



Allozyme revealed substantial genetic diversity between hatchery stocks of Siamese fighting fish, *Betta splendens*, in the province of Nakornpathom, Thailand

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Received 25 November 2004; received in revised form 9 March 2005; accepted 11 March 2005

Abstract

This study was conducted to quantify genetic variation within and between stocks of hatchery reared Siamese fighting fish, *Betta splendens*, which is an economically important ornamental fish in Thailand. Our preliminary study showed that a natural population of Siamese fighting fish had average number of alleles/locus of 1.5, 45.5% polymorphic loci and $H_o=0.065$. Fourteen stocks of Siamese fighting fish comprising seven populations each of hatcheries with closed and open broodstock management practices were collected from Nakornpathom Province, Thailand. Thirteen isozyme systems and one protein system were analyzed and resulted in 19 loci being resolved that included seven polymorphic loci. Most populations conformed to Hardy–Weinberg equilibrium ($P>0.0002$) after Bonferroni correction. Genetic variation within hatchery populations with closed and open systems was not different. All had relatively low average numbers of alleles per locus which were between 1.32–1.42 (averaged 1.38) whereas observed and expected heterozygosities were relatively high ($H_o=0.081$ – 0.125 , averaged 0.099; $H_e=0.091$ – 0.142 , averaged 0.113). A high value of F_{st} ($F_{st}=0.0754$; $P<0.0002$) indicated strong population structuring. Average Cavalli-Sforza and Edwards genetic distance was 0.081. The populations were not strongly grouped according to the neighbor-joining tree. It is concluded that sufficient genetic diversity among populations existed although allele diversity and proportion of polymorphic loci were reduced relative to wild population.

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Keywords: Genetic diversity; Allozymes; *Betta splendens*; Siamese fighting fish

1. Introduction

Siamese fighting fish or Betta (*Betta splendens*) is a popular ornamental fish worldwide. Domestication for over a hundred years by unknown farmers in Thailand has resulted in two domesticated forms: the

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long-finned Siamese fighting fish for ornamental purposes and the sport Siamese fighting fish, which has short and rounded fins similar to its wild form but with improved fighting ability. Only the long-finned male Siamese fighting fish is exported and comprises approximately 10% of the quantity of exported ornamental fish of Thailand (approximately 3.6 million fish/year) (Wiwatchaisaet, 2000). The long-finned form is the subject of this study due to its economic importance.

The long history of domestication of Siamese fighting fish has raised concerns that genetic variation of broodstock may have been lost as has been reported for several fish and shellfish species, such as cutthroat trout, *Salmo clarki* (Allendorf and Phelps, 1980), rainbow trout, *Onchorynchus mykiss* (Koljonen, 1986; Stahl, 1983), Guppy, *Poecillia reticulata* (Barinova et al., 1997; Barinova and Nakajima, 1999), Atlantic salmon, *Salmo salar* (Koljonen et al., 2002), giant clam, *Tridacna gigas* (Benzie and Williams, 1996), Pacific abalone, *Haliotis discus hannai* (Li et al., 2004), abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*) (Evans et al., 2004). Genetic variation of hatchery populations of these species decreased due to the small effective number of founders and/or genetic drift that occurred during the maintenance of the broodstock (Allendorf and Phelps, 1980; Norris et al., 1999).

In addition to unintentional inbreeding caused by reduction of effective population size (N_e) (Falconer, 1983), intentional inbreeding has been occasionally practiced to extract desired traits of ornamental fish and hence led to reduction of genetic variation (Falconer, 1983).

In order to retain healthiness of the stocks, stock translocation has been practiced in many Siamese fighting fish hatcheries (Meejui, pers. com.). Despite the benefit of elevating genetic variation within stocks, stock translocation can have an adverse effect by reducing genetic diversity among stocks, which can be a useful element for genetic improvement programs.

The objectives of the present study were (1) to quantify genetic variation within hatchery stocks of Siamese fighting fish and (2) to study genetic diversity among stocks. The knowledge obtained from this study will facilitate proper broodstock management of Siamese fighting fish in order to

retain genetic diversity within and between stocks of cultured Siamese fighting fish in Thailand.

