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Morphological variations and phylogenesis of four strains in *Cyprinus carpio*

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Abstract: Traditional morphological data and truss network data were combined to conduct a multivariate analysis to study the morphological variations and phylogenesis of four strains in red common carp (*Cyprinus carpio*): *C. c.* var. *color*, *C. c.* var. *singguonensis*, *C. c.* var. *wananensis* and *C. c.* var. *wuyuanensis*. The results indicate that: (1) In the aspect of meristic characters, there were no significant differences ($P > 0.05$) among the four strains; (2) In the aspect of traditional measurable characters plus truss network characters, there were significant differences ($P < 0.05$) among the four strains. Cluster analysis showed that *C. c.* var. *wuyuanensis* was separated from the other three strains which are close to one another; discriminate functional analysis based on 28 ratio variables showed that there were highly significant differences among the four strains ($P < 0.01$); discriminate functional analysis with 8 selected morphological ratio variables gave a synthetic discriminating accuracy of 92%; principal component analysis indicated that morphological differences among the four strains were mostly due to variations in the frontal part of the body. (3) Using various multivariate analysis techniques to analyze the combination of traditional morphological data and truss network data greatly enhances the study capacity for morphological variations and phylogenesis among populations or strains under a species of fish.

Key words: morphology; multivariate analysis; variation; phylogenesis; red common carp; strain

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1 Introduction

Common carp (*Cyprinus carpio*) is the oldest cultured fish species in the world. It is one of the principal aquaculture species , with the production of 2.37 million ton in 2004 accounting for 12.5% of total freshwater aquaculture production in China①.

Red common carp is a special group of common carp, with red color as the common character. The three red common carp strains, Xingguo red common carp (*C. carpio* var. *singguonensis*), purse red common carp (*C. carpio* var. *wuyuanensis*), glass red common carp (*C. carpio* var. *wananensis*), generally called "Three Kinds of Red Common Carp in Jiangxi Province", were established by the Chinese fish aquaculturists in Jiangxi Province[1,2]②. Besides sharing of the red color, Xingguo red common carp is characterized by a spindle shape with a ratio of 3.38 in body length/depth. Purse red common carp is characterized by a purse shape with a ratio of 2.0 - 2.3 in body length/depth and a shorter caudal peduncle length. Glass red common carp is very close to Xingguo red common carp in spindle shape, but with some transparency, especially in juvenile stage.

Since the 1970s, "Three Kinds of Red Common Carp in Jiangxi Province" have been introduced to many parts of China. Besides being used in aquaculture, they are widely used as excellent genetic

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① China Fisheries Bureau, 2004. Yearbook of Fisheries in China. China Fisheries Bureau. 2005.

② Breeding farm of Xingguo red common carp and Biology Department of Jiangxi University, 1985.

and breeding materials, many hybridization combinations were produced between these red common carps and local strains of common carp or crucian carp, some of them have played a significant role in increasing production of carp culture and economic development in the local areas[3].

Oujiang color common carp is a regional species in Oujiang River basin in Zhejiang Province. It has been cultured for more than 1200 years, mostly in paddies and yard ponds. Recently it is found that it could be an excellent candidate also as an ornamental species because of variety of colors[4].

In order to reveal the biodiversity of the red common carps, and to exploit their genetic potential for edible fish and ornamental species, a comprehensive study has been conducted since 2000 to compare the morphology, aquaculture performance, biochemical and molecular variations of the four strains[4,5]. This paper reports the results on morphological variations and phylogenesis.

2 Materials and Methods

2.1 Measurements

Over 30 fish from each of the four strains of red common carp all two years old were collected from Aquatic Genetic Resources Research Station of Shanghai Fisheries University. Their body weights were measured to the nearest 0.01 g (Tab.1).

