グッピーの実験集団から作成した分集団における遺伝子頻度の変化

誌名	日本水産學會誌
ISSN	00215392
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巻/号	57巻12号
掲載ページ	p. 2223-2227
発行年月	1991年12月

Nippon Suisan Gakkaishi 57(12), 2223–2227 (1991)

Fluctuation of Gene Frequency in Sub-populations Originated from One Guppy Population

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(Received June 6, 1991)

Larger fluctuation of gene frequencies among cultured fish populations has been generally explained by the founder and/or bottleneck effect in the process of artificial propagation. However, the experimental support for this explanation in fish has not been reported.

To obtain evidence of the fluctuation of gene frequencies due to sampling, sub-populations formed from one guppy population were examined using the loci *AAT-1** and *PGM-1**.

The *AAT-1** and *PGM-1** gene frequencies of an experimental population composed by 500 individuals, included 200–300 adult fishes, were stable throughtout generations. On the other hand, gene frequencies fluctuated within a wide range among 16 sub-populations, each made from one parent. The expected number of the sub-population in which the allele was fixed or lost nearly coincided witht he observed number. Thus, it was suggested that the large fluctuation of gene frequencies among cultured populations resulted from sampling errors of the parents.

Larger fluctuations of gene frequencies among cultured fish populations than among natural populations has been demonstrated in masu salmon,[1] black rock fish[2] and red sea bream.[3,4] Moreover, the larger fluctuation was observed among cultured populations under strong artificial selection, such as rainblw trout.[5] In cultured rainbow trout populations, fluctuations of gene frequencies increased with an increase of the pressure of artificial selection. The reason of this phenomenon was explained to be the founder and/or bottleneck effect in the process of artificial propagation, and concluded that a cultivation is the subdivision of original population and fixation of specific genes. Such conclusion is supported by several simulation studies. Kimura[6] pointed out that the population which had a small effective population size fluctuated in a very wide range after a small number of generations from the initial population, and the number of fixed loci will increase, using computer simulation. There were also many reports in which the change of gene frequencies of cultured stocks in fish was explained by genetic drift at the founder individual,[1-5,7-10] however, there is little experimental support for this explanation in fish.

Knowlege of the fluctuation of gene frequencies in the sub-populations formed from one population is very important for the management of cultured fish populations.

The purpose of this study is to confirm the fluctuation of the gene frequency in sub-populations formed from one experimental population of the guppy.

Materials and Methods

Guppy (*Poecilia reticulata*) was used as experimental material. The guppy used was a standard (S) strain, and was maintained as a closed colony in our laboratory. Experimental populations I, II and III were made by the isolates from the original S strain population. They are maintained in 60 *l* aquaria at a temperature of 23±2°C. The population size is about 500 individuals which includes 200–300 adult fishes. This number of individuals were kept in each generation. For the genetic markers, isozymic loci, *AAT-1** and *PGM-1** which are already known as polymorphic loci, were used.[11] Procedures of detection of isozyme followed Fujio.*2

Two experiments were made, one to examine the fluctuation of the gene frequency at each generation in a closed colony kept at reasonable density of about 500 individuals per aquarium, and the other to determine the distribution of

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*2 Y. Fujio: Study on Genetic Characteristics of Fish and Shellfishes in Isozymic Analysis, Nosuisho Tokubetu Shiken Houkusho, 1984, pp. 1–65.

Table 1. The fluctuation of gene frequency at each generation in the closed colony of guppy

Sampling Time	Experimental population I					Experimental population II				
	Number of Individuals	*AAT-1**		*PGM-1**		Number of Individuals	*AAT-1**		*PGM-1**	
		*q*a*	*q*b*	*q*a*	*q*b*		*q*a*	*q*b*	*q*a*	*q*b*
I	105	0.257	0.743	0.779	0.221	105	0.355	0.645	0.710	0.290
II	150	0.233	0.767	0.770	0.230	150	0.317	0.683	0.707	0.293
III	60	0.275	0.725	0.783	0.217	60	0.283	0.717	0.725	0.275
IV	60	0.233	0.767	0.717	0.283	60	0.333	0.667	0.767	0.233

the gene frequency at several sub-populations obtained from the same original population.