2. Materials and methods

2.1. A preliminary study

A preliminary study was conducted to quantify genetic diversity of a natural population of Siamese fighting fish ($n=23$) collected from Chainat Province, north of central Thailand. Nine isozyme systems were analyzed and resulted in 11 loci being resolved including 5 polymorphic loci. Parameters for genetic variation within population were estimated following the protocol described for the following study.

2.2. Fish samples

Live male (25 fish/population) and female Siamese fighting fish (25 fish/population) were collected from 14 farms in Nakornpathom Province, which is located 60 km south of Bangkok. It is a main production site of exported Siamese fighting fish. We selected only farms that have been operated for more than 10 years. At each particular location we selected two farms, one with closed and one with open broodstock recruitment. The closed practice was characterized by using male and females produced in a farm as broodstock. Introduction of broodstock from outside was limited (once in 2–3 years or never). Farms practicing open broodstock recruitment would bring in males and female broodstock from other hatcheries every generation (Fig. 1). Information on the locations and frequencies of broodstock introduction to the farms where samples were collected is given in Table 1.

The live fish were transported to the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok where they were subsequently sacrificed. Liver, muscle and eye tissues were collected and kept in $-40\text{ }^{\circ}\text{C}$ until analyzed.

2.3. Isozyme analyses

Thirteen enzyme systems and one protein system were analyzed following methods described by Hara and Na-Nakorn (1996). Chemical visualization fol-

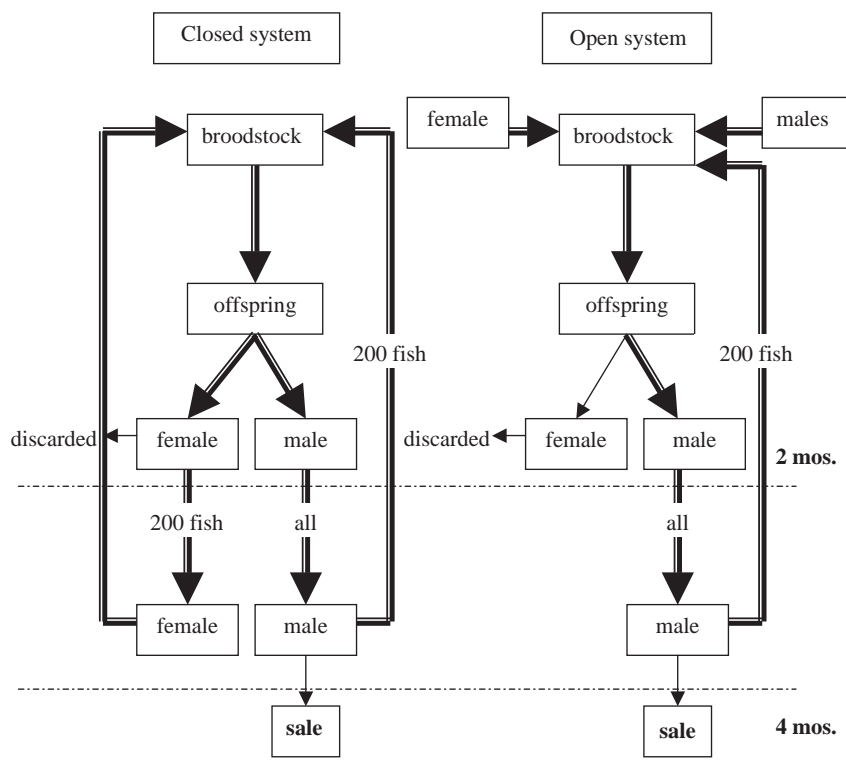


Fig. 1. A diagram showing broodstock recruitment of Siamese fighting fish in farms in Nakornpathom Province, Thailand.

lowed Morizot and Schmidt (1990). Details of enzyme systems, E.C. numbers, tissue and buffer used, and the resolved loci are shown in Table 2. Gene nomenclature followed Shaklee et al. (1990).

