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评　述　　鱼类生物学和生物技术专辑

鱼类性别异形和性别决定的遗传基础及其生物技术操控

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摘要　鱼类养殖对世界食品特别是动物蛋白的持续供给做出了至关重要的贡献. 鱼类生殖对策的多样性, 特别是单性雌核生殖方式的利用已开创了鱼类遗传育种的典型范例. 不少鱼类在生长和个体大小等重要经济性状上表现出显著的性别异形. 性别特异分子标记的开发和性别控制生物技术的发展为增加鱼产量及其经济价值提供了重要的技术途径. 随着基因组学和分子遗传学技术的迅速发展, 鱼类性别异形的遗传基础逐步被揭示, 鱼类性别决定机制及其性别决定相关基因的鉴定已经取得了重大进展. 本文对此进行了概述, 以期为该领域的深入研究提供一些方向性和目标性思考.

关键词
生殖对策
性别异形
性别决定
性别决定基因
性染色体
性控育种

长期以来, 性别一直是生命科学研究的重大命题之一. 大多数脊椎动物为雌雄异体, 且在形态和生理上表现出显著的雌雄两性性别差异, 也正是这些性别差异使生命变得复杂而精彩, 以致进化生物学家认为“性别是进化生物学中问题的皇后”[1]. 在鱼类中, 自刘建康先生 1944 年首次报道黄鳝(*Monopterus albus*)存在雌雄同体和性反转现象[2]并由此打开鱼类性别机制研究之门[3]以来, 鱼类性别和相关研究不但在基础方面发展迅速、成果迭出, 而且应用价值厚实, 一些研究成果已为水产养殖所用[4]. 由于鱼类在动物系统进化中处于承前启后的地位, 其物种数量在已知的脊椎动物中占了 1/2 以上[5], 因而其性别差异和性别决定的遗传基础及其机制研究一直广受关注, 特别是性染色体和性别相关基因的鉴定也取得了重要进展[6~8].

联合国粮食和农业组织发布的 2014 年世界渔业及水产养殖报告显示, 在过去 50 年中, 世界食用水产品供应量的增长速度超过了全球人口的增速, 鱼类等水产动物养殖已成为人类获取动物蛋白的一个主要途径[9]. 特别是进入 21 世纪后, 水产养殖对世界水产品供应的作用已成共识[10,11], 围绕鱼类性别差异的性别控制育种生物技术等基础和应用研究也取得了重要突破[12,13]. 本文将着重回顾近 20 年来有关鱼类性别差异和性别决定的遗传基础及其调节机制研究的突破性进展以及性别控制育种生物技术的成功事例, 并由此分析和展望未来的发展前景.

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1　鱼类生殖对策的多样性及其遗传育种应用

1.1　鱼类生殖对策的多样性

目前自然界中已记录认识的鱼类共约 32700 种(www.fishbase.org). 作为较原始的脊椎动物, 为了适应所栖居水环境的变化, 鱼类的生殖对策(reproduction strategy)呈现出丰富的特殊性和多样性[14]. 依据其生殖对策的不同, 鱼类可分为单性(unisexuality)鱼类、雌雄同体(hermaphroditism)鱼类和雌雄异体(gonochorism)鱼类[15]. 绝大多数鱼类表现为雌雄异体, 雌雄同体现象也较常见, 已在黄鳝、鲷(*Acanlhopagrus schegeli*)和石斑鱼(*Epinephelus coioides*)等鱼类中有报道[2,16~18]. 即使在雌雄异体的虹鳟(*Oncorhynchus mykiss*)中, 也有研究发现在其性别决定的关键时期, 性类固醇激素水平的改变可导致兼性个体产生, 并由其形成的精子和卵子自我受精产生 YY 雄鱼[19].

1.2　多倍体银鲫单性和有性多重生殖方式的发现及其遗传育种利用

自 1932 年美国鱼类学家 Hubbs 等人发现第一种行雌核生殖的单性鱼亚马逊莫莉(*Poecilia formosa*)以来, 已陆续发现有约 30 种鱼类存在全雌性种群, 并发现这些单性鱼类可采用雌核生殖(gynogenesis)、杂种生殖(hybridogenesis)和雄核生殖(androgenesis)等方式繁衍后代[20~23]. 特别是多倍体银鲫(*Carassius auratus gibelio*), 由于发现其既具有单性雌核生殖又具有有性生殖多重生殖方式的克隆系[23], 因而引起了更多关注[12,24]. 用银鲫作为研究对象, 不仅鉴定出一批已用于其进化和生态遗传学研究的具有丰富多态性的基因组 DNA 标记[25~33], 也克隆筛选出一批可用于发育遗传学及其生殖调控机制研究的功能基因[34~40]. 此外, 在揭示其单性生殖和有性生殖双重生殖方式的基础上, 通过遗传背景差异较大的两个银鲫克隆系间的有性交配, 筛选出优良个体经 5 代以上单性雌核生殖扩群, 培育出一个新的银鲫核质杂种克隆系[41]. 该克隆系由于比已推广的高背鲫生长快且对一种寄生于肝脏的碘泡虫(*Myxobolus wulii*)有较强的抗性[41,42], 已作为新品种异育银鲫“中科 3 号”在中国迅速推广养殖.

