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虹鳟倍性鉴定 SSR 标记筛选与应用研究*

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摘要 虹鳟(*Oncorhynchus mykiss*)是我国冷水性鱼类的主要养殖品种。与二倍体相比,虹鳟三倍体具有生长速度快、肉质好和不育等优点。目前,鉴定虹鳟倍性主要是通过流式细胞术进行 DNA 含量分析,但这种方法需要采集鱼类血液或组织样本,会对其造成伤害。为了实现利用微创方法收集样本鉴定虹鳟倍性,本研究利用 PCR 扩增以及电泳分离技术对 153 个微卫星标记(SSR 标记)进行分析,其中 139 个 SSR 标记能够在二倍体和三倍体中成功扩增,筛选出 132 个 SSR 标记具有多态性,最终鉴定出 7 个标记在三倍体中表现出较高的变异性。通过在 52 个已知倍性水平的参考样本和 48 个未知倍性的样本上进行验证,进一步从中筛选出 3 个 SSR 标记(SSR1054、SSR1056 和 SSR1468)足以区分倍性水平,并且根据 3 个标记序列重新设计了 3 对具有良好稳定性的引物序列进行倍性分析应用。本研究所筛选的 SSR 标记解决了现有虹鳟倍性鉴定中存在的技术流程复杂、耗材昂贵的问题,并有助于不同倍性虹鳟的遗传多样性研究。

关键词 虹鳟; 鲑科鱼类; 微卫星; 三倍体; 倍性检测

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虹鳟(*Oncorhynchus mykiss*)是目前世界上养殖范围最广的冷水性经济鱼类之一。我国自 20 世纪 60 年代起,陆续从朝鲜、美国、丹麦等国家引进虹鳟种质,在种质保存、鉴定、养殖和品种选育等方面开展了系统研究,目前,已选育出生长速度快、适合我国养殖模式的虹鳟“水科 1 号”新品种(王炳谦等, 2021)。随后虹鳟“全雌 1 号”通过了全国水产原种和良种审定委员会审定,实现了核心种源自主可控。三倍体虹鳟饲料转化率高、生长优势明显,肉质优于二倍体,备受国内外养殖户和消费者的欢迎。同时,三倍体性腺

发育不全(Han *et al*, 2010; Huang *et al*, 2021; Krisfalusi *et al*, 1999; Nynca *et al*, 2022),避免了其产卵期死亡率高和肉质品质下降的养殖风险(Cleveland *et al*, 2016; Manor *et al*, 2012、2014; Werner *et al*, 2008),并且规避了养殖逃逸造成外来物种入侵等生态风险(Glover *et al*, 2016; Karlsson *et al*, 2016; Nie *et al*, 2014; Xu *et al*, 2022),因此,养殖三倍体虹鳟具有更为显著的市场效益和生态效益。

由于虹鳟二倍体和三倍体在形态学上非常相似,很难通过形态学来区分虹鳟倍性。以往多倍体通过细

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胞学分析直接计数染色体数目来确定,但这种方法往往会导致虹鳟死亡(苏泽古等, 1984; 尹洪滨, 2001; 印杰等, 2005)。近年来,多采用流式细胞术测定细胞中 DNA 的相对含量来确定样本的倍性。然而,使用流式细胞术对样本血细胞分析来检测倍性,采集血液的鱼类体长至少需 5 cm,并且这可能会对鱼体造成伤害(Allen, 1983; Lecommandeur *et al*, 1994; 齐嫣然等, 2022)。此外,这两种方法操作精细、技术流程复杂、耗材昂贵,因此,虹鳟三倍体倍性鉴定的普及应用受到了极大制约。

