DOI: 10.1577/T05-130.1

Association between Growth and *Pan I** Genotype within Atlantic Cod Full-Sibling Families

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Abstract.—Studies of the pantophysin (*Pan I**) locus in Atlantic cod *Gadus morhua* and other marine gadoids indicate that the locus is under positive selection; in Atlantic cod, genotypic variation at this locus has been linked to differences in growth. Here, we present preliminary data comparing the growth and condition of different Atlantic cod *Pan I** genotypes *within* families held under seminatural mesocosm conditions. Larvae from three full-sibling families carrying *Pan I*bb* or *Pan I*ab* genotypes were reared for 10 weeks in two mesocosms. Multivariate analysis of variance indicated that larvae carrying the *Pan I*ab* genotype exhibited significantly higher standard length, dry weight, and RNA: DNA ratio (condition factor) than did larvae that carried the *Pan I*bb* genotype, potentially indicating selection. The influence of linked loci cannot be excluded; indeed, the absence of a significant correlation between genotype and growth in one family may substantiate this. The lack of differences in survival among genotypes indicates that moderate selective effects are acting primarily through size-specific mortality and fecundity. The proposed putative fitness effects, together with documented marked geographic differentiation in the wild, have implications for Atlantic cod population structure, effective migration rates, recruitment, and local adaptation, which are of particular relevance in a species threatened by continuing exploitation and rising sea temperatures.

Traditionally, research on population genetics has focused mainly on neutral molecular markers to estimate genetic divergence between populations and assess levels of migration and gene flow (Wright and Bentzen 1995; Carvalho and Hauser 1998). However, new opportunities to investigate

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Received May 11, 2005; accepted August 23, 2005
Published online January 9, 2006

relationships between genotype and the environment have led to an increased interest in molecular markers that are under selection (Schulte 2001; Schlötterer 2002; Guinand et al. 2004). Such an approach can provide direct insights into the importance and nature of local adaptation (Moran 2002). Furthermore, selected markers may be a powerful alternative to more commonly used neutral molecular markers (e.g., microsatellites, mitochondrial DNA) in species with high gene flow, where migratory exchange between essentially self-recruiting populations often maintains genetic homogeneity and thus masks stock structure relevant to management (Hauser and Ward 1998; Waples 1998). An understanding of local adaptation and selective pressures, although difficult to obtain empirically (Imsland and Jónsdóttir 2003), may have considerable implications for fishery management and is gaining increasing significance as evidence for global climate change accumulates (Wood and McDonald 1997; Conover 1998). Continuing technological advances have facilitated the detection and screening of variation at candidate genes that are likely to be under selection, although at present research in fish has focused primarily on salmonids (Moran 2002). However, such approaches are equally applicable to marine species, particularly in the context of overexploitation, climate change (O'Brien et al. 2000; Beaugrand and Reid 2003; Platt et al. 2003; Edwards and Richardson 2004), and recent evidence of genetic structuring at surprisingly fine scales (Ruzzante et al. 2000; Hutchinson et al. 2001; Knutsen et al. 2003), which offers increased potential for local adaptation.

One such candidate gene implicated to be under selection (Pogson and Mesa 2004) is the pantophysin (*Pan I**) locus in Atlantic cod *Gadus morhua*. The *Pan I** locus was first identified by Pogson et al. (1995) using restriction fragment length polymorphism techniques (Dowling et al. 1996) and exhibited highly significant differences in allele frequency among populations of Atlantic cod that far exceeded those revealed for the additional 10 anonymous loci studied simultaneously (Pogson et al. 1995). The locus was subsequently sequenced and identified as part of the synaptophysin gene (Fevolden and Pogson 1997) and was later revised to pantophysin (*Pan I**; Pogson 2001), an integral membrane protein expressed in cytoplasmic transport vesicles (Haass et al. 1996; Windoffer et al. 1999; Brooks et al. 2000). Exceptionally large differences in allele frequency have since been observed among geographically proximate populations of Norwegian coastal and northeast Arctic Atlantic cod (Fevolden and Pogson 1997) and among Icelandic populations (Jónsdóttir et al. 1999, 2001). Natural selection was thought to be partly responsible for the distribution of allele frequencies, but because of the unreasonably high levels of selection that would be required to explain such patterns it was concluded that limited gene flow must also be influential in maintaining such structuring. Consequently, *Pan I** was proposed to be a useful population genetic marker for population structure analysis (Fevolden and Pogson 1997).

