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# A genome scan of a four-way tilapia cross supports the existence of a quantitative trait locus for cold tolerance on linkage group 23

**Thomas Moen[1], Jeremy J Agresti[2]*, Avner Cnaani[3]†, Hillary Moses[2], Thomas R Famula[2], Gideon Hulata[3], Graham A E Gall[2] & Bernie May[2]**

[1]AKVAFORSK (Institute of Aquaculture Research), Aas, Norway

[2]Department of Animal Science, University of California, Davis, CA, USA

[3]Institute of Animal Science, Agricultural Research Organization, Bet Dagan, Israel

**Correspondence:** T Moen. (E-mail: thomas.moen@akvaforsk.nlh.no). B May, Department of Animal Science, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA. E-mail: bpmay@ucdavis.edu

***Present address:** MRC Laboratory of Molecular Biology, Cambridge, UK.

†**Present address:** Hubbard Center for Genome Studies, University of New Hampshire, Durham, USA.

## Abstract

A genome scan, searching for quantitative trait loci (QTL) for the traits cold tolerance and body weight in tilapia, was performed on a cross between a (*Oreochromis niloticus* × *Sarotherodon galilaeus*) male and a (*O. mossambicus* × *O. aureus*) female. Fifty-four microsatellites and 23 amplified fragment length polymorphism (AFLP) primer combinations were genotyped and tested for marker–trait associations. Sex-specific linkage maps were constructed from this data. Twenty-three point-wise significant marker–trait associations were found in the genome scan, and putative QTL were subsequently tested in another (*On* × *Sg*) × (*Om* × *Oa*) family. None of the putative QTL from the first experiment were significant in the second experiment. However, one microsatellite, UNH130, found to be associated to weight in the first experiment, was found to be strongly associated to cold tolerance in the second experiment. Since QTL for cold tolerance and body weight were recently found on the linkage group containing UNH130 (linkage group 23) in another study, this linkage group was investigated more closely using interval mapping. The results provide indications, but not conclusive evidence, of a QTL for cold tolerance on linkage group 23.

**Keywords:** tilapia, QTL, cold tolerance, body weight, linkage map

## Introduction

Tilapias are cichlid fishes native to Africa and the Jordan Valley. The tolerance for wide ranges of water quality, diets and farming systems has ensured the adoption of these species for aquaculture in virtually all tropical and subtropical regions of the world. The growth rates of tilapias are, however, reduced if water temperatures fall below those found in their native habitat (26–28 °C) (Pullin 1991). In some regions tilapia producers suffer great losses due to cold temperatures in the winter months, and some temperate regions are excluded from the culture of tilapia entirely because of climate (Sarig 1993; Wu, Hwang, Hew & Wu 1998). Because of the otherwise excellent aquaculture qualities of these fishes, many regions of the world would benefit from a cold-tolerant strain of tilapia.

Several studies have compared the degrees of cold tolerance in the different tilapia species and interspecific hybrids (Khater & Smitherman 1988; Behrends, Kingsley & Bulls 1990; Tave, Jayprakas & Smitherman 1990; Cnaani, Gall & Hulata 2000; Sifa, Chenhong, Dey, Gagalac & Dunham 2002). All studies have indicated that there is a genetic component to the variation in cold tolerance. There does not seem to be a correlation between cold tolerance and fish size (Behrends *et al.* 1990; Cnaani *et al.* 2000). If genetic markers linked to quantitative trait loci (QTL) affecting cold tolerance could be found, this information would accelerate the development of a cold-tolerant strain of Tilapia.

A linkage map of molecular markers is a pre-requisite for QTL studies. When compared with livestock and model species, genomic research on tilapia species is still in its infancy. Nevertheless, three genetic

 

maps of tilapia have been published. Kocher, Lee, Sobolewska, Penman and McAndrew (1998) made an AFLP/microsatellite map using gynogenetic offspring from a single *O. niloticus* female. The same group has also made a more extensive map based on the $F_2$ of a (*On* × *Oa*) cross (http://hcgs.unh.edu/comp/). This latter map (referred to as the UNH map) is the most comprehensive tilapia map at the present stage. Agresti, Seki, Cnaani, Poompuang, Hallerman, Umiel, Hulata, Gall and May (2000) made AFLP/microsatellite maps from a cross between an *Oreochromis mossambicus* (*Om*) female and a (*O. aureus* × Red *O. niloticus*) (*Oa* × R*On*) male. Partial maps of *O. aureus* and *O. niloticus* were made by McConell, Beynon, Leamon and Skibinski (2000), using an inter-specific backcross population.

A few marker–trait associations have previously been found in tilapia species. Markers associated with genes with deleterious alleles (Palti, Shirak, Cnaani, Hulata, Avtalion & Ron 2002) and distorted sex ratios (Shirak, Palti, Cnaani, Korol, Hulata, Ron & Avtalion 2002) were found in an inbred gynogenetic line of *O. aureus*. Variation in a microsatellite was found to be associated with expression of the prolactin gene and growth in salinity-challenged fish from the $F_2$ of a (*Om* × *On*) cross (Streelman & Kocher 2002). A QTL mapping study was recently done by Cnaani, Hallerman, Ron, Weller, Indelman, Kashi, Gall and Hulata (2003), aiming at the identification of QTL for cold tolerance and body weight. In this experiment, a (*Om* × *Oa*) $F_2$ family was first screened with 20 microsatellites, whereupon putative QTL were tested for replication in another (*Om* × *Oa*) $F_2$ family. One QTL for cold tolerance and one QTL for growth were found to be significant in both trials, and both QTL were located on UNH linkage group 23.

