THE CAVEFISH *OREONECTES JIARONGENSIS* CAN BE INDUCED TO DIFFERENTIATE AND RECOVER UNDER THE LIGHT CONDITION

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ABSTRACT

This research indicated that one cave fish species of *Oreonectes jiarongensis* can recover the transparent to black under the light condition, this species belongs to the *Oreonectes*, Nemacheilinae, and distributes in Libo County, Guizhou Province, China. The changing process time was 14 days. This is the first time that suggests the cave vertebrates which lived in the dark environment not longer time could change the body color in the light environment, and has a new adaptive strategy for the darkness condition. The result may indicate that this species entrance the underground river not so long time, and the genes not mutation, which control the melanin express, it still has the physiological regulation mechanism under the light condition.

RESUMEN: El pez de las cavernas *Oreonectes jiarongensis* puede ser inducido a la diferenciación y recuperación bajo condiciones de iluminación.

En esta investigación se encuentra que una especie de pez que habita en cavernas, *Oreonectes jiarongensis*, puede alternar su apariencia de transparente a negra, bajo distintas condiciones de iluminación. Esta especie pertenece al género *Oreonectes*, Nemacheilinae, y se distribuye en el condado Lobo, provincia de Guizhou, en China. El proceso de cambio tomó 14 días. Esta es la primera vez que se sugiere que un vertebrado que habita en cavernas, viviendo en un ambiente de oscuridad, puede cambiar de color bajo condiciones de iluminación, lo que puede representar una una nueva estrategia adaptativa. Los resultados pueden indicar que esta especie ingresa no por mucho tiempo al ambiente subterráneo en los rios, de manera que los propios genes, y no una mutación, que controlan la expresión de la melanina, siguen siendo el mecanismo fisiológico de regulación bajo condiciones de iluminación.

REZUMAT: Inducerea diferențierii și a recuperării pigmentării la lumină la peștele cavernicol *Oreonectes jiarongensis*.

Prezentul articol arată că una din speciile cavernicole de pește *Oreonectes jiarongensis* poate să își recupereze la lumină pigmentarea pielii, de la transparent la negru. Această specie aparține genului *Oreonectes*, fam. Nemacheilinae și se întâlnește în ținutul Libo, provincia Guizhou, China. Procesul de refacere a pigmentării a durat 14 zile. Este prima citare care sugerează că vertebratele cavernicole ce trăiesc în întuneric de puțină vreme își pot modifica culoarea corpului în cazul expunerii la lumină și au o strategie adaptativă nouă pentru viața în lipsa luminii. Rezultatul poate indica că această specie a pătruns în râul subteran de relativ puțină vreme, mutația genelor care controlează expresia melaninei neavând timp să se fixeze, mecanismul de reglare fiziologică în condiții fotice fiind încă prezent.

INTRODUCTION

Body colour of teleost fish plays a role in concealing, disguising, alerting, and mating by Salopek and Jimbow (1996). Fish body colour is determined by melanocytes, xanthophore, erythrophores, and iridocytes in the dermis by Feng et al. (2014). Therefore, freshwater teleost fishes present a variety of body colours. Of these, a majority of fishes are black, owing to the presence of abundant, stable melanocytes in their skin that selectively absorb light of specific wavelength and reflect light of other wavelengths by Ye et al. (2003).

In typical cave-dwelling fishes, body colour disappears, and they appear white or translucent, in order to adapt to completely dark environments by Zhao and Zhang (2006). The pathway for production of melanin in freshwater teleost is Phenylalanine → L-tyrosine → 3,4-dihydroxyphenylalanine (dopa) → dopaquinone → melanin by McCauley et al., 2004; Jeffery et al. (2016). Two ligands of MC1R, α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH), bind to MC1R on the melanocyte membrane. This makes the G-protein coupling to the receptor transform from inactive guanosine diphosphate (GDP) to active guanosine triphosphate (GTP), thereby activating the adenylate cyclase system present on the membrane, and transforming adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Further, cAMP activates tyrosine kinase and tyrosinase (Tyr). Tyr catalyzes tyrosine in the melanocytes to produce dopa, and subsequent release of melanin after it accumulates in the melanocytes to a certain extent by Yu et al. (2010). Studies on *Astyanax fasciatus mexicanus* found that it failed to form melanin the mutation of *occuloalbinism 2* (*Oca2*) by McCauley et al. (2004) and *melanocortin 1 receptor* (*Mc1r*) genes by Gross et al. (2009). The main function of *Oca2* gene is to code for tyrosinase related transporter protein by Gross and Wilkens (2013). Meanwhile, *Mc1r* mutation significantly reduces the melanin content and decreases the number of melanocytes by Gross et al. (2009). Tyr is the major rate-limiting enzyme for the conversion of tyrosine to melanin by Jeffery et al. (2016). Too little Tyr will cause tyrosine to transform into cysteamine dopa, thereby hindering the production of melanin.

