Genetic mapping of Tilapiine fishes

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Introduction

Selective breeding is a powerful tool for improving the performance of domesticated species, and has been employed with spectacular success to improve production of both animal and plant crops. Selective breeding is also essential to maintain the performance of superior stocks, as these stocks will tend to decline over time. This is particularly true in fish, where opposing selective pressures frequently reduce growth rate, and encourage early reproduction.

Most production traits are not controlled by single Mendelian genes. Rather, they depend on the effects of a number of genes, each of which contributes to the phenotype. The large number of highly polymorphic genetic markers which can now be developed for any species make it possible to identify the genes contributing to particular phenotypic traits. This information can be used to more directly select for gene contributing to high performance.

The goal of our study was to develop a comprehensive map of *O. niloticus* using DNA polymorphisms, which might be suitable for analysis of quantitative traits. Our approach was to study the segregation of these polymorphisms in the haploid progeny of a single female *O. niloticus*.

Materials and methods

Haploid gynogenesis

Milt was collected from, *O.niloticus* into glass capillary tubes and diluted to 2.5x 10^7 sperm/ml (HUSSAIN *et al.*, 1993) in modified fish Ringers solution (0.1M NACl; 40mM KCl; 1.4mM $CaCl_2$ $2H_20$; 2mM $NaHCO3$, pH adjusted to 8.0). One ml of diluted milt was

Typing of microsatellites

Genotypes were obtained by automated sizing of fluorescently

Linkage analysis

We used the Macintosh porting (ver. 2.0) of Mapmaker (LANDER *et al.*, 1987) to identify linkage groups and determine marker order. An initial grouping of markers was performed with a LOD cutoff of 3.0. Because of the high levels of interference observed, final map distances were calculated using the Kosambi function (OTT, 1991).

Result

Genotypes

The parent female and six haploid progeny were screened for a total of 147 microsatellites. The mother was heterozygous for 63 (43%)

per group. Twenty-four linkage groups contain at least one microsatellite polymorphism.

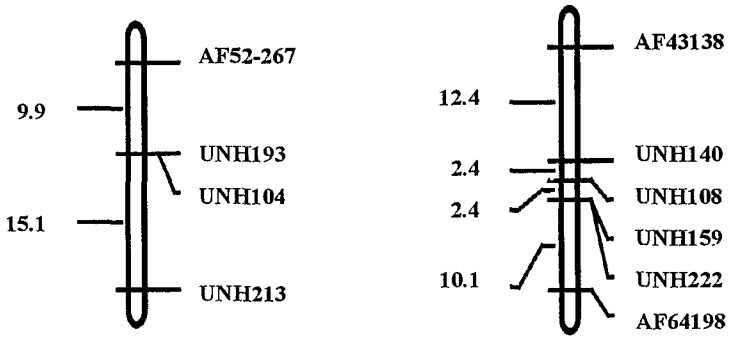

Figure 1
Part of the current linkage map for *Oreochromis niloticus* (two from the thirty linkage groups). The DNA markers fall in 30 linkage groups. Microsatellite loci (in bold) are identified with a combination of letters and numbers to designate the institution which developed the marker (UNH=University of New Hampshire). AFLP markers are designated AF, followed by two digits to indicate the primer combination and three digits to indicate the size of the scored fragment. Numbers to the left of each interval indicate the recombination distance (cM) between the markers.

Estimates of genome size

HULBERT *et al.* (1988) suggest that the ultimate map length can be estimated by observing the proportion of locus pairs linked at specific distances, and comparing this to an expectation based on the assumption that the loci are distributed randomly across the map. We performed these calculations separately for each marker type at four distances. When we analysed the proportion of pairs exhibiting less than 5% recombination, all combinations of marker pairs gave similar estimates of genome size, ranging from 412cM for the AFLP to 668cM for AFLP/micro pairs. These estimates are all smaller than the spanned length of our map. For larger intervals, the estimates are less consistent, and for recombination fractions of

20%, the genome size estimates range from 740 to 1,719cM. Our best estimate is that the genome is about 1,200cM in length.

Discussion

Strategies for QTL mapping

Microsatellites have become the preferred marker for animal gene mapping because of their high heterozygosity and ease of typing via PCR. AFLP is a new approach which offers rapid marker development and typing, but which has a higher error rate, and is less comparable across experiments than microsatellites. It may be possible to use a mixed strategy for mapping quantitative trait loci (QTL). High-density AFLP maps may be anchored with a much smaller set of microsatellite loci. We have already mapped at least one microsatellite on 24 of the 30 linkage groups, and it seems likely that we have mapped at least one 1 microsatellite on each chromosome. The 62 microsatellites we have characterized ensure a 95% probability of uniquely identifying each chromosome with a microsatellite locus in an MS-AFLP map. These anchor loci will allow comparison of AFLP maps produced for QTL analyses in different laboratories.

The Next Step

We have several goals in continuing this line of research. The first is the identification of QTL in different strains of tilapia which might be usefully combined to produce a faster growing tilapia. The map we have constructed is adequate for that purpose. Although we cannot expect that all 62 of these microsatellite markers will be variable in other crosses, we will continue to score the other 84 microsatellites already characterized, and hope eventually to incorporate all of them into the map. Inclusion of 50-60 microsatellites in each experimental cross will be sufficient

to identify homologous chromosomes. Marker density is most

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