

GENETICS OF LATERAL PLATE AND GILLRAKER PHENOTYPES IN A RAPIDLY EVOLVING POPULATION OF THREESPINE STICKLEBACK

by

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Summary

Twenty-seven crosses were used to study the genetics of rapidly evolving traits in a recently founded population of threespine stickleback in Loberg Lake, Alaska. Lateral plate morph segregation ratios were inconsistent with all published models of lateral plate morph genetics except Avise's (1976) general two-locus model. Incompatibility of the results of our plate morph crosses with those of most previous studies suggests that the genetic architecture underlying lateral plate morphs differs among populations or is more complex than presently recognized. Segregation ratios for lateral plate morphs indicate that consistently low frequencies of partial morphs observed in the Loberg Lake population at least partly reflect genetic architecture. Gillraker number and probably low morph lateral plate number are highly heritable and correlated with each other. Lateral plate asymmetry was high but not significantly heritable. Low and complete morph lateral plate number do not appear to be genetically correlated, indicating a significant element of independent genetic control.

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Introduction

The evolutionary response to natural selection depends in large part on the genetic determination of a trait. Variation must include a substantial genetic component for a phenotype to evolve in response to selection. However, the nature of that response may depend strongly on the trait's genetic architecture, including the number and interactions of alleles at loci and number of loci and interactions among them (Lande, 1979; Lynch & Walsh, 1998). Thus, genetics may provide crucial insights into evolutionary patterns.

Interpretation of phenotypic variation in the threespine stickleback species complex, *Gasterosteus aculeatus*, has a long and checkered past (reviewed by *e.g.* Wootton, 1976; Bell, 1984, 1995; Bell & Foster, 1994a). Phenotypic variation was used to diagnose 42 nominal species and to recognize regional subspecies and hybrid zones between them, interpreted as non-heritable phenotypic plasticity, and finally interpreted as heritable, rapidly evolving, adaptive variation (reviewed by Bell, 1995). The modern era of research on three-spine stickleback evolution was heralded by Hagen & McPhail's (1970) critique of Miller & Hubbs' (1969) claim that variation among western North American populations of *G. aculeatus* simply reflects existence of subspecies that hybridize in sympatry. Shortly thereafter, Hagen & Gilbertson (1972) demonstrated that population differentiation for armor and trophic morphology is correlated with local environmental conditions. Their geographical analysis was soon buttressed by evidence that the variable traits are heritable (Hagen, 1973; Hagen & Gilbertson, 1973a) and can experience intense, local, directional selection and rapid evolution (Moodie, 1972; Hagen & Gilbertson, 1973b). Overwhelming evidence that stickleback variation is heritable and adaptive has now accumulated (See reviews in Bell & Foster, 1994b).

It is also evident that the same phenotypes have evolved independently, innumerable times from anadromous or marine populations of *G. aculeatus* after they colonized fresh water (*e.g.* McPhail & Lindsey, 1970; Bell, 1976; Bell & Foster, 1994a; Taylor & McPhail, 2000). Therefore, it is possible that similar phenotypes are produced by different genetic architectures in different populations. This has been documented in other taxa. For example, eye reduction in blind cave fish derived independently from the surface ancestor, *Astyanax mexicanus*, depends on different genes (Wilkins, 1971, 1988; Dowling *et al.*, 2002). For threespine stickleback, separate studies of lateral

plate morph (LPM; *sensu* Hagen & Gilbertson, 1972) genetics, using different populations, have produced inconsistent results (Bañbura & Bakker, 1995). Thus, interpretation of variation in specific stickleback populations requires genetic analyses using those populations.

The threespine stickleback population in Loberg Lake, Alaska was founded by anadromous stickleback between 1983 and 1989. Annual samples from 1990 to 2001 reveal dramatic evolution of lateral plate (LP), gillraker (GR) (Bell, 2001; Bell *et al.*, 2004), and body form phenotypes (Aguirre, unpubl. data). LPM frequencies shifted from near-monomorphism of the ancestral complete morph to numerical dominance by the low morph, without the partial morph becoming as frequent as expected under some genetic models (see Bañbura & Bakker, 1995). LP number for both low and complete morphs and GR number declined significantly. The genetic basis of these rapidly evolving traits has been studied in other populations (Hagen, 1973; Hagen & Gilbertson, 1973a; Hermida *et al.*, 2002) but is unknown in this rapidly evolving population. We performed a series of genetic crosses to evaluate the applicability of existing genetic models of LPM determination, estimate the heritability of LP number, LP asymmetry, and GR number, and test for correlations between traits in *G. aculeatus* from Loberg Lake, Alaska.

