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Environmental induced methylation changes associated with seawater adaptation in brown trout

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ABSTRACT

Migratory trout (sea trout) and sedentary trout (freshwater trout) coexist and interbreed. However, the mechanisms underlying the development of the migrant morphotype are still unclear. At one point parr–smolt transformation is initiated and, as a result, juvenile trout are ready to leave the river and migrate to the sea. The smoltification process has been linked to various factors such as body size, growth rate and the physiological state of the fish. In addition, the process is also strongly influenced by environmental factors such as year, seasonality, water temperature and flow rate. For example, hatchery environment can depress the natural parr–smolt transformation and consequently, the success of the seawater migration of reared trout from these hatchery programmes might be adversely affected. We have investigated whether changes in DNA methylation, by means of MSAP (methylation-sensitive amplified polymorphism), could be involved in anadromy. We identified dramatic differences in genome-wide methylation patterns between hatchery reared and seawater brown trout. Furthermore, we demonstrated that salt enriched diets can trigger short-term genome-wide methylation changes in hatchery reared trout. However, these changes only lasted for a short period of time. Determining the duration of this effect could result in increased survival of hatchery-reared trout in seawater when fed on salt-enriched diets. Altogether, these results suggest that salt-induced alterations in DNA methylation patterns could play an important role in enabling fish acclimation to seawater conditions, potentially with important economic consequences for fish farming.

1. Introduction

Two different morphotypes of brown trout (*Salmo trutta* L.) can be found in rivers from the European Atlantic coast and Baltic Sea: the migratory trout (sea trout) and the resident trout (freshwater trout). Although both life-history forms present morphological, demographic and ecological differences (Bagliniere et al., 2000), they coexist and interbreed (Ruzzante et al., 2001). Juveniles from both morphotypes are indistinguishable. To date, genetic differences between sea trout and sedentary brown trout inhabiting the same river have not been reported (Charles et al., 2005; Cross et al., 1992; Hindar et al., 1991; Pettersson et al., 2001). Brown trout has little importance in commercial fisheries but an extraordinary value for recreational fisheries and hereby for the tourist industry. Consequently, many hatcheries are now focusing on raising stocks from wild parent fish. Under hatchery conditions some stocks of brown trout undergo a domestication process and can be successfully reared in captivity. However, domestication can result in lower fitness when individuals are returned back to the wild. The contribution of hatchery reared trout to natural populations has been repeatedly shown to be rather small (reviewed by Hansen, 2002). Moreover, the artificial environment in which the anadromous brown trout has been reared can also depress the natural parr–smolt transformation and adversely affect the success of seawater migration and the survival of the fish in the long-term (Sundell et al., 1998). This could represent an important failure of these expensive practices, especially when breeding migratory sea trout populations represent the main goal of the enhancement programme. As an illustrative example, the cost of the Atlantic salmon breeding expenditure relative to the number of captured salmons in a Spanish river has been estimated to be 4000 Euros per captured individual (Horreo et al., 2012).

In natural conditions, the two trout morphotypes (i.e. migrant and resident) become well established during the second year of their lives thanks to the ability of some juveniles to undergo smoltification (Økland et al., 1993). This process involves important physiological and morphological adaptations to seawater, thus preparing the trout for the ocean life prior to its downstream migration. Changes in their external morphology include variations in body shape and coloration patterns (Björnsson et al., 2011), those referring to physiological features involve increased salinity tolerance, olfactory sensitivity and changes in its metabolic and growth rates, as well as alterations in haemoglobin and visual pigment concentrations (McCormick et al., 1998).

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What natural selection mediated mechanism does underline the migration decision? Trout smoltification has been linked to various interrelated threshold factors such as body size, growth rate and physiological conditions that could activate migratory pathways (Acolas et al., 2012; Jonsson and Jonsson, 1993). In addition, the smoltification process is also strongly influenced by environmental factors, such as year, season, water temperature and flow rate (Jensen et al., 2012). The smoltification process involves high individual costs but also high benefits in reproductive performance (Jonsson and Jonsson, 1997, 1998).

Increasing salinity tolerance requires differentiation and activation of the epithelial transport and the synthesis of new transport proteins (McCormick, 2001). Changes in Na^+/K^+-ATPase, $Na^+/K^+/2Cl^-$ co-transporter and in the distribution of the mitochondrial-rich cells (MRCs) have been recorded in the gills of the freshwater salmon (*Salmo salar*) when compared to the seawater morphotype (Hiroi and McCormick, 2007). This is the result of MRCs being distributed on the primary filaments and secondary lamellae of the freshwater salmon but completely absent in those of the seawater salmon (Hiroi and McCormick, 2007). In concordance with physiological changes, differences in gene expression of several genes such as transaldolase 1, constitutive heat-shock protein HSC70-1 and endozepine have also been described between migratory and sedentary brown trout belonging to different populations (Giger et al., 2008). Similar findings have also been reported in populations of Atlantic salmon (Seear et al., 2010).