微卫星标记(SSR 标记)是广泛分布在真核生物基因组中的简单重复序列,已广泛应用于鱼类的倍性和谱系分析(Ohara *et al*, 1999、2000; Mishina *et al*, 2014; Nie *et al*, 2014)。微卫星分析的优势包括重现性高、样本需求量低及群体遗传分析快速等。微卫星分析已应用于亚马逊帆鳍鲳(*Poecilia formosa*) (Lampert *et al*, 2006)、银鲫(*Carassius auratus*) (Bai *et al*, 2011)、大菱鲂(*Scophthalmus maximus*) (Hernández-Urcera *et al*, 2012)、大西洋鲑(*Salmo salar*) (Glover *et al*, 2016)和泥鳅(*Misgurnus anguillicaudatus*) (Feng *et al*, 2018)的多倍体鉴定。因此,本研究采用微卫星技术分析虹鳟二倍体群体和三倍体群体间的差异,以期养殖条件下区分二倍体和三倍体虹鳟提供理论依据。

1 材料与方法

1.1 样本采集

实验鱼来自中国水产科学研究院黑龙江水产研究所渤海冷水性鱼试验站,鱼体长(15±2) cm,体重(30±5) g。共采集 52 尾已知倍性(26 尾二倍体, 26 尾三倍体)和 48 尾未知倍性(二倍体和三倍体混合比例未知)虹鳟幼鱼血液及鳍条组织作为样本。其中 52 尾虹鳟幼鱼的倍性通过使用流式细胞仪分析红细胞 DNA 含量来确定(图 1)。

1.2 DNA 的提取与检测

使用 DNA 提取试剂盒(Tiagen)从虹鳟尾鳍中提取 DNA。在紫外分光光度计上测定纯度($OD_{260/280}=1.8\sim2.0$)和浓度,并将浓度稀释到 50 ng/μL,使用 1%琼脂糖凝胶电泳检测质量。

1.3 PCR 扩增及检测

153 个虹鳟微卫星标记由 Rexroad、Coulibaly 和 Phillips 等开发(Rexroad *et al*, 2001、2002a、b、c; Coulibaly *et al*, 2005; Phillips *et al*, 2006),利用

primer3 对其中部分 SSR 标记重新设计了稳定的引物(Robinson *et al*, 2004),由生工生物(上海)工程公司合成。

PCR 总体系为 10 μL,包括 2×*Tap Mix* 5 μL;上、下游引物(10 μmol/L)各 0.5 μL;DNA 模板 1 μL;ddH₂O 3 μL。在 ABI 9700 型 PCR 仪进行如下 PCR: 94 °C 预变性 5 min; 94 °C 变性 30 s,退火温度 58 °C 复性 30 s, 72 °C 延伸 30 s, 25 个循环;最后 72 °C 延伸 7 min。扩增产物经 8%聚丙烯酰胺凝胶电泳分离、银染法显色,数码相机拍摄保存。

1.4 筛选出的 SSR 标记在倍性鉴定中的有效性验证

使用选择的 SSR 标记对来自中国水产科学研究院黑龙江水产研究所渤海冷水性鱼试验站的 48 个未知倍性的虹鳟样本进行验证。每个个体采集尾鳍提取 DNA;尾静脉穿刺法采集血液进行流式细胞术分析。利用筛选所得的 SSR 标记进行 PCR 扩增。根据每个个体在 SSR 位点上的最大等位基因数来估计倍性。

为了验证倍性检测的准确性,采用 sysmex 流式细胞仪测量每个个体的 DNA 含量,并以 2n=30 的虹鳟红细胞作为二倍体对照。

1.5 数据统计与分析

使用 Gel-Pro Analyzer (Version 6.3)软件进行图像数据分析,获得特异等位基因及基因型数据,并统计等位基因片段大小,分析和计算每个标记在二倍体、三倍体群体的等位基因数(A)、观测杂合度(H_o)。观测杂合度计算公式:观测杂合度(H_o)=观察得到杂合个体数/样本个体数总数。

2 结果与分析

2.1 微卫星标记对虹鳟基因组 DNA 的扩增结果及遗传差异

本研究使用的 153 个 SSR 标记,139 个(90.85%) SSR 标记在二倍体虹鳟基因组 DNA 中扩增得到清晰条带;141 个(92.16%) SSR 标记在三倍体虹鳟基因组 DNA 中扩增得到清晰条带;139 个(90.85%) SSR 标记在二倍体和三倍体虹鳟基因组 DNA 中均扩增得到清晰条带,其中,132 个(86.27%)标记在个体间表现出多态。

