus provides structural support for the spines and dispersal of forces during predator manipulation (Reimchen, 1983). All metric measurements were made to 0.010mm using a dissecting scope and micrometer.

After preliminary measurements were completed, 12 samples were randomly chosen for analysis of measurement error. From each sample, 20 individuals were randomly selected and re-measured. A two-way mixed model ANOVA was run for each trait with side (fixed) and individual (random) as factors and trait as the dependent (Palmer, 1994) in order to determine whether measurement error variance contributed significantly to between sides variance. One of the traits (AP6) had significant measurement error after Bonferroni correction (Table 1) and was therefore excluded from further analysis.

Directional asymmetry and anti-symmetry among juveniles were examined with *t*-tests of the signed asymmetry (R-L) of each trait against a mean of zero, and by calculating the kurtosis of the R-L distribu-

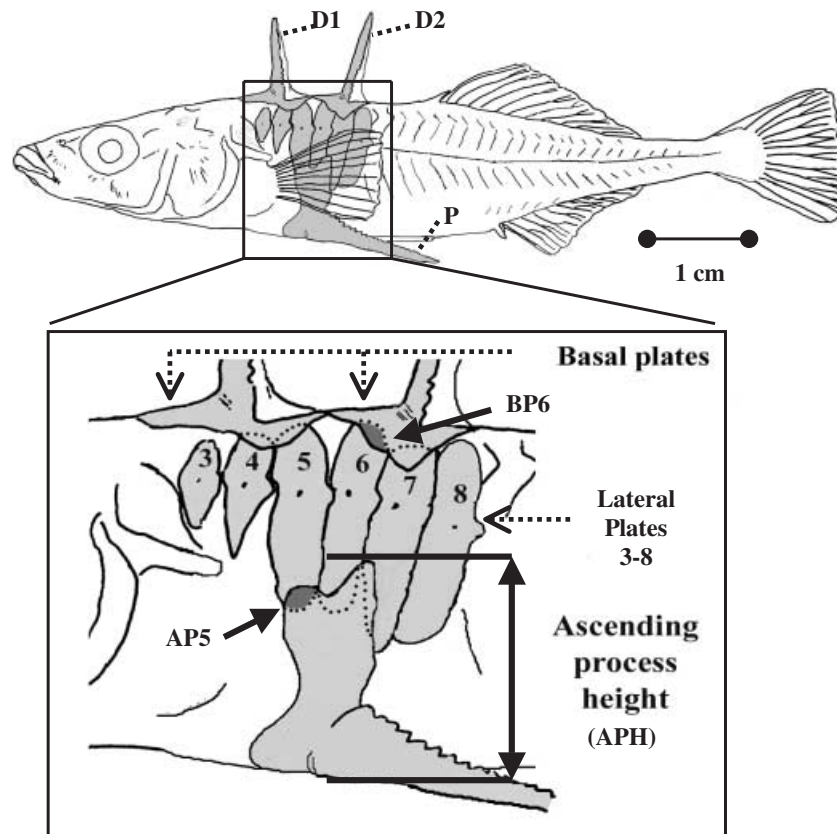


Figure 2. Camera lucida sketch of the left side of a *G. aculeatus* showing the first dorsal spine (D1), second dorsal spine (D2), left pelvic spine (P), bony lateral plates number 3 through to 8 highlighted in grey, basal plates, and ascending process. Inset shows traits used in the preliminary FA analysis: BP4, BP5, BP6, BP7 shown in dotted lines designating overlap between lateral plates 4–7 and the basal plates. AP5, AP6 and AP7 shown in dotted lines designating overlap between lateral plates 5–7 and the ascending process. Number of forks (FORKS) on dorsal edge of the ascending process ranged from 1 to 4 (2 in this individual). Ascending process width (APW) measured at the widest point of the ascending process, anterior to posterior. Ascending process height (APH) measured at the highest point, dorsal to ventral. Final traits used in the FA analysis (designated by heavy solid arrows) were: ascending process height (APH), overlap between lateral plate 6 and the basal plate (BP6), and overlap between lateral plate 5 and the ascending process (AP5).

Table 1. Two-way mixed model ANOVA of Sides (fixed) by Individual (random). Results are from the subset of re-measured populations only. Significant Side effects are evidence of directional asymmetry, significant Individual effects are evidence of size variance in the trait among individuals, and significant Side×Individual effects are evidence that FA contributes significantly more to between sides variance than measurement error (Palmer, 1994). Significance values are before Bonferroni corrections

Trait	Side		Individual		Side×Individual	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
FORKS	2.687	0.103	5.173	<0.001	2.481	<0.001
BP4	0.250	0.617	5.877	<0.001	8.847	<0.001
BP5	0.056	0.813	4.435	<0.001	3.828	<0.001
BP6	0.494	0.483	10.872	<0.001	4.271	<0.001
BP7	0.127	0.722	4.160	<0.001	13.756	<0.001
AP5	3.193	0.075	12.143	<0.001	2.059	<0.001
AP6	0.221	0.639	33.572	<0.001	1.234	0.038
AP7	0.038	0.845	15.447	<0.001	2.047	<0.001
APH	0.029	0.865	74.097	<0.001	3.397	<0.001
APW	5.652	0.018	29.766	<0.001	3.147	<0.001

Table 2. *t*-tests (two-tailed) against a mean of zero and kurtosis (g^2) of (R-L) distributions (all juveniles from the data set included). A significant result is evidence of directional asymmetry. Kurtosis values greater than 0 are evidence of the absence of anti-symmetry (platykurtosis). Significance values are before Bonferroni corrections

Trait	<i>t</i>	<i>P</i>	g^2
FORKS	3.059	0.002	n/a
BP4	-0.742	0.458	6.772
BP5	-0.749	0.454	14.251
BP6	-0.114	0.909	1.792
BP7	1.039	0.299	113.350
AP5	-1.210	0.227	5.991
AP6	-1.861	0.063	101.505
AP7	0.846	0.398	34.580
APH	-0.533	0.594	8.807
APW	5.583	<0.001	n/a

tions (Table 2). Two traits showed significant directional asymmetry (FORKS and APW were both right side dominant) and were thus excluded from further analysis. Kurtosis values (g^2) for each trait were positive, evidence that the R-L distributions were unimodal and leptokurtic (Palmer & Strobeck, 1986).