Methods

Sexually mature males and gravid females were collected using minnow traps on June 14 and 15, 2000 in Loberg Lake, near Palmer, Alaska (61°33'35"N, 149°15'30"W). The characteristics of Loberg Lake and sampling procedures were described in Bell (2001) and Bell *et al.* (2004). Note, however, that the outlet from Loberg Lake does not discharge to a stream but terminates blindly, so the means by which anadromous stickleback entered Loberg Lake is unknown.

Thirty-five genetic crosses were performed within 12 hours of capture using Hagen's (1973) methods. Males and females were anesthetized with MS-222 (Tricaine Methanesulfonate). Eggs were gently squeezed out of the females and both testes were removed through a lateral incision on the males' abdomens. Both parents were immediately preserved in 10% buffered formalin. Testes were minced and sperm was spread over the eggs with a pipette. After the fertilization membrane had formed, eggs were transferred to incubators made from a short section of polyvinyl chloride pipe covered by fine nylon mesh at one end. The incubator was suspended in 18°C water near an air stone to aerate the eggs until shipment to the laboratory in Stony Brook. Two or three days after fertilization, the eggs were transferred to a 50 ml tube, chilled to 5-10°C in a water bath, aerated briefly, and sealed with about 2 ml of air. They arrived in the laboratory about 12 hours later, and the tubes with eggs were gradually warmed by immersion in an aquarium to 18°C and returned to an aerated incubator. Infected eggs were culled from the incubators each day and clutches were dipped (10 sec) in Fungus

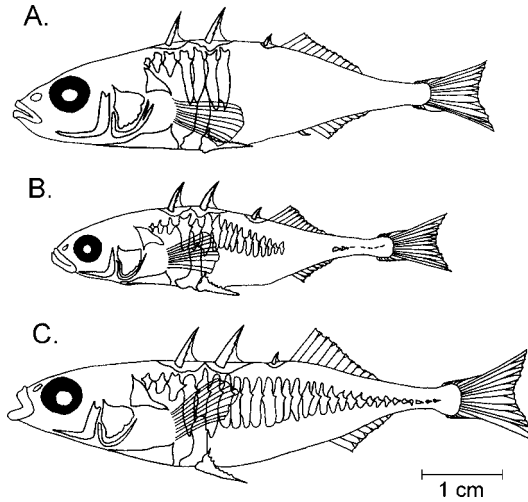


Fig. 1. Lateral plate morphs. A. Low, B. Partial, C. Complete. Modified from Francis *et al.* (1985).

Cure[®] as needed to suppress infection. Fry hatched about six days after fertilization. They were fed brine shrimp nauplii until they were large enough to eat frozen adult brine shrimp. In September 2001, the offspring of the twenty-seven surviving crosses were anesthetized with MS-222 and sacrificed. The parents and progeny were individually labeled, fixed in formalin, stained with alizarin red S, and stored in 50% isopropyl alcohol.

Parents and offspring were scored for LPM on both sides of the body, and the number of LP on both sides of the body and of GR on the first right gill arch were counted under a dissecting microscope. LP number asymmetry was also scored. Mean parental LP number asymmetry was calculated by summing the difference between left and right plate counts for each parent and dividing by two. Mean offspring LP asymmetry was calculated by summing the difference between left and right LP number among all offspring in a family and dividing by the number of offspring.

Hagen & Gilbertson's (1972, 1973a) criteria for lateral plate morphs were generally used (Fig. 1). The complete morph has a continuous row of plates beginning just behind the head and terminating on the caudal peduncle, where it forms a keel. However, specimens with a one-myomere gap on one side were also classified as completes (Hagen & Gilbertson, 1973a). The partial morph has a row of abdominal plates separated by an unplated gap of at least two consecutive unplated myomeres from a posterior plate row. Specimens with an unplated gap plus additional short interruptions in the plate row were classified as partials. Low morphs usually have up to 10 plates restricted to the abdomen, but one specimen with 13 plates per side but no posterior plates was classified as a low morph. Segregation ratios of LPM for the 23 families that had at least 11 offspring were tested against predictions from five genetic models (reviewed in Bañbura & Bakker, 1995) using *G*-tests (Sokal & Rohlf, 1995). Avise's (1976) one locus model was considered a special case of his two-locus model with the B allele fixed at the B locus (Bañbura & Bakker, 1995) and not evaluated separately.

