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Fluctuating allelic equilibria in a hybrid swarm population of the cyprinids *Gnathopogon caerulescens* and *G. elongatus* in Lake Ono, Yamaguchi Prefecture, Japan

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Abstract : Allozyme allelic frequency fluctuations in a hybrid swarm population of *Gnathopogon caerulescens* and *G. elongatus* (Cyprinidae) in Lake Ono, Yamaguchi Prefecture, Japan, monitored for twenty years from 1988 through 2007, indicated a fluctuating hybrid swarm equilibrium. Although future difficulty in the elimination of introgressant alleles was indicated, the possibility of future extinctions of some alleles at particular loci through genetic drift was also suggested.

Key words : Cyprinidae, Lake Ono, transplantation, hybrid swarm, allozyme

Introduction

The pelagic cyprinid minnow *Gnathopogon caerulescens* (Sauvage) has been transplanted from Lake Biwa to many ponds and reservoirs over several decades because of its high commercial value[1]. Consequently, the species has colonized several freshwater systems[2]. In many cases, however, resulting progeny have appeared intermediate between *G. caerulescens* and a brooklet-dwelling relative *G. elongatus* (Temminck & Schlegel)[3]. Such an example occurs in Lake Ono, Yamaguchi Prefecture, a reservoir which appeared in 1950 following the construction of the Koto River Dam, and to which *G. caerulescens* was transplanted in 1953[4]. Present-day *Gnathopogon* fish in Lake Ono are intermediate morphologically and genetically between *G. caerulescens* and *G. elongatus*, being a hybrid swarm of the two species[5, 6].

Neither the pelagic *G. cearulescence* nor the brooklet-dwelling *G. elongatus* were native to the limited lacustrine environment of Lake Ono[5-7]. The eventual outcome of the hybrid swarm, therefore, may be extremely relevant to predictions of the result of artificial fish stocking in an exotic habitat; in particular, which genes of which species are eliminated, or whether or not gene elimination actually occurs.

Allozyme allelic frequency fluctuations in the hybrid swarm population of *Gnathopogon* in Lake Ono were monitored for twenty years from 1988 through 2007, and the findings discussed below.

Materials and Methods

A total of 875 hybrid *Gnathopogon* fish (Fig. 1) were collected from Lake Ono in April, May or September

Fig. 1. *Gnathopogon* from Lake Ono, 66.5 mm SL (standard length).

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from 1988 through 2007 (Table 1, Fig. 2). All samples were frozen immediately after collection for allozyme analysis. Samples taken in 1988, including *G. caerulescence* and *G. elongatus* from the Lake Biwa basin, were analyzed by Sakai et al.5). The 1989 sample was reported by Sakai & Yonehana6).

Small pieces of muscle and liver were removed from the frozen samples for allozyme analysis. Gene products of five allozyme or protein coding loci which were variable in the Lake Ono population5) were investigated by standard horizontal starch gel electrophoresis and zymogram methods8): Glycerol-6-phosphate isomerase (E.C. 5.3.1.9, *Gpi-1**, *Gpi-2**), phosphogluconate dehydrogenase (E.C. 1.1.1.44, *Pgdh**), phosphoglucomutase (E.C. 5.4.2.2, *Pgm**), and general protein (*Prot-2**). The number of analyzed loci varied from one to five in different years, although all five loci were analyzed in samples from 1988, 1992, 1999, 2001, 2003 and 2006.

Observed and expected heterozygosity (*Ho* and *He*) and allelic frequencies were calculated from observed genotypes. Hardy-Weinberg expectation was tested by chi-square test (X^2 test), summing minor genotypes to number five or more.

Allelic frequency fluctuations were also tested by X^2 test for independence between number of alleles and years over twenty years, summing minor alleles numbering fewer than five.