Tab.1 Body weights and sample size of analyzed four strains in red common carp

strain	*C. c.* var. *color* (Oujiang color common carp)	*C. c.* var. *singguonensis* (Xingguo red common carp)	*C. c.* var. *wananensis* (glass red common carp)	*C. c.* var. *wuyuanensis* (purse red common carp)
body weight (mean ± SD, g)	774.95 ± 148.60	965.32 ± 385.86	891.65 ± 144.82	185.37 ± 49.59
range(g)	532 – 1100	490 – 1514	350 – 1140	103.6 – 304.2
sample size	30	33	31	33

Two sets of data were collected from each fish. The first set of data was the traditional morphological data, including meristic characters and measurable characters. The meristic group includes 8 characters: scale number above lateral line (SCA), scale number beneath lateral line (SCB), scale number on lateral line (SCL), gill rake number on first gill arch (GRF), spine number on dorsal fin (SPD), soft ray number on dorsal fin (SFD), spine number on anal fin (SPA) and soft ray number on anal fin (SRA); The measurable group includes 9 characters which were measured to the nearest 0.1 cm: total length (TL), body length (BL), body depth (BD), head length (HL), snout length (SL), eye diameter (ED), inter-orbital width (IW), caudal peduncle length (CPL), and caudal peduncle depth (CPD). The second set of the data was composed of 20 truss network[6,7] data (Fig. 1). Landmark points for truss measurements (Fig. 1) were followed by Li[8].

2.2 Analytical methods

The differences of meristic characters among the four strains were analyzed using χ^2-test[9].

Three kinds of multivariate analyses, including cluster analysis, discriminate analysis and principal component analysis were conducted on the combination of 9 measurable parameters and 20 truss network parameters. All analyses were performed using the statistical package SYSTAT, version 4[10]. Considering the fish sizes were quite different even when they were at the same age (Tab. 1), all parameters were converted to the ratios of total length to reduce the bias from statistical analysis, totally 28 ratios.

Cluster analysis Euclidean nearest neighbor of joining hierarchical cluster[11] was used for the analysis.

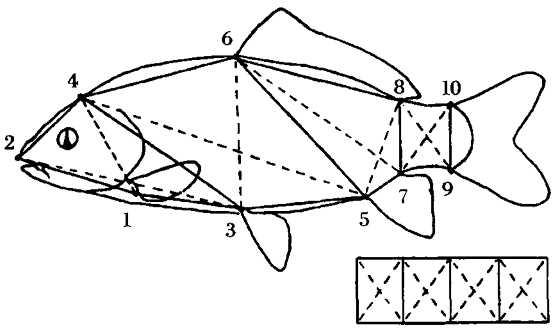

Fig.1 Truss network of common carp

The truss parameter measurements are the distances between two of the 10 landmark points. For example. D3 – 4 denotes the distance between landmark points 3 and 4. The frame on the down right indicates the philosophy of truss network

1.most posterior point of maxillary; 2. tip of snout; 3. origin of pelvic fin; 4. most anterior of scales on skull; 5. origin of anal fin; 6. origin of dorsal fin; 7. terminus of anal fin; 8. terminus of dorsal fin; 9. ventral origin of caudal fin; 10. dorsal origin of caudal fin

Discriminate analysis Stepwise discriminate analysis[10] was used. Eight ratios, which showed the most contribution to the total variation, were chosen from above 28 ratios, to establish a simple discriminate formula to distinguish the four strains.

Principal component analysis Their contribution rate and accumulating contribution rate were calculated in accordance with Wilkinson[12].

Discriminating accuracy ($P1$) = fish number discriminated correctly for i strain/ observed fish number of i strain

Discriminating accuracy ($P2$) = fish number discriminated correctly for i strain/ total fish number discriminated into i strain from all strains

$$\text{Synthetic discriminate rate}(\%) = \sum_{i=1}^{n} A_i / \sum_{i=1}^{n} B_i$$

A_i refers to fish number discriminated correctly in i strain, B_i refers to the total fish number in i strain, n refers to the total number of fish studied.

