Quillet, E.; Krieg, F.; Dechamp, N.; Hervet, C.; Bérard, A.; Le Roy , P.; Guyomard, R.; Prunet, P.; Pottinger, T.G. 2014. **Quantitative trait loci for magnitude of the plasma cortisol response to confinement in rainbow trout**. *Animal Genetics*, 45 (2). 223-234. 10.1111/age.12126

QTL for magnitude of the plasma cortisol response to confinement in rainbow trout

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Key words : QTL, cortisol, stress, growth, rainbow trout

ABSTRACT

Better understanding of the mechanisms underlying inter-individual variation in stress responses and their links with production traits is a key issue for sustainable animal breeding. In this study, we searched for QTL controlling the magnitude of the plasma cortisol stress response and compared them to body size traits in five F2 full-sib families issued from two rainbow trout lines divergently selected for high or low post- confinement plasma cortisol level. Approximately 1000 F2 individuals were individually tagged and exposed to two successive acute confinement challenges (one month interval). Post-stress plasma cortisol concentrations were determined for each fish. A medium density genome scan was carried out (268 markers, overall marker spacing less than 10cM). QTL detection was performed using QTLMap software, based on an interval mapping method (http://www.inra.fr/qtlmap). Overall, QTL of medium individual effects on cortisol responsiveness (<10% of phenotypic variance) were detected on nineteen chromosomes, strongly supporting the hypothesis that control of the trait is polygenic. While a core array of QTL controlled cortisol concentrations at both challenges, several QTL seemed challenge specific, suggesting that responses to the first and to a subsequent exposure to the confinement stressor are distinct traits sharing only part of their genetic control. Chromosomal location of the steroidogenic acute regulatory protein (*StAR*) makes it a good potential candidate gene for one of the QTL. Finally, comparison of body size traits QTL (weight, length and body confirmation) with cortisol-associated QTL did not support evidence for negative genetic relationships between the two types of traits.

INTRODUCTION

In fish as in terrestrial farm animals, repeated or chronic exposure to stressors has negative impact on both production traits and health and welfare traits (see reviews by Wendelaar Bonga 1997; Segner *et al*. 2012). Farmed fish are unavoidably exposed to many environmental perturbations, such as changes in water quality or handling and manipulation. A better understanding of stress responses, including regulatory mechanisms at the individual level and the links with major production traits, is thus a key issue for animal breeding. Glucocorticoid hormones (cortisol in most mammals and fish, corticosterone in rodents and birds) are released into the bloodstream when animals are exposed to stressful stimuli. In fish, cortisol production is mediated by the activation of the hypothalamo-pituitary-interrenal (HPI) axis. Cortisol is considered as the cornerstone of the primary (neuroendocrine) stress response, and cortisolemia following exposure to a stressor is commonly used as a tractable indicator of the magnitude and thus severity of the stress response.

Furthermore, cortisol directly affects numerous behavioural traits and physiological processes associated with production and robustness phenotypes. Cortisol is well known for its negative effect on growth physiology. In fish, cortisol inhibits energy consumption, decreases condition factor and feed efficiency, though the effect may depend on age and/or rearing conditions (Pickering 1990; Wendelaar Bonga 1997; Fevolden *et al*. 2002; Pottinger 2006 ; Øverli *et al*. 2006, in rainbow trout, *Oncorhynchus mykiss*; Hori *et al*. 2012, in Atlantic cod *Gadus morhua*; Martins *et al*. 2011, in Nile tilapia, O*reochromis niloticus*). However, paradoxically, some genetic studies in rainbow trout have shown a positive correlation between cortisol responsiveness to acute stress and growth performance (Lankford & Weber 2006; Weber & Silverstein 2007). High cortisol-responsiveness is also associated with a greater susceptibility to a range of common aquacultural stressors like hypoxic conditions (Hoglund *et al*. 2008; Laursen *et al*. 2011) or long duration transportation (Ruiz-Gomez *et al*.

2008). Cortisol is implicated in the immunosuppressive effects of stress, though inconsistent results have been reported according to species and diseases (Fevolden *et al*. 1992, 1993a, 1933b, 1994; Refstie 1982; Kittilsen *et al*. 2009; Weber *et al*. 2008). Morphological and molecular indicators of heart pathology in rainbow trout and zebrafish have also been associated with high levels of cortisol (Johansen *et al*. 2011a; Nesan & Vijayan 2012).

There is strong evidence that the magnitude of the cortisol response to stressors is under genetic control (Mormède *et al*. 2011). In fish, moderate to high heritability estimates for the cortisol response to confinement were recorded in rainbow trout (Fevolden *et al*. 1999; Pottinger & Carrick, 1999; Weber *et al*. 2008; Vallejo *et al*. 2009), brook charr (Crespel *et al.* 2011), Atlantic cod (Kettunen *et al.* 2007) and carp (Tanck *et al*. 2001). The existence of one or more major genes governing the plasma cortisol response to a crowding stressor was suspected using segregation analyses in a domestic population of rainbow trout (Vallejo *et al*. 2009). Finally, significant Quantitative Trait Loci (QTL) for post-stressor cortisol responsiveness were found in the rainbow trout genome (Drew *et al*. 2007; Rexroad *et al*. 2012, 2013) and suggestive ones described in sea bass (Massault *et al*. 2009) and sea bream (Boulton *et al*. 2011).

QTL discovery constitutes a step toward the molecular dissection and deeper biological understanding of complex phenotypes. It may also help with implementing Marker Assisted Selection (MAS) which is particularly relevant where seeking to enhance selection efficiency for traits that are difficult to assess in practice. The detection of QTL associated to stress response could therefore facilitate the introduction of adaptation and robustness traits in breeding programmes. In this study, we searched for QTL controlling cortisol responsiveness in rainbow trout, using a F2-family design issued from two lines divergently selected for high or low post-confinement plasma cortisol level. Confinement is a reliable non-invasive means of triggering a neuroendocrine stress response in fish and is also analogous to stressors

commonly encountered in aquaculture. Analyses were carried out screening data from two successive exposures to the same standardized confinement. Results were compared to those of previous studies in rainbow trout that have investigated the core characteristics of cortisol responsiveness in trout. QTL for body size and conformation were mapped in the same F2 families in order to provide further insight into the possible links between stress response and production traits.

MATERIAL AND METHODS

Experimental design

Experimental design and QTL families were the same as in Le Bras *et al.* (2011). Briefly, F0 grand-parents belonged to two lines of rainbow trout divergently selected for their blood cortisol response to an acute confinement stressor. 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 (Pottinger & Carrick, 1999, Øverli *et al*., 2005), confirming the existence of a substantial genetic control of the trait. F1 parents were produced by crossing F0 individuals of selected HR and LR lines. The next generation, five F1 males and five F1 females were single pair mated to produce five F2 full-sib families. Fish from F0, F1 and F2 generations were all reared at the CEH (Centre for Ecology & Hydrology) experimental fish facilities (Windermere, UK). The experimental work was carried out under the UK Animals (Scientific Procedures) Act 1986, Project Licence no. 40/2600.

In the first rearing period, each family was kept in one or two holding tanks according to the family size. When fish were about 11 months old, 215 individuals per family were randomly sampled, tagged with passive integrated transponders (PIT; Trovan ID100A) and

fin clipped for further DNA extraction. Individual body mass (BM_1, g) and fork length (L_1, mm) were recorded. After measurement, fish were redistributed into ten holding tanks (1000 litres, circular GPR, constant flow of lake water 20 litres/min), with each family held in two tanks (107 and 108 fish/tank). During the whole period of survey, the fish were fed as normal (approx 2% body mass, 3 days per week, Skretting Standard Expanded 40) until the commencement of phenotyping.

Confinement challenge and blood collection

The confinement stressor was basically the same as that applied during the selection of the grand parental HR and LR lines.The first round of confinement stress challenges took place when fish were about 15 months old. For every holding tank, twenty-five fish were netted on day 1 and transferred to five 50-liter confinement tanks, five fish per tank. Each confinement tank was covered with a lid and supplied with a constant flow of lake water (15 L/min). After 1h of confinement, each batch of five fish was anaesthetized (2-phenoxyethanol, 1:2000). Fish were identified by reading the PIT tag (Trovan GR-250 RFID Reader) and body mass (BM_2, g) and length (L_2) were recorded. Blood samples (0.2 ml) were collected from the Cuverian sinus into a syringe containing EDTA (0.4 mg) as anticoagulant. Each batch was subsequently placed in a new holding tank with each family ultimately split into four holding tanks (50 fish per tank). Due to the large number of fish to be tested, the confinement stressor process was conducted over several days. To avoid any modification of the response to confinement due to prior disturbance in holding tanks, each holding tank was sampled only once each day (a single netting of 25 fish) and was revisited at 2-3 day intervals. The complete process for the ten holding tanks required fifteen days. The second round of confinement stress challenges was carried out one month later. The procedure followed was exactly as for the first round with the exception that body mass and length were not recorded,

and the fish were held now in four tanks of 50 fish per family. When fish were sacrificed a few months later, sex was recorded for the remaining individuals (macroscopic examination of the gonads).

Assays for cortisol analysis

Blood samples were immediately placed on ice and each batch of 25 samples was then centrifuged at 4°C and the plasma transferred to two tubes that were immediately frozen at -80°C before transfer to – 20°C until required for cortisol measurements.

Individual plasma cortisol concentrations ($ng.mL^{-1}$) were determined according to the radioimmunoassay procedure described in Pottinger and Carrick (2001). For every fish, cortisol plasma concentrations after the two rounds of confinement were referred to as Cort1 and Cort2 respectively.