2　鱼类性别异形及其产生的遗传基础

2.1　鱼类性别异形的多样性

性别异(二)形(sexual dimorphism), 广泛意义上指同一物种雄性和雌性个体之间的差异. 鱼类的性别异形既包括个体大小异形(size dimorphism)、形态异形(shape dimorphism)和颜色异形(color dimorphism), 又包括已生理和行为等差异[43,44]. 水产养殖和野外调查发现, 许多鱼类的雌雄个体间存在显著差异, 表 1 列出了迄今已报道的具有性别大小异形的 20 多种鱼类. 如在鲤鱼(*Cyprinus carpio*)、虹鳟、日本牙鲆(*Paralichthys olivaceus*)和半滑舌鳎(*Cynoglossus semilaevis*)等 10 多种鱼类中, 卵巢成熟比精巢晚, 性成熟期间大部分营养物质转化为身体的增长, 因而雌性个体比雄性大; 相反, 尼罗罗非鱼(*Oreochromis niloticus*)、黄颡鱼(*Pelteobagrus fulvidraco*)、斑点叉尾鮰(*Ictalurus punctatus*)和锈色鹦嘴鱼(*Scarus ferrugineus*)等 10 多种鱼类的精巢成熟比卵巢晚, 雄性生长速率比雌性快, 因而雄性个体大. 特别是在一些先雌后雄的性变鱼类中, 其雄性的快速生长更为明显, 已被认为与其对交配权的垄断有关[70]. 此外, 在一些观赏鱼类中雌雄个体的形态和体色有明显的差异, 如雄性鳍条剑尾鱼(*Xiphophorus helleri*)的尾鳍下缘延伸出一针状鳍条, 比雌性更具有观赏价值[71]; 雄性鳑鲏(*Rhodeus ocellatus*)在繁殖季节体色变得非常鲜艳, 而雌性的鳑鲏则全身为银白色[72]. 研究还发现, 鱼类的雌雄个体在神经系统和感觉器官的生理反应水平上也明显不同, 进而导致个体行为上的差异[73].

2.2　鱼类性别异形的遗传基础

性别异形在整个动物界中普遍存在, 但人们对其产生机制的了解还极为有限. 大量研究表明, 脊椎动物的性别异形主要是进化过程中的遗传选择造成的[74], 是胚胎、幼体和成体发育和生长过程中基因差异表达的产物[75,76]. 理论上, 性染色体及位于其上的基因在性别异形中可能起了重要的作用[77,78], 但性染色体有时也被发现并不是性别异形的主导者, 因为在性别异形的数量遗传研究中, 性染色体的作用尚不明确[79]. 三刺棘鱼(*Gasterosteus aculeatus*)的许多特征虽表现出显著的性别异形, 但其两性间的遗传构架非常相似, 意味着对性别异形进化的遗传约

表 1　雌雄个体间存在大小异形的已知鱼类物种

性别大小异形	种名	性别决定类型	参考文献
雄性个体大	黄颡鱼(*Pelteobagrus fulvidraco*)	XX-XY	[45]
	尼罗罗非鱼(*Oreochromis niloticus*)	XX-XY	[46]
	奥利亚罗非鱼(*Oreochromis aureus*)	ZW-ZZ	[47]
	乌苏里拟鲿(*Pseudobagrus ussuriensis*)	XX-XY	[48]
	斑点叉尾鮰(*Ictalurus punctatus*)	XX-XY	[49]
	乌鳢(*Channaargus Cantor*)	XX-XY	[50]
	蓝鳃太阳鱼(*Lepomis macrochirus*)	XX-XY	[51]
	背斑拟鲈(*Parapercis snyderi*)	?	[52]
	蓝鳍金枪鱼(*Thunnus maccoyii*)	?	[53]
	沙塘鳢(*Odontobutis obscura*)	?	[54]
	锈色鹦嘴鱼(*Scarus ferrugineus*)	?	[55]
雌性个体大	鲤鱼(*Cyprinus carpio*)	XX-XY	[56]
	牙鲆(*Paralichthys olivaceus*)	XX-XY	[57]
	漠斑牙鲆(*Paralichthys lethostigma*)	XX-XY	[58]
	虹鳟(*Oncorhynchus mykiss*)	XX-XY	[59]
	大西洋鲑(*Salmo salar*)	XX-XY	[60]
	欧洲鲈鱼(*Dicentrarchus labrax*)	XX-XY	[61]
	黄金鲈(*Perca flavescens*)	XX-XY	[62]
	大西洋庸鲽(*Hippoglossus hippoglossus*)	XX-XY	[63]
	大鳞大麻哈鱼(*Oncorhynchus tshawytscha*)	XX-XY	[64]
	银鲑(*Oncorhynchus kisutch*)	XX-XY	[65]
	半滑舌鳎(*Cynoglossus semilaevis*)	ZW-ZZ	[66]
	欧洲鳗鲡(*Anguilla Anguilla*)	?	[67]
	金钱鱼(*Scatophagus argus*)	?	[68]
	银腹鲃(*Puntius gonionotus*)	?	[69]

束力(genetic constraints)并非像通常认为的那样严重和绝对[80].

