金万昆论文集

金万昆 等 著



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金万昆论文集

著作委员会

金万昆 沈俊宝 王民生 赵建英 高永平

虽然我国是世界淡水鱼类养殖大国,养殖历史悠久,但已开发的养殖品种仅有100余种。新中国成立后,特别是改革开放以来,国家对鱼类遗传育种的研究和开发工作高度重视,曾先后将其列入"十五"、"十一五"渔业发展规划。鱼类育种科学工作者更是以高度的责任感和使命感,从多方面、多渠道探索鱼类的遗传变异,并通过品种选育、杂交选育和基因工程育种等途径与技术,创新选育出不少高产、优质、抗病、抗逆良种。

金万昆同志自 20 世纪 50 年代任天津市换新水产良种场场长以来,一直致力于淡水鱼类良种的选育工作。特别是 2002 年以来,带领科研团队进行了鲤鱼、鲫鱼、鳊鱼、鲂鱼、鲌鱼和观赏鱼等多种鱼类新品种的选育研究,先后培育出红白长尾鲫、蓝花长尾鲫、墨龙鲤、乌克兰鳞鲤、津新鲤、黄金鲫、津鲢、芦台鲂鲌、津新乌鲫、津新鲤 2 号(超级鲤)10 个经全国水产原种和良种审定委员会审定,农业部批准在全国推广养殖的新品种。更为可贵的是,这些新品种经全国推广后,许多品种已成为全国重要的养殖品种。如黄金鲫和被广大养殖者誉为"超级鲤"的津新鲤 2 号等已推广到全国 29 个省区市,获得了显著的经济、社会效益,其产值占到全国同类产品 30%的市场份额。同时在新品种的研究开发中,还创新出一批实用新技术,其中,有 10 项实用新型和发明专利获得国家知识产权局授权。金万昆同志及其团队,在淡水鱼类新品种的选育研究与创新上所做的大量工作,对不断提高淡水鱼类良种覆盖率,推动我国淡水鱼类养殖业的健康、高效和可持续发展,做出了突出贡献。

康、高效和可持续发展,做出了突出贡献。

在淡水鱼类新品种的研发上,他们共进行了600 余项目间、科间、亚科间、属间的远缘杂交试验,获得了一批有生命力的远缘杂交子代。选育出多项具有育种前景的组合,为进一步开展鱼类遗传育种和分子生物育种奠定了良好基础。在此期间,他们先后将实验研究和取得的结果,以论文形式写成科研报告,先后出版了《淡水鱼类远缘杂交种染色体图谱》《淡水鱼类远缘杂交实验报告》《淡水养殖鱼类种质资源库》和《淡水鱼类杂交种胚胎发育图谱》4部专著。

《金万昆论文集》是将金万昆同志在国内有关期刊、杂志上发表的论文收集整理,以论文集形式出版,以便为广大水产养殖及科技工作者提供参考。

北京

2015年9月16日

前 言 PREFACE

天津市换新水产良种场场长金万昆同志带领其科研团队,自2002年以来,在淡水鱼类遗传育种和健康高效养殖技术方面进行了大量深入系统的研究和实践。先后培育出红白长尾鲫、蓝花长尾鲫、墨龙鲤、乌克兰鳞鲤、津新鲤、黄金鲫、津鲢、芦台鲂鲌、津新乌鲫、津新鲤2号(超级鲤)10个经全国水产原种和良种审定委员会审定、农业部批准在全国推广养殖的新品种。在此期间,还进行了600余项目间、科间、亚科间、属间远缘杂交试验,获得了一批有生命力的远缘杂交子代,并为以后进一步深入研究提供了宝贵的育种材料。

上述新品种的部分育种试验研究、淡水鱼类远缘杂交试验以及一些品种的育种素材、亲本培育和健康养殖技术研究中积累的基础资料,已经整理,并在国内相关水产刊物上发表。为了将这些淡水鱼类新品种培育研究方面所做的工作整理存档,并将这些新品种培育及远缘杂交试验的资料提供给水产科学研究和养殖工作者参考,现将这些文章以论文集形式出版。

本文集根据内容分为品种、杂交组合、品种育种及养殖技术3部分,共收录论文46篇。鉴于淡水鱼类品种变异复杂,相关研究亟待进一步深入和持续,同时由于水平所限,本文集资料中的疏漏和不当之处在所难免,敬请读者批评指正。对本文集中所引用参考文献的作者,在此一并致谢!

