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学 位 論 文 題 目　　　Studies on Genetic Changes Occurred During Pre-
　　　　　　　　　　servation of Strains in the Guppy, *Poecilia reticulata*
　　　　　　　　　　（グッピーの継代飼育系統における遺伝的変化）

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Introduction

The conservation of genetic variation is a component of many species management programs. To manage any biological resource effectively, researchers must identify the level of genetic variation within and among populations. In general, several conditions are needed for constant gene frequencies and genetic variability in a population: random mating, large population size, no migration, no mutation, natural selection should not affect locus under consideration. However, always there are some processes which change the gene frequencies in the population. For the detailed investigations of causes which lead to the genetic changes in the populations, cultured populations should be used. Comparison of the genetic variability and differentiation between cultured and natural populations of the same species could provide valuable opportunities to explore the influence of artificial selection on a population. Loss of genetic variability and increase of genetic differences were observed in the comparison of cultured stocks with the natural populations in cutthroat trout, masu salmon, black rockfish, rainbow trout and Pacific herring. In each case, the genetic drift and/or founder effect were assumed as a cause of the changes and because the initial stock size and the number of individuals involved in reproduction in each stock were usually small compared with that of the natural populations.

The guppy, *Poecilia reticulata*, is one of the useful model for study of genetics because of its short life cycle, large reproductive capacity, and ease of breeding as an aquarium fish. The guppy is native to the coastal streams and rivers of Venezuela, Guyana, Surinam, Barbados, Trinidad, Tobago and parts of Brazil. It has been the focus of much evolutionary interest and as models of theoretical interest. By aquarists many varieties of the guppy were created, differing in body color patterns, in shape and color of fins and tail in adult guppy males. Now, many cultured varieties are created and cultivated in Japan and also introduced from foreign countries into Japan. Furthermore, some guppies adapted to the various natural locations in Japan, such as streams and ponds in the tropical zone or near of hot springs. The varieties, which were created based on morphological differences, also have genetic differences detected by biochemical genetic markers and some physiological traits. For the population genetic analysis the enzymes detected by electrophoresis should be selected as markers. Allozyme electrophoresis permit quantitative estimates of the amount and distributions of genetic variation between and among populations.

The aim of this work is the analysis of genetic changes and their main causes during the strain preservation in the guppy, *Poecilia reticulata*, as a fish model, by allozyme electrophoresis. An investigation of the changes it should be done

because the strains are the genetic resources by themselves and maintaining of the variability of the strain is an important task for the successful management program. For this purpose, the investigation was divided into five parts.

1. The mode of inheritance of isozyme genes in the guppy.

The mode of inheritance of enzyme genes, which were used for the study was investigated. It was done in order to select the enzymes, which could be easily used as markers for the study of genetic features of the guppy. Using the enzyme loci, which located on the sex chromosomes, linked loci or loci which have null allele makes the calculation more complicated and could also lead to the incorrect results. Several cultured strains of the guppy, maintained as closed colonies in our laboratory, were used for the experiment. The 16 enzymes were used and variation was observed in 7 out of 16 enzymes. Total of 32 loci scored polymorphism was observed at the 8 loci, namely *Aat-1*, *Ak-2*, *Ck-1*, *Gpi-2*, *Mdh-4*, *Pgm-1*, *Pgm-2* and *Sod-2* (Table 1). It was confirmed again that the MDH enzyme variation is due to a null allele at the *Mdh-4* locus and it was assumed that the AK enzyme variation is also due to a null allele at the *Ak-2* locus. It was suggested do not use these enzymes for the future research. The results of cross experiments agreed with expected numbers at the *Aat-1*, *Ck-1*, *Gpi-2*, *Pgm-1*, *Pgm-2* and *Sod-2* loci, which suggested that these loci are under the Mendel's law. Same results were observed in reciprocal crosses at these loci, which suggested them to be located on autosomes. It was also indicated that inheritance of these polymorphic loci is independent of each other. The 6 polymorphic loci (*Aat-1*, *Ck-1*, *Gpi-2*, *Pgm-1*, *Pgm-2* and *Sod-2*) and 24 monomorphic loci were the loci which were selected as a markers for the investigation.

