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Note

Size and *MEP-2** variation in juvenile Atlantic salmon (*Salmo salar*) in the River North Esk, Scotland

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Abstract — The relationship of size to *MEP-2** genotype was investigated in 0+ juvenile Atlantic salmon (*Salmo salar*) in the River North Esk, Scotland. Significant temperature-associated *MEP-2** heterogeneity was observed among tributaries and, in the two tributaries studied, size and *MEP-2** genotype were significantly associated. No size association, or heterogeneity among tributaries, was found for the other allozyme polymorphisms screened. The results suggest that the association between size and *MEP-2** variation, noted by others, is likely to be widespread in wild populations, and potentially linked to habitat temperature. Crown © 1999 Published by Éditions scientifiques et médicales Elsevier SAS

Atlantic salmon / *Salmo salar* / malic enzyme / *MEP-2 genotype / growth**

1. INTRODUCTION

The importance of natural selection, as opposed to mutation and genetic drift, in maintaining enzyme polymorphisms in populations of fish and other species remains unclear. In Atlantic salmon, a role for selection has been indicated for the malic enzyme ($NADP^+$) locus polymorphism, *MEP-2**. Genotype variation at this locus has been found to be associated with differences in juvenile growth [8, 13], and with sea-age at maturity [9]. It may also be linked to the observed associations of *MEP-2** allele frequencies with latitude in both Europe and North America [21], with water temperature both among and within river systems [21, 22], and with the significant correlation at the locus between geographic and genetic distance [2, 10]. An association with growth could also explain the significant allele frequency differences observed at this locus between wild and farmed salmon [4, 23] and could lie behind part of the observed association in Atlantic salmon between allozyme heterozygosity and size [1].

While supported by experimental work, the evidence for a link between *MEP-2** and growth (i.e. fish size at age) in wild salmon comes from a single study of fish in one tributary of the River Dee, Scotland [8]. To determine whether this relationship was widespread, the association between *MEP-2** and size of 0+ salmon parr was examined in tributaries of another Scottish river, the North Esk (*figure 1*). The tributaries of the North Esk were of particular interest as their populations have previously been shown to display major life history differences (*table I*).

2. MATERIALS AND METHODS

2.1. Samples and temperature data

Juvenile Atlantic salmon parr in their first year of life (0+) were collected in August 1992 from the Water of Mark ($n = 61$) and the Corscarie Burn ($n = 70$) tributaries (*figure 1*) in areas with high densities of spawners to ensure samples were representative of the populations from which they derived. Fork lengths were recorded, and carcasses stored at –80 °C until analysis. The rank order of temperature conditions in the tributaries was established for the winter months using reported data [17] and for the summer months

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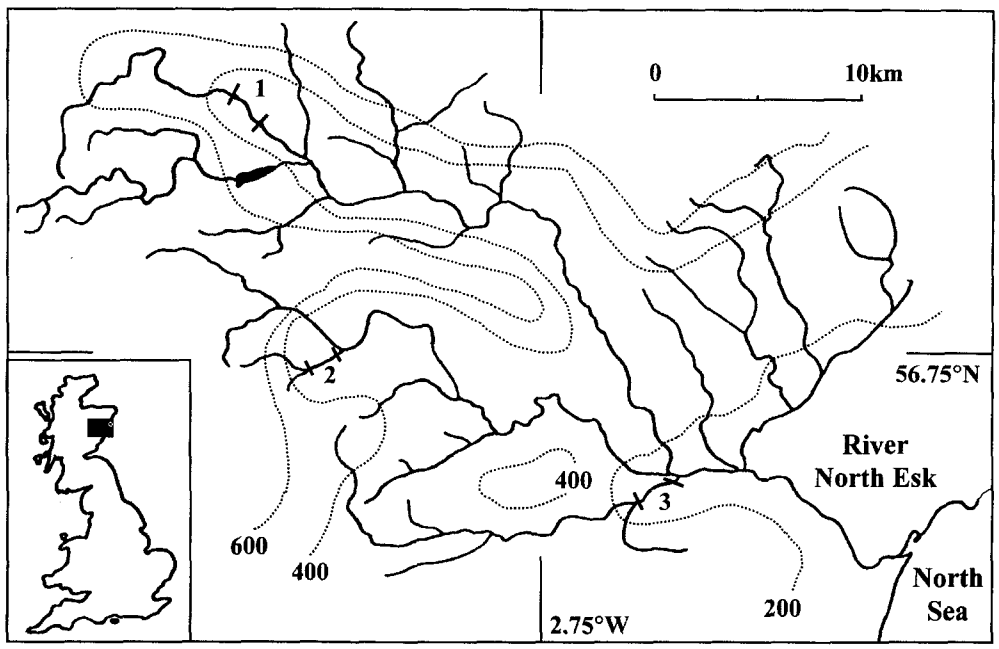

