BRIEF COMMUNICATIONS

GENETICALLY BASED PHENOTYPE-ENVIRONMENT ASSOCIATION FOR SWIMMING BEHAVIOR IN LAKE WHITEFISH ECOTYPES (*COREGONUS CLUPEAFORMIS* MITCHILL)

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Abstract.—Studies of phenotype-environment associations in adaptive radiation have focused largely on morphological traits related to resource-based phenotypic differences. The genetic basis of adaptive behaviors implicated in population divergence remains poorly understood, as few studies have tested the hypothesis of behavioral phenotype-environment associations. We provide evidence of a phenotype-environment association for differential adaptive swimming behaviors through experiments conducted on dwarf, normal, and hybrid lake whitefish (*Coregonus clupeaformis*). Highly significant differences were observed for depth selection, directional changes, and burst swimming, implicating a genetic basis for these behaviors. Hybrid crosses revealed that depth selection is under additive genetic control, while dominance effects were suggested for directional changes and burst swimming. Estimates for the genetic basis of behavioral differentiation from an animal model were consistent with these observations. Comparative estimates of behavioral differentiation (Q_{ST}) against neutral expectations (F_{ST}) revealed pronounced departures from neutral expectations in all three behavioral phenotypes, consistent with the hypothesis that directional selection has driven the divergence of behavior in dwarf and normal lake whitefish ecotypes.

Key words.—Adaptive radiation, behavior, *Coregonus clupeaformis*, divergent natural selection, phenotype-environment association, population divergence.

Investigating the evolution of natural populations that diverge to exploit different ecological resources represents a main objective of evolutionary biology (Ayala and Fitch 1997; Howard 1998). In this view, perhaps the most comprehensive, predictive concept available to evolutionary biologists is the ecological theory of adaptive radiation (Orr and Smith 1998). This theory holds that adaptive radiation, including both phenotypic divergence and speciation, is ultimately the outcome of divergent natural selection stemming from resource heterogeneity and competitive interactions (Huxley 1942; Mayr 1942; Lack 1947; Dobzhansky 1951; Simpson 1953; Schluter 2000). In order to define an adaptive radiation and assess the role of divergent natural selection within the process (reviewed in Schluter 2000), populations must exhibit genetically based phenotype-environment associations that fulfill the adaptive criterion. Evidence must also be provided that the phenotype-environment association is driven by divergent natural selection.

Such studies of phenotype-environment associations have focused largely on morphological traits related to resource-based phenotypic differences, such as body/trait size versus prey/habitat size relationships (e.g., Grant 1986; Price 1991; Garland and Losos 1994; but see Schluter 2000). In comparison, very few studies have directly tested the hypothesis of phenotype-environment associations for behavioral traits (Skúlason et al. 1993, 1999). Yet, evolutionary changes in behavior are often considered integral in initiating adaptive shifts whereby populations or species may elicit a variety of habitat selection strategies to use resources, and may differ in the behavioral traits used to exploit those resources (Mayr 1963; Skúlason et al. 1999). Behavior may even be the first trait to evolve as a result of divergent natural selection, as morphology is often relatively conservative to change when

compared to the diversity of behavioral phenotypes (Dill 1983; Noakes 1989; Malmquist 1992; Skúlason et al. 1999).

The objective of this study was to investigate the extent of heritable behavior phenotype-environment associations in lake whitefish ecotypes (*Coregonus clupeaformis*). Dwarf and normal whitefish ecotypes co-inhabit several lakes of the St. John River drainage in northern Maine, and southeastern Québec, Canada (Bernatchez and Dodson 1990; Pigeon et al. 1997). Strong bimodal size distributions of sexually maturing fish are the primary indication for the existence of sympatric dwarf and normal ecotypes (Chouinard et al. 1996). The dwarf form matures by the age of one or two years and seldom exceeds 20 cm in length and 100 g in weight while the normal form does not generally mature until two years of age and commonly exceeds 40 cm and 1000 g. A phenotype-environment association between morphology and diet has been documented whereby dwarf ecotypes with higher gill-raker numbers were associated with a diet of smaller, limnetic zooplanktonic prey, whereas normal ecotypes with fewer gill rakers fed mainly on larger, epibenthic preys (Bodaly 1979; Bernatchez et al. 1999). These results have qualified the lake whitefish as a broadly limnetic/benthic dichotomy whereby increased efficiency for small prey capture may be more determinate on fitness for the dwarf than the normal ecotype (Bernatchez 2003).