Evidence of selection at the *Pan I** locus is provided by direct molecular analysis of synonymous (d_S) and nonsynonymous (d_N) mutations in both Atlantic cod (Pogson and Mesa 2004) and walleye pollock *Theragra chalcogramma* (Canino and Bentzen 2004). Normally, synonymous and nonsynonymous substitutions would be equally prevalent at a presumptive neutral locus, while a locus under diversifying selection would exhibit a d_N:d_S ratio exceeding unity. Indeed, the d_N:d_S ratio at the *Pan I** loci of Atlantic cod and walleye pollock significantly exceeds unity, providing strong evidence that the operation of natural selection has favored nonsynonymous changes. Furthermore, in wild populations, the *Pan I*a* allele has been linked to increased size at age (Fevolden and Pogson 1995; Pogson and Fevolden 1998; Jónsdóttir et al. 2002), although *Pan I** could simply be acting as a population marker, identifying distinct populations with differing life histories. It remains unclear whether differences in growth performance between genotypes are manifested when fish are reared under common conditions (Imsland and Jónsdóttir 2002) and against a common genetic background.

Here, we present preliminary data on fitness variation associated with genotypic differences at the *Pan I** locus. Specifically, we examine the relationship between genotypic and phenotypic variation in full siblings with different *Pan I** genotypes reared in common, predator-free mesocosms. By combining mesocosm rearing with microsatellite-based genetic parental assignment, we were able to rear larvae from multiple families under identical environmental conditions, thus minimizing error due to the inherent environmental variability found in comparative experiments in which families are separated. The experiment was conducted for the European Union project MACOM (Demonstration of Maternal Effects of Atlantic Cod: Combining the Use of Unique Mesocosm and

TABLE 1.—Temporal variation in the number of Atlantic cod larvae possessing the *Pan I*bb* or *Pan I*ab* genotype within three families that exhibited variation at the *Pan I** locus in two experimental mesocosms in 2000. ''Ratio'' is the number of *Pan I*bb* homozygotes divided by the total for each family at each date. The ''*P*'' columns contain one-tailed *P*-values indicating the cumulative binomial probability of deviation from the expected 50:50 ratio.

			Family 5c (*ab*♂ × *bb*♀)				Family 6a (*bb*♂ × *ab*♀)				Family 6b (*bb*♂ × *ab*♀)			
Mesocosm	Date	Week	*bb*	*ab*	Ratio	*P*	*bb*	*ab*	Ratio	*P*	*bb*	*ab*	Ratio	*P*
2,500 m^3	6 Apr	1	22	22	0.50	0.56	4	3	0.57	0.77	26	25	0.51	0.61
	21 Apr	3	9	11	0.45	0.41	6	1	0.86	0.99	11	10	0.52	0.67
	3 May	5	12	10	0.55	0.74	3	0	1.00	1.00	22	24	0.48	0.44
	8 Jun	10	21	22	0.49	0.50	9	4	0.69	0.95	21	28	0.43	0.20
4,400 m^3	7 Apr	1	3	2	0.60	0.81	22	15	0.59	0.91	11	18	0.38	0.13
	21 Apr	3	7	6	0.54	0.71	22	23	0.49	0.50	20	18	0.53	0.69
	27 Apr	4	4	5	0.44	0.50	30	22	0.58	0.89	19	16	0.54	0.75
	9 Jun	10	5	12	0.29	0.07	32	21	0.60	0.95	22	27	0.45	0.28

Novel Molecular Techniques) (T. Svåsand and coworkers, unpublished report available at http://macom.imr.no), which was not aimed specifically at investigating *Pan I** effects. The presence of three heterozygote individuals among the broodstock allowed an assessment of the effect of genotype on growth and condition *within* families sharing a comparatively uniform genetic background and a common environment. Although our data were necessarily restricted to these three families, they strongly suggested that selection is acting on *Pan I** (or a linked locus), and are thus valuable for designing more targeted experiments with the potential of generating a linkage map for fitness traits in Atlantic cod.

