Mapping QTLs affecting Cortisol Response to Confinement Stress in Rainbow Trout

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Introduction

Animal welfare and health has become an increasing concern for both terrestrial livestock rearing and aquaculture. In fish, exposure to stress has been reported to have detrimental effects on production traits like growth, reproduction and disease resistance (Portz, D.E., Woodley, C.M., and Cech Jr, J.J. (2006)). Defining practices that reduce stress and implementing efficient selection for reduced stress responsiveness are two complementary approaches to improve welfare in domesticated fish.

Confinement is a common stressor in fish farming. It is also well-established as a reliable means of triggering a neuroendocrine stress response. In rainbow trout (*Oncorhynchus mykiss*) the plasma cortisol response to this stressor has been used to characterize the relative magnitude of the stress response (inter-individual variation in stress responsiveness) and provided the basis of the trait by which divergent selection for high- and low-responding lines of rainbow trout (Pottinger, T.G. and Carrick, T.R. (1999)) was accomplished. After 2 generations of selection, fish from the high-responding (HR) line exhibited a post-challenge blood cortisol level up to twice as high as the individuals from the low-responding (LR) line (Øverli, Ø., Winberg, S. and Pottinger, T.G. (2005)).

In this study, we use a QTL design to investigate the genetic architecture of variation in the blood cortisol response to an acute confinement stressor using F2 progeny from a cross between HR and LR grand-parents from the second generation of selection. The objective was to create the foundation for future selective breeding and for a better understanding of the physiological response associated with stress and its variation, and the identification of underlying genes.

Materials and methods

Experimental design. HR and LR grand-parental broodstock was maintained at the CEH experimental fish facility (Windermere, UK). F1 parents were produced by mating single individuals within the second generation of selected rainbow trout HR and LR lines, one from each line. The next generation, 5 individual F1 males and 5 F1 females (originating from different F1 crosses) were mated in order to produce 5 F2 full-sib families. When the fish were about 11 months old, 210 individuals per family were randomly sampled, individually tagged with passive integrated transponders (PIT; Trovan ID100A), fin clipped

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for further DNA extraction and distributed into holding tanks until the commencement of phenotyping.

Phenotyping. Phenotyping started when fish were about 15 months old. For each round of confinement stress challenge, 25 individuals were netted from a holding tank and transferred to five 50-liter confinement tanks, 5 fish per tank. After 1h confinement, the fish were anaesthetized (2-phenoxyethanol; 1:2000) and a sample of blood removed for cortisol analysis. Fish were then redistributed into new holding tanks. Due to the large number of fish to be tested, the confinement stressor process was repeated over several days. To avoid any modification of the response to confinement due to prior disturbance in holding tanks, each tank was revisited at 2-3 day intervals. A second round of confinement following the same procedure was carried out at one month interval.
Blood samples were immediately centrifuged and plasma collected and frozen. Cortisol levels were subsequently determined by RIA (Pottinger, T.G., and Carrick , T.R. (2001)).

Genotyping. A set of 198 microsatellites and 95 SNPs was used for genotyping. Microsatellites were chosen according to the level of polymorphism and the location on the genetic map previously published by Guyomard, R., Mauger, S., Tabet-Kanale, K. *et al.* (2006). SNPs were designed in a set of genes of interest for other purposes. They were genotyped by Genoscope using the SNPlex Genotyping System (Applied Biosystem). Genetic linkage maps were rebuilt for the families of the QTL design, using the Carthagene software (De Givry, S., Bouchez, M., Chabrier, P. *et al*. (2005)).

Statistical analyses and QTLs detection. QTLmap software was used for QTL detection (Filangi, O., Elsen, J.M., Gilbert, H. *et al*. (2010)). An interval mapping method described by Elsen, J.M., Mangin, B., Goffinet, B. *et al*. (1999) was applied for a set of non-related full-sib families design, making no assumption about allele numbers or allele frequencies at QTL within founder populations. The statistical test used to compare the hypotheses of the presence of one QTL (H1) *vs.* no QTL (H0) at one location was an approximate likelihood ratio (LR) (Le Roy, P., Elsen, J.M., Boichard, D. *et al*. (1998)). Significance thresholds were obtained from simulations under H0. For each round of challenge, individual cortisol levels were adjusted for fixed environmental effects, i.e. holding tank, day of challenge and sex, jointly to the QTL detection. Because of external reasons, fish were sacrificed several months only after the challenge, and sex of a number of challenged individuals could not be recorded. In the end, QTL analyses were performed with about 780 individuals (129 to 173 per family).

