Loss of genetic variability in the sub-strains which were established from one strain of the guppy Poecilia reticulata

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Loss of genetic variability in the sub-strains which were established from one strain of the guppy *Poecilia reticulata*

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Abstract

The changes of gene frequencies and genetic variabilities in sub-strains which were experimentally subdivided from the same strain of the guppy *Poecilia reticulata* was examined. Each sub-strain was established by a small number of pairs (3-10) and maintained as a closed colony at a size of 200-500 individuals per aquarium.

Of the 30 loci scored, variations were observed at three loci, *Aat-1*, *Pgm-1* and *Pgm-2*. At these three polymorphic loci, the allele frequencies of each sub-strain fluctuated around the allele frequency of the original strain. However, there were no significant differences between the allele frequency of the original strain and the mean allele frequency of sub-strains at each locus. Average heterozygosities of each sub-strain also fiuctuated and were less than that of the original strain. There was no correlation between the decrease of the average heterozygosity and the initial number of pairs in each sub-strain, and the time after subdivision.

These results suggest that the change of allele frequencies and loss of genetic variability would be determined by the genetic constitution of initial individuals of sub-strains.

Introduction

In the Mendelian population, it was expected that gene frequencies and genetic variabilities of the population would be constant. In general, several conditions are needed for constant gene frequencies and genetic variabilities. These are : mating is random, population size is very large, migration is negligible, mutation can be ignored and natural selection does not afiect the locus under

consideration *etc.* (1). But, processes that change gene frequencies are always present.

Loss of genetic variabilities and increase of genetic differences among cultured strains were observed in rainbow trout cultured in Japan. Such phenomenons were due to the increase of artificial processes during the maintenance of the strains (2). Loss of genetic variabilities were also observed in the comparison of natural populations and cultured stocks in cutthroat trout (3), masu salmon (4), black rockfish (5), Pacific herring (6) and guppy (7). In each case, the genetic drift and/or founder effect was assumed as a cause of genetic differences and the loss of genetic variabilities, because the initial population size and the number of individuals involved in reproduction in each stock were usually small compared with the natural populations.

Nakajima *et al.* (2) demonstrated that the fluctuation of allele frequencies in sub-populations which were established from one pair was widely distributed around the allele frequency of the original population of the guppy. Strains or cultured stocks were subdivisions of original population. Small numbers of individuals were used for first generations, but there were a few cases where only one pair was used.

The purpose of this study is to examine the change of allele frequencies and genetic variabilities in the sub-strains which were experimentally established by subdivision of a small number of parents from the same strain.

Materials and Methods

Establishment of sub-strains

The guppy *Poecilia reticulata* was used for the experimental fish. The S3 strain which had been maintained as a closed colony in the laboratory since 1981 was used for the original strain, and 11 sub-strains were established by the subdivision of a small number of parents. The process of the establishment of each sub-strains is represented in Fig. 1. The S3HL strain was the earliest sub-strain which was separated from S3 strain in 1990. It was established from the offspring which were obtained from 6 females who survived for 24 h in 35°C water temperature (8). The S3SWSW strain and S3SWFW strain were the strains established from 3 females and 8 females, respectively, which were acclimated to 35 ppt artificial sea water (9). After acclimation, the S3SWSW strain was maintained in 35 ppt artificial sea water, and the S3SWFW strain was maintained in fresh water. S3SWR1, S3SWR2, S3SWS1 and S3SWS2 were the sub-strains which were selected to sea water resistant (SWR) and sea water sensitive (SWS). Each sub-strain was established from the offsprings obtained from six selected females (10). S3-20, S3-23, S3-26 and S3-29 were the sub-strains which were maintained at 20°C, 23°C, 26°C and 29°C water temperature, respectively. The

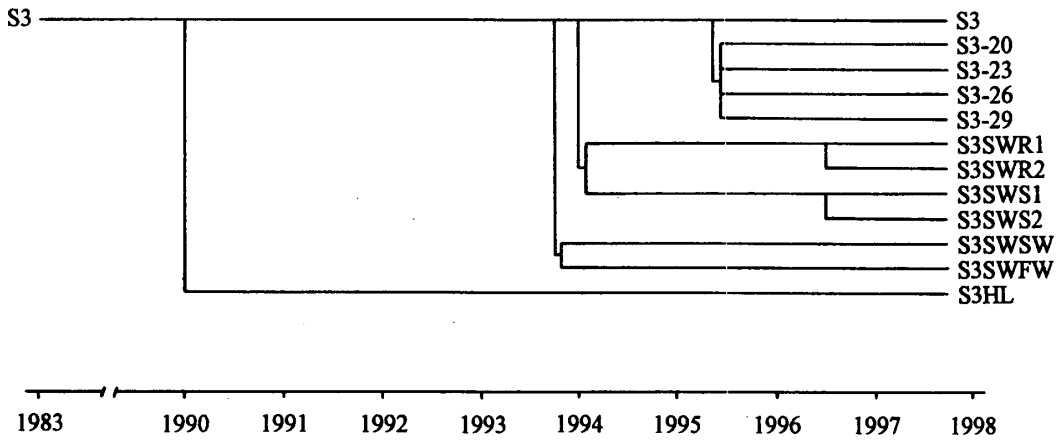

FIG. 1. The process of the establishment of each sub-strain in this study.

initial number of pairs of each sub-strain was 10.