3 Results

3.1 Meristic characters

Means and standard deviations of 8 meristic characters of the four strains of red common carp are shown in Tab.2. The values of χ^2-test are shown in Tab.3. All of them were less than the critical value of 2.17 ($\chi^2_{0.95;7}$). It indicates that there is no significant difference in the meristic characters among the four strains of red common carp.

3.2 Measurable characters and truss network characters

Cluster analysis *C. c. var. wuyuanensis* was separated away from the other three strains which are close to one another (Fig.2).

Tab.2 Means and standard deviations of meristic characters of four strains in red common carp

character	means of meristic characters(mean ± SD)			
	C. c. var. *color*	*C. c.* var. *singguonensis*	*C. c.* var. *wananensis*	*C. c.* var. *wuyuanensis*
scale number above lateral line	5.57 ± 0.53	5.00 ± 0.00	5.40 ± 0.55	6.00 ± 0.24
scale number beneath lateral line	5.43 ± 0.53	5.67 ± 0.58	5.60 ± 0.55	5.50 ± 0.46
scale number on lateral line	35.57 ± 0.53	36.67 ± 0.58	35.80 ± 0.84	36.50 ± 0.62
gill rake number on first gill arch	19.43 ± 1.27	20.67 ± 2.31	20.60 ± 0.55	21.50 ± 0.83
spine number of dorsal fin	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
soft ray number of dorsal fin	18.71 ± 0.49	17.00 ± 0.00	18.20 ± 0.45	17.00 ± 0.21
spine number of anal fin	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
soft ray number of anal fin	5.86 ± 0.38	5.00 ± 0.00	6.00 ± 0.00	5.00 ± 0.11

Tab.3 Values of χ^2 test of meristic characters between two strains of red common carp

strain	*C. c.* var. *color*	*C. c.* var. *singguonensis*	*C. c.* var. *wananensis*	*C. c.* var. *wuyuanensis*
C. c. var. *color*	–			
C. c. var. *singgonensis*	0.5024	–		
C. c. var. *wananensis*	0.0960	0.2977	–	
C. c. var. *wuyuanensis*	0.5746	0.2048	0.3976	–

Notes: All values in the table are less than the critical χ^2 value of 2.17($\chi^2_{0.95;7}$). It denotes no significant difference between each two strains.

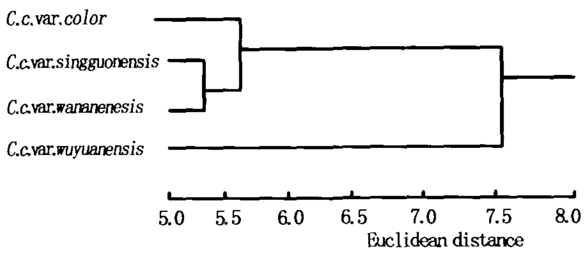

Fig.2 Cluster dendrogram of four strains in red common carp

Discriminate analysis When 28 ratios of morphological parameters, were used, the discriminating accuracy reached 98.06% and the discriminating efficiency was highly significant ($P<0.01$).

When 8 ratios (D1-4/TL, CPD/TL, IW/TL, D7-8/TL, D7-9/TL, D5-7/TL, D6-8/TL, D2-4/TL; Tab.4) were selected from the above 28 ratios, much simple discrimination formulas were produced. The discriminating efficiency was 92% (Tab. 5). In this case, the discriminating efficiency lost 6% than 28 ratios were used, but about 70% time and labor can be saved because of simplicity.

By above formula, any red common carp can be discriminated into their own strain, just input the required 8 ratio variables into above formula, and calculate $Y1$ value, the greatest Y1 denotes which strain it belonges to.

Principal component analysis Four strains in red common carp were separated from each other clearly (Fig.3). There was some overlap between the *C. c.* var. *wananenesis* and *C. c.* var. *singguonensis*, and between the *C. c.* var. *color* and *C. c.* var. *singguonensis* respectively. *C. c.* var. *wuyuanensis* was separated from above three.