132 个具有多态性的 SSR 标记的样本 PCR 扩增片段在 79~482 bp,每个标记检测到的等位基因数在 1~18 之间,共检测到 588 个等位基因,二倍体群体 410 个,三倍体群体 402 个。二倍体群体在 139 个标

记的等位基因数(A)在 1~14 之间, 平均为 3.11, 三倍体群体的 A 值介于 1~16 之间, 平均为 3.05。二倍体群体观测杂合度(H_o) 在 0.00~1.00 之间, 平均为 0.66; 三倍体群体的 H_o 在 0.00~1.00 之间, 平均为 0.71。两个群体遗传多样性处于较高水平。

2.2 虹鳟倍性鉴定特征性位点筛选

首先, 通过使用流式细胞仪分析红细胞 DNA 含量来确定 52 尾虹鳟幼鱼的倍性, 区分出 26 尾二倍体样本和 26 尾三倍体样本(图 1)。使用 153 个 SSR 标记对 4 个(2 个二倍体和 2 个三倍体)DNA 样本进行 PCR 扩增, 产物经聚丙烯酰胺凝胶分离后, 检测到 132 个标记具有多态性。通过观察等位基因数目来计算等位基因实际数目。对表现出多态的 132 个标记进行倍性鉴定特征性位点的第一次筛选。倍性鉴定特征性位点的筛选标准为: 所有二倍体样本的图谱条带数目 ≤ 2 , 三倍体样本中包含个体图谱条带数目 ≥ 3 。经过图像数据分析, 13 个 SSR 标记符合筛选标准。

随后将进行 PCR 的 DNA 样本数量增加到 48 个(24 个二倍体和 24 个三倍体), 对 13 个符合初次筛选

标准的 SSR 标记进行第二次筛选。结果显示, 7 个 SSR 标记(表 1)符合, 其余 6 个 SSR 标记不符合筛选标准的主要原因是个别二倍体样本的图谱条带数目 >2 。

从符合倍性鉴定特征性位点筛选标准的 7 个 SSR 标记中选择 3 个 SSR 引物(SSR1054、SSR1056 和 SSR1468)作为鉴别倍性水平的标记(表 2), 它们不仅具有较多的等位基因数和较高的观测杂合度, 而且在这 3 个标记均检测出三倍体特有的等位基因(表 3), 如图 2 所示, SSR1054-305bp、SSR1056-463bp 和 SSR1468-363bp 3 条条带在虹鳟三倍体群体中占比分别为 62.50%、58.33%和 70.83%, 而在虹鳟二倍体群体中占比均为 0%。使用所选的 3 个引物通过观察等位基因数目和 3 条特异等位基因条带来鉴定虹鳟样本的倍性(表 4)。

2.3 特异 SSR 标记在虹鳟三倍体鉴定中的应用

用所选的 3 个鉴定虹鳟倍性水平的标记对 48 个未知倍性虹鳟样本进行 PCR 扩增, 其中, 扩增出现特异标记条带或 3 条条带的样本均为三倍体, 其余个体判定为虹鳟二倍体。如表 5 所示, SSR1054 样本扩

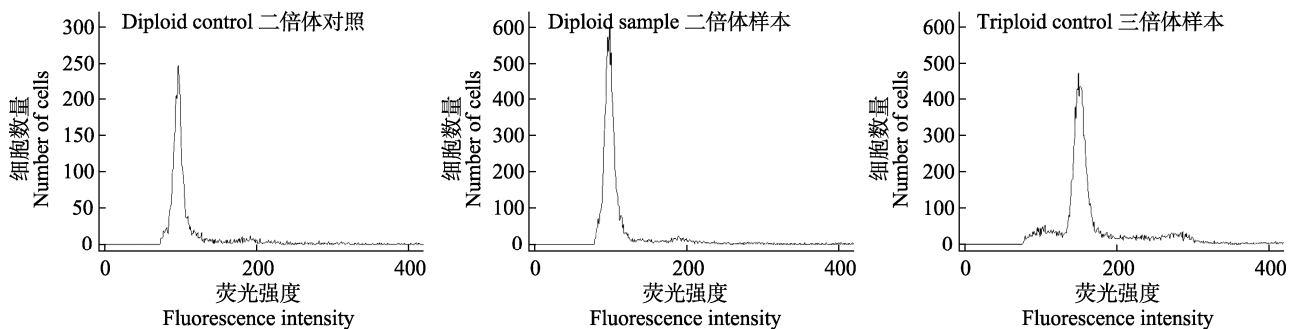


图 1 二倍体和三倍体样本(细胞 DNA 含量)的流式细胞仪检测结果(以 $2n=30$ 的虹鳟红细胞作为二倍体对照)