越来越多的模式鱼类和养殖鱼类基因组的破译以及转录组和蛋白组数据的积累[12], 为探索性别异形的遗传和分子机制提供了前期基础. 众所周知, 脊椎动物的个体大小主要是由生长速率不同导致的, 且生长由下丘脑-垂体-性腺轴和其他组织分泌的生长激素/类胰岛素生长因子等调控[81]. 目前在鱼类中已发现的促生长激素轴因子包括生长激素(growth hormone, GH)/生长激素受体(growth hormone receptor, GHR)/胰岛素生长因子(insulin-like growth factor, IGF)[82]和促肾上腺皮质激素释放激素(corticotropin-releasing hormone, CRH)/阿黑皮素(proopiomelanocortin, POMC)/黑皮质素受体(melanocortin receptor, MCR)等[83]. 在*Xiphophorus*属的剑尾鱼中, *Mc4r*基因被发现在XX雌性或雄性、小XY雄性、中和大XY雄性个体中的表达水平呈递增趋势, 其B等位基因没有胱氨酸基且仅在XY雄鱼中表达, 其表达与雄鱼的个体大小密切相关[84]. 在尼罗罗非鱼中, 精巢和卵巢转录组比较分析也揭示出性别分化过程中的基因表达差异[85]. Jing等人[86]采用solexa测序比较了XX雌性、XY雄性和YY超雄黄颡鱼性腺组织中的基因和microRNA表达情况, 并试图通过分析生长相关基因在成体雌性和雄性下丘脑和垂体中的表达差异来揭示其性别生长异形的分子基础(未发表资料).

此外, 一些鱼类的颜色、体型、生理和行为等在雌雄之间的差异也被发现受一些关键基因的控制, 如马拉维湖慈鲷的雌雄颜色异形与*pax7*的表达密切相关[87], 而三刺棘鱼的新性染色体与雌雄鱼的生理和繁殖行为异形有明显的关联[88].

3　鱼类性别决定的遗传基础

3.1　鱼类性别决定系统的多样性

在硬骨鱼类中, 性别决定一般是由遗传因素(遗传性别决定)和环境因素(环境性别决定)共同作用的结果, 且这种作用是一个可塑性过程[89,90]. 多种环境因素如激素、温度、pH、密度、光照强度、低氧等都可能影响性别决定途径. 研究表明, 对于许多具备

遗传性别决定的鱼类来说，环境如温度等因素也有可能在温度耐受阀值的边缘地带压倒遗传性别决定的作用[91,92].

在大多数两性鱼类中，遗传性别决定的作用机制通常是由位于性染色体或常染色体上的单基因或多基因所决定[8]. 在单基因系统中，性别是由性染色体上的特定基因所决定[6]. 鱼类的性染色体决定型主要表现为XX/XY和ZZ/ZW两大系统，少数也表现为XX/XO, XX/XY1Y2, X1X2X1X2/X1X2Y, X1X2X1X2/X1X2X1，或ZZ/ZO和ZZ/ZW1W2等类型，许多鱼类还没有发现具有明显异形的性染色体[93]. 即使在已确定的有性染色体的鱼类中，大多数性染色体的分化程度较低，难以通过细胞遗传学和荧光原位杂交技术鉴定出异形的性染色体[4,94].

研究表明，一些鱼类的性别是由分布于基因组中多个基因座的等位基因联合决定的，或者是由一对优势的(性)染色体对上的若干等位基因累积效应所决定的，这两类情况已被称为多基因性别决定(polygenic sex determination, PSD)系统[95,96]. 在斑马鱼(*Danio rerio*)[97,98]、马拉维湖丽科鱼(*Metriaclima zebra*)[99]和欧洲鲈鱼[100]等鱼类中，它们不具有典型的XX/XY或者ZZ/ZW性别决定系统，性别由位于不同染色体上的多个基因共同决定. 有时在同种鱼类的不同品系或种群中，性别决定位点也有异变，会存在超过2种类型的性别决定系统，这种情况可能是由常染色体和现有性染色体之间的重组或融合形成新的性染色体等原因造成的[101~103].