There also were some studies demonstrated that melanocyte proliferation and melanin secretion were positively correlated with ultraviolet radiation in embryonic *Xenopus laevis* (Yu et al., 1987). Thus, melanin expression in vertebrates might be subject to a combined action of molecular basis and external light environment.

MATERIAL AND METHODS

Experimental species

In 6th-14th, 2016, nine individuals of *Oreonectes jiarongensis*, body length 52-116 mm, were collected from a cave in Shuijingwan Village, Latan group, Jiarong, Libo, Guizhou, China (25°28’12.89” N, 108°06’34.35” E; altitude-634 m) and reared in a dark condition. Lasted for 30 days in the ecology laboratory of the College of Life Science, Guizhou Normal University, From April 15 to May 15, 2016, during which three fish died.

Feeding conditions

Five fish tanks (45 cm × 30 cm × 29 cm), numbered 1, 2, 3, 4, and 5, were used for feeding the *O. jiarongensis*. Another ultra clear glass tank (22 cm × 15 cm × 18 cm) was used for photographing the fish body colour change every day.

The feeding conditions of this cave fish species cited from Richard (2008) mainly, and a little rearrangement based on the origin condition that the fish live in. In order to make the fish were alive. After being collected from the cave environment, the water was applied 24 h with cycle oxygen. During feeding, the water was oxygenated and circulated continuously.

According to Ye et al. (2003), Earthworms, procured from the flower and bird market in Youzha Street, Guiyang, were kept alive before being fed to the fish. During feeding, the earthworms were cut into one-two mm segments. The fish were fed once a day, in the evening, and the unconsumed earthworms were taken out the following evening to maintain water quality.

Experimental methods

Light and other environmental conditions

The fluorescent lamp was used as the experimental light instead of the normal sunlight, and turned on at 08:30 am and turned off at 17:30 pm everyday during the experiment. Meanwhile, the sunrise time was 06:27-06:07 h and the sunset time was 19:17-19:31 h. Illumination was monitored using an illuminometer (VICTOR 1010A, SHENZHEN VICTOR HI-TECH CO., LTD, CHINA), and the illumination interval was 94.0-130.3 lux. The air temperature was 8-28°C and the atmospheric pressure was 1000.9-1014.1 hPa.

Grouping

Nine specimens were divided into two groups by the standard body length of the fish, which body length > 70 mm (range: 73-116 mm) were assigned to group A, and coded number as A1, A2, A3 and A4 (numbered by the decreasing order of body length); if body length < 70 mm (range: 52-63 mm), then belonged to group B, also numbered by the decreasing order of body length as B1, B2, B3, B4, it might reveal that the different body size fished might have the recovery speed on the body colour. In addition, one spared fish with a body length of 67 mm was assigned to B group and denoted as B5 (Fig. 1; Tab. 1).

Nine *O. jiarongensis* were included before and after the experiment. At the start of the experiment, A1 and B1, A2 and B2, A3 and B3 as well as A4 and B4 were fed in tanks 1, 2, 3, and 4 (Tab. 1). Four days later, A2, A3 and B3 died successively. Therefore, B5 that was simultaneously fed under the light environment was considered as the observatory object.

Table 1: Feeding in fish tanks by Body length of fish.

Group	Tank number				
	1	2	3	4	5
A (> 70 mm)	A1 (116 mm)	A2 (95 mm)	A3 (80 mm)	A4 (73 mm)	
B (< 70 mm)	B1 (63 mm)	B2 (61 mm)	B3 (56 mm)	B4 (52 mm)	B5 (67 mm)

Observation and record

An ultra-clear glass aquarium with a scale plate (length 22 cm, least count 0.5 cm) was used for observing and photographing. Each fish was observed once every other day or at noon on two consecutive days in the following order: A1 → B1 → A2 → B2 → A3 → B3 → A4 → B4 → B5. The blackened part of the body was carefully observed. At 20:30-21:00 h on the same day, changes in body colour was photographed and recorded in the same order as that at noon. Each fish was taken out from the tank with a hand-held net, and was placed into the ultra clear glass aquarium. The fish were photographed using a camera (Nikon D810, Nikon Corporation, Japan) when its head turned left, tail turned right and body was parallel to the scale plate.

Determination of degree of blackening

From the photographs of *O. jiarongensis*, taken on the 14th day of light exposure, the degree of blackening at selected points were measured using Adobe Photoshop CC2014 (32bit) operation system based on windows7, use Photoshop CC2014 to measure the extent of blackening with the following settings: graphics mode: CMYK colour, 32 bit; and sample size: average 101*101.