Methods

Production and Rearing of Larval Atlantic Cod

Two-hundred adult Atlantic cod were collected from near Bear Island (Norway) in the Barents Sea during August 1998 and transferred to net-cages at the Parisvatnet Experimental Station near Bergen, Norway. Each fish was tagged with an internal passive integrated transponder tag, and fin clippings were collected for microsatellite genotyping. Maturity was determined by biopsy during the 1999 spawning season.

From the original 200 individuals, 30 spawning pairs were established during the 2000 spawning season in ten 3-m-diameter tanks, each divided into three compartments. Pairs were established according to maturity, health, and microsatellite genotype (enabling the accurate assignment of larvae to their parental origin based on a minimum of microsatellite loci). Eggs were collected daily between 18 February and 25 April, counted, and assessed for size, quality, and fertilization rate. Eggs were incubated under low-light conditions in cylindrical, black plastic containers (250–500 L) with continuous aeration and a constant supply of clean seawater. Larvae from 25 families, hatched within 2–3 d of each other, were selected for transfer to the mesocosms.

On 28, 29, and 30 March 2000, newly hatched larvae from the 25 families were flown to Flødevigen Marine Station near Arendal, Norway. Larvae from each family were counted by hand and were released into two adjacent mesocosms. Approximately 4,000 larvae from each family were released into a 2,500-m^3 mesocosm, and 8,000 larvae from each family were released into a 4,400-m^3 mesocosm.

The smaller (2,500 m^3) of the two mesocosms had a 600-m^2 surface area and a maximum depth of 5 m. The larger (4,400 m^3) mesocosm had a 1,700-m^2 surface area and a maximum depth of 4.5 m. The mesocosms were predator-free and contained a natural zooplankton community that had developed since December 1999, when the mesocosms were filled with coarsely filtered seawater from a nearby fjord. Temperature was monitored daily at depths of 0, 0.5, 1, 2, 3, and 4 m from 1 March to 5 June 2000. Estimates of zooplankton density were obtained twice weekly from 80-L pumped samples taken at a depth of 3.0 m.

Sampling of Larval and Juvenile Atlantic Cod

Six-hundred larvae were sampled from each mesocosm at 1, 3, 4 or 5, and 10 weeks postrelease, and were stored at −80°C for subsequent RNA: DNA quantification and genotyping. To minimize net avoidance, sampling was conducted at night using a two-chambered net with a 0.3-m^2 opening and 180-μm mesh. After the first 3 weeks, the mesh size was increased to 350 μm. Nets were towed at a constant speed of 1 m/s at a depth of 2 m and over distances of 35 and 50 m in the 2,500- and 4,400-m^3 mesocosms, respectively. At week

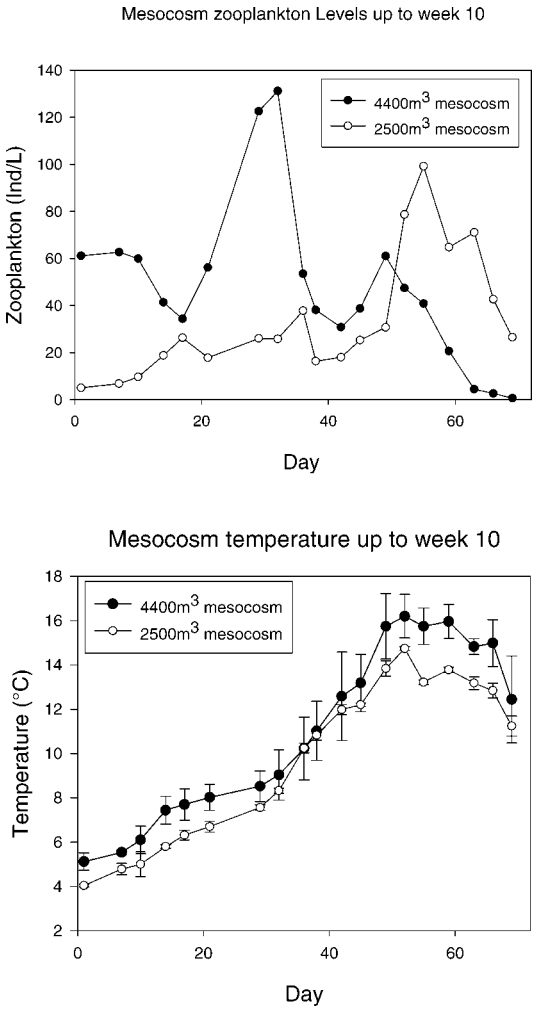

Figure 1.—Zooplankton levels (upper panel) and mean water temperature (lower panel) for two experimental mesocosms during the first 10 weeks of Atlantic cod larval development.

