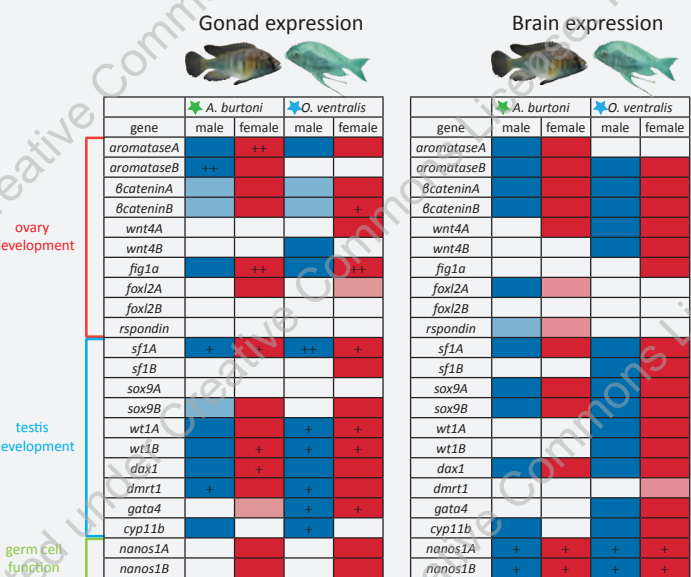# The evolution of sex determination and sexual differentiation in the species-flock of cichlid fishes of Lake Tanganyika, East Africa

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Introduction

The East African Great Lakes Tanganyika, Malawi and Victoria are home to one of the most fascinating examples of adaptive radiations: more than 1500 endemic cichlid species evolved in a few million years only, making these cichlid species-flocks prime model systems in evolutionary biology. Yet, the abiotic and biotic factors that triggered explosive speciation in these cichlids remain elusive. One hypothesis is that turnovers in sex-determining systems could be coupled with speciation. We investigate sex determination and sexual differentiation in the genetically, morphologically and ecologically most diverse radiation, the one in Lake Tanganyika.
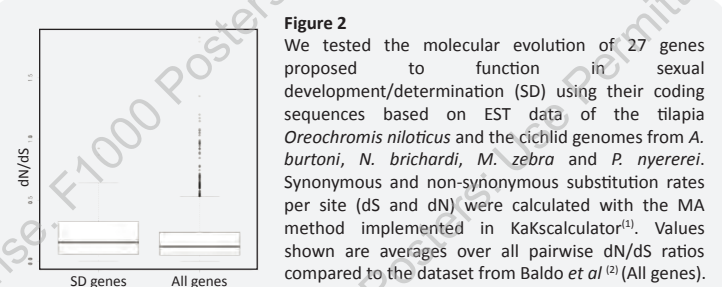
The Lake Tanganyika cichlid assemblage

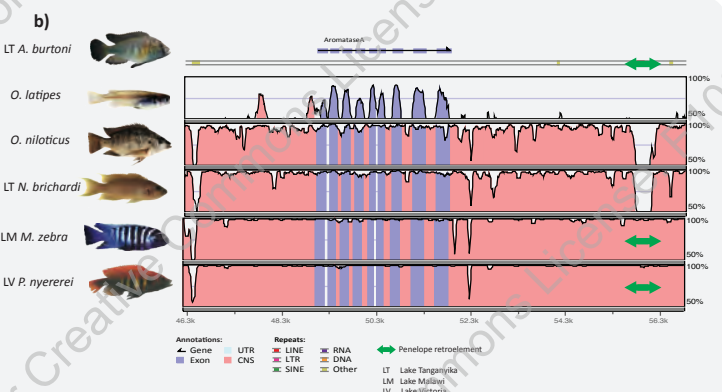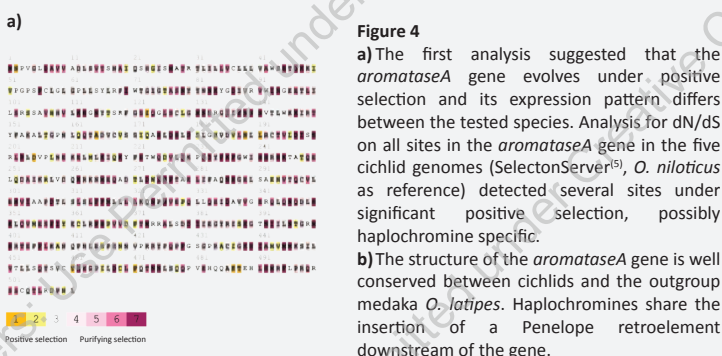Muschick, Indermaur and Salzburger, unpublished