2. Genetic variability and genetic differentiation in the cultured strains in comparison with naturally propagated populations of the guppy.

The aim of this Chapter was to characterize genetically, in term of genetic variability and genetic differentiation, based on enzyme polymorphism cultured strains of the guppy in comparison with naturally propagated populations of the guppy of Japan. This comparison could be useful for an understanding of the influence of artificial selection on the strain creating and preservation. Out of 30 loci, 5 polymorphic loci were observed in the 13 cultured strains and 6 polymorphic loci were observed in the 10 naturally propagated populations. In spite of the fact that ranges of variability estimations in the cultured strains and in the naturally propagated populations showed overlapping, all average estimations of genetic variability in the 13 cultured strains were lower than those in the 10 naturally propagated populations (Table 2). It was suggested

that the changes were because of the founder effect and/or bottleneck, which occur during the strain creating. Variability of the Trinidadian wild populations of the guppy was at the similar level with that in the naturally propagated populations (Table 2). The results of this study showed that the cultured strains (G_{ST} was 0.263) were more diversified than the naturally propagated populations (G_{ST} was 0.146) and the degree of the genetic differentiation in the wild Trinidadian populations (G_{ST} was 0.400) also was significantly more than that in the naturally propagated populations, used in this study (Table 3). The remarkable differences between the naturally propagated populations and cultured strains, and between the wild and naturally propagated populations are considered to be the results of the influence of the different selection types and the introductions from the different origins.

3. Genetic changes occurred during the preservation of the strains of the guppy.

This Chapter concerned with genetic changes occurred during the strain preservation of the guppy. Investigation whether the decrease of genetic variability and increase of genetic differentiation in the cultured strains compare with those in the natural populations caused by founder effect and/or it was because of the long maintenance in the laboratory, genetic features of the cultured strains were investigated in term of time of strain preservation. The comparison of the genetic variability and genetic differentiation in the 6 old cultured strains (collection was made in 1993, when these strains have been maintained in our laboratory for about ten years), with those in the 7 new cultured strains (which have been maintained in our laboratory for two to three years when collection was made) could answer the question did the genetic features of cultured strains change during the maintenance or the present level of the genetic variability and differentiation was the same with that at the point of strain introduction. In spite of the fact that ranges of estimations of variability in the 6 old cultured strains and in the 7 new cultured strains showed overlapping, all average estimations of genetic variability in the new cultured strains were higher than those in the old cultured strains, however were lower than those in the naturally propagated populations (Table 4). Comparing of the genetic variability for the 6 old cultured strains (collection data of 1993), with that for the same 6 old cultured strains (collection data of 1987), which have been maintained in our laboratory for two years when collection was made (data was recalculated on the bases of allele frequencies at the 22 loci) showed lower estimations for the 6 old cultured strains, collection data of 1993 (Table 4). Negative correlation between heterozygosity and time of maintenance (r=-

0.546, n=13, P<0.10), was found by using data of the old cultured strains and new cultured strains, collection data of 1993 (Figure 1). This indicates that during the long strain preservation, genetic variability will be decrease, if during the maintenance it has been done nothing to increase the genetic variability. It could be explained by genetic drift, which was caused by decreasing of effective population size, which occur during the long maintenance of these strains in the laboratory. The G_{ST} estimation for the 6 old cultured was higher (G_{ST} was 0.310) than that for the 7 new cultured strains (G_{ST} was 0.122) (Table 5). In spite of the fact that G_{ST} estimations for the 6 old cultured, collection data of 1987 (G_{ST} was 0.328) and for the 6 old cultured strains, collection data of 1993 (G_{ST} was 0.325) did not change during maintenance (Table 5), the increasing of genetic differentiation for several cultured strains during the long preservation was found (r=0.866, n=6) (Figure 2).

