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An Experimental Study on Genetic Drift in Hatchery Population of Red Sea Bream

Ketut Sugama,*1 Nobuhiko Taniguchi,*1 and Susumu Umeda*2

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Genetic analyses of red sea bream hatched from eggs spawned on different dates were carried out by using electrophoretic biochemical markers. Eggs for seeding were collected from natural spawning in tank 5 times at 5 day intervals during spawning season. We have detected significant differences in allele frequencies among the offsprings spawned on different dates. It is argued that the difference in allelic frequency caused by genetic drift is due to too few number and different individuals of parents spawned on each spawning date. With this argument, we estimated that the actual number of parents genetically contributing in each spawning date varied from 8 to 16, although the total number of parents placed in tank was 130. A reduction of genetic variability was also found in the pooled hatchery samples when comparing to the wild population. The magnitude of reduction was 40% in the number of polymorphic loci, 12.2% in the number of allele per locus and 18.0% in observed heterozyosity. These results have to be considered in the artificial production of red sea bream for enhancement of natural stock.

Red sea bream *Pagrus major* is the most important and popular among the marine species mass-produced in Japan. The total production has markedly increased following establishment of techniques for natural spawning. The artificial propagation of this species for both put-and-take fisheries and aquaculture has evolved to a very large scale. Throughout Japan 39 hatcheries produced seed for releasing and commercial culture.[1]

Artificial seed production may cause genetic change in the next generation. Several studies have reported genetic change in hatchery stocks of cutthroat trout,[2] brown trout,[3] atlantic salmon[4] and black sea bream.[5] Loss of genetic variability have been argued to be due to the use of only a few individuals as broodstock.[2,4] The undesirable effects of reduced genetic variability have been recognized and discussed.[2,6]

In genetics, the ideal population is infinitely large but broodstock must work with finite population, and genetically the size of finite population is expressed as effective number of parents (*Ne*).[7,8] *Ne* is influenced by several factors,[7-9] thus it is not easy to measure the number of parents that should be used to maintain genetic variability. The FAO and UNEP[6] recommended an *Ne* of 50 for short term programs and an *Ne* of 500 for long term programs. Kincaid[10] proposed an *Ne* of 500 to keep inbreeding to "negligible" levels. Taniguchi *et al.*[5] and Sbordoni *et al.*[11] pointed out the importance of multiple egg collection from spawning tanks during spawning season to increase *Ne*. That point is especially relevant to the artificial propagation of red sea bream because of the very high fecundity of this species and it's long period of spawning. No data are available concerning genetic characteristic of offspring hatched from eggs spawned at different dates, therefore that may be useful as concept in the hatchery management.

In this paper we report a variation of allelic frequency of offsprings hatched from eggs spawned on different dates during spawning season using electrophoretic biochemical markers. The actual number of contributing parents in each spawning date was estimated based on the magnitude of random drift in allele frequency of offsprings from those of broodstock. Here we also compare the genetic variability of offsprings produced with those of wild population.

Materials and Methods

The seed production of red sea bream for put-and-take fisheries was carried out at the Prefec-

*1 Department of Cultural Fisheries, Faculty of Agriculture, Kochi University, Nankoku, Kochi 783, Japan (ケツットスガマ，谷口順彦：高知大学農学部栽培漁業学科).

*2 Usa Marine Biological Institute, Kochi University, Tosa, Kochi 781-04, Japan (楳田　晋：高知大学).

Table 1. Source of red sea bream samples used in electrophoretic analysis

Sample number	Spawning date 1986	No. of fish examined	Average body length (Cm)±SD*	Average body weight (g)±SD*
I	May 10	200	4.04±0.433	1.43±0.499
II	May 15	200	3.63±0.617	1.13±0.723
III	May 20	200	3.91±0.585	1.37±0.715
IV	May 25	200	4.06±0.507	1.47±0.745
V	May 30	200	4.28±0.443	1.79±0.592

* Standard deviation.