Figure 1
Phylogeny of the cichlid species flock of the Lake Tanganyika. Two main breeding modes exist in Lake Tanganyika cichlids: substrate spawning (in the Lamprologini) and mouth breeding. Mouth breeding species often show strong sexual colour dimorphism whereas substrate spawners do not or very little. Stars mark species that have been tested here.

Genes involved in sexual development

Gonad expression

		A. burtoni		O. ventralis	
	gene	male	female	male	female
ovary development	aromataseA		++		
	aromataseB	++			
	ßcateninA				
	ßcateninB				+
	wnt4A				
	wnt4B				
	fig1a		++		++
	foxl2A				
	foxl2B				
	rspondin				
testis development	sf1A	+		++	+
	sf1B				
	sox9A				
	sox9B				
	wt1A			+	+
	wt1B		+	+	+
	dax1		+		
	dmrt1	+		+	
	gata4			+	+
	cyp11b			+	
germ cell function	nanos1A				
	nanos1B				

Brain expression

		A. burtoni		O. ventralis	
	gene	male	female	male	female
	aromataseA				
	aromataseB				
	ßcateninA				
	ßcateninB				
	wnt4A				
	wnt4B				
	fig1a				
	foxl2A				
	foxl2B				
	rspondin				
	sf1A				
	sf1B				
	sox9A				
	sox9B				
	wt1A				
	wt1B				
	dax1				
	dmrt1				
	gata4				
	cyp11b				
	nanos1A	+	+	+	+
	nanos1B		+	+	+

Figure 2
We tested the molecular evolution of 27 genes proposed to function in sexual development/determination (SD) using their coding sequences based on EST data of the tilapia *Oreochromis niloticus* and the cichlid genomes from *A. burtoni*, *N. brichardi*, *M. zebra* and *P. nyererei*. Synonymous and non-synonymous substitution rates per site (dS and dN) were calculated with the MA method implemented in KaKscalculator[1]. Values shown are averages over all pairwise dN/dS ratios compared to the dataset from Baldo *et al* [2] (All genes).

Figure 3
We tested 22 of the genes for their expression in two Tanganyika cichlids: *Astatotilapia burtoni* (Haplochromini) and *Ophthalmotalipa ventralis* (Ectodini, see figure 1) . Where present we tested the expression of gene duplicates derived from the teleost specific whole genome duplication[3] (marked A and B). Gonad expression could be shown for 15 genes. Almost all genes are expressed in brain as expected at least for genes with a role in the hypothalamic-pituitary-gonad axis. Results shown are the summary of QPCR-data from six males and females normalized to *rpl7* using the ΔΔCt-method[4] and a mixed tissue pool for each individual as reference. Red/blue colour: female/male expression, + and ++: tissues with high expression.

AromataseA gene evolution

a)

Positive selection   Purifying selection

Figure 4
a) The first analysis suggested that the *aromataseA* gene evolves under positive selection and its expression pattern differs between the tested species. Analysis for dN/dS on all sites in the *aromataseA* gene in the five cichlid genomes (SelectonServer[5], *O. niloticus* as reference) detected several sites under significant positive selection, possibly haplochromine specific.
b) The structure of the *aromataseA* gene is well conserved between cichlids and the outgroup medaka *O. latipes*. Haplochromines share the insertion of a Penelope retroelement downstream of the gene.

b)

References

1. Zhang *et al*. 2006 Genomics Proteomics Bioinformatics
2. Baldo *et al*. 2011 Genome Biology and Evolution
3. for review see Meyer and Van de Peer 2005 Bioessays
4. Livak and Schmittgen 2001 Methods