

凤鲚两群体线粒体细胞色素 *b* 基因片断多态性及进化特征

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摘要:为研究凤鲚(*Coilia mystus*)的长江群体和珠江群体的遗传多态性、遗传关系及进化特征, 使用线粒体DNA(mitochondrial DNA, mtDNA)的细胞色素 *b* (cytochrome *b*, *cytb*) 基因片断序列进行分析。在该基因片断上共检测到44个变异位点, 总变异率为11.5%。凤鲚长江群体内部的遗传距离为0.3%~1.0%, 珠江群体内部的遗传距离为0.5%~0.8%, 长江群体与珠江群体之间的遗传距离为9.4%~10.9%。这2个群体具有完全不同的单倍型, 其中长江群体5个个体中共检测到5种不同的单倍型, 单倍型多样性指数为1.000, 核苷酸多样性指数为0.677%; 珠江群体5个个体中共检测到4种单倍型, 单倍型多样性指数和核苷酸多样性指数分别为0.900和0.573%。对两者序列的自然选择检验分析表明, 自然选择在两者差异形成过程中起重要的作用。[中国水产科学, 2006, 13(3): 337~343]

关键词:凤鲚; 细胞色素 *b*; 遗传多样性; 遗传差异; 进化特征; 自然选择

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凤鲚(*Coilia mystus*)是一种小型鱼类, 在林奈氏分类系统中属于鲱形目(*Clupeiformes*)、鲱科(*Engraulidae*)、鲱属。凤鲚主要分布于印度洋北部沿海和太平洋西部沿海, 东至中国、朝鲜、日本, 南至印度尼西亚。中国沿海均有产量^[1-2]。凤鲚是短距离洄游鱼类, 一般是从近海向河口溯河洄游。在繁殖季节, 凤鲚长江群体成熟个体以崇明、长兴、横沙3个岛附近的南、北港一带最为密集, 通常白茆河向上数量会逐渐减少, 到达镇江的个体就非常少了^[1]。凤鲚珠江群体也是主要分布于广东沿海及河口^[3]。尽管长江凤鲚和珠江凤鲚是凤鲚的不同种群, 都具有河口短距离洄游的习性, 但是两者所生存的水域有很大的差别, 且珠江口和长江口距离遥远, 长期的隔离加上适应于不断演化的环境, 所以两者的遗传结构和进化有可能呈现出不同的特点。

为阐明两者之间的遗传多样性和进化特点, 有必要使用合适的相对稳定的分子标记来研究。线粒体DNA(mitochondrial DNA, mtDNA)是核外DNA, 由于它具有分子结构稳定、母系遗传、替换速率相对快、缺少重组等特征, 已被证明其为动物群体进化研究中的一种有用的分子标记^[4-6]。mtDNA

可以为群体遗传结构、地理变异及物种特性的研究方面提供有效的分子标记^[7]。mtDNA做为群体遗传分析的工具具有广泛的优点^[8-9]。

细胞色素 *b* (cytochrome *b*, *cytb*) 基因是mtDNA上唯一在结构和功能了解得较为清楚的蛋白编码基因, 其进化动力学及其蛋白质产物的生物化学特性都比其他的分子系统要清楚^[10-11]。*cytb* 基因在分类和进化上具有很强的适用性^[9], 在鱼类群体与进化遗传学中有广泛的应用^[12-14]。

迄今, 将mtDNA用于凤鲚不同群体的遗传多态性和进化特征研究尚未见报道。本研究从长江凤鲚和珠江凤鲚线粒体 *cytb* 基因片断序列分析入手, 探讨两者遗传多样性水平、遗传差异及进化特征, 研究结果将有助于深入了解凤鲚不同群体的变异特点。

1 材料与方法

1.1 样本

凤鲚长江群体样本取自上海长江口(N31°37', E121°80'), 珠江群体样本取自广州珠江口(N22°51', E113°82')。采样地点见图1。共采集10尾鱼,