2.4. Data analyses

The presumed genotypes were used for calculation of allele frequencies. Populations were tested

Table 1

Details of abbreviation, locations of Siamese fighting fish farms included in this study and information on the frequency of broodstock introduction

No.	Abbreviation	Location	Frequency of broodstock introduction
1	A	Tambol Wangtakoo	3 times/year
2	B	Tambol Wangtakoo	Once every 2–3 years
3	C	Tambol Prongmadea	1 time/year
4	D	Tambol Prongmadea	No introduction
5	E	Tambol Nongpaklong	3 times/year
6	F	Tambol Nongpaklong	Once every 2–3 years
7	G	Tambol Maabkae	1 time/year
8	H	Tambol Maabkae	Once every 2–3 years
9	I	Tambol Boplab	1 time/year
10	J	Tambol Boplab	No introduction
11	K	Tambol Samkwaiepeug	1 time/year
12	L	Tambol Samkwaiepeug	No introduction
13	M	Tambol Laembua	1 time/year
14	N	Tambol Laembua	Once every 2–3 years

Table 2

Names of enzyme/protein systems, their E.C. number, tissue and buffer used, and the resolved loci

Names of enzyme/protein	E.C. number	Tissue	Buffer	Loci
Aspartate aminotransferase	2.6.1.1	Liver	TC pH8	<i>sAAT*</i>
Acid phosphatase	3.1.3.2	Muscle	CAPM pH7	<i>ACP*</i>
Alcohol dehydrogenase	1.1.1.1	Liver	TC pH8	<i>ADH*</i>
Esterase	3.1.1.1	Liver	CAPM pH7	<i>EST*</i>
Glucose-6-phosphate isomerase	5.3.1.9	Muscle	CAPM pH7	<i>GPI-1*</i> , <i>GPI-2*</i>
Isocitrate dehydrogenase	1.1.1.42	Liver	TC pH8	<i>IDHP-1*</i>
		Muscle	TC pH8	<i>IDHP-2*</i>
Leucine aminopeptidase	3.4.11.1	Muscle	CAPM pH7	<i>LAP*</i>
L-Lactate dehydrogenase	1.1.1.27	Muscle	CAPM pH7	<i>LDH*</i>
Malate dehydrogenase	1.1.1.37	Muscle	TC pH8	<i>sMDH-1*</i>
				<i>sMDH-2*</i>
Malic enzyme	1.1.1.40	Liver	CAPM pH7	<i>MEP*</i>
Mannose phosphate isomerase	5.3.1.8	Liver	TC pH8	<i>MPI-1*</i>
				<i>MPI-2*</i>
				<i>PGDH*</i>
12-Phosphogluconate dehydrogenase	1.1.1.44	Muscle	TC pH8	<i>PGDH*</i>
Phosphoglucomutase	5.4.2.2	Muscle	TC pH8	<i>PGM*</i>
Sarcoplasmic protein		Muscle	CAPM pH7	<i>PROT-1*</i>
				<i>PROT-2*</i>

for departure from Hardy–Weinberg equilibrium using the Markov chain method. Genetic variation within populations, percentage of polymorphic loci-P (a locus was considered polymorphic if the highest allele frequency did not exceed 0.95), number of alleles per locus, observed and expected heterozygosity (H_o and H_e , respectively) were calculated following formulae provided by Hedrik (1985). T-tests (Sokal and Rohlf, 1995) were performed to compare H_o , P and average numbers of alleles per locus between the farms with closed and open broodstock recruitment both across locations and between a pair within a location.

Wright's F -statistic approach (Wright, 1951, 1978) and its exact test were calculated to test for genetic population structure. Population differentiation and genotypic disequilibrium were tested using the Markov chain method. All calculations were done with the software program GENEPOP version 3.3 (Raymond and Rousset, 1995). Significance levels were Bonferroni corrected (Rice, 1989).

Genetic distance of Cavalli-Sforza and Edwards (1967) was calculated using a software program PHYLIP version 3.5c (Felsenstein, 1993). The same program was also used to construct a neighbor-joining dendrogram. We used the Cavalli-Sforza and Edwards (1967) genetic distance because it assumes no mutation and allele frequency differences among

population were caused by genetic drift. Bootstrapped values were computed over 1000 replications.