Fig.1 Diploid and triploid samples (cell DNA content) were detected by flow cytometry, red blood cells of rainbow trout ($2n=30$) as diploid control

表 1 符合虹鳟倍性鉴定特征性位点筛选标准的 SSR 引物及遗传参数
Tab.1 SSR primers and genetic parameters conforming to the screening criteria of characteristic sites for ploidy identification of rainbow trout

引物 Primer	GenBank 注册号 GenBank No.	片段长度 Segment length/bp	A		H_o	
			2n	3n	2n	3n
OMM1045	AF346692.1	143~285	8	7	0.92	0.96
OMM1053	AF352738.1	237~345	8	10	0.88	0.96
SSR1054	AF352739.1	220~365	13	13	0.83	1.00
SSR1056	BV722065.1	248~463	14	12	0.83	0.96
SSR1468	BV079609.1	220~363	14	11	0.79	1.00
OMM1669	BV212161.1	206~250	4	4	0.50	0.17
OMM3142	BV718475.1	171~189	4	3	0.58	0.63
平均值 Mean	0	206~323	9.29	8.57	0.76	0.81

注: A 为等位基因数, H_o 为平均观测杂合度。

Notes: A : Allele number; H_o : Mean observed heterozygosity.

表 2 鉴别虹鳟三倍体的特异标记信息
Tab.2 Specific microsatellite markers for identification of triploid rainbow trout

引物 Primer	序列 Sequence (5'~3')	核心重复序列 Repeat motif	GC 含量 GC content/%	退火温度 Annealing temperature/℃
SSR1054F	CACGCCATACCAACTGAGC	(ATCT) ₂₀	57.89	56
SSR1054R	TCACCACACATCAGCTACCG		55.00	
SSR1056F	CCAGTCAGTGTGTTGCTGCAT	(AGAT) ₁₇	50.00	58
SSR1056R	ACCTGACCATGAAAGGATGG		50.00	
SSR1468F	TGTTGATGTCAAAGCGTGTG	(ATCT) ₂₁	45.00	56
SSR1468R	GGTTGGTTTCATCTCGGAGT		50.00	

表 3 虹鳟二倍体、三倍体在 3 个标记中出现的比例差异
Tab.3 The differences in proportional distribution of the 3 microsatellite markers
between diploid and triploid groups of rainbow trout

标记名称 Marker name	扩增产物大小 Product size/bp	二倍体 Diploids	三倍体 Triploids	二倍体、三倍体分布比例差值 Difference of distribution ratio between diploid and triploid/%
SSR1054	349	1 (4.17%)	0	4.17
	322	2 (8.33%)	0	8.33
	305	0	15 (62.50%)	62.50
	287	0	4 (12.50%)	12.50
	261	1 (4.17%)	0	4.17
SSR1056	463	0	14 (58.33%)	58.33
	413	2 (8.33%)	0	8.33
	372	2 (8.33%)	0	8.33
	311	0	2 (8.33%)	8.33
SSR1468	363	0	17 (70.83%)	70.83
	350	1 (4.17%)	0	4.17
	258	4 (12.50%)	0	12.50
	242	1 (4.17%)	0	4.17
	220	1 (4.17%)	0	4.17

注：括号中的百分数表示扩增出该等位基因的个体数占群体总个体数(24 个)的百分比。
Note: The percentage in parentheses represents the percentage of individuals whose alleles were amplified to the total population (24).