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即每个群体各取5尾。所有的个体通过干冰运至实验室,储存至 -80°C 冰箱备用。



图1 采样位置图

1. 广州珠江口; 2. 上海长江口

Fig.1 Locations of sampling sites

1. Pearl River estuary, Guangzhou; 2. Yangtze River estuary, Shanghai

1.2 DNA提取

从肌肉组织中提取总基因组DNA。采用通常的酚氯仿法抽提^[15]。抽提出的DNA用0.8%的琼脂糖凝胶电泳检测,然后稀释到合适的浓度(约100 ng/ μL),用于PCR扩增。

1.3 PCR扩增及测序

凤鲚两群体的mtDNA *cytb* 基因,通过使用通用引物 L14724 和 H15915^[16] 扩增而获得。引物序列为: L14724, 5'-GAC TTG AAA AAC CAC CGT TG-3'; 和 H15915, 5'-CTC CGA TCT CCG GAT TAC AAG AC-3'。PCR 扩增反应混合物包括以下成分: 100 ng DNA 模板, 0.2 mmol/L dNTPs, 每个引物各 1.0 $\mu\text{mol/L}$, 4.0 mmol/L MgCl_2 , 5.0 μL 10 $\times$ reaction buffer, 2U *Taq* 聚合酶, 然后加蒸馏水至终体积 50 μL 。PCR 扩增在 PE2400 型 PCR 仪上进行。扩增程序如下: 94 $^{\circ}\text{C}$ 起始变性 4 min; 然后是 35 个循环, 每个循环包括 94 $^{\circ}\text{C}$ 变性 50 s, 55 $^{\circ}\text{C}$ 退火 1 min, 72 $^{\circ}\text{C}$ 延伸 1 min 30 s; 最后是 72 $^{\circ}\text{C}$ 再延伸 7 min。PCR 扩增产物在 1% 琼脂糖凝胶电泳检测, 然后溴化乙锭(EB)染色, 凝胶成像系统中拍照。

PCR 扩增产物用 1% TAE 琼脂糖凝胶电泳回收。回收步骤按照试剂盒说明(上海中科开瑞公司产品)。回收纯化后产物用 ABI3730 测序仪测序。为保证所测序列的可靠性, 每一个样品均用正反 2 个引物分别测序核对。测序所用引物为 H15915 和 L15519^[16]。

1.4 数据分析

双向测序后得到的结果用 DNASTAR 软件包进行拼接、核对序列。DNA 序列碱基组成、转换颠换特点以及单倍型之间遗传距离用 MEGA2.1^[17] 进行计算。

计算群体内基因型多样性(haplotype diversity, H)、核苷酸多样性(nucleotide diversity, π)^[18-19]。分子水平自然选择作用的检测采用 K_a/K_s (Li-Wu-Luo 模型^[20]) 和 MK 检验(McDonald and Kreitman^[21])。

2 结果

2.1 细胞色素 *b* 基因片段序列和差异

双向测序的结果经拼接核对后, 共获得长江凤鲚与珠江凤鲚样本的 *cytb* 基因 384 bp 的一致序列(consensus sequence)。图 2 是所测得的凤鲚两群体 *cytb* 基因片段序列的排序图。结果显示, 序列有 44 个变异位点, 总变异率为 11.5%。其中长江凤鲚有 5 种单倍型(haplotype), 而在珠江凤鲚中检测到 4 种单倍型。两群体之间的遗传距离和碱基的转换、颠换见表 1。

可以看出, 长江凤鲚内部的遗传距离为 0.3%~1.0%, 珠江凤鲚内部的遗传距离为 0.5%~0.8%, 长江凤鲚与珠江凤鲚之间的遗传距离为 9.4%~10.9%。