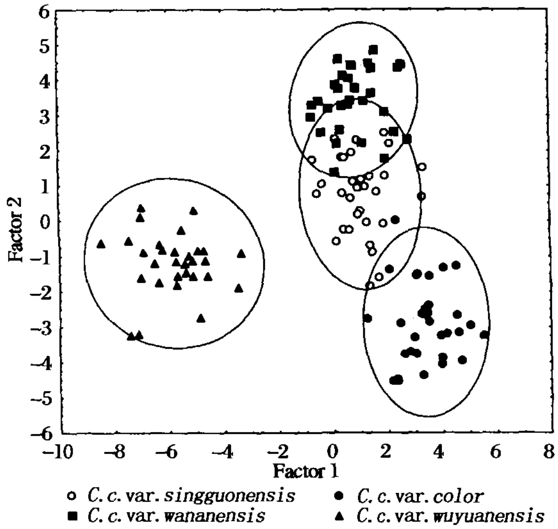

Fig.3 Map of principal component analysis of four strains in red common crap

Tab.4 Ratio variables with high contribution in discrimination analysis

ratio variables	V12	V9	V7	V21	V22	V18	V28	V26
meaning in morphology	D1-4/TL	CPD/TL	IW/TL	D7-8/TL	D7-9/TL	D5-7/TL	D6-8/TL	D2-4/TL
F test value	80.988	47.075	40.063	36.887	34.426	32.556	29.779	27.610

Notes: F value line denotes F test values of ratio variables, ranged by values

Tab.5 Results of discrimination analysis of four strains in red common carp

strains	*C. c.* var. *color*	*C. c.* var. *singuonensis*	*C. c.* var. *wananensis*	*C. c.* var. *wuyuanensis*	discriminating accuracy $P1$ (%)	discriminating accuracy P2 (%)	synthetic discriminating accuracy (%)
C. c. var. *color*	28	1	1	0	93.33	87.50	
C. c. var. *singguonensis*	2	29	2	0	87.88	90.63	92.13
C. c. var. *wananensis*	2	2	27	0	87.10	90.00	
C. c. var. *wuyuanensis*	0	0	0	33	100.00	100.00	

(1) For *C. c.* var. *color* $Y1 = -346.259 + 555.980V12 - 38.807V9 + 270.664V7 + 912.235V21 + 789.484V22 + 931.519V18 + 526.620V28 + 566.711V26$

(2) For *C. c.* var. *singguonensis* $Y1 = -354.456 + 792.374V12 + 31.235V9 + 76.927V7 + 703.330V21 + 772.850V22 + 1075.698V18 + 456.372V28 + 671.961V26$

(3) For *C. c.* var. *wananenesis* $Y1 = -338.620 + 660.938V12 - 135.667V9 + 263.831V7 + 651.713V21 + 836.390V22 + 1167.934V18 + 512.386V28 + 573.782V26$

(4) For *C. c.* var. *wuyuanensis* $Y1 = -393.076 + 1055.406V12 + 145.245V9 - 77.580V7 + 690.097V21 + 560.439V22 + 1285.818V18 + 418.371V28 + 692.425V26$

Truss network The truss dendrograms for 4 strains in red common carp were constructed as Fig. 4, in which the similarity and diversity can be seen clearly. Again, *C. c.* var. *wuyuanensis* is slightly far-away from the other three.

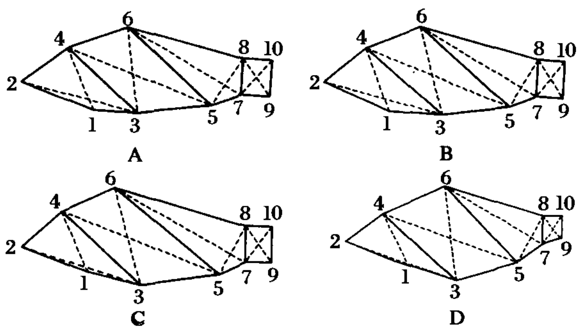

Fig.4 Fitted truss network of four strains in red common carp

A: *C. c.* var. *singguonensis*, B: *C. c.* var. *wananensis*, C: *C. c.* var. *color*, D: *C. c.* var. *wuyuanensis*

