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 placed into a small petri dish and irradiated with a UV dose of 290-295 microWatts/cm squared for exactly 2 minutes using a 254 nm UV lamp. 600-1000 eggs were stripped from an *O.niloticus* female and fertilized with the irradiated milt. Fry were collected 2-3 days post-hatching, at which point 75 haploids were recovered.

Genomic DNA extraction

After removal of the yolk-sac, fry were placed into individual sterile 1.5ml microcentrifuge tubes containing 150µl TEN buffer (100mM Tris-HCl, pH8.0; 10mM EDTA; 250mM NACl), 10 microliters 20% SDS and 5µl proteinase K (10mg/ml stock). Tubes were placed in water bath at 37°C overnight or at 55°C for a few hours. Two phenol and one chloroform/IAA (24: 1) extractions were carried out. DNA was precipitated using isopropanol. Approximately 1 µg of DNA was obtained from each embryo.

Microsatellite markers

The majority of microsatellite loci scored consisted of 139 di- and tri-nucleotide repeats isolated from an enriched *O. niloticus* genomic DNA library (LEE and KOCHER, 1996). An additional six loci isolated from *O. shiranus* (AMBALI, 1996) were also tested. Four markers isolated from Lake Malawi haplochromines were examined, including two loci from *Pseudotropheus zebra* (PARKER and KORNFIELD, 1996) and two from *Melanochromis auratus* (KELLOGG *et al.*, 1995).

Typing of microsatellites

Genotypes were obtained by automated sizing of fluorescently-tagged alleles amplified via PCR. We used a 25µl reaction volume containing 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.4mM $MgCl_2$, 0.16mM each dNTP, and 0.16µM each primer, to which we added 20 ng of haploid genomic DNA. The PCR conditions were as follows: 95°C 1min, 50-58°C 2min, 72°C 2min, and 25-30 thermal cycles according to the efficiency of amplification. For sizing, 1 µl from as many as three different PCR reactions were combined into a new tube, and dried in a speed-vac. The pellet was resuspended with both 0.3 µl of GeneScan 500 Tamra size standard (Applied Biosystems Inc., Foster City, CA) and 2.7 µl of formamide loading buffer. After denaturation at 90°C for 2min, the entire solution was loaded on a 6% acrylamide gel on an ABI 373A automated DNA sequencer. ABI GeneScan software (ver. 2.02) was used to analyze the genotypes of the micro-satellite loci.

AFLP markers

Vos *et al.* (1995) described a new technique for analysis of Anonymous Fragment Length Polymorphisms. We used the Perkin-Elmer AFLP plant mapping kit (Rev. A). A total of 250 ng of haploid genomic DNA was used in the initial ligation step. For the selective amplification step, we tested 22 primer pairs on a panel of six haploid progeny. One µl of each selective amplification product, together with the GeneScan 500 Rox size standard was loaded in each lane of the 6% gel on the automated sequencer. The frequency of variable bands per primer pair ranged from 0 (ACT+CAC and ACC+CAG) to 15 (AGG+CTT). Those primer combinations generating more than 5 variable markers were selected for typing the remaining 35 haploids. These primer pairs were [EcoRI+MseI (number of variable bands)]: ACT+CTA (7), AGG+CTG (10), ACA+CAA (11), AGG+CTT (15), ACA+CAC (10), ACT+CAT (13), AGC+CTA (10), ACC+CAA (6), ACT+CAG (11), AGC+CAT (6), AGG+CTA (7), and AGC+CAG (9).

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 62 (42%) of these markers. An additional 36 haploids were scored for these 62 microsatellites. We also scored the 41 haploids for 12 AFLP primer combinations, which identified 112 AFLP polymorphisms. The final data set consisted of genotypes of 62 microsatellites and 112 AFLP polymorphisms for 41 haploid progeny of the single female.