10, the mesocosms were drained, leaving 2,927 and 11,400 juveniles in the 2,500- and 4,400-m^3 mesocosms, respectively. Six-hundred fish from each mesocosm were randomly selected for analysis and were frozen; the remaining fish were transferred to indoor tanks (Clemmesen et al. 2003).

Analytical Procedures

Standard length, dry weight, and RNA:DNA ratio.—After the fish were thawed, standard length (SL) was measured to the nearest 0.1 mm. Dry mass was measured to the nearest 0.1 μg after 24-h freeze-drying. For RNA:DNA analysis, which is a measure of nutritional condition (Buckley 1984; Clemmesen 1988, 1994; Belchier et al. 2004), whole larvae (up to week 5) were homogenized following a procedure modified from Clemmesen (1993) (Clemmesen et al. 2003). A fragment of dorsal muscle was homogenized for analysis of week-10 samples.

Microsatellite analysis.—We used a standard phenol–chloroform method (Taggart et al. 1992) to extract DNA from fin clippings taken from the broodstock. The DNA was amplified by polymerase chain reaction (PCR) at three microsatellite loci (*Gmo2,* Brooker et al. 1994; *Gmo8* and *Gmo19,* Miller et al. 2000) and was analyzed by use of an ALFexpress (Pharmacia) automated DNA sequencer. Where necessary, a fourth locus (*Gmo132,* Brooker et al. 1994) was included to ensure unambiguous family assignment. Larval microsatellite DNA was PCR amplified directly from the homogenates obtained for the RNA:DNA ratio analysis.

Restriction fragment length polymorphism.—A 313-base-pair fragment of the *Pan I** gene was PCR amplified with primers redesigned by D. O'Leary (Department of Biochemistry, University College, Cork, Ireland, personal communication) from *Pan I** sequences published on GenBank (accession numbers AF288943–AF288977). These primers flank the diagnostic *Dra I** restriction site, which differentiates the *Pan I*a* and *Pan I*b* alleles; the primers amplify a shorter fragment than that used by Fevolden and Pogson (1997), thereby enhancing PCR and screening efficiency (Coughlan 2001). Each 12-μL PCR reaction mix contained 20 ng of template DNA, 1 × NH_4 reaction buffer (Bioline), 200 μM of deoxynucleotide triphosphates (dNTPs), 50 mM of KCl, 0.1 mg of bovine serum albumin (BSA) per milliliter, 250 nM of each primer (one primer end-labeled with Cy5 fluorescent dye), and 0.45 units of *Taq* polymerase (Bioline; enzyme number 2.7.7.7; IUBMB 1992). The PCR was carried out on an MJ Research Tetrad thermocycler in the following manner: 1 cycle at 95°C for 60 s; 5 cycles at 94°C for 45 s, 60°C for 30 s, and 72°C for 60 s; 35 cycles of 94°C for 45 s, 58°C for 30 s, and 72°C for 60 s; and a final annealing step of 72°C for 180 s. Three microliters of PCR product were subsequently digested at 37°C for 6 h in a solution containing five units of *Dra I** restriction endonuclease (Promega), 14.3 μL of double-distilled H_2O, 1 × Promega Buffer B, and 0.1-mg/mL BSA; a final enzyme-denaturing step of 65°C for 5 min was then performed. Fragments were size separated by elec-