The remaining traits varied in the frequency of their occurrence in individuals. Some of the populations have reduced numbers of structural lateral plates and therefore it is not possible to measure overlap with the spine supports when plates are missing. The plates at

positions 5 and 6 are the most conserved, and consequently, for this study, we used the amount of overlap between lateral plate 5 and the ascending process (AP5), the amount of overlap between lateral plate 6 and the basal plates (BP6), as well as the ascending process height (APH) (Fig. 2). None of the samples demonstrated significant trait size/asymmetry associations for these traits. Asymmetries from these three traits were coalesced into a composite FA index (CFA). Because the variables represent both continuous and discrete distributions, we used the sum of the ranks of all three traits (see Leung, Forbes & Houle, 2000). Each individual in the pooled data set was given a rank according to the absolute (R-L) score for each of the 3 traits, and the ranks were then added to give a summed CFA score for each individual. There were no significant differences in mean CFA between sexes in any of the samples after sequential Bonferroni corrections ($P > 0.100$ in all cases), and the number of samples that had greater mean CFA for males did not differ significantly from those that had greater mean CFA for females ($N = 37$ and 43 , respectively; $\chi^2 = 0.450$; d.f. = 1; $P > 0.500$; 7 samples had only one sex present). Sexes were therefore pooled for the remaining analyses.

Selection differentials (i) between sub-adults and adults for CFA and for FA of each trait separately were calculated for 73 localities that had both age classes represented in the samples. Selection differentials were calculated by subtracting the mean juvenile FA from the mean adult FA, and dividing this difference by the standard deviation of juvenile FA (see

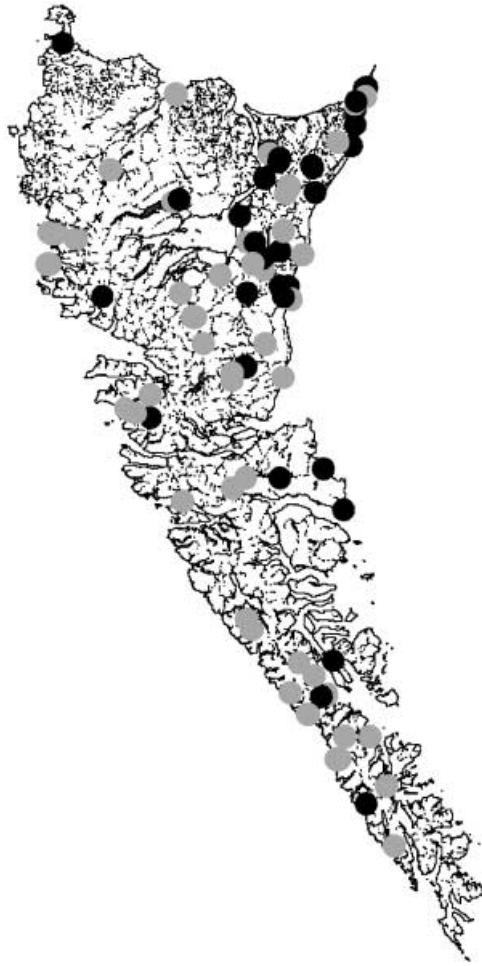


Figure 3. Distribution of mean total CFA among populations. Black circles designate populations with mean CFA scores greater than 6000 (see Appendix 1) and grey circles designate populations with mean CFA scores less than 6000. The populations with black circles on the north-west coast are Bruin L., Seal Inlet L. and Dawson L.

Endler, 1986). We tested for the presence of intra-individual correlation of asymmetry among traits in juveniles only, in order to reduce the potential impact of selection on asymmetry. Individuals were ranked (within each sample) based on the relative asymmetry of each trait, and the ranks of individuals were tested for concordance among all 3 traits. Comparisons of the relative asymmetry among traits were performed using $|\log(L/R)|$ (L=value on left side, R=value on right side), in order to express the asymmetry scores on a common scale (Clarke, 1998b).

RESULTS

Mean total CFA (adults and juveniles combined) varied significantly among *G. aculeatus* samples col-

Table 3. Multiple regression of total and juvenile sample mean CFA by pH, T400, specific conductance, maximum water depth and lake area. Model results for total samples: $R^2=0.007$, $P=0.115$; model results for juveniles: $R^2=0.009$, $P=0.304$

Factor	Total		Juvenile	
	Partial <i>r</i>	<i>P</i>	Partial <i>r</i>	<i>P</i>
pH	0.031	0.276	-0.020	0.659
T400	0.016	0.569	0.067	0.132
Specific conductance (log)	0.038	0.176	0.057	0.205
Maximum water, depth (log)	0.006	0.822	0.043	0.337
Lake area (log)	0.034	0.233	0.002	0.972

lected from the 87 localities on Haida Gwaii (Kruskal–Wallis: $\chi^2=365.68$; d.f.=86; $P<0.001$). This differed among the three geographical regions (Kruskal–Wallis: $\chi^2=9.210$; d.f.=2; $P=0.010$), with lowland *G. aculeatus* samples having the highest FA and mountain samples having the lowest. In the plateaux and mountains, the most symmetric populations tended to be on the west coast (Fig. 3). There were no significant differences between mean total CFA of creeks and their adjoining lakes (Paired *t*-test: $t=0.630$; d.f.=10, $P=0.540$).

Mean total CFA differed with respect to limnological factors among the lakes. For bivariate comparisons, it was inversely correlated with lake pH ($r=-0.460$; $P=0.003$) and water colour ($r=-0.390$; $P=0.001$) but not with maximum water depth ($r=-0.090$; $P=0.390$), lake area ($r=-0.060$; $P=0.590$) or specific conductance ($r=-0.180$; $P=0.270$). Water colour (T400) and pH are strongly positively correlated among localities ($r=0.790$; $P<0.001$) and both increase in value from the lowlands to the mountains, tending to be highest on the west coast of the archipelago, which also contains the least asymmetric samples. Of the ten localities with the highest CFA, nine were from lowland habitats, which are characterized by very shallow, acidic, and dystrophic lakes (low values of T400). The three west coast lakes with the highest asymmetry (Bruin L., Seal Inlet L. and Dawson L.; Fig. 3; Appendix 1) also have the highest levels of water staining in this area (pH was not collected from these three localities). However, the independent residual effects of pH, T400, specific conductance, maximum water depth and lake area on total CFA were insignificant (Table 3).