In general, the genetic resources of farmed tilapia have been poorly managed, with some exceptions (Eknath, Dey, Rye, Gjerde, Abella, Sevilleja, Tayamen, Reyes & Bentsen 1998 and references therein). Aquaculture stocks have often been based on a few wild progenitor animals, serially distributed from farm to farm, and high levels of inbreeding depression have been found (Eknath, Tayamen, Palada-de Vera, Danting & Reyes 1993). Aiming at developing a broader genetic base on which future genetic improvements can be built, an artificial centre of origin (ACO) was developed (Agresti *et al.* 2000). The ACO consists of numerous three-way and four-way crosses between four species of tilapia; *O. aureus*, *O. mossambicus*, *O. niloticus* and *Sarotherodon galilaeus* (*Sg*). The ACO contains wide genetic diversity and presents opportunities for genes to recombine and interact with other genes originating from different species. We report here the mapping of QTL for body weight and cold tolerance performed on two four-way cross (4WC) families from the ACO. Identified QTL for important traits could be implemented through marker-assisted selection (MAS), with the aim of producing rapidly growing, cold-tolerant Tilapia.

## Materials and methods

### Experimental design

This study consisted of three consecutive experiments. Experiment 1 is a genome-scan of a 4WC family of tilapia, aiming at the identification of QTL for cold tolerance and body weight. In experiment 2, significant marker–trait associations from experiment 1 were tested in another 4WC family, to see if the associations were replicable. In experiment 3, the position of a putative QTL was investigated through interval mapping using a larger number of microsatellites and the family from experiment 2, with the aim of mapping the detected QTL to a more precise location.

### Mating scheme

Eggs stripped from an *Sg* female were artificially inseminated with sperm from an *On* male to produce a (*On* × *Sg*) F1 generation (Fig. 1). At the same time, an *Oa* male was mated with a *Om* female to produce a parallel (*Oa* × *Om*) F1 generation. A (*On* × *Sg*) male was mated to two (*Oa* × *Om*) females to produce the two (*On* × *Sg*) × (*Oa* × *Om*) 4WC families used in this study. One of the 4WC families, termed 'family 1', was used in the genome scan (experiment 1), while the other ('family 2') was used in experiments 2 and 3. There were 54 offsprings in family 1 and 44 offsprings in family 2. At the time experiments 2 and 3 were carried out, DNA and tissue from family 1 had been discarded.

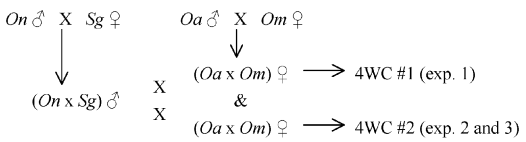


**Figure 1** The pedigree structure of the tilapia families used in this study. *Oa*, *Oreochromis aureus*; *On*, *O. niloticus*; *Om*, *O. mossambicus*; *Sg*, *Sarotherodon galilaeus*; 4WC = four-way cross.

  

### Trait measurements

The fish were born in January 1998 and the cold challenge was done in August 1998. Body weight was recorded at the time of the challenge test. Each of the two 4WC families was reared in a separate aquarium, but they were sharing the same water system. They were fed daily with commercial tilapia feed, containing 30% protein (Zemach Feed Mills, Israel). Throughout these months the temperatures in the aquaria were 22–28 °C. The families used were part of a larger experiment aimed at studying the genetic variation for cold tolerance in different tilapia species and hybrids (Cnaani *et al.* 2000). Thus, when the experiment was set, the extremes of the size distribution were not used for the cold challenge.

Complete details of the cold temperature challenge test can be found in Cnaani *et al.* (2000). In short, water temperature was gradually reduced from ambient temperature to 16 °C over 7 days and held for 4 days; then the temperature was reduced at a rate of 0.5 °C at 2-day intervals to a lower limit of 8 °C. Each day, comatose fish were removed from the tank, weighed and a sample of fin tissue was taken for genomic DNA extraction.

The parameter, 'cooling-degree-days' (CDD) was calculated to provide an empirical estimate of the cold tolerance of each fish. The parameter represents the sum over days of the difference between the daily temperature and the initial temperature (16 °C):

$$\mathrm{CDD} = \sum_{i=1}^{k} (t_0 - t_i)$$

where $i$ = days, $t_0$ = initial temperature (16 °C), $t_i$ = temperature at the $i$th day and $k$ = day of mortality.

### DNA extraction

Genomic DNA was extracted from fin tissue (0.5–1 $cm^2$) of 54 progeny from family 1, 44 progeny from family 2, parents and maternal grandparents. Tissue from the paternal grandparents was not available. Two different protocols were used: the PureGene kit (Gentra Systems, Minneapolis, MN, USA) was used for most of the progeny, while the organic extraction procedure of Doyle and Doyle (1987) was used on some parental tissue.

### Microsatellite and AFLP genotyping

The protocols used for microsatellite and AFLP genotyping were the same as in Agresti *et al.* (2000), with one change; a three-base rather than a two-base extension was used on the *EcoRI* primer for the selective amplification of AFLPs. Amplified fragment length polymorphism bands were named by their three-base *EcoRI* extensions followed by their three-base *MseI* extension followed by the size of the band in base pairs. Amplified fragment length polymorphism markers were scored as dominant markers, and individual AFLPs were not considered if both parents had the band present phenotype. In the text, 'allele A' refers to the AFLP band present allele, or the microsatellite allele with highest molecular weight (coming from the parent in question). 'Allele B' refers to the AFLP band absent allele, or the microsatellite allele with lowest molecular weight (coming from the parent in question).