Overall, behavioral differentiation between ecotypes may be directly influenced by the limnetic and benthic niches they inhabit and the resources they consume. For example, brook charr (*Salvelinus fontinalis*) exhibit intrapopulation diversity with respect to foraging behavior reflecting variable microhabitat use such as column depth and diet selection (McLaughlin et al. 1994). These findings suggest that the development and maintenance of specializations in water-column use and diet reported for several salmonids, including

arctic charr (*Salvelinus alpinus*), may be critical toward understanding the initial stages of population divergence (see Skúlason et al. 1999).

Given these observations, specific predictions may be made about the swimming behavior of dwarf and normal lake whitefish ecotypes. First, the dwarf ecotype, which is associated with pelagic prey, should occupy higher positions in a water column (McLaughlin et al. 1994). Second, these ecotypes should also display more swimming behaviors than the normal form associated with the capture of small, pelagic prey, such as burst swimming (Webb 1983; Weihs and Webb 1984; McLaughlin et al. 1994). Finally, given evidence for the increased susceptibility of pelagic fish to predation within limnetic environments (Vamosi and Schluter 2002), dwarf ecotypes should exhibit more mechanisms for predator evasion to compensate for this increased susceptibility, such as significant biases in directional swimming turns (Heuts 1999).

We tested the null hypothesis of no heritable phenotype-environment associations in the swimming behavior of lake whitefish. Rejection of the null hypothesis would be achieved if dwarf and normal experimental groups raised under identical environmental conditions differed with respect to their swimming behavior in a manner predicted by differences in their use of habitats in nature. Given that this null hypothesis was rejected, a second objective was to compare dwarf and normal groups with F_1 hybrids reared under the same conditions to gain insight into the nature of the genetic basis for behavior. A third objective was to test the null hypothesis of no selective response from observed behavioral-environment associations by comparing the extent of behavioral differences with neutral expectations. We also compared the extent of deviation from neutral expectations observed in the behavioral traits relative to morphological differences in order to gauge the relative response to selection for behavior and morphology in the context of evolution in lake whitefish ecotypes.

MATERIALS AND METHODS

Experimental Families

The parental generation of dwarf and normal whitefish ecotypes was sampled in 1996 from Temiscouata Lake (47°36′N, 68°45′W) and Aylmer Lake (45°50′N, 71°26′W), respectively. The F_1 generation was produced the same year and consisted of reciprocal pure dwarf, pure normal, and hybrid dwarf/normal crosses with the initial goal of generating families to study hybrid inviability, as described in Lu and Bernatchez (1998). The pure dwarf F_1 generation was made factorial using 20 males and 20 females, and three males and three females were used to generate the normal crosses. The use of different numbers of fish for dwarf and normal ecotypes was needed to equilibrate the number of gametes for previous experiments of hybrid inviability (Lu and Bernatchez 1998). Half of the eggs and milt from the dwarf and normal crosses were maintained to generate the reciprocal hybrid crosses (N_FD_M and D_FN_M). All experimental groups were reared under identical environmental parameters that simulated natural lake conditions. Families within groups were maintained in the same tanks and as such family identification

was unknown. Behavior experiments started when fish were 18 months old.