2.2 凤鲚两群体的遗传多态性

凤鲚两群体的遗传多态性如表 2 所示。

结果显示两者的单倍型多样性和核苷酸多样性水平都较高。

2.3 分子水平自然选择作用检验

采用 K_a/K_s (Li-Wu-Luo method) 检验和 MK (McDonald-Kreitman test) 检验检测 DNA 水平选择作用的结果分别见表 3 和表 4。

K_a/K_s 均小于 1, 提示在该基因位点上可能存在净化选择(Purifying selection)。

采用 McDonald 和 Kreitman 提出的检验方法进行检验(两者群体间差异和群体内多态见表 4), 结果显示: 中立指数(Neutrality Index)为 11.333, G -test 的 G 值为 6.715, P 值为 0.009 56 ($0.001 < P < 0.01$), 差异极显著。该极显著差异的结果提示: 长江凤鲚和珠江凤鲚之间的错义突变数目大于基于群体内多态性估计得到的期望值, 说明细胞色素 *b* 基因在两群体的进化中受到了选择作用。

CJ1	CCA	GAA	TGA	TAC	TTC	CTC	TTT	GCC	TAC	GCC	ATT	CTA	CGA	TCG	ATT	OCT	AAT	AAA	TTA	GGA	GGG	GTA
CJ2	...	...	...	...	...	...	...	...	...	T	...	...	...	...	...	...	...	G	...	...	...	...
CJ3	...	...	...	...	...	...	...	...	...	T	...	...	...	...	...	...	...	...	...	...	...	...
CJ4	...	...	...	...	...	...	...	...	...	T	...	...	...	...	...	...	...	...	...	...	...	...
CJ5	...	...	...	...	...	...	...	...	...	T	...	...	...	...	...	...	...	G	...	...	...	...
ZJ1	...	G	...	...	...	...	...	...	...	...	...	...	...	A	C	C	...	C	...	A	...	
ZJ2	...	G	...	...	...	...	...	...	...	...	...	...	...	A	C	C	...	C	...	A	...	
ZJ3	...	G	...	...	...	...	...	...	...	...	...	...	...	A	C	C	...	C	...	A	...	
ZJ4	...	G	...	...	...	...	...	...	...	...	...	...	...	A	C	C	...	C	...	A	...	
CJ1	TTA	GCG	CTA	TTA	TTC	TCA	ATC	CTT	GTC	CTC	ATA	GTA	GTA	CCA	ATT	CTT	CAT	AOC	TCT	AAA	CAA	CGG
CJ2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G
CJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZJ1	...	A	...	...	...	...	T	...	T	T	...	C	...	C	C	...	T	A	G	...	A	
ZJ2	...	A	...	...	...	...	T	...	T	T	...	C	...	C	C	...	T	A	G	...	A	
ZJ3	...	A	...	...	...	...	T	...	T	T	...	C	...	C	C	...	T	A	G	...	A	
ZJ4	...	A	...	...	...	...	T	...	T	T	...	C	...	C	C	...	T	A	G	...	A	
CJ1	GGC	CTA	AOC	TTC	OGA	CCC	CTC	ACA	CAA	TTC	CTT	TTC	TGA	ACT	CTG	GTC	GCA	GAC	GTA	ATT	ATC	TTA
CJ2	...	...	...	...	G	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ5	...	...	...	...	G	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZJ1	...	T	...	...	...	A	C	...	...	...	...	...	C	T	...	...	...	C	T	...	...	
ZJ2	...	T	...	...	...	A	C	...	...	...	...	...	C	C	...	...	...	C	T	...	...	
ZJ3	...	T	...	...	C	A	C	...	...	...	...	...	C	T	...	...	...	C	T	...	...	
ZJ4	...	T	...	...	...	A	C	...	...	...	...	...	C	T	...	...	A	...	C	T	...	
CJ1	ACA	TGA	ATC	GGA	GGA	ATA	OCT	GTT	GAA	CAC	OCT	TTC	ATT	ATT	ATT	GGT	CAA	ATC	GCA	TCA	GTA	CTC
CJ2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZJ1	...	T	...	...	G	C	...	...	...	...	...	...	C	C	C	...	...	C	...	...	...	
ZJ2	...	T	...	...	G	C	...	...	...	...	...	...	C	C	C	...	...	C	...	...	...	
ZJ3	...	T	...	...	G	C	...	...	...	...	...	...	C	C	C	...	...	C	...	...	...	
ZJ4	...	T	...	...	G	C	...	...	...	...	...	...	C	C	C	...	...	C	...	...	...	
CJ1	TAC	TTC	AOC	CTA	TTT	TTA	GTA	CTA	AGT	CCC	CTA	GCA	GGC	TGA	CTA	GAA	AAC	AAA	GCG	CTC	AAC	TGA
CJ2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CJ5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZJ1	T	...	...	G	...	GG	...	C	T	T	...	...	...	...	...	...	...	...	...	...	...	...
ZJ2	T	...	...	G	...	G	...	C	T	T	...	...	...	...	...	...	...	...	...	...	...	...
ZJ3	T	...	...	G	...	G	...	C	T	T	...	...	...	...	...	...	...	...	...	...	...	...
ZJ4	T	...	...	G	...	G	...	C	T	T	...	...	...	...	...	...	...	...	...	...	...	...
CJ1	AAC	TOC	OCT	AGT	AGC	TTA	AAT	TAA	AGC	GOC	GGT	CTT	GTA	ATC	CGG	AGA	TCG	GAG				
CJ2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
CJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
CJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
CJ5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
ZJ1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
ZJ2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
ZJ3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				
ZJ4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...				