In experiment 1, 54 microsatellites were chosen from the map of Agresti *et al.* (2000). The selection of these 54 microsatellites was based on their genomic distribution (attempting an even distribution over the genome), as well as their genotype qualities. In the second experiment, a subset of eight microsatellites was used. For the third experiment, nine additional microsatellites were chosen from linkage group 23 of the UNH map (http://hcgs.unh.edu/comp/).

Markers were tested for non-Mendelian segregation using a $\chi^2$-test. Markers that tested positive for non-Mendelian segregation at $P < 0.05$ were excluded from the QTL analysis.

### Linkage analysis

Linkage analysis was performed separately on the male and female data sets, using Joinmap 3.0 (Van Ooijen & Voorips 2001). The default settings of the program were used. The grouping of markers was done at LOD > 3.0. As usual, a marker was defined to belong to a linkage group if it was found to be linked to at least one other member of the group. Kosambi's mapping function was used. To ease comparison with other published studies, we investigated the linkage groups for connections to the UNH map. Linkage groups for which connections were found were named accordingly (Fig. 2).

Once the correct marker order had been found, the data were checked for double recombinants, using a Visual Basic program (T. Moen, unpublished). Whenever double recombinants were found, the gel images were rechecked and the individuals in question were genotyped once more if necessary.

### QTL analysis

*Single marker analysis*

In experiments 1 and 2, each marker was tested individually for association to cold tolerance and body