Behavioral Experiments

Experiments were conducted in isolated 1500-l cylindrical aquaria. Aquaria have been employed in many studies of fish behavior and are a viable means to test the null hypothesis of no heritable phenotype-environment associations for behavior. These aquaria provided the sufficient environmental conditions necessary to study the behavioral parameters of interest but moreover maintained minimal environmental variance critical toward comparing groups and determining the genetic basis (Webb 1983; Skúlason et al. 1993; Bakker 1999). For each experimental group, four separate trials were performed and behavioral observations were recorded at the same time of day per trial. The day before each trial, 10 fish of a given experimental group were randomly chosen and moved to the isolated observation tank. The following morning, five consecutive series of observations recorded three behavioral traits (depth selection, directional changes, burst swimming).

Depth selection was defined as the observed distance of each fish in the water column from the bottom of the tank and was measured by sectioning a 1500-l experimental tank into 5-cm increments (up to 70 cm) whereupon the position of the fish could be recorded to the nearest 5-cm increment. Directional changes were defined as single, immediate turns greater than 90°. Burst swimming was defined as the number of instantaneous accelerations invoking a short, strong use of the caudal fin to increase speed.

For depth selection, five observations for a group of 10 fish per trial were recorded resulting in 50 measures of depth selection per trial and 200 overall for each experimental group. For burst swimming and directional changes, one fish was chosen at random during each of the five observations and the number of times the behavior was observed during 1 min was recorded, resulting in 20 observations overall per experimental group for these behaviors. An analysis of variance (ANOVA) first assessed the significance of behavioral measures between the four experimental trials within the experimental groups. For depth selection, this was performed using mean values observed for 10 fish in each observation. A second ANOVA and post-hoc analyses using least significant difference (LSD) planned comparisons were performed to test the null hypothesis of no differences in depth selection, directional changes, or burst swimming behavior between dwarf, normal, and hybrid groups.

Genetic Basis for Behavioral Differentiation

The extent of the genetic basis for behavioral phenotypes between ecotypes was estimated using the DFREML 3.0 software package (Meyer 1998). DFREML estimates variance components for continuous traits by restricted maximum likelihood, fitting a linear mixed model and allows for both progeny and parents without pedigree assignments to be included in the analysis (Meyer 1998). Dwarf and normal groups were treated as unrelated ''parental'' groups and the hybrid experimental groups as ''full-siblings'' of these parental groups. Each behavioral observation recorded in the exper-

imental trials was used as one measure of a continuous trait. The error of the variance was estimated directly from the residual sum of squares and the likelihood was maximized with respect to the remaining variance components expressed as proportion of the total phenotypic variance (Meyer 1998). The absence of parental data and differential number of families limits strict estimates given that phenotypic variance components may vary between the P_1 and F_1 generation (Roff 1997). Therefore, these estimates must be interpreted cautiously and were used only to complement observations of hybrid intermediacy to more fully test the null hypothesis of no additive genetic variation in the hybrid groups.

Behavioral and Morphological Differences versus Neutral Expectations

The hypothesis that the phenotype-environment association is driven by divergent natural selection was tested by comparing the extent of differentiation at phenotypic traits (Q_{ST}) with that of neutral expectations (quantified at neutral molecular markers, F_{ST}; Spitze 1993). Under the influence of migration, mutation, and genetic drift, the among-population proportion of total genetic variance in phenotypic traits is expected to equal that of ''neutrally evolving'' nuclear marker loci (Lande 1992). The prediction is that divergent selection will cause Q_{ST} to be larger than that expected from neutral expectations. Q_{ST} analyses based on the use of phenotypic variance as a surrogate for additive genetic variance must be interpreted cautiously. However, the fact that estimates derived from phenotypic and genotypic variance have not differed in their general patterns of F_{ST}-Q_{ST} relationships (Merilä et al. 1997; Lynch et al. 1999; Schluter 2000; Merilä and Crnokrak 2001) suggests that the approach based on phenotypic variance is not strongly biased.

Behavioral and meristic data were used untransformed, whereas the univariate residual method was used to adjust each morphometric character for size heterogeneity among individuals (Flemming et al. 1994). Components of phenotypic variance were estimated by performing an ANOVA whereupon phenotypic variance was equal to twice the observational component of variance for individuals within populations and used as a surrogate for $2\sigma_{GW}^2$. The phenotypic variance between populations was equated to the observational variance component for populations and used as a surrogate for σ_{GB}^2. In the case of the morphological characters only two populations were used and as such 95% confidence intervals could not be calculated. In the case of behavioral characters, Q_{ST} was calculated for each of the four experimental trials such that mean trait Q_{ST} and subsequent 95% confidence intervals could be calculated.

