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A microsatellite-based genetic linkage map and putative sex-determining genomic regions in Lake Victoria cichlids

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ABSTRACT

Cichlid fishes in East Africa have undergone extensive adaptive radiation, which has led to spectacular diversity in their morphology and ecology. To date, genetic linkage maps have been constructed for several tilapias (riverine), *Astatotilapia burtoni* (Lake Tanganyika), and hybrid lines of Lake Malawi cichlids to facilitate genome-wide comparative analyses. In the present study, we constructed a genetic linkage map of the hybrid line of Lake Victoria cichlids, so that maps of cichlids from all the major areas of East Africa will be available. The genetic linkage map shown here is derived from the F_2 progeny of an interspecific cross between *Haplochromis chilotes* and *Haplochromis sauvagei* and is based on 184 microsatellite and two single-nucleotide polymorphism (SNP) markers. Most of the microsatellite markers used in the present study were originally designed for other genetic linkage maps, allowing us to directly compare each linkage group (LG) among different cichlid groups. We found 25 LGs, the total length of which was 1133.2 cM with an average marker spacing of about 6.09 cM. Our subsequent linkage mapping analysis identified two putative sex-determining loci in cichlids. Interestingly, one of these two loci is located on cichlid LG5, on which the female heterogametic ZW locus and several quantitative trait loci (QTLs) related to adaptive evolution have been reported in Lake Malawi cichlids. We also found that *V1R1* and *V1R2*, candidate genes for the fish pheromone receptor, are located very close to the recently detected sex-determining locus on cichlid LG5. The genetic linkage map study presented here may provide a valuable foundation for studying the chromosomal evolution of East African cichlids and the possible role of sex chromosomes in generating their genomic diversity.

1. Introduction

The species flocks of the East African great lakes—Lakes Tanganyika (LT), Malawi (LM), and Victoria (LV)—with their several hundred endemic cichlid species, which are ecologically and morphologically highly diverse, are the textbook example of adaptive radiation (Fryer and Iles, 1972; Turner et al., 2001; Kocher, 2004). Phylogenetic and geographical studies suggest that the radiation of the cichlids in LT, LM, and LV occurred independently in different time periods. The ages of the cichlids in LT, LM and LV basin were estimated to be 10–12 million years ago (Cohen et al., 1993), less than 5 million (Genner et al., 2007) and less than 100,000 years ago (Verheyen et al., 2003). In particular, phylogenetic and population genetic studies indicate little differentiation among LV cichlid species based on the analysis of mitochondrial DNA (Meyer et al., 1990; Nagl et al., 2000; Mzighani et al., 2010), nuclear-encoded genes (Seehausen et al., 2003; Watanabe et al., 2004; Samonte et al., 2007; Kobayashi et al., 2009; Magalhaes et al., 2009; Takeda et al., 2013), and short interspersed elements (Terai et al., 2004). The genome-wide comparison of genetically similar but morphologically diverse cichlids is an excellent model for understanding the genetic mechanism of morphological diversification.

So far, genetic linkage maps using microsatellite markers have been constructed for the following cichlid groups: several tilapias (*Oreochromis niloticus* and *Oreochromis* spp.; Kocher et al., 1998; Lee et al., 2005), which are the basal lineages in the phylogenetic tree of the East African cichlids; the LT cichlid *Astatotilapia burtoni* (Sanetra et al., 2009); and a hybrid line of LM cichlids *Metriaclima zebra*/

Abbreviations: QTL, quantitative trait loci; SNP, single-nucleotide polymorphism; LV, Lake Victoria; LM, Lake Malawi; LT, Lake Tanganyika; LG, linkage group; cM, centimorgan; LOD, log-likelihood.

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Labeotropheus fuelleborni (Albertson et al., 2003; Albertson et al., 2005). In addition to the microsatellite-based genetic linkage maps, a restriction site-associated DNA linkage map of a hybrid line of LM cichlids, *Pseudotropheus elongatus*/*Cynotilapia afra*, was recently constructed (Parnell and Streelman, 2013). Furthermore, Guyon et al. (2012) constructed a high-resolution radiation hybrid (RH) map of *O. niloticus*, in which the whole genome of this species was organized into 22 LGs, which can be used as representative LGs of cichlids for deciphering their chromosomal evolution.

The genetic linkage maps are quite useful for understanding the chromosomal evolution, as well as genotype–phenotype relationships when QTL analyses are incorporated. In fact, several QTL analyses of LM cichlids have revealed the genomic regions associated with evolutionarily adaptive traits (Albertson et al., 2003; Albertson et al., 2005; Roberts et al., 2009) and sex determination (Roberts et al., 2009; Ser et al., 2010; Parnell and Streelman, 2013). The sex-determining regions of tilapias were identified by bulked segregation analysis, which was also based on the information from genetic linkage maps (Lee et al., 2003; Lee et al., 2004). The genetic linkage map-based analyses have provided valuable insight into the genetic mechanism of adaptive radiation of East African cichlids.