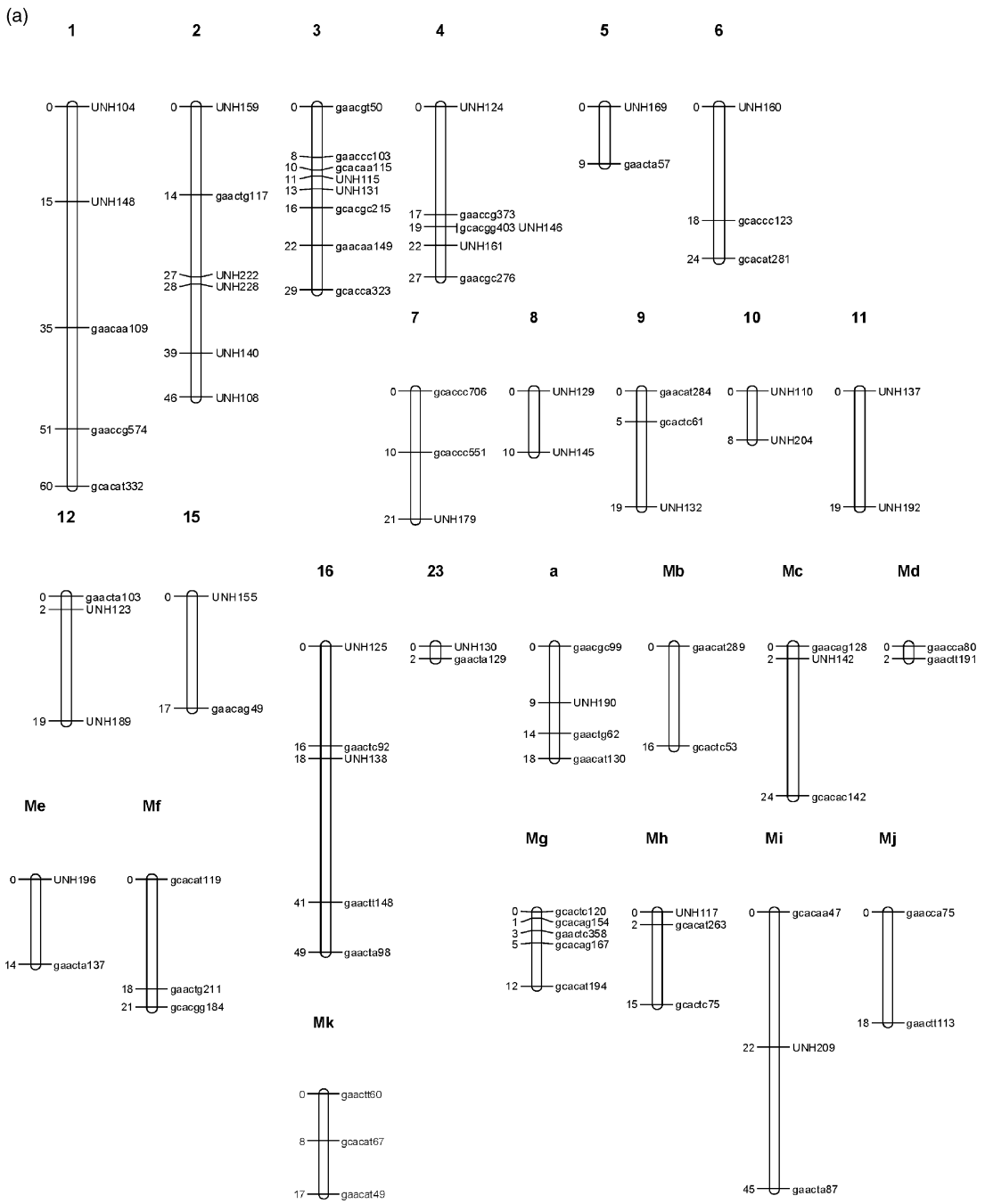


**Figure 2** AFLP/microsatellite maps made from (a) meioses in one (*S. galilaeus* × *O. niloticus*) individual, the male parent of family 1, and (b) meioses in one (*O. mossambicus* × *O. aureus*) individual, the female parents of family 1. Any linkage group named with a number corresponds to the linkage group in the UNH map having the same number. If two linkage groups corresponded to the same UNH linkage group, then they were designated by the UNH linkage group number, followed by 'a' or 'b'. For example, linkage groups 3a and 3b both contain markers found in UNH linkage group 3. There was correspondence between the male and female map for one other linkage group (linkage group a). For the other linkage groups, no links were found between the maps.

  

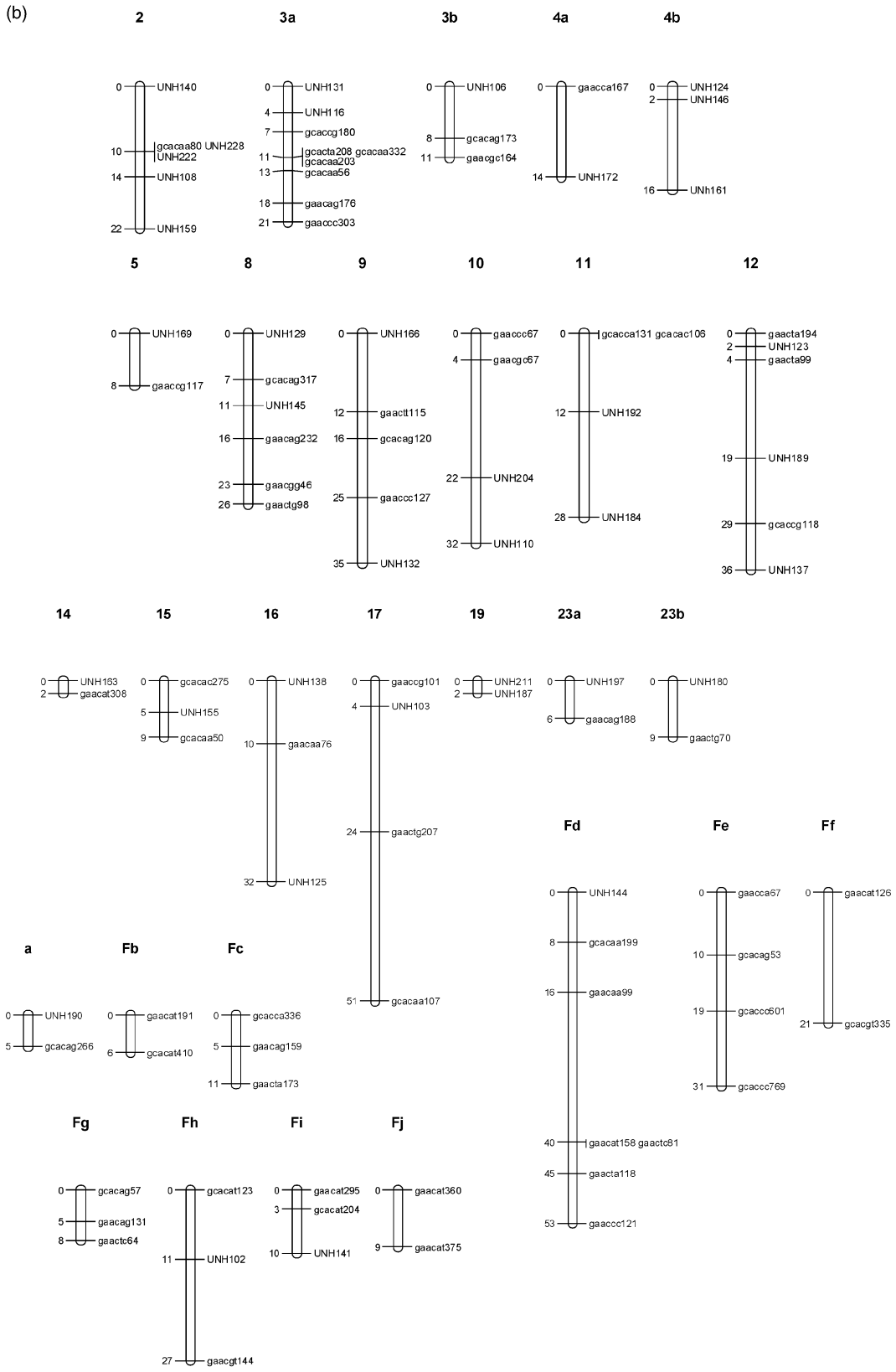


**Figure 2** (Continued).

weight, using *t*-tests. The male and female data sets were analysed separately. The *t*-test statistic was

$$t = \frac{\bar{y}_A - \bar{y}_B}{S_{pooled}\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}},\ \text{d.f.} = n_A + n_B - 2$$

where $\bar{y}_A$ and $\bar{y}_B$ are the trait means of offspring having inherited allele A or allele B, respectively, from the parent in question, $n_A$ and $n_B$ are the number of offsprings in the corresponding groups, and $S_{pooled}$ is the estimator for the pooled standard deviation.