4. Genetic variability in the 11 sub-strains originated from the one cultured strain of the guppy.

The information about initial number of individuals used for the strain creation and genetic changes occurred at the beginning of this process could be very useful for the breeding management and program of genetic conservation. In our laboratory the sub-division of the 11 sub-strains from the one cultured strain of the guppy was made experimentally by artificial selection. The aim of this Chapter was to analyze the genetic variability in the 11 sub-strains originated from the one cultured strain of the guppy. It was found that in the 11 sub-strains, at the three polymorphic loci, the allele frequencies of the each sub-strain fluctuated widely. 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 (Table 6). The average heterozygosities of each sub-strain fluctuated and were less or the same level than that of the original strain (Table 6). No correlation was observed between the time after subdivision and decrease of the genetic variability (Figure 3). It was shown that the establishment of the guppy sub-strain by small number of parents leads to the strong founder effect, which affects on the changes of the allele frequencies at the polymorphic loci of this sub-strain.

5. Genetic differentiation in the 11 sub-strains originated from the one cultured strain of the guppy.

Information about the level of the genetic differentiation in the populations which were made from the same origin could be useful for the breeding

management and program of genetic conservation. In this Chapter the genetic differentiation in sub-strains originated from the one cultured strain of the guppy and causes, which lead to the genetic changes in cultured strains were analyzed. G_{ST} between the S3 strain and the mean of 11 sub-strains was 0.083 and estimation among the 11 sub-strains estimation was 0.171 (Table 7). The H_T value for the S3 strain and the mean of 11 sub-strains was the same level with that for the 11 sub-strains, however the H_S value for the 11 sub-strains was lower, which lead to the larger G_{ST} value. This phenomenon indicates that the genetic constitution of the mean of the 11 sub-strains was not different from that of the original strain, however, gene frequencies in each sub-strain were widely fluctuated. G_{ST} among the 11 sub-strains was the same level with the estimation for the new cultured strains (Table 5). The establishment of the guppy strain by small number of parents leads to the strong founder effect, which affects on the changes of the allele frequencies at the polymorphic loci of this strain. In the case of establishment of the guppy strain by the large number of parents, during maintenance of this strain by genetic drift the changes of the allele frequencies at the polymorphic loci in the strain will occur.

Conclusion

At the present study it was suggested that a creating of the strain by a large number of parents leads to the founder effect, which influence on the genetic features of the strain would be smaller than that of the genetic drift during maintenance. On the other hand, it was shown that a creating of the strain by a small number of parents leads to the strong founder effect, which influence on the genetic features of the strain was suggested to be greater than that of the genetic drift which could occur during the preservation of the strain (Figure 4).

Stock enhancement programs can use principles of population genetics and animal husbandry to manage resources and maintain genetic variation, while conservation programs can use the principles to evaluate and maintain genetic diversity of wild stocks. It is often difficult to acquire a large number of individuals to found a new hatchery population, but to ignore the expected reduction in genetic variation due to small population size is to increase the probability that the hatchery program will fail to achieve its objectives. Restriction of population size and genetic drift result in loss of genetic diversity and fixation of undesirable genes within stocks, leading to increased inbreeding levels. To improve the gene pool and minimize the undesirable effects of inbreeding, fish farmers must understand the importance of practicing genetic broodstock management.

Table 1. Full names, abbreviation, E.C. numbers and genetic variation of enzymes used as markers in the study.