tural Fisheries Experimental Station of Kochi. The broodstock used for propagation is the first generation (F_1) derived from natural stock raised at hatchery. The gene frequency of the fish belonging to the same group with the broodstock used in this study was previously examined by Taniguchi (Table 2, unpublished data). One hundred and thirty matured fishes (65 males and 65 females) were placed in 60 ton tank and spawned naturally during spawning season from late April to May 1986. Fertilized eggs were collected 5 times at 5 day intervals in May 1986 (Table 1). The

Table 2. Allele frequencies at polymorphic loci and χ^2 values for difference between observed and expected frequencies of phenotypes in offspring spawned at different dates, broodstock and wild population of red sea bream

Locus	Allele	Offspring					Pooled	Broodstock*	Wild population
		I (200)	II (200)	III (200)	IV (200)	V (200)	(1000)	(80)	(40)
Adh	A	0.098	0.165	0.050	0.105	0.098	0.103	0.275	0.200
	B	0.603	0.445	0.728	0.593	0.633	0.506	0.425	0.563
	C	0.270	0.390	0.223	0.303	0.270	0.292	0.300	0.238
	χ^2	2.07	7.09	1.95	3.46	5.23	8.45[a]	—	1.14
Est	D	0.035	0.038	0.050	0.078	0.048	0.049	0.031	0.150
	E	0.555	0.475	0.558	0.565	0.642	0.559	0.563	0.588
	F	0.323	0.435	0.292	0.307	0.238	0.319	0.257	0.200
	X	0.087	0.052	0.101	0.050	0.072	0.073	0.150	0.063
	χ^2	10.68	7.27	6.18	17.45	2.59	15.13[a]	—	1.89
6-Pgd	A	0.125	0.123	0.153	0.123[b]	0.158	0.136	0.169	0.138
	B	0.873	0.875	0.847	0.873	0.838	0.861	0.813	0.838
	C	0.003	0.003	—	0.005	0.005	0.003	0.019	0.025
	χ^2	0.15	1.90	0.54	4.12	4.91	3.92	—	1.13
Pgm-1	A	0.863	0.923	0.923	0.923	0.978	0.922	0.837	0.938
	B	0.138	0.078	0.078	0.078	0.023	0.078	0.163	0.063
	χ^2	0.02	0.04	0.64	45.22[b]	0.10	14.93[b]	—	0.04
Pgm-2	A	0.030	0.023	0.048	0.035	0.013	0.030	NS	—
	B	0.970	0.978	0.953	0.965	0.988	0.970		1.000
	χ^2	3.97	0.12	0.49	0.26	0.32	0.02	—	—
Mdh-1	A	0.983	0.985	0.943	0.970	0.940	0.940	0.956	0.975
	B	0.018	0.015	0.058	0.030	0.060	0.036	0.044	0.025
	χ^2	0.06	0.05	0.74	0.19	0.82	1.51	—	0.00
Gpi-1	A	—	—	—	—	—	—	—	—
	B	1.000	1.000	1.000	1.000	1.000	1.000	0.987	0.962
	C	—	—	—	—	—	—	0.013	0.038
	χ^2								0.04
Aat-2	A	—	—	—	—	—	—	0.007	0.025
	B	1.000	1.000	1.000	1.000	.1000	1.000	0.993	0.983
	C	—	—	—	—	—	—	—	0.037
	χ^2								1.46
Aat-3	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988
	B	—	—	—	—	—	—	—	0.013
	χ^2								0.00
α-Gpd	A	1.000	1.000	1.000	1.000	1.000	1.000	NS	0.988
	B	—	—	—	—	—	—		0.00
Sdh	A	—	—	—	—	—	—	—	0.038
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.963
	χ^2								0.00
Idh	A	—	—	—	—	—	—	0.013	—
	B	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000

Number of fish examined in parenthesis, NS=Not studied.

* Taniguchi, 1982 (Unpublished). a) $P<0.05$; b) $P<0.01$.

eggs were transferred to Usa Marine Biological Institute of Kochi University, and were hatched and reared in different tanks. Details concerning fry rearing methods are described by Fukuhara.[12] The fishes were raised for 50 days except sample May 15 which was raised for 45 days. The fish samples were immediately stored at −30°C, body length (fork length) and body weight were measured prior to electrophoretic analysis (Table 1).