Results and discussion

Mean values of cortisol levels for each family and each round of challenge are shown in table 1. The second confinement resulted in higher cortisol levels than the first one (more than 40% relative increase). Family and sex effects were significant (mean cortisol concentration was lower in males). The correlation between individual cortisol levels after the two challenges was moderate to low, within family and for all individuals (Table 1).

Table 1: Mean values and standard deviations of plasma cortisol (ng. mL $^{-1}$) at the end of the first (CORT 1) and the second (CORT 2) round of confinement challenge in the 5 QTL families and Pearson coefficient of correlation between individual values of CORT 1 and CORT 2 (Pearson).

F2 family	X3	X4	X8	X14	X17	All
CORT 1	150 ± 42	110 ± 42	108 ± 39	134 ± 39	89 ± 30	118 ± 44
CORT 2	192 ± 48	152 ± 49	162 ± 51	166 ± 57	154 ± 52	166 ± 53
Pearson [α]	0.09 ns	0.23	0.26	0.34	0.29	0.32

[α] ns : non significant (P~0.20). Otherwise: significant at P<0.001.

Table 2: Location and significance of the QTLs detected for CORT 1 and CORT 2 after the genome scan.

Linkage group[α]	CORT 1[β]	CORT 2[β]
1		P<0.05
3	P< 0.10	P<0.10
6	**P<0.001**	
8	P<0.10	P<0.10
15	P<0.05	
20		P<0.10
21		P<0.05
22	P<0.01	
23	P<0.10	
27	P<0.05	
30	P<0.01	
31		P<0.05

[α] Nomenclature from Guyomard *et al.* (2006).

[β] Bold characters : significant at the genome-wide level. Otherwise, significant at the chromosome-wide level.

Overall, thirteen putative QTLs distributed on twelve linkage groups were identified for CORT 1 and CORT 2. Three significant (P<0.05) QTLs were identified for CORT 2, and two significant and three highly significant (P<0.01) QTLs were detected for CORT 1. None of the most significant QTLs explained both CORT 1 and CORT 2 variations. Nevertheless, the low to moderate correlation between CORT 1 and CORT 2 together with the fairly different mean cortisol levels at the end of the two challenges indicate that fish responded differently during the second challenge. The difference may come from the fact that fish were no longer 'naive' regarding confinement when they were exposed to the second stress or may have been introduced by an unaccounted-for environmental perturbation. Water temperature is known to modulate the stress response in fish, with higher cortisol levels occurring in response to the same stressor at higher temperatures (e.g. Sumpter, J.P.,

Pickering, A.D., and Pottinger, T.G. (1985); Pottinger, T. G., Yeomans, W. E. and Carrick, T. R. (1999)). In the present study mean water temperatures during the period of CORT 1 (6.8°C, range 6.05 –7.6) were lower than those during CORT 2 (10.4°C, range 8.5 – 13.6°C). However, while this difference in temperature may account for a shift in overall stress-induced plasma cortisol levels it does not offer an explanation for the low individual correlation between the two test periods, or the lack of agreement in attributing QTLs for the two tests. Thus, different mechanisms may have been triggered during the two rounds of challenges, resulting in the detection of different QTLs.

Conclusion

The findings clearly show that several QTLs explain the cortisol response to stress in domestic rainbow trout and agree with previous studies reporting genetic variation for this trait. They also emphasize that response to stress is a highly complex trait, and that plasma cortisol may reflect a range of underlying physiological mechanisms. Thus, further investigations are needed to optimize the use of stress-induced plasma cortisol concentration as a possible selection trait to improve resistance to the stressors the fish face in farming conditions.

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