Genotyping and linkage analysis

The genome scan was performed by genotyping grand-parents, F1 parents and F2 progeny with 268 markers (184 microsatellite markers and 84 SNP or indel, details in Supplementary material Table S1). There were between 206 to 222 informative markers per family. The overall mean polymorphism information content (PIC) was 0.45, ranging from 0.33 to 0.56 per linkage group (Table S1). The genetic consensus linkage map was rebuilt for the QTL families with CathaGène software (de Givry *et al*., 2005; http://www.inra.fr/mia/T/CartaGene/). Map total length was 2592 cM, with a mean overall spacing for genome coverage less than 10 cM. Linkage groups were named according to Danzmann *et al.* (2008) with RT04 and RT25 artificially merged to form a metacentric linkage group as described in Guyomard *et al*. (2012). Correspondence with physical

chromosomes (Phillips *et al.* 2006) is indicated. In the rebuilt linkage map, markers from linkage group RT2 remained split into two independent sub-groups (named RT2a and RT2b).

Statistical analyses and QTL detection

Prior to QTL mapping analyses, traits were checked for normality. All were normally or approximately normally distributed. Trait values were adjusted for fixed effects and covariables using the SAS GLM procedure. Plasma cortisol concentrations (Cor1, Cor2) were adjusted for sex, date of confinement and holding tank as fixed effects and for body mass (BM_2) and fork length (L_2) as covariables. Due to the short time span between the two confinement challenges, and in order to minimise the manipulations of fish, size traits measured at the first confinement test were used as covariates for both challenges. Relative growth of the lines was inferred at 11 months old using body mass (BM_1) and fork length (L_1). Size data at the time of challenge were not used in order to avoid any environmental differences induced by the stress confinement protocol among fish of the same family. To analyse body conformation at 11 months old, we searched for an indicator independent of absolute body size. The Fulton coefficient of condition did not meet this condition (correlated to body mass and to length, data not shown). The conformation index (Cf) measured as the residual of the linear regression of $\log_{10}$ transformed body mass on $\log_{10}$ transformed fork length was preferred, though it was still slightly correlated to body mass (Table 2). Prior to QTL analysis raw data (BM_1, L_1 and Cf_1) were adjusted for sex and for rearing tank as fixed effects.

QTL detection was performed with QTLMap software (Filangi *et al*. 2010). An interval mapping method described by Elsen *et al*. (1999) was applied considering a set of non-related full-sib families and making no assumption about allele numbers or allele

frequencies at QTL within the two grand-parental lines. For every cM along a linkage group, the hypothesis of the presence of one QTL (H1) *vs* no QTL (H0) was tested with an approximate likelihood ratio test (LRT, Le Roy *et al*., 1998). Significance thresholds for H0 rejection were estimated according to Harrel & Davis (1982) from the empirical distribution of LRT obtained by simulation from under the null hypothesis, with a trait heritability fixed to 0.5. At the chromosome-wide level, a QTL was considered as significant for P-value < 0.05 (1000 simulations). Significance at the genome-wide level (P<0.05) was tested using the Bonferroni correction (Knott *et al*. 1998) using 10,000 simulations. The 95%-confidence intervals of QTL positions were calculated according to the method by Li H-G (2011) which is based on a distribution of QTL position approximated from likelihood. Under H1, QTL effects were estimated for each sire and dam as the allelic substitution effects and were tested using a Student's t-test to determine the status of each parent (heterozygous *vs* homozygous at QTL, P<0.05). The origin of alternative alleles (HR or LR) was determined from the pedigree.

Univariate (trait by trait) analyses were first carried out. Multitrait (two traits) analyses were performed in a second step, applying a multivariate model with a multinomial penetrance distribution (Gilbert & Le Roy, 2003).

RESULTS

Mean performances and correlations

Summary statistics of recorded traits are summarized in Table 1. The plasma cortisol response was higher overall at the second confinement challenge than at the first one (+40% mean increase, P<0.001) though families responded differently (+28 to +73% increase, family-challenge interaction significant at P<0.001 in two-way ANOVA). The Pearson coefficients of phenotypic correlation (SAS CORR procedure) among the different traits are

shown in Table 2. Correlation between individual plasma cortisol concentrations at the two successive confinement exposures was moderate (R=0.34, P<0.0001). A negative correlation between post-stressor plasma cortisol concentrations and conformation index at time of challenge (Cf_2) was detected, especially at the first challenge.

QTL associated to plasma cortisol concentrations after confinement stress

Results of QTL detection are summarized in Tables 3 and S3 and illustrated in Figure S1. For Cor1, unitrait analyses detected eight significant QTL (RT03, RT06, RT08, RT10, RT22, RT23, RT27 and RT30). For Cor2, five significant QTL (RT01, RT05, RT21, RT30 and RT31) were identified. One linkage group only (RT30) was shared between Cor1 and Cor2, QTL locating at overlapping positions. Further testing the two-QTL hypothesis *vs* the one-QTL hypothesis (Gilbert & Le Roy, 2007) on this linkage group did not support the two-QTL hypothesis for any of the traits. Average effects of individual QTL explained up to 8.3% of phenotypic variance (up to 13% in some F1 progenies). An increasing effect of the HR alleles at QTL was the general rule (Table 3), with the exception of RT01 and RT06.

Multitrait analyses (Cor1-Cor2) confirmed the existence and approximate position of five out of the twelve QTL detected by unitrait analyses, namely QTL on RT03, RT06, RT08, RT30 and RT31. They also supported the existence of the QTL detected on RT21 and RT22, with likelihood ratios just below the suggestive threshold (P~0.05, data not shown). Additionally, two-traits analyses detected a novel suggestive QTL on RT02a, leading to a total of thirteen significant cortisol responsiveness QTL. Some of those QTL consistently affected plasma cortisol values across challenges while others seemed challenge-specific.

QTL associated to body size and body conformation

Unitrait analyses detected fifteen significant QTL for size on fourteen linkage groups, among which six influenced body mass, and nine influenced body length (Table 4). Five QTL were length specific, two were body mass specific and four affected both BM_1 and L_1 (RT02a, RT06, RT25, RT30) with very close positions for the two traits except on RT06 where, despite overlapping confidence intervals, distinct QTL positions suggested that several QTL may locate on the linkage group.

Two-traits analyses with length and body weight confirmed existence and position of QTL for body size on RT02b, RT06, RT12, RT21 and RT30. They also supported the suggestive QTL on RT19 and RT25 (likelihood ratios were just below the 5% significance threshold). On RT06, the two-traits analysis confirmed the QTL for length previously identified at 87 cM. The differing positions of QTL for BM_1 after unitrait and multitrait analyses suggested the existence of several QTL for body size on this linkage group. Further testing of multi-QTL hypotheses (2 or 3 QTL) indicated that RT06 likely harbours up to three size-QTL (data not shown). Multitrait analyses also revealed four novel QTL significantly affecting both BM_1 and L_1 (on RT08, RT11, RT26 and RT31). Taking together the fifteen QTL detected for body size, LR alleles tended to have a positive effect on body mass or length (about two out of three cases of significant allele substitution effects at QTL).

Results of QTL detection for body conformation are summarized in Table 5. Twelve significant QTL were found by unitrait analyses, with no obvious directional effect of HR *versus* LR alleles. Many of those QTL were found at similar locations to the body size QTL, suggesting pleiotropic effects of the QTL. Two-traits analyses further supported the hypothesis on many linkage groups (details in supplementary material Table S2). Finally, three QTL only (RT11, RT18, RT20) were found to be specific of body conformation. At QTL that influenced both body size and conformation, there was no evidence for common directional effects (increasing or reducing) of QTL alleles on the two traits (Table S2).

Comparison of QTL associated to body traits and plasma cortisol responsiveness

Seven linkage groups were identified after unitrait analyses for QTL detection for size traits (BW_1 and/or L_1) and for QTL detection for plasma cortisol concentrations (RT02a, RT03, RT06, RT08, RT21, RT30 and RT31). In order to further investigate whether the same QTL may govern the two types of traits, we performed two-traits analyses combining size traits (L_1 or BW_1) and cortisol traits (Cor1 or Cor2 respectively). Results are detailed in Table S3. They confirmed the location of several QTL initially detected in cortisol analyses (on RT01, RT02a, RT03, RT06, RT08, RT21, RT22 and RT30). Presence of cortisol-QTL on RT31 was also confirmed, though locations depended on the analysis. Most of the linkage groups initially identified in size analyses were also confirmed. Two-traits analysis identified a new cortisol-QTL on RT07, and suggested the existence of two cortisol -QTL with differing positions on RT03. However, the test of the two-QTL hypothesis *vs* the one-QTL hypothesis was not significant. Altogether, those results strongly support the hypothesis that a number of QTL control both juvenile size and stress-induced plasma cortisol in our families. However, according to the distribution of allelic effects (Table S3), there was no clear evidence of overall directional effect of those common QTL on the two types of traits. Finally, comparison of the different analyses revealed several QTL that seemed to be size specific (RT11, RT14, RT19, RT25) or cortisol specific (RT05, RT10, RT22, RT23, RT27).