Two hundred fishes from each batch were used for electrophoresis. Electrophoretic techniques, staining procedures, interpretation of electrophoretic banding pattern and designation of loci are according to Taniguchi and Okada.[13] The enzymes and proteins examined in this study were: Alcohol dehydrogenase (ADH, E.C. 1.1.1.1), Esterase (EST, E.C. 3.1.1.3), α-Glycerophosphate dehidrogenase (α-GDH, E.C. 1.1.1.8), Isocitrate dehydrogenase (IDH, E.C. 1.1.1.44), Asparate aminotransferase (AAT, E.C. 2.6.1.1), Lactate dehydrogenase (LDH, E.C. 1.1.1.27), Malate dehydrogenase (MDH, E.C. 1.1.1.37), 6-Phosphogluconate dehydrogenase (6-PGD, E.C. 1.1.1.44) Phosphoglucomutase (PGM, E.C. 2.7.5.1), Glucosphate isomerase (GPI, E.C. 5.3.1.9) and Sorbitol dehydrogenase (SDH, E.C. 1.1.1.14).

Wild population of red sea bream for comparison with the offspring produced in this hatchery were collected in April 1986.

Results

Twelve enzymes coded by 24 loci were resolved clearly in all samples spawned at different dates. Six loci were polymorphic, i.e., *Mdh-1*, *Pgm-1*, *Pgm-2*, *Adh*, *6-Pgd* and *Est*. Allelic frequencies and chi-square values for discrepancy between observed and expected frequencies of phenotypes are shown in Table 2. Statistically significant difference was detected at Pgm-2 locus in sample I ($\chi^2=3.97$; df=1; $P<0.05$) and at Pgm-1 and Est locus in sample IV ($\chi^2=45.22$; df=1; $P<0.01$ and $\chi^2=17.45$; df=6, $P<0.01$, respectively). The significant differences observed here were caused by homozygote excess in these samples. A significant deviation from Hardy-Weinberg expectation caused by homozygote excess were also observed in Adh, Pgm-1 and Est locus when all samples were pooled (Table 2). The interpretation of the homozygote excess is not so simple, however, since one of the assumptions of the Hardy-Weinberg equilibrium an infinitely large population size. The deviation observed here is due probably to the unrandom mating or inbreeding of the fishes bred. The differences in allelic frequencies among samples were examined by the heterogeneity test. Statistically significant allele frequency differences were found at ten out of 13 isozyme loci (Table 3). A matrix under the diagonal in Table 4 shows the result of test of significant heterogeneities between every pair of 5 samples examined. Fig. 1 demonstrates fluctuation of allelic frequency of offspring spawned at different dates. A change in the allelic frequency of offspring was observed in the different spawning dates although we used the same breeders and tank. Loss of rare allele was also observed in this study. No individual with the *6-Pgd-c* was observed in sample III (Table 2).

Table 3. Heterogeneity test of allelic frequencies of isozyme loci among red sea bream samples spawned at different dates

Isozyme allele	Chi-square value (d.f.=4)	Isozyme allele	Chi-square value (d.f.=4)
Mdh-1 A	21.41*2	6-Pgd A	4.19
Pgm-1 A	36.61*2	6-Pgd B	3.56
Pgm-2 B	9.68*1	6-Pgd C	2.27
Adh A	29.08*2	Est D	9.96*1
Adh B	70.57*2	Est E	22.72*2
Adh C	29.84*2	Est F	38.49*2
		Est X	11.21*1

*1 $P<0.05$; *2 $P<0.01$.

Table 4. Test of significant heterogeneities in gene frequencies under the diagonal, between every pair of red sea bream samples spawned at different dates

Sample	I	II	III	IV	V
I					
II	+6				
III	+4	+7			
IV	+3	+6	+4		
V	+4	+7	+5	+5	

The sign +3 mean significant difference at 3 locus.