3. Results

3.1. Genetic variation of a natural population of fighting fish from Chainat

Based on 5 polymorphic loci, the natural population had mean numbers of alleles per locus of 1.50 (± 0.2), percentage of polymorphic loci=45.5%, mean observed and expected heterozygosity (H_o , H_e)=0.065 (± 0.032) and 0.095 (± 0.039), respectively.

3.2. Genetic diversity of hatchery populations of Siamese fighting fish

Analyses of 13 enzyme systems and one protein system resulted in 19 loci being resolved. Seven loci were polymorphic (a locus was considered polymorphic if a frequency of most common allele did not exceed 0.95- $P_{.95}$), *EST**, *GPI-1**, *IDHP-1**, *sMDH-1**, *MEP**, *MPI-1**, and *PGM**. Allele frequencies of the polymorphic loci are shown in Table 3.

Among the polymorphic loci, fixation of alleles was observed at *MPI-1** (in 4 of 14 populations) and *PGM** (in 8 of 14 populations).

Table 3

Allele frequencies of seven polymorphic allozyme loci in fourteen hatchery populations of *B. splendens* in Nakornpathom Province, Thailand

Loci	Population													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
<i>EST*</i>														
*100	0.760	0.460	0.790	0.776	0.790	0.459	0.420	0.480	0.640	0.810	0.490	0.450	0.120	0.300
*82	0.240	0.540	0.210	0.224	0.210	0.541	0.580	0.520	0.360	0.190	0.510	0.550	0.880	0.700
<i>GPI-1*</i>														
*280	0.140	0.060	0.210	0.140	0.170	0.290	0.240	0.390	0.210	0.375	0.220	0.160	0.300	0.260
*-100	0.670	0.680	0.570	0.750	0.530	0.510	0.610	0.510	0.700	0.500	0.650	0.500	0.490	0.540
*-380	0.190	0.260	0.220	0.110	0.300	0.200	0.150	0.100	0.090	0.125	0.130	0.340	0.210	0.200
<i>IDHP-1*</i>														
*127	0.130	0.060	0.030	0.150	0.250	0.180	0.143	0.122	0.190	0.214	0.090	0.270	0.094	0.420
*100	0.870	0.940	0.970	0.850	0.750	0.820	0.857	0.878	0.810	0.786	0.910	0.730	0.906	0.580
<i>sMDH-1*</i>														
*100	0.400	0.410	0.370	0.470	0.510	0.570	0.350	0.480	0.750	0.510	0.640	0.490	0.730	0.440
*60	0.600	0.590	0.630	0.530	0.490	0.430	0.650	0.520	0.250	0.490	0.360	0.510	0.270	0.560
<i>MEP*</i>														
*124	0.210	0.090	0.070	0.153	0.316	0.347	0.100	0.448	0.190	0.078	0.130	0.296	0.309	0.320
*100	0.790	0.910	0.930	0.847	0.684	0.653	0.900	0.552	0.810	0.922	0.870	0.704	0.691	0.680
<i>MPI-1*</i>														
*110	0.130	0.030	0.010	0	0.020	0.020	0.031	0.115	0.090	0.021	0	0	0	0.130
*100	0.870	0.970	0.990	1.000	0.980	0.980	0.969	0.885	0.910	0.979	1.000	1.000	1.000	0.870
<i>PGM*</i>														
*120	0	0	0.070	0.010	0	0	0.060	0	0.100	0.010	0	0.020	0	0
*100	1.000	1.000	0.930	0.990	1.000	1.000	0.940	1.000	0.900	0.990	1.000	0.980	1.000	1.000

Number of samples analyzed per locus is 45–50.

3.3. Departure from HW equilibrium and linkage disequilibrium

Significant departure from the HW equilibrium ($P < 0.05$) was observed in 11 out of the 14 populations. However, after Bonferroni correction only 4 populations, E, F, L, and N did not conform to the HW equilibrium ($P < 0.0002$) as indicated by significant F_{is} values (Table 4). Linkage disequilibrium was significant ($P < 0.0002$) between *IDHP-1** and *MEP** in A and I, *IDHP-1** and *MPI-1** in A, H, I, N, and *MEP** and *MPI-1** in A and I.