DISCUSSION

Fish are subject to a broad variety of stressors in aquaculture production environments including crowding, handling and fluctuations in water quality. Deciphering the genetic architecture of an animal's response to stressors is an important factor in implementing

sustainable management of aquaculture broodstocks. Cortisol is the cornerstone of the non-specific endocrine response to acute stressors of this nature. In this study, using acute confinement as a model stressor and a moderate density genome scan, we identified ten significant or highly significant and ten suggestive QTL contributing to individual variability in post-stressor plasma cortisol concentration (Summary in Table S4). Altogether, individual QTL explained no more than 10% of phenotypic variance. A number of QTL with moderate effects were also detected in other studies (Rexroad *et al*., 2012, 2013) suggesting a multigenic control of the trait. However, some QTL explaining a large proportion of the phenotypic variance were also identified in those studies and in Drew *et al*. (2001) which is in line with the conclusion by Vallejo *et al*. (2009) that a few major genes control the cortisol response in some populations. Several factors, including differences among populations, differences in the experimental stressor or in QTL design and analytical methods may have contributed to the differing results among studies. Altogether, those results support the hypothesis of a complex genetic control of cortisol response.

Chromosomal locations of the QTL detected in the present study were compared to those of QTL detected after testing cortisol response to similar stressors in other populations of rainbow trout (Drew *et al*., 2007; Rexrorad *et al*., 2012; 2013). Because of differences in linkage maps among studies and moderate precision of QTL positions, comparison was performed at the level of the chromosome. Our results support several of the previously identified QTL and also detected novel ones (Table S4). Overall, QTL were detected on many different chromosomes, which reinforces the hypothesis that the trait is under a complex genetic control. Notably, several QTL were shared among populations, and should be the focus of further studies aiming to dissect more precisely the genes involved in the regulation of this trait. However, many other QTL were population specific. This may be due to differences among the experimental designs as previously suggested, but may also correspond

to differences in the genetic polymorphisms determining the control of cortisolemia in different populations.

The comparison of results for the first and second challenges in the present study highlighted the complexity of cortisol responsiveness and of its significance. As commonly observed in similar tests, the phenotypic correlation (R=0.34) between cortisol responses to the first and second challenge was moderate. For instance, it ranged from 0.18 to 0.48 after submitting rainbow trout to four successive episodes of crowding stress (Rexroad *et al*. 2012) and it was 0.18 after two low water confinement stressors in Atlantic cod (Kettunen 2008). Nevertheless, in both studies, the estimated genetic correlations between challenges were high (0.87±0.5 in Atlantic cod, >0.84 in trout between responses to the second exposure to stressor and the subsequent ones), indicating that the successive traits do share common genetic bases. The exception was the response to the first challenge in trout experiment by Rexroad and co-authors that appeared genetically distinct from responses to subsequent exposures (lower heritability and genetic correlations). Our results, identifying a core array of QTL consistently affecting cortisol across the first and second challenge together with challenge specific QTL are similar. One cannot exclude the possibility that the limited power of the design prevents consistent detection of QTL across the two successive challenges in our experiment. Differences in attributing QTL for the two tests may also have been induced by an unaccounted-for environmental perturbation, such as changes in mean water temperatures, that were higher during the second challenge (10.4°C, range 8.5 – 13.6°C) than in the first one (6.8°C, range 6.05 –7.6). Temperature is known to modulate the stress response in fish, with higher cortisol levels occurring in response to the same stressor at higher temperatures (Sumpter *et al*.1985; Pottinger *et al.* 1999). However, the consistency of our observations and those by Rexroad and co-authors suggests that, at least in rainbow trout, responses to the first and to subsequent exposures to stressor are distinct traits sharing only part of their genetic

control. In this perspective, it is noteworthy that two of the Cor1-specific QTL we detected (RT23/Omy8 and RT27/Omy2) were also identified by Drew *et al*. (2007) after a single exposure to stressor. No QTL was found on those linkage groups in the study by Rexroad *et al*. (2012) using cortisol values at the second exposure and successive ones only, and a suggestive one was found on Omy8 in related families taking values of the first exposure together with those of the three subsequent exposures (Rexroad *et al*., 2013. Investigating those QTL in a separate analysis of response to the first exposure in those two studies would be interesting.

The response of each individual to a stressor depends on genetic factors and on individual life history. In wild animals like birds or amphibians, differences in glucocorticoid responses are commonly observed between the first capture and the subsequent occasions (Cockrem *et al*. 2009; Narayan *et al.* 2012), suggesting that the appraisal of the stimulus contributes to the variability of the response. Fish possess sophisticated cognitive capabilities, including memory and learning (see Ebbesson and Braithwaite, 2012) and this, together with the relatively short interval between successive confinement episodes, may account for the differing responses to the second challenge in the present study. Such habituation to repeated acute stressors has previously been observed in salmon (Schreck *et al*. 1995). Furthermore, the appraisal of the subsequent exposures likely depends on individual genotype, as suggested by the significant interaction observed at the family level between cortisol response after the first and the second challenges. For instance, it is reasonable to suggest that differences between high *versus* low responsive individuals for traits like time to resumption of feeding after an environmental change (Øverli *et al.* 2005), learning flexibility (Ruiz-Gomez *et al.* 2011) or memory retention (Moreira *et al.* 2004) influence the way individuals will appraise the subsequent exposure to a repeated stressor. In this context, the results observed in the present study, a cortisol response to a first acute stressor which appears to be controlled

differently than the response to a second challenge suggest some possible specific neuroendocrine mechanisms which still need to be clarified. Obviously, there is a need for further understanding of the origin and plasticity of the individual cortisol response to repeated exposure to acute stressor and its significance.

The hypothalamic-pituitary-interrenal (HPI) axis is a pivotal element in the initiation and regulation of the neuroendocrine response to stressors in fish. Hypothalamic neurohormones (vasopressin and corticotrophin-releasing hormone, *CRH*) control the release of adrenocorticotrophin hormone (*ACTH*) by the anterior pituitary gland. In its turn, *ACTH* stimulates the biosynthesis of cortisol within the interrenal and its release into the circulation. Further steps determine the ultimate effects of cortisol on its targets, including the activity of converting and binding enzymes, the presence and affinity of receptors and post-receptor mechanisms (Mormède *et al*. 2012; Johansen *et al.* 2011b; Kiilerich & Prunet 2011). In order to assess whether the QTL we detected could harbour relevant candidate genes involved in the up-stream regulation of cortisol, we checked for annotation of Sigenae EST contigs (SIGENAE [http://www.sigenae.org/]) associated with markers used for the genome scan or that were mapped close to the QTL positions on the INRA reference linkage map (Guyomard *et al.* 2012). Interestingly, one potentially significant candidate gene was identified, the *StAR* protein that locates in the centromeric region of RT10/Omy6 (at marker *OmyS00583INRA* between *OMM5013* and *OMM1294* that flank the suggestive cortisol-QTL identified on this chromosome). The *StAR* protein (steroidogenic acute regulatory protein) mediates a key rate-limiting step of cortisol synthesis, by transporting cholesterol, the precursor of cortisol, between the outer and inner mitochondrial membrane before it can be further converted. Expression of the gene encoding for that protein has been shown to be highly correlated to plasma cortisol levels after acute stress (Geslin & Auperin 2004). Hence, the *StAR* protein

appears as a relevant functional and positional candidate mediator of variability of post-stressor plasma cortisol concentrations in our families.

The *SGK1* gene (serine/threonine-protein kinase Sgk1, alternative name serum/glucocorticoid-regulated kinase 1, UniProt) was also found at the QTL position on RT03/Omy14 (marker *OmyS00560INRA*). *SGK1* is under the regulation of glucocorticoid and mineralocorticoid hormones and the protein is ubiquitously expressed in all tissues in mammals. *SGK1* is a potent regulator of metabolism, transport, transcription and enzyme activity and thus participates in the regulation of diverse functions such as epithelial transport, excitability, cell proliferation and apoptosis (Lang *et al*. 2006). In fish, *SGK1* is implicated in adaptation to seawater (Notch *et al*. 2011), a process in which cortisol also plays a role. Interestingly, in a recent study aiming at analyzing genetic variations that influence glucocorticoid-mediated regulation of transcription and protein secretion, cis-regulatory polymorphisms upstream of the *SGK1* gene were suggested to play an important role (Luca *et al*. 2009; Maranville *et al.* 2011). Hence, the hypothesis that clusters including genes influencing the regulation of plasma cortisol levels and regulation factors of the downstream effects of cortisol would deserve further studies.

Detrimental effects of exposure to stress on production traits like growth, reproduction and disease resistance have been reported (Portz *et al*. 2006) and possible trade-offs between the response to stressors and production traits is an issue in implementing breeding strategies in domestic fish broodstock. Moreover, cortisol has been shown to inhibit somatic growth by stimulating energy consumption, gluconeogenesis and lipolysis (Wendelaar Bonga 1997). The joint analysis of cortisol responsiveness and production traits QTL aimed at improving knowledge on the genetic relationships between the two types of traits.

The detection of numerous QTL for size and body conformation in the present study is consistent with previous studies (Wringe *et al*. 2010). In rainbow trout, rotund body shape,

not a preferred character, has been associated with large body mass (Kause *et al.* 2003) which may be an issue for production purposes. However, the estimated genetic correlation was moderate suggesting that body shape partly relies on a distinct genetic control. The QTL detected in the present study underpin this picture of the genetic links between the two traits. These results open up new prospects for an efficient control of the undesired correlation between growth and body shape if necessary.