Based on the magnitude of random genetic drift in allele frequencies of offspring from those of broodstock used in this study, the actual number of contributing parents (*Ne*) in each spawning date was estimated by Taniguchi's formula:

$$Ne=(2Ns-1)/2(2NsV_{\Delta}p-1)$$

Where Ns and $V_{\Delta}p$ stand for the number of samples used for estimating isozyme frequency in each spawning date and the variance of standarized fluctuation in marker frequency at various loci

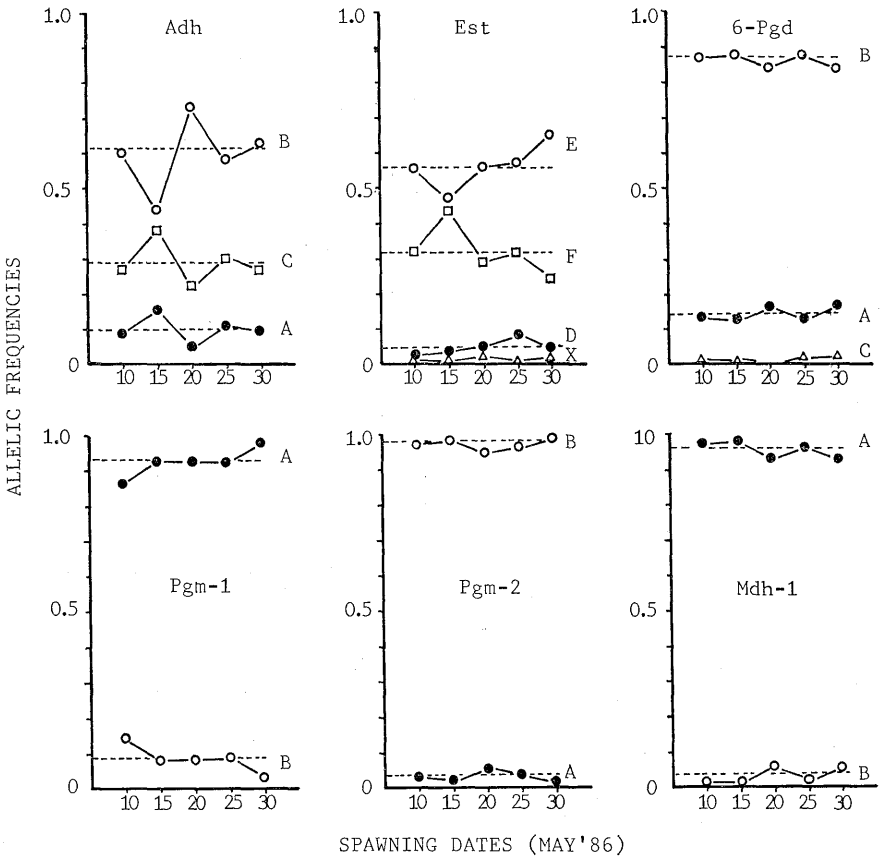

Fig. 1. Genetic variations in red sea bream offspring hatched from eggs spawned at different dates in the spawning season. The broken lines represent the allele frequency of pooled sample.

Table 5. Estimates of the effective number of contributing parents (*Ne*) in each spawning date with a range of confidence at 95% probability level

Spawning date	d^2	$V_{\Delta p}=\sum d^2/12$	*Ne**	Range
I	0.417995	0.034833	15.5	4.7<Ne<30.1
II	0.501618	0.041802	12.3	3.9<Ne<24.4
III	0.782071	0.065173	7.9	2.5<Ne<15.0
IV	0.534017	0.044501	11.9	3.6<Ne<22.8
V	0.590054	0.049175	10.7	3.3<Ne<20.4

* $Ne=(2Ns-1)/2(2Ns V_{\Delta p}-1)$.
Ns = samples size.