3.2　鱼类性别分化的可塑性

在黄颡鱼、青鳉(*Oryzias latipes*)和鲤鱼等大部分雌雄异体鱼类中，性腺的发育过程是从未分化的原始状态发育分化成卵巢或者精巢；而在斑马鱼和欧洲鳗等少数鱼类中，未分化的性腺首先发育成卵巢样状态，到稚鱼期，约1/2的个体其性腺的卵巢样组织退化并最终发育成正常的精巢[104,105]. 鱼类性别分化以性别决定为前提，但鱼类胚胎发育及其性别决定和分化过程暴露在一个可变因素较多的外界环境中，在进化中通过多样的性别决定机制和可塑的性别分化方式逐渐适应了其生存环境[6,92,106].

在绝大部分鱼类中，无论是否有性别决定基因，性类固醇浓度或温度的改变能够使之发生性反转，从而导致遗传型和生理型不一致[44,92,93]. 温度是影响鱼类性别分化的主要因素，对于大部分温度敏感性鱼类，雄性后代的比例会随着温度升高增加，低温则诱导卵巢的发育[107~109]. 雌激素和雄激素对于雌性和雄性个体的性别分化和性腺发育必不可少. 在鱼类性别分化早期，当体内的内源性类固醇激素还未产生或含量不足时，使用足够剂量的外源性类固醇激素能够改变性腺的分化方向[6,92]. 在雌性先成熟的石斑鱼中，甲基睾酮能诱导雌性向雄性转变[110]，使得*Dmrt1*的表达量上调[111,112], *Cyp19a1a*和*Sox3*的表达量下调[113,114]. 雌激素能诱导罗非鱼发生性反转，并导致*Dmrt1*的表达受到抑制[115]. XX基因型青鳉在高温下能被诱导性反转为生理雄鱼，此时*Cyp19a1a*的表达量会降低，而用雌二醇共同处理时会抑制性反转[116]. 除了性激素外，芳香酶抑制剂(Fadrozole和Letrozole)能抑制芳香化酶活性从而雄性化，因此能够诱导罗非鱼和青鳉发生性反转[117,118]. Godwin[119]分析了社会因素对珊瑚礁鱼类性别决定和分化的影响，并由此探讨了这种可塑性机制在进化中的优势. 在性变的双带锦鱼中，已有学者发现其个体是直接形成雄性还是先发育成雌性再发生性逆转成雄性取决于性别决定初期接收到的环境信号[120].

3.3　鱼类性别决定基因

(1) XX/XY性别决定系统鱼类的性别决定基因. 性别决定基因主要是指在未分化性腺中早期瞬时表达的基因，由于其表达并行使其功能，它指导具有双向性潜能的性腺发育成精巢或是卵巢. *Sry*基因是在脊椎动物中最早发现的雄性性别决定基因，其定位于哺乳动物Y染色体上并决定精巢发育[121,122]. 鱼类中首次鉴定到的性别决定基因是日本青鳉Y染色体连锁的*Dmy/Dmrt1bY*基因[123]. 随后，在其他XX/XY性染色体决定型鱼类，如吕宋青鳉(*Oryzias luzonensis*)、恒河青鳉(*Oryzias dancena*)、河豚(*Takifugu rubripes*)、牙汉鱼(*Odontesthes hatcheri*)和虹鳟中分别鉴定出了性别决定相关基因*Gsdf*, *Sox3*, *Amhr2*, *Amhy*和*SdY*. 在精巢分化过程中，这5个鱼类性别决定基因产物都定位于初始分化的精巢组织细胞中. 研究还进一步证实，*Dmy*, *Sox3*, *Amhr2*和*SdY*基因的突变以及*Amhy*基因的敲降都导致了XY型雌鱼，而*Sox3*, *SdY*和*Gsdf*的转基因过表达则产生XX型雄鱼，说明他们是雄性决定基因，在雄性决定过程中起着关键作用[124~128]. 大量研究已经证实，包括青鳉*Dmy*在内

的多数雄性性别决定基因一般是 *Dmrt1*(*dsx* and *mab-3* related transcription factor 1)的重复基因[129,130]. 在青鳉中已经观察到, *Dmrt1* 基因的突变会引起雄性向雌性逆转, 表明 *Dmrt1* 对于维持精巢分化有着类似于 *Dmy* 在雄性决定信号通路中至关重要的作用[131,132]. 事实上, 自第一个鱼类性别决定基因 *Dmy* 在青鳉中鉴定后, 很快发现该基因并不是鱼类中普遍存在的主要性别决定基因, 鱼类的性别决定基因似乎存在多源性. 在吕宋青鳉和恒河青鳉中已发现其 *Gsdf* 和 *Sox3* 取代 *Dmy* 成为性别决定基因[124,125]. 通过全基因组连锁分析和关联分析策略, Kamiya 等人[126]发现 *Amhr2* 基因的激酶结构域中的一个 SNP(single nucleotide polymorphism)(C/G)多态性位点仅与河豚和 2 种多纪鲀(*Takifugu*)的性别表型相关, 但与四齿鲀属(*Tetraodon*)鱼类无相关性. 这些资料显示, 即使同一属下的不同鱼类, 性别决定机制仍存在多样性. 此外, 日本青鳉的 *hotei* 突变体在 Amhr2 激酶结构域中发生的纯合子突变, 也引起了 XY 雌性的产生[133]. 因此, *Amhr2* 主要通过其激酶域的功能, 在河豚和青鳉的性别决定机制中起着关键的作用.