Linkage map

Overall, 93.1 % of the markers tested showed detectable linkage to another marker. 59 of the 62 microsatellites (95%), and 103 of 112 (92%) of the AFLP's were detectably linked to another polymorphism. The final linkage map consists of 30 linkage groups spanning 704cM (Figure 1 shows two of them). A total of 162 polymorphisms are included, for an average spacing of 4.3cM. The size of the linkage groups ranges from 0 to 73.6 cM (mean 23.5cM). The number of markers per linkage group varies from 2 to 28, with an average of 2 microsatellites and 3.4 AFLP markers

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

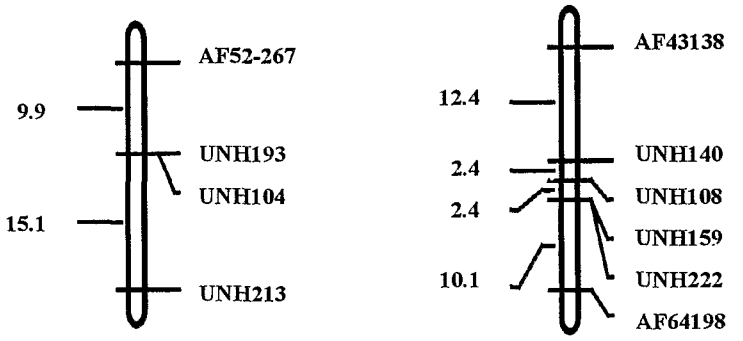

Figure 1
Part of the current linkage map for *Oreochromis niloticus* (two from the thirty linkage groups). The DNA markers fall in 30 linkage groups. Microsatellite loci (in bold) are identified with a combination of letters and numbers to designate the institution which developed the marker (UNH=University 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 conveniently increased in each cross through the typing of AFLP markers.

A second goal is to use these genetic markers to characterize germplasm resources of tilapia. Preliminary work suggests that microsatellites are a useful way to estimate heterozygosity of stocks, and will be very useful for tracking parentage in selection experiments. Preliminary AFLP data suggests that this technique will be useful for classifying tilapia strains to species or identifying their probably hybrid origins.

Finally, we plan to extend our mapping efforts to other groups of cichlids, particularly the species flock of Lake Malawi haplochromines. A large proportion of our tilapia markers also amplify Lake Malawi cichlids, and it will be interesting to determine the extent of synteny with this group. A genetic map for these fish would allow QTL mapping of traits associated with speciation and adaptive radiation in this group.

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References

AMBALI (A.), 1996 — *The relationship between domestication and genetic diversity of* Oreochromis *species in Malawi:* Oreochromis shiranus shiranus *(BOULENGER) and* Oreochromis shiranus chilwae *(TREWAVAS)*. Ph.D. thesis, Dalhousie University, Halifax, NS.

HULBERT (S. H.), ILOTT (T. W.), LEGG (E. J.), LINCOLN (S. E.), LANDER (E. S.), MICHELMORE (R. W.), 1988 — Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. *Genetics*, 120: 947-958.

HUSSAIN (M.G.), PENMAN (D.J), MCANDREW (B.J), JOHNSTONE (R.), 1993 — Suppression of first cleavage in the Nile tilapia, *O.niloticus* L.- a comparison of the relative effectiveness of pressure and heat shocks. *Aquaculture*, 111: 263-270.

KELLOGG (K.A.), MARKERT (J.A.), STAUFFER JR. (J.R.), KOCHER(T.D.), 1995 — Quantifying multiple paternity in Lake Malawi cichlid fish. *Proc. Roy. Soc. London Ser. B.*, 260: 79-84.

LANDER (E.), GREEN (P.), ABRAHAMSON (J.), BARLOW (A.), DALEY (M.), LINCOLN (S.), NEWBURG (L.), 1987 — MAPMAKER: An Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. *Genomics*, 1: 174-181.

LEE (W.J.), KOCHER (T. D), 1996 — Microsatellite DNA markers for genetic mapping in the tilapia, *Oreochromis niloticus. J. Fish Biology*, 49: 169-171.

OTT (J.), 1991 — *Analysis of human genetic linkage.* John Hopkins University Press, Baltimore ND.

PARKER (A.), KORNFIELD (I.), 1996 — Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Env. Biol. Fish.*, 47: 345-352.

VOS (P.), HOGERS (R.), BLEEKER (M.), REIJANS (M.), VAN DE LEE (T.), HORNES (M.), FRIJTERS (A.), POT (J.), PELEMAN (J.), KUIPER (M.), ZABEAU (M.), 1995 — AFLP : a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23: 4407-4414.