Narrow sense heritabilities (h^2) were calculated for GR number, low morph LP number, and LP number asymmetry as the slope of the regression of the mean progeny value on mid-parent value for each trait (Lynch & Walsh, 1998). Standard errors were calculated using univariate methods for slopes (Sokal & Rohlf, 1995). Heterogeneity of means and variances of these traits was evaluated in male and female parents. Differences between male and female mean parental low morph LP number were tested with a *t*-test. GR number and LP asymmetry were not normally distributed, and attempts to normalize the data using the Box-Cox transformation (Sokal & Rohlf, 1995) failed, so Mann-Whitney *U*-tests were used. No heterogeneity of variances between male and female parents was found using the Levene test. The h^2 for LP's was based on averages of left and right LP counts in the 11 Low \times Low (L \times L) crosses, whereas h^2 estimate for LP asymmetry and GR number are based on counts for all families. The 11 L \times L families were relatively homogeneous in offspring number ranging from 11 to 25 offspring, so differences in the precision of the estimate of mean offspring plate number were considered unimportant (Roff, 1997), and h^2 of low morph LP number was estimated through unweighted least squares regression. However, family size was more variable across the 27 crosses used to estimate h^2 of GR number and LP asymmetry, with several families having less than ten offspring and four having five or fewer offspring (Table 1). For these traits, h^2 was estimated through a weighted least squares regression, with weighting coefficients calculated as the inverse of the variance of the estimate error for each family, as described by Roff (1997).

LP number h^2 was evaluated using only low morphs because LPM's are typically discrete Mendelian phenotypes, and LP number within different morphs may be controlled by different genetic systems. To evaluate this possibility, a correlation analysis was carried out on mean LP number among offspring from 12 crosses that produced both complete and low morph offspring. If the same genetic system controls LP number in both morphs, mean LP number of low and complete morphs should be correlated among families. We also tested whether loci determining LPM phenotypes influence low morph LP number by assessing whether low morph offspring produced from crosses involving a low morph parent and a partial or complete morph parent have a significantly higher LP number than low morph offspring from crosses in which both parents are low morphs. LP number of the parents of the 11 L \times L crosses (mean \pm SE = 6.886 ± 0.171) and low morph parents of the ten mixed crosses (mean = 6.700 ± 0.186) that produced low morph offspring in this study were tested for significant differences, and none was found ($F_{1,30} = 0.432$, $p = 0.516$). Thus a significant difference among low morph offspring of these different cross types would suggest that the partial/complete parents have an effect on the LP number of their low morph offspring.

Finally, because GR and LP are functionally important traits, a correlation analysis was carried out to test whether there was an association between them. Genetic correlations are notoriously difficult to estimate (Lynch & Walsh, 1998), and the number of families in this study was not adequate to attempt this estimation. So instead, a phenotypic correlation analysis was carried out on all offspring across families with mean family values subtracted from both variables for each offspring, and mean plate number calculated separately for low and complete morphs within each family. Since individuals were scored as deviations from family means and reared in a relatively homogeneous environment, a significant phenotypic correlation would suggest a genetic correlation between these traits. Statistical analyses were carried out with SPSS 9.0 (SPSS, Inc., 1998) and BIOMstat 3.301 (Applied Biostatistics, Inc., 2002).

TABLE 1. *Segregation ratios for Loberg Lake genetic crosses*

Cross #	Parents		Offspring			
	♂	♀	L	P	C	N
1	L	L	14			14
2	L	L	15			15
3	L	L	20			20
4	L	L	14			14
5	L	L	16			16
6	L	L	17			17
7	L	L	17			17
8	L	L	12			12
9	L	L	13			13
10	L	L	24			24
11	L	L	11			11
12	L	P	1	1		2
13	L	P	7		10	17
14	L	P	14	3	6	23
15	P	L	9	2	8	19
16	C	L	13	1	9	23
17	C	L			2	2
18	C	L	5	1	5	11
19	C	L	6	1	6	13
20	C	L	7	1	2	10
21	L	C	10	1	6	17
22	L	C	2	1	2	5
23	C	P			1	1
24	P	C	4	7	6	17
25	C	C		6	15	21
26	C	C	2	4	14	20
27	C	C	5	2	18	25

L, low morph; P, partial morph; C, complete morph; and N, number of offspring in the family.