增图谱中含有三倍体特异等位基因 305 bp 或 3 条带基因型的个体判定为虹鳟三倍体个体,共检测出三倍体个体 29 个, SSR1056 共检测出三倍体个体 27 个, SSR1468 共检测出三倍体个体 35 个。结合特异标记 SSR1054、SSR1056 和 SSR1468 判定为虹鳟三倍体为 46 尾,与流式细胞仪检测倍性结果一致。

3 讨论

本研究通过对 153 个虹鳟 SSR 标记筛选,结合计算和实验方法,最终选择 3 个特异 SSR 标记用于虹鳟三倍体鉴定。在实验过程中发现,这 3 个 SSR 引物在正规实验操作的情况下频繁出现失活的现象,

因此,利用 Primer3 重新在 SSR 位点的侧翼区域设计引物对,最终得到 3 对稳定的引物序列。结果显示,所有 52 个虹鳟参考样本以及 48 个倍性未知样本的倍性水平均被正确区分开,这证实了本研究所筛选的特异 SSR 标记和相关分析方法的可靠性和实用性。

在选择用于倍性鉴定的 SSR 标记时,本研究优先选择具有高变异性的扩增位点,这将大大提高 SSR 辅助倍性鉴定的效率(Guo *et al.*, 2016)。对扩增位点变异性的区分主要考虑表达的等位基因数量,以及这些等位基因的相对频率。此外,具有易于观察分析的清晰图谱条带也是筛选优质特异 SSR 标记的重要标准之一。因为通过统计清晰的图谱条带可以正确地判断