(2) ZW/ZZ 性别决定系统鱼类的性别决定基因. 鸟类性别决定系统为 ZZ(雄性)/ZW(雌性), 且第一个 ZW/ZZ 系统的性别决定基因首先从鸟类被鉴定, 其也是由位于 Z 染色体上的雄性决定基因 *Dmrt1* 决定[134]. *DM-W* 基因, 一个 W 染色体连锁的 *Dmrt1* 旁系同源基因, 能够抑制 *Dmrt1* 的转录活性, 随后在 ZZ/ZW 性别决定型的非洲爪蟾中被证实是雌性决定基因[135,136]. 尽管在 ZZ/ZW 型硬骨鱼中还没有鉴定出性别决定基因, 但一些性别决定的候选基因已开始呈现.

Chen 等人[137]报道了一种 ZZ/ZW 型鱼类半滑舌鳎的全基因组序列. 通过系统进化分析, 发现半滑舌鳎的 ZW 染色体对与鸡的 ZW 染色体对有高度的同源性. 他们在 Z 染色体上发现了具有功能的 *Dmrt1*, 而在 W 染色体上仅发现其已失活的假基因. 与前面提到的性别决定基因的表达模式相似, 在性别决定时期的未分化性腺中, *Dmrt1* 特异高表达于舌鳎的雄性生殖细胞和前体细胞, 而且在精巢发育时期 *Dmrt1* 一直维持较高的表达水平. *Dmrt1* 的启动子在 ZW 雌鱼中发生甲基化而被沉默, 但在 ZW 伪雄鱼中, *Dmrt1* 的启动子发生去甲基化而激活它的转录, 通过剂量补偿效应使 *Dmrt1* 在 ZW 雄性中的表达量上调到与正常发育的 ZZ 雄性一致, 且雌性特异的 W 染色体上基因发生甲基化从而受到抑制[138,139]. 这些发现表明, 鱼类同鸟类类似, 不仅 Z 染色体上的部分基因发生了有效的、可变的剂量补偿效应, W 染色体也包含了性别决定的一些组成成分或剂量补偿机制[139]. 显然, 这一来自于养殖鱼类全基因组序列分析的见解对 ZW/ZZ 型性别决定基因的深入研究[140,141]将是一个令人振奋的刺激.

此外, 通过性别决定区段的分子标记和 QTL 连锁分析, 在 ZZ/ZW 型的大菱鲆(*Scophthalmus maximus*)和罗非鱼中发现 *Amh* 和 *Dmrta2* 定位于性别决定区域, 是潜在的性别决定基因[142,143]. Xu 等人[144]在斑马鱼中也观察到 *Dmrta2* 有调控精子发生的作用.

3.4 鱼类性别决定的网络模块

已知的鱼类性别决定基因如 *Dmy* 和 *Amhy* 被证实分别是常染色体上的 *Dmrt1* 和 *Amh* 基因在 Y 染色体上的重复基因[123,127]. 由于在硬骨鱼类祖先的进化过程中整个基因组发生了复制, 基因复制也是一个很常见的现象[32,145~147]. 事实上, 脊椎动物的主要性别决定基因, 如 *Sry*, *Dmy*, *Amhr2*, *Amhy* , *Gsdf* 都是性腺分化的关键因子或它们的重复基因. 在虹鳟和多种鲑科鱼类中, *SdY* 为常染色体上的干扰素调节因子 9(*Irf9*)在性染色体上的重复基因[148]. 近期一些新的性别决定基因的发现更有力地诠释了一个假说, 不仅是性腺分化的下游关键因子及相关基因, 很多参与其他发育过程的基因直接或重复复制后能被招募到性别决定通路的上层信号通路中[15,149].

一般认为, 鱼类未分化的性腺最初具有向精巢或卵巢发育的双向潜能, 当性别决定的“总开关”通过性别决定基因起始后, 一类保守的性别决定和分化的遗传网络随之激活(图 1). 这类下游性别相关基因能调控性类固醇激素的表达, 从而控制着性腺发育最终分化为一个有功能的性腺并与性别表型相对应[150~152]. 有观点认为, 鱼类性别决定不是一个单一的遗传级联反应, 也不是遗传网络分级呈现的结果, 而是不同遗传模块(genetic modules)相互交联并以模块化的形式发挥功能[15,153]. 如图 1 所示, 在 XX/XY 性别决定鱼类中, 位于 Y 染色体上的主雄性决定基因如 *Dmy*, *Sox3*, *Amhr2*, *Sdy* 或 *Amhy* 的高度表达可以启动不同的基因网络模块从而激活精巢发育必需的基因如 *Dmrt1*, *Sox9* 和 *Amh*, 并由此形成雄性; 相反,

