

A genomic survey on the immune differences among *Sinocyclocheilus* fishes

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ABSTRACT

In our previous work, we reported comparative genomics studies on 3 *Sinocyclocheilus* fishes, including the surface-dwelling *S. graham* (Sg), the semi-cave-dwelling *S. rhinoceros* (Sr) and the cave-restricted *S. anshuiensis* (Sa). Here, we performed a genomic survey on the immunity differences among the 3 fishes by combination of the genome and transcriptome data. Our results demonstrate that immune-related genetic pathways in Sa and Sr present more similarities than Sg. The innate immune activity in Sa seems to be higher than Sg and Sr, while Sr owns more copy numbers of MHC-related genes (related with the acquired immune system) than Sg and Sa. These differential immune activities in the 3 *Sinocyclocheilus* species may be due to their differential habitats. Generally speaking, most immunity genes transcribe the lowest levels in Sa when compared with Sg and Sr, which may be associate with a less various microbial environment in the cave-restricted habitat. However, Sr has more members of MHC-related genes than those in Sg and Sa, suggesting a great contribution from the semi-cave-dwelling condition.

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The freshwater *Sinocyclocheilus* (Cypriniformes: Cyprinidae) fishes are endemic to China's massive karst area, i.e., the eastern part of Yungui Plateau and the surrounding region (including northwest Guangxi Province and east Yunnan Province).¹ The genus *Sinocyclocheilus* includes surface-dwelling, semi-cave-dwelling and cave-restricted species, which makes it a special group for investigating the molecular mechanisms of adaptive evolution in cavefishes.² In our previous study, we compared the whole genome sequences of 3 *Sinocyclocheilus* species, including the surface-dwelling *S. graham* (Sg), the semi-cave-dwelling *S. rhinoceros* (Sr) and the cave-restricted *S. anshuiensis* (Sa). By comparative genomics analyses of the 3 genomes, we provided genetic evidence for the cave adaption of cavefishes, such as eye degeneration, albinism and hearing loss.³

Interestingly, immune activities in the 3 *Sinocyclocheilus* fishes are obviously different. For example, Sa is more susceptible to disease in captivity (Jiang et al., unpublished data). In our previous work,³ we annotated all predicted genes and calculated their expression levels in the skin of the 3 *Sinocyclocheilus* fishes. Here, we picked out 800 "1:1:1" orthologous immune-

related genes, and identified 396 of them are differentially expressed genes (DEGs; changes over 2 folds). Different from Sg, Sa and Sr present similar gene expression patterns (Fig. 1), which is consistent with the similar cave-dwelling behaviors of Sa and Sr. This also suggests difference of immunity system between surface-dwelling and cave-dwelling fishes. Simultaneously, most immunity genes transcribe the lowest levels in Sa when compared with Sg and Sr,⁴ which may be associate with a less various microbial environment in the cave-restricted habitat.

TLR (toll-like receptor) gene family is an important group of innate immune receptors located on the surface of membrane.⁵ They are divided into 13 families and named as TLR1 to TLR13.⁶ Most of them play a significant role in immunity by detecting pathogen-associated molecular patterns (PAMPs).⁵ We analyzed the TLR gene family among the 3 *Sinocyclocheilus* fishes, and identified 2 copies of Tlr8 and Tlr18 genes in the Sa genome while only one copy in the Sg and Sr genomes. This difference may tell us that the innate immune activity in cave-dwelling fishes is higher than that in surface-dwelling and cave-restricted fishes.

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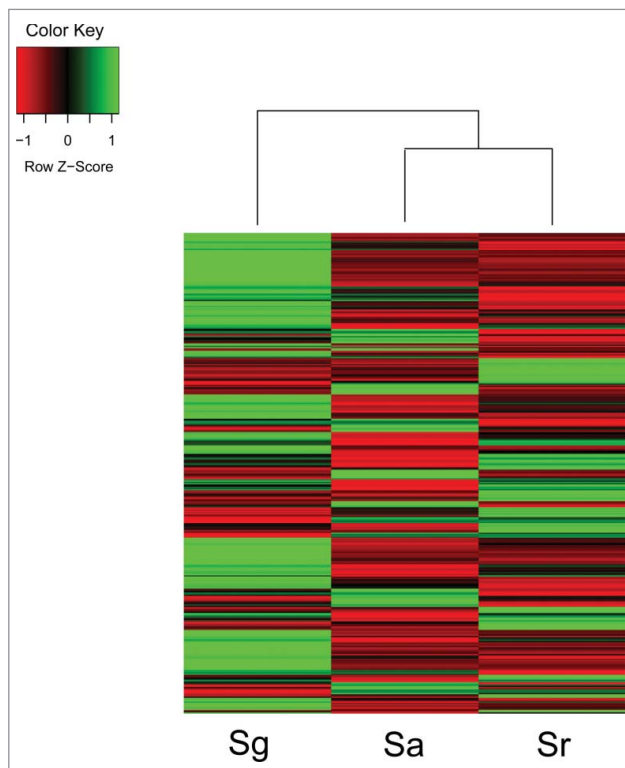


Figure 1. Expression patterns of immune-related gene in the 3 *Sinocyclocheilus* fishes.