3.4. Genetic variation within populations

The number of alleles per locus within populations ranged between 1.32 (± 0.13) and 1.42 (± 0.14) and averaged 1.38; percentage of polymorphic loci ranged between 26.32% and 36.84% (averaged 28.58%), and observed heterozygosities were quite high (Table 4). H_o ranged between 0.081 (± 0.038) and 0.125 (± 0.051) and averaged 0.099 while expected heterozygosities (H_e) ranged

between 0.091 (± 0.040) and 0.142 (± 0.051) and averaged 0.113.

There were no differences in H_o , percentage of polymorphic loci and number of alleles per locus between closed and open populations from the same locality or the pooled data for each category.

Most of the locus-wise F_{is} values within each population (Table 5) were not different from 0 ($P > 0.0002$ after Bonferroni correction). Among the significant tests two were at *EST** and *MEP** and one at *MPI-1**. The F_{is} values for *EST** and *MPI-1** were relatively high in most populations.

3.5. Genetic variation between populations

F_{st} (0.0754) showed significantly population differentiation among the hatchery populations included in this study (Table 6). All loci contributed to population structuring. Pair-wise genetic differentiation was significant in all but 20 of the population pairs (Table 7). It is likely that population differ-

Table 4

Genetic variation within populations of hatchery populations of *B. splendens* in Nakornpathom Province, Thailand

Population	No. samples/locus	No. alleles/locus (SE)	Polymorphic loci (%)	Average heterozygosity		F_{is}^*
				H_o (\pm SE)	H_e (\pm SE)	
A	50	1.37 (0.14)	31.58	0.098 (0.040)	0.113 (0.042)	0.113
B	50	1.37 (0.14)	26.32	0.081 (0.038)	0.095 (0.041)	0.147
C	49.9	1.42 (0.14)	26.32	0.082 (0.039)	0.091 (0.040)	0.099
D	49.8	1.37 (0.14)	26.32	0.089 (0.038)	0.095 (0.039)	0.063
E	49.9	1.37 (0.14)	26.32	0.105 (0.048)	0.121 (0.048)	0.132**
F	49.9	1.37 (0.14)	26.32	0.107 (0.048)	0.127 (0.051)	0.157**
G	49.6	1.42 (0.14)	31.58	0.083 (0.033)	0.111 (0.043)	0.252
H	49.7	1.37 (0.14)	31.58	0.125 (0.051)	0.132 (0.050)	0.053
I	50	1.42 (0.14)	36.84	0.116 (0.042)	0.112 (0.040)	0.033
J	49.5	1.42 (0.14)	26.32	0.100 (0.046)	0.103 (0.044)	0.029
K	50	1.32 (0.13)	26.32	0.087 (0.039)	0.099 (0.043)	0.121
L	49.8	1.37 (0.14)	26.32	0.108 (0.050)	0.130 (0.051)	0.169**
M	49.7	1.37 (0.14)	26.32	0.091 (0.043)	0.097 (0.043)	0.062
N	50	1.37 (0.14)	31.58	0.113 (0.049)	0.142 (0.051)	0.204**
Average		1.38	28.57	0.099	0.113	

* $F_{is} = (H_e - H_o) / H_e$.** Statistically significant ($P < 0.0002$ -Bonferroni correction).

entiation occurred regardless of localities or types of broodstock management. For example, 5 of 7 population pairs that came from the same locations were significantly different. While the open population I was significantly different from the others, 4 of 13 pairs including K, which was also the open type, were significantly different. Similar trends were shown for the closed populations.

The pair-wise genetic distance of Cavalli-Sforza and Edwards (1967) ranged between 0.044 (F–H) and 0.137 (C–M) (Table 7).