For the first experiment, the experimental populations I and II, which have the same population size as mentioned above, were used. Sampling was done randomly, four times at intervals of 90 days, which is the generation time of the guppy, and the gene frequency compared between each sampling time.

In the second experiment, 16 sub-populations were prepared from the original population, experimental population III. From the original population, one female which was corssed to one male was collected and an offspring taken. From this offspring, a sub-population was prepared in 60 *l* aquarium. So, each sub-population was made from one pair.

After more four generations have passed and the number of individuals increased to more than 500, including 200–300 adult fishes, sampling was made in a random fashion.

Results

Gene frequency throughtout generations in a closed colony.

The gene frequency of *AAT-1** and *PGM-1** at each sampling time were shown in Table 1. In *AAT-1** locus, gene frequency of **a* allele fluctuated 0.233 to 0.275 in experimental population I, and 0.283 to 0.355 in experimental population II. Between each combination of each sampling time, no significant differences were observed. In *PGM-1** locus, gene frequency of **a* allele fluctuated 0.717 to 0.783 and 0.707 to 0.767. In this locus, no significant differences were observed between each conbination of sampling time. In each locus, the observed number of individuals at each genotype well agreed with the expected number calculated for Hardy-Weinberg equilibrium. Therefore, an increase of the inbreeding coefficient was not detected. These results suggest that the population in which the number of individuals were maintained around 500, including 200–300 adult fishes, keeps the gene frequency constant from one generation to another.

Distribution of gene frequency in sub-populations.

The gene frequency of each sub-population is shown in Table 2.

In the *AAT-1** locus, gene frequency of **a* allele was distributed 0.125 to 1.000, against the gene frequency of the original population was 0.581. The result of the T-test, reveals the difference of gene frequencies, 11 out of 16 sub-populations indicated a significant difference compared with the original population. Gene frequency of **a* and **b* allele, calculated from the total number of individuals was 0.561 and 0.439, respectively. These values were influenced by the number of individuals at each sub-population. However, there were no significant differences of gene frequencies compared to the original population. Nonweighted values, calculated from the overall mean of 16 subpopulations was 0.566 and 0.434, respectively. These values were very near to the gene frequency of the original population.

In the *PGM-1** locus, the gene frequency of **a* allele was distributed 0.131 to 0.917, against the gene frequency of the original population was 0.605. In the results of the T-test, 8 out of 16 sub-populations indicated a significant difference of allele frequencies with the original population. The range of gene frequencies at each sub-population was more than nine times of the standard error of the original population. Gene frequencies of **a* and **b* allele calculated from a total number of individuals were 0.633 and 0.367, respectively. There were no significant differences compared to the original population. The gene frequency calculated from the overall mean of 16 subpopulations was 0.644 and 0.356, respectively.

Table 2. Gene frequency of the original population and each sub-populations

	*AAT-1**			*PGM-1**		
	Number of Individuals	Gene Frequency		Number of Individuals	Gene Frequency	
		*q*a*	*q*b*		*q*a*	*q*b*
Original Population	62	0.581	0.419	62	0.605	0.395
S- 1	96	0.521	0.479	96	0.677	0.323
S- 2	61	0.738	0.262*	60	0.600	0.400
S- 3	61	0.377	0.623*	61	0.131	0.869*
S- 4	41	0.915	0.085*	41	0.939	0.061*
S- 5	138	0.645	0.355	138	0.638	0.362
S- 6	21	0.571	0.429	21	0.548	0.452
S- 7	28	0.946	0.054*	30	0.833	0.167*
S- 8	23	0.174	0.826*	22	0.341	0.659*
S- 9	30	0.617	0.383	30	0.500	0.500
S-10	25	0.820	0.180*	25	0.640	0.360
S-11	30	0.667	0.333	30	0.917	0.083*
S-12	48	0.219	0.709*	48	0.531	0.469
S-13	37	1.000	0 *	37	0.838	0.162*
S-14	44	0.250	0.750*	34	0.853	0.147*
S-15	48	0.375	0.625*	50	0.740	0.260*
S-16	46	0.152	0.848*	49	0.582	0.418
Total	767	0.561	0.439	772	0.633	0.367
Overall Medn		0.566	0.434		0.644	0.356

* indicates the sub-population which shows significant differences between the original population at each locus.