We also examined mean CFA for juvenile fish in relation to limnological factors among lakes. While there were significant differences in mean CFA among populations (Kruskal–Wallis: $\chi^2=173.96$; d.f.=70;

Table 4. Multiple regression of mean absolute asymmetry for three traits, APH, AP5 and BP6 by pH, T400, specific conductance, maximum water depth and lake area. Model results for APH: $R^2=0.011$, $P=0.297$; model results for AP5: $R^2=0.005$, $P=0.733$; model results for BP6: $R^2=0.036$, $P=0.001$

Factor	APH		AP5		BP6	
	Partial r	P	Partial r	P	Partial r	P
pH	-0.015	0.730	0.005	0.730	0.032	0.452
T400	0.061	0.149	0.061	0.149	-0.047	0.266
Specific conductance (log)	-0.058	0.170	-0.058	0.170	-0.146	0.001
Maximum water depth (log)	-0.067	0.112	-0.067	0.112	0.073	0.086
Lake area (log)	0.054	0.206	0.054	0.206	-0.063	0.137

$P < 0.001$), there were no differences among geographical regions (Kruskal–Wallis: $\chi^2=0.380$; d.f.=2; $P=0.830$), or between creeks and their adjoining lakes (Paired t -test: $t=0.518$; d.f.=9; $P=0.620$). There were no significant bivariate correlations between juvenile CFA and any limnological variable (pH: $r=-0.070$; $P=0.690$; T400: $r=-0.050$; $P=0.680$; specific conductance: $r=-0.260$; $P=0.120$; maximum water depth: $r=-0.020$; $P=0.850$; lake area: $r=-0.010$; $P=0.930$) and no significant residual effects from these variables resulting from multiple regression analysis (Table 3).

In order to determine if asymmetry of the three individual traits used to compute the CFA index varied in their sensitivity to environmental fluctuations, we ran multiple regressions of the five environmental variables and mean juvenile absolute asymmetry for each trait. While APH and AP5 demonstrated no significant associations with the environmental variables, the regression model for BP6 was significant and this was primarily due to a negative correlation with specific conductance (Table 4).

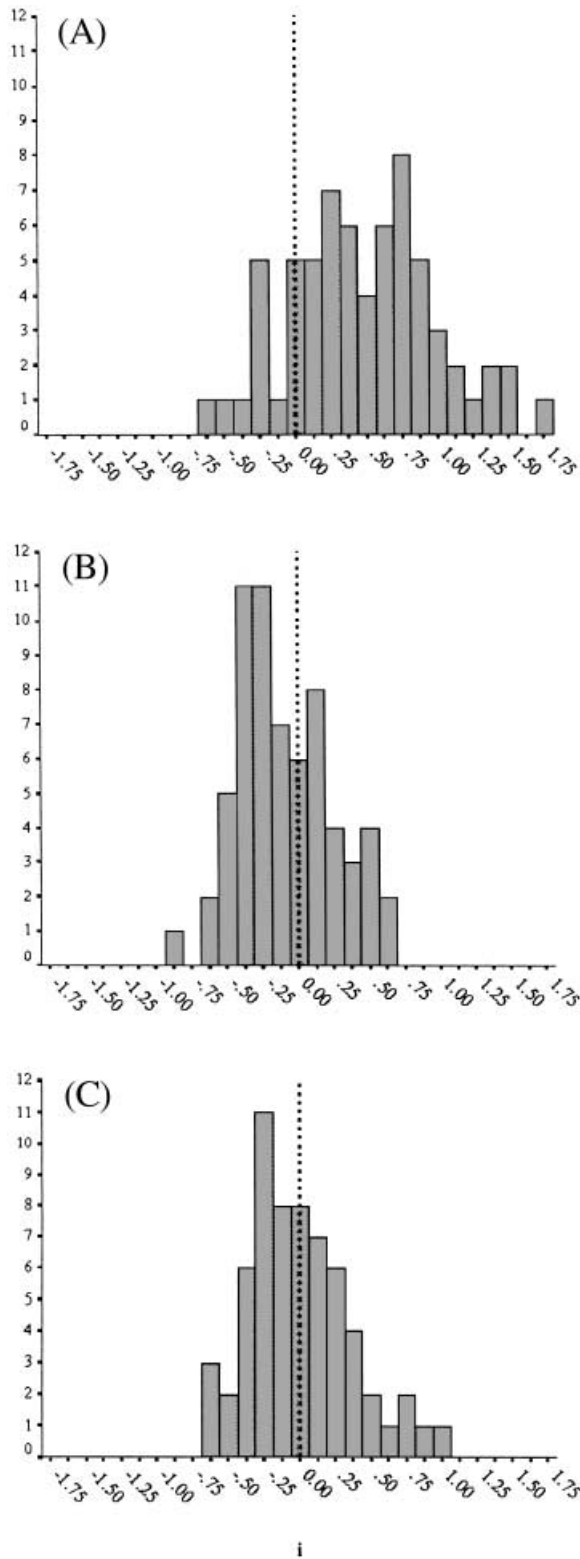
We examined whether some traits were consistently more asymmetric than others among individuals. Six populations showed significant concordance (Kendall's W) of trait asymmetry rank after sequential Bonferroni corrections (Escarpment L., Gowgaia West L., Gudal L., Inskip Lagoon, Lutea L. and Yakoun River). In each of these populations, ascending process height ranked highest for asymmetry relative to the other two traits. Consistent with this pattern among individuals, the rank of mean trait asymmetries showed significant concordance among all populations (Kendall's $W=0.29$; $\chi^2=41.000$; d.f.=2; $P < 0.001$), with APH having the highest mean asymmetry rank (2.480) and AP5 having the lowest (1.440).