RESULTS AND DISCUSSION

We observed the blackening process in six individuals of *O. jiarongensis*, all of which gradually blackened to resemble surface fish after receiving light exposure for 14 days. In group B (body length < 70 mm), the rate of blackening was generally fast, and the extent of blackening was remarkable. Meanwhile, in group A the rate of blackening was faster than that in group B during the first three days, before becoming slower than that in group B. In addition, the overall extent of blackening was lighter than that in group B (Tabs. 2 and 3). The blackening process progressed in the following order: upper part of the snout → cranium → back → tail → caudal fin → lower part of the snout → dorsal fin → pectoral fin and other appendages.

Table 2: Change in body colour of *O. jiarongensis* with different sizes under light condition.

Time	Group A	Group B
1st day	Translucent	Translucent
5th day	Nostril outside, cranium, upper part of the snout, central part of the head, dorsal fin base, and maxillary barbell base became black; black spots were visible on the back.	Nostril outside, cranium, upper part of the snout, central part of the head, dorsal fin base, most part of maxillary barbell base and caudal-peduncle became black.
8th day	Superior and inferior caudal fins appeared black, whereas the back, cranium, and upper part of the snout became significantly black.	Superior and inferior caudal fins and barbells became black, whereas caudal-peduncle, upper part of the snout, cranium, and back were significantly black.
14th day	Black spots appeared at the lower part of the snout, black spots increased at the base of pectoral rays and caudal fin, cranium, upper part of the snout, the back became black as a whole, the pelvic fin and caudal fin became black.	Blackening became significant as a whole, where dorsal part of the head and the back become completely black, black spots increased in the lower part of snout, the black spots on the caudal fin increased and deepened; the fish looked like a surface fish.

Table 3: Comparison of the extent of blackening in *O. jiarongensis* with different sizes on the 14th day of light exposure.

Part	Group A	Group B
Nostril outside	K = 45%	K = 64%
Cranium	K = 40%	K = 56%
Upper part of snout	K = 44%	K = 63%
Back	K = 36%	K = 53%
Central part of the head	K = 37%	K = 55%
Gill	K = 33%	K = 52%
Middle part of the lateral side	K = 27%	K = 52%
Lower part of the lateral side	K = 18%	K = 50%
Lower part of the snout	K = 40%	K = 61%
Base of dorsal fin	K = 41%	K = 58%
Junction of caudal-peduncle and caudal fin	K = 63%	K = 70%
Caudal-peduncle	K = 62%	K = 68%
Barbell	K = 47%	K = 64%

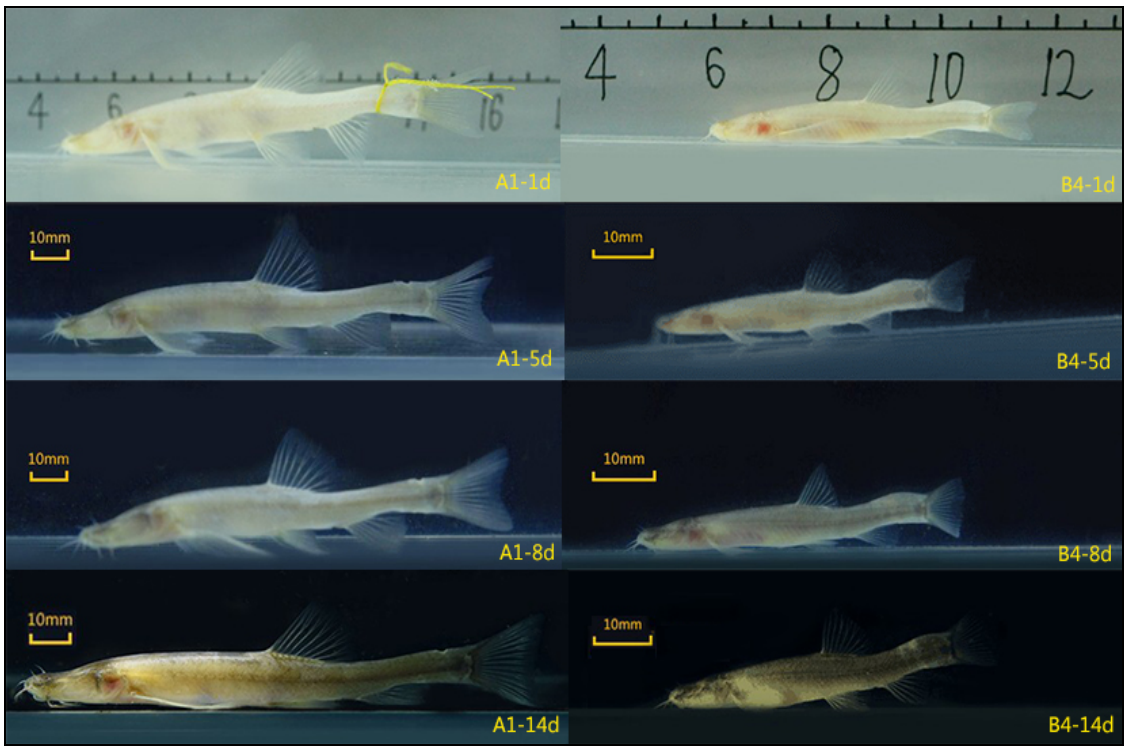

Figure 1: Body colour changes of *O. jiarongensis* with different sizes before and after light exposure.
A-group A (body length > 70 mm), B-group B (body length < 70 mm), d-days.