More than 18 months had passed after sub-populations were first established. Sub-strains were maintained at 23°C with a density of 200 to 500 individuals per aquarium.

Isozyme analysis

Electrophresis and staining procedures were based on Fujio (11). Thirty one loci controlling sixteen enzymes were already reported by Barinova *et al.* (12, 13), were used for genetic characterization of the S3 strain and 11 sub-strains. The average heterozygosity (He) was calculated from the allele frequencies as the indicator of genetic variability. To quantify the genetic differences among sub-strains, coefficient of gene differentiation (G_{ST}) (14) was calculated from the allele frequencies.

Results

1) Fluctuation of allele frequencies

Three polymorphic loci, *Aat-1*, *Pgm-1* and *Pgm-2*, were observed from the electrophoretic analysis of 31 isozymic loci, in which two alleles were observed at each of the three polymorphic loci. The allele frequencies of each of the three loci in the S3 strain and 11 sub-strains are represented in Table 1. In the *Aat-1* locus, the variation was observed in the S3 strain and 11 sub-strains, and the *A* allele frequency was distributed from 0.190 in S3-20 to 0.889 in S3-29 with a mean of 0.422. Significant differences of allele frequencies were observed in 8 out of the 11 comparisons between the S3 strain and 11 sub-strains, however, there was no significant difference between the S3 strain and the mean of the 11 sub-strains. In the *Pgm-1* locus, the *A* allele frequency was distributed from 0.183 in S3HL to

TABLE 1. *Genetic characteristics of the S3 strain and 11 sub-strains*

Locus	Allele	S3	Sub-strains										
			mean of sub-strains	S3HL	S3 SWSW	S3 SWFW	S3 SWR1	S3 SWR2	S3 SWS1	S3 SWS2	S3-20	S3-23	S3-26	S3-29
Aat-1	*A*	0.395	0.422	0.274	0.363	0.582	0.551	0.510	0.342	0.038	0.190	0.600	0.297	0.889
	B	0.605	0.578	0.726	0.637	0.418	0.449	0.490	0.658	0.962	0.810	0.400	0.703	0.111
	N	119		53	102	67	157	50	139	39	145	209	96	54
Pgm-1	*A*	0.528	0.512	0.183	0.681	0.864	0.661	0.355	0.429	0.478	0.654	0.336	0.840	0.231
	B	0.472	0.488	0.817	0.319	0.136	0.339	0.645	0.571	0.522	0.346	0.664	0.160	0.769
	N	127		63	102	70	158	55	140	45	149	216	97	54
Pgm-2	*A*	0.906	5.941	0.857	0.938	1.000	0.984	0.900	1.000	1.000	0.842	0.998	1.000	0.833
	B	0.094	0.059	0.143	0.062	0	0.016	0.100	0	0	0.158	0.002	0	0.167
	N	127		63	105	70	158	55	140	45	149	216	97	54
He		0.037	0.029	0.030	0.033	0.021	0.031	0.037	0.030	0.018	0.033	0.030	0.022	0.027

0.864 in S3SWFW with a mean of 0.512. Significant differences of allele frequencies were observed at 10 out of the 11 comparisons between the S3 strain and 11 sub-strains, however, there was no significant difference between the S3 strain and the mean of the 11 sub-strains. In the *Pgm-2* locus, the *A* allele frequency was distributed from 0.842 in S3-20 to 1.000 in S3-26, S3-SWS1, S3-SWS2 and S3-SWFW with a mean of 0.941. Significant difference of allele frequencies were observed at 6 out of the 11 comparisons between the S3 strain and 11 sub-strains, however, there was no significant difference between the S3 strain and the mean of the 11 sub-strains. The allele frequencies fluctuated widely around the allele frequency of the S3 strain, however, there were no significant differences of allele frequencies between the S3 strain and the mean of the 11 sub-strains at each of the three loci. Significant deviations from Hardy-Weinberg's low were observed at two out of 36 cases: at the *Aat-1* locus in S3-SWR2 and at the *Pgm-1* locus in S3-SWR1, former was the heterozygote excess and latter was homozygote excess.