In order to determine if developmental stability had a genome-wide effect, we assessed the degree of concordance of FA among these three traits within individuals in each sample using Kendall's coefficient of concordance (W). We found no significant intra-

individual correlation of asymmetry among traits in any sample, implying that the relative asymmetry of one individual was not predictable among traits in any sample.

There were no significant differences between parasitized and non-parasitized fish in CFA or in individual trait FA within any population (t -tests: all $P > 0.050$). However, parasitized fish had a higher CFA than non-parasitized fish in a significant majority of populations containing parasites (41 of the 63 populations, Binomial test: $P < 0.025$) although this did not occur for comparisons with individual trait FA (Binomial test: all $P > 0.100$). Parasitism did not appear to cause greater FA in infected fish, as fish with increasing numbers of parasites did not show greater FA than fish with fewer parasites (all $P > 0.200$).

We assessed whether there was evidence of a correlation between survivorship and CFA in the samples by calculating selection differentials between the age classes, and determined whether there was any geographical predictability to the distribution of these selection differentials. Among the 73 localities, no significant (Bonferroni corrected) selection differentials were detected, but contrary to our predictions, there were significantly more positive than negative differentials ($N=52$ and 21, respectively; Binomial test: $P < 0.001$). This trend occurred in each of the three geographical areas but was strongest in the lowland samples (lowlands 20 vs. 6, respectively; Binomial $P < 0.050$; plateaux: 13 vs. 7; Binomial $P=0.260$; mountains: 19 vs. 8; Binomial $P=0.050$). We found no relationships among the selection differentials between creeks and their adjoining lakes (Paired t -test: $t=1.410$; d.f.=9; $P=0.190$) or with any environmental variables using bivariate comparisons and multiple regressions. We also examined mortality selection on asymmetry in the three individual traits. While there were no significant selection differentials for FA in any population, differentials varied significantly among traits (Kruskal–Wallis $\chi^2=1096.142$; d.f.=2



$P < 0.001$; Fig. 4). The differentials of the three traits did not differ among the three geographical areas and were not correlated with the limnological variables (all $P > 0.050$).

DISCUSSION

Our results have demonstrated high levels of variance in multi-trait bilateral asymmetry among natural populations of freshwater *G. aculeatus* residing in pristine habitats. Inter-population variance in CFA differed geographically with highest values occurring in lakes with increased acidity and increased water staining, these tending to occur in the lowland regions of the archipelago. In contrast, low levels of CFA were most prevalent in the oligotrophic lakes with higher pH and higher water clarity that were most prevalent in mountain regions. The abiotic associations were detected only in the complete samples (adults and juveniles) and did not occur in the subset of juvenile fish from each locality.

Previous studies have found elevated levels of FA in organisms subjected to stress during embryonic development, including acidic conditions (Jago & Haines, 1985; Ostbye *et al.*, 1997; Mazzi & Bakker, 2001), exposure to UV radiation (Midgley *et al.*, 1998), food deprivation (Swaddle & Witter, 1994; Roy & Stanton, 1999; Stoks, 2001) and temperature fluctuations (Beardmore, 1960; Gest *et al.*, 1986; Clarke & McKenzie, 1992; Leary *et al.*, 1992; Hosken *et al.*, 2000). Since these factors can generate FA in newly emerging young, we expected to see elevated FA in juvenile stickleback in the small bog lakes of the lowlands, as these are acidic, dystrophic and shallow. That we did not observe this suggests that the specific environmental variables we measured were not generating detectable FA in the young. However, several other factors may account for these results.

Other studies that have investigated environmental impacts on population FA in the wild have focused on short-term disturbances caused by human activity (Valentine & Soulé, 1973; Valentine *et al.*, 1973; Sanchez-Galan *et al.*, 1998; Kirchhoff *et al.*, 1999). Stickleback populations on Haida Gwaii may have adapted their developmental buffering capabilities to these limnologically extreme but historically stable

Figure 4. Population selection differential (*i*) frequency histograms for: (A) ascending process height asymmetry (APH), (B) lateral plate 6 overlap asymmetry with basal plate (BP6), and (C) lateral plate 5 overlap asymmetry with ascending process (AP5). Selection differentials for the three traits vary significantly (Kruskal–Wallis $\chi^2 = 1096.142$; d.f. = 2; $P < 0.001$; APH mean = 0.42, AP5 mean = 0.03, BP6 mean = -0.05).

habitats. However, this still does not explain the large variance in juvenile FA among populations. It is likely that developmental stability in these populations was not sensitive to the particular variables we measured, but rather to other ecological and/or abiotic sources of stress. In addition, the environmental variance among the pristine habitats used in this study may not have been great enough to generate detectable differences in FA. The ranges of environmental conditions in the laboratory that generate differences in FA are in many cases greater than environmental fluctuations in the wild (Zakharov, 1992). Comparing FA among populations encompassing a greater range of conditions may reveal some more general associations.

Our study involving 87 natural populations found no clear evidence that asymmetric individuals were suffering reduced survivorship. While several studies have demonstrated a negative correlation between FA and survivorship (Møller, 1994; Ueno, 1994; Nosil & Reimchen, 2001), others have not (Bjorksten, Pomiankowski & Fowler, 2001) and many have failed to find a correlation between FA and other fitness measures (Eggert & Sakaluk, 1994; Swaddle & Cuthill, 1995; Tomkins & Simmons, 1998). In some cases, a positive correlation between FA and reproductive success or functional performance has been reported (Moodie & Moodie, 1996; Seligmann, 1998). What was unique to our study was the positive correlation between survivorship and asymmetry in some of the populations. High metabolic costs of developmental stability could sustain epigenetic errors in populations (Schlichting & Pigliucci, 1998). Survivorship may be the result of interactions between the metabolic costs of maintaining symmetry and the fitness benefits associated with that symmetry. In habitats where the fitness benefits of symmetry are minimal, this might result in a reduction of survivorship in highly symmetric individuals.