著作委员会 2015年9月30日

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一、品种

(一)津鲢

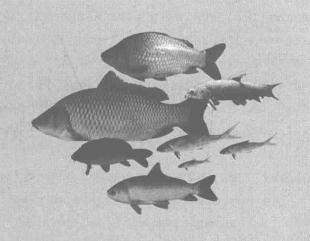
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一、品种

(一)津 鲢



金万昆论文集

Isozyme Analysis of Jin Silver Carp (Hypophthalmichthys molitrix Var Jin)

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 - 3. Tianjin Tianxiang Aquatic Co., Ltd., Tianjin 301500, China)

Abstract: [Objective] The aim was to carry out isozyme analysis of jin silver carp (Hypophthalmichthys molitrix Var Jin). [Method] The isozyme of AAT, EST, α – GPD, GPI, IDH, LDH, MDH, ME, PGM and PROT of muscles and liver in two populations of the silver carp (Hypophthalmichthys molitrix): Jin silver carp (a breed through selective breeding) and artificially propagated population bought from Jingzhou city, Hubei Province were examined by horizontal starch gel electrophoresis. [Result] Eighteen loci were observed in two populations. Two loci of GPI* and PGM* in Jin silver carp population and the locus of GPI* in Jingzhou population were polymorphic. The proportions of polymorphic loci (maximum allele frequency \leq 0.99) of Jin silver carp and Jingzhou populations were 11.11% and 5.56% respectively, expected heterozygosity were 0.0150 and 0.0011 respectively. The Nei's genetic distances were 0.00059 between two populations. The result of chi – square test of the GPI* gene in two populations showed that their genetic structure has very significant difference. [Conclusion] This study provided a theoretical basis for large – scale extension of Jin silver carp.

Key words: Silver carp (Hypophthalmichthys molitrix); Isozyme; Genetic diversity

Silver carp (*Hypophthalmicthys molitrix*), as one of Chinese "Four Fish", belongs to Cyprinidae of Cypriniformes^[1]. It is also one of the most important freshwater fish in China. The Yangtse River is our country's important produce place of silver carp. However, due to recent changes in the natural environment, the production of silver carp of Yangtze River has declined sharply. At the same time, the proportion of silver carp also produced corresponding change^[2], which is dropped from 26. 1% to 3. 9%^[3].

The substantially change in natural output of silver carp has a close relationship with their own genetic material structure changes. Therefore, many scholars have carried out variety of studies on the genetic variation of silver carp by many methods such as morphology, isozyme, RAPD, mtDNA RFLP, D –

loop segment sequencing of mtDNA and microsatel-lite^[4~14].

In recent years, artificial propagation has been used to increase the yield of silver carp, but the unreasonable genetic resources management and propagation method used in this process has significantly reduced the growth performance, disease resistance, stress resistance and genetic diversity of silver carp. Jin silver carp, a breed through selective breeding, is obtained by closed breeding of silver carp collected from Yangtze River with the method of the combination of population propagation and hybrid breeding in "National Level Tianjin Huanxin High Quality Fish Farm". In 2010, it has been approved as new variety by the National Aquatic Species and Varieties Committee. Jin silver carp has many advanta-

ges such as fast growth, good adaptability, strong resistance, high fecundity, high economic benefits and so on. It is the first variety in "Four Fish" which is obtained by artificial breeding in our country^[16]. In this study, the isozyme analysis technology was used for genetic diversity analysis of Jin silver carp so as to provide theoretical basis for large – scale spread of jin silver carp.

I Materials and Methods

1 Materials

Fifty jin silver carp from National Level Tianjin Huanxin High Quality Fish Farm with the average weight of (73.6 ± 14.1) g was collected on Nov. 17, 2010, which belonged to artificial breeding fries of June 2010;50 Jingzhou silver crap with the average weight of (73.4 ± 9.9) g were collected from Tianjin Tianxiang Aquatic Co., Ltd. on Sep 21, 2011, which was purchased from Daming aquaculture farms in

Jingzhou City of Hubei Province on May 15, 2011. All materials were collected and then saved at −20℃.

