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SCIENCE DIRECT
Gene 373 (2006) 126–133
GENE
SECTION
EVOLUTIONARY GENOMICS
www.elsevier.com/locate/gene

magp4 gene may contribute to the diversification of cichlid morphs and their speciation

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Received 22 September 2005; received in revised form 19 January 2006; accepted 22 January 2006
Available online 6 March 2006
Received by T. Sekiya

Abstract

Lake Victoria harbors more than 300 species of cichlid fish, which are adapted to a variety of ecological niches with various morphological species-specific features. However, it is believed that these species arose explosively within the last 14,000 years and transcripts among Lake Victoria cichlid species are almost identical in sequence. These data prompted us to develop a DNA chip assay to compare patterns of gene expression among cichlid species. We prepared a DNA chip spotted with 6240 elements derived from cichlid expressed sequence tag (EST) clones and successfully characterized gene expression differences between the cichlid species *Haplochromis chilotes* and *Haplochromis* sp. "rockkribensis". We identified 14 transcripts that were differentially expressed between these species at an early developmental stage, 15 days post-fertilization (dpf), and several were further analyzed using quantitative real-time PCR (qPCR). One of these differentially expressed transcripts was a homolog of microfibril-associated glycoprotein 4 (*magp4*), a putative causative gene for the human inherited disease, Smith–Magenis syndrome (SMS), for which facial defects are among the phenotypic features. Further analysis of *magp4* expression showed that *magp4* was expressed in the jaw portion of cichlid fry and that expression profiles between *Haplochromis chilotes* and *Haplochromis* sp. "rockkribensis" differed during development. These data suggest that the differential expression of a gene associated with human cranial morphogenesis may be involved in the diversification of cichlid jaw morphs.

Keywords: Jaw and teeth; DNA chip; Human inherited disease; Lake Victoria

1. Introduction

Cichlids are freshwater fishes widely distributed in tropical areas of the world and are especially numerous in the East African Great Lakes, Victoria, Malawi and Tanganyika (Fryer and Iles, 1972; Greenwood, 1981; Keenleyside, 1991). These lakes harbor more than 1000 cichlid species, which have developed many distinct morphological features, including body size, color pattern, and jaw and tooth shape. The shape of the jaw and teeth varies widely among cichlids, correlating strongly with feeding ecology (Greenwood, 1974; Leim, 1991). For example, a certain plankton-eating species has a very short, broad head and jaw, whereas a fish-eating species has a long snout and jaw. Other insect-eating species, such as *Haplochromis chilotes*, used in this study, have specialized lobed lips (Fig. 1a; Greenwood, 1974). With regard to tooth shape, bicuspid dentition is sometimes observed in cichlid species that feed on 'aufwuchs', the general biocover on the rocks, which consists of algae, bacteria and invertebrates. This is the case for *Haplochromis* sp. "rockkribensis", the second species used in this study (Fig. 1d; Seehausen, 1996). In contrast, some of the

Abbreviations: cDNA, DNA complementary to RNA; dpf, days post-fertilization; ECM, extracellular matrix; EST, expressed sequenced tag; qPCR, quantitative real-time PCR; SMS, Smith–Magenis syndrome.

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doi:10.1016/j.gene.2006.01.016