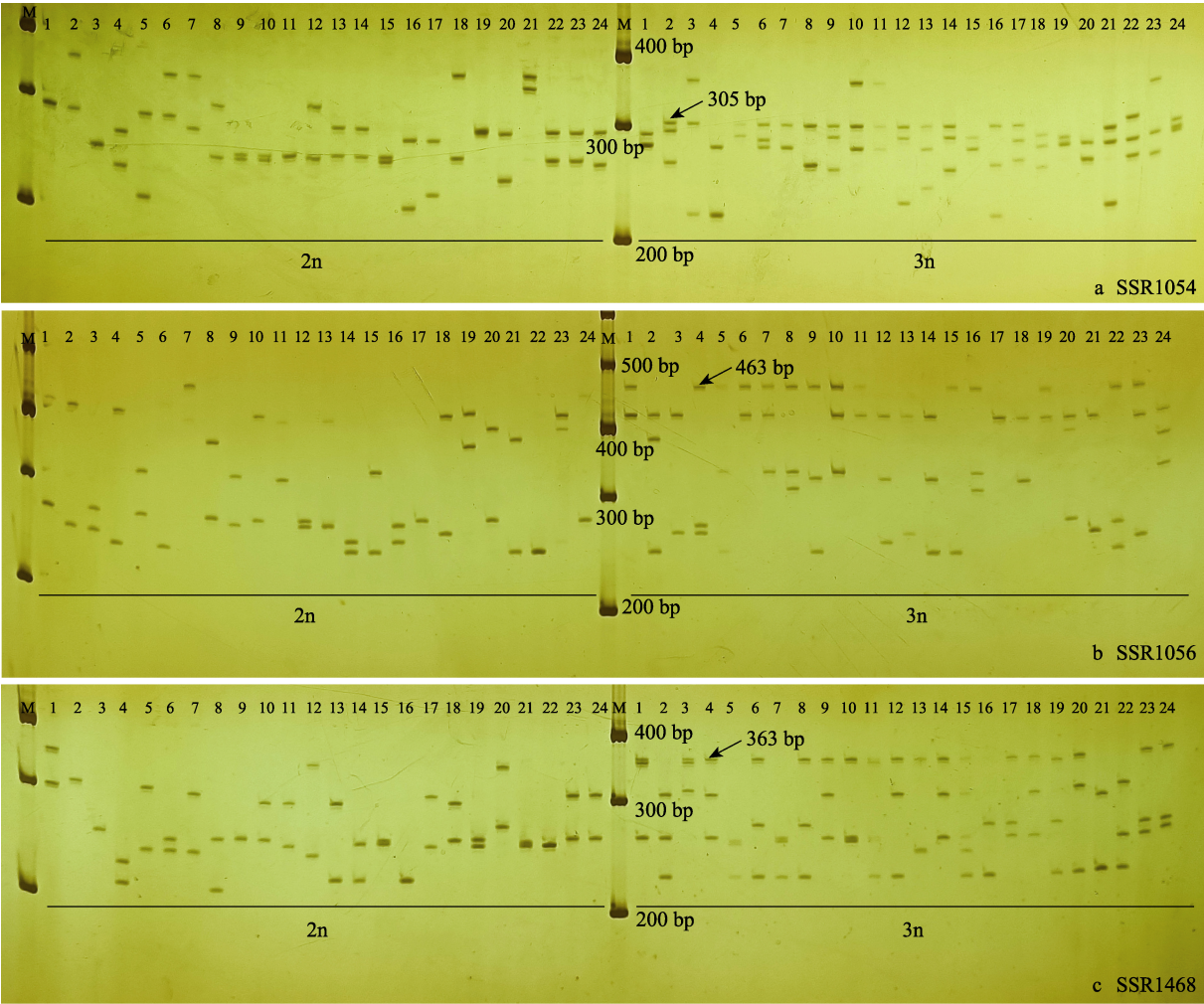


图 2 引物 SSR1054(a)、SSR1056(b)和 SSR1468(c)扩增虹鳟基因组 DNA 的电泳
Fig.2 Electrophoretic patterns of primer SSR1054 (a), SSR1056 (b) and SSR1468 (c)
in multiplication of genomic DNA in rainbow trout

M: 分子量标准 DL1000; 2n: 虹鳟二倍体个体; 3n: 虹鳟三倍体个体
M: Molecular weight standard DL1000; 2n: Diploid individual rainbow trout; 3n: Individual triploid rainbow trout

表 4 3 个特异标记组合对三倍体虹鳟个体的鉴别结果

Tab.4 Identification results of three specific microsatellite markers for individual triploid rainbow trout

特异标记 Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
SSR1054		√	√		√	√	√	√	√	√	√	√	√		√	√	√				√	√	√	√
SSR1056	√	√		√	√	√	√	√	√	√	√	√		√	√	√			√	√		√	√	√
SSR1468	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√		√	√	√
三倍体 Triploids	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

注: √表示通过观察等位基因数目和特异等位基因判定个体为三倍体。表 5 同
Note: √ indicates that an individual is triploid by observing the number of alleles and specific alleles. Same in Tab.5.

每个位点上的等位基因构型,从而确定虹鳟的倍性水平。
利用 SSR 标记对多倍体物种进行群体遗传学研究是具有挑战性的,因为当电泳图谱中显示的微卫星 DNA 等位基因数量少于该多倍体物种倍性水平可能

出现的最大等位基因数量时,很难通过直接观察等位基因数量来准确判断其倍性(Ferrante *et al*, 2010; Palop-Esteban *et al*, 2011)。在二倍体个体中,高度杂合的 SSR 在一个特定的位点上最多只能扩增 2 个等位基因。在三倍体和四倍体个体中,则可观察到 3 个

表 5 3 个 SSR 特异标记在倍性鉴定中的有效性验证结果
Tab.5 Validation results of three specific microsatellite markers in ploidy identification

特异标记 Locus	SSR1054	SSR1056	SSR1468	三倍体 Triploids	特异标记 Locus	SSR1054	SSR1056	SSR1468	三倍体 Triploids
1	√	√		√	25	√	√	√	√
2	√		√	√	26		√	√	√
3	√	√	√	√	27		√		√
4		√	√	√	28		√		√
5	√	√	√	√	29				
6			√	√	30	√	√		√
7	√	√	√	√	31		√		√
8	√	√	√	√	32		√	√	√
9	√		√	√	33			√	√
10	√			√	34				
11	√	√	√	√	35		√	√	√
12	√			√	36	√	√		√
13	√		√	√	37	√		√	√
14			√	√	38	√		√	√
15	√		√	√	39			√	√
16	√	√	√	√	40			√	√
17	√	√	√	√	41		√	√	√
18	√	√	√	√	42	√			√
19	√		√	√	43		√		√
20			√	√	44	√	√	√	√
21	√		√	√	45	√	√	√	√
22		√		√	46	√		√	√
23	√	√	√	√	47	√	√	√	√
24		√	√	√	48	√		√	√