Genetic differentiation at neutral markers between dwarf ($n = 40$) and normal ($n = 40$) was assessed using six microsatellite loci developed for *Coregonus* (BFW1, BFW2, C2–157, C4–157, CoCl-22, and CoCl-23) as detailed in Lu and Bernatchez (1999). The extent of genetic differentiation was first quantified by a pairwise fixation index based on variance in allele frequencies (θ of Weir and Cockerham 1984) using Fstat version 1.2 (Goudet et al. 1996). The 95% confidence intervals for θ were calculated by bootstrapping over loci whereupon values for morphological Q_{ST} were

deemed different from neutral expectations if they were outside the 95% confidence interval (CI) of θ, whereas behavioral Q_{ST} were considered significantly different when their 95% confidence intervals did not overlap with the 95% CI of θ.

RESULTS

Experimental Families

At the time of experiments, significant growth differences had manifested between groups, in conformity to observations in nature. The dwarf group (21.25 cm, CI = 21.25–22.03; 88.41 g, CI = 77.98–98.84) was significantly smaller in length and weight than the normal and hybrid groups. The normal group (26.08 cm, CI = 25.31; 213.10 g, CI = 190.99–235.21) did significantly differ in weight compared to $D_F N_M$ (24.23 cm, CI = 22.56–25.91; 149.60 g, CI = 120.10–179.09), but not compared to $N_F D_M$ (26.48 cm, CI = 25.51–27.45; 210.28 g, CI = 181.08–239.47).

Swimming Behavior Traits

Analysis of variance for depth selection revealed significant differences between trials ($P < 0.001$ for all experimental groups), suggesting that periodic differences for depth selection within experimental groups persisted for the duration of the study (Table 1). Despite trial differences within experimental groups, mean and range values followed the same trend; that is, the dwarf group was consistently higher than the normal group whereas the hybrid groups were consistently intermediate (Table 1). Trial data was therefore pooled for further analysis assuming that these data should not introduce bias into final interpretations on general trends in differences among experimental groups. ANOVA among trials for burst swimming and directional change traits showed no significant differences persisted between trials for these behaviors (Table 1).

An ANOVA among groups detected significant differences for all three behavioral traits ($P < 0.001$; Table 2). For depth selection, the dwarf experimental group maintained an average distance nearest to the surface (mean = 44 cm, range = 34–62 cm). In contrast, the normal experimental group was consistently recorded nearest to the bottom (mean = 16 cm, range = 10–22 cm) (Table 1). Least significant difference comparisons indicated a significant difference ($P < 0.001$) between dwarf and normal for depth selection (Table 2; Figure 1A). Both dwarf and normal exhibited the behavior of changing direction spontaneously but dwarf whitefish performed more turns per minute (mean = 4.1, range = 1–8) than the normal whitefish (mean = 2.0, range = 0–4; Table 1). Least significant difference comparisons showed a significant difference for this behavior ($P < 0.001$) between dwarf and normal (Table 2; Figure 1B). The dwarf group also revealed a higher frequency of burst swims (average = 1.3, range = 0–4) when compared to the normal group (average = 0.2, range = 0–3; Table 1). Although not as pronounced as the previous two behaviors, LSD comparisons indicated significant differences ($P < 0.001$) between dwarf and normal (Table 2; Figure 1C).

Comparative analyses of the hybrid groups with respect to

TABLE 1. Summary of behavioral data among four experimental trials for depth selection (cm from bottom of tank), directional changes (number observed per minute), and burst swimming (number observed per minute). For each trial, the mean and range of trait values is presented. An analysis of variance tested the significance of behavioral measures among trials. An asterisk represents significant differences after Bonferroni adjustments (α = 0.05, k = 4; Rice 1989).