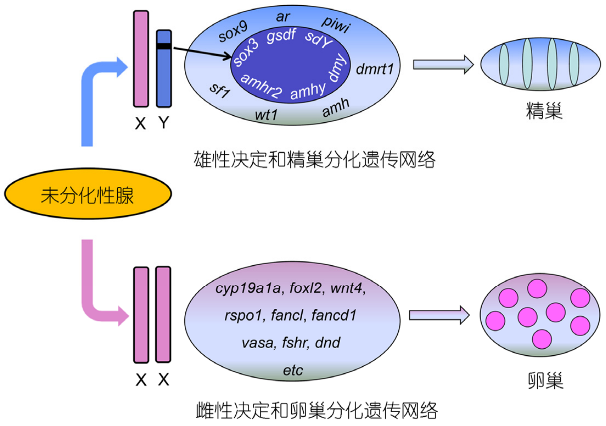

图 1　XX/XY 性别决定系统鱼类的性别决定和性腺(精巢或卵巢)分化的示意图

由于 X 染色体上没有主雄性决定基因则导致雌性性别决定遗传网络中其它模块激活, 由此诱导 *Cyp19a1*, *Foxl2*, *Sf1* 和 *Wnt4* 等卵巢形成相关基因的表达[154,155].

研究发现, 在性变鱼类中一些性别分化相关基因也受到表观修饰的调控[156,157]. 在欧洲鲈鱼中, 雄性幼鱼的芳香化酶(*Cyp19a*)启动子甲基化的水平被发现是雌性的 2 倍, 且高温暴露会增加雌性 *Cyp19a* 启动子甲基化程度, 并抑制 *SF-1* 和 *Foxl2* 对它的转录调控[156]. 同样在雌雄同体和性变的黄鳝中, *Cyp19a1a* 启动子甲基化程度也被发现在间性性腺和精巢中比卵巢中高得多, 且 DNA 甲基化抑制剂 5-aza-2′-deoxycytidine 能够逆转自然状态下黄鳝的性转变[157].

4　鱼类性别控制生物技术及其在育种上的应用

生长是鱼类遗传改良最有价值的经济性状之一. 由于一些鱼类雌雄个体具有不同的生长速度和大小差异, 培育全雌或全雄的养殖群体对渔业生产具有重要意义. 如上所述, 鱼类遗传育种学家们已经对多种雌雄间存在生长和大小异形的养殖鱼类进行了大量研究. 通过这些研究, 已成功开发出性别连锁遗传标记或 X 染色体和 Y 染色体连锁遗传标记辅助的鱼类性别控制生物技术, 为鱼类性控遗传育种的应用提供了便利而实用的技术路线[12,158,159]. 近 5 年来, 仅中国学者就已成功培育出黄颡鱼“全雄 1 号”、全雌牙鲆“北鲆 1 号”、全雌牙鲆“北鲆 2 号”和罗非鱼“鹭雄 1 号”等单性水产新品种. 基于这些重要的突破性进展, 本节将在概述单性鱼类生产技术途径的基础上, 结合成功先例, 提出更为有效的思路和技术措施.

4.1　种间杂交与全雄或全雌鱼的生产

种间杂交不仅可以用来提高鱼类经济性状[160,161], 如生长率、存活率、抗病力、耐寒和耐低氧等, 还可以用来生产高雄性率或高雌性率的杂交子代. 最具有代表性的范例是罗非鱼不同种间杂交可获得全雄子代. 早在 1960 年, Hickling[162]首次报道用 XX/XY 性别决定型的莫桑比克罗非鱼(*Oreochromis mossambicus*)作为母本, ZW/ZZ 性别决定型的霍诺鲁姆罗非鱼(*O. hornorum*)作为父本, 两者杂交产生了全雄性后代群体. 随后, 人们进行了大量的罗非鱼种间杂交的研究, 发现尼罗罗非鱼和奥利亚罗非鱼的杂交组合在生长、存活和雄性比例上都具有优势[163,164]. 进而, 当将通过群体选育技术培育的“夏奥 1 号”奥利亚罗非鱼与尼罗罗非鱼杂交时, 其奥尼杂交子代的雄性率在 93%以上, 已在中国用于大规模生产养殖[165]. 在鲈鱼中, 不同种类海鲈之间的杂交可以产生 100%的雌性后代[166], 但其性别决定型及其产生机制尚不清楚[167].

4.2　人工雌核生殖与全雌鱼的生产

人工雌核生殖是获得单性鱼类种群的又一重要手段, 一般是指单倍体卵子经异源或遗传失活的精子激活, 并经过染色体加倍而发育成二倍体后代, 其遗传信息基本来自其母本[4]. 在进行人工雌核生殖时, 如果是 XY 性别决定系统的鱼类, 就会得到 XX 全雌性子代; 在 ZW 性别决定类型的鱼类中, 理论上只能产生 ZZ 雄性和 WW 雌性, 不可能产生全雌性后代.

