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Linkage mapping of the blond locus in Nile tilapia (*Oreochromis niloticus* L.) and preliminary analysis of its effect on blotching in red Nile tilapia

Amy H. Rajaee [1], Christos Palaiokostas, Ved P. Saini [2], Brendan J. McAndrew, David J. Penman *

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom

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ABSTRACT

Black blotching of the skin in red tilapia is undesired by tilapia producers since it affects their marketability. This study was carried out with the aims of mapping the blond locus and testing whether the homozygous recessive blond gene reduces or removes blotching in red tilapia by studying the interaction between the red and blond genes. Two types of mapping families (two intraspecific and one interspecific) were used for the mapping of the blond locus. Genome-wide mapping using microsatellites was first carried out to identify molecular markers that were linked to the blond locus. The locus was mapped in linkage group (LG) 5 in an interval that varied between 7.5 and 20.8 cM, depending on the tested family. In the intraspecific families the blond locus was closely linked to the microsatellites UNH309, UNH169 and GM017 ($P < 10^{-11}$), while in the interspecific family microsatellite GM636 ($P < 10^{-8}$) was also found to be significantly associated, in addition to the above three microsatellites. Linked markers were used to identify the genotype of individual fish for the blond and red loci from the specifically designed crosses so the effect of recessive blond gene on blotched fish could be assessed. The underlying blond trait did not significantly reduce the area of blotching but did reduce the saturation (paler blotching) and enhanced the redness of body colour in the homozygous blond fish compared to the heterozygous (wild type) group.

1. Introduction

Tilapias belong to the family Cichlidae and are abundant in tropical and subtropical regions. Tilapias are farmed close to 140 countries (FAO, 2014). Nile tilapia (*Oreochromis niloticus*) is the most important farmed species with a production exceeding 2.8 million metric tonnes in 2010 (FAO, 2012). Red coloured tilapia are more appreciated than the wild type coloured fish in some markets (Mather et al., 2001; Thodesen et al., 2013). The red body colour originates from *O. niloticus* (McAndrew et al., 1988) or *O. mossambicus* (Mather et al., 2001), the latter commonly present in red tilapia hybrids used in brackishwater aquaculture. Linkage mapping places the gene controlling red body colour from each species in an overlapping region in linkage group 3 (Howe, 2004). The red colour allele in *O. niloticus* is dominant to the wild type colour allele but in *O. mossambicus* the effect is additive, although this might be due to different genes or mutations in the melanin biosynthesis pathway (Howe, 2004; Mather et al., 2001; McAndrew et al., 1988).

A significant problem in the case of red tilapias (Nile, Mozambique and various hybrids) is the appearance of black blotches on the surface of the fish. These blotches reduce market acceptability (Mather et al., 2001; Thodesen et al., 2013). The blotches can cover up to 25% of the fish surface, including the peritoneum, and coverage is greater in heterozygous than in homozygous red *O. niloticus* (McAndrew et al., 1988). Selective breeding to reduce blotching has been attempted in different populations of red tilapia. Garduño-Lugo et al. (2004) applied mass selection in red *O. niloticus*. They observed significant reduction in the degree of blotching from the first generation to the fifth generation, at least part of which was due to elimination of the wild type allele (r) as wild type (rr genotypes) were removed in each generation. Mather et al. (2001) also used mass selection to reduce black blotching, but in a Fijian stock originating from a hybrid between *O. niloticus* and *O. mossambicus*, in which the red colour originated from *O. mossambicus*. They also succeeded in reducing blotching, in this case primarily in the heterozygous Rr genotype (RR in this case is pink, with reduced vigour), and concluded that this was done without any correlated negative effect on growth performance. However, Thodesen et al. (2013) found a positive correlation between growth and black blotches in a breeding programme based on a red tilapia population derived from eight different stocks (four of Asian origin and four of South American origin: no details given of the species origin of the red alleles).

Scott et al. (1987) and McAndrew et al. (1988) reported a third, recessive colour variant in the Stirling strain of Nile tilapia, known as

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* Corresponding author. Tel.: +44 1786467901; fax: +44 1786 472133.
E-mail address: d.j.penman@stir.ac.uk (D.J. Penman).

[1] Present address: Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97000 Bintulu, Sarawak, Malaysia.

[2] Present address: Aquaculture Research Unit, Directorate of Research, MPUAT Udaipur, 313001, Rajasthan, India.