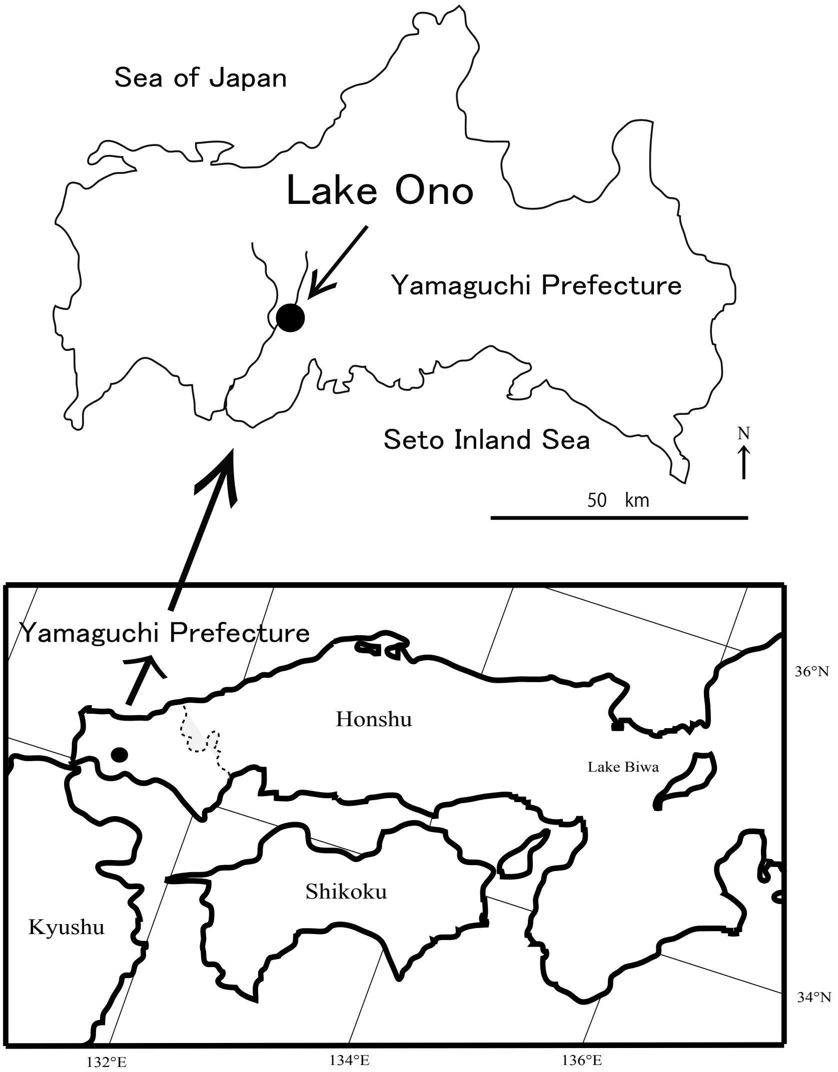

Fig. 2. Map of western Japan showing Lake Ono, a dammed reservoir on the Koto River system, Yamaguchi Prefecture.

Table 1. Allelic fequency at five loci in *Gnathopogon* fish from Lake Ono from 1988 through 2007 and *G. caerulescence* and *G. elongatus* from Lake Biwa in 1988, and results of X^2 test for Hardy-Weinberg equiribrium