The neighbor-joining tree (Fig. 2) did not separate populations into distinct groups. The populations were not clustered according to geographic localities of the farms nor broodstock management schemes (open or closed). Only seven

Table 5

Locus-wise F_{is} within each of 14 populations of *B. splendens* in Thailand

Population	<i>EST</i> *	<i>GPI-I</i> *	<i>IDHP-I</i> *	<i>sMDH-I</i> *	<i>MEP</i> *	<i>MPI-I</i> *	<i>PGM</i> *
A	0.242	0.041	-0.140	-0.157	0.465	0.565	-
B	0.124	-0.019	0.300	0.142	0.398	0.662	-
C	0.465	-0.089	-0.021	0.109	0.241	-	-0.065
D	0.306	-0.074	0.147	-0.074	0.144	-	-
E	0.465	-0.123	-0.324	0.090	0.582*	1.000	-
F	0.717*	-0.127	-0.074	-0.010	0.199	1.000	-
G	0.596*	0.170	0.010	0.175	0.147	0.662	-0.054
H	0.527	-0.341	-0.129	-0.032	0.294	-0.119	-
I	0.401	-0.301	0.165	-0.324	0.295	0.155	-0.101
J	0.295	-0.042	-0.020	-0.151	0.236	1.000	-
K	0.488	-0.005	-0.089	-0.119	0.214	-	-
L	0.242	-0.074	-0.259	0.010	0.952*	-	1.000
M	0.252	-0.077	-0.093	0.117	0.361	-	-
N	0.437	-0.122	-0.189	0.117	0.548	0.913*	-

* Statistically significant ($P < 0.0002$ -Bonferroni correction).

Table 6
Values for F -statistics of hatchery populations of *B. splendens* in Nakornpathom Province, Thailand

Loci	F_{is}	F_{st}
<i>EST</i> *	0.4086*	0.1681*
<i>GPI-1</i> *	-0.0855	0.0296*
<i>IDHP-1</i> *	-0.0786	0.0630*
<i>sMDH-1</i> *	-0.0150	0.0524*
<i>MEP</i> *	0.4030*	0.0702*
<i>MPI-1</i> *	0.5005*	0.0470*
<i>PGM</i> *	0.0137	0.0466*
Average	0.1218*	0.0754*

* Statistically significant F_{is} and F_{st} ($P < 0.0002$ -Bonferroni correction).

of 12 nodes were supported by the bootstrapping values of more than 50%.

4. Discussion

4.1. Genetic variation within populations

In order to interpret the level of the genetic variation of the hatchery stocks we used data of the natural population obtained from the preliminary study as a baseline although that population was from a different location than our hatchery samples. Anecdotal information suggests that the origin of the commercial form of fighting fish could have been populations in central Thailand although the specific

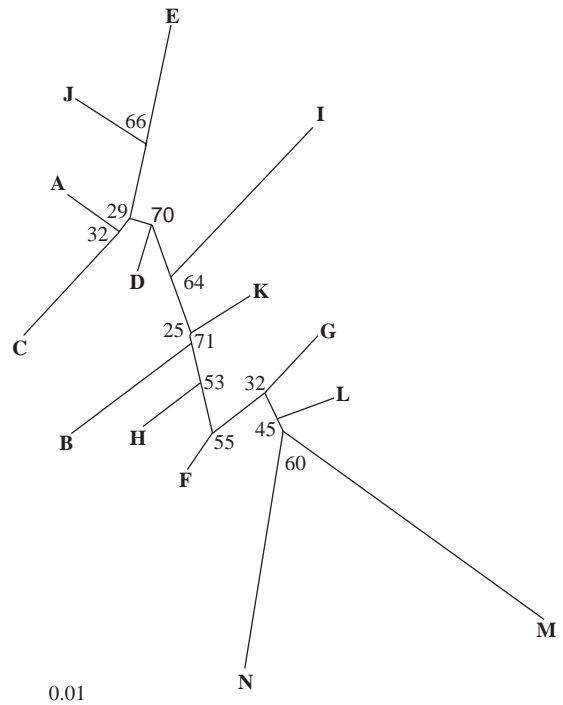


Fig. 2. Neighbor-joining tree based on Cavalli-Sforza and Edwards genetic distance between 14 hatchery populations of Siamese fighting fish in Nakornpathom Province, Thailand.

location was unknown. Therefore we were confident that our reference natural population would give relatively reliable baseline information for our study.