Figure 1. Map of North Esk River system showing relative geographical position of tributaries sampled, height above sea level (metres) and limits of sampling areas (bars). Water of Mark, 1; Corscarie Burn, 2; Cruick Water, 3.

using data provided by Julian McLean of the FRS Freshwater Fisheries Laboratory, Montrose station (*figure 2*).

Table I. Comparison of length of 0+ Atlantic salmon parr, and selected population life history characteristics, in three tributaries of the River North Esk, Scotland.

Character	Water of Mark	Corscarie Burn	Cruick Water
Min.–max. fork length (cm)	5.5–8.5	7.5–10.9	n.a.
Mean fork length (cm)	6.83	9.29	n.a.
– with *MEP-2*125*	6.96	9.10	n.a.
– without *MEP-2*125*	6.58	9.49	n.a.
River age of smolts[a]	Number of fish (%)		
1	0	2	20
2	23	69	80
3	70	29	0
4	7	0	0
Sea-age of mature fish[a]	Number of fish (%)		
1 (grilse)	26	100	100
2 (salmon)	74	0	0
Spawning period[b]	mid Oct.–mid Nov.	late Oct.–late Nov.	mid Dec.–mid Jan.

River age = time in fresh water; sea-age = time at sea; [a] Summers [17]; [b] Summers [18]; n.a. = not available.

2.2. Genetic analysis

Fish were screened for variation at *MEP-2** (E.C.1.1.1.40) locus as well as for three other known allozyme polymorphisms – *AAT-4** (EC.2.6.1.1), *IDDH-2** (EC.1.1.1.14) and *IDHP-3** (EC.1.1.1.42) by standard starch-gel electrophoresis [22]. Allele frequency data from 0+ juveniles (n = 50) collected in November 1995 from a third tributary, the Cruick Water (*figure 1*) and presented elsewhere [2], were also included in assessing levels of inter-tributary variation.

2.3. Data analysis

Statistical tests used (ANOVA, heterogeneity X^2, Fisher's test for combining probabilities, Bartlett's test for the homogeneity of variances, Kolmogorov-Smirnov test) are described by Sokal and Rohlf [16]. ANOVA analyses were carried out using MINITAB statistical software (Minitab Inc., State College, USA). F_{ST} values were calculated from allelic frequencies using Arlequin genetic data analysis software [14] based on a random model [24].

3. RESULTS

Genotype frequencies in all samples at the loci screened conformed to Hardy-Weinberg expectations (see [2] for Cruick analysis). Overall allozyme heterogeneity among tributaries was significant (X^2 = 15.57, P = 0.049). Individually, this was only the case for the heterogeneity X^2 for *MEP-2** (*table II*) and genetic