2 Methods

Horizontal starch gel electrophoresis method was used in this study with the citric acid – aminopropyl morpholine (C – APM, pH = 6) as buffer. Electrophoresis and staining methods were according to the method of Taniguchi and Dong heart, muscle, liver and kidney of two populations of silver carp were used as samples for pre test to distinguish the suitable isozymes and organization for individual genotypes. Then, the isozymes detection of two populations of silver carp was carried out, and the isozyme types, No., locus and organization were shown in Table 1. Isozymes abbreviated name, No., locus, allele and genotype name were according to the method of Shaklee [19].

Table 1 The detected isozyme, No., locus

Isozyme	Abbreviated name	No.	Locus	Tissue
Aspartate aminotransferase	AAT	2. 6. 1. 1	AAT*	Muscle
Esterase	EST	3. 1. 1 -	EST*	Liver
Glucosephosphate isomerase	GPI	5. 3. 1. 9	GPI*	Muscle
Glyceraldehyde phosphate dehydrogenase	α – GPD	1. 2. 1. 12	α – GPD *	Muscle
Isocitrate dehydrogenase	IDH	1. 1. 1. 42	IDH - 1 *	Muscle
			IDH -2*	Liver
			IDH - 3 *	Liver
Lactate dehydrogenase	LDH	1. 1. 1. 27	LDH - 1 *	Muscle
			LDH - 2.*	Muscle
Malate dehydrogenase	MDH	1. 1. 1. 37	MDH - 1 *	Muscle
			MDH −2 *	Muscle
			MDH - 3 *	Muscle
			MDH -4 *	Muscle
Malic enzyme	ME	1. 1. 1. 40	ME -1 *	Muscle
			ME - 2 *	Muscle
			ME - 3 *	Liver
Phosphoglucomutase	PGM	5. 4. 2. 2	PGM *	Muscle
Sarcoplasmic protein	PROT		PROT*	Muscle

3 Data processing and analysis

According to the electrophoretic band, the genotypes of each isozyme of each fish were judged, and then the allele frequency of each allele was calculated. According to the method of Wang Zhongren^[20] and PopGen32 software, proportions of polymorphic loci(P), No. of allele gene(A), No. of effective alleles(Ne), Observed heterozygosity(Ho) and Expected heterozygosity(He) were calculated. And the Hardy – Weinberg equilibrium χ^2 test was carried out

on the varied locus of each population. At the same time, the χ^2 test between two populations on the highest allele frequency of the varied locus was performed^[21].

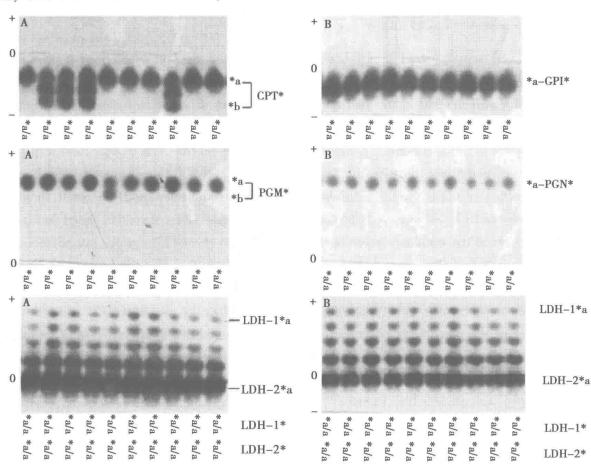
II Results and Analysis

1 Allele frequency of two populations

The pre test result showed that the activity of AAT, a - GPD, GPI, IDH, LDH, MDH, ME, PGM and PROT in muscle tissue was strong and the bands were clear. The locus of EST, IDH, ME in liver was significantly different from those in muscle, and the

bands were clear. The bands of other tissues were the same as those in liver or muscle, or can't be distinguished. Therefore, liver and muscle were selected as test materials.

In the ten detected isozymes, there were a total of 18 locus, in which the GPI* and PGM* were the varied loci in Jin silver carp and the GPI* was the varied loci in Jingzhou silver carp. The allele frequency of GPI* and PGM* loci was shown in Table 2. Parts of isozyme electrophoresis of two populations of silver carp were shown in Figure.