Table 6. Summary of genetic variability in red sea bream samples spawned at different dates based on electrophoretic analysis of 24 loci

Items	Sample					
	I	II	III	IV	V	Pooled
Number of sample examined	200	200	200	200	200	1000
Number of loci examined	24	24	24	24	24	24
Number of polymorphic loci	6	6	6	6	6	6
Proportion of polymorphic loci	0.25	0.25	0.25	0.25	0.25	0.25
Number of allele per locus	1.46	1.50	1.46	1.46	1.50	1.50
Heterozygosity						
Observed (H^o)	0.063	0.068	0.067	0.060	0.063	0.064
Expected (H^e)	0.069	0.069	0.067	0.066	0.063	0.067
H^o/H^e	0.915	0.979	1.000	0.913	1.000	0.955

Table 7. Comparison of genetic variability between natural and hatchery populations of red sea bream

Items	Natural population	Hatchery population	Reduction of variability (%)
Number of sample examined	40	1000	
Number of loci examined	24	24	
Number of polymorphic loci	10	6	40.0
Proportion of polymorphic loci	0.417	0.250	40.0
Number of allele per locus	1.708	1.500	12.2
Heterozygosity			
Observed (H^o)	0.078	0.064	18.0
Expected (H^e)	0.078	0.067	
H^o/H^e	1.000	0.955	

respectively. The estimated numbers of contributing parents (*Ne*) were 16, 12, 8, 12 and 11 in sample I, II, III, IV and V, respectively (Table 5).

Genetic variability of the samples spawned at different date are summarized in Table 6. There was no clear difference among the samples in that value, except the ratio of H^o/H^e were less than 1 in samples I, II, IV and in the pooled sample, suggesting that inbreeding had a little effect in these sample. In the wild population of red sea bream we found ten polymorphic loci consistently scorable in liver tissue, such as *Adh*, *Est*, *6-Pgd*, *α-Gpd*, *Aat-2*, *Aat-3*, *Gpi-1* and *Sdh* (Table 2). The genetic variability of the wild population is summerized in Table 7. Comparison of genetic variability of pooled sample produced in this hatchery to the wild population are shown in Table 7. We have found a reduction of genetic variability in hatchery population, as shown in Table 7 the magnitude of reduction was 40.0% in the number of polymorphic loci, 12.2% in the number of allele per locus and 18.0% in the observed heterozygosity.

Discussion

The genetic changes associated with artificial seed production can result from unconcious selection in artificial environment, genetic drift and inbreeding in small population. All of these genetic phenomena have been reviewed recently as they apply to aquaculture.[14-18]

In the present study we have detected significant difference in allelic frequency among the offspring spawned at different dates. The allelic frequency would not be expected to change among the samples if all of breeders present in the tank have contributed in each spawning date and they were bred random mating. This phenomenon is well known as random genetic drift. Random change is difficult to discuss, since we can not say whether or not that some specific change will happen, we can only say that change is probable or improbable.[19] In this case it is rather difficult to determined wether or not the allelic frequency change was caused by unconcious selection, sampling error or genetic drift. As we analyzed 200 fishes from each batch, we assumed that samples were sufficient to minimized sampling error in observed allelic frequencies. However, on the basis of our experience working in red sea bream hatchery, from 65 pairs of broodstock present in the tank not all of them spawned everyday. We felt that the present result could be explained by genetic drift due to too few number and different number of broodstock involved in each spawning date. It was estimated that only 8 to 16 of spawners have contributed in each spawning dates although the total number of spawners used was 130 (Table 5). There is possibility that the value of *Ne* was under estimation because the sampling error of broodstock (ancestor effect) was neglected.

Big difference between the actual number of parents used and actual number of parent contributing to the next generation was observed in artificial propagation of black sea bream,[5] i.e. which was only 16 out of 102 breeders genetically spawned. It was explained that the difference observed was due to the collection of eggs for seeding was done only on a single day. Significant allelic frequency difference between broodstock and offspring spawned at different dates in artificial propagation of cod has also been reported.[20] This was also because only a fraction of the breeders present in the pond spawned in a single day.