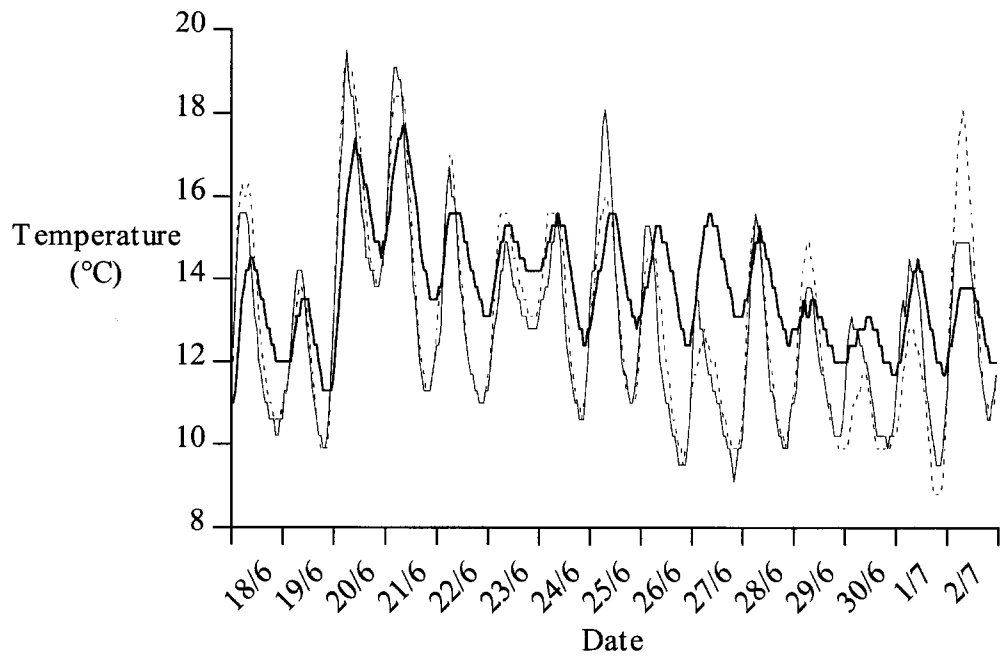

Figure 2. 1998 summer water temperatures in the three River North Esk tributaries sampled in the study. ----, Water of Mark; —, Corscarie Burn; —, Cruick Water.

differentiation among tributaries, as measured by F_{ST}, was over seven times greater for *MEP-2** than the mean F_{ST} for the other three polymorphic loci (*table III*).

Fork lengths of the fish in the Mark (7.5–10.9 cm) and Corscarie (5.5–8.5 cm) tributaries were normally distributed (Kolmogorov-Smirnov normality test, $P > 0.15$ in both tributaries) and the variances homogeneous (Bartlett's test, $P = 0.67$). The mean fork lengths in the two tributaries (*table I*) were significantly different ($F = 330.31$, $P < 0.001$), with the Water of Mark fish averaging 26.5 % smaller. Juveniles in the Water of Mark with a *MEP-2*125* allele (i.e. **125/125* and **125/100* genotypes) were significantly larger ($F = 3.87$, $P = 0.05$) than fish without **125* (i.e. **100/100* genotypes) while in the Corscarie Burn, fish with a **125* allele were significantly smaller ($F = 4.41$, $P = 0.04$). Combining the ANOVA probabilities [16] for the two independent tributary tests, the null hypothesis of no association of *MEP-2** genotype with fork length can be rejected ($X^2 = 12.43$, df = 4, $P = 0.014$). There was no evidence of associations with size for any of the other polymorphic loci.

Mean temperature from mid June to early July, 1998 (*figure 2*), was significantly higher ($F = 25.53$, $P < 0.001$) in the Cruick Water (13.8 °C) than in either the Water of Mark (12.9 °C) or Corscarie Burn (12.8 °C). The difference between the latter two was not significant but the Water of Mark had the lowest

Table II. Comparison of allele frequencies of 0+ Atlantic salmon parr from three tributaries of the River North Esk, Scotland.

Allele	Tributary		
	Water of Mark	Corscarie Burn	Cruick Water[a]
No. of fish	61	70	50
*AAT-4**			
**25* and **50*[b]	0.21	0.17	0.17
**100*	0.79	0.83	0.83
Heterogeneity $X^2 = 0.96$, df = 2, $P = 0.619$			
*IDDH-2**			
**0* and **-90*[b]	0.28	0.37	0.38
**100*	0.72	0.63	0.62
Heterogeneity $X^2 = 3.35$, df = 2, $P = 0.187$			
*IDHP-3**			
**100*	0.79	0.76	0.86
**116*	0.21	0.24	0.14
Heterogeneity $X^2 = 3.88$, df = 2, $P = 0.144$			
*MEP-2**			
**100*	0.58	0.71	0.74
**125*	0.42	0.29	0.26
Heterogeneity $X^2 = 7.39$, df = 2, $P = 0.025$*			

[a] Bourke et al. [2].
[b] Frequencies of rare alleles combined to allow comparison of all tributaries.
* Significant.

