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Genetic diversity of domesticated stocks of striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878), in Thailand: Relevance to broodstock management regimes

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ABSTRACT

Thai stocks of striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878), have a relatively long domestication history (>20 generations) that began with fish of Chaophraya River origin. The genetic diversity of this species was studied in six hatchery populations with two different broodstock management regimes (without introduction of either wild or domesticated stocks vs. with occasional introduction of either original or non-original populations) and in three wild populations (from the Chaophraya River and its tributary and from the Mekong River). The results, based on five microsatellite loci, revealed high allele diversity of the hatchery populations (A_r = 6.53–8.06) with the introduction of non-original stock (from the Mekong population) relative to those with the introduction of original stock (from the Chaophraya population) or without introduction (A_r = 3.18–6.06). Notably, heterozygosity was high in the majority of populations (H_o = 0.633–0.763; H_e = 0.593–0.834). Genetic introgression from the Mekong population was observed in every population, as revealed by Bayesian population assignment. The wild populations showed unexpectedly low allele diversity (A_r = 4.89–5.98) and were not genetically differentiated from the hatchery populations (as revealed by AMOVA).

1. Introduction

The booming catfish culture and export industry in Vietnam [the export value increased by 42.9% over 8 years, reaching $US 1.6 billion in 2008 (Hau, 2008)] has drawn global attention to striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878), one of the major species within this industry. The stocks used for culture in Vietnam were recently introduced from wild populations of the Mekong River (Ha et al., 2008), yet genetic change did not occur (Ha et al., 2009). Genetic variation within populations of hatchery and wild populations of striped catfish in Vietnam are relatively homogenous and moderate (allelic richness, A_r = 4.10–5.06; H_o = 0.61–0.66; H_e = 0.61–0.64). In contrast, striped catfish, which have been bred in captivity in Thailand since 1967 (Boonbrahm et al., 1967; Sidthimonk and Pinyoying, 1968) and now are domesticated (from Chaophraya stock), have not reached the commercial scale of culture (e.g., annual production was only 28,000 t in 2006; FAO, 2006) and the product is locally consumed without exportation. According to anecdotal information, a major drawback of the culture business of this species in Thailand is the deteriorated traits of the stock(s) (e.g., slow growth and yellow meat), which may have been the result of domestication. Altering species traits via domestication can be either desirable or undesirable. For example, reduction of survival rate and increase of the abnormality rate of *Heterobranchus longifilis* (Agnèse et al., 1995) and reduced growth performance (Brummett et al., 2004) and improved breeding success (Osure and Phelps, 2006) of domesticated Nile tilapia, *Oreochromis niloticus*, depended on broodstock management regimes.

Domestication may lead to genetic deterioration of stocks due to genetic drift, inbreeding, and selection (direct or indirect) if poor broodstock management is applied (Doyle, 1983; Falconer and Mackay, 1996). Allendorf and Phelps (1980) reported that the most apparent signature of domestication was allele loss caused by genetic drift which has been reported in a wide range of aquatic species (e.g., *H. longifilis*, Agnèse et al., 1995; turbot, *Scophthalmus maximus*, Coughlan et al., 1998; Japanese flounder, *Paralichthys olivaceus*, Sekino et al., 2002; common carp, *Cyprinus carpio*, Kohlmann et al., 2005; Nile tilapia, *O. niloticus*, Aho et al., 2006; kuruma prawn, *Marsupeneus japonicus*, Luan et al., 2006; and Pacific oyster, *Crassostrea gigas*, Appleyard and Ward, 2006). In contrast, Falconer and Mackay (1996) stated that the effect of genetic drift on heterozygosity was minor.

Due to concern about genetic deterioration, some striped catfish hatcheries in Thailand introduced novel stocks, e.g., from wild Mekong population(s), and bred them with domestic stocks to increase genetic variation (Boplanarong Farm, pers. comm.). The introduction of genetically distinct stocks increased the mean heterozygosity of a

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farmed population of African catfish, *Clarias gariepinus* (Van der Bank et al., 1992; Grobler et al., 1997); retained the genetic variation of Siamese fighting fish (Meejui et al., 2005), and improved the mean phagocytosis activity and variation at the alpha region of the MHC class I gene over those of parental strains of African catfish (Wachirachaikarn et al., 2009). However, introduction of nonlocal stocks may result in loss of the genetic integrity of local stocks which may have some advantageous traits (e.g., disease resistance and hardiness).