	Trial 1		Trial 2		Trial 3		Trial 4		ANOVA (between trials)		
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	F	P-value
Depth selection											
Dwarf	41	(36–46)	41	(39–48)	37	(34–42)	58	(58–62)	44	22.14	0.0001*
Normal	17	(14–20)	11	(10–13)	19	(17–22)	17	(16–18)	18	10.45	0.0001*
$D_F N_M$	37	(33–41)	27	(22–30)	28	(24–34)	18	(16–21)	28	14.95	0.0001*
$N_F D_M$	25	(21–28)	29	(24–32)	24	(21–27)	47	(44–49)	31	64.12	0.0001*
Directional changes											
Dwarf	4.2	(3–6)	3	(1–5)	5.4	(2–8)	3.6	(2–7)	4.1	1.35	0.30
Normal	2.4	(1–3)	1.6	(1–2)	1.8	(0–4)	2.2	(1–4)	2.0	0.59	0.63
$D_F N_M$	2.2	(0–5)	2.4	(0–5)	1.2	(0–2)	1	(0–3)	1.7	0.63	0.61
$N_F D_M$	2.8	(0–6)	0.6	(0–2)	3.6	(1–6)	3	(2–4)	2.5	2.89	0.08
Burst swimming											
Dwarf	1	(0–2)	1.6	(0–3)	1.2	(0–3)	1.4	(0–4)	1.3	0.18	0.91
Normal	0.6	(0–3)	0	(0–0)	0.2	(0–1)	0	(0–0)	0.2	0.76	0.54
$D_F N_M$	0.6	(0–1)	0.4	(0–1)	0.4	(0–2)	0.4	(0–1)	0.5	0.09	0.96
$N_F D_M$	0.4	(0–1)	0.4	(0–1)	0.2	(0–1)	0.6	(0–1)	0.4	0.52	0.68

depth selection revealed that both hybrid groups selected intermediate depths ($D_F N_M$ average = 28 cm, range 16–41 cm; $N_F D_M$ average = 31 cm, range = 21–49 cm) when compared to dwarf and normal (Table 1). Under purely additive genetic control (Lynch and Walsh 1998), an absolute intermediate in depth selection given the average depth values for the dwarf and normal groups was expected to be 30 cm from the bottom. The average depth selected by hybrid groups combined was 29 cm from the bottom. Post-hoc LSD comparisons revealed that intermediate depth selection values were significantly different ($P < 0.001$) from their dwarf and normal counterparts; however, no significant difference existed between the hybrid groups (Table 2; Figure 1A).

For the directional change behavior, the $D_F N_M$ hybrid group performed fewer changes in direction (mean = 1.7, range = 0–5) when compared to the normal group (average = 2.0) while the $N_F D_M$ group maintained an intermediate number of directional changes (mean = 2.5, range = 0–6; Table 1). Given an intermediate expectation of 3.0 turns per minute as predicted from dwarf and normal values for a purely additive genetic influence, the mean of hybrid groups (mean = 2.1) was below this prediction. Overall, hybrid directional change behavior was closer to the normal than to the dwarf group (Figure 1B). Least significant difference comparisons reflected this result where significant differences existed between the dwarf and hybrids, but the normal group did not significantly differ from the hybrids (Table 2). A similar pattern was observed in the hybrid groups for burst swimming ($D_F N_M$ = 0.5; $N_F D_M$ = 0.4; Table 1). The average number of burst swims performed by hybrids was 0.43, below the intermediate value and closer to the normal group (Table 1; Figure 1C). Overall, the number of burst swims in hybrids was significantly different from the dwarf experimental group, yet the normal group did not differ significantly from the hybrids (Table 2).

Genetic Basis for Behavioral Differentiation

Estimates for the genetic basis of behavioral traits indicated that depth selection had the strongest heritable component (h^2 = 0.73, SE = 0.55), relative to both burst swimming (h^2 = 0.38, SE = 0.40) and directional changes (h^2 = 0.40, SE = 1.46). Given the large standard error estimates, however, only depth selection could be deemed as being significantly different from zero.