表 6 虹鳟二倍体和三倍体所有等位基因构型的预期电泳条带数

倍性 Ploidy	二倍体 Diploids		三倍体 Triploids		
等位基因型式 Allele patterns	1 个等位基因 One allele	2 个等位基因 Two alleles	1 个等位基因 One allele	2 个等位基因 Two alleles	3 个等位基因 Three alleles
可能的等位基因型 Possible allele configurations	AA	AB	AAA	AAB ABB	ABC
电泳图谱条带数目 Electrophoretic-band numbers	1	2	1	2 2	3

和 4 个等位基因(Feng *et al*, 2018)。然而，在一些特殊情况下，无法通过直接观察等位基因数目来正确判断等位基因的实际数量。当只有 1 个独特等位基因时，无法准确判断其基因型。同理，倍性未知的个体显示 2 个等位基因数目也有多种可能的基因型，二倍体(AB)和三倍体(AAB 或 ABB)都可能发生这种情况(表 6)。本研究通过结合多个特异 SSR 标记的分析结果来提高倍性检测的准确性。如图 2b 中三倍体的 17 号参考样本在图谱中仅显示 1 条条带，但在图 2a 和图 2c 中三倍体的 17 号样本显示 3 条条带。此外，本

研究发现了 3 条可以辅助区分虹鳟二倍体和三倍体的特异标记条带，分别是 SSR1054-305bp、SSR1056-463bp 和 SSR1468-363bp。这 3 条特异性标记条带将大大提升使用 SSR 标记鉴定虹鳟三倍体的准确率。

微卫星无效等位基因是 SSR 在倍性鉴定中的另一个潜在的限制，它是由于 SSR 侧翼序列突变等导致基因无法正常扩增，它可以通过改变引物与侧翼序列的结合位点来消除或避免(Ellis *et al*, 2007; Hernández-Urcera *et al*, 2012; Nie *et al*, 2014)。在使用

SSR 标记倍性鉴定时, 无效等位基因可能会导致实验统计的等位基因数目比实际等位基因数目低。在本研究中, 筛选的 3 个特异 SSR 标记在分析的 100 个虹鳟样本过程中没有受到无效等位基因影响。这可能由于这些微卫星是从虹鳟物种本身基因组中开发的, 如果将这 3 个 SSR 引物应用于近缘物种时, 会提高无效等位基因的出现频率(Palop-Esteban *et al*, 2011)。此外, 已有研究表明, 当采用适当数量的具有高度可变性的 SSR 标记时, 无效等位基因在倍性鉴定中不会产生严重影响, 因为 SSR 位点的大量变异性补偿了由任何一个位点引入的潜在偏差影响(Biasi *et al*, 2015; Lemer *et al*, 2011)。虽然本研究的结果不能在未来的样本中完全摒弃无效等位基因的存在, 但目前的实验数据表明, 无效等位基因出现的频率非常低, 它们对多倍体鉴定的影响将是微乎其微的。

综上所述, 本研究所筛选出的一组 3 个虹鳟倍性鉴定特异 SSR 标记, 为区分虹鳟二倍体、三倍体提供了一种微创、经济、可靠和可批量操作的分子方法。该方法可用于虹鳟三倍体制种过程对发眼卵、幼鱼等进行倍性监测的质控环节。

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Screening and Application of SSR Markers for Identification of Ploidy in *Oncorhynchus mykiss*