At the phenotypic level, we observed no adverse correlation between cortisol responsiveness and juvenile size ($R=0.07$, with limit significance at $P<0.05$), whilst low cortisol responsiveness was associated to a higher conformation index (more rotund fish), especially at the first challenge. At the genetic level, the detection of numerous QTL for size and body conformation in the present study is consistent with previous studies (Wringe *et al*. 2010). QTL with possible pleiotropic effects on both growth-associated traits and cortisol response were identified, but there were no consistent effects of QTL alleles on the two types of traits. Hence our results do not support evidence for negative genetic relationships between early growth traits and cortisol responsiveness. Similarly, Drew *et al.* (2001) observed a positive relationship between cortisol levels and growth on very young fish. However, in the present study, rearing operations and disturbance were as reduced as possible during the period of growth survey (no anaesthesia and handling). Thus, the relative sizes recorded here may not be representative of growth potential under more adverse conditions and further confirmation of the results in a range of rearing environments and larger sized fish is needed.

In summary, significant QTL for plasma cortisol responsiveness after a standardized confinement stress were found on eighteen different chromosomes in rainbow trout genome. The comparison of two successive exposures to confinement challenge underlined the complexity of the cortisol response to stressors in terms of individual life history. Further investigations are needed to fine-tune the traits to target to get a sensible assessment of fish

adaptation to farming situations. The identification of functionally relevant QTL will create a foundation for better understanding of the physiological and genetic control of the response to stressors in finfish. The present study allowed us to characterize several significant QTL regions, some of which offer particular promise, having already been observed in similar QTL analysis using a different experimental design (Drew *et al.* 2007; Rexroad *et al.* 2012; 2013). Finally, the steroidogenic acute regulatory protein (*StAR*), a mediator of a key rate-limiting step of cortisol biosynthesis was identified as a relevant candidate gene for one of the QTL. Hopefully, the ongoing development of rainbow trout markers and the generation of a reference genome assembly will help confirm this finding and facilitate further investigation of significant genes within the other QTL regions. Finally, these results did not support the hypothesis of major negative genetic links between growth traits (size) and cortisol responsiveness in the tested population. However, further confirmation of this result in a range of situations (such as rearing conditions, age, strains) is needed. Indeed, relationship between cortisol response and other economic traits will be one of the critical points to take into account in the design of future breeding objectives and management practices aiming at improving welfare and robustness together with production traits in aquaculture stocks.

Acknowledgement

Authors acknowledge the helpful contributions of H. Bovenhuis (Animal Breeding and Genetics Group, Wageningen University, The Netherlands) for advising about the QTL design; LABOGENA (http://www.labogena.fr/) and S. Mauger, K. Tabet-Aoul, A. Launay and L. Laffont (INRA) for genotyping; A. Neau and M. Boussaha (INRA) for help in data management; D. Abel for technical assistance at CEH (Windermere, UK); T. Wang (Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen,

UK) for supervising the sex records when fish were sacrificed in the Scottish Fish Immunology Research Centre facilites.

This study was funded by the European Commission (project AQUAFIRST, contract number FP6-STREP-2004-513692), the Natural Environment Research Council of the United Kingdom and INRA (Institut National de la Recherche Agronomique, France).

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TABLES

Table 1. Summary statistics of the traits measured in the five F2 crosses of the QTL design.

Traits	Family means					Joint mean	n
	X3	X4	X8	X14	X17		
BM_1	61± 16	55± 14	69± 22	52± 10	72± 17	62 ± 18	1005
L_1	17± 2	16± 2	17± 2	17± 1	18± 2	17 ± 2	1004
BM_2	131± 31	121± 33	134± 38	113± 20	130± 26	126 ± 31	983
L_2	23± 2	22± 2	22± 2	22± 1	22± 2	22 ± 2	970
Cor1	150 ± 42^a	110 ± 42^c	109 ± 39^c	134 ± 39^b	89 ± 30^d	118 ± 44	981
Cor2	192 ± 48^a	153 ± 49^b	162 ± 51^b	166 ± 57^b	154 ± 52^b	166 ± 53	928

BM_1, L_1: body mass (g) and fork length (mm) at 11 months old; BM_2, L_2 : body mass and fork length at the first confinement challenge (around 15 months old). Cort1, Cort2: plasma cortisol concentrations ($ng.mL^{-1}$) after the first and second confinement stress respectively; n : total number of observations. Values are means ± standard deviations; different letters indicate different values within each challenge ($P<0.05$).

Table 2. Pairwise coefficients of phenotypic correlations between the measured and calculated traits

		Cor1	Cor2	BM_1	L_1	Cf_1	BM_2	L_2	Cf2
Cor1	R	x	**0.34**	**0.07**	**0.07**	0.04	-0.02	0.05	**-0.19**
	P		*<0.0001*	*0.05*	*0.04*	*0.26*	*0.56*	*0.21*	*<0.0001*
Cor2	R		x	**0.07**	**0.07**	0.05	0.02	0.04	-0.06
	P			*0.05*	*0.05*	*0.18*	*0.68*	*0.23*	*0.09*
BM_1	R			x	**0.96**	**0.24**	**0.81**	**0.79**	**0.12**
	P				*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.001*
L_1	R				x	-0.01	**0.76**	**0.81**	-0.03
	P					*0.89*	*<0.0001*	*<0.0001*	*0.44*
CI_1	R					x	**0.23**	0.05	**0.56**
	P						*<0.0001*	0.23	*<0.0001*
BM_2	R						x	**0.85**	**0.38**
	P							*<0.0001*	*<0.0001*
L_2	R							x	-0.01
	P								*0.96*

Pearson's coefficients of correlation (R) and associated P-values (*P*); correlations are calculated between individual records corrected for fixed effects (722 to 820 pairs, according to traits). BM_1, L_1, Cf_1: body mass (g), fork length (mm) and conformation index at 11 months old; BM_2, L_2, Cf_2: body mass, fork length and conformation index at the first confinement challenge. Cort1, Cort2: plasma cortisol concentrations ($ng.mL^{-1}$) at the first and second confinement challenge respectively.

Table 3. Results of QTL analyses for plasma cortisol concentrations after a standardized confinement stress (two successive challenges)

LG	Chr	Analysis	LR	Position	CI (cM)	Cor1				Cor2			
						n_H		effect	range	n_H		effect	range
						HR	LR			HR	LR		
RT01	Sex	Cor2	25.2*	0	0-106	-	-	-	-	1	5	0.41	0.21-0.68
RT02a	Omy13	two-traits	37.3*	24	12-32	4	0	0.38	0.21-0.70	4	1	0.33	0.21-0.49
RT03	Omy14	Cor1	30.1*	100	91-107	3	2	0.38	0.24-0.72	-	-	-	-
		two-traits	40.9*	99	0-116	3	1	0.43	0.27-0.79	2	1	0.28	0.20-0.39
RT05	Omy22	Cor2	25.1*	78	57-78	-	-	-	-	5	4	0.34	0.23-0.43
RT06	Omy1	Cor1	44.1*, g	42	31-58	1	4	0.47	0.31-0.67	-	-	-	-
		two-traits	48.8*	39	32-48	1	6	0.40	0.20-0.72	1	3	0.29	0.23-0.39
RT08	Omy5	Cor1	26.8*	98	55-115	7	1	0.46	0.27-0.61	-	-	-	-
		two-traits	45.7*	101	85-115	7	1	0.48	0.25-0.74	5	1	0.40	0.21-0.78
RT10	Omy6	Cor1	22.2*	22	13-37	1	3	0.40	0.23-0.60	-	-	-	-
RT21	Omy9	Cor2	29.9*	28	0-39	-	-	-	-	2	2	0.45	0.25-0.61
RT22	Omy16	Cor1	25.9*	122	106-128	3	2	0.44	0.25-0.65	-	-	-	-
RT23	Omy8	Cor1	24.4*	17	0-53	3	3	0.44	0.22-0.60	-	-	-	-
RT27	Omy2	Cor1	25.6*	43	0-78	6	0	0.43	0.23-0.77	-	-	-	-
RT30	Omy23	Cor1	30.8*	15	0-23	5	0	0.40	0.21-0.56	-	-	-	-
		Cor2	21.2*	1	0-9	-	-	-	-	2	1	0.48	0.29-0.60
		two-traits	48.7*	0	0-8	4	0	0.55	0.27-0.77	2	1	0.46	0.29-0.57
RT31	Omy3	Cor2	28.5*	30	1-48	-	-	-	-	4	1	0.40	0.21-0.77
		two-traits	40.3*	29	2-39	2	1	0.31	0.22-0.47	3	2	0.42	0.22-0.77

Unitrait (Cor1 or Cor2 respectively) and two-traits analyses were performed for every linkage group; only those having detected one QTL are reported. LG: linkage group labelled according to Guyomard *et al.* (2012) with corresponding chromosome (Chr); LR: likelihood ratio; * = significant at the chromosome-wide level at P<0.05; ^{g}= significant at the genome-wide level at P<0.05; CI: 95% confidence interval of the QTL position; n_H: number of F1 parents segregating at the QTL (P<0.05) with HR-LR, the origin of the grand-parental allele with an increasing effect on the trait; effect is estimated as the average allele substitution effect for segregating F1 parents (in phenotypic standard deviation).

Table 4. Results of QTL analyses for growth (BM_1, body mass and L_1, fork length) at 11 months.

