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Morphological and Genetic Variation in Japanese Populations of the Anemonefish *Amphiprion clarkii**

L. J. Bell [1, **], J. T. Moyer [1] and K. Numachi [2]

[1] **Tatsuo Tanaka Memorial Biological Station; Ako, Miyake-jima, Izu Islands 100-12, Japan**
[2] **Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo; Akahama, Otsuchi 028-11, Japan**

Abstract

Geographic populations of the anemonefish *Amphiprion clarkii* (Bennett) from 6 widely separated locations off the coast of southern Japan are morphologically different, exhibiting (1) latitudinal clinal patterns in color pattern and meristics and (2) degrees of genetic differentiation. Electrophoretic examination of 7 polymorphic loci (95% level) among 6 populations collected between July 1979 and January 1980 revealed an average genetic distance value of 0.008. Significance was demonstrated for 39 out of 105 (37%) tests of heterogeneity. Averaged over the 6 populations, the percentage of polymorphic loci ($\bar{P}_{0.95}$) was 24.6% and the proportion of heterozygous loci per individual ($\bar{H}$) was 0.0613. The results indicate that genetic clines are absent and suggest that the morphological variation may not be genetic. Relative isolation of genetic populations may be maintained by (1) localized larval dispersal resulting from a relatively short larval stage, and (2) current gyres tending to trap larvae, increasing the return of juveniles to their adult coastal habitat. Morphological clines may be due to clines in ecological parameters related to latitude.

Introduction

Fishes of the pomacentrid genus *Amphiprion,* known as anemonefish, are widely distributed, ranging from the Caroline Islands westward to the Red Sea (Allen, 1972). In his reviews of the genus, Allen (1972, 1980) used morphological similarities to designate 6 taxonomic complexes of closely related species. Geographically, color patterns within a species can be more variable than color patterns among species in a single complex. Probably the extreme example of such intraspecific color variation occurs in *A. clarkii* (Allen, 1972; Moyer, 1976), a characteristic which has doubtless contributed to the numerous synonyms for this species (Allen, 1972). Moyer (1976), for example, distinguished 4 different color "types" of *A. clarkii* from localities within Japan. Other distinct types have since been observed in Western Australia and Guam (Allen, 1980; Moyer, personal observation). The factors underlying this color variation have not been identified. Moyer (1976), in his original description of Japanese types, suggested that each might represent a partially isolated population. Subsequently, Moyer (1980) emphasized that ecological factors (possibly related to temperate waters) may contribute to color variation in *A. clarkii* from Miyake-jima, Japan. In view of this pronounced polymorphism observed among different localities of the Japanese *A. clarkii,* one can ask whether such variation has a genetic basis.

Considerable research has focused on the genetic basis of populations (see reviews by Ayala, 1976; Nevo, 1978). Although several population genetic studies have involved temperate and warm-temperate marine fishes (e.g. Utter, 1969; Utter and Hodgins, 1969; Utter *et al.,* 1970, 1973; Aspinwall, 1974; Okazaki, 1978; Smith *et al.,* 1978), similar studies of more tropical species are uncommon (e.g. Graves and Rosenblatt, 1980; Winans, 1980). Using the technique of starch-gel electrophoresis, we examined the extent of genetic differentiation among specimens of *Amphiprion clarkii* from 6 localities within Japan. The main objective was to determine if genetic differences exist between previously designated types of *A. clarkii.* A secondary objective was to compare the amount of genetic variability in this species with that of other fishes previously studied.

* Contribution No. 39 of the Tatsuo Tanaka Memorial Biological Station

** Present address: Mid-Pacific Research Laboratory, Enewetak, Marshall Islands, c/o Hawaii Institute of Marine Biology, Box 1346, Kaneohe, Hawaii 96744, USA

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Materials and Methods

Between July 1979 and January 1980, we collected a total of 171 specimens of *Amphiprion clarkii* (Bennett) off the coast of Southern Japan. The following numbers of individuals were collected at each of the following 6 locations: 31 from Miyake-jima (Izu Islands; 34°05′N,139°30′E), 37 from Sukumo (Shikoku; 32°52′N, 132°40′E), 33 from Kagoshima (Kyushu; 31°35′N, 130°34′E), 30 from Kakeroma (Amami Islands; 28°15′N, 129°20′E), 30 from South Okinawa (Ryukyu Islands; 26°05′N, 128°00′E), and 10 from Chichi-jima (Bonin Islands; 26°38′N, 142°09′E) (Fig. 1). All locations, except the Bonin Islands, are influenced by the Kuroshio Current, which moves northward along the southern coast of Japan. Approximately 1 000 km separate the most distant study sites.

The abundance of *Amphiprion clarkii* varied among study sites. In areas of low density, every fish greater than 40 mm standard length (SL) was collected, while in high-density areas collection was limited to the larger individuals (min. SL = 57 mm). Morphological data collected for each specimen included number of dorsal spines and rays, and number of pectoral rays, width of body bars at the widest point (expressed as percentage of standard length), detailed color descriptions, and standard length (SL). Sexual maturity was determined by characteristic adult color patterns (Moyer, 1976, 1980), and verified by visual inspection of the gonads. For data analysis we utilized fin-ray counts from all specimens, but we considered adults only for body bar width, color description and standard length.

Fish were transported live to the laboratory and then sacrificed. Liver and white muscle were dissected from each specimen and placed in liquid nitrogen. Samples were subsequently stored at −80 °C (for up to 6 mo) until use. Horizontal starch-gel electrophoresis was performed upon cell lysates produced by thawing small portions of each sample (procedures described by Numachi, 1971). Three buffer systems were used. The first buffer was TBE (tris, boric acid, EDTA), pH 8.7 (Kraus and Neely, 1964). The second buffer was CAEA (citric acid, *N*-(3-aminopropyl)diethanolamine), pH 7.0 (Clayton and Tretiak, 1972) with the following modifications: the gel buffer was 0.002 *M* citric acid adjusted to pH 7.0 with *N*-(3-aminopropyl)diethanolamine (Aldrich Chemical Co., Milwaukee, Wisconsin, USA) and the electrode buffer was 0.04 *M* citric acid adjusted to pH 7.0 with the same amine (Numachi *et al.*, 1979). The third buffer was discontinuous LiOH, pH 8.1/8.4, with the gel buffer diluted 1/5 (Selander *et al.*, 1971).