图2 凤鲚两群体线粒体cytb基因片断序列排序图

CJ:凤鲚长江群体;ZJ:凤鲚珠江群体

Fig.2 Alignment of mitochondrial cytb gene segment sequences from two populations of *C. myzus*CJ: *C. myzus* of the Yangtze River population; ZJ: *C. myzus* of the Pearl River population

表1 凤鲚两群体的遗传距离 p-distance(左下角)和转换/颠换(右上角)

Tab.1 Genetic distance(below the diagonal) and substitution(above the diagonal) between two populations of *C. mystus*

	CJ1	CJ2	CJ3	CJ4	CJ5	ZJ1	ZJ2	ZJ3	ZJ4
CJ1		3/1	1/0	2/0	4/0	31/7	30/6	31/7	32/6
CJ2	0.010		2/1	3/1	1/1	34/8	33/7	33/8	35/7
CJ3	0.003	0.008		1/0	3/0	32/7	31/6	32/7	33/6
CJ4	0.005	0.010	0.003		2/0	33/7	32/6	33/7	34/6
CJ5	0.010	0.005	0.008	0.005		35/7	34/6	34/7	36/6
ZJ1	0.099	0.109	0.102	0.104	0.109		2/1	0/2	1/1
ZJ2	0.094	0.104	0.096	0.099	0.104	0.008		2/1	3/0
ZJ3	0.099	0.107	0.102	0.104	0.107	0.005	0.008		1/1
ZJ4	0.099	0.109	0.102	0.104	0.109	0.005	0.008	0.005	

注: CJ - 凤鲚长江群体; ZJ - 凤鲚珠江群体。

Note: CJ - *C. mystus* of the Yangtze River population; ZJ - *C. mystus* of the Pearl River population.