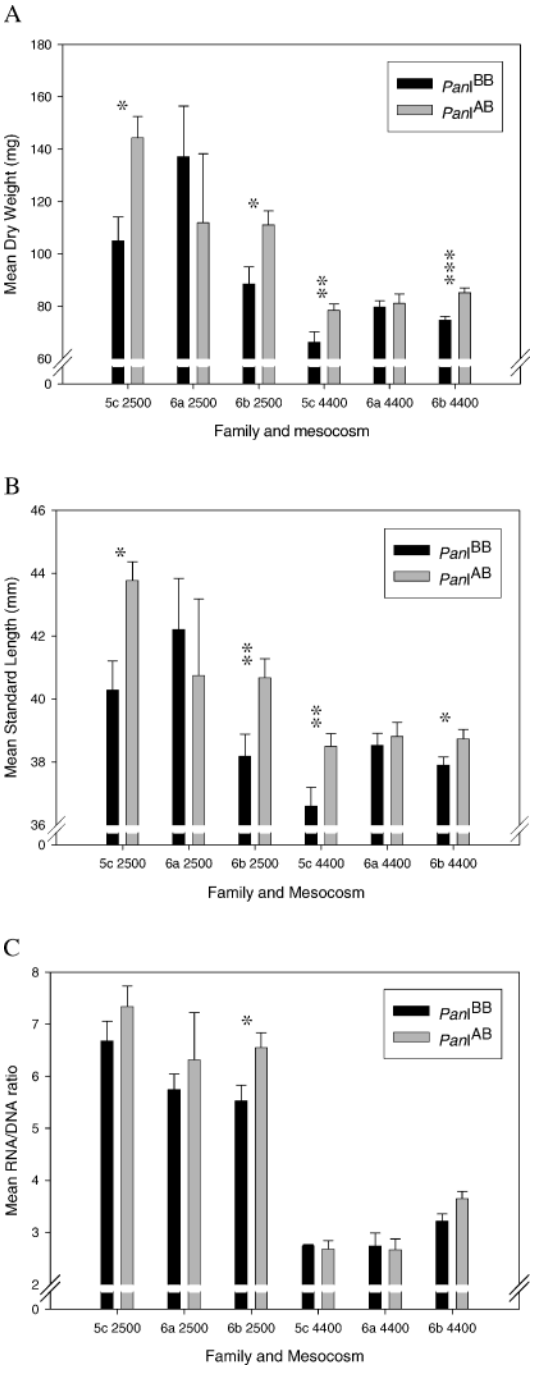

Figure 2.—Mean + SE **(A)** dry weight, **(B)** standard length, and **(C)** RNA:DNA ratio in 10-week-old Atlantic cod larvae differing in *Pan I** genotype. Asterisks indicate the significance of individual Mann–Whitney *U*-tests comparing the differences between genotypes within families (5c, 6a, and 6b) within 2,500- and 4,400-m^3 mesocosms ($P < 0.05$*; $P < 0.01$**; $P < 0.001$***).

trophoresis on 1.5% agarose gels and were visualized by staining with ethidium bromide.

The DNA from broodstock fish was screened for polymorphism at the *Pan I** locus to identify adults differing in *Pan I** genotype. This enabled the selection of families containing variation at the *Pan I** locus for further analysis of growth and nutritional condition. The relationship between *Pan I** genotype and larval growth and condition was determined by comparing the mean SL, dry mass, and RNA:DNA ratio of each *Pan I** genotype within families at each sampling date. All 600 larvae from each weekly sample were microsatellite genotyped and assigned to their parental origin. Larvae belonging to families varying at the *Pan I** locus were then screened to identify their *Pan I** genotypes.

Statistical Analysis

The data were separated by mesocosm, and multivariate analysis of variance (MANOVA) was used to examine the effects of family and *Pan I** genotype on SL, dry weight, and RNA:DNA ratio in larvae sampled at 1, 3, 4 or 5, and 10 weeks postrelease. *Pan I** genotype was a fixed factor, and family was a random factor. Growth data (weight and length) were inverse transformed, and condition data (RNA:DNA) were $\log_e$ transformed to normalize the distribution of the data. With the exception of dry weight in the 2,500-m^3 mesocosm, the data conformed to multivariate normality and were homoscedastic after transformation. Mann–Whitney *U*-tests were also used to examine the effect of *Pan I** genotype on growth and condition by comparing individual pairs of medians within families for both mesocosms at each weekly sampling. These tests enabled assessment of the performance of separate families. The results of the Mann–Whitney *U*-tests were combined by use of Fisher's method for combining probabilities (Sokal and Rohlf 2003) and were used to confirm the MANOVA results. Associations between *Pan I** genotype and survival were examined by comparing the observed ratio of homozygotes to heterozygotes at each sampling date with the expected ratio predicted from Mendelian genetics. The cumulative binomial probability of deviation from the expected ratio was calculated to indicate whether the observed ratios deviated significantly from expected ratios (Sokal and Rohlf 2003).