Enzymes	Abbreviation	E.C. number	Loci	Variation
Aspartate Aminotransferase	(AAT)	2.6.1.1.	*Aat-1*	P
			Aat-2, Aat-3	M
Adenylate Kinase	(AK)	2.7.4.3.	*Ak-1*	M
			Ak-2	P*
Creatine Kinase	(CK)	2.7.3.2.	*Ck-1*	P
			Ck-2	M
Fumarase	(FH)	4.2.1.2.	*Fh*	M
α-Glycerophosphate Dehydrogenase	(αGPD)	1.1.1.8.	*αGpd-1*	M
			αGpd-2	M
Glucosephosphate Isomerase	(GPI)	5.3.1.9.	*Gpi-1*	M
			Gpi-2	P
Isocitrate Dehydrogenase	(IDH)	1.1.1.42.	*Idh-1*	M
			Igh-2	M
Lactate Dehydrogenase	(LDH)	1.1.1.27.	*Ldh-1*	M
			Ldh-2, Ldh-3	M
Malate Dehydrogenase	(MDH)	1.1.1.37.	*Mdh-1, Mdh-2*	M
			Mdh-3	M
			Mdh-4	P*
Malic Enzyme	(ME)	1.1.1.40.	*Me*	M
Mannosephosphate Isomerase	(MPI)	5.3.1.8.	*Mpi*	M
Octanol Dehydrogenase	(ODH)	1.1.1.73.	*Odh-1, Odh-2*	M
6-Phosphogluconate Dehydrogenase	(6PGD)	1.1.1.44.	*6Pgd*	M
Phosphoglucomutase	(PGM)	5.4.2.2.	*Pgm-1*	P
			Pgm-2	P
Sorbitol Dehydrogenase	(SDH)	1.1.1.14.	*Sdh-1, Sdh-2*	M
Superoxide Dismutase	(SOD)	1.15.1.1.	*Sod-1*	M
			Sod-2	P

* Locus was not used for the study

Table 2. Genetic variability in cultured strains, naturally propagated and wild populations of the guppy.

	Group	Number of strains or locations	No. of loci	P	V	P+V	A/L	H
Japan	Cultured strains	13	30	0.082 (0.033-0.100)	0.008 (0-0.033)	0.090 (0.067-0.133)	1.10 (1.07-1.13)	0.029 (0.006-0.043)
	Naturally propagated populations	10	30	0.133 (0.067-0.200)	0.010 (0-0.033)	0.143 (0.100-0.200)	1.16 (1.10-1.23)	0.041 (0.022-0.059)
Trinidad*	Wild populations	11	24	0.121 (0.042-0.167)	0.038 (0-0.083)	0.159 (0.083-0.208)	1.23 (1.13-1.33)	0.048 (0.018-0.083)

*Data from Carvalho et al. (1991), Shaw et al. (1991) and Shaw et al. (1992).
Range is enclosed in ( ).

Table 3. Genetic differentiation in cultured strains, naturally propagated and wild populations of the guppy.

	Group	Number of strains or locations	No. of loci	H_T	H_S	G_{ST}**
Japan						
	Cultured strains	13	30	0.038	0.028	0.263
	Naturally propagated populations	10	30	0.048	0.041	0.146
Trinidad*						
	Wild populations	11	24	0.080	0.048	0.400

*Data from Carvalho et al. (1991), Shaw et al. (1991) and Shaw et al. (1992).
** $G_{ST}=(H_T - H_S)/H_T$, where H_T represents the genetic diversity in the total population and H_S represents the average gene diversity within populations.

Table 4. Genetic variability in cultured strains and naturally propagated populations of the guppy.

Group	Collection date	Number of strains or locations	No. of loci	P	V	P+V	A/L	H
Old cultured strains	1993	6	30	0.067 (0.033-0.100)	0.006 (0-0.033)	0.073 (0.067-0.100)	1.08 (1.07-1.10)	0.020 (0.006-0.035)
	1993	6	22	0.091 (0.045-0.136)	0.008 (0-0.045)	0.099 (0.091-0.136)	1.09 (1.09-1.11)	0.027 (0.008-0.048)
	1987*	6	22	0.106 (0.091-0.181)	0.008 (0-0.045)	0.114 (0.091-0.181)	1.13 (1.09-1.23)	0.043 (0.033-0.061)
New cultured strains	1993, 1997	7	30	0.095 (0.067-0.100)	0.009 (0-0.033)	0.105 (0.067-0.133)	1.11 (1.07-1.13)	0.036 (0.023-0.043)
Naturally propagated populations	1993,1996 1997	10	30	0.133 (0.067-0.200)	0.010 (0-0.033)	0.143 (0.100-0.200)	1.16 (1.10-1.23)	0.041 (0.022-0.059)

*Data from Macaranas and Fujio (1990).
Range is enclosed in ( ).

Table 5. Genetic differentiation in cultured strains and naturally propagated populations of the guppy.