3 讨论

3.1 凤鲚长江群体和珠江群体的遗传关系

在以往的文献中, 只有对凤鲚物种的一般描述^[1-3], 而对凤鲚不同地理群体的遗传差异和遗传关系没有进行研究。对凤鲚不同地理群体的形态差异, 也很少研究, 仅见 Cheng 等^[22]报道, 长江凤鲚和珠江凤鲚在可量形态性状上有很大的差异。通常, 鱼类的形态变异更易受到环境影响^[23-24]。因此, 在应用形态学特征进行研究的同时, 还应该采用较为稳定的 DNA 分子标记进行研究。

表2 凤鲚两群体的单倍型多样性和核苷酸多样性

Tab.2 Haplotype diversity and nucleotide diversity of two populations of *C. mystus*

凤鲚群体 <i>C. mystus</i> populations	单倍型多样性 Haplotype diversity (h)	核苷酸多样性/% Nucleotide diversity (π)
长江群体 Yangtze River population	1.000	0.677
珠江群体 Pearl River population	0.900	0.573
平均 Mean	0.950	0.625

表3 凤鲚两群体的同义替换和非同义替换

Tab.3 Synonymous(K_s) and nonsynonymous(K_a) substitution of two populations of *C. mystus*

Pairwise	K_s	K_a	K_s/K_a	Pairwise	K_s	K_a	K_s/K_a
CJ1 - CJ2	0.030 4	0.003 5	0.115 1	CJ3 - ZJ2	0.468 7	0.007 1	0.015 1
CJ1 - CJ3	0.010 0	0.000 0	0.000 0	CJ3 - ZJ3	0.508 7	0.007 1	0.013 9
CJ1 - CJ4	0.010 0	0.003 5	0.350 0	CJ3 - ZJ4	0.487 6	0.010 7	0.021 9
CJ1 - CJ5	0.030 3	0.003 5	0.115 5	CJ4 - CJ5	0.020 1	0.000 0	0.000 0
CJ1 - ZJ1	0.468 7	0.010 7	0.022 8	CJ4 - ZJ1	0.487 6	0.014 2	0.029 1
CJ1 - ZJ2	0.450 4	0.007 1	0.015 8	CJ4 - ZJ2	0.468 7	0.010 7	0.022 8
CJ1 - ZJ3	0.489 3	0.007 1	0.014 5	CJ4 - ZJ3	0.508 7	0.010 6	0.020 8
CJ1 - ZJ4	0.468 7	0.010 7	0.022 8	CJ4 - ZJ4	0.487 6	0.014 2	0.029 1
CJ2 - CJ3	0.020 1	0.003 5	0.174 1	CJ5 - ZJ1	0.527 4	0.014 2	0.026 9
CJ2 - CJ4	0.020 1	0.007 1	0.353 2	CJ5 - ZJ2	0.507 5	0.010 7	0.021 1
CJ2 - CJ5	0.000 0	0.007 1	—	CJ5 - ZJ3	0.529 3	0.010 6	0.020 0
CJ2 - ZJ1	0.528 6	0.014 2	0.026 9	CJ5 - ZJ4	0.527 4	0.014 2	0.026 9
CJ2 - ZJ2	0.508 7	0.010 6	0.020 8	ZJ1 - ZJ2	0.020 3	0.003 5	0.172 4
CJ2 - ZJ3	0.530 6	0.010 6	0.019 9	ZJ1 - ZJ3	0.010 1	0.003 5	0.346 5
CJ2 - ZJ4	0.528 6	0.014 2	0.026 9	ZJ1 - ZJ4	0.000 0	0.007 1	—
CJ3 - CJ4	0.000 0	0.003 5	—	ZJ2 - ZJ3	0.030 7	0.000 0	0.000 0
CJ3 - CJ5	0.020 1	0.003 5	0.174 1	ZJ2 - ZJ4	0.020 3	0.003 5	0.172 4
CJ3 - ZJ1	0.487 6	0.010 7	0.021 9	ZJ3 - ZJ4	0.010 1	0.003 5	0.346 5

注: CJ - 凤鲚长江群体; ZJ - 凤鲚珠江群体。

Note: CJ - *C. mystus* of the Yangtze River population; ZJ - *C. mystus* of the Pearl River population.