Results

Twenty-seven of the 35 crosses produced surviving progeny. Some clutches failed to develop, and some families were lost or greatly reduced in size by a virulent strain of *Glugea* sp. (Micosporidia). All L × L crosses yielded only low morphs (Table 1), which fit the 1:0:0 ratio predicted by all models (Table 2). Low × Partial (L × P) and Low × Complete (L × C) crosses typically yielded mostly low and complete individuals, with few partials (Table 2). Bimodality of the LP phenotypes within families was even more apparent when LP number rather than morph was evaluated because many in-

TABLE 2. *Fit of Loberg Lake crosses with 10 or more offspring to possible ratios from published genetic models summarized in Bañbura & Bakker (1995)*

Cross type	Expected ratio (L:P:C)	Loberg Lake crosses that fit expectations	Model
L × L	1:0:0	All (11/11)	A, M, HG, Z, B
L × P	1:0:1	13 (1/3)	A
	2:1:1	14, 15 (2/3)	A, Z, B
	4:3:1	None (0/3)	HG, Z, B
L × C	2:1:1	18, 19, 20, 21 (4/5)	A, Z, B
	1:2:1	None (0/5)	HG
	4:1:3	16, 18, 19, 20, 21 (5/5)	A
P × C	1:2:1	24 (1/1)	Z, B
	1:1:2	24 (1/1)	Z, B
	1:3:4	24 (1/1)	HG
	2:3:3	24 (1/1)	A
C × C	0:1:1	25 (1/3)	Z, B
	0:1:3	25 (1/3)	A, HG, Z, B
	2:1:5	26, 27 (2/3)	A
	4:3:9	26, 27 (2/3)	A

Models from papers in the literature cited: A, Avise (1976); B, Bañbura (1994); HG, Hagen & Gilbertson (1973a); M, Münzing (1959), and Z, Ziuhanov (1983).

individuals scored as partials had high plate counts (Fig. 2, *e.g.* crosses 15, 16, 18). Partial × Complete (P × C) and Complete × Complete (C × C) crosses produced a greater frequency of partials than the crosses involving lows, with the C × C crosses being dominated by completes but producing some lows. There were no significant differences between observed segregation ratios and those expected from Avise's (1976) general two-locus LPM genetics model (Table 2). Two C × C crosses (26 and 27) produced all three morphs, which is predicted only by Avise's model, and two other crosses (13 and 16) produced segregation ratios that differed significantly from expectations based on every model except Avise's. Ziuhanov's (1983) and Bañbura's (1994) models performed relatively well, providing an adequate fit for 19 of the 23 crosses evaluated. Hagen & Gilbertson's (1973a) model performed poorly, providing a fit for only 13 of the 23 crosses, and Münzing's (1959) model performed the worst, providing an adequate fit for only the 11 L × L crosses.