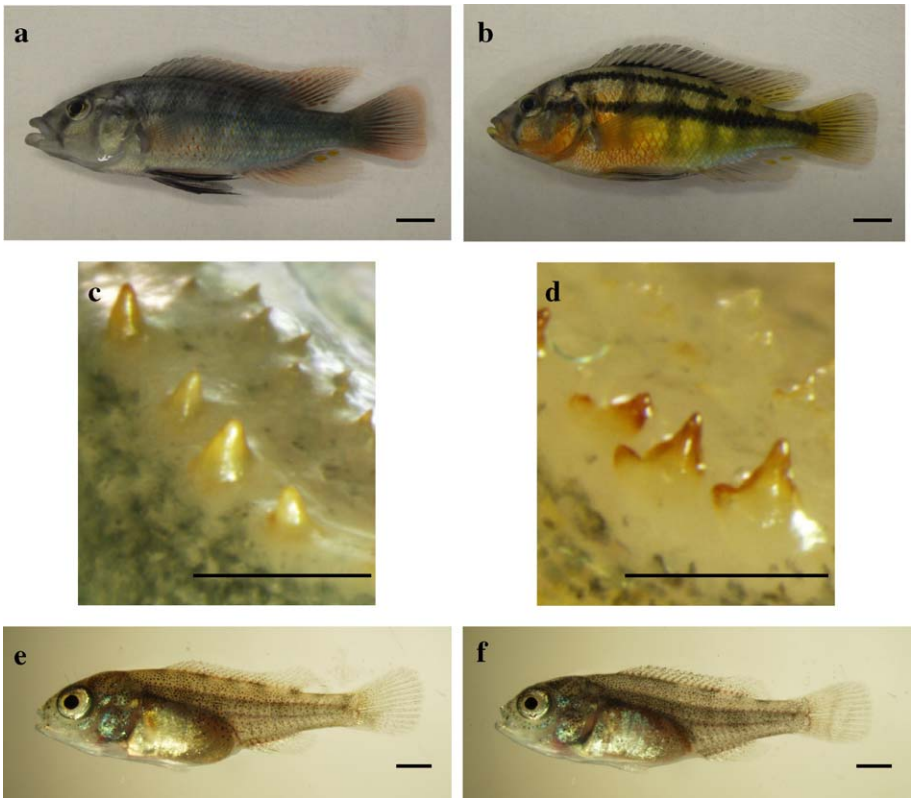

Fig. 1. Cichlid fishes used in this study. Cichlid specimens *Haplochromis chilotes* (a, c, and e) and *Haplochromis* sp. "rockkribensis" (b, d, and f). Adult male fish have blue/yellow nuptial colors on their body (a, b). The development of cichlid tooth shape correlates strongly with feeding ecology. *H. chilotes* has unicuspid dentition (c), whereas *Haplochromis* sp. "rockkribensis" has bicuspid dentition (d). mRNAs for DNA chip hybridization were prepared from the jaw portions of cichlid fry at 15 dpf (e, f). Scale bars in panels a and b indicate 10 mm and scale bars in panels c, d, e and f indicate 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other insect-eating species including *H. chilotes* have unicuspid dentition (Fig. 1c).

Among the cichlids species in the African Great Lakes, those from Lake Victoria serve as a model of vertebrate evolution because of their rapid and explosive speciation. It is believed that Lake Victoria dried up completely for thousands of years and refilled about 14,000 years ago (Johnson et al., 1996). The hundreds of cichlid species endemic to Lake Victoria are thought to have arisen within this short period. Molecular phylogenetic studies based on mitochondrial DNA, nuclear-encoded genes, and Short Interspersed Element (SINE) insertions have elucidated close phylogenetic relationships among the Lake Victoria cichlids (Meyer et al., 1990; Nagl et al., 1998, 2000; Terai et al., 2004). In addition, it was recently shown that expressed sequence tag (EST) sequences are nearly identical among cichlid species in the lake and that the genetic distance between these species is small (Watanabe et al., 2004). Still, some neutral intra-specific polymorphisms and inter-specific variations have been maintained. Accordingly, if specific mutations in certain genes are responsible for speciation between two closely related species, then these mutations must constitute the fixed difference between the two respective species. This provides an excellent system for identifying genetic changes that contribute to species-specific characteristics in cichlids. To identify genes that contribute to variations in oral and/or jaw shape, Albertson et al. (2003) performed quantitative trait loci (QTL) analysis for Lake Malawi cichlids, and localized more than ten loci that contribute to the diversity among cichlid species. In our present study, we have taken a different approach by identifying differences in gene expression that underlie the actual morphological differences between two closely related species. We had previously constructed cDNA libraries derived from the jaws of Lake Victoria cichlids and sequenced >20,000 cDNA clones (Watanabe et al., 2004, and unpublished data). We also constructed a BAC library for *H. chilotes* (Watanabe et al., 2003). Furthermore, we have constructed a cichlid DNA chip that can detect differences in gene expression between closely related species and screened for genes that are differentially expressed between two cichlid species (Kijimoto et al., 2005). In the present study, we constructed a new version of the cichlid DNA chip on which 6240 EST clones were spotted.

Because sequences of transcripts among the different Lake Victoria cichlids are almost identical and we can thus assume that they have the same hybridization efficiency, cichlids are especially advantageous for inter-species comparisons using a DNA chip assay. We chose two Lake Victoria cichlid species, *H. chilotes* and *Haplochromis* sp. "rockkribensis", for DNA chip analysis (Fig. 1). *H. chilotes* is a model Lake Victoria cichlid species that offers the following advantages for DNA chip analysis: 1) the species can easily be identified by morphologic characteristics; 2) *H. chilotes* is easy to breed due to its calm demeanor in the tank and its ability to thrive on commercial