表4 群体内和群体间的同义替换数和非同义替换数

Tab.4 Number of nonsynonymous and synonymous substitutions for fixed differences between populations and polymorphisms within populations

	固定位点 (群体间差异) Fixed site	多态位点 (群体内多态) Polymorphic site
非同义替换 Nonsynonymous	2	4
同义替换 Synonymous	34	6

本研究显示,长江凤鲚内部单倍型之间的遗传距离为 0.3%~1.0%,珠江凤鲚内部单倍型之间的遗传距离为 0.5%~0.8%,长江凤鲚与珠江凤鲚之间的遗传距离为 9.4%~10.9%。Billington 和 Hebert^[25]报道种内单倍型趋异一般在 10% 的水平内。Avice^[9]认为同一种的个体间一般有 0.1%~5% 的趋异。对其他一些动物的 *cytb* 基因序列的分析表明,种内个体间的序列差异一般在 0%~4.06%,差异超过 6% 的个体间已有明显的亚种或种的分化^[26-28]。

根据两者的 *cytb* 基因的差异程度,本研究认为,长江凤鲚与珠江凤鲚之间的分化可能已经达到亚种水平,并且本课题的形态学研究结果^[22]更加支持这种推断。然而,还应该使用更多的分子标记,例如 D-loop、COI 等来验证这一推断。

长江凤鲚和珠江凤鲚两者之间极显著的差异,可能是跟它们的短距离河口洄游习性以及相互隔离、适应环境有关。它们主要是从近海向河口洄游,因此两者之间很少相互交流,并且长江口和珠江口的水域环境、气候条件有着很大的差别,而且珠江口和长江口距离约 1 689 km,长期的隔离加上适应于不断演化的环境,所以两者的遗传差异很大是有可能的。

3.2 长江凤鲚和珠江凤鲚的遗传多态性

核苷酸多样性(π)是表示每个群体内,各个单倍型的两两配对差异的平均值。 π 值是一个群体的遗传多样性指标。凤鲚长江群体和珠江群体的 π 值分别为 0.677% 和 0.573%,表明凤鲚两群体在 mtDNA 水平上存在较丰富的遗传多样性。

有效群体的大小对遗传多样性有很大的影响。就凤鲚长江群体来说,由于长时期的无节制添船加网捕捞,特别是对幼鱼的捕捞,导致凤鲚补充群体逐年减少,凤鲚资源下降^[29],其有效群体数目减少。

近年来,由于环境恶化、水体污染加剧、航运增加等不利于凤鲚生长繁殖的因素,以及凤鲚资源所呈现的小型化现象,更导致凤鲚产量较低。这说明,应该切实加强凤鲚资源保护,通过限制作业船只数、作业时间等手段降低捕捞强度,以确保凤鲚资源的恢复和可持续利用^[30]。

鉴于此,农业部发布了《长江刀鲚凤鲚专项管理暂行规定》^[31],对捕捞许可证发放、捕捞作业时间、渔具网目等都进行专项管理。研究结果显示,这种保护措施对恢复凤鲚种质资源的遗传多样性水平起到了积极的作用。珠江凤鲚资源所受的破坏程度可能较之长江凤鲚要小,因此其遗传多样性水平仍然是比较丰富的。

3.3 DNA 水平自然选择作用的检测

在分析长江凤鲚和珠江凤鲚的序列时,发现有些变异只是在其中一个群体内特有的。由此推测可能由于自然选择的作用,使得差异已经固定。为此,本研究检测了自然选择是否在此两群体的 DNA 水平变异中起作用。所采用的方法主要有 2 种,即: K_a/K_s 检验和 MK 检验。一般来说, K_a/K_s 的值大于 1,提示可能存在正向达尔文选择(Positive selection);小于 1 则提示可能存在负选择(Negative selection)或者叫 Purifying selection;等于 1 则表明该位点没有选择,是中性进化的(Neutral site)。MK 检验是检验种间(或种群间)选择作用的有效方法。MK 检验思路简捷,计算简单,在检验中性假说时很有说服力。该检验不需要很多假设限制,重组和群体大小的动态对检验结果没有影响,因此该方法应用较为广泛。