Different experiments have demonstrated the genetic basis of some components of anadromy. In rainbow trout (Le Bras et al., 2011) and Arctic charr (Norman et al., 2011), several QTLs (quantitative trait loci) on three linkage groups have been associated with fish performance in the seawater environment. However, the seawater morphotype can be externally induced and various features of the seawater gill phenotype could be induced by feeding freshwater rainbow trout with a salt enriched diet (Perry et al., 2006). In addition, it is well known that hatchery conditions substantially reduce smoltification (Aarestrup et al., 2000) and seawater migration compared to natural conditions (Marco-Ríus et al., unpublished results). Thus, genetic and environmental factors underlie life-history strategies, which have led to the consideration of anadromy in brown trout as a threshold quantitative trait (Ferguson, 2007).

Epigenetics can help in understanding the smoltification process in the brown trout. Smoltification is a reversible process that can be stimulated by external conditions, for example, full-sib individuals might show different behaviours. Thus, we hypothesised that methylation might be part of the acclimation mechanisms of the brown trout to the seawater conditions, since the methylated state is usually associated with gene expression inactivation and conversely, gene activation is associated with demethylation (reviewed by Mazzio and Soliman, 2012).

Environmental stimuli are known to alter cytosine methylation throughout the genome at specific loci. For example, Navarro-Martín et al. (2011) have shown that methylation of the gonadal aromatase promoter is involved in temperature-dependent sex ratios in sea bass. In addition, emerging evidences strongly suggest that certain dietary bioactive food components can change gene expression via alterations in DNA methylation and histone modifications (reviewed by Tammen et al., in press). In humans, it has been suggested that the bioactive nutritional component (also called epigenetic diet) could be incorporated into our regular dietary regimen and therapeutically used for medical purposes (Hardy and Tollefsbol, 2011).

In this study, we investigated whether changes in DNA methylation might be involved in anadromy and whether the level of DNA methylation might change in response to osmotic stresses induced by environmental stimuli such as a salt enriched diet. Understanding external stimuli effects will allow for better fish management, and in turn, increasing or decreasing the smoltification rates.

To achieve this, we designed a hatchery experiment where one-year-old trout were fed a salt enriched diet for a period from 2 to 28 days. Since gill is one of the most sensitive tissues responding to the transition between freshwater and the marine environment, we employed the methylation-sensitive amplified polymorphism (MSAP) methodology that allows detecting the methylation state of a particular recognition sequence. Thus, any methylation changes in the gill tissues in response to different diets and/or salinity concentrations could be measured. Physiological changes promoted by a salt enriched diet were monitored in the gill tissue by inmunocytochemistry analysis using the Na^+/K^+-ATPase antibody. This was done at different critical phases of the experiment. In order to test the seawater proficiency of the hatchery trout fed with a salt enriched diet, these fish together with a control group were transferred to seawater and the survival rates compared.

The final goal of the study was to gain further knowledge about the time from the hatchery until the onset of the migration to the sea, and to try to reduce mortality at this critical period by rearing trout under the most optimal conditions.

2. Materials and methods

2.1. Experimental design

The experimental design included two stages. The first one aimed to explore the differences in methylation induced by a salt enriched diet during one month. One-year-old hatchery reared trout were used in this experiment. One hundred full-sibling individuals were bred in a circular fibreglass tank at the Carballedo hatchery facilities (Northwest Spain) and fed daily with commercial trout pellets. Two weeks later six specimens were separated representing the freshwater control group (FW-control). The remaining trout were fed using a diet supplemented with 11% NaCl as described in Perry et al. (2006). Thereafter, six individuals were sampled 2, 4, 6, 8, 10, 14, 21 and 28 days after the beginning of the treatment. Samples were labelled as follows: FW-2, FW-4 FW-6, FW-8, FW-10, FW-14, FW-21, and FW-28. No mortality was recorded during the whole experimental period. All fish involved in the experiment were euthanized using MS-222 (Sigma) and the gill tissue removed. Those individuals which were not euthanized during the experiment were returned to their normal diet and no mortalities were recorded after one week of daily checks. Gills were fixed in ethanol for DNA extraction. For immunocytochemistry, gills were fixed in 4% paraformaldehyde in phosphate buffer 0.1 M pH 7.4 (PB) for two days at 4 °C and then stored in PB.

The second part of the experiment intended to explore the differences in seawater survival in relation to the diet. Following the same procedures described above, 100 fish of the same stock and age were used. Fish were separated in two tanks (50 individuals per tank) and reared under the same environmental conditions. In one tank, trout were fed with commercial trout pellets whereas in the other they were fed using a diet supplemented with 11% NaCl as previously described. After 4 days, the adipose fin of those trout fed with commercial trout pellets was removed for identification purposes. Then, the two groups of trout were transported to the O Grove aquarium to be transferred to the same seawater environment. Fish were acclimated to salty water in two phases, starting from 0 ppt to 15 ppt during 24 h followed by a second increase from 15 ppt to 37 ppt by filling the tank with natural sea water. During 24 days, the tank was checked in the morning and mortality was recorded. Survival differences were calculated using a mixed effect linear model with a Poisson distribution (Zuur et al., 2009) in the *lme4* package (Pinheiro and Bates, 2009) implemented in R 2.15.1 (R Development Core Team, 2012) where treatment (fed with salt or not) was a fixed factor and time (days of survival) a random effect.

Forty individuals (20 of each group), reared in seawater for a period of 10 to 12 days, were analysed further using MSAP (group labelled as GROVE).