Group	Collection date	Number of strains or locations	No. of loci	H_T	H_S	G_{ST}**
Old cultured strains	1993	6	30	0.029	0.020	0.310
	1993	6	22	0.040	0.027	0.325
	1987*	6	22	0.064	0.043	0.328
New cultured strains	1993, 1997	7	30	0.041	0.036	0.122
Naturally propagated populations	1993,1996 1997	10	30	0.048	0.041	0.146

*Data from Macaranas and Fujio (1990).
** $G_{ST}=(H_T - H_S)/ H_T$, where H_T represents the genetic diversity in the total population and H_S represents the average gene diversity within populations.
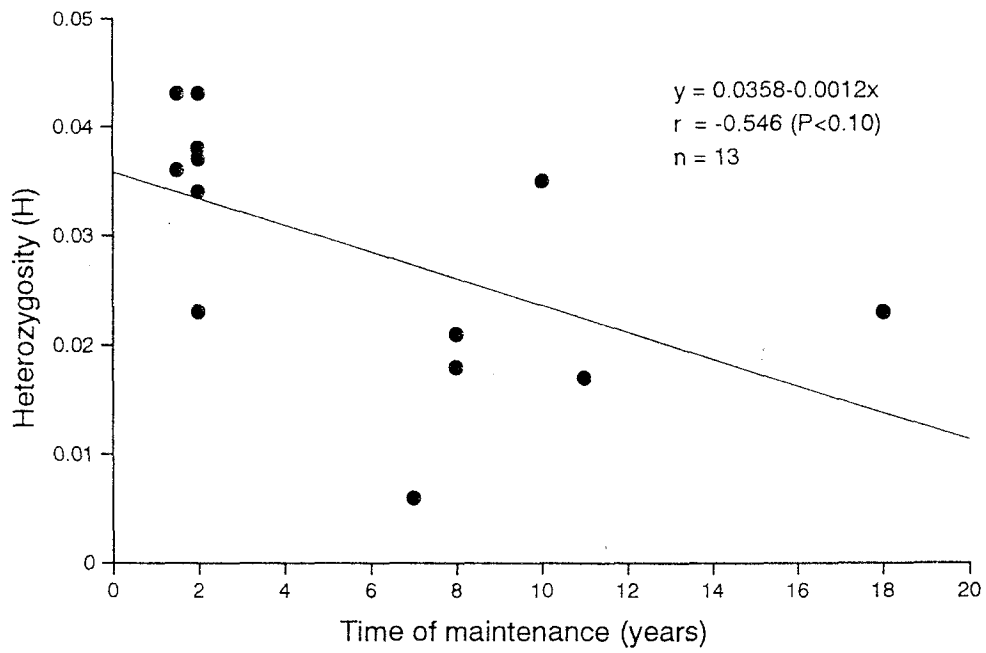
Fig. 1. Correlation between heterozygosity and time of maintenance
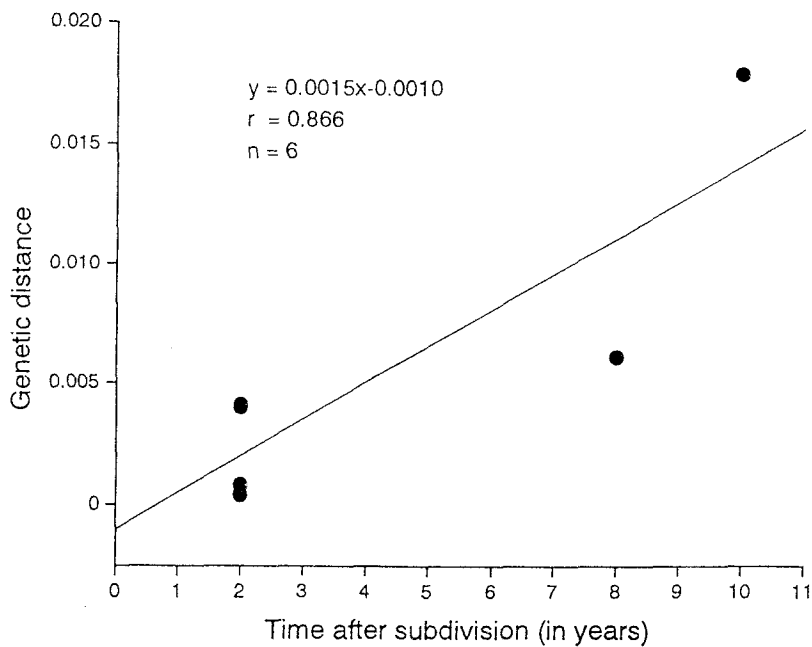in the 13 cultured strains of the guppy, *Poecilia reticulata.*