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Abstract Rainbow trout (*Oncorhynchus mykiss*) is one of the world's most widely farmed cold-water economic fish. It is also the primary cold-water fish species in China. Since the 1960s, the rainbow trout germplasm has been introduced from North Korea, the United States, Denmark, and other countries, and a systematic study has been carried out on germplasm preservation, identification, breeding, and variety breeding. Compared with rainbow trout diploid, the triploid has the advantages of a high feed conversion rate, fast growth rate, and good meat quality. Moreover, the triploid gonadal hypoplasia can mitigate risks associated with high mortality and meat quality decline during spawning and avert the ecological risk of invasive alien species caused by breeding escape. Therefore, breeding triploid rainbow trout has more significant market and ecological benefits. As diploid and triploid rainbow trout are very similar in terms of morphology, it is difficult to distinguish the ploidy of rainbow trout by morphology alone. Currently, DNA content analysis by flow cytometry is the main method used to identify the ploidy of rainbow trout, but this method necessitates the collection of fish blood or tissue samples, and the body length of the fish to collect the blood must be at least 5 cm. Additionally, these sampling operations may harm the fish. This method also involves a delicate operation, complicated technical process, and expensive consumables, thus, the popularization and application of triploid identification of rainbow trout are greatly restricted. Considering the limitations of the technical test for the ploidy of rainbow trout, a minimally invasive and economical method with only a small amount of sampling is needed to identify and analyze the ploidy of rainbow trout in batches. Microsatellite markers (SSR markers) are simple repeated sequences widely distributed in eukaryotic genomes and have been widely used in ploidy and pedigree analyses of fish. The advantages of microsatellite (SSR) analysis include high reproducibility, low sample requirements, and rapid population genetic analysis. In order to achieve the use of a minimally invasive method to collect samples for trout ploidy identification, this study analyzed 153 SSR markers using PCR amplification and electrophoresis separation technology. Among them, 139 SSR markers could be successfully amplified in diploid and triploid, and 132 SSR markers were screened for polymorphism. Finally, seven markers showed high variability in triploid. By verifying 52 reference samples with a known ploidy level and 48 samples with an unknown ploidy level, three SSR markers (SSR1054, SSR1056, and SSR1468) were further screened to distinguish the ploidy level. Moreover, three pairs of primer sequences with good stability were redesigned according to the three marker sequences for ploidy analysis application. When SSR markers were selected for ploidy identification, amplification sites with high variability were preferred because they greatly improve the efficiency of SSR-assisted ploidy identification. Differentiation of the variability of amplification sites mainly considers the number of alleles expressed and the relative frequency of these alleles. Furthermore, having clear map bands that are easy to observe

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and analyze is an important criterion for screening high-quality specific SSR markers. Statistical clear map bands can correctly judge the allelic configuration at each locus, thus, determining the ploidy level of rainbow trout. However, when the number of microsatellite DNA alleles shown in the electrophoretic map was less than the maximum number of alleles that may occur at the ploidy level of the polyploid species, it was difficult to accurately judge the ploidy by direct observation of the number of alleles. In diploid individuals, highly heterozygous SSR amplifies at most two alleles at a given site. Similarly, three and four alleles can be observed in triploid and tetraploid individuals, respectively. However, in some rare cases, the actual number of alleles cannot be correctly determined by looking directly at the number of alleles. For example, individuals with unknown ploidy show multiple possible genotypes for two alleles, which can occur with both diploids (AB) and triploids (AAB or ABB). Therefore, this study combined the analysis results of multiple specific SSR markers to improve the accuracy of ploidy detection. In addition, this study found three specific labeling bands that can assist in the differentiation of diploid and triploid rainbow trout, namely, SSR1054-305bp, SSR1056-463bp, and SSR1468-363bp. These three specific markers will greatly improve the accuracy of using SSR markers to identify triploid rainbow trout. Microsatellite null alleles are another potential limitation in SSR ploidy identification, which can be eliminated or avoided by changing the binding site of primers and flanking sequences due to the failure of normal gene amplification due to mutations in SSR lateral sequences. Although the results of this study cannot eliminate the presence of null alleles in future samples, the present experimental data suggest that the frequency of null alleles is so low that their impact on polyploid identification is minimal. SSR markers screened in this study solve the existing problems of a complex technical process and expensive consumables in the identification of rainbow trout ploidy, providing a minimally invasive, economical, reliable, and batch-operable molecular method and contributing to the study of the genetic diversity of rainbow trout with different ploidy.

Key words Rainbow trout; Salmonidae; Microsatellite; Triploid; Ploidy detection