While we found no negative correlation between survivorship and FA, we found that parasitized fish were slightly more asymmetric than unparasitized fish among two-thirds of the populations. Other studies have found elevated FA in parasitized individuals from diverse taxa (Escos *et al.*, 1995; Bonn *et al.*, 1996; Hunt & Allen, 1998) as well as in stickleback (Reimchen, 1997; Reimchen & Nosil, 2001), suggesting that FA is a reasonably consistent indicator of susceptibility of the immune system to parasite infestation. The variability in FA/fitness associations in this and other studies implies that the association is specific to particular fitness components.

We observed that asymmetry in one trait was a poor predictor of asymmetry in another trait in the same individual, indicating no suggestion of genome-wide developmental instability. Rather, FA appeared to be trait specific. For example, asymmetry of overlap

between the basal plate with the 6th lateral plate (BP6) in juveniles was inversely associated with specific conductance, while neither ascending process height asymmetry (APH) nor asymmetry of overlap between the ascending process and the 5th plate (AP5) showed correlations with any habitat parameter in our analysis. BP6 could be more sensitive to environmental stress than the other traits, possibly due to differences in the developmental stability or timing of development among these three traits.

Estimates of the health of populations based on the relative asymmetry of traits should be interpreted with caution. This study provides an example of how conclusions regarding conservation priorities may be erroneously reached by measuring FA in one trait versus another. We also found large variance in the degree of both individual trait FA and a composite FA measure among natural, anthropogenically unstressed populations. As suggested by Palmer (1996), knowledge of baseline levels of FA in natural populations is crucial to informed applications of FA to conservation efforts. While FA may be reflecting differences in homozygosity among populations from this archipelago, we have found no evidence that it is responding to natural fluctuations in abiotic factors. That this study found large FA variance in natural populations that was for the most part uncorrelated with natural environmental variation suggests that there may be a large level of background FA variance that is not necessarily a reflection of population 'health'.

Asymmetry among the three traits varied significantly and demonstrated strong concordance in their relative levels of asymmetry among the populations. Ascending process height (APH) had the highest level of FA among populations and ascending process overlap asymmetry with lateral plate 5 (AP5) had the lowest. That the ranking of asymmetry among these three traits is similar among populations implies a common developmental process among localities and potentially a common ranking of the functional importance of these three traits in different habitats. Consistent differences in asymmetry among specific lateral plates exist as well, as lateral plate asymmetries in the structural plates that support the spines are relatively rare compared to asymmetry in the other plates (Bergstrom & Reimchen, 2000), presumably due to increased functional costs. In the present study, there may be more serious costs of asymmetry in BP6 or AP5 than of APH, resulting in relatively stronger selection for developmental stability in BP6 and AP5 over the last 12 000 years. If this is correct, then there should be less evidence for selection against asymmetry in APH than in BP6 or AP5 and this is indeed the case, as selection differentials for APH asymmetry are significantly more positive than for the other two traits (Fig. 4).

In summary, this study has demonstrated a large variance in CFA among 87 natural populations of *G. aculeatus*. We found significantly elevated FA in adults from lowland dystrophic localities that tend to be highly acidic, stained and shallow, and significant but weak negative bivariate correlations between adult CFA and pH and T400. However, mean CFA in juveniles was randomly distributed across the archipelago and not strongly statistically associated with pH, T400, conductance or water body size. The differences in the distribution and habitat correlates of mean CFA between these two age classes implies that there are other potential selective factors associated with the major geographical areas of the archipelago that are generating differences in asymmetry in adults, but not juveniles. We found little evidence of reduced survivorship in asymmetric individuals or for correlations in asymmetry among traits within individuals, although we did find that the significant majority of samples had slightly elevated CFA in parasitized fish. Relative asymmetry among the individual traits was consistent among populations, and only one of the traits showed an association between FA and one limnological variable. The variance in selection differentials for FA among the three traits may reflect variability in their functional importance. We have previously shown (Bergstrom & Reimchen, 2000) that there are differences in developmental stability among lateral plate positions, while current investigations suggest that symmetry in some plates is more critical in habitats where predatory trout are a dominant source of mortality. These cumulative data imply that FA/fitness associations are character-specific and may be the result of interactions between the costs and benefits of symmetric development.

ACKNOWLEDGEMENTS

We would like to thank S. Douglas for assistance in collecting the samples, P. Nosil for discussion and helpful suggestions, J. Broadhead for use of the satellite image of Haida Gwaii, and two anonymous referees for suggestions that improved the manuscript. Financial support was provided by a King-Platt Fellowship awarded to C.A.B and an NSERC research grant (#A2354) awarded to T.E.R.

REFERENCES

- Allen GR & Simmons LW. 1996.** Coercive mating, fluctuating asymmetry and male mating success in the dung fly *Sepsis cynipsea*. *Animal Behaviour* **52**: 737–741.
- Ames LJ, Felley JD, Smith MH. 1979.** Amounts of asymmetry in centrarchid fish inhabiting heated and nonheated reservoirs. *Transactions of the American Fisheries Society* **108**: 489–495.
- Balmford A, Jones IL, Thomas ALR. 1993.** On avian asymmetry: evidence of natural selection for symmetrical tails and wings in birds. *Proceedings of the Royal Society of London B* **252**: 245–251.
- Beardmore JA. 1960.** Developmental stability in constant and fluctuating temperatures. *Heredity* **14**: 411–422.
- Bergstrom CA & Reimchen TE. 2000.** Functional implications of fluctuating asymmetry among endemic populations of *Gasterosteus aculeatus*. *Behaviour* **137**: 1097–1112.
- Bjorksten TA, Pomiankowski A, Fowler K. 2001.** Temperature shock during development fails to increase the fluctuating asymmetry of a sexual trait in stalk-eyed flies. *Proceedings of the Royal Society of London B* **268**: 1503–1510.
- Bonn A, Gasse M, Rolff J, Martens A. 1996.** Increased fluctuating asymmetry in the damselfly *Coenagrion puella* is correlated with ectoparasitic water mites: implications for fluctuating asymmetry theory. *Oecologia* **108**: 596–598.
- Brown AS. 1968.** *Geology of the Queen Charlotte Islands*. Victoria: Department of Mines and Petroleum Resources.
- Bryden C & Heath D. 2000.** Heritability of fluctuating asymmetry for multiple traits in chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 2186–2192.
- Clarke GM. 1993.** The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. *Genetica* **89**: 15–23.
- Clarke GM. 1995.** Relationships between developmental stability and fitness: application for conservation biology. *Conservation Biology* **9**: 18–24.
- Clarke GM. 1998a.** Developmental stability and fitness: the evidence is not quite so clear. *American Naturalist* **152**: 762–766.
- Clarke GM. 1998b.** The genetic basis of developmental stability. IV. Individual and population asymmetry parameters. *Heredity* **80**: 553–561.
- Clarke GM & McKenzie LJ. 1992.** Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. *Journal of Economical Entomology* **85**: 2045–2050.
- Crespi BJ & Vanderkist BA. 1997.** Fluctuating asymmetry is vestigial and functional traits of a haplodiploid insect. *Heredity* **79**: 624–630.
- Dufour KW & Weatherhead PJ. 1996.** Estimation of organism-wide asymmetry in red-winged blackbirds and its relation to studies of mate selection. *Proceedings of the Royal Society of London B* **263**: 769–775.
- Eggert AK & Sakaluk SK. 1994.** Fluctuating asymmetry and variation in the size of courtship food gifts in decorated crickets. *American Naturalist* **144**: 708–716.
- Endler JA. 1986.** *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
- Escos J, Alados C, Emlen J, Alderstein S. 1995.** Developmental instability in the pacific hake parasitized by *Myxosporeans kudoa* spp. *Transactions of the American Fisheries Society* **124**: 943–945.