Fig. 2. Correlation between genetic distance (Nei, 1972) and time
after subdivision in the 9 cultured strains of the guppy,
Poecilia reticulata.

Table 6 Allele frequencies at 3 polymorphic loci in S3 strain and 11 sub-strains of the guppy.

LOCUS	Allele	S3 strain	Sub-strain										
			S3-20	S3-23	S3-26	S3-29	S3-SWR1	S3-SWR2	S3-SWS1	S3-SWS2	S3-SWSW	S3-SWFW	S3HL
Aat-1	A	0.395	0.190	0.600	0.297	0.889	0.551	0.510*	0.342	0.038	0.363	0.582	0.274
	B	0.605	0.810	0.400	0.703	0.111	0.449	0.490	0.658	0.962	0.637	0.418	0.726
	(N)	(119)	(145)	(209)	(96)	(54)	(157)	(50)	(139)	(39)	(102)	(67)	(53)
Pgm-1	A	0.528	0.654	0.336	0.840	0.231	0.661*	0.355	0.429	0.478	0.681	0.864	0.183
	B	0.472	0.346	0.664	0.160	0.769	0.339	0.645	0.571	0.522	0.319	0.136	0.817
	(N)	(127)	(149)	(216)	(97)	(54)	(158)	(55)	(140)	(45)	(105)	(70)	(63)
Pgm-2	A	0.906	0.842	0.998	1.000	0.833	0.984	0.900	1.000	1.000	0.938	1.000	0.857
	B	0.094	0.158	0.002	0	0.167	0.016	0.100	0	0	0.062	0	0.143
	(N)	(127)	(149)	(216)	(97)	(54)	(158)	(55)	(140)	(45)	(105)	(70)	(63)
H		0.038	0.034	0.031	0.023	0.028	0.032	0.038	0.031	0.019	0.034	0.024	0.031

Thirty loci were scored; 27 were monomorphic for the same allele - Aat-2, Aat-3, Ak-1, Ck-1, Ck-2, Fh, αGpd-1, αGpd-2, Gpi-1, Gpi-2, Idh-1, Idh-2, Ldh-1, Ldh-2, Ldh-3, Mdh-1, Mdh-2, Mdh-3, Me, Mpi, Odh-1, Odh-2, 6Pgd, Sdh-1, Sdh-2, Sod-1, Sod-2.
(N) refers to the number of individuals used for experiment.
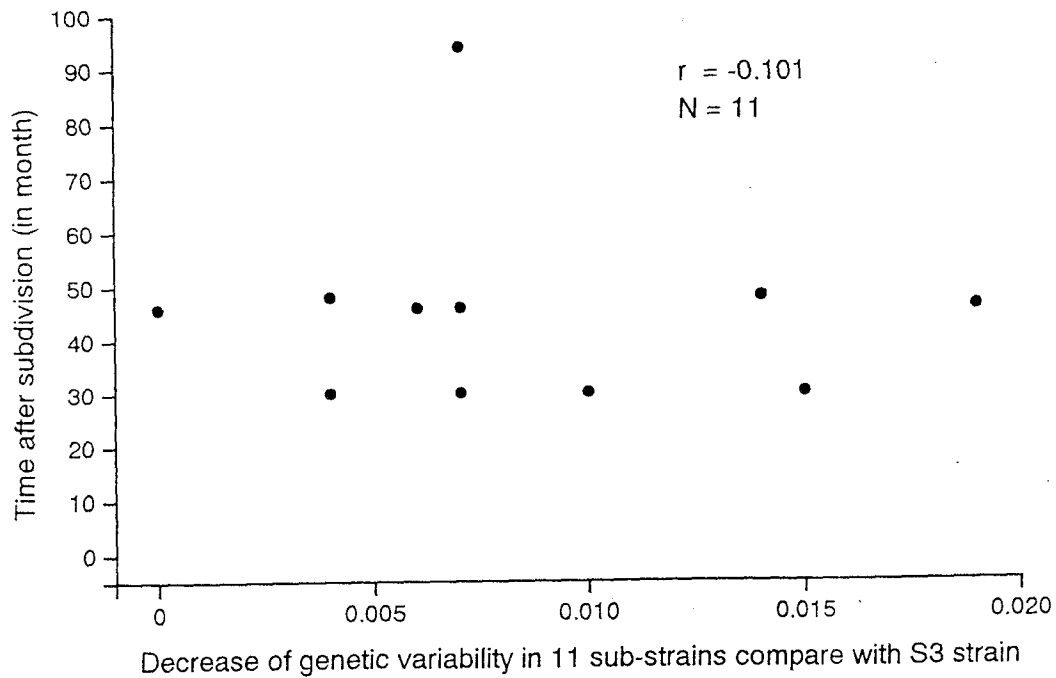* deviation from Hardy-Weinberg's expectations.