Table 3. Expected number of populations which calculated by the probability of the genotype combination at the original population, and the observed number of populations at sub-populations

Locus	Combination of Genotypes of Parents at Original Population	Probability of Combination of the Parents	Expected Number of Sub-populations	Observed Number of Sub-populations	
*AAT-1**	*a/a*×*a/a*	0.073	1.2	1	
	a/a×*a/b*, *a/a*×*b/b* *b/b*×*a/b*	0.914	14.6	15	
	b/b×*b/b*	0.013	0.2	0	$\chi^2=0.244$
*PGM-1**	*a/a*×*a/a*	0.149	2.4	0	
	a/a×*a/b*, *a/a*×*b/b* *b/b*×*a/b*	0.826	13.2	16	
	b/b×*b/b*	0.025	0.4	0	$\chi^2=3.394$

The number of sub-populations which indicated significant differences between the original population were 11 in *AAT-1**, and 8 in *PGM-1**. The number of sub-populations which indicated significant differences in both loci were 7, and significant differences only at one locus were 5.

These sub-populations are progenies of the parents, or their offspring which could spawn or survive. It is needed to determine if selection exists on these processes. For the purpose of determining the existence of selection, the observed number of populations in which the allele fixed or lost at each locus was counted and compared with expected number of populations (Table 3). The expected number was calculated from the genotype frequency of the original population. For example, the probability in which the genotype of both parents of a sub-population is *AAT-1***a/a* is 0.073 in *AAT-1** locus, and in this subpopulation **a* allele in *AAT-1** locus will be fixed. Such subpopulation is expected 1.2 out of 16 sub-populations, against observed number of 1. In this way, other genotypes were compared at *AAT-1** and *PGM-1** locus, respectively. There are no significant differences between expected and observed numbers of sub-population. Thus,

Table 4. The number of combinations which indicate significant differences between the original population at each number of sub-populations to combine

Number of Subpopulation to Combine	Number of Combinations	Number of Combinations Which Indicate Significant Differences With The Original Population	
		*AAT-1**	*PGM-1**
1	16	11	8
2	120	64	58
3	560	234	232
4	1820	384	263
5	4368	1265	1231
6	8008	1870	1759
7	11440	2078	1936
8	57915	9444	6571
9	11440	1119	1166
10	8008	513	634
11	4368	162	234
12	1820	33	60
13	560	2	12
14	120	0	0
15	16	0	0
16	1	0	0

it can be said that fluctuation occurred due to the sampling error of the parents.

Discussion

It was confirmed that the sampling error during the culture leads to the change of gene frequency from an original population. This fact is important for the management of cultured populations, especially for the population which is maintained as a gene bank or a special strain.

The number of pairs which are needed to maintain the gene frequency of an original population can be calculated from the data which were obtained in this study (Table 4). One sub-population is a progeny of one pair, thus, if we want to obtain the gene frequency of population which started from the progeny of two pairs, it will be obtained from the total data of two sub-populations. 120 combinations can be made from 16 sub-populations. 64 out of 120 combinations indicated significant differences between original population at the *AAT-1** locus. On the other hand, 58 out of 120 combinations indicated significant differences between the original population at the *PGM-1** locus. And so on, in each combination in each number of sub-population to combine, differences of gene frequencies between each combination and original populations were tested. When the number of sub-populations to combine was 14, there was no combination which indicated significant differences between the original population at *AAT-1** and *PGM-1**. It suggests that at least 14 pairs, 14 females and 14 males, are needed to mainatain the same gene frequency level of the original population in regard to two isozymic loci.

In recent studies, the number of individuals needed to mainatin the population were simulated, according to it, a founding population of, at least, 25 females and 25 males, is a reasonable absolute minimum.[9,12,13] The number of pairs calculated in this study is less than in recent studies, because this study did not refer to genetic variability. However, polymorphism at two loci was maintaned more than theory in experimental sub-populations. There are several reports which suggest the relationship between genotype and quantiative characters.[14,15] It is necessary to investigate the change of genotype or genetic variability in other quantitative characters.

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