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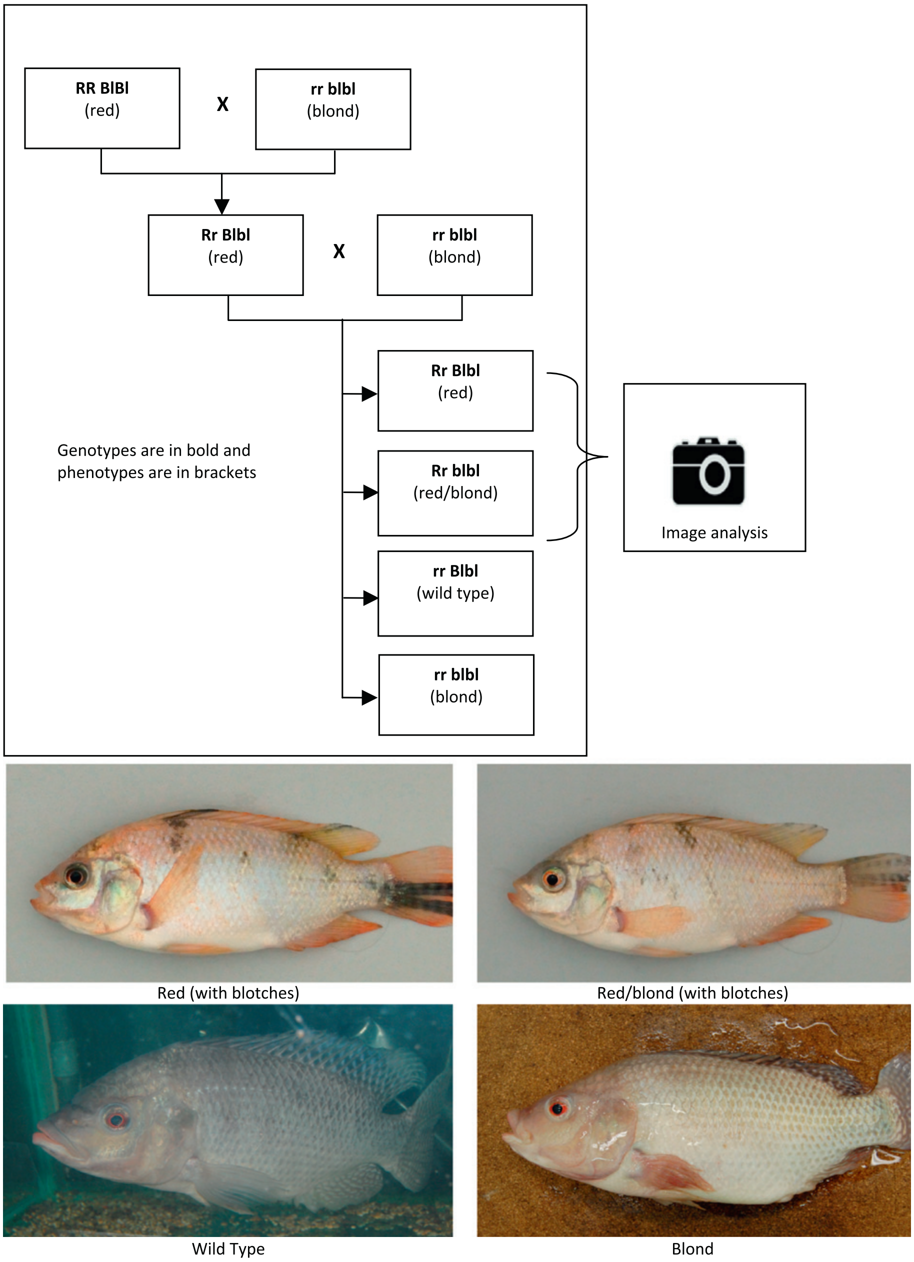

Red (with blotches) Red/blond (with blotches)

Wild Type Blond

Fig. 1. The upper part shows the experimental design for image analysis, while the lower part shows pictures of fish with the four different genotypes (note that for the red and red/blond fish, individuals with heavy blotching were selected to illustrate the effect of blond on the blotches).

blond (or bronze). The normal wild type colouration of Nile tilapia is dark gray in colour with vertical stripes containing melanophores. The blond tilapia can be confused with the normal wild type colour: it is paler with fainter stripes than the wild type (Scott et al., 1987). The blond phenotype can be distinguished easily under anaesthesia or by exposing the fish to a darker background as the wild type usually darkens, while the blond tends to stay paler than the wild type. In blond tilapia, melanophores are smaller in size compared to the wild type and red blotched fish (McAndrew et al., 1988). While an all-blond Stirling strain is already used for aquaculture in Mexico (personal observation, B.J. McAndrew), combining the blond and red phenotypes may help in reducing the black blotches or spots found in the latter.

Currently no information exists concerning the genomic location of the blond locus. Since the blond phenotype may be masked by red, a study on the interaction between these genes would be assisted by molecular markers linked to the blond locus. A microsatellite-based linkage map of the tilapia has been published by Lee et al. (2005) and linkage mapping for this study was carried out using markers from this linkage map. The objectives of this study were (i) to map the blond locus and (ii) to study the effect of blond on blotching in red Nile tilapia.

2. Materials and methods

2.1. Mapping families

Two intraspecific families (*Oreochromis niloticus* blond female × wild type male; backcrossed to a blond female) and one interspecific family (*O. aureus* female × *O. niloticus* blond male; backcrossed to a blond *O. niloticus* male) were produced for mapping the blond locus. The offspring from these crosses were scored either as blond (blbl; 20 offspring on each family) or wild type (Blbl; 20 offpsring on each family). The interspecific family (20 offspring scored as blond and 14 offspring scored as wild type) was produced to provide additional polymorphic markers for the linkage map. Caudal fin biopsies were collected from the offspring