Point-wise significance levels were calculated using tabulated thresholds. Experiment-wise significance levels were calculated using a Bonferroni correction to account for the four parent–trait combinations tested (assumed to be independent of each other), and using permutation tests (Churchill & Doerge 1994) with 1000 iterations to account for the testing of multiple markers within each of these parent–trait combinations.

### *Interval mapping*

Interval mapping was performed using the web-based version of the QTL express software (Seaton, Haley, Knott, Kearsey & Visscher 2002; http://qtl.cap.ed.ac.uk/). Allele contributions from the two parents were analysed separately using the half-sib analysis option, based on the method of Knott, Elsen and Haley (1996). A one-QTL model was assumed, and calculations were performed at every 1 cM. Chromosome-wide permutation tests were performed, with 2000 iterations. For the calculation of confidence intervals for QTL position, bootstraps (Visscher, Thompson & Haley 1996) with 1000 iterations were performed. For ease of presentation, the linkage map used for the interval mapping was a sex-averaged one, combining the information from the two parents.

## Results

### Experiment 1

Table 1 displays the test statistics from experiment 1. A correlation test showed that the variables were not correlated ($r = -0.14$, $P = 0.31$). The cold-tolerance data were approximately normally distributed, while the body weight data were not (Table 1).

Thirty-seven microsatellites were male informative, 41 were female informative and 31 were informative for both parents. Twenty-three AFLP primer combinations were done, resulting in 71 male-informative AFLPs and 76 female-informative AFLPs. Nineteen percent of the markers were segregating in a non-Mendelian fashion at $P < 0.05$, higher than expected by chance.

The male map (Fig. 2a) length was 561 cM, distributed on 26 linkage groups. Eighty-eight markers were integrated into the male map, 33 microsatellites and 53 AFLPs. The female map (Fig. 2b) length was 524 cM, distributed on 28 linkage groups. The female map consisted of 99 markers; 36 microsatellites and 63 AFLPs. Based on shared microsatellites, the corresponding linkage groups in the UNH map could be found for 15 male linkage groups and 17 female linkage groups. There was generally a one-to-one relationship between linkage groups found in this study and UNH linkage groups, although in a few cases two linkage groups corresponded to the same UNH group.

A total of 23 marker–trait associations were found at a point-wise significance level of $P < 0.05$ (Table 2a). The false discovery rate (Weller, Song, Heyen, Lewin & Ron 1998) was 0.87, meaning that most of the significant associations would be expected to be due to chance. No markers were found to be associated to the same trait in both parents. One marker, UNH124 (on linkage group 4), showed association to both traits, but the two associations were not found in the same parent.

The above statements on the statistical significance of the tests did not take into account the fact

**Table 1** Descriptive statistics on cold-day-degrees (cold tolerance) and body weight from experiment 1 and 2

| Experiment | Trait | *N* | Mean | Std. dev. | Test for normality |
|---|---|---|---|---|---|
| 1 | Cold-day-deg. | 54 | 65.25 | 10.79 | $P > 0.15$ |
| 1 | Body wt. (g) | 54 | 7.92 | 3.75 | $P < 0.01$ |
| 2 | Cold-day-deg. | 44 | 67.02 | 9.52 | $P > 0.15$ |
| 2 | Body wt. (g) | 44 | 6.60 | 2.57 | $P < 0.05$ |

The test for normal distribution was a Kolmogorov–Smirnov test.

  

that a large number of tests were performed. On an experiment-wise level, none of the marker–trait associations were significant at $P < 0.05$.

## Experiment 2

The family tested in the second experiment (family 2) came from a cross very similar to the cross performed in the first experiment (family 1); the two families had a common father, and their mothers were full sibs. The mothers of the two families had identical genotypes at all eight microsatellite markers investigated in both families, implying that the maternal grandparents had low levels of heterozygosity.

Table 1 displays the test statistics from experiment 2. As in experiment 1, there was no significant correlation between the variables ($r = -0.24$, $P = 0.10$), and the cold-day-degrees data were approximately normally distributed, while the body weight data were not (Table 1).

The eight microsatellites that showed association to either trait in experiment 1 were genotyped for family 2. Each of the eight microsatellites were tested for association to either trait on both parents, meaning that other associations were tested in addition to the ones that were found to be significant in experiment 1. At a point-wise significance level of $P < 0.05$, only one marker–trait association was significant, namely the association of cold tolerance to alleles in the male parent at marker UNH130. The association was not experiment-wise significant, but it was close to being so (Table 2b). The association was, however, not anticipated from experiment 1, since no significant association between UNH130 and cold tolerance was found in that experiment. UNH130 had instead