LG	Chr	Analysis	LR	Position	CI (cM)	BM_1				L_1			
						n_H		effect	range	n_H		effect	range
						HR	LR			HR	LR		
RT02a	Omy13	BM_1	23.1*	20	4-30	1	3	0.46	0.30-0.71	-	-	-	-
		L_1	25.4*	20	5-30	-	-	-	-	1	3	0.47	0.29-0.76
RT02b		L_1	27.5*	44	26-68	-	-	-	-	2	5	0.47	0.29-0.76
		two-traits	43.0*	44	28-68	1	3	0.46	0.22-0.96	2	5	0.40	0.21-1.05
RT03	Omy14	L_1	27.0*	117	28-117	-	-	-	-	5	1	0.37	0.24-0.62
RT06	Omy1	BM_1	26.7*	17	4-91	2	3	0.35	0.25-0.51	-	-	-	-
		L_1	25.2*	87	2-91	-	-	-	-	1	3	0.42	0.31-0.67
		two-traits	39.5*	90	2-91	2	3	0.37	0.24-0.68	2	3	0.38	0.27-0.67
RT08	Omy5	two-traits	50.0*	49	36-115	0	3	0.26	0.23-0.31	0	4	0.30	0.25-0.38
RT09	Omy12	L_1	23.8*	78	0-133	-	-	-	-	3	2	0.34	0.22-0.49
RT11	Omy27	two-traits	28.0*	2	0-17	0	2	0.33	0.24-0.43	0	2	0.31	0.30-0.32
RT12	Omy7	BM_1	25.0*	107	0-125	2	3	0.38	0.21-0.59	-	-	-	-
		two-traits	50.2*	110	80-123	2	3	0.36	0.20-0.55	3	2	0.34	0.21-0.51
RT14	Omy19	L_1	25.1*	17	0-110	-	-	-	-	2	4	0.44	0.28-0.59
RT19	Omy11	L_1	24.6*	82	0-99	-	-	-	-	2	3	0.35	0.26-0.43
RT21	Omy9	BM_1	24.9*	28	0-75	0	4	0.37	0.21-0.57	-	-	-	-
		two-traits	38.1*	65	3-76	0	3	0.41	0.24-0.58	0	3	0.34	0.25-0.45
RT25	Omy29	BM_1	20.6*	20	0-20	2	2	0.34	0.26-0.40	-	-	-	-
		L_1	21.5*	20	0-20	-	-	-	-	3	2	0.30	0.20-0.45
RT26	Omy24	two-traits	48.3*	31	5-39	1	4	0.36	0.21-0.45	0	4	0.41	0.32-0.48
RT30	Omy23	BM_1	36.5*, g	21	0-23	5	2	0.35	0.20-0.51	-	-	-	-
		L_1	32.4*, g	20	0-23	-	-	-	-	4	2	0.37	0.24-0.46
		two-traits	53.3*, g	6	0-22	4	2	0.40	0.33-0.60	4	2	0.39	0.27-0.54
RT31	Omy3	two-traits	51.2*, g	68	60-88	1	3	0.30	0.25-0.42	4	1	0.31	0.21-0.45

Unitrait (BM_1 or L_1 respectively) and two-traits analyses were performed for every linkage group; only those having detected QTL are reported. LG: linkage group according to Guyomard *et al*. (2012) with corresponding chromosome (Chr); LR: likelihood ratio; * =

significant at the chromosome-wide level at P<0.05; [g] = significant at the genome-wide level at P<0.05**;** CI: 95%-confidence interval of the QTL position; n_H: number of F1 parents segregating at the QTL (P<0.05), with HR/LR the origin of the grand-parental allele with an increasing effect on the trait; effect is estimated as the average allele substitution effect for segregating F1 parents (in phenotypic standard deviation).

Table 5. Results of QTL analyses for body conformation (Cf_1) at 11 months.

LG	Chr	LR	Position	CI (cM)	n_H		effect	range
					HR	LR		
RT05	Omy22	29.8*	78	18-78	0	4	0.49	0.20-1.00
RT08	Omy5	27.9*	46	27-59	4	1	0.40	0.23-0.54
RT11	Omy27	15.6*	2	0-28	1	2	0.39	0.32-0.48
RT12	Omy7	33.4*	97	62-121	1	5	0.40	0.21-0.53
RT13	Omy28	33.0*, [g]	58	21-79	3	1	0.88	0.39-2.23
RT17	Omy20	38.8*, [g]	0	0-9	7	1	0.33	0.20-0.60
RT18	Omy26	22.8*	32	0-36	1	3	0.34	0.20-0.62
RT20	Omy10	23.7*	69	0-120	3	5	0.45	0.22-0.87
RT21	Omy9	24.6*	67	0-90	1	6	0.31	0.20-0.54
RT26	Omy24	39.4*, [g]	3	0-34	3	1	0.59	0.39-0.86
RT30	Omy23	22.5*	16	0-21	4	3	0.28	0.20-0.47
RT31	Omy3	32.6*	68	59-96	3	2	0.43	0.22-0.67

LG: linkage group according to Guyomard *et al.* (2012) with corresponding chromosome; LR: likelihood ratio; * = significant at the chromosome-wide level at P<0.05; [g] = significant at the genome-wide level at P<0.05; CI: 95% confidence interval of the QTL position; n_H: number of F1 parents segregating at the QTL (P<0.05), with HR/LR the origin of the grand-parental allele with an increasing effect on the trait; effect is estimated as the average allele substitution effect for segregating F1 parents (in phenotypic standard deviation).

Supporting information

Additional information may be found in the online version of this article.

Table S1. List of markers used for the genome scan.

Table S2. QTL detection after multitrait analyses for growth and conformation traits.

Table S3. QTL detection after multitrait analyses for growth and cortisol traits.

Table S4. Summary of QTL detection for plasma cortisol in rainbow trout.

Figure S1. Results of unitrait (Cor1) and multitrait (Cor1-Cor2) detection of QTL for plasma cortisol concentrations on RT06 (Omy1) and RT08 (Omy5).

Representative plots of the likelihood ratios (LR) values (Y-axis) according to chromosome location (X-axis, cM). Thresholds for the null hypothesis rejection at relevant thresholds are shown.

Table S2. QTL detected after multitrait analyses for growth and conformation traits.

LG	Chr	LR	Position	CI (cM)	$n_{H\ Growth}$		$n_{H\ Conformation}$		$n_{H\ Both}$	
					HR	LR	HR	LR	Same allelic effect	Opposite allelic effect
RT02a	Omy13	34.0*	19	2-29	1	3	2	0	-	-
RT02b		39.0*	44	27-68	2	2	8	0	2	2
RT05	Omy22	37.8*	78	59-78	0	2	0	3	-	-
RT08	Omy5	41.6*	48	31-115	1	3	3	2	1	1
RT12	Omy7	56.4*, g	97	78-120	3	3	1	5	1	1
RT13	Omy28	39.7*	58	19-62	3	1	3	2	0	4
RT17	Omy20	43.1*	0	0-10	1	1	7	1	1	0
RT21	Omy9	42.1*	65	0-77	0	3	1	4	2	0
RT25	Omy29	41.4*	20	4-20	2	2	0	5	1	1
RT26	Omy24	54.5*, g	29	9-40	3	1	4	0	1	1
RT30	Omy23	52.6*, g	19	0-22	3	3	4	3	3	1
RT31	Omy3	53.4*, g	69	60-94	2	3	3	2	2	2

Two-trait analyses (BM_1-Cf_1) were performed separately for every linkage group; only those having detected QTL are reported. LG: linkage group according to Guyomard *et al*. (2012) with corresponding chromosome (Chr); LR: likelihood ratio; * = significant at the chromosome-wide level at P<0.05; ^{g} = significant at the genome-wide level at P<0.05**;** 95% CI: confidence interval of the QTL position (one LOD 'drop off' method); $n_{H\ Growth,}$ $n_{H\ Conformation,}$: number of F1 parents segregating at the QTL (P<0.05) for BW_1 and Cf1 respectively. HR-LR indicate the lineage origin of the grand-parental allele with an increasing effect on the trait. $n_{H\ Both}$: number of F1 parents segregating at the QTL for the two traits, according to the effect of each QTL allele on trait values (same effect: a given QTL allele increases or decreases both traits).

Table S3. QTL detected after multitrait analyses for growth and cortisol traits.

LG	Chr	Analysis	LR	Position	CI (cM)	$n_{H\ Growth}$		$n_{H\ Cortisol}$		$n_{H\ Both}$	
						HR	LR	HR	LR	Same allelic effect	Opposite allelic effect
RT01	Sex	L_1-Cor2	39.6*	0	0-11	5	3	3	3	4	0
RT02a	Omy13	L_1-Cor1	39.4*	22	10-32	1	3	3	0	0	0
		L_1-Cor2	36.7*	23	12-32	1	3	4	1	0	2
RT03	Omy14	L_1-Cor1	48.4*	100	92-112	3	2	5	1	0	2
		L_1-Cor2	43.8*	59	38-70	4	1	5	2	4	1
RT06	Omy1	L_1-Cor1	61.0*, g	46	31-59	2	2	2	4	1	1
RT07	Omy15	L_1-Cor2	37.9*	42	5-61	3	1	3	3	2	0
RT08	Omy5	L_1-Cor1	51.1 *	94	80-110	0	6	3	6	5	1
		L_1-Cor2	41.0*	95	81-113	0	4	4	0	0	0
RT09	Omy12	L_1-Cor2	42.8*	0	0-133	4	1	3	2	1	2
RT12	Omy7	L1-Cor1	37.2*	87	76-125	5	2	2	3	2	1
RT21	Omy9	L_1-Cor2	49.7*	28	4-39	0	4	2	2	0	1
RT26	Omy24	L_1-Cor1	35.9*	22	8-44	3	2	3	3	2	0
RT29	Omy17	L_1-Cor2	38.3*	0	0-9	1	3	5	2	1	2
RT30	Omy23	L_1-Cor1	59.6*, g	19	0-23	4	2	5	0	2	0
		L_1-Cor2	51.2*, g	1	0-9	4	2	2	1	1	0
RT31	Omy3	L_1-Cor1	35.3 *	77	0-122	2	3	1	3	1	0
		L_1-Cor2	39.5*	0	0-85	5	3	3	3	4	0

For every linkage group, multitrait analysis (L_1 joined with Cor1 or Cor2 and BM_1 joined with Cor1 or Cor2) were performed separately. L_1 or BM_1 used as growth traits provided very similar results. Hence, only joined analyses performed with L_1 as growth trait and having detected QTL are reported. LG: linkage group according to Guyomard *et al*. (2012) with corresponding chromosome (Chr); LR: likelihood ratio; * = significant at the chromosome-wide level at P<0.05; ^{g} = significant at the genome-wide level at P<0.05 ; CI: 95% confidence interval of the QTL position; $n_{H\ Growth,}$ $n_{H\ Cortisol,}$: number of F1 parents segregating at the QTL (P<0.05) for each type of traits (L_1 or BW_1 as growth traits, and Cor1 or Cor2 as cortisol traits according to the analysis). HR/LR indicate the lineage origin of the grand-parental allele with an increasing effect on the trait. $n_{H\ Both}$: number of F1 parents segregating at the QTL for the two traits, according to the effect of each QTL allele on trait values (same effect: a given QTL allele increases or decreases both traits).