A, Jin silver carp; B, Jingzhou silver carp.

Figure Parts of the isozymes electrophoresis of two populations of silver carp

Table 2 The allele frequency of two populations of silver carp

Locus	Allele -	Allele frequency				
		Jin silver carp	Jingzhou silver carp			
GPI*	* a	0. 8900	0. 9900			
	* b	0.1100	0. 0100			
PGM*	* a	0. 9700	1. 0000			
	* b	0. 030 0	0			

Other 16 loci are without variation.

2 Genetic variation within and among groups

The calculated variation indexes were shown in Table 3. It could be concluded that the calculated P, A, Ne, Ho and He of Jin silver carp were higher than that of Jingzhou silver carp. The ratio of Ho to He of two populations was close to 1. The χ^2 test on the varied locus of each population showed that P > 0.05,

which was in line with Hardy – Weinberg equilibrium. The Nei genetic distance between two populations was 0.00059, with the χ^2 value of a allele frequency on GPI * was 9.95, P < 0.01. It suggested that these two populations had certain genetic differences. The χ^2 value of a allele frequency on PGM* was 3.05, P > 0.05.

Table 3 The genetic variation between two populations of silver carp

Population	Number of locus	Number of polymorphic loci	P	A	Ne	Но	Не	Но/Не
Jin silver carp	18	2	0.1111	1.1111	1. 0183	0.0167	0. 0150	1, 1133
Jingzhou silver carp	18	1	0.0556	1.0556	1.0011	0.0011	0.0011	1.0000

The highest allele frequency of variant loci≤0. 99.

III Discussion

The natural distribution area of silver carp is fromHeilongjiang to Red River. Silver carp has been introduced to 71 countries or regions [22]. The Yangtze River and the Pearl River are the birthplace of fries and propagation of silver carp (including the Aristichthys nobilis and *Ctenopharyngodon idellus*) in our country and around the world, are very important fish gene pool and germplasm resources [23]. In recent years, natural resources of silver carp appear a severe recession, and the number of fry was drastically reduced [6]. Therefore, the breeding of silver carp new varieties appears to be particularly important. However, till 2009, there is not a silver carp breeding varieties in China.

Many scholars in our country have carried out studies on the population structure of silver carp by the application of multiple genetic markers^[2-14]. Zhao and Li^[2], Li *et al.* [4], Wu and Nang^[7], Wang and Liu^[24], Zou *et al.* [25] and others had studied on the genetic structure of multiple population of silver carp or individual polymorphism and so on using isozyme detection technology. In these studies, LDH and EST show variation loci, and there also a small number of groups showed the ADH, MDH and IDH have gene variation. Zhao Jinliang *et al.* [2] detected 10 kinds of isozymes and sarcoplasmic proteins, and

they found that four populations of silver carp of middle and lower reaches of Yangtze River were populations with no significant genetic differentiation.

In this study, 10 kinds of isozymes (four of them are different from that of Zhao) and sarcoplasmic protein (PROT) of Jin silver carp and Jingzhou silver carp were detected, and the result showed that the GPI* and PGM* were the varied loci in Jin silver carp and the GPI* was the varied loci in Jingzhou silver carp. The variation degree of Jin silver carp was higher than the Jingzhou silver carp. The χ^2 test on an allele frequency of GPI* of two kinds of silver carp showed that there were significant difference between these two populations. Wang^[26] analyzed the RFLP of D - loop section of mtDNA of Jin carp and three populations of silver carp of Yangtse River, the results also showed that haplotype diversity index, nucleotide diversity index of Jin silver carp were the highest, which was consistent with the results in this study.

Generally, the genetic diversity of artificial propagation population will have a certain decline degree. However, there are some exceptions. Taniguchiet al. [27] analyzed the 10 kinds of *Plecoglossus altivelis* by isozyme analysis technology, and the results showed that the allele frequency and genetic variability of artificial breeding populations were significantly different from wild populations and that some genes

may be directly or indirectly associated with growth factor. The reason for high variation degree of Jin silver carp may be due to low variation degree of Jingzhou silver carp, or that Jin silver carp has retained the original genetic diversity.

IV References

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