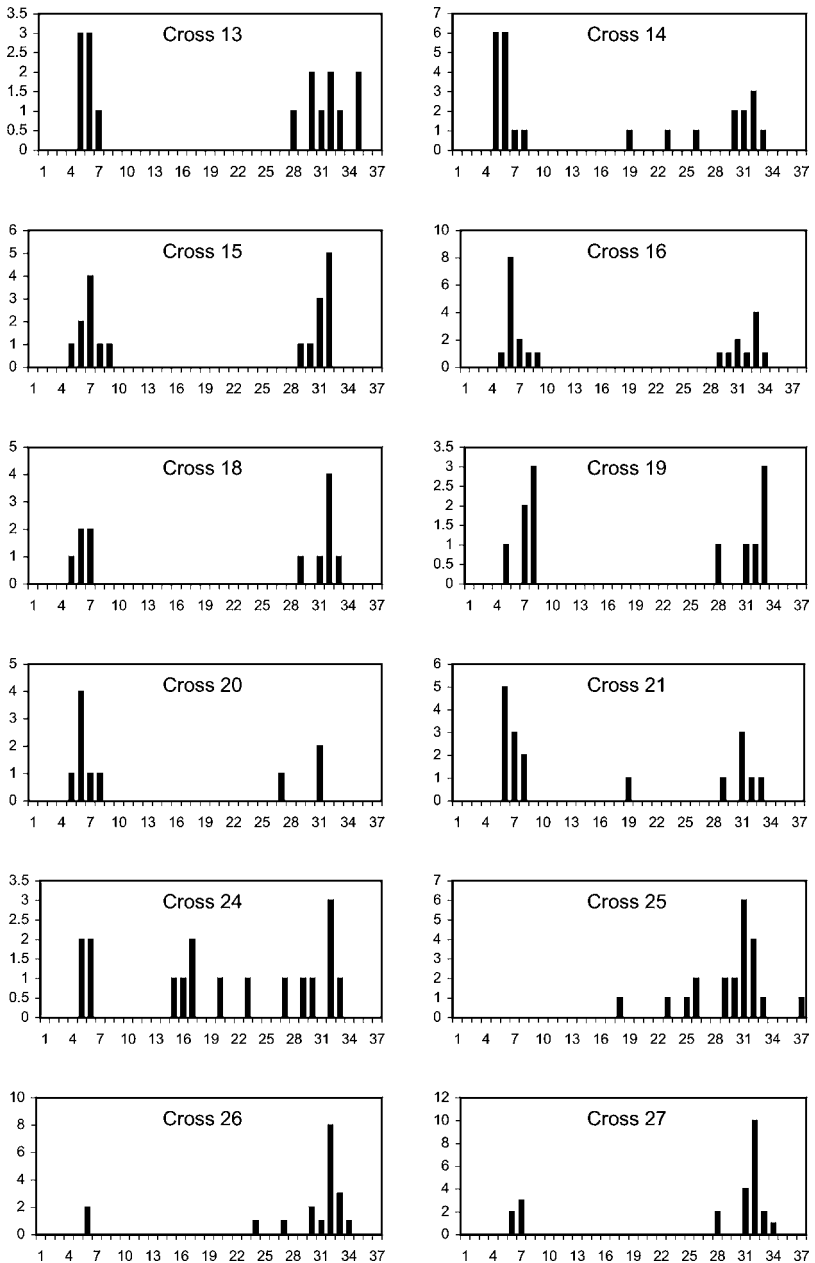


Fig. 2. LP number frequency of progeny from mixed morph crosses with ten or more offspring. Individual LP number is the average of left and right sides. Crosses 13-15 are $L \times P$, 16-21 are $L \times C$, 24 is $C \times P$, and 25-27 are $C \times C$.

Regression of mean progeny LP number on mid-parent LP number for $L \times L$ gave an h^2 estimate of 0.879, which was marginally non-significant ($F_{1,9} = 4.522$, $p = 0.0622$). However, an association is apparent when the data are plotted (Fig. 3). The marginal probability value may be due to a lack of power resulting from the small number of $L \times L$ crosses (11). LP number asymmetry was highly variable among families, but generally common. Mean family asymmetry ranged from 0.154 to 1.250 plates per fish. Although there appears to be a weak association between mean parent and offspring LP asymmetry for most crosses when the data are plotted (Fig. 4), the regression is not significant ($F_{1,25} = 0.179$, $p = 0.679$). This was in large part due to three crosses with high mean offspring asymmetry values.

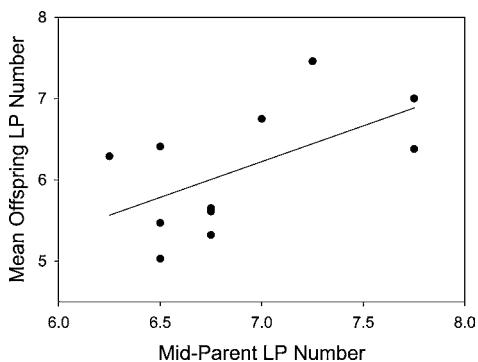


Fig. 3. Relationship between mid-parent and family means for lateral plate (LP) number in low morph crosses.

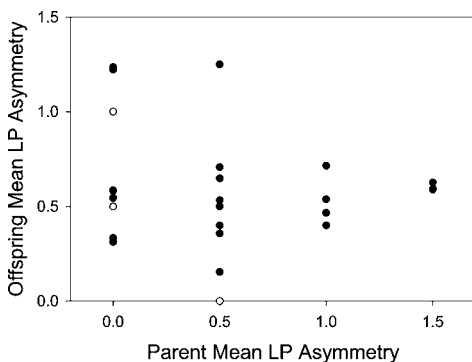


Fig. 4. Relationship between mid-parent and family means for lateral plate (LP) asymmetry. Open circles depict families with ≤ 5 offspring.