Locus	Allele	Lake Biwa in 1988		*Gnathopogon* fish from Lake Ono																
		G. caerulescence	*G. elongatus*	1988	1989	1991	1992	1993	1994	1996	1997	1999	2000	2001	2002	2003	2004	2005	2006	2007
		N=100	78	90	200	39	31	44	50	20	45	45	39	35	32	50	47	31	48	29
*Pgm**	**a*	0.605	0.957	0.767	-	0.885	0.742	-	0.760	0.800	0.834	0.767	0.898	0.800	-	0.710	0.755	0.726	0.719	0.828
	**b*	0.215	0.006	0.155	-	0.102	0.129	-	0.150	0.100	0.089	0.144	0.038	0.071	-	0.180	0.202	0.081	0.104	0.069
	**c*	0.155	0.006	0.056	-	0.013	0.081	-	0.070	0.050	0.033	0.033	0.026	0.086	-	0.070	0.011	0.161	0.156	0.086
	**d*	0.025	0.000	0.022	-	0.000	0.048	-	0.020	0.050	0.044	0.056	0.038	0.043	-	0.040	0.032	0.032	0.021	0.017
	Ho	0.550	0.026	0.422	-	0.231	0.484	-	0.360	0.350	0.333	0.378	0.154	0.286	-	0.460	0.383	0.387	0.333	0.276
	He	0.563	0.084	0.384	-	0.206	0.424	-	0.395	0.345	0.293	0.387	0.190	0.346	-	0.457	0.388	0.439	0.447	0.302
	X^2 value	3.992	0.023	1.662	-	0.038	0.401	-	0.223	0.009	0.306	0.319	0.059	0.045	-	0.152	0.890	1.002	2.434	0.002
	p	0.407	0.880	0.645	-	0.846	0.818	-	0.894	0.926	0.858	0.853	0.808	0.833	-	0.927	0.641	0.606	0.487	0.968
*Gpi-1**	**a*	0.000	0.000	0.000	-	0.038	0.048	-	-	-	-	0.055	-	0.029	0.000	0.040	0.032	0.048	0.052	0.034
	**b*	0.930	0.904	0.867	-	0.808	0.790	-	-	-	-	0.878	-	0.900	0.875	0.790	0.851	0.790	0.677	0.742
	**c*	0.070	0.096	0.133	-	0.154	0.162	-	-	-	-	0.067	-	0.071	0.125	0.170	0.117	0.162	0.271	0.224
	Ho	0.140	0.167	0.200	-	0.385	0.387	-	-	-	-	0.244	-	0.200	0.250	0.300	0.319	0.355	0.333	0.448
	He	0.130	0.174	0.231	-	0.322	0.347	-	-	-	-	0.222	-	0.184	0.219	0.354	0.261	0.347	0.466	0.398
	X^2 value	0.021	0.008	0.101	-	0.255	0.022	-	-	-	-	0.062	-	0.030	0.044	0.450	0.957	0.022	3.202	0.139
	p	0.884	0.930	0.751	-	0.614	0.882	-	-	-	-	0.804	-	0.863	0.835	0.502	0.328	0.882	0.074	0.709
*Gpi-2**	**a*	0.005	0.006	0.039	-	-	0.048	-	-	-	-	0.044	-	0.014	0.047	0.050	0.011	0.048	0.021	0.069
	**b*	0.950	0.872	0.922	-	-	0.872	-	-	-	-	0.934	-	0.972	0.937	0.940	0.915	0.936	0.969	0.897
	**c*	0.045	0.122	0.028	-	-	0.048	-	-	-	-	0.022	-	0.014	0.016	0.010	0.074	0.016	0.010	0.017
	**d*	0.000	0.000	0.011	-	-	0.032	-	-	-	-	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	Ho	0.100	0.192	0.133	-	-	0.194	-	-	-	-	0.133	-	0.057	0.333	0.120	0.170	0.129	0.063	0.207
	He	0.095	0.225	0.147	-	-	0.234	-	-	-	-	0.125	-	0.055	0.120	0.114	0.157	0.121	0.060	0.190
	X^2 value	0.010	0.203	0.829	-	-	0.348	-	-	-	-	0.018	-	-**	-**	0.008	0.014	-**	-**	0.020
	p	0.919	0.652	0.376	-	-	0.555	-	-	-	-	0.893	-	-**	-**	0.930	0.906	-**	-**	0.889
*Pgdh**	**a*	0.048	0.009	0.000	-	-	0.016	0.000	0.000	0.000	0.000	0.011	0.000	0.000	-	0.000	-	-	0.000	-
	**b*	0.606	0.089	0.267	-	-	0.306	0.284	0.270	0.300	0.356	0.167	0.346	0.286	-	0.360	-	-	0.354	-
	**c*	0.346	0.830	0.733	-	-	0.678	0.716	0.730	0.700	0.644	0.822	0.654	0.643	-	0.600	-	-	0.636	-
	**d*	0.000	0.072	0.000	-	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	-	0.040	-	-	0.010	-
	Ho	0.519	0.304	0.422	-	-	0.419	0.341	0.420	0.600	0.444	0.311	0.487	0.514	-	0.560	-	-	0.521	-
	He	0.551	0.298	0.391	-	-	0.446	0.407	0.394	0.420	0.459	0.296	0.453	0.500	-	0.430	-	-	0.450	-
	X^2 value	0.003	0.109	0.569	-	-	0.064	0.178	0.039	2.660	0.038	0.151	0.203	0.493	-	0.511	-	-	0.511	-
	p	0.998	0.947	0.752	-	-	0.801	0.673	0.843	0.103	0.981	0.698	0.652	0.782	-	0.775	-	-	0.775	-
*Prot-2**	**a*	0.000	0.994	0.717	0.715	0.756	0.710	0.739	0.760	0.750	0.767	0.767	0.615	0.743	0.703	0.720	0.787	0.790	0.792	0.742
	**b*	1.000	0.006	0.283	0.285	0.244	0.290	0.261	0.240	0.250	0.233	0.233	0.385	0.257	0.297	0.280	0.213	0.210	0.208	0.258
	Ho	0.000	0.013	0.411	0.370	0.282	0.516	0.432	0.400	0.400	0.311	0.289	0.564	0.457	0.406	0.360	0.298	0.290	0.417	0.379
	He	0.000	0.012	0.406	0.408	0.369	0.412	0.386	0.365	0.375	0.357	0.357	0.474	0.382	0.418	0.403	0.335	0.332	0.329	0.383
	X^2 value	-**	-**	0.014	1.671	0.303	1.363	0.092	0.066	0.018	0.059	0.207	1.188	0.195	0.005	0.097	0.073	0.067	0.393	0.000
	p	-**	-**	0.993	0.434	0.582	0.243	0.762	0.797	0.892	0.807	0.650	0.276	0.659	0.944	0.756	0.787	0.795	0.531	1.000
Mean *Ho* at 5 loci		0.262	0.140	0.318			0.400					0.271		0.303		0.360			0.333	
Mean *He* at 5 loci		0.260	0.158	0.312			0.373					0.274		0.293		0.352			0.350	
Ho/He		1.008	0.886	1.019			1.072					0.989		1.034		1.023			0.951	