Table 7

A lower matrix shows Cavalli-Sforza and Edwards (1967) genetic distance between population pairs of *B. splendens* in Nakornpathom Province, Thailand

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A	***	.0000	.0000	.0525	.0083	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000
B	.066	***	.0000	.0000	.0000	.0000	.0091	.0000	.0000	.0000	.0031	.0000	.0000	.0000
C	.071	.074	***	.0036	.0000	.0000	.0007	.0000	.0000	.0001	.0000	.0000	.0000	.0000
D	.060	.071	.057	***	.0109	.0000	.0002	.0000	.0000	.0226	.0008	.0000	.0000	.0000
E	.052	.084	.084	.057	***	.0035	.0000	.0000	.0000	.0003	.0000	.0194	.0000	.0000
F	.072	.076	.097	.075	.058	***	.0000	.0546	.0000	.0000	.0126	.2518	.0007	.0004
G	.075	.059	.067	.071	.088	.069	***	.0000	.0000	.0001	.0004	.0001	.0000	.0000
H	.072	.095	.108	.095	.079	.044	.081	***	.0000	.0000	.0000	.0000	.0000	.0000
I	.078	.096	.089	.075	.083	.076	.076	.085	***	.0001	.0001	.0000	.0000	.0000
J	.066	.091	.063	.052	.063	.078	.073	.091	.073	***	.0001	.0000	.0000	.0000
K	.080	.059	.080	.057	.078	.053	.064	.082	.075	.071	***	.0001	.0001	.0000
L	.085	.075	.094	.074	.060	.046	.066	.083	.088	.087	.067	***	.0000	.0003
M	.130	.102	.137	.122	.119	.068	.099	.092	.114	.129	.074	.082	***	.0000
N	.090	.097	.130	.110	.089	.060	.081	.066	.098	.105	.097	.071	.096	***

P -values for population differentiation are in the upper matrix ($P = 0.0002$ -Bonferroni correction).

We found little reduction in allele diversity in the hatchery populations relative to data from the natural population from Chainat (8% difference in number of alleles/locus). However, the proportion of polymorphic loci showed a large decline (37.4% change) with relatively high heterozygosity compared with the natural population.

The loss of rare alleles is quite common in hatchery populations. It would occur initially because of a founder effect, the situation in which small number of brooders were taken from the natural populations for domestication (Allendorf and Phelps, 1980; Norris et al., 1999). The populations keep facing allele loss during the domestication process due to inbreeding and/or genetic drift (Allendorf and Phelps, 1980; Fujio et al., 1999). Recently with the aid of highly polymorphic markers the effects of domestication on allele loss have been clearly demonstrated in hatchery stocks of fish and shellfish; for example, Atlantic salmon, *S. salar* (Koljonen et al., 2002); flounder, *Paralichthys olivaceus* (Sekino et al., 2002); abalone, *H. rubra* and *H. midae* (Evans et al., 2004), and Pacific abalone (Li et al., 2004).

Observed heterozygosities (mean $H_o = 0.099$; ranged between 0.081 and 0.125) obtained in this study were quite high as compared to H_o of a natural population of Siamese fighting fish ($H_o = 0.065$) and hatchery populations of other species, based on the same marker type, such as black sea bream, *Acanthopagrus schlegeli* ($H_o = 0.048$ – 0.052); Japanese char, *Salvelinus leucomaenis* ($H_o = 0.052$); *Oreochromis mossambicus* in Japan and Philippines ($H_o = 0.022$); *Oreochromis niloticus* in Japan, Philippines, Taiwan and Thailand ($H_o = 0.073$); common carp in Japan ($H_o = 0.074$); guppy in Japan ($H_o = 0.054$) (Macaranas and Fujio, 1990); common carp in France ($H_o = 0.003$ – 0.029), and Czech Republic ($H_o = 0.026$ – 0.058) (Desvignes et al., 2001). Generally heterozygosity of hatchery populations tend to decline due to increased inbreeding rate which was the result of small effective population size (Falconer, 1983). Empirical data based on microsatellite loci in Atlantic salmon showed 1.7% rate of heterozygosity loss in a short-term breeding program (Koljonen et al., 2002).