Q_{ST}–F_{ST} Comparisons

The level of genetic differentiation between dwarf and normal ecotypes estimated from θ at microsatellite loci was 0.24 (95% CI = 0.13–0.33). The Q_{ST} estimate was highest for depth selection (0.98, 95% CI = 0.96–1.00), intermediate for directional changes (0.68, 95% CI = 0.45–0.91) and lowest for burst swimming (0.53, 95% CI = 0.11–0.96). Both depth selection and directional changes were significantly different from neutral expectations, but not burst swimming (Figure 2). The Q_{ST} estimates for morphological traits ranged from 0.06 (maxillary length) to 0.66 (gill-raker counts). Concordant with a previous study (Bernatchez 2003), gill rakers were the only morphological trait significantly higher than neutral

TABLE 2. Analysis of variance and post-hoc least significant difference comparisons of means between experimental groups for depth selection (cm from bottom of tank), directional changes (number per minute), and burst swimming (number per minute). An asterisk represents significant differences after Bonferroni adjustment (α = 0.05, k = 6; Rice 1989).

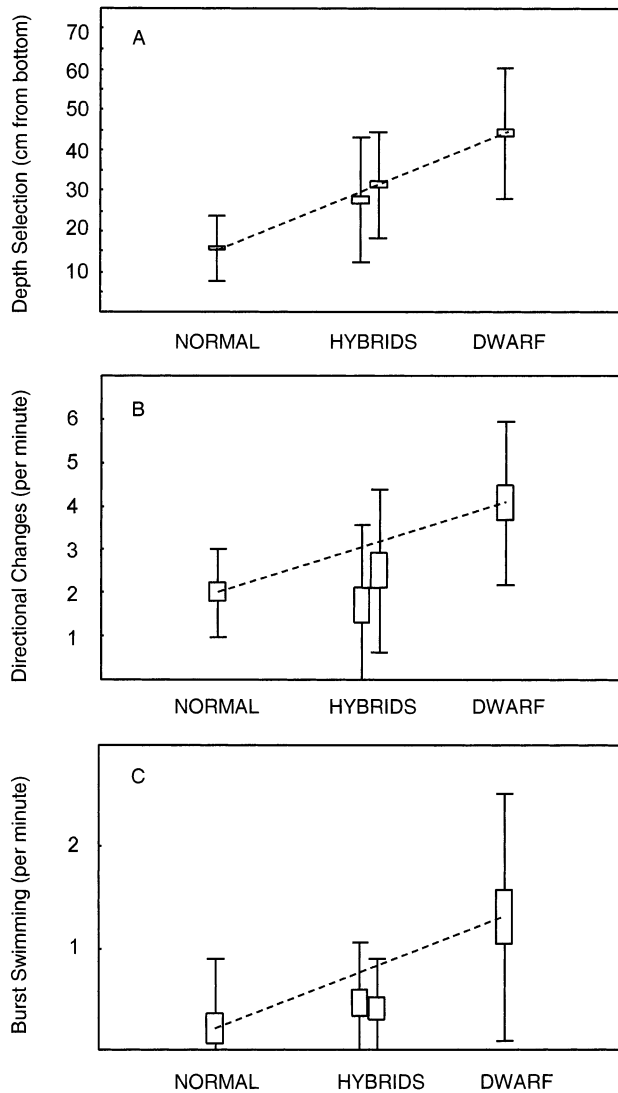| Experimental group		Depth selection	Directional changes	Burst swimming
Overall		0.0001*	0.0002*	0.0002*
dwarf	normal	0.0001*	0.0003*	0.000046*
dwarf	$D_F N_M$	0.0001*	0.00004*	0.0013*
dwarf	$N_F D_M$	0.0001*	0.0052*	0.0007*
normal	$D_F N_M$	0.0001*	0.579	0.329
normal	$N_F D_M$	0.0001*	0.356	0.434
$D_F N_M$	$N_F D_M$	0.012	0.141	0.845