分析的结果表明, K_a/K_s 的值均小于 1(除了 3 个 K_s 等于 0 无法计算外),而 MK 检验的 G 值为 6.715($P < 0.01$),差异极显著。两种检验结果都表明自然选择在长江凤鲚和珠江凤鲚的序列差异形成过程中起了重要作用。

鱼类基因的进化过程中,遗传因素和选择因素都会起作用。一般来说,承担重要功能的基因,由于要承担功能,往往会受到较强的选择限制。细胞色素 *b* 基因是线粒体自身的能够编码蛋白质的少数基因之一,是合成 ATP 的电子呼吸链的重要成员^[32]。一旦该基因发生错义突变,可以导致一些严重的疾病,由此提示这个基因在功能上是很重要的。一旦有害的错义突变发生,那么这个错义突变个体

就由于选择的作用而被淘汰,从而这种错义突变就不能在群体中固定下来。可见能够检测到选择作用的存在是比较合理的。

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Genetic diversity and evolutionary characters in two populations of *Coilia mystus* inferred from cytochrome *b* gene segment sequence of mitochondrial DNA

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Abstract: *Coilia mystus* is distributed mainly in the northern Indian Ocean and the western Pacific Ocean, extending eastward to China, Korea and Japan, and Southward to Indonesia. It is classified into genus *Coilia* (Gray, 1831), Engraulidae, Clupeiformes. Some differences maybe exist between different populations of *C. mystus* due to isolation and adaption. The great morphological differences between two geographical populations of *C. mystus*, Yangtze River population and Pearl River population, were revealed by multivariate analysis. In general, fishes demonstrate greater variance in morphometric traits both within and between populations than other vertebrate, and are more susceptible to environmentally - induced morphological variation. Thus, it is necessary to reveal the differences between different geographical populations by using stable molecular markers.

Genetic diversity and evolutionary characters in two populations of *Coilia mystus*, Yangtze River population and Pearl River population, were investigated at the mtDNA level in this study. A total of ten fish, i.e., five fish at each site, were collected. Sequences of cytochrome *b* (*cytb*) gene segments amplified by PCR was used. Forty-four variable sites were detected, accounting for 11.5% of total sequence. Genetic distances within the Yangtze River population were between 0.3% and 1.0%, within the Pearl River population were between 0.5% and 0.8%, and genetic distances between Yangtze River population and Pearl River population were from 9.4% to 10.9%. The large genetic distances between these two populations suggest their divergence maybe arrived at subspecies level. Five different haplotypes were detected within Yangtze River *C. mystus* and four within Pearl River *C. mystus*. The haplotype diversity index of Yangtze River *C. mystus* and Pearl River *C. mystus* were 1.000 and 0.900, respectively. The nucleotide diversity of Yangtze River population and Pearl River population were 0.677% and 0.573%, respectively. These indexes suggest that these two populations of *C. mystus* are abundant in genetic diversity.

Two methods, K_a/K_s test and McDonald-Kreitman test, were used to detect if natural selection operated at DNA level during the formation of divergence between these two populations. Significant results were revealed by the two methods (the ratios of K_a/K_s are all less than 1 and the G value and P value of MK test is equal to 6.715 and 0.009 56, respectively), and it suggests that natural selection operates on this gene during its evolution. The *cytb* gene is an important gene in mitochondrial DNA, and severe diseases can be caused once the nonsynonymous substitution happens in this gene. Due to natural selection, the individual which has this damaged gene can not be fixed in the population and was removed from this population. Thus, it is reasonable to observe the action of natural selection. [Journal of Fishery Sciences of China, 2006, 13(3): 337 - 343]

Key words: *Coilia mystus*; cytochrome *b*; genetic diversity; genetic differentiation; evolution characteristics; natural selection

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