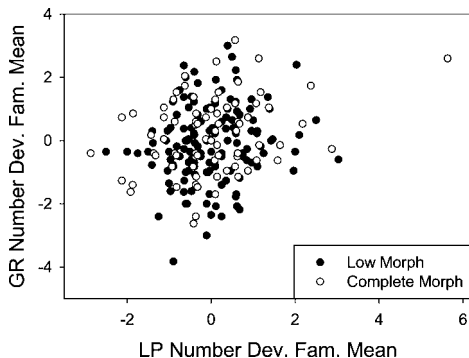


Fig. 7. Relationship of gillraker (GR) and lateral plate (LP) number plotted as deviations from family means.

the parents. LP number and GR number exhibited a significant positive correlation (Fig. 7). This correlation held for low and complete morphs evaluated together ($r = 0.196$, $p = 0.001$, $N = 268$) and separately (lows: $r = 0.148$, $p = 0.046$, $N = 182$; completes: $r = 0.274$, $p = 0.011$, $N = 86$). A complete morph specimen that was an extreme outlier contributed substantially to the correlation in complete morphs. It had deviations of +5.63 and +2.60 from the family mean LP and GR numbers, respectively. Excluding this individual, the correlation for complete morphs declined to 0.183, which was marginally non-significant ($p = 0.093$, $N = 85$). However, the overall correlation across morphs was still significant ($r = 0.157$, $p = 0.01$, $N = 267$). Although significant, the association explained only a small fraction of the phenotypic variance.

Discussion

Interpretation of the results of our genetic crosses is hindered both by the limited number and small size of the families. Nevertheless, we can draw some important conclusions about the genetics of rapidly evolving traits in the Loberg Lake population, partially explain the pattern of LPM evolution during 12 years of observation, and add a new insight into LP number genetics.

The results of our genetic crosses using Loberg Lake stickleback allowed falsification of all published models except Avise's (1976) general two-locus model. This is consistent with the findings of Cresko *et al.* (2004) based on

crosses of anadromous and lake populations located near Loberg Lake in the Mat-Su valley. The Avise model was developed through interpopulation crosses that included an unusual population in Friant, California, which has a similar LPM frequency distribution and mean low morph LP count to the Loberg Lake population. Both populations are trimorphic but have strongly bimodal LP number distributions that are dominated by completes and lows with partials occurring at low frequencies (about 5% in Friant and usually about 10% in Loberg; Bell *et al.*, 2004). Although Avise's (1976) model is older than Ziuganov's (1983) and Bañbura's (1994) models, the latter two models are basically straightforward extensions of Münzing's (1959) one locus model with incomplete dominance, in which the dominance relations for the major plate locus A/a (or $A/a/a_k$ in Bañbura's model) are modified by a second 2 allele locus C/c . Although Ziuganov and Bañbura's models were a major improvement over existing models (including Avise's two-locus model) in some respects (Bañbura & Bakker, 1995), they do not account for the production of individuals of all three morphs from the $C \times C$ crosses obtained in our study, whereas Avise's model could. The incompatibility of our results with those of previous authors (Bañbura & Bakker, 1995), and the inconsistency of Avise's (1976) two-locus model with results from other populations suggests that the genetic architecture controlling LPM either differs among populations or is more complex than presently recognized. It would not be surprising if the genetic architecture controlling LPM's indeed differs among populations because the low morph has arisen independently in many post-glacial freshwater populations in western North America from marine and anadromous ancestors that are monomorphic for the complete morph (*e.g.* McPhail & Lindsey, 1970; Bell, 1976, 1995; Bell & Foster, 1994a; Taylor & McPhail, 2000).

The genetic architecture of the Loberg Lake population can partially account for the low frequency of partials during most of the rapid evolution of LPM frequency in Loberg Lake. This population went from numerical domination by completes in 1990 (complete morph frequency = 96%) to domination by lows in 2001 (low morph frequency = 75%) with partials consistently between 5 and 15%, except in 1993. In 1993, completes and lows had similar frequencies and partials reached a frequency of approximately 30%. The low frequency of partial offspring produced among the laboratory crosses involving low morph parents suggests that a genetic bias against the production of partials may be responsible for the low frequency of partials in latter years.

This is consistent with Cresko *et al.* (2004) in which few partials were produced in crosses between complete morph anadromous and low morph lake populations.