FIG. 1. Box plots (mean/standard error/standard deviation) illustrating depth selection (A), directional changes (B), and burst swimming (C) behaviors for normal, hybrid (D_FN_M and N_FD_M, respectively), and dwarf experimental groups. Dotted lines between normal and dwarf phenotypic mean values intersecting the hybrid groups schematically illustrate the level of intermediacy observed in the hybrid crosses.

expectations. However, the behavioral Q_{ST} values were in turn higher than the gill-raker Q_{ST} but only in the case of depth selection was the Q_{ST} significantly higher than that of gill-raker counts (Figure 2).

DISCUSSION

This study provided evidence of a behavioral phenotype-environment association in dwarf and normal lake whitefish ecotypes supporting the working hypothesis that ecological divergence in the lake whitefish has been driven by divergent natural selection acting on behavioral swimming traits. We found significant swimming behavioral differences between dwarf and normal groups leading to the conclusion that these behavioral phenotypes are genetically based (Skúlason et al. 1989, 1993). The intermediate level of depth selection in

hybrid crosses offered additional evidence that the genetic basis consisted of an additive component (Noakes 1986; Lynch and Walsh 1998). In the case of burst swimming and directional change behavior, the hybrids had trait values closer to the normal group suggesting that dominance in addition to additive genetic variation is likely affecting the genetic basis (Roff 1997). Estimates for the genetic basis of the traits using DFREML supported observed patterns of hybrid intermediacy where only in the case of depth selection could we conclude the existence of an additive genetic component given a positive h^2 value under standard error consideration. In contrast, standard error estimates were high for burst swimming and directional changes such that the positive estimates were not conclusive. Dominance factors can typically contaminate behavioral h^2 estimates under a full-sib breeding design (Roff 1997), while the differential number of potential families generated between experimental groups likely also contributed to the high standard error estimates observed. The fraction of dominance versus additive genetic variance differs pending the nature of the trait explored (Crnokrak and Roff 1995), but our results suggest that dominance may play more of a role in predator-prey behaviors than in the case of depth selection.

Upper departures of Q_{ST} from neutral expectations were pronounced in all three behavioral phenotypes, rejecting the null hypothesis of no selective effect for observed behavioral phenotype-environment associations. Gill-raker counts most strongly deviated from neutral expectations compared to other morphological traits but were still significantly lower than depth selection. Bernatchez (2003) showed that of Q_{ST} estimates for 17 of 18 morphological characters, gill-raker counts were the only character between dwarf and normal whitefish to differ significantly from neutral expectations suggesting that it most likely evolved under directional selective pressures. The Q_{ST} results should be interpreted with caution given that phenotypic rather than genetic variance was used. Yet, they leave little doubt that differences in behavior are mainly driven by divergent natural selection given that the magnitude of effect on this trait is at least comparable to gill-raker counts, a known selected trait (Bernatchez 2003).

These data still do not strictly rule out alternative explanations for the role of such behavioral processes. For this, positive correlations between the extent of divergent natural selection and behavioral specialization in different environments must be further investigated throughout their ontogeny, in additional lakes and ultimately to other species complexes. Finally, critical experiments designed to test the hypothesis of a potential trade-off in fitness versus behavioral specialization still needs to be performed.