Results

Due to low frequencies of the *Pan I*a* allele in natural populations of Barents Sea Atlantic cod,

TABLE 2.—Multivariate ANOVA table showing effects of genotype, family, and mesocosm on standard length (SL), dry weight, and RNA:DNA ratio of 10-week-old Atlantic cod larvae in a 2,500-m^3 experimental mesocosm. The upper section shows the overall effect of each factor on growth and condition, while the lower section shows the effect of each factor on individual variables. For the multivariate test results, *F* represents Pillai's trace.

Effect	Dependent variable	df	*F*	*P*
	Multivariate tests			
Genotype		6	28.093	<0.001
Family		3	6.697	<0.001
Genotype × family		3	0.316	0.814
	Between-factor effects			
Genotype	SL	4	674.340	<0.001
	Dry weight	4	28.389	<0.001
	RNA:DNA ratio	4	500.819	<0.001
Family	SL	2	11.371	<0.001
	Dry weight	2	7.918	0.006
	RNA:DNA ratio	2	8.106	0.006
Genotype × family	SL	1	0.001	0.973
	Dry weight	1	0.000	0.986
	RNA:DNA ratio	1	0.817	0.369
Error	SL	84		
	Dry weight	84		
	RNA:DNA ratio	84		

only 3 of 50 parental broodstock were heterozygous (*Pan I*ab*) at the *Pan I** locus and none were *Pan I*aa* homozygous. These heterozygotes (one male, two females) were paired with *Pan I*bb* homozygotes, resulting in three families with expected genotype frequencies of 50% *Pan I*bb* and 50% *Pan I*ab* (Table 1).

The two mesocosms differed in food availability and temperature (Figure 1). Zooplankton levels in the 4,400-m^3 mesocosm, though relatively high for much of the first 8 weeks, subsequently declined from 61 individuals/L on 16 May 2000 to 0.6 individuals/L in the final week. In the 2,500-m^3 mesocosm, zooplankton levels rose steadily from an initially low density of 5.04 individuals/L to 99 individuals/L on 23 May 2000 before declining to 26.5 individuals/L in the final week. The mean temperature in each mesocosm was calculated from readings taken at different depths. Over all sampling dates, the 4,400-m^3 mesocosm was, on average, 1.25°C warmer than the 2,500-m^3 mesocosm (*t*-test: $P < 0.0001$; Figure 1).

TABLE 3.—Multivariate ANOVA table showing effects of genotype, family, and mesocosm on standard length (SL), dry weight, and RNA:DNA ratio of 10-week-old Atlantic cod larvae in a 4,400-m^3 experimental mesocosm. The upper section shows the overall effect of each factor on growth and condition, while the lower section shows the effect of each factor on individual variables. For the multivariate test results, *F* indicates Pillai's trace.

Effect	Dependent variable	df	*F*	*P*
	Multivariate tests			
Genotype		6	30.000	<0.001
Family		3	5.456	0.002
Genotype × family		3	0.616	0.606
	Between-factor effects			
Genotype	SL	2	2,030.020	<0.001
	Dry weight	2	268.651	<0.001
	RNA:DNA ratio	2	32.249	<0.001
Family	SL	1	0.019	0.890
	Dry weight	1	1.107	0.296
	RNA:DNA ratio	1	15.214	<0.001
Genotype × family	SL	1	0.014	0.907
	Dry weight	1	0.636	0.427
	RNA:DNA ratio	1	0.202	0.654
Error	SL	93		
	Dry weight	93		
	RNA:DNA ratio	93		

TABLE 4.—*P*-values from Mann-Whitney *U*-tests indicating the significance of differences between Atlantic cod larvae with genotypes *Pan I*bb* and *Pan I*ab* in week 10 of an experiment in which larvae were reared in 2,500- and 4,400-m^3 mesocosms. The final column shows the *P*-values for the combined effect (i.e., across all families) on dry weight, standard length, and RNA:DNA ratio calculated using Fisher's method for combining probabilities (Sokal and Rohlf 2003). The asterisks denote the significance before sequential Bonferroni correction ($P < 0.05$*, $P < 0.01$**, $P < 0.001$***). Values that remained significant after sequential Bonferroni correction at the level of each factor are given in bold italics.