Only two previous studies produced h^2 estimates for low-morph LP number. Hagen (1973) obtained h^2 estimates ranging from 0.50 to 0.84 for samples raised at two different temperatures. Hermida *et al.* (2002) obtained values ranging from 0.34 to 0.90 using three different methods to estimate heritabilities. The estimate they obtained with the regression method (the method employed in our paper) was 0.34. Our h^2 estimate of 0.88 was similar to that obtained by Hagen (1973) for fish reared under similar conditions. The relatively large h^2 estimated for the Loberg Lake population is in accordance with the substantial evolution of low morph LP number observed in the lake from 1990-2001. In addition, the different estimates of h^2 for plate number in low morphs are reasonably consistent with the observation of rapid evolution (Bell, 2001) and extensive population differentiation (Reimchen, 1994) of low morph plate number in natural populations of *G. aculeatus*. The marginal probability value obtained for the statistical significance of our h^2 estimate may be due to a lack of statistical power resulting from the small sample size.

Fluctuating asymmetry is random variation between homologous structures on the right and left sides of otherwise bilaterally symmetrical organisms (Van Valen, 1962; Palmer & Strobeck, 1986). Hagen (1973) detected a large component of heritable variation for LP number asymmetry in low morphs. In contrast, Hermida *et al.* (2002) found little evidence for h^2 of fluctuating asymmetry of meristic traits, including LP number. Inspection of the scatter plot of mean fluctuating asymmetry of progeny on the mid-parent value (Fig. 4) suggests a general association in our crosses, but statistical analysis did not support this interpretation. However, the statistical results are strongly influenced by three crosses (8, 16, 20) in which relatively symmetrical parents (*i.e.* one asymmetrical plate among six parents) produced the most strongly asymmetrical offspring. Our results may indicate general heritability of LP fluctuating asymmetry plus possible segregation at a major locus (*i.e.* with large phenotypic effects) with a dominant allele favoring plate symmetry. Variation of the results from Hagen (1973), Hermida *et al.* (2002) and our Loberg Lake crosses may represent differences in the genetics of plate number fluctuating asymmetry among populations or of the conditions under which the field-caught parents and laboratory-reared progeny developed in different studies.

Previous h^2 estimates for GR number also exist. Hagen (1973) estimated the h^2 of GR number at 0.58. Hermida *et al.* (2002) divided the GR counts into upper (epibranchial) and lower (ceratobranchial) sets, and using the regression method estimated their h^2 at 0.13 and 0.35 respectively, with the estimate for upper gillraker number not being significant. Other methods they employed yielded estimates ranging from 0.34 to 0.47 for lower GR number. Our estimate of GR number h^2 is 0.511, which is generally comparable to that of Hagen (1973) and estimates of lower GR number in Hermida *et al.* (2002). These relatively high estimates are in accordance with the characteristic divergence in GR number observed in post-glacial freshwater populations (*e.g.* Hagen & Gilbertson, 1972; Moodie & Reimchen, 1976), and the decline observed in the Loberg Lake population between 1990 and 2001 (Bell *et al.*, 2004).

All models for LPM genetics involve a few alleles at one or two genetic loci (Bañbura & Bakker, 1995), indicative of control by loci of large effect. However, substantial intrapopulation variation exists for LP number in all morphs (reviewed by Bell, 1984; Reimchen, 1994), and LP number within low morphs is significantly heritable (Hagen, 1973; Hermida *et al.*, 2002), indicating that it is controlled by a polygenic system. These observations suggest a model of LP genetics in which alleles at major loci determine plate morphs (*i.e.* distribution of plates on the body and general range of plate number), and minor loci with small, additive effects adjust plate number up or down within each morph. Recently, research applying QTL methods to different sets of populations provided evidence in favor of this model. Two different studies indicate that LPM is controlled by a single major locus (Colosimo *et al.*, 2004; Cresko *et al.*, 2004), and one of these studies reported that modifiers of small effect contribute semiadditively to LP number (Colosimo *et al.*, 2004). If this model is correct, the number of plates of low and complete morphs within families should be positively correlated. We did not find this correlation. This may be due to lack of statistical power since our results are based on only 12 families. Nevertheless, our results suggest that a significant component of genetic variance for LP number differs between low and complete morphs. If true, this would not be too surprising. Spatial and temporal expression patterns of regulatory elements along the trunk are complex and it is not difficult to envision genetically based elements that can add or subtract one or a few LP but are specific to a particular region of the trunk. Not surprisingly, the polygenetic system determining low morph LP

number did not seem to be affected by having a partial or complete morph parent, which is expected if major loci determine LPM and an independent polygenic system determines LP number within morphs.