- Evans AS & Marshall M. 1996.** Developmental instability in *Brassica campestris* (Cruciferae): fluctuating asymmetry of foliar and floral traits. *Journal of Evolutionary Biology* **9**: 717–736.
- Gangestad SW & Thornhill R. 1999.** Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. *Journal of Evolutionary Biology* **12**: 402–416.
- Gest TR, Siegel MI, Anistranski J. 1986.** The long bones of neonatal rats stressed by cold, heat, and noise exhibit increased fluctuating asymmetry. *Growth* **50**: 385–389.
- Gomendio M, Cassinello J, Rolden ERS. 2000.** A comparative study of ejaculate traits in three endangered ungulates with different levels of inbreeding: fluctuating asymmetry as an indicator of reproductive and genetic stress. *Proceedings of the Royal Society of London B* **267**: 875–882.
- Graham JJ. 1992.** Genomic coadaptation and developmental stability in hybrid zones. *Acta Zoologica Fennica* **191**: 121–131.
- Gummer DL & Brigham RM. 1995.** Does fluctuating asymmetry reflect the importance of traits in little brown bats (*Myotis lucifugus*)? *Canadian Journal of Zoology* **73**: 990–992.
- Hosken DJ, Blanckenhorn WU, Ward PI. 2000.** Developmental stability in yellow dung flies (*Scathophaga stercoraria*): fluctuating asymmetry, heterozygosity and environmental stress. *Journal of Evolutionary Biology* **13**: 919–926.
- Hunt J & Allen GR. 1998.** Fluctuating asymmetry, call structure and the risk of attack from phonotactic parasitoids in the bushcricket *Sciarasaga quadrata* (Orthoptera: Tettigoniidae). *Oecologia* **116**: 356–364.
- Jago CH & Haines TA. 1985.** Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. *Canadian Journal of Zoology* **63**: 130–138.
- Kirchhoff S, Seigny J, Couillard C. 1999.** Genetic and meristic variations in the mummichog *Fundulus heteroclitus*, living in polluted and reference estuaries. *Marine Environmental Research* **47**: 261–283.
- Leary RF, Allendorf FW, Knudsen KL. 1992.** Genetic, environmental, and developmental causes of meristic variation in rainbow trout. *Acta Zoologica Fennica* **191**: 79–95.
- Lens L, VanDongen S, Galbusera P, Schenck T, Matthysen E, VanDeCastele T. 2000.** Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. *Journal of Evolutionary Biology* **13**: 889–896.
- Leung B, Forbes MR, Houle D. 2000.** Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *American Naturalist* **155**: 101–115.
- Manning JT & Chamberlain AT. 1993.** Fluctuating asymmetry in gorilla canines: a sensitive indicator of environmental stress. *Proceedings of the Royal Society of London B* **255**: 189–193.
- Mason LG, Ehrlich PR, Emmel TC. 1967.** The population biology of the butterfly, *Euphydryas editha*. V. Character clusters and asymmetry. *Evolution* **21**: 85–91.
- Mather K. 1953.** Genetical control of stability in development. *Heredity* **7**: 297–336.
- Mazzi D & Bakker TCM. 2001.** Acid stress increases pelvic spine asymmetry in juvenile three-spine sticklebacks. *Journal of Fish Biology* **59**: 582–592.
- Midgley GF, Wand SJE, Musil CF. 1998.** Repeated exposure to enhanced UV-B radiation in successive generations increases developmental instability (leaf fluctuating asymmetry) in a desert annual. *Plant, Cell and Environment* **21**: 437–442.
- Mitton J. 1995.** Enzyme heterozygosity and developmental stability. *Acta Theriologica* **3**: 33–54.
- Møller AP. 1994.** Sexual selection in the barn swallow (*Hirundo rustica*). IV. Patterns of fluctuating asymmetry and selection against asymmetry. *Evolution* **48**: 658–670.
- Møller AP & Pomiankowski A. 1993.** Fluctuating asymmetry and sexual selection. *Genetica* **89**: 267–279.
- Møller AP & Swaddle JP. 1997.** *Asymmetry, developmental stability, and evolution*. Oxford: Oxford University Press.
- Moodie GEE. 1977.** Meristic variation, asymmetry, and aspects of the habitat of *Culea inconstans* (Kirtland), the brook stickleback, in Manitoba. *Canadian Journal of Zoology* **55**: 398–404.
- Moodie GEE & Moodie PF. 1996.** Do asymmetric sticklebacks make better fathers? *Proceedings of the Royal Society of London Series B* **263**: 535–539.
- Moodie GEE & Reimchen TE. 1976.** Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. *Systematic Zoology* **25**: 49–61.
- Nosil P & Reimchen TE. 2001.** Tarsal asymmetry, nutritional condition, and survival in water boatmen (*Callicorixa vulnerata*). *Evolution* **55**: 712–720.
- Ostbye K, Oxnevad S, Vollestad L. 1997.** Developmental stability in perch (*Perca fluviatilis*) in acidic aluminium-rich lakes. *Canadian Journal of Zoology* **75**: 919–928.
- Packer C & Pusey AE. 1993.** Should a lion change its spots? *Nature* **362**: 595.
- Palmer AR. 1986.** Inferring relative levels of genetic variability in fossils: the link between heterozygosity and fluctuating asymmetry. *Paleobiology* **12**: 1–5.
- Palmer AR. 1994.** Fluctuating asymmetry analyses: a primer. In: Markow TA, ed. *Developmental instability: its origins and evolutionary implications*. Dordrecht: Kluwer, 335–364.
- Palmer AR. 1996.** Waltzing with asymmetry. *Bioscience* **46**: 518–532.
- Palmer AR & Strobeck C. 1986.** Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* **17**: 391–421.
- Pankakoski E, Koivisto I, Hyvarinen H. 1992.** Reduced developmental stability as an indicator of heavy metal pollution in the common shrew *Sorex araneus*. *Acta Zoologica Fennica* **191**: 137–144.
- Pelabon C & van Breukelen L. 1998.** Asymmetry in antler size in roe deer (*Capreolus capreolus*): an index of individual and population conditions. *Oecologia* **116**: 1–8.
- Rantala MJ, Koskimaki J, Taskinen J, Tynkkynen K, Suhonen J. 2000.** Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens*.

