580. QTLs controlling swimming performance and their effect on growth in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Critical swimming speed (U_{crit}) is an important measurement of swimming performance as well as a good indicator for cardio-respiratory health. It offers a new opportunity to select fish with better fitness. Here we present results from a GWAS study on critical swimming speed and growth in Nile tilapia under conditions of recurrent hypoxia. Heritability for U_{crit} was moderate (0.31 ± 0.04). Large fish generally swim faster than smaller fish, whereas fish with better swimming performance tend to show slower growth later in life. Nine suggestive SNPs associated with swimming performance were identified under a hypoxic environment. A clear pleiotropic effect on growth traits was found for some of the SNPs associated with swimming performance, while other SNPs had only an effect on swimming performance, but not on growth. Our analyses sheds a first light on the genomic mechanisms of swimming performance and growth in Nile tilapia.

Introduction

Swimming performance is an important feature that correlates with fitness, survival, and metabolism in many aquacultural fish species (Palstra and Planas, 2012). Swimming performance in fish can be measured in a critical swimming challenge test, by which U_{crit} can be assessed. U_{crit} is the speed at which maximum oxygen uptake occurs. In tilapia, U_{crit} has been shown to be highly correlated with maximum metabolic rate (McKenzie *et al.*, 2003). However, it is unknown which QTLs are associated with U_{crit} in tilapia.

Nile tilapia (*Oreochromis niloticus*) is one of the most important farmed fish species in the world. Farming is mostly done in ponds and cages. However, for most smallholder farmers aeration of fishponds is not available or too expensive. In non-aerated ponds extreme hypoxia can occur, especially during the night when algae become net oxygen consumers (Mengistu *et al.*, 2020). Therefore, it is crucial to select tilapia that grow better under conditions where dissolved oxygen is limited.

The main objectives in this study were: (1) to estimate the genetic correlations between U_{crit} with body size traits at the swimming test, and with growth traits using a genomic relationship matrix, (2) to identify SNPs and genes associated with swimming performance and (3) to estimate the effect of candidate SNPs for U_{crit} on the growth traits.

Materials & methods

Experimental design and traits collection. The experimental design and data collection is described in full detail in Mengistu *et al.* (2021). Nile tilapia were collected as part of the GIFT selective breeding program. U_{crit} and body weight at test (BWtest) during the swimming test were recorded. After the swimming test, fish were stocked in non-aerated ponds for growing-out and harvested after 145 or 146 days. The stocking and harvest weight (Harw) were recorded, by and daily growth coefficient (DGC) was calculated.

SNP genotyping and quality control. The DNA was extracted and genotyped by Identigen (Dublin, Ireland) using an Axiom® SNP array, which contains 65K SNP markers dispersed over the Nile tilapia reference genome (Peñaloza *et al.*, 2020). Data was filtered to meet a dish quality control >0.82 and call rate for samples >0.93, respectively. Next, a second quality control step was applied based on per SNP call rate and minor allele frequency statistics.

Genetic parameters estimation. Variance components and heritability for swimming performance traits including U_{crit}, BWtest, Harw and DGC were estimated using univariate models based on residual maximum likelihood method with the following model was applied:

$$y = Xb + Z_1a + Z_2c + e \tag{1}$$

Phenotypic and genetic correlations between U_{crit} and traits BWtest, Harw and DGC were estimated based on a bivariate linear model. The fixed effects were the same as in the univariate models for U_{crit}, BWtest, Harw and DGC traits.

Association analysis and candidate genes in QTLs. Animal phenotypes and genotypes were jointly analysed to identify genomic regions associated with U_{crit}. The following model was applied:

$$y = W\alpha + Z\mu + x\beta + \varepsilon \tag{2}$$

LD and haplotype blocks were analysed with LDBlockShow (Dong *et al.*, 2020), while $r^2 > 0.8$ as cut-off for blocks. Putative candidate genes associated with swimming performances were characterized within a 200 kb window size (100 kb upstream and downstream) flanking the candidate SNPs.

The effect of candidate SNPs in swimming performance on growth. We estimated the genetic association between SNPs and DGC based on generalized linear model. The best fitting genetic model was evaluated based on Akaike Information Criterion score. The significant threshold was defined as adjusted *P*-value<0.05.