Limited data are available about the genetic diversity of striped catfish. So et al. (2006a) studied the mitochondrial DNA among wild populations of striped catfish in the Cambodia Mekong River and found no evidence of population structure. Later, the presence of cryptic wild populations was revealed using microsatellite DNA markers (So et al., 2006b). Ha et al. (2009) reported genetic homogeneity between domesticated and wild stocks of striped catfish in Vietnam, whereas genetic variation within populations was moderate. Prior to this study, information about the genetic diversity of the Thai stock of striped catfish was limited. No studies have been performed on the Chaophraya stocks, except for those introduced from Thailand to Bangladesh. Of those, based on three polymorphic isozyme loci the mean number of alleles per locus (1.3 ± 0.3) was low and they showed moderate heterozygosity (0.06 ± 0.05) (Barua et al., 2004). Therefore, this study was conducted to explore the genetic diversity of six hatchery stocks and three wild populations from the Mekong and Chaophraya Rivers and a tributary. The objectives of the study were to quantify the genetic variation within populations of hatchery stocks having different stock introduction histories and to assess the impacts of stock translocation on the genetic integrity of the Chaophraya stocks. Based on the results, we provide recommendations on genetic broodstock management of striped catfish in Thailand. Moreover, the information obtained in this study will facilitate efficient use of these stocks for further genetic improvement. More importantly, the use and exchange of genetic resources have captured global interest and the mutual benefit of owners and users of the resources is an important issue (Network of Aquaculture Centres in Asia-Pacific, 2009). As such, genetic diversity data would be very useful for ownership clarification and for monitoring of translocation impacts.

2. Materials and methods

2.1. Sample collection

In this study, six populations of striped catfish broodstock were sampled from hatcheries with a long history (>10 years) of fingerling production and that varied in their broodstock recruitment practices (see Table 1 and Fig. 1). Three wild populations were sampled as well: two from the Chaophraya River Basin and one from the Mekong River. Due to the low incidence of adult wild fish caught by fishermen, the wild samples (1–3 kg) were collected from ponds and cages stocked with wild mixed Pangasiid catfishes. Table 1 provides details about the sampling localities and sample sizes. A piece of caudal fin tissue (approximately 50 mg) was cut from individual fish and preserved in 95% alcohol until DNA extraction was performed.

Table 1
Details of sampling locations, population abbreviation, history of the stocks and sample sizes of striped catfish in Thailand.

Pop. abbrev.	Sampling localities/name and location of hatcheries	History of stocks	Sample sizes
PC 1	Pocharoen Farm, Amphur Muang, Chachoengsao Province (male)	Newly recruited (3–4 generations) from Mekong River, used as male brooders for cross breeding with PC 2	40
PC 2	Pocharoen Farm, Amphur Muang, Chachoengsao Province (female)	Originally recruited from domesticated Chaophraya River stock from a hatchery in Nakornsawan Province, used as female brooders for cross breeding with PC 1	40
SP	Songpinong Farm, Amphur Songpinong, Suphanburi Province (male and female)	Broodstock of mixed origin comprising of domesticated stock introduced from a hatchery in Nakornsawan Province some 10 years ago and wild Chaophraya River stock and possibly some introduced wild Mekong River population.	50
NI	Nakornsawan Fisheries Research and Development Institute, Amphur Muang, Nakornsawan Province	Originally domesticated from wild population of Chaophraya River stock for about 20 generations (40 years) with occasional reintroduction of wild Chaophraya River population.	46
NR	Boplanarong Farm, Choomsang District, Nakornsawan Province	Founded from NI stock with some newly introduced wild Mekong River population.	56
HS	Hereshare Farm, Choomsang District, Nakornsawan Province	Founded from NI stocks about 30 years ago without any introduction.	52
AY-W	Chaophraya River, Sena District, Ayudhya Province (lat.14°19′32″N/long.100°24′25″E)	Wild population collected from a pond stocked with fingerling of wild origin.	51
UT-W	Sakaekrang River, Muang District, Uthaithani Province (lat.15°17′46″N/long.100°4′40″E)	Wild population collected from a pond stocked with fingerling of wild origin.	20
NP-W	Mekong River, Muang District, Nakornphanom Province (lat.17°23′45″N/long.104°47′58″E)	Wild population collected from a cage stocked with fingerling of wild origin.	20
		Total	375

2.2. DNA extraction and amplification

The DNA extraction procedure described by Taggart et al. (1992) with slight modification was used in this study. Each individual DNA sample then was used as a template in PCR reactions primed by each of five microsatellite primer pairs (*Pg-1*, *Pg-2*, *Pg-3*, *Pg-13*, and *Pg-14*) developed for *Pangasianodon gigas* (Na-Nakorn et al., 2006). Each 20 μl reaction mixture contained 10 ng DNA template, 10 × PCR buffer (100 mM Tris–HCl pH 9.0, 500 mM KCl), 0.5 pmol each of the forward and reverse primers, 0.2 U Tag DNA polymerase, and distilled water. PCR profiles were generated as follows: denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s; optimum annealing temperature for each primer (52–60 °C) for 30 s; extension at 72 °C for 1 min; and a final extension at 75 °C for 5 min. Next, 5 μl of loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol FF, 0.5 M EDTA pH 8, and 99% formamide) was added to each PCR product and stored at 4 °C until the next step. Scoring of the PCR products was done by electrophoresis on 4.5% polyacrylamide gel and subsequent silver staining. Sizes of the PCR products were determined relative to the M13 sequence ladder.

2.3. Test for Mendelian inheritance of microsatellites

Due to a concern about null alleles (alleles yielding no PCR products), which often occur with cross-species amplification, three full-sib families (a male and a female parent and 20 offspring/family) of striped catfish were used to test for Mendelian inheritance of the alleles recognized by the primers. DNA extraction, PCR amplification, and scoring procedures were the same as previously described. The χ^2 test was used to test the departure of the segregation ratio in offspring against the expected ratio.