Fig. 3. Relationship between genetic variability and time after subdivision in the S3 strain and 11 sub-strains of the guppy, Poecilia reticulata.

Table 7. Genetic differentiation in 11 sub-strains originated from the one cultured strain in the guppy.

Group	Number of strains or sub-strains	H_T	H_S	G_{ST}^*
S3 strain and 11 sub-strains	2	0.036	0.033	0.083
11 sub-strains produced from S3 strain	11	0.035	0.029	0.171

* $G_{ST}=(H_T - H_S)/ H_T$, where H_T represents the genetic diversity in the total population and H_S represents the average gene diversity within populations.

Conclusion:

Decrease of genetic variability and increase of genetic differentiation during the long preservation were found for cultured strains of the guppy. Genetic changes occurred during the preservation of cultured strains were suggested to be caused by founder effect and/or genetic drift.
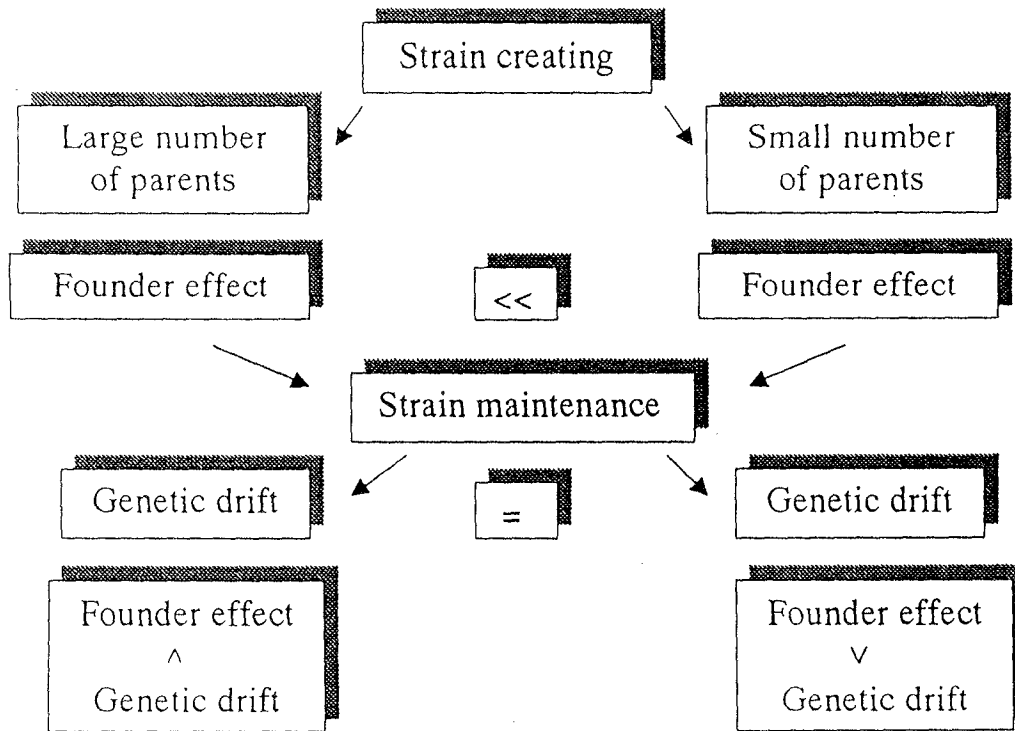