银鲫因具有单性雌核生殖的能力, 用异源精子刺激其雌核生殖就可大量生产全雌性后代, 已成为鱼类性别控制在水产养殖中应用的一个典型范例[23]. 用人工雌核发育和性反转相结合的技术路线, 也曾培育出全雌鲤并已用于水产养殖实践[60]. 近年来, 人工雌核生殖已在牙鲆[168]、真鲷(*Pagrosomus major*)[169]、欧洲鲈鱼[170]、圆斑星鲽(*Verasper variegatus*)[171]和半滑舌鳎[172]等海水养殖鱼类中大量尝试过. 特别是在牙鲆中, 多个人工雌核生殖克隆系已被诱导获得[173~175], 在此基础上, 中国水产科学研

究院北戴河实验站还先后培育出牙鲆养殖新品种“北鲆 1 号”和“北鲆 2 号”.

一般来说, 雌核生殖产生的后代都为雌性, 但在一些远缘杂交导致的雌核生殖发育而来的花鳉(*Poecilia formosa*)和红鲫(*Carassius auratus* red var.)等鱼类中, 也会出现一定比例的雄性个体[176~178], 这些雄性个体在鱼类遗传育种中可能有重要的开发价值.

4.3 性别连锁位点和性染色体连锁标记的鉴定

大多数鱼类性染色体分化程度较低, 从染色体形态上难以识别; 而且鱼类性别易受环境因素特别是温度的影响[179], 导致性别生理表型与基因型不一致. 因此, 寻找到可以快速识别鱼类性别遗传组成的简捷方法在水产养殖中具有十分重要的意义. 在一些鱼类中, 鱼类遗传学家通过不同的分子标记技术如扩增片段长度多态性技术(amplified fragment length polymorphism, AFLP), 单核苷酸多态性技术(SNP), 随机扩增多态性 DNA 标记技术(random amplified polymorphic DNA, RAPD), 微卫星标记技术(simple sequence repeats, SSR)和数量性状位点技术(quantitative trait locus, QTL)成功筛选到一批性别特异的或性染色体连锁的位点和DNA标记(表 2), 这些标记为鱼类遗传性别的确定和性染色体的鉴定以及全雄或全雌鱼的批量生产奠定了基础[12,180].

表 2　鱼类性别特异标记、性染色体连锁标记和相关基因的鉴定

种名	性别决定类型	性别连锁位点鉴定方法	标记类型及数量	关联基因	参考文献
黄颡鱼(*Pelteobagrus fulvidraco*)	XX/XY	扩增片段长度多态性	X, Y 连锁标记各 2 个		[49]
		基因组步移	X, Y 连锁标记各 2 个; 1 个 X, Y 长度不同标记		[180]
斑点叉尾鮰(*Ictaluru spunctatus*)	XX/XY	测序分析	雄性特异标记 1 个		[181]
非洲鲶(*Clarias gariepinus*)	XX/XY	随机扩增多态性 DNA 标记	雄性特异标记 2 个		[182]
虹鳟(*Oncorhynchus mykiss*)	XX/XY	扩增片段长度多态性	雄性特异标记 15 个		[183]
		基因表达	Y 连锁标记 1 个	*sdY*	[128]
牙汉鱼(*Odontesthes hatcheri*)	XX/XY	扩增片段长度多态性	雄性特异标记 1 个		[184]
		基因表达	Y 连锁标记 1 个	*amhy*	[127]
红鳍东方纯(*Takifugu rubripes*)	XX/XY	单核苷酸多态性	Y 连锁标记 1 个	*Amhr2*	[126]
日本清鳉(*Oryzias latipes*)	XX/XY	基因表达	Y 连锁标记 1 个	*dmy*	[123]
吕宋青鳉(*Oryzias luzonensis*)	XX/XY	基因表达	Y 连锁标记 1 个	*gsdf*	[124]
恒河青鳉(*Oryzias dancena*)	XX/XY	基因表达	Y 连锁标记 1 个	*sox3*	[125]
剑尾鱼(*Xiphophorus maculatus*)	XX/XY	序列分析	Y 连锁标记 1 个	*MC4R*	[84]
肥头鲤(*Pimephales promelas*)	XX/XY	扩增片段长度多态性	雄性特异标记 8 个 雌性特异标记 1 个		[185]
多刺鱼(*Pungitius pungitius*)		微卫星标记	雄性特异标记 1 个		[186]
鲤(*Cyprinus carpio*)	XX/XY	随机扩增多态性 DNA 标记	雄性特异标记 1 个		[187]
尼罗罗非鱼(*Oreochromis niloticus*)	XX/XY	扩增片段长度多态性	雄性特异标记 3 个		[188]
		随机扩增多态性 DNA 标记, 扩增片段长度多态性	X 连锁标记 4 个 Y 连锁标记 5 个		[189]
大菱鲆(*Scophthalmus maximus*)	ZZ/ZW	随机扩增多态性 DNA 标记	雌性特异标记 1 个	*wnt4*, *foxl2*	[190]
		随机扩增多态性 DNA 标记	雄性特异标记 1 个	*amh*, *dmrta2*	[191]
圆斑星鲽(*Verasper variegatus*)	ZZ/ZW	扩增片段长度多态性	雌性特异标记 2 个		[192]
狭鳞庸鲽(*Hippoglossus stenolepis*)		微卫星标记	雌性特异标记 3 个		[193]
半滑舌鳎(*Cynoglossus semilaevis*)	ZZ/ZW	扩增片段长度多态性, 微卫星标记	雌性特异标记 7, 1 个		[70,174]
		基因组测序	Z 连锁标记 1 个	*dmrt1*	[137]
鰤(*Seriola quinqueradiata*)	ZZ/ZW	微卫星标记	雌性特异标记 1 个		[194]
条石鲷(*Oplegnathus fasciatus*)	$X_1X_1X_2X_2/X_1X_2Y$	扩增片段长度多态性	雄性特异标记 4 个		[195]
亚马逊石脂鲤(*Brycon amazonicus*)		随机扩增多态性 DNA 标记	雌性特异标记 1 个	*PIGW*	[196]