**Table 2** Pointwise significant ($P < 0.05$) associations with body weight (BW) or cold tolerance (CDD) in (a) experiment 1 and (b) experiment 2

| Trait | Marker | Parent | LG | Mean A | Mean B | *t* | Point-wise *P*-value | Experiment-wise *t*-threshold ($P < 0.05$) |
|---|---|---|---|---|---|---|---|---|
| (a) | | | | | | | | |
| BW | UNH124 | ♂ | 4 | 9.42 ± 0.76 | 6.68 ± 0.67 | 2.71 | 0.01 | 4.188 |
| BW | UNH130 | ♂ | 23 | 6.77 ± 0.66 | 9.25 ± 0.71 | 2.54 | 0.014 | 4.188 |
| BW | gaacta129 | ♂ | 23 | 6.86 ± 0.72 | 9.14 ± 0.72 | 2.24 | 0.029 | 4.188 |
| BW | gcacac60 | ♂ | – | 6.20 ± 0.68 | 9.90 ± 0.66 | 3.90 | 0.001 | 4.188 |
| BW | UNH138 | ♂ | 16 | 6.52 ± 0.71 | 8.78 ± 0.69 | 2.29 | 0.026 | 4.188 |
| BW | gaacta98 | ♂ | 16 | 5.23 ± 1.06 | 8.70 ± 0.54 | 2.91 | 0.005 | 4.188 |
| BW | gaactt148 | ♂ | 16 | 5.88 ± 1.03 | 8.60 ± 0.61 | 2.26 | 0.029 | 4.188 |
| BW | UNH104 | ♀ | 1* | 9.30 ± 0.74 | 6.76 ± 0.65 | 2.57 | 0.013 | 5.182 |
| BW | gaaccg358 | ♀ | – | 6.20 ± 0.83 | 9.04 ± 0.66 | 2.69 | 0.01 | 5.182 |
| BW | gcacat410 | ♀ | Fb | 9.12 ± 0.76 | 6.64 ± 0.61 | 2.53 | 0.015 | 5.182 |
| CDD | UNH123 | ♂ | 12 | 71.71 ± 2.47 | 62.21 ± 1.75 | 3.14 | 0.003 | 4.130 |
| CDD | gaacta103 | ♂ | 12 | 70.24 ± 2.34 | 62.54 ± 1.73 | 2.64 | 0.011 | 4.130 |
| CDD | UNH179 | ♂ | 7 | 69.14 ± 1.85 | 60.67 ± 2.03 | 3.19 | 0.003 | 4.130 |
| CDD | gaacat289 | ♂ | Mb | 60.80 ± 2.72 | 68.05 ± 1.99 | 2.15 | 0.037 | 4.130 |
| CDD | gcaccc706 | ♂ | 7 | 68.68 ± 1.83 | 59.18 ± 2.30 | 3.23 | 0.002 | 4.130 |
| CDD | gaacaa109 | ♂ | 1 | 61.00 ± 2.10 | 68.69 ± 1.91 | 2.71 | 0.009 | 4.130 |
| CDD | UNH228 | ♂ | 2 | 68.12 ± 2.19 | 62.19 ± 1.96 | 2.02 | 0.049 | 4.130 |
| CDD | gaacat49 | ♂ | Mk | 62.04 ± 2.22 | 68.74 ± 2.27 | 2.11 | 0.041 | 4.130 |
| CDD | gcacat67 | ♂ | Mk | 61.23 ± 1.99 | 68.98 ± 1.92 | 2.80 | 0.007 | 4.130 |
| CDD | UNH124 | ♀ | 4b | 69.39 ± 2.41 | 62.73 ± 1.99 | 2.15 | 0.037 | 4.623 |
| CDD | UNH169 | ♀ | 5 | 62.50 ± 1.89 | 70.68 ± 2.37 | 2.70 | 0.01 | 4.623 |
| CDD | gaacat158 | ♀ | Fd | 62.29 ± 2.13 | 70.20 ± 2.18 | 2.60 | 0.013 | 4.623 |
| CDD | gaacta118 | ♀ | Fd | 68.66 ± 2.09 | 62.59 ± 1.97 | 2.11 | 0.040 | 4.623 |
| (b) | | | | | | | | |
| CDD | UNH130 | ♂ | 23 | 63.15 ± 1.98 | 71.26 ± 1.68 | 3.09 | 0.004 | 3.208 |

The linkage group (LG) numbers refer to the group numbers in the female or male maps, according to which parent is tested. Mean A and Mean B are the trait means in the groups of offspring having inherited marker allele A or B, respectively, from the parent in question. The *t*-threshold is the threshold value for rejecting the null hypothesis of no QTL at experiment-wise critical value $P < 0.05$ (meaning that the threshold in each of the four bootstraps within an experiment was at $P < 0.0125$).

*Linkage group 1 in the male map; the marker was unlinked in the female map.

been included in experiment 2 because of a significant association to weight in the experiment 1 (Table 2a).

Because of the association between UNH130 and cold tolerance detected in experiment 2, the corresponding data from experiment 1 was re-investigated. It was found that the *P*-value of the corresponding point-wise test in experiment one was 0.16. When data from the two families were combined into one *t*-test, the association was point-wise significant ($P < 0.05$).

The grandparental origin of alleles segregating in the male parent at UNH130 could unfortunately not be determined, since the genotypes of the *O. niloticus* and *S. galilaeus* grandparents were not available.

### Experiment 3: interval mapping

It was decided that linkage group 23 should be investigated more closely, by interval mapping. The reason for the decision was the identification of a putative QTL for growth on this linkage group in experiment 1, and the identification of a putative QTL for cold tolerance in experiment 2. An additional motivating factor was the recent report of QTL for the same traits being found on the same linkage group in another study (Cnaani *et al.* 2003).

Nine additional microsatellites from linkage group 23 were selected from the UNH map. Family 2 was then genotyped for these microsatellites, and interval mapping was performed. The likelihood curve (Fig. 3, Table 3) indicates that there are two QTL for cold tolerance on this linkage group, segregating in the male parent. However, the 95% confidence interval for QTL position covers the whole linkage group, meaning that neither the positions of the QTL peaks nor the indicated number of QTL should be given too much weight. In lack of stronger evidence, we therefore maintained the hypothesis of one QTL. The cold-tolerance QTL segregating in the male parent was chromosome-wide significant at $P < 0.05$, meaning that it remained significant after multiple testing across the linkage group was taken into account.