Fig. 4. Conclusion of the studies on genetic changes occurred during preservation of strains in the guppy, *Poecilia reticulata*.

論 文 審 査 結 果 要 旨

現在，様々な魚介類が養殖化され，生涯を人為管理下におかれるものも存在するようになった。人為管理下では再生産に用いる親魚数が減少するため遺伝的多様性が低下する事が指摘されているが，実験集団を用いての遺伝的変化とその要因を実証する試みはなされていない。本研究は，魚介類養殖における継代飼育にともなう遺伝的多様性低下現象を念頭において，グッピーをモデル魚としてそれらの系統維持過程での遺伝的変化とその要因を明らかにすることを目的としている。

第一章では，本研究で遺伝マーカーとして用いる，アイソザイムの遺伝様式の検証を試みている。グッピーの16酵素を支配する30遺伝子座のうちの多型的6遺伝子座に関して交配実験を行い，いずれの遺伝子座もメンデルの法則から期待される期待値と観察値は一致しており，集団遺伝学的分析を行う上で有効マーカーであることを確認している。

第二章では，グッピーの継代飼育系統と自然繁殖集団の遺伝的多様性の比較研究を行っている。継代飼育系統の遺伝的変異性は平均ヘテロ接合体率，多型遺伝子座の割合などでみるかぎり，自然繁殖集団よりも低い値を示した。系統間および採集地間での遺伝的分化を比較すると継代飼育系統間の遺伝的分化の方が自然繁殖集団間よりも大きな値を示した。

第三章では，グッピーの継代飼育に伴う遺伝的変化を追跡研究している。研究室に導入された品種をクローズドコロニーとして継代飼育したところ，クローズドコロニーとして維持されている期間と遺伝的変異性との間には弱い負の相関が観察され，維持されている期間が長いほど遺伝的変異性が低下する傾向が見られ，系統間の遺伝的分化は分割されてからの時間が長いほど大きかった。

第四章ではグッピーの系統作成に伴う遺伝的変異性の低下を追跡研究している。一つの系統を起源として11の副次系統を作成し，遺伝的変異性の変化を調べ，それぞれの遺伝子頻度が元系統の遺伝子頻度を中心としてばらついていること，さらに，元集団と有意に異なる対立遺伝子頻度を持つ遺伝子座が観察された。しかし，全ての副次系統の遺伝子頻度を平均すると元系統の遺伝子頻度とほぼ同じ値となることが判明した。また，平均ヘテロ接合体率は全ての副次系統が元系統よりも低い値を示すとともに，創始者効果も観察された。

第五章では，グッピーの系統作成における遺伝的分化の要因について解析している。一系統を起源として副次系統を作成したところ，副次系統間の遺伝的距離は地方品種レベルの分化程度を示すケースが見られた。作成されてからの時間と副次系統間の遺伝的距離との関係を調べたところ有意な相関は観察されず，ここでも創始者効果が確認された。

継代飼育に際して，親魚数が少ない場合創始者効果が大きく影響し，時間とは無関係に大きな遺伝的変化が生じること，多くの親魚を用いる場合は時間の経過に伴いゆっくりとした変化が生じることが解明された。

以上の研究結果は，水産遺伝・育種学分野の学問的向上に寄与するだけでなく，水産増養殖における再生産に関する遺伝的管理技術の改善において極めて有用な情報を提供することは明らかである。よって，審査委員一同は本論文の著者を博士(農学)の学位を授与するに値するものと判定した。