Results

Genetic parameters estimation with different traits at swimming test. The heritability for U_{crit} was 0.31 with standard error 0.043, while the heritability for body weight at swimming test was 0.27 (Table 1). The common environmental effects were not significant for both traits. The phenotypic and genetic correlations between U_{crit} and BWtest were 0.43 and 0.36 (Table 2), indicating that fish with better swimming capacity usually show big size. The estimated r_g between U_{crit} and harvest weight (-0.13±0.13) and U_{crit} and DGC (-0.26±0.13) were slightly negative but not different from zero, suggesting that U_{crit} might have a negative genetic correlation to body weight at harvest and growth until harvest.

Table 1. Variance components for U_{crit} and body weight traits.

Traits	σ^2_a	σ^2_e	σ^2_p	h^2
U_{crit}	5.15	11.72	16.88	0.31 (0.043)
BWtest	1.23	3.38	4.61	0.27 (0.043)

Table 2. Genetic and phenotypic correlations between U_{crit} and body weight traits.

Traits	r_g	r_p
BWtest	0.43±0.10	0.36±0.03
Harw	-0.13±0.13	0.06±0.04
DGC	-0.26±0.13	-0.01±0.04

Genome-wide association study for swimming performance. Nine SNPs located on LG12, LG13, LG14 and LG19 exceeded the genome-wide suggestive threshold for U_{crit} (Figure 1). All genes in the up- and down-stream 100 kb regions of the 9 suggestive SNPs in a high LD ($r^2>0.8$) were investigated, which were *limk1a, elna, lsamp, aipl1* and *dner* on LG14, *pprc1* and *rrp12* on LG13, *hectd1 and smyd1* on LG19 and LG12.

Pleiotropic SNPs with swimming performance and growth. In this study, we investigated the impact of candidate nine SNPs associated with U_{crit} on growth traits. Three of these nine SNPs were significantly associated with both harvest weight and DGC, and SNP genotypes that presented a low mean U_{crit} had a high mean harvest weight and DGC.

Discussion

The observed genetic correlation between U_{crit} and Harw, and U_{crit} and DGC, suggests an negative interaction between growth and critical swimming speed under hypoxic environment.

Our results show several QTLs involved in the swimming performance in Nile tilapia. Overall, 16 genes in high LD with the identified SNPs, could be interesting candidate genes in regulation of swimming performance. For instance, the *rrp12* gene has been associated with adaptation to low oxygen (Foll *et al.*, 2014) and *pprc1* is potentially involved in cardiac and exercise-related muscle activity (Uguccioni *et al.*, 2010).

Our results show only part of the SNPs to be significantly associated with harvest weight and DGC, which located on several genes (*hip1, hectd1, dner*). They potentially can be applied as biomarkers to select fish with good cardio-respiratory health and good growth.
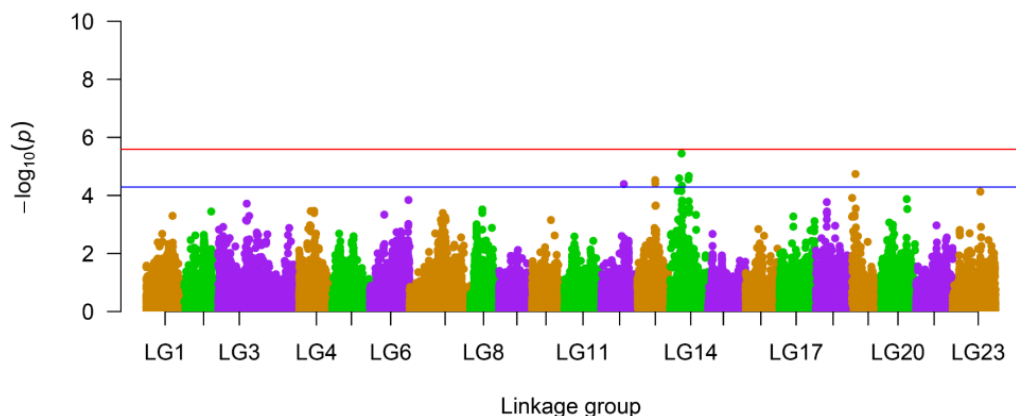

Figure 1. Manhattan plot for U_{crit}. The orange and blue horizontal line represent the genome-wide significance (2.57E-06) and suggestive significance threshold value (5.15E-05), respectively.

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