	Family 5c		Family 6a		Family 6b		
Factor	2,500 m^3	4,400 m^3	2,500 m^3	4,400 m^3	2,500 m^3	4,400 m^3	Overall *P*
Dry weight	0.0365*	***0.0026****	0.4140	0.8415	***0.0109****	<***0.001*****	<***0.001*****
Standard length	0.0268*	***0.0073*****	0.7105	0.7401	***0.0095*****	0.0337*	<***0.001*****
RNA:DNA ratio	0.6835	0.5750	0.5167	0.5899	0.0267*	0.0586	0.0783

No significant effect of *Pan I** genotype on growth or condition was detected in Atlantic cod larvae sampled during weeks 1, 3, 4, and 5 (MANOVA: $P > 0.05$). However, in the 2,500-m^3 mesocosm at week 10, MANOVA (Table 2) indicated that genotype significantly affected weight ($P < 0.001$, Figure 2a), length ($P < 0.001$, Figure 2b), and RNA:DNA ratio ($P < 0.001$, Figure 2c); fish that shared the *Pan I*ab* genotype were significantly heavier and longer and displayed a higher RNA:DNA ratio than those with the *Pan I*bb* genotype. Families also differed significantly in length ($P < 0.001$), weight ($P = 0.006$), and RNA:DNA ratio ($P = 0.006$). In the 4,400-m^3 mesocosm at week 10, MANOVA (Table 3) indicated that larvae with the *Pan I*ab* genotype also exhibited a significantly greater weight ($P < 0.001$, Figure 2a), length ($P < 0.001$, Figure 2b), and RNA:DNA ratio ($P < 0.001$, Figure 2c) than those with the *Pan I*bb* genotype. There were no significant differences between families in length ($P = 0.890$) or weight ($P = 0.296$), but families did display significantly different RNA:DNA ratios ($P < 0.001$). Multivariate ANOVA showed no significant genotype × family interaction in either mesocosm, indicating that, overall, genotype had a consistent effect on growth and condition across all families.

Meta-analyses based on Fisher's method for combining probabilities (Sokal and Rohlf 2003) integrated the results of the Mann–Whitney *U*-tests and indicated that the overall effects of genotype on length ($P < 0.001$) and weight ($P < 0.001$) were highly significant (Table 4), while effects on RNA:DNA ratio were nonsignificant ($P = 0.078$). With the exception of the nonsignificant overall effect of genotype on RNA:DNA ratio, the results of these meta-analyses substantiated those of the MANOVA (Tables 2–4).

The cumulative binomial probability of deviation from the expected 50:50 ratio of homozygotes to heterozygotes was not significant ($P > 0.05$) at any sampling date (Table 1), suggesting that *Pan I** had no effect on survival during the first 10 weeks of development.

Discussion

The MANOVA indicated that *Pan I*ab* genotypes exhibited greater length and weight and better condition (RNA:DNA ratio) than did *Pan I*bb* genotypes. With the exception of condition, which was not significantly affected by genotype ($P = 0.0783$), the meta-analysis of Mann–Whitney *U*-tests confirmed the MANOVA results. Such associations may be due to population differentiation if samples consist of several populations (Lynch and Walsh 1998). However, in our experiment, differences in performance between genotypes occurred within full-sibling families and thus within groups of animals that shared additive and dominance genetic variation (Lynch and Walsh 1998) as well as maternal effects and common environments during incubation and rearing in the mesocosms. However, the limited number of families, the exclusive use of *Pan I*ab* heterozygotes and *Pan I*bb* homozygotes, and the small sample size in some families complicates the interpretation. Nevertheless, we can draw some conclusions that are pertinent for future, more targeted mating and rearing experiments.

Due to the rarity of chromosomal crossing-over in single crosses, we lack the experimental rigor to exclude the influence of closely linked loci. Nevertheless, the lack of a significant genotype × family interaction in the MANOVA suggested that *Pan I** variation may be responsible for the observed variation in growth rate. On the other hand, genotypic effects on growth were significant in families 5c and 6b but not in family 6a, despite comparable sample sizes in the larger mesocosm.