A lower amount of genetic variability in hatchery population as compared to wild population was also observed in this study. As observed in cutthroat trout,[2] brown trout,[3] black sea bream[5] and Penaeid shrimp,[11] in all of these cases bottleneck effect and genetic drift was well explained to be due to the use of small effective number of parents in producing the next generation. The reduction in genic variability observed here suggests a

similar explanation.

Based on the value of H^o/H^e (Table 7), inbreeding seemed to be of a little effect in the reduction of genetic variability. In addition the broodstock used in this hatchery was first generation raised at hatchery from natural stocks, mating between related individuals may also be of importance in considering the observed genetic change.

The effect of unintentional inbreeding and genetic drift due to the use of small effective number of parents in producing the next generation have been discussed in several meetings.[2,4,6,18,21] Several authors have emphasized the importance of using a large number of parent fish for the maintenance of genetic variability.[3,6,8] However, using a large number of parents alone is not sufficient for a successful propagation program. As pointed out by Taniguchi[18] and Jorstad,[20] detailed understanding of genetic characteristic of offspring spawned at different times during spawning season would be useful for the formulation of hatchery management.

Present data show that even in the case all of the samples spawned at different dates were pooled the genetic variability was not increased (Table 6). This was due to used of the first generation (F_1) as spawner, which have already reduced in genetic variability through bottleneck or drift. The allele with low frequencies in broodstock are likely to be lost in F_2 due to drift or breeding (Table 2). Such findings have to be considered in the management of hatchery e.g. it is important that hatcheries do not use F_1 as broodstock and periodic replacement of hatchery fish with those from natural populations is also advised to prevent the lost of genetic variability caused by drift and inbreeding.

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References

1) JSFFA: Farming Fisheries, Seed production Procurement and releasing Statistics for 1983. Pub. Tokyo, 1983, 298 p.
2) F. W. Allendorf and S. T. Phelps: *Trans. Am. Fish. Soc.*, **109**, 537–543 (1980).
3) N. Ryman and G. Stahl: *Can. J. Fish. Aquat. Sci.*, **37**, 82–87 (1980).
4) T. V. Cross and J. King: *Aquaculture*, **33**, 33–40 (1983).
5) N. Taniguchi, K. Sumantadinanta, and S. Iyama: *Aquaculture*, **35**, 33–40 (1983).
6) FAO/UNEMP: FAO Fisheries Technical Paper, 217 (1981).
7) R. W. Doyle and A. J. Talbot: *Aquaculture*, **57**, 27–35 (1986).
8) D. Tave: *Prog. Fish-Cult*, **46**, 262–268 (1984).
9) J. F. Crow and M. Kimura: An introduction to population genetics theory. Harper and Row, New York, 1970, 519 p.
10) H. L. Kincaid: *Trans. Am. Fish. Soc.*, **108**, 457–461 (1979).
11) V. Sbordoni, E. De Matthaeis, M. Coblli Sbordoni, G. La Rossa, and M. Mattoccia: *Aquaculture*, **57**, 239–251 (1986).
12) O. Fukuhara: *Nippon Suisan Gakkaishi*, **51**, 731–743 (1985).
13) N. Taniguchi and Y. Okada: *Nippon Suisan Gakkaishi*, **46**, 437–443 (1980).
14) H. Fredeen: *Aquaculture*, **57**, 1–26 (1986).
15) G. F. Newkirk: *Aquaculture*, **19**, 209–228 (1980).
16) R. W. Doyle: *Aquaculture*, **33**, 167–185 (1983).
17) N. P. Wilkins: *Aquaculture*, **22**, 209–228 (1980).
18) N. Taniguchi: In "Sea Farming Technology of Red Sea Bream" (ed. by M. Tanaka and Y. Matsumiya), Koseisha Koseikaku, Tokyo, 1986, pp. 37–58.
19) J. S. Gale: Population Genetics, Thomson Litho Ltd., East Kilbride, Scotland, 1980, 189 p.
20) K. Jorstad: *Aquaculture*, **57**, 227–238 (1986).
21) G. A. E. Gall: *Aquaculture*, **33**, 383–394 (1983).