High H_o accompanying low allele diversity is possible (Allendorf and Phelps, 1980; Norris et al., 1999) and might be due to the fluctuation of gene frequencies in small populations (Leberg, 1992;

Shikano and Taniguchi, 2001). It is also possible that the high H_o in this study was a result of hybridization between populations as a consequence of broodstock translocation. The significant linkage disequilibrium observed in 3 pairs of genes in A and I provided evidence for exchange between differentiated populations. A number of studies reported no decrease of heterozygosity of hatchery populations compared to wild populations while decreased allele diversity was observed, for example, in Atlantic salmon (Norris et al., 1999) and abalone in Australia and Africa (Evans et al., 2004).

Generally inbreeding is expected in hatchery stocks due to small effective population size (Allendorf and Phelps, 1980) and/or intentional inbreeding. However, no evidence of inbreeding was observed in Siamese fighting fish stocks included in this study as revealed by high heterozygosity and lack of population specific nature of locus-wise F_{is} . This is because the farmers use large numbers of broodstock (200 males+200 females) in each generation with occasional introduction of new breeders from different farms. It was recommended that inbreeding could be minimized if N_e exceeded 50 (Kapusinski and Jacobson, 1979) or by using a number of brooders at between 263 and 344 fish/generation (Tave, 1986).

Loss of alleles is a major concern for sustainability of the stocks in the long term because rare alleles were frequently associated with fitness traits (Allendorf and Phelps, 1980). Moreover, there were no private alleles observed in this study. Therefore, crossing between these populations would not increase allele diversity of a population. In case farmers observe decline in fitness traits and want to increase allele diversity we would suggest bringing in more diversity from wild stocks which are still available at present.

The “closed” and the “open” populations did not show differences in genetic variation; however, it should be noted that the “closed system” was not completely closed. Our results implied that occasional introduction of broodstock from different populations efficiently restored genetic variation within hatchery populations of Siamese fighting fish in Thailand. Therefore, it may also imply that reduction of allele diversity and percent of polymorphic loci observed in these populations could have occurred in the past rather than being a result of recent management.

Most of the hatchery populations (10 of 14) of Siamese fighting fish conformed to HW equilibrium despite selection and hybridization being practiced in most of the farms. Theoretically, selection should not cause disequilibrium of molecular markers due to their neutral nature while hybridization would. However, no matter how HWE was violated it can be resumed after a single generation of random mating (Falconer, 1983) whereas linkage disequilibrium would exist for a longer period.

4.2. Genetic differentiation among populations

Population structuring existed among hatchery stocks of Siamese fighting fish in Nakornpathom Province. This implied that stock translocation, although widely practiced, could have been done in systematic manner so that gene flow was limited and resulted in genetic integrity within populations. The F_{st} of 0.075 indicated moderate genetic differentiation among populations (Wright, 1978). It was slightly higher than F_{st} among hatchery strains of French common carp ($F_{st}=0.05$) (Bárfai et al., 2003) and black sea bream ($F_{st}=0.049$) (Taniguchi et al., 1983) and lower than Czech populations of common carp ($F_{st}=0.20$) (Bárfai et al., 2003). However, the degree of differentiation would mainly depend on whether they originated from a common ancestor and on the level of gene flow that occurred between them.

4.3. Conclusion and recommendation

The genetic diversity of hatchery populations of Siamese fighting fish in Thailand was marked with a slight decline in allele diversity and proportion of polymorphic loci while heterozygosity increased. Sufficient genetic diversity existed among populations and would greatly benefit genetic improvement programs of this species. We would recommend keeping genetic integrity of each population by minimizing stock translocation between genetically distinct populations.

Acknowledgement

We would like to express our sincere appreciation to Kasetsart University Research and Devel-

opment Institute for supporting this study through the project entitled “Genetic Study and Genetic Improvement of *Betta splendens*” (Grant No. KIP122.44). This study was partially supported by the Agriculture Biotechnology Center, Kasetsart University through a scholarship awarded to Oamduen Meejui. Partial support was also provided by “Thesis and Dissertation Support Fund”, Graduate School, Kasetsart University. We thank Professor Fred Allendorf, PhD, University of Montana, U.S.A. for his invaluable comments. We also thank the anonymous referees for their constructive comments that significantly improved the paper.

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