Table S4. Comparison of chromosomal locations of QTL for post-stressor plasma cortisol detected in the rainbow trout genome in four different studies.

LG	Chr	S1	S2	S3	Present study			
					QTL (all)	Cor1	Cor2	Cor1-Cor2
RT01	Sex		**x**		**x**		*	
RT02	Omy13				**x**			*
RT03	Omy14		**x**		**x**			*
RT04-25	Omy25			**x**				
RT05	Omy22		**x**		**x**		*	
RT06	Omy1				**x**			*
RT07	Omy15				**x**		*	
RT08	Omy5				**x**			*
RT09	Omy12		**x**	**x**	**x**		*	
RT10	Omy6		**x**		**x**	*		
RT12	Omy7				**x**	*		
RT14	Omy19		**x**					
RT20	Omy10		**x**					
RT21	Omy9			**x**	**x**			*
RT22	Omy16		**x**		**x**	*		
RT23	Omy8	**x**			**x**	*		
RT26	Omy24				**x**	*		
RT27	Omy2	**x**			**x**	*		
RT29	Omy17				**x**		*	
RT30	Omy23				**x**			*
RT31	Omy3				**x**			*

x: QTL retained as significant in the different studies ($P<0.05$ at the chromosome-wide level in the present study)

S1: Drew *et a*l., 2007. Genome-scan of DH offspring from of a cross between two clonal lines with differing level of domestication.

S2: Rexroad *et al*., 2012. Genome-scan of 7 full-sib families. Families are F1 crosses from high and low responding parents selected on phenotypes in the NCCCWA broodstock under selection for growth

S3: Rexroad *et al*., 2013. Genome-scan of 2 full-sib families. Families are F2 generation from the F1 individuals obtained after crossing high and low responsive grand-parents selected on phenotype in the NCCCWA broodstock

Table S1. Markers and genetic map used for the genome scan						
Linkage group	cM	Name	type	genebank ref	PIC	Mean for PIC by LG (*sd*)
RT01	0	*OmyS00371INRA*	indel	rs162764430	0.3353	0,4371 *(0,168)*
Sex	41	*Omy1200INRA*	µsat	BV681488	0.5538	
	43.5	*OMM1118*	µsat	BV212292	0.5538	
	43.6	*OMM1665*	µsat	BV212292	0.5720	
	47	*OmyS00603INRA*	SNP	ss#538786295	0.3318	
	63	*OmyD00405INRA*	indel	rs162764429	0.2225	
	104	*Ots516NWSC*	µsat	AY042706	0.2591	
	106	*OMM1026*	µsat	AF346683	0.6681	
RT02a	0	*OMM3006*	µsat	G73806	0.3810	0,4558 *(0,066)*
Omy 13	12	*Omy1126/1INRA*	µsat	BV681391	0.4824	
	32	*OMM1064/1*	µsat	AF352744	0.5039	
RT02b	0	*Omy1297/1INRA*	µsat	BV681402	0.5511	0,4403 (*0,115*)
Omy 13	4	*Omy1513INRA*	µsat	BV681449	0.5270	
	31	*OmyD00029INRA*	indel	rs162764431	0.3515	
	68	*Omy1192/1*	µsat	CA376300	0.3318	
RT03	0	*OmyD00353INRA*	indel	rs162764440	0.3515	0,3986 *(0,128)*
Omy 14	10	*OmyS00551INRA*	SNP	rs162764439	0.1638	
	48	*OmyS00550INRA*	SNP	rs162764432	0.3318	
	56	*Omy1137INRA*	µsat	BV681523	0.6746	
	75	*OmyS00238INRA*	SNP	rs162764433	0.3515	
	76	*Ogo1*	µsat	AF007827	0.4064	
	77	*OmyS00569INRA*	SNP	rs162764434	0.3318	
	80	*Omy1263INRA*	µsat	BV681572	0.4064	
	85	*OMM1230*	µsat	AF470010	0.6324	
	92	*OmyS00037INRA*	SNP	rs162764437	0.3648	
	95	*OmyS00401INRA*	SNP	rs162764438	0.3047	
	100	*OmyS00560INRA*	SNP	rs162764436	0.3318	
	105	*OMM1346*	µsat	G73577	0.3750	
	115	*Omy1347INRA*	µsat	BX306955	0.5009	
	117	*Omy1333/1INRA*	µsat	BV681575	0.4523	
RT04	0	*Omy1351INRA*	µsat	BV681610	0.6428	0,4499 *(0,169)*
Omy 25	28	*OmyS00555INRA*	SNP	rs162764279	0.3318	
	35	*OmyD00553INRA*	indel	rs162764277	0.3750	
RT05	0	*OmyS00398INRA*	SNP	rs162764269	0.3047	0,4126 *(0,131)*
Omy 22	4	*Omy1296INRA*	µsat	BV212208	0.3047	
	30	*OMM1728*	µsat	BV212208	0.4918	
	36	*Ots249b*	µsat	BV725417	0.4757	
	39	*OmyS00558INRA*	SNP	rs162764282	0.3318	

	53	*OMM1032*	µsat	AF352737	0.5870	
	64	*Oki29*	µsat	AF055453	0.3470	
	65	*Omy1096INRA*	µsat	BV681429	0.6116	
	78	*Omy1270INRA*	µsat	BV681540	0.2591	
RT06	0	*OmyS_00273INRA*	SNP	ss#749616234	0.3750	0,5588 *(0,209)*
Omy 1	17	*OMM1081*	µsat	AF352752	0.9555	
	21	*Omy1143INRA*	µsat	BV681517	0.3810	
	25.6	*Omy1185INRA*	µsat	BV681622	0.6804	
	34.8	*OMM1780*	µsat	BV212247	0.6515	
	37.7	*OMM1454*	µsat	BV079598	0.6324	
	48.9	*Omy1276INRA*	µsat	BV681512	0.5781	
	65.2	*OmyS00044INRA*	SNP	rs162764435	0.2688	
	75.9	*OMM1776*	µsat	BV212244	0.7002	
	90.9	*OmyS00572INRA*	SNP	ss#538786286	0.3648	
RT07	0	*Omy1105INRA*	µsat	BV686450	0.4102	0,4377 *(0,142)*
Omy 15	30	*Omy3DIAS*	µsat	AF113668	0.5720	
	31	*OmyRGT17TUF*	µsat	AB087594	0.6035	
	42	*OMM1351*	µsat	G73581	0.3047	
	47	*Omy7INRA*	µsat	Pr009689137	0.3047	
	52	*OMM1764*	µsat	BV212233	0.5478	
	66	*OMM1112*	µsat	AF375024	0.5870	
	77	*Omy1474INRA*	µsat	BV681632	0.2688	
	83	*OmyD00567INRA*	indel	rs162764290	0.3047	
RT08	0	*OMM1075*	µsat	AF352746	0.8469	0,4302 *(0,177)*
Omy 5	6	*Oki26*	µsat	AF055450	0.2225	
	17	*OmyS00020INRA*	SNP	rs162764255	0.3047	
	24	*OmyS00135INRA*	SNP	rs162764261	0.3648	
	25	*OMM5205*	µsat	CA348745	0.4359	
	45	*OmyUW1198*	µsat	AY505310	0.3725	
	51	*OmyFGT12TUF*	µsat	Pr009689164	0.3515	
	63	*Omy1169INRA*	µsat	BV681435	0.4415	
	72	*Omy1435INRA*	µsat	BV681439	0.5129	
	111	*OMM1009*	µsat	AF346671	0.4415	
	114	*Omy1236INRA*	µsat	BV681468	0.6454	
	115	*OmyS00424INRA*	SNP	rs162764272	0.2225	
RT09	0	*OMM1128*	µsat	AF375030	0.7224	0,4669 *(0,172)*
Omy 12	30	*Omy1192/2INRA*	µsat	CA376300	0.1638	
	41	*OMM1161*	µsat	AY039643	0.4102	
	51	*Omy1297/2INRA*	µsat	BV681402	0.5511	
	56	*OMM1711*	µsat	BV212192	0.4918	
	60	*Omy1287/2INRA*	µsat	CO805129	0.3680	
	63	*OmyS00370INRA*	SNP	rs162764268	0.3318	
	67	*OMM1450*	µsat	BV079594	0.6609	
	74	*Ots215*	µsat	AJ534364	0.6609	
	80	*OmyS00309INRA*	SNP	rs162764267	0.3047	