LP function in defense against predation and GR for food acquisition. Benthic-feeding (benthics) stickleback forage on large prey, close to shelter from predatory fish, and tend to have a reduced number of LP and GR. In contrast, planktivorous stickleback (limnetics) feed on small prey in the open where they are more vulnerable to fish predation, favoring higher numbers of both GR and LP than in benthics (McPhail, 1994; Bell & Foster, 1994a; Vamosi, 2002). We observed a positive phenotypic correlation (taking LPM into account) between LP and GR number, with siblings within families having higher LP counts also tending to have higher GR counts. The basis of this correlation is unknown but could be the result of genetic correlation, since individuals were reared in a relatively homogenous environment and individual scores were assigned based on comparisons to siblings. In addition, phenotypic correlations are surprisingly good indicators of genetic correlations when the relationship between them has been evaluated (Chevarud, 1988; Roff, 1995). The correlation documented here results in phenotypes with an adaptive combination of LP and GR number (*i.e.* both high or both low) in both benthic and limnetic populations. It seems improbable, however, that this correlation is an adaptation to produce integrated phenotypes (*e.g.* Olson & Miller, 1958; Chevarud, 1996) because it is difficult to envision how selection could fashion such an adaptive correlation in the anadromous ancestor of the Loberg Lake population. Anadromous stickleback spend most of their life cycle at sea feeding on plankton far from cover (Cowen *et al.*, 1991; Mecklenberg *et al.*, 2002). Rather, the correlation appears to be an exaptation (Gould & Vrba, 1982), a historical accident, unrelated to, but appropriate for conditions in Loberg Lake. Although somewhat surprising, this correlation is consistent with the decline of both traits in the Loberg Lake population since 1990.

In summary, the results of this study shed light on the genetic basis of evolution observed in Loberg Lake stickleback since 1990. Our estimate of low morph LP number and GR number h^2 were both relatively high, indicating ample genetic variation for the rapid evolution of both traits. We also documented a correlation between these two traits, which may have a large genetic component, and thus could have facilitated the concerted decline of these traits. Although LP number asymmetry was high, we did not detect a

significant heritable component to it. We also found some evidence in favor of independent control of LP number in different morphs and that having a partial or complete morph parent does not seem to affect LP number in low morph offspring. The results of our crosses were mostly unimodal for lows (in $L \times L$ crosses) or bimodal for lows and completes (in mixed crosses) suggesting a genetic bias against the production of partials. These results may help explain the low frequency of partials observed in Loberg Lake during the transition from dominance by completes to dominance by lows. Perhaps most importantly, it is clear from our results that simple Mendelian models are reaching the limits of their utility to decipher the genetic architecture of natural polymorphisms of LP phenotypes. Our results were inconsistent with four of the previous five models of LPM genetics and suggested that LPM genetics differs among populations or is more complex than presently recognized. In addition, although the results of our crosses agreed with Avise's (1976) two-locus model, that does not mean that this model is correct for the Loberg population. It is merely the simplest model that can explain the cross results. Without detailed knowledge of the actual genetic architecture of this trait it will be difficult to tell whether the simplest model that fits is actually the correct one.

Development of new molecular genetic methods, such as QTL mapping, promise to help answer many of the questions raised in this paper through the identification of the actual loci responsible for adaptively important traits. Indeed, important progress in this direction has already been made (Pechel *et al.*, 2001; Kingsley *et al.*, 2004, Cresko *et al.*, 2004; Colosimo *et al.*, 2004). A one locus model with modifiers, as has recently been proposed would actually fit the Loberg Lake population relatively well, assuming that most of the completes used as parents in our study were heterozygotes (which is likely given that the frequency of completes in Loberg Lake in 2000 was only 17.1% and that two of the three $C \times C$ crosses produced some low morph offspring) and that complete morph alleles are dominant. Dominance of the complete morph has been observed in crosses of populations that are monomorphic for LPM phenotypes (Cresko *et al.*, 2004; Bell, unpubl. data) as well as in the polymorphic Friant population (Colosimo *et al.*, 2004). Mapping of QTL could also potentially help explain the correlation between LP and GR number and the lack of an association between low and complete morph plate number reported here by assessing whether elements involved in the polygenic systems controlling these traits are shared or linked.

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