The major histocompatibility complex (MHC) is a set of cell surface proteins essential for acquired immune system to recognize foreign substances in vertebrates.⁷ MHC genes include MHC class I and MHC class II; MHC class I genes are responsible for protection against viral pathogens, while MHC class II genes are eligible for

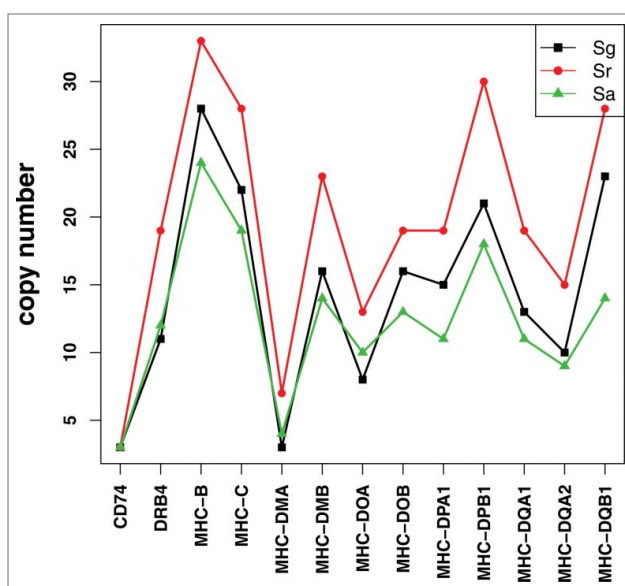


Figure 2. Copy numbers of MHC-related genes in the 3 *Sinocyclocheilus* fishes.

Table 1. The GO enrichment analysis of contraction gene families in Sa.

GO ID	GO Term	GO Class	Pvalue
GO:0042613	MHC class II protein complex	CC	1.12E-08
GO:0007601	visual perception	BP	5.37E-05
GO:0019882	antigen processing and presentation	BP	7.10E-05

detection of bacterial pathogens in vertebrates.⁸ Therefore, elevation of MHC gene numbers will facilitate pathogen detection and improve immunity. Here we identified MHC gene family in the 3 *Sinocyclocheilus* fishes, and observed that Sr owns the highest number of MHC genes while Sa has the least (Fig. 2). In our previous genome paper,³ we analyzed contraction and expansion of gene families in the 3 *Sinocyclocheilus* fishes and all the predicted proteins were subjected to GO/KEGG/IPR enrichment analyses. Among these gene families, we discovered that the GO terms of visual perception, MHC class II protein complex and antigen processing presentation were undergoing contraction in Sa (Table 1). The GO terms related to acquired immunity in Sr, such as MHC protein complex and immune response, are undergoing expansion (Table 2). These results suggest that the acquired immunity of cavefishes may be lower than that of their surface-dwelling counterparts, because they live in a subterranean habitats with less microbial and food. Sr has more members of MHC-related genes than those in Sg and Sa, suggesting a great contribution from the semi-cave-dwelling condition. Hence, we deduce that Sr should have more intense immune defense against pathogens than those encountered in cave or surface alone. This phenomenon is similar to that in the reported amphibious mudskippers.⁹

In summary, our current results provide new insights into the genetic basis for immunity in cavefishes, and reach a novel conclusion that the 3 *Sinocyclocheilus* fishes have developed differential immune activities that may be related to their differential habitats. We also provide a solid foundation for further investigation of the biological differences in immunity of the 3 *Sinocyclocheilus* fishes.

Table 2. The GO enrichment analysis of expansion gene families in Sr.

GO ID	GO Term	GO Class	Pvalue
GO:0006955	immune response	BP	1.87E-105
GO:0042611	MHC protein complex	CC	3.85E-57
GO:0019882	antigen processing and presentation	BP	3.85E-57
GO:0042612	MHC class I protein complex	CC	1.53E-42
GO:0042613	MHC class II protein complex	CC	1.26E-15

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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