	81	*OMM1130*	µsat	AF375031	0.6950	
	86	*OmyS00464INRA*	SNP	rs162764273	0.3725	
	132	*Omy1133INRA*	µsat	BV681528	0.4992	
	133	*OmyS00006INRA*	SNP	rs162764253	0.3047	
RT10	0	*OMM1179*	µsat	AF469966	0.5870	0,4524 *(0,097)*
Omy 6	10	*OMM5004*	µsat	CO805110	0.3470	
	12	*OmyS00564INRA*	SNP	rs162764287	0.3725	
	13	*Omy1332INRA*	µsat	BV681574	0.5870	
	20	*Omy1288INRA*	µsat	BV681472	0.4244	
	21	*OMM5013*	µsat	CA348663	0.4244	
	33	*OMM1294*	µsat	AF470054	0.4244	
RT11	0	*OmyS00582INRA*	SNP	rs162764441	0.1638	0,3279 *(0,101)*
Omy 27	2	*Omy1017INRA*	µsat	BX313739	0.2469	
	17	*Omy1179INRA*	µsat	BV681537	0.4401	
	23	*OmyS00011INRA*	SNP	rs162764442	0.3047	
	24	*OmyS00254INRA*	SNP	rs162764443	0.3318	
	25	*Omy7Dias*	µsat	AF239043	0.4401	
	28	*OMM1172*	µsat	AF469960	0.3680	
RT12	0	*OmyD00574INRA*	indel	rs162764487	0.3318	0,4237 *(0,114)*
Omy 7	46	*OMM1468*	µsat	BV079609	0.6276	
	50	*OmyS00081INRA*	SNP	rs162764447	0.3648	
	76	*OmyS00049INRA*	SNP	rs162764446	0.3047	
	78	*Omy1440INRA*	µsat	BV681551	0.3750	
	80	*OmyS00574INRA*	SNP	rs162764448	0.3750	
	91	*OMM1381*	µsat	BV212278	0.5720	
	93	*OmyS00013INRA*	SNP	rs162764444	0.3318	
	101	*OmyS00016INRA*	SNP	rs162764445	0.3725	
	113	*OMM5098*	µsat	BV722093	0.5870	
	117	*OMM1006*	µsat	AF346668	0.4910	
	125	*OmyS00517*	SNP	rs162764532	0.3515	
RT13	0	*Omy1013UW*	µsat	AY518336	0.3515	0,4495 *(0,118)*
Omy 28	42	*OMM1020*	µsat	AF346679	0.5862	
	46	*Omy1479INRA*	µsat	BV686475	0.4244	
	64	*OmyRGT46TUF*	µsat	AB087612	0.3515	
	78	*OmyS00397INRA*	SNP	rs162764451	0.3318	
	89	*OmyS00225INRA*	SNP	en cours	0.3750	
	99	*Omy1039INRA*	µsat	BV681337	0.5720	
	136	*OMM1216*	µsat	AF469998	0.6035	
RT14	0	*OMM1241*	µsat	AF470021	0.7009	0,5063 *(0,192)*
Omy 19	22	*OmyD00554INRA*	indel	rs162764278	0.2688	
	24	*OmyD00415INRA*	indel	rs162764271	0.1103	
	27	*OMM1279*	µsat	AF470043	0.5039	
	34	*Omy1214INRA*	µsat	BV681478	0.6113	
	35	*OMM1086*	µsat	AF352755	0.5270	

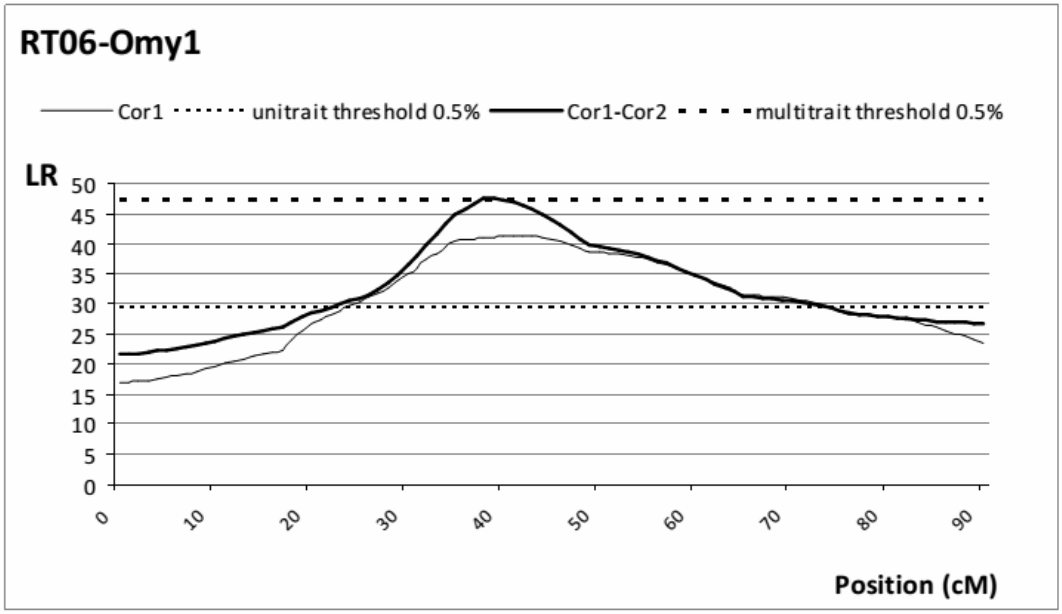
	37	*OmyD00021INRA*	indel	rs162764256	0.3725	
	44	*Omy1182INRA*	µsat	BV681504	0.4415	
	60	*Omy1374INRA*	µsat	BV681404	0.4425	
	68	*Omy1242/2INRA*	µsat	BV681390	0.7649	
	70	*Omm1174/2*	µsat	AF469962	0.6856	
	110	*Omy1407INRA*	µsat	BV681637	0.6463	
RT15	0	*Omy1383INRA*	µsat	BV681442	0.5261	0,5016 *(0,193)*
Omy21	22	*Omy1248INRA*	µsat	BV681382	0.6197	
	31	*OmyS00008INRA*	SNP	rs162764254	0.1638	
	38	*Ots1BML*	µsat	AF107029	0.5711	
	51	*OMM1036*	µsat	AF346686	0.6272	
RT16	0	*OMM1352*	µsat	BV005145	0.2469	0,3755 *(0,206)*
Omy 18	31	*Omy1038INRA*	µsat	BV681522	0.6035	
	52	*Omy1216INRA*	µsat	BV681613	0.4359	
	59	*Omy77DU*	µsat	Probe|9689151	0.5009	
	78	*Omy1499INRA*	µsat	BV681360	0.0905	
RT17	0	*OtsG85*	µsat	AF393190	0.7457	0,4644 *(0,258)*
Omy 20	13	*OmyS00476INRA*	SNP	rs162764454	0.2225	
	18	*OmyD00565INRA*	indel	rs162764453	0.1638	
	34	*Omy1376INRA*	µsat	BV681462	0.6454	
	40	*Omy1108INRA*	µsat	BV681362	0.5444	
RT18	0	*Omy1427/1INRA*	µsat	BV686471	0.3750	0,6003 *(0,178)*
Omy 26	15	*OMM1159*	µsat	AY039641	0.5339	
	24	*OMM1384*	µsat	BV078070	0.7083	
	25	*Omy1001UW*	µsat	AY518324	0.7112	
	35	*Omi187TUF*	µsat	AB105857	0.4415	
	38	*Omy1163/2INRA*	µsat	BX888425	0.8316	
RT19	0	*OmyS00090INRA*	SNP	ss#538786287	0.3725	0,5042 *(0,129)*
Omy 11	38	*Ocl8UW*	µsat	AF028697	0.5597	
	60	*Omi174TUF*	µsat	AB105854	0.6454	
	61	*OMM1375*	µsat	BV078061	0.5270	
	74	*Ots209*	µsat	AJ534367	0.6116	
	76	*Omy1542INRA*	µsat	KC906187	0.6278	
	78	*Omy1279INRA*	µsat	BV681437	0.5444	
	80	*OMM1313*	µsat	G73553	0.5594	
	82	*OmyD00259INRA*	indel	rs162764475	0.3725	
	85	*Omy1363INRA*	µsat	BV681324	0.3318	
	87	*OMM1008*	µsat	AF346670	0.6575	
	114	*OmyUW1052*	µsat	AY505331	0.4757	
	119	*OmyS00268INRA*	SNP	rs162764265	0.2688	
RT20	0	*Omy1348INRA*	µsat	CR372971	0.6896	0,4950 *(0,173)*
Omy 10	27	*OMM1050*	µsat	AF346694	0.6191	
	55	*SsaN82LEE*	µsat	U86706	0.5632	