There are some studies on the body colour of the vertebrates animals recently, such as: fishes (Maan and Sefc, 2013; Sköld et al., 2013; Nyboer et al., 2014), amphibians (Kindermann and Hero, 2016a, b), Reptiles (Junko and Tsutomu, 2009; Takeo et al., 2009) these researches all indicated that the external body colour changes in amphibians and other colour changing animals are possible due to different distributions of pigment cells (chromatophores) and the movement of pigment within them, meanwhile, also have some genetic foundation. It was suggested that the fishes body colour regulation mechanisms have two kinds, one was the physiological regulation, this mechanism means that the pigment granules could gather and disperse rapidly by the nerve regulating (Leah and Catherine, 1994); another one was the morphological regulation mechanism, that means, the body colour was regulated by the endocrine system (Tripathi et al., 2008). More studies often concern on the river surface or ground vertebrate animals, just a few researches focused on the cave vertebrate animals, for example, the cave fish of *A. fasciatus mexicanus*. As for this fish species which dwelling in the different underground rivers in Mexico, more studies were done on the synthesis and express pathway of the *A. fasciatus mexicanus* based on the biochemistry and genetic, and showed if the main control genes Mc1r and Oca2 had mutated, then this species cannot has the black colour on its' skin (Gross and Wilkens, 2013; Bierbach et al., 2013).

As for the fresh water fish species, the body colour has the very important means on the phenotypic and the fitness in the different subpopulations. In the present study, the body colour of *Oreonectes jiarongensis* was found to transform from translucent to black when exposed to light. It was inferred that the melanin-controlling genes did not undergo mutation. At the same time, the genetic study did not show the genes mutations occurred in the species of *O. jiarongensis* by amplification of *Mc1r*, *Oca2*, and *Tyr* genes, which controlling the synthesis and express of the melanin, meanwhile, it indicated that these three genes could express normally by transcriptome analysis (different study which belongs to the same project, the paper submitted), the results showed the three genes of *O. jiarongensis* had the normal functions. In dark environments that lack ultraviolet radiation, it appears translucent, owing to decreased function of the melanocytes. In contrast, *Mc1r* and *Oca2* genes in *Astyanax fasciatus mexicanus*, incur mutations, thereby reducing the number of melanocytes significantly. In addition, transport of the substrate of melanin, i.e., tyrosine, into the melanocyte nuclei fails, thus preventing melanin synthesis. That means the *Astyanax fasciatus mexicanus* was unable to recover its body colour under light condition, because of the genes mutations.

O. jiarongensis started blackening after being exposed to the selected light regime for three days, and its body colour changed completely after 14 days. The order of blackening was: upper part of the snout → cranium → back → tail → caudal fin → lower part of the snout → dorsal fin → pectoral fin → other appendages. Owing to physiological regulation, this species appeared white when living in the cave but turned black after being exposed to light for a short time. This physiological regulation might reflect imperfect adaptation to the dark cave environment, probably owing to the fact that this species has not been living in the dark cave for a long time. The cave for sampling was formed 91,000-163,300 years ago by Zhang et al. (2000). Meanwhile, *A. fasciatus mexicanus* started to live in caves 2,800,000-6,700,000 years ago by Gross and Wilkens (2013), which implies that the time was sufficiently long for the mutation of their *Oca2* and *Mc1r* genes controlling synthesis of melanin. Thus, the body colour degradation in *A. fasciatus mexicanus* is the morphological regulation mechanism. In summary, two different fish species, *Oreonectes jiarongensis* and *Astyanax fasciatus mexicanus*, present albino phenotypes in a dark environment, but their mechanisms of body colour changing are considerably different.

CONCLUSIONS

In the present study, we investigated the process of body colour change in cave-dwelling fishes under regulated light conditions, which indicates that it takes a long time for the mutation of the functional genes controlling body colour. Regarding the body colour, a significant difference exists in the mechanism of adaptation to the dark cave environment among different fish species.

It is the first time that report and demonstrate the cave fish species can recover the body colour similar to the surface species in short time under the light condition, and suggests that the gene mutation of the body colour expressing of the cave fishes need more longer time, meanwhile, the melanin express of the fish need the environment stimulation factor.

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