- dens* L. *Proceedings of the Royal Society of London B* **267**: 2453–2457.
- Reimchen TE. 1983.** Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution* **37**: 931–946.
- Reimchen TE. 1989.** Loss of nuptial colour in threespine sticklebacks (*Gasterosteus aculeatus*). *Evolution* **43**: 450–460.
- Reimchen TE. 1994.** Predators and morphological evolution in threespine stickleback. In: Bell, MA, Foster, SA, eds. *The evolution of threespine stickleback*. Oxford: Oxford University Press, 240–276.
- Reimchen TE. 1997.** Parasitism of asymmetrical pelvic phenotypes in threespine stickleback. *Canadian Journal of Zoology* **75**: 2084–2094.
- Reimchen TE & Nosil P. 2001.** Dietary differences between phenotypes with symmetrical and asymmetrical pelvis in the threespine stickleback. *Canadian Journal of Zoology* **79**: 533–539.
- Reimchen TE, Stinson EM, Nelson JS. 1985.** Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Canadian Journal of Zoology* **63**: 2944–2951.
- Roy BA & Stanton ML. 1999.** Asymmetry of wild mustard, *Sinapis arvensis* (Brassicaceae), in response to severe physiological stresses. *Journal of Evolutionary Biology* **12**: 440–449.
- Sanchez-Galan S, Linde A, Izquierdo J, Garcia-Vazquez E. 1998.** Micronuclei and fluctuating asymmetry in brown trout (*Salmo trutta*): complementary methods to biomonitor freshwater ecosystems. *Mutation Research–Genetic Toxicology and Environmental Mutagenesis* **412**: 219–225.
- Schlichting CD & Pigliucci M. 1998.** *Phenotypic Evolution: A Reaction Norm Perspective*. Massachusetts: Sinauer Associates, Inc.
- Seligmann H. 1998.** Evidence that minor directional asymmetry is functional in lizard hindlimbs. *Journal of Zoology* **245**: 205–208.
- Simmons L & Ritchie M. 1996.** Symmetry in the songs of crickets. *Proceedings of the Royal Society of London B* **263**: 1305–1311.
- Soulé ME. 1967.** Phenetics of natural populations. II. Asymmetry and evolution in a lizard. *American Naturalist* **101**: 141–160.
- Soulé ME. 1979.** Heterozygosity and developmental stability: another look. *Evolution* **33**: 396–401.
- Soulé ME & Baker B. 1968.** Phenetics of natural populations. IV. The population asymmetry parameter in the butterfly *Coenonympha tullia*. *Heredity* **23**: 611–614.
- Stoks R. 2001.** Food stress and predator-induced stress shape developmental performance in a damselfly. *Oecologia* **127**: 222–229.
- Swaddle JP. 1997.** Developmental stability and predation success in an insect predator–prey system. *Behavioural Ecology* **8**: 433–436.
- Swaddle JP & Cuthill IC. 1995.** Asymmetry and human facial attractiveness: symmetry may not always be beautiful. *Proceedings of the Royal Society of London B* **261**: 111–116.
- Swaddle JP & Witter MS. 1994.** Food, feathers and fluctuating asymmetry. *Proceedings of the Royal Society of London B* **255**: 147–152.
- Tomkins JL & Simmons LW. 1998.** Female choice and manipulations of forceps size and symmetry in the earwig *Forficula auricularia* L. *Animal Behaviour* **56**: 347–356.
- Ueno H. 1994.** Fluctuating asymmetry in relation to two fitness components, adult longevity and male mating success in a ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Ecological Entomology* **19**: 87–88.
- Valentine DW & Soulé M. 1973.** Effect of, p, p'-DDT on developmental stability of pectoral fin rays in the grunion, *Leuresthes tenuis*. *Fishery Bulletin* **71**: 921–926.
- Valentine DW, Soulé ME, Samollow P. 1973.** Asymmetry analysis in fishes: a possible statistical indicator of environmental stress. *Fishery Bulletin* **71**: 357–370.
- Van Valen L. 1962.** A study of fluctuating asymmetry. *Evolution* **16**: 125–142.
- Vrijenhoek RC & Lerman S. 1982.** Heterozygosity and developmental stability under sexual and asexual breeding systems. *Evolution* **36**: 768–776.
- Waddington CH. 1942.** Canalisation of development and the inheritance of acquired characters. *Nature* **150**: 563–565.
- Watson PJ & Thornhill R. 1994.** Fluctuating asymmetry and sexual selection. *Trends in Ecology and Evolution* **9**: 21–25.
- Wayne RK, Modi WS, O'Brien SJ. 1986.** Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*), a genetically uniform species. *Evolution* **40**: 78–85.
- Zakharov VM. 1992.** Population phenogenetics: analysis of developmental stability in natural populations. *Acta Zoologica Fennica* **191**: 7–30.