3) Genetic differences among 11 sub-strains

The degree of genetic differences was measured by the coefficient of gene differentiation (G_{ST}). G_{ST} was calculated in two different components of the sub-strains, one was the S3 strain and the mean of 11 sub-strains, and the other was among 11 sub-strains (Table 2). G_{ST} between the S3 strain and 11 sub-strains was 0.083, and among 11 sub-strains was 0.171. This phenomenon indicates that the genetic constitution of the mean of 11 sub-strains was not different from that of the original strain, however, gene frequencies fluctuated in each sub-strains. G_{ST} among 11 sub-strains was more than two times larger than that between the

TABLE 2. *Genetic differences among each compornents of strains, sub-strains, cultured strains and introduced populations in the guppy.*

Group	Number of compornents to compare	H_T	H_S	G_{ST}
S3 strain and 11 sub-strains	2	0.036	0.033	0.083
11 sub-strains	11	0.035	0.029	0.171
New cultured* strains	7	0.041	0.036	0.122
Old cultured* strains	6	0.029	0.020	0.310
Cultured strains*	13	0.038	0.028	0.268
Introduced* populations	10	0.048	0.041	0.146

*: Barinova *et al.* 1998

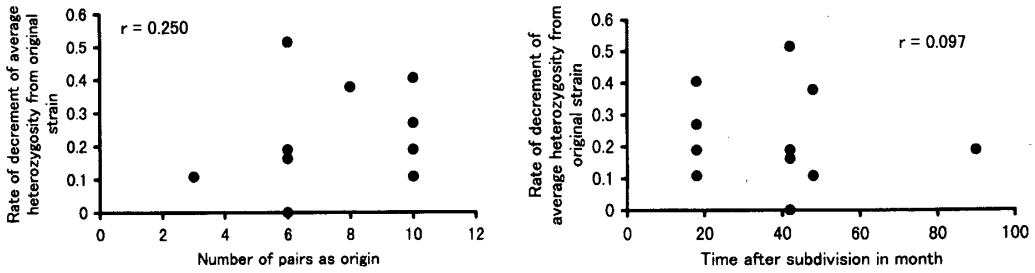

FIG. 2. Relationship between amount of decrease of average heterozygosity and number of pairs as origin in each sub-strain, and time after subdivision.

S3 strain and the 11 sub-strains and that value was also larger than that in the new cultured strains, indicating that subdivision can lead to genetic differences as well in the cultured strains. The large value obtained from 11 sub-strains was caused by the loss of genetic variability within each sub-strains which was observed as a loss of H_S.

2) Decrement of average heterozygosity

The average heterozygosity of the S3 strain was 0.037, and in the 11 sub-strains the average heterozygosities were distributed from 0.018 in S3-SWS2 to 0.037 in S3-SWR2 with a mean of 0.029. The distribution of the average heterozygosities within the sub-strains was less than that of the S3 strain and the mean of the average heterozygosity of 11 sub-strains were reduced to 21.6% less than that of S3 strain. Loss of genetic variability was observed in the newly established 11 sub-strains.

The amount of decrease of average heterozygosity and the time after subdivision in each sub-strain, the amount of decrement of the average heterozygosity and the initial sumber of pair of each sub-strain were compared (Fig. 2). There was no correlation between them.

Discussion

Genetic differentiation and loss of genetic variabilities caused by the founder effect were demonstrated by experimentally subdivided sub-strains of the guppy. The same phenomenon was observed in the case of sub-populations which were established by one pair from the same strain (2). Nakajima *et al.* (2) suggested that at least 14 pairs were need to make the allele frequencies reappear in the newly established population. According to this suggestion, the initial number of pairs needed to establish sub-strains in this study was too small to make the allele frequencies of the original strain reappear. This study supports the suggestion by Nakajima *et al.* (2).

The decrement of average heterozygosity was observed in each sub-strains

and compared to the original strain. The amount of decrease of average heterozygosities from the original strain was fluctuated from 0 to 0.019 with a mean of 0.009. The rate of this decrease of average heterozygosities was about 21.6% which is one of the indicators of the coefficient of inbreeding. This decrement of average heterozygosity is close to the coefficient of inbreeding of the ful-sib mating.

Loss of genetic variability was also reported in the comparison between wild populations (15-17) and introduced populations, and between old and new cultured strains of the guppy (7). As shown by Nei *et al.* (18), the effect of population size reduction on average heterozygosity is expected to last for hundreds of thousands of generations after the recovery of population size. The population size of each sub-strain did not fluctuat during the maintenance of the sub-strains, and no correlation was observed between the time after subdivision, the amount of decrement of average heterozygosity, and the initial number of parents, indicating that the genetic constitution was determined by the initial individuals of each sub-strain.

It can be neglect that the fluctuation of gene frequencies and genetic variability during the maintenance of sub-strains in this study which less than 48 months and passed after subdivision. During the long period of maintenance, fluctuation of population size would be occurred and effective population size will be decreased (19). In the case of a long period of maintenance of the strain, it is necessary have to consider the possible effect of bottleneck during the maintenance of the strain.

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