Although several genetic linkage maps are available for the cichlids of phylogenetically and geographically broad lineages (Fig. 1A, B, C), a genetic linkage map was not previously available for the LV cichlids. In this study, we constructed a microsatellite-based genetic linkage map derived from the F_2 hybrid line of two ecologically and morphologically different LV cichlids (*Haplochromis chilotes* and *Haplochromis sauvagei*; Fig. 1C). *H. chilotes* is the most unmistakable species among the rock-dwelling cichlids of LV (Seehausen, 1996), with its long snout, narrow teeth, and hypertrophied lip. This species is an insect larvae picker, although how the hypertrophied lip in *H. chilotes* is related to this dietary habit remains disputed (e.g., Greenwood, 1974). In contrast, *H. sauvagei* is a trophic generalist and feeds on 'aufwuchs', the biocover on the rocks, which consists of algae, bacteria, and invertebrates (Seehausen, 1996). The lip of *H. sauvagei* is not hypertrophied that is normal case in most fish including cichlids. Our group has focused on the above two cichlid species to understand the genetic mechanism underlying their differences in jaw morphology. Previous studies from our laboratory have included the construction of the bacterial artificial chromosome (BAC) library for *H. chilotes* that was used here (Watanabe et al., 2003), the generation of expressed sequence tags (ESTs) for *H. chilotes* (Watanabe et al., 2004; Kobayashi et al., 2009), and a DNA chip assay to characterize gene expression differences in the jaw tissues of *H. chilotes* and *H. sauvagei* (Kobayashi et al., 2006). In the present study, we constructed the linkage map of *H. chilotes*/*H. sauvagei*. The microsatellite linkage map of the *H. chilotes*/*H. sauvagei* hybrid consists of 25 LGs. Most of the microsatellite markers used in this map were originally designed for *M. zebra*/*L. fuelleborni* (Albertson et al., 2003) and *A. burtoni* (Sanetra et al., 2009). As most markers had been used in the previously published linkage maps, we were able to reveal correspondence of each LG of *H. chilotes*/*H. sauvagei* to the maps of the other cichlid species. We also performed a preliminary QTL analysis for putative sex-determining loci in *H. chilotes*/*H. sauvagei* and showed that sex determination in this hybrid might be controlled by at least two genomic regions, one of which was located on cichlid LG5, which was shown to be the ZW heterogametic sex chromosome in LM cichlids (Ser et al., 2010). Based on the comparison of linkage maps, we show here the correspondence relationships of each chromosome among East African cichlids and discuss the potential role of the sex chromosome in adaptive evolution.

2. Materials and methods

2.1. Population genetic analysis

To evaluate the degree of genetic differentiation between LV cichlids including *H. chilotes* and *H. sauvagei*, we estimated the genetic differentiation (F_{ST}) by the frequency of the haplotypes. We also estimated the genetic divergence between species pairs by calculating the average distances between species. We used the nucleotide sequences of the mitochondrial d-loop regions of *H. sauvagei*, *Lithochromis rubripinnis*, *Lithochromis rufus*, *Haplochromis pyrrhocephalus*, and *Haplochromis laparogramma*, which were published in Takeda et al. (2013). Additionally, we determined the d-loop sequences of *H. chilotes* (accession numbers AB973402–AB973428). Sampling localities, the number of samples of each cichlid individual used in the analysis is summarized in Supplementary Table S1. LV cichlid specimens used in the present study were collected during an exploration from 2005 to 2007 under the permission of Tanzania Commission for Science and Technology (COSTECH).

Tissues from fresh-caught fish were fixed in 100% ethanol and stored at 4 °C. DNA was extracted using the DNeasy Tissue kit (QIAGEN). Polymerase chain reaction (PCR) amplification and sequencing were performed as described (Maeda et al., 2009; Terai et al., 2004). The d-loop sequences were edited and visually inspected using the Sequencing Analysis version 3.7 software (Applied Biosystems) and Genetyx version 7.0 software (Genetyx Corporation). The F_{ST} values and genetic divergence between species were calculated using DNA Sequence Polymorphism (DnaSP) version 5.0 (Librado and Rozas, 2009) and MEGA version 6 (Tamura et al., 2013), respectively.

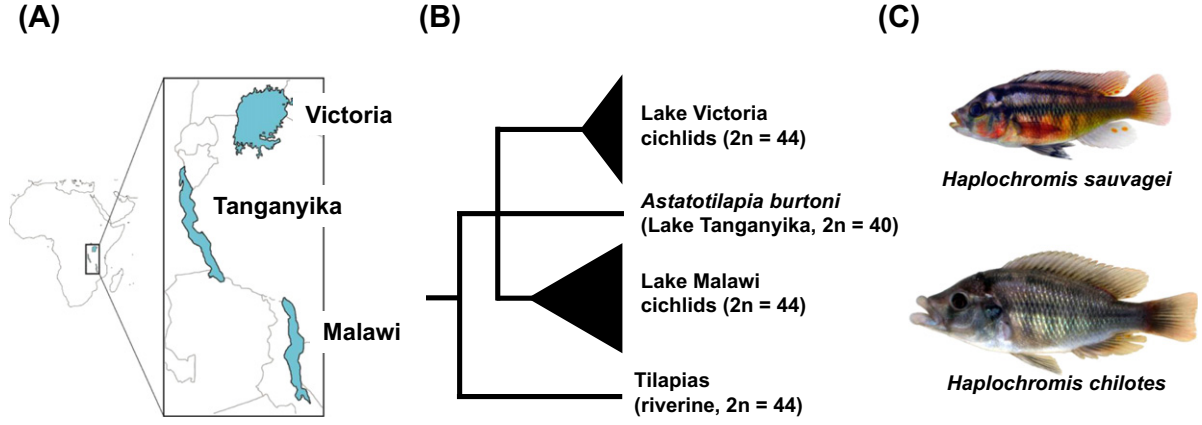

Fig. 1. The cichlids of East African great lakes. (A) The map of East African great lakes (lakes are shown in light blue). (B) Phylogenetic relationships among tilapias, *A. burtoni*, Lake Malawi cichlids, and Lake Victoria cichlids, which are based on mitochondrial DNA sequences (Salzburger et al., 2005). The number of diploid chromosomes was based on this study and previous studies (Poletto et al., 2010; Yoshida et al., 2011). (C) The two Lake Victoria cichlids (*Haplochromis sauvagei* and *H. chilotes*) used for construction of the genetic linkage map.