4 Discussion

4.1 Morphological differences and phylogenetic relationship among the four strains of red common carp

From the point of view of genetic diversity, qualitative study of morphology is not enough to study the genetic variation within species, such as the four strains of red common carp. Quantitative data and analysis are necessary to understand their differences and then phylogenetic relationships. This study reveals that *C. c.* var. *color*, *C. c.* var. *singguonensis* and *C. c.* var. *wananenesis* are close to each other, and *C. c.* var. *wuyuanensis* is far from them.

The variations in morphology could be related to evolution history of species. *C. c.* var. *singguonensis* is a local strain of common carp produced from natural selection first and then artificial selection[12,13]. *C. c.* var. *wananensis* might originate from natural mutant of *C. c.* var. *singguonensis* and occasionally found and then selected. *C. c.* var. *wuyuanensis* was introduced into Wuyuan County, northern part of Jiangxi Province, 300 years ago, and inbred for a long period, then stabilized through selection for short body shape. *C. c.* var. *color* came from the Oujiang River originally, where it is isolated from the area (Yangtze River basin) of other red carps.

Considering that the morphological characters are usually more influenced by the environment, it may not be enough to study the phylogenetic relationships among populations/strains. Therefore, a parallel study on biochemical genetics and molecular genetics was carried out[5]. It is encouraging that the results from morphology are in agreement with genotypic study.

4.2 Value of multivariate analysis when it is used for studying subspecies relation

Three multivariate analyses, namely, cluster analysis, discrimination analysis and principal component analysis, were used in this study. These three methods have been widely used in many fields because of their strong capacity in solving realistic problems[11].

Objects could be clarified preliminarily and their differences could be quantified by cluster analysis. So it is often used for analyzing degree of similarity of objects analyzed. This work demonstrated that morphological distances among four strains of red common carp could be found and clarified by using cluster analysis. Discriminate analysis is a method that searching objective discriminate evidence for classification under category known[14]. It has been used for identifying wild populations of blunt snout bream (*Megalobrama amblycephala*) successfully[15]. This study demonstrated once again that this method is very effective($P < 0.01$) for identifying population/strain of red common carp with close phylogenetic relationship. Principal component analysis is a statistical method optimizing more indexes into fewer indexes[11]. Results of principal component analysis in this study indicated that morphological differences among four strains of red common carp are mostly caused by variations in front part of body, then by the caudal bar.

From this work, it can be seen that analytical results were basically identical by these three analytical methods. But they couldn't be substituted because they reflected morphological differences from different angles and depths. The synthetical results acquired by these three methods used synthetically could have a stronger demonstration power.

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红鲤四品系的形态差异和种系关系

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摘要：综合传统形态学数据和框架测定数据，通过多元变量分析，研究了我国红鲤四个品系（兴国红鲤、玻璃红鲤、荷包红鲤及瓯江彩鲤）的形态差异和种系关系。主要结果：(1)可数性状方面，4品系红鲤无显著差异($P>0.05$)。(2)传统可量性状和框架测定数据合在一起，4品系红鲤间有显著差异($P<0.05$)；聚类分析表明，瓯江彩鲤、兴国红鲤及玻璃红鲤三者形态相近，而荷包红鲤与这三种红鲤的形态差异明显。在28项比例变量基础上所作判别分析表明，红鲤4品系间形态差异极显著($P<0.01$)，用优选的8项比例变量判别，综合判别准确率达92%；主成分分析表明，4品系红鲤间形态差异主要系躯体前半部差异所致。(3)把传统可量性状数据和框架测定数据结合在一起，使用多种多元变量分析技术，可大大增强鱼类种内不同群体间差异和亲缘关系的研究能力。

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