This difference between families is not due to maternal effects, as the female was the heterozygote individual in families 6a and 6b but not in family 5c. If such an inconsistency between families is confirmed in further experiments, a gene linked to *Pan I** or an epistatic modification of *Pan I** effects may be responsible for the observed variation.

The low frequency of the *Pan I*a* allele in the broodstock prevented a direct comparison of all three genotypes. Without *Pan I*aa* genotypes, the observed superiority of *Pan I*ab* heterozygotes could be explained by (associative) overdominance, partial dominance, or additivity of fitness effects of alleles (Lynch and Walsh 1998). Studies of wild Atlantic cod caught off the southern Norwegian coast and in the northeastern Arctic have shown that *Pan I*aa* homozygotes exhibit higher mean growth than heterozygotes, which in turn exhibit higher mean growth than *Pan I*bb* homozygotes (Fevolden and Pogson 1995). Such data indicate that the *Pan I*a* allele is likely to be either completely additive or directionally dominant, and is associated with faster growth in natural populations. Further experiments that use parents with all three genotypes (*Pan I*aa, Pan I*ab, Pan I*bb*) are required to test this hypothesis.

Despite the considerable differences in environmental conditions between the mesocosms, especially in food availability (Figure 1), the effect of the *Pan I** genotype on growth and condition was consistent and significant in both mesocosms for families 5c and 6b (MANOVA; Tables 2, 3). However, environmental differences may explain the superiority of *Pan I*ab* heterozygotes over *Pan I*bb* homozygotes. Adult broodstock were caught near Bear Island in the Barents Sea, where *Pan I*a* allele frequencies are extremely low (Jónsdóttir et al. 2003; Case et al. 2005). Larvae were transferred to mesocosms in southern Norway, near a latitude and temperature regime where *Pan I*a* allele frequencies exceed 95% (Case et al., in press). Faster growth of fish carrying this allele may therefore be expected. What is more notable is that this transfer from the Barents Sea to southern Norway did not cause a detectable effect on survival, suggesting that selective effects are limited, at least within specific cohorts.

Despite significant differences in growth and condition between different *Pan I** genotypes, there was no significant difference in survival across the 10 weeks of larval monitoring (Table 1). Such observations support the notion that selection at the *Pan I** locus may be fairly limited in early larval stages, where stochastic effects arising from environmental heterogeneity may play a primary role (Cushing 1990; Beaugrand et al. 2003; Platt et al. 2003). However, unlike growth and condition, survival is not a continuous variable, and as such it may encompass significant variance in fitness-related characters without displaying any variation in mortality. Furthermore, with the exception of cannibalism, the predator-free mesocosm environment reduces the mortality of the smallest and most vulnerable fish that might occur in the wild (Bailey and Houde 1989; but see Rijnsdorp and Jaworski 1990 and Litvak and Leggett 1992). In addition, the apparently similar estimates of survival may arise from the relatively short duration of the experiment, which may have concealed the potential effect of genotypic differences on survival in the long term.

Growth and survival are polygenic traits with generally moderate to low heritability and strong susceptibility to maternal and environmental effects (Lynch and Walsh 1998). Atlantic cod follow this general pattern, exhibiting a within-population heritability (h^2) of 0.29 $\pm$ 0.27 (mean $\pm$ SE) for growth and 0.00 $\pm$ 0.12 for survival (Gjerde et al. 2004). Molecular genetic effects on these traits are therefore difficult to demonstrate, especially under natural (or semi-natural) conditions, and to our knowledge there is currently no linkage map available for Atlantic cod. In addition to their potential direct involvement in growth and survival, candidate genes such as *Pan I** could be used to identify genomic regions central to growth regulation (Tao and Boulding 2003). While we could not demonstrate conclusively that growth effects were due to *Pan I** variation, our findings indicate the feasibility of genetic mapping of quantitative traits for selective breeding programs and for the molecular investigation of adaptive variation in the wild.

Acknowledgments

We are grateful to Cock van Oosterhout and David Weetman for their valuable comments. This study was funded by a Ph.D. scholarship from the Ferens Educational Trust and the European Union under the program, ''Quality of life and living resources'' (QLK5-CT1999–01617).

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