Cumulatively, these results imply that behavioral differentiation between dwarf and normal whitefish ecotypes is likely directly influenced by the environments they inhabit and the resources they consume, a critical component within the framework of adaptive radiation (Schluter 2000). The number of gill rakers has historically stood out as the main phenotypic trait for which such a correlation has defined differential use of resources in sympatric fish species complexes (Bodaly 1979; Svärdson 1979; Schluter 1993; Snorrason et al. 1994; Skúlason et al. 1999; Bernatchez 2003). These mor-
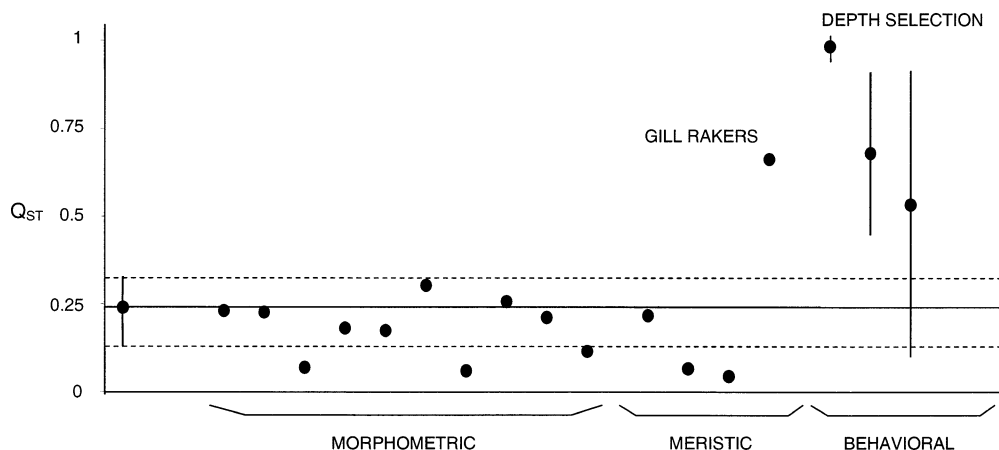
FIG. 2. Q_{ST}-estimates for ten morphological (in order: preorbital length, orbital length, trunk length, dorsal fin length, caudal peduncle length, maxillary width, maxillary length, body depth, head depth, interorbital width), four meristic (suprapelvic scales, dorsal ray count, pectoral ray count, gill-raker count), and three behavioral characters with 95% confidence intervals (depth selection, directional changes, burst swimming). The first point represents a θ-value of 0.24, whereas the 95% CI of this neutral expectation is delineated by dotted lines.

phological observations have generally established the limnetic/benthic dichotomy of sympatric fish species complexes.

However, our results support the hypothesis that behavior is likely the first responsive character to selection in divergence towards limnetic habitats concordant with the consensus that expansion to new resources and environments remains the dominant ecological theme in adaptive radiation (Skúlason et al. 1999; Schluter 2000). Within this context, the expansion to new resources typically follows a recognizable sequence of events involving an initial habitat separation followed by divergence with respect to prey size and food types (Diamond 1986). Within new environments, morphological characters such as gill rakers may only have a clear utility when or as the specialization to habitat increases and intrapopulation competition leads to morphological phenotype-environment associations (Snorrason et al. 1994). In whitefish, the current experimental results suggest the possibility of a similar scenario whereby the shift into a limnetic trophic niche results from a depth selection phenotype-environment association followed by intrapopulation competition for resources, ultimately leading to the divergence of an increased number of gill rakers associated with feeding efficiency. The result of such competitive interactions within the derived dwarf ecotype may in turn lead to phenotypic interactions among other traits, namely physiological costs associated with a limnetic environment; that is, a feeding regime of smaller prey coupled with an increased risk of predation lending to differential bioenergetic costs. We observed growth differences for our experimental groups with the dwarf group being significantly smaller after only 18 months of being reared in the same environment as the normal groups. Under natural conditions dwarf whitefish consume 40–50% more food than normal ecotypes, yet their respective conversion efficiency of these resources is reported to be two to three times lower than the normal ecotype (Trudel et al. 2001). These results were consistent with observations for lake cisco (*Coregonus artedi*), another coregonine species that feed in a limnetic environment (Trudel et al. 2001). Altogether, there is little doubt that the habitat selection of a

limnetic environment incurs a substantial bioenergetic cost to the dwarf form.

The interaction between behavioral, morphological, and physiological phenotype-environment associations during population divergence remains unknown (Futuyma 1986; Losos 1990; Skúlason et al. 1993), but may best be elucidated through studies of their genetic architecture (Rogers et al. 2001). The empirical incorporation of behavior phenotype-environment associations as part of this synthesis will complement finer scenarios of the role of divergent natural selection in the process.

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