-: No data

-**: Sum of minor genotypes being less than five, X^2 test was not performed

Results and Discussion

Allelic frequencies, heterozygosities and results of chi-square tests for Hardy-Weinberg expectation are shown in Table 1. The number of observed genotypes at all loci of all samples showed insignificant departure from the Hardy-Weinberg equilibrium (p = 0.074-1.000). Although *Ho* and *He* varied with both years and loci, mean values at five loci were relatively stable, being higher than those of *G. caerulescence* and *G. elongatus*. *Ho/He* were around 1.000 (0.951-1.072). These results indicated that the Lake Ono *Gnathopogon* hybrid swarm had been a Mendelian population over the twenty year period.

Fluctuations in the number of alleles over twenty years are shown in Table 2. The independence test between number of alleles and years indicated that the former was relatively stable and independent from the latter for Pgm*, Gpi-2*, Pgdh* and Prot-2* (p = 0.070-0.680) except for Gpi-1*, in which allelic numbers differed significantly over time (p = 0.007), even after Bonferroni correction. Although the Prot-2* alleles are almost displaced between *G. caerulescence* (*a) and *G. elongatus* (*b) (Table 1), the *Gnathopogon* population in Lake Ono has been having both alleles stably for twenty years (p = 0.680, Table 2), strongly indicating the Lake Ono population is a hybrid swarm between the two species.

The overall results indicated that the *Gnathopogon* population in Lake Ono was in a rough hybrid swarm equilibrium, although the allelic frequency of each locus had been fluctuating to some extent. In particular, Gpi-1* exhibited significant fluctuations, possibly indicating that the effective population size of the hybrid swarm was not so large as to maintain a stable Mendelian population.

Judging from the allelic fluctuating extent of the Lake Ono population as discussed above, the *Gnathopogon* hybrid swarm is likely to continue in the lake, complete elimination of genes of one or other species being difficult. However, allelic frequencies at such loci as Gpi-1* have been fluctuating significantly, probably because of the relatively small effective population size. This indicates a possibility of future extinction of some alleles at particular loci through genetic drift, resulting in a reduction of genetic variability in the population.

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Table 2. Chi-squear test for independence between number of alleles (minor alleles less than 5 are summed) and years observed in samples of Gnathopogon fish from Lake Ono from 1988 through 2007

Locus	Allele	Number of alleles																	Independence test
		1988	1989	1991	1992	1993	1994	1996	1997	1999	2000	2001	2002	2003	2004	2005	2006	2007	Pearson's X^2
		180	400	78	62	88	100	40	90	90	78	70	64	100	94	62	96	58	p
Pgm*	*a	138	-	69	46	-	76	32	75	69	70	56	-	71	71	45	69	48	21.2
	*b+*c+*d	42	-	9	16	-	24	8	15	21	8	14	-	29	23	17	27	10	0.070
Gpi-1*	*a+*c	24	-	15	13	-	-	-	-	11	-	7	8	21	14	13	29	15	24.2
	*b	156	-	63	49	-	-	-	-	79	-	63	56	79	80	49	65	43	0.007**
Gpi-2*	*a+*c+*d	14	-	-	8	-	-	-	-	6	-	2	4	6	8	4	3	6	9.2
	*b	166	-	-	54	-	-	-	-	84	-	68	60	94	86	58	93	52	0.422
Pgdh*	*a+*b+*d	48	-	-	20	25	27	12	32	16	27	20	-	36	-	-	34	-	14.2
	*c	132	-	-	42	63	73	28	58	74	51	45	-	60	-	-	61	-	0.164
Prot-2*	*a	129	286	59	44	65	76	30	69	69	48	52	45	72	74	49	76	43	12.9
	*b	51	114	19	18	23	24	10	21	21	30	18	19	28	20	13	20	15	0.680

**: the number of alleles and years not independent (p<0.01: after Bonferroni collection).

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山口県小野ダム湖のホンモロコとタモロコの雑種群における浮動的な対立遺伝子平衡

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山口県小野ダム湖のホンモロコとタモロコの雑種群におけるアロザイム対立遺伝子頻度の変動を1988年から2007年の20年間にわたって追跡した結果，この雑種群は浮動的な平衡集団であることが推測された．一方の種の遺伝子が排除されることは困難ながら，特定の遺伝子座における対立遺伝子の削減の可能性が示唆された．