From the likelihood plots, there are also indications of a QTL for cold tolerance segregating in the female parent. The QTL is not chromosome-wide significant at $P < 0.05$, but when the microsatellite located closest to the QTL peak (UNH180) was analysed using a point-wise *t*-test, the test was significant at $P < 0.05$. Thus, there were indications of a cold-tolerance QTL on linkage group 23 segregating in the female parent as well (female segregation on this linkage group was not investigated in the earlier *t*-test because the only microsatellite from the linkage group genotyped in experiment two – UNH130 – was not informative in the female parent).

From the likelihood plots, it is also evident that there is no QTL for body weight on linkage group 23 segregating in this family.

## Discussion

Since AFLPs are dominant markers, alleles generally cannot be traced from offspring to parent if both

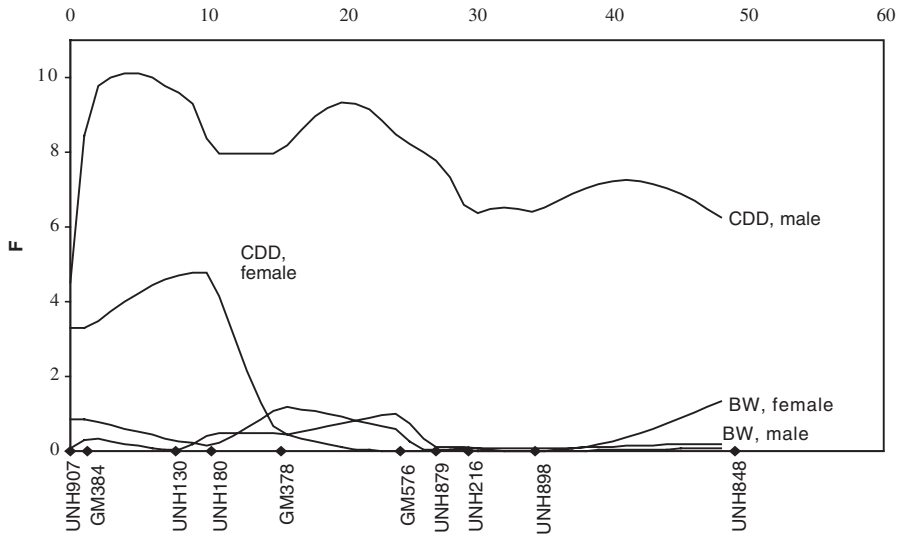


**Figure 3** Interval mapping of chromosome 23, with marker points indicated.

  

**Table 3** Interval mapping, output from QTL express

| Parent | Trait | Position of QTL | *F* | *F*-statistic threshold, $P < 0.05$ | *F*-statistic threshold, $P < 0.01$ |
|---|---|---|---|---|---|
| ♂ | CDD | 5 cM | 10.11 | 6.39 | 10.35 |
| ♀ | CDD | 10 cM | 4.79 | 6.61 | 9.86 |
| ♂ | BW | – | 0.48 | 6.71 | 11.12 |
| ♀ | BW | – | 1.32 | 6.65 | 10.69 |

The position of the QTL is defined as the position with the highest test statistic (*F*) in the test for QTL. The test is chromosome-wise significant (i.e. significant after testing of multiple markers across the linkage group has been taken into account) if *F* is greater than the *F*-statistic threshold. CDD, cold-day-degrees; BW, body weight.

parents are heterozygous. The usefulness for linkage analysis of AFLP markers having these parental genotypes is therefore limited, particularly if the family sizes are small. As a consequence, we chose to use only AFLP markers that had one heterozygous parent and one that was homozygous for the null allele. These AFLP markers, however, are only informative for one parent at a time, so that the microsatellites represented the only bridges between the maps. Since the set of markers informative in both sexes therefore became rather small, we decided to make separate male and female maps.

The lengths of the maps are rather short, 561 cM (male) and 524 cM (female). At the same time, 20% of the male informative markers and 15% of the female informative markers were not mapped. Due to the simple nature of the data set and the fact that every marker used in the linkage mapping carried about the same amount of information, it is likely that the unlinked markers were too far away from other markers to be connected to the map. The length of the UNH map is 1311 cM, so it is likely that less than half of the tilapia genome has been covered by the maps presented here. Although there was generally a one-to-one relationship between linkage groups in our maps and the UNH map, in some cases two linkage groups in our maps corresponded to the same UNH linkage group. This could be due to chromosome polymorphism, but a more likely reason is that some linkages were not detected in our data due to the small family size.

The multiple testing problem is a recurring issue in QTL mapping. The more tests performed, the more likely it is that the outcome of some will be positive just by chance. One way to deal with this problem is to see if the QTL can be replicated in additional, independent trials (Lander & Kruglyak 1995). Consequently, if one regards experiment-wise significance in both subexperiments as the criteria for significance in this study, no QTL has been found here. However, these are very strict criteria, and there are reasons for considering the cold-tolerance QTL identified on linkage group 23 as a putative QTL. First, it was close to being experiment-wise significant in the male parent in experiment 2. Second, it was point-wise significant in the female in experiment 2. Third, the *P*-value for the male parent in experiment one was quite low ($P = 0.16$), though not significant. Fourth, a QTL for the same trait on the same linkage group was recently reported in another study (Cnaani *et al.* 2003).