APPENDIX 1

Localities in ascending order from lowest mean CFA to highest, showing mean CFA (including both age classes), pH, T400, specific conductance, maximum water depth and lake area. All variables are untransformed.

Locality	<i>N</i>	Geographical area**	CFA	pH	T400 (%)	Specific conductance ($\mu\text{mhos cm}^{-1}$)	Water depth (m)	Lake area (ha)
Inskip	75	M	4810.77		99		<1	
Ain	20	L	4826.30	5.1	87	37	5	78
Hidden	2	M	5039.25		93	80	50	18
Gowgaia W.	78	M	5115.61		78		8	25
Lumme Swp.	10	L	5128.95				1	<1
Capeball Rv.*	26	L	5257.65		47	118	<1	
Yakoun Rv.*	88	P	5257.82	6.5	90		<1	
Kiokathli	85	P	5262.31	6.0	75	45	15	9
Lutea	92	M	5296.91	6.8	94	2200	2	3
Peter	20	P	5354.28	6.9	92		20	41
Poque	65	M	5396.37	6.0	91		25	17
Mosquito	17	M	5418.68		95		30	340
Gowgaia E.	76	M	5419.09		78	2200	8	25
Amber	34	L	5437.08	6.3	87	46	4	72
Wright	80	M	5519.77				25	36
Woodpile	84	L	5560.34	4.9	61	71	2	4
Cedar	37	L	5564.34				4	4
Marie	65	P	5572.90	7.0	89		20	373
Dam	10	L	5580.33	5.3	52	560	3	9
Spence	20	L	5585.24	7.0	67		30	95
Stellata	70	L	5622.79		76	180	3	3
Pontoon Center	95	L	5625.43	6.7	88	85	<1	1
Gudal	82	M	5644.32	7.4	95		30	25
Tlell Estuary	41	L	5664.51				1	
Coates	44	P	5667.37	6.0	95	49	30	90
Otter N.	91	L	5668.32	4.6	46	118	2	10
Desolate	78	P	5396.37		82	35	5	3
Yakoun	88	P	5676.10	6.5	90		60	679
Stiu	40	M	5691.01	7.2	93	78	30	24
Darwin	59	M	5699.50		90		15	14
White Swan	75	M	5710.19	6.9	89	87	2	<1
Pontoon Tlell	62	L	5747.85		75			<1
Blackwater Ck.*	57	P	5749.16					<1
Elk Survey Ck.*	20	P	5754.08					<1
Van Inlet	71	M	5754.71				20	23
Escarpment	86	M	5758.41		94	36	50	97
Kumdis Pd.	25	L	5769.52	5.7		120	2	<1
Gold Ck.*	45	L	5770.50		50	98	2	
Puffin	94	M	5787.21		83	57	10	5
Survey Ck.*	59	L	5829.38		84			<1
Florence Ck.*	35	L	5837.06	6.6	76	125		<1
Otter S.	91	L	5879.45	4.6	45	160	3	36
Fairfax	74	M	6020.84		90	49	4	1
Snub	15	M	6034.23	6.7	83		20	2
Irridens	71	M	6034.67		87		5	18
Eden	97	P	6069.38				50	513
Drizzle Inlet*	4	L	6096.63		38		2	
Goski	76	M	6097.62	7.3	88	64	5	10
Krajina	62	P	6099.06	6.0	87	47	10	16
Vaccinium	23	L	6100.17				4	8

APPENDIX 1 *Continued*

Locality	N	Geographical area**	CFA	pH	T400 (%)	Specific conductance ($\mu\text{mhos cm}^{-1}$)	Water depth (m)	Lake area (ha)
Kumara	27	L	6114.50	5.5	62		4	15
Menyanthes	81	P	6127.57	6.3	82	17	5	6
Dawson	88	M	6180.45		82	55	4	1
Oeanda Rv.*	33	L	6251.47				3	
Seal Inlet	33	P	6303.36		82	34	5	2
Mayer	30	L	6311.60	4.9	57	90	20	373
Blue Danube	13	L	6336.35		65		<1	8
Drizzle	75	L	6337.81	5.1	67	69	16	97
Anser	56	L	6451.92	5.3	76		4	18
Sundew	54	M	6467.30	6.3	87	46	4	5
Downtree	23	L	6468.98	6.2	84	40	3	27
Pure	67	L	6566.41	4.5	77	76	10	34
Skidegate	55	P	6579.31		94		30	545
Cumshewa	38	P	6593.93				2	8
Lower Victoria	80	M	6668.66				25	149
Kumdis L.	2	L	6790.50				5	28
Geikie 3*	16	L	6810.44	4.9	63	91		<1
Grus	43	L	6840.17				3	14
Mica	17	L	6920.24				5	11
Geikie 2*	18	L	6927.94	4.7	63	109		<1
Hickey	12	L	6996.96				20	122
Anderson S.	53	P	7085.11	7.1	91	78	4	14
New Years	8	L	7088.25	4.7	69	70	2	7
Bruin	2	L	7146.25				10	9
Debris	15	P	7300.77	5.3	57	128	4	4
Anderson N.	47	P	7370.86	7.1	88	83	4	3
Clearwater	13	L	7441.65		76		10	53
Parkes	5	L	7476.00	4.8	61		5	16
Skonun	5	L	7532.00	4.5	68		15	51
Loon	2	L	7866.25				3	11
Richter	1	L	8369.00	4.3	52		5	12
Lumme L.	1	L	8445.50				10	39
Gross	7	L	8456.50		65		2	2
Wegner	1	M	8723.00				3	3
Bigfish	11	L	8750.82	4.5			2	2
Wiggins	2	L	9129.25				3	24
Eriophorum	2	L	9219.75		45		1	1

* =Stream sample. ** L=Lowland, P=Plateau, M=Mountain