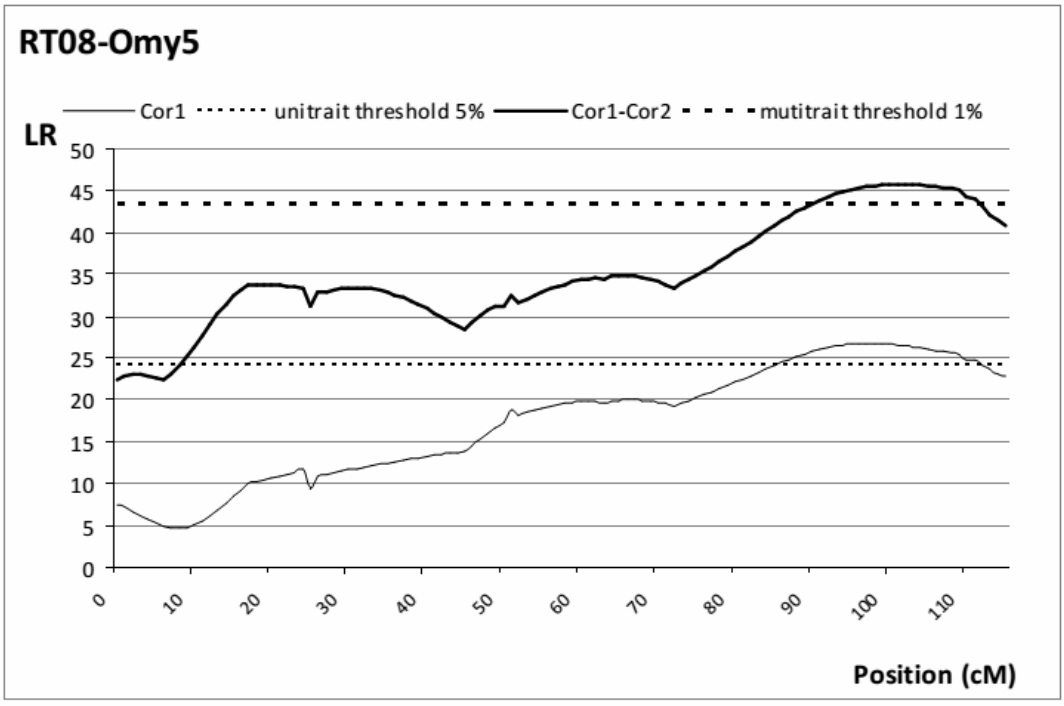
	58	*OmyS00604INRA*	SNP	ss#538786296	0.3047	
	67	*OMM1544*	µsat	BV212073	0.7622	
	69	*OmyD00576INRA*	indel	rs162764457	0.3648	
	99	*OMM1174/1*	µsat	AF469962	0.4956	
	104	*OmyS00160INRA*	SNP	rs162764456	0.3515	
	120	*Omy1242/1INRA*	µsat	BV681390	0.3047	
RT21	0	*OMM5132*	µsat	BX076842	0.6116	0,5301 *(0,151)*
Omy 9	22	*Omy1359INRA*	µsat	BV681626	0.6569	
	28	*OmyFGT2TUF*	µsat	Pr009689160	0.5511	
	46	*OMM1145*	µsat	AF375040	0.4064	
	49	*OMM1736*	µsat	BV212213	0.6272	
	57	*OmyD00306INRA*	indel	rs162764266	0.3047	
	65	*OmyUW1090*	µsat	AY505318	0.5441	
	72	*OMM5197*	µsat	BX086448	0.5781	
	78	*OmyD00173INRA*	SNP	rs162764263	0.3047	
	88	*OMM5126*	µsat	CO805128	0.4523	
	90	*Omy1252INRA*	µsat	BV686463	0.7943	
RT22	4	*OmyS00387INRA*	SNP	rs162764459	0.2688	0,4467 *(0,173)*
Omy16	43	*OmyS00038INRA*	SNP	rs162764462	0.3318	
	55	*OMM1362*	µsat	BV005154	0.7002	
	63	*OmyS00168INRA*	SNP	rs162764464	0.3318	
	73	*OmyS00581INRA*	SNP	rs162764458	0.3648	
	80	*Omi20TUF*	µsat	AB105829	0.4918	
	85	*Str58CNRS*	µsat	U60223	0.5840	
	90	*OmyS00379INRA*	SNP	rs162764465	0.3648	
	94	*OmyS00078INRA*	SNP	rs162764461	0.3725	
	112	*OMM5133*	µsat	BV211864	0.7680	
	119	*Ssa420UOS*	µsat	AJ402737	0.5594	
	128	*OmyD00499INRA*	indel	rs162764460	0.2225	
RT23	0	*Omy1125INRA*	µsat	BV681399	0.3810	0,4013 *(0,144)*
Omy 8	8	*OMM1459*	µsat	BV079603	0.6675	
	38	*Omy1475INRA*	µsat	BV681589	0.4401	
	44	*OmyS00051INRA*	SNP	rs162764257	0.1638	
	48	*Omy1358INRA*	µsat	BX871675	0.4916	
	62	*OMM1354*	µsat	BV005150	0.4757	
	63	*Ots212*	µsat	AJ534362	0.4757	
	100	*Omy1361INRA*	µsat	BV681353	0.2591	
	130	*OMM5010*	µsat	CO805116	0.3894	
	140	*OmyRGT9TUF*	µsat	AB087590	0.2688	
RT24	0	*Omy1393INRA*	µsat	BV681550	0.4502	0,3804 *(0,137)*
Omy 4	25	*Omy1287/1INRA*	µsat	BV681583	0.5094	
	59	*OmyS00442INRA*	SNP	rs162764471	0.3318	
	60	*OmyS00274INRA*	SNP	rs162764469	0.3318	
	64	*Omy1233INRA*	µsat	BV681466	0.5009	
	70	*OmyRGT36TUF*	µsat	AB087605	0.5261	

	72	*OmyS00426INRA*	SNP	rs162764473	0.0905	
	79	*OmyS00252INRA*	SNP	rs162764468	0.3318	
	80	*OmyS00361INRA*	SNP	rs162764470	0.3515	
RT25	0	*OMM1389*	µsat	BV078075	0.5720	0,5504 *(0,135)*
Omy 29	12	*OMM1797*	µsat	BV212257	0.5781	
	16	*OmyS00559INRA*	SNP	rs162764283	0.3648	
	20	*OMM1054*	µsat	AF352739	0.6869	
RT26	0	*Omy1321INRA*	µsat	BV681520	0.7700	0,4606 *(0,235)*
Omy 24	20	*OmyRGT39TUF*	µsat	AB087607	0.3788	
	31	*OmyS00570INRA*	SNP	rs162764520	0.3725	
	20	*OmyFGT24TUF*	µsat	Pr009689169	0.5511	
	54	*Omy1350INRA*	µsat	BX085137	0.6005	
	55	*OmyD00563INRA*	indel	rs162764286	0.0905	
RT27	0	*OmyS00557INRA*	SNP	rs162764281	0.2688	0,3463 *(0,125)*
Omy 2	6	*Omy25INRA*	µsat	Pr009689147	0.3525	
	12	*OmyS00498INRA*	SNP	rs162764276	0.3047	
	20	*Omy1264INRA*	µsat	BV681587	0.3470	
	25	*OmyS00562INRA*	SNP	rs162764285	0.2688	
	28	*OmyS00266INRA*	SNP	rs162764455	0.3047	
	46	*OMM1039*	µsat	AF346689	0.4796	
	63	*OMM1070*	µsat	AF375019	0.4102	
	76	*Omy1300/1INRA*	µsat	BV681381	0.5261	
	104	*Oke04*	µsat	AF330221	0.2469	
	111	*OMM5000/1*	µsat	CO805106	0.0905	
	112	*Oke12*	µsat	AF330228	0.3470	
	129	*OMM5270*	µsat	BX082395	0.5547	
RT29	0	*OmyS00568INRA*	SNP	rs162764289	0.3047	0,4765 *(0,155)*
Omy 17	16	*OmyS00477INRA*	SNP	rs162764275	0.3648	
	20	*OmyRGT19TUF*	µsat	AB087595	0.5339	
	22	*OmyS00099*	SNP	rs162764260	0.3725	
	31	*Omy1271INRA*	µsat	BV681378	0.6030	
	40	*Omy1040INRA*	µsat	BX866010	0.3515	
	46	*OtsG43*	µsat	AF393186	0.6077	
	48	*OMM5043*	µsat	CA349167	0.4205	
	53	*OmyD00096INRA*	indel	rs162764259	0.3725	
	54	*Omy21INRA*	µsat	Pr009689145	0.7188	
	58	*OmyS00556*	SNP	rs162764280	0.3318	
	69	*OMM1064/2INRA*	µsat	AF352744	0.7358	
RT30	0	*Omy1380INRA*	µsat	BV686469	0.3648	0,4234 *(0,183)*
Omy 23	13	*Omy005DIAS*	µsat	AF239041	0.3725	
	16	*OMM1019*	µsat	AF346678	0.6876	
	23	*OmyD00082INRA*	indel	rs162764472	0.2688	
RT31	0	*OMM5000/2*	µsat	CO805106	0.3515	0,4506 *(0,133)*

Omy 3	4	*OmyS00561INRA*	SNP	rs162764284	0.3515
	25	*Omy1300/2INRA*	µsat	BV681381	0.3725
	37	*Omy1027INRA*	µsat	BV681350	0.6953
	48	*OMM1058*	µsat	AF352741	0.6569
	72	*OmyS00399INRA*	SNP	rs162764270	0.3750
	77	*OMM1053*	µsat	AF352738	0.5511
	105	*Omy1241INRA*	µsat	BV681482	0.5307
	112	*OmyS00566INRA*	SNP	rs162764288	0.3750
	119	*OmyS00172INRA*	SNP	rs162764262	0.3318
	122	*Omy1392INRA*	µsat	BX861189	0.3648

Figure S1.


RT06-Omy1
Cor1
unitrait threshold 0.5%
Cor1-Cor2
multitrait threshold 0.5%
LR
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
Position (cM)


RT08-Omy5
Cor1
unitrait threshold 5%
Cor1-Cor2
mutitrait threshold 1%
LR
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100
110
Position (cM)