In Cnaani *et al.* (2003), linkage group 23 was also found to harbour a QTL for body weight. In our study, we found a putative QTL for growth on linkage group 23 in the family 1, but there were no sign of any body weight QTL in the family 2. Furthermore, since the association detected in experiment 1 was not highly significant, we conclude that there is little evidence of a body weight QTL in our study. It is not surprising that a QTL for body weight was not found, given that the extremes of the size distribution were not used for the cold challenge.

When the results from this paper are viewed together with the results from Cnaani *et al.* (2003), there does seem to be quite good indications that there is one or more QTL for cold tolerance on linkage group 23. The positions of the QTL, however, do not correspond well between the two studies. Cnaani *et al.* (2003) reported the peak of their QTL to be located very close to the marker UNH879, which is located at 38 cM in their map but at 27 cM in the present paper. This is approximately the same position as the second highest peak found in the male parent in the present study, but almost 20 cM away from the highest peak. Thus, it is possible that we have in fact detected two cold-tolerance QTL on this linkage group, with one of them being the same as the QTL reported in Cnaani *et al.* (2003). Alternatively, it could be that we have detected only one QTL, which may or may not be the same as the one detected in the earlier

study. We could not distinguish between these two options given the statistical power of this experiment.

Cnaani *et al.* (2003) detected the cold-tolerance QTL on linkage group 23 in a $F_2$ population resulting from a *O. aureus* × *O. mossambicus* cross. In the present study, the QTL was detected primarily in the male parent, coming from a cross between *O. niloticus* and *S. galilaeus*, but there were also indications of a QTL segregating in the female parent of family two, coming from a *O. aureus* × *O. mossambicus* cross. Thus, if the same QTL has been detected in the different crosses, it is not restricted to any particular cross. Unfortunately, the origin of the alleles segregating in the male parent could not be determined, since grandparental genotypes were not available on the paternal side. On the maternal side, the cold-tolerance allele seemed to come from the *O. mossambicus* rather than the *O. aureus* grandparent. Cnaani *et al.* (2003) also found the cold-tolerance allele to come from *O. mossambicus*. These findings are somewhat unexpected, since studies have shown *O. mossambicus* to be less cold tolerant than *O. aureus* (Behrends *et al.* 1990; Cnaani *et al.* 2000).

The families used in this study were of small sizes, giving reduced power for QTL detection and small chances of locating the QTL to a precise area. On the other hand, the small family sizes indicate that the putative QTL have large effects, since they would else not have been detected (Lynch & Walsh 1998). A follow-up of this study should be performed, to confirm the existence of the putative QTL with more certainty, and to map it with more precision. The follow-up study should include trials with much larger family sizes and denser marker maps. Then, the confidence interval of the QTL could typically be narrowed down to a fraction of a linkage group using traditional QTL mapping methods. After that, fine-mapping methods, like combined linkage and linkage disequilibrium mapping (Meuwissen, Karlsen, Lien, Olsaker & Goddard 2002) could be used to narrow down the QTL region further, but it would require data from multiple families and even denser marker maps. If the confidence interval could be made as low as a few cM, one could consider the positional cloning of the QTL, that is, the identification and cloning of the underlying gene(s). There are now many examples of successful positional cloning studies (e.g. Freking, Murphy, Wylie, Rhodes, Keele, Leymaster, Jirtle & Smith 2002; Grisart, Coppieters, Farnir, Karim, Ford, Berzi, Cambisano, Mni, Reid, Simon, Spelman, Georges & Snell 2002; Takeda, Takami, Oguni, Tsuji, Yoneda, Sato, Ihara, Itoh, Kata, Mishina, Womack, Moritomo, Sugimoto & Kunieda 2002), but since the genomic resources in tilapia and other aquaculture species are far less developed than such resources in the major livestock and model species, positional cloning in tilapia would be a big task. However, such attempts would be very valuable since they would generate much genetic information and resources along the way. Furthermore, if gene(s) responsible for variation in cold tolerance are identified, it will be important for our understanding of the genetics behind the trait.

Given further research, this QTL could be used as a tool in MAS or marker-assisted introgression (MAI) (Davis & DeNise 1998; Dekkers & Hospital 2002). There are prospects for the use of these methods in breeding on aquaculture species (Poompuang & Hallerman 1997; Davis & Hetzel 2000; Fjalestad, Moen & Gomez-Raya 2003), but in general, more research is needed before the methods can safely and cost-efficiently be taken into use in aquaculture. In addition to a more precise mapping of any QTL, one needs to know more about their modes of inheritance (additive, dominant), their effects on other traits (pleiotropy), the interactions between different QTL (epistasis), the magnitude of their effects and the amount of linkage disequilibrium in their genomic surroundings. However, cold tolerance is an example of a trait where MAS and MAI could in the end prove to be useful, since the alternative, challenge testing for cold tolerance, is a quite large and expensive operation.

In conclusion, we have presented in this paper supportive evidence for the existence of a QTL for cold tolerance on tilapia linkage group 23. If strict criteria of experiment-wise significance are applied, the QTL is not significant. Using less strict criteria, there are indications that the QTL is segregating in two out of three parents investigated in this study. This, together, with the identification of this QTL in a previous study, gives much credit to the existence of a cold-tolerance QTL on this linkage group.

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