Table III. F_{ST} estimates for 0+ Atlantic salmon parr from three tributaries of the River North Esk, Scotland.

Locus	F_{ST}	*P*-value
*AAT-4**	–0.0044	0.6178
*IDDH-2**	0.0056	0.1867
*IDHP-3**	0.0078	0.1496
*MEP-2**	0.0224	0.0215*

* Significant.

daily maximum (11.7 °C cf. 13.1 °C) and minimum (8.8 °C cf. 9.5 °C) temperatures as well as the highest percentage of days under 10 °C (50 % cf. 42 %). Data for October through January 1990/1991 [18] show a similar rank order of temperature conditions in the tributaries, with the Water of Mark being the coldest and the Cruick Water being the warmest. Temperature and *MEP-2*100* rankings were positively correlated for the tributaries and a significant linear change in genotype proportions is detectable across the temperature ranked tributary frequencies (X^2 = 6.54, df = 1, P = 0.02).

4. DISCUSSION

Significant associations of size with *MEP-2** variation in juvenile Atlantic salmon were detected in two independent tributaries of the North Esk River. Combined with the previous report of associations in juveniles in the neighbouring River Dee [8], this suggests that a widespread relationship exists between these two variables in wild populations. However, the nature of the relationship is likely to involve a complex genotype–environment interaction with variable outcomes depending on the specific rearing circumstances. Just as the relationships observed in the North Esk differed between the two tributaries, *MEP-2* 100/100* juveniles in the Girnock tributary of the River Dee were significantly larger than other genotypes but the opposite was true among tank-reared mature parr from the same tributary population [8]. Other hatchery studies have shown fish with *MEP-2*125* to have higher specific growth rates and food conversion efficiencies under cold water conditions than *MEP-2*100/100* genotypes [13]. These growth associations may lie behind the observation that among salmon collected from the Rivers Tay and Dee in Scotland, *MEP-2** heterozygosity has been found to be associated with sea-age [9], which is influenced by maturation and growth performance. While this life-history association was not tested here, salmon spawning in the North Esk tributaries studied do show major differences in life history traits including sea-age at maturity (*table I*).

*MEP-2** variation has also been linked to environmental temperature [21, 22] with an association of higher frequencies of *MEP-2*100* with warmer conditions. While in the present study the highest *MEP-2*125* frequency was not associated with the lowest mean summer temperature, it was found to be associated with the 'harshest' thermal regime (colder in winter, greater number of colder days in summer and colder absolute temperatures). This is broadly consistent with previous findings [21, 22]. This is also true of the observation that *MEP-2** divergence among North Esk tributaries was over seven times higher than for the other allozyme loci; the pattern of genetic variance (F_{ST}) among locations for the *MEP-2** locus across the species range has generally been observed to be anomalous compared to other loci [10]. This suggests that differentiation at the locus is affected by different forces than are other loci. If so, the most likely cause is selection.

The associations of *MEP-2** variation with temperature, size at age and other life history traits are all potentially linked, and selection acting directly on the *MEP-2** locus itself is plausible. Malic enzyme, coded for by *MEP-2**, is involved in pyruvate formation in the citric acid cycle [11], carbon flux in the Krebs cycle [12], fatty acid oxidation [15] and glycogen synthesis [7]. Furthermore, Godolphin [6] found that mitochondria from heart muscle of *MEP-2*125/125* homozygotes had significantly higher rates of malate oxidation than *MEP-2*100/100* homozygotes at lower temperatures, which would result in a greater amount of energy being available for somatic growth under these conditions [13]. However, more experimental studies are required to establish how *MEP-2** genotype interacts with temperature to affect juvenile growth.

The present study shows that the association of *MEP-2** with growth in juvenile Atlantic salmon is likely to be widespread. Consistently among allozyme polymorphisms in the species, only *MEP-2** shows associations with either temperature or growth-related variables, and only spatial patterns of *MEP-2** differentiation are anomalous. This is unlikely unless the *MEP-2** polymorphism, or one closely linked to it, is adaptively relevant and subject to selection. Thus, the findings presented provide further support for the widespread existence of locally adapted Atlantic salmon populations within river systems [3, 5, 19–21].

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