4.4　鱼类性别控制育种的生物技术路线

在性腺分化早期, 外源的类固醇激素处理能够诱导鱼类性逆转. 由于目前尚不能确定激素代谢过程中的衍生物是否能很快代谢掉, 因而激素诱导性反转直接生产单性鱼苗必须加以限制[4,12,197]. 出于食品安全考虑, 可行的办法是将激素诱导的性逆转鱼作为亲鱼使用, 由此培育出单性子代才可用于生产养殖.

在鲤和鲫中, 中国学者已将人工雌核生殖和性反转技术结合起来建立了快速生产全雌鱼的育种技术[198,199]. 黄颡鱼“全雄 1 号”是在鉴出 Y 染色体特异标记和 X 染色体特异标记[49,180]的基础上, 采用性染色体特异标记遗传鉴定和性激素诱导性逆转相结合的性别控制育种技术培育出来的养殖新品种[12,200]. 由此还提出了一条在 XX/XY 型鱼类中通过雌二醇(17α-ethinylestradiol, EE2)处理和 Y 染色体特异标记与 X 染色体特异标记遗传鉴定大规模生产全雄鱼的集成性控技术路线[12]. 在 XX/XY 型鱼类中, 全雌鱼生产的技术路线更为简单. 它可通过人工雌核生殖加性逆转得到 XX 生理雄鱼, 也可直接用甲基睾酮(17α-methyltestosterone, MT)诱导子代性逆转再通过 Y 染色体特异标记(Y chromosome-specific marker, YSM)和 X 染色体特异标记(X chromosome-specific marker, XSM)筛选出 XX 生理雄鱼. 接着, 将 XX 生理雄鱼和 XX 雌鱼产生的后代再次用 MT 处理批量扩增出 XX 生理雄鱼. 最后, 批量的 XX 生理雄鱼和雌鱼交配就能够大规模生产全雌鱼(图 2).

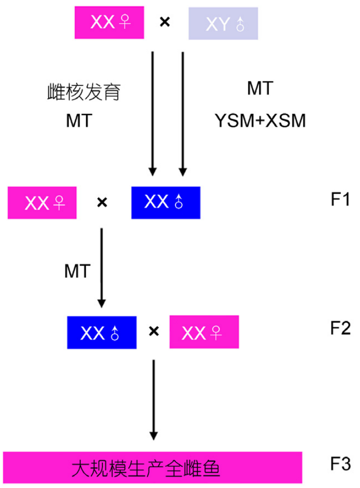

图 2　通过雌核生殖、甲基睾丸酮(MT)处理和 X 染色体特异标记(XSM)与 Y 染色体特异标记(YSM)遗传鉴定大规模生产全雌鱼的集成技术路线示意图

5　结论和展望

过去几十年来, 鱼类性别决定基因和性染色体的发现进展缓慢, 主要是由于遗传信息和基因组资源短缺. 最近, 高通量测序技术、鱼类基因组和转录组的大数据信息以及比较系统学方法极大地推进了鱼类性别决定遗传基础研究的节奏[201]. 大量的性别连锁或性染色体特异的分子标记也被迅速地开发出来, 并开始在水产养殖中得到有效利用. 此外, 基因打靶和编辑技术如 TALEN(transcription activator-like effector nuclease)和 CRISPR(clustered regularly interspaced short palindromic repeats)/Cas9(CRISPR-associated 9)的出现使在鱼类中进行功能性研究相对简单[202~204]. 可以预期, 一个关于鱼类性别异形与性别决定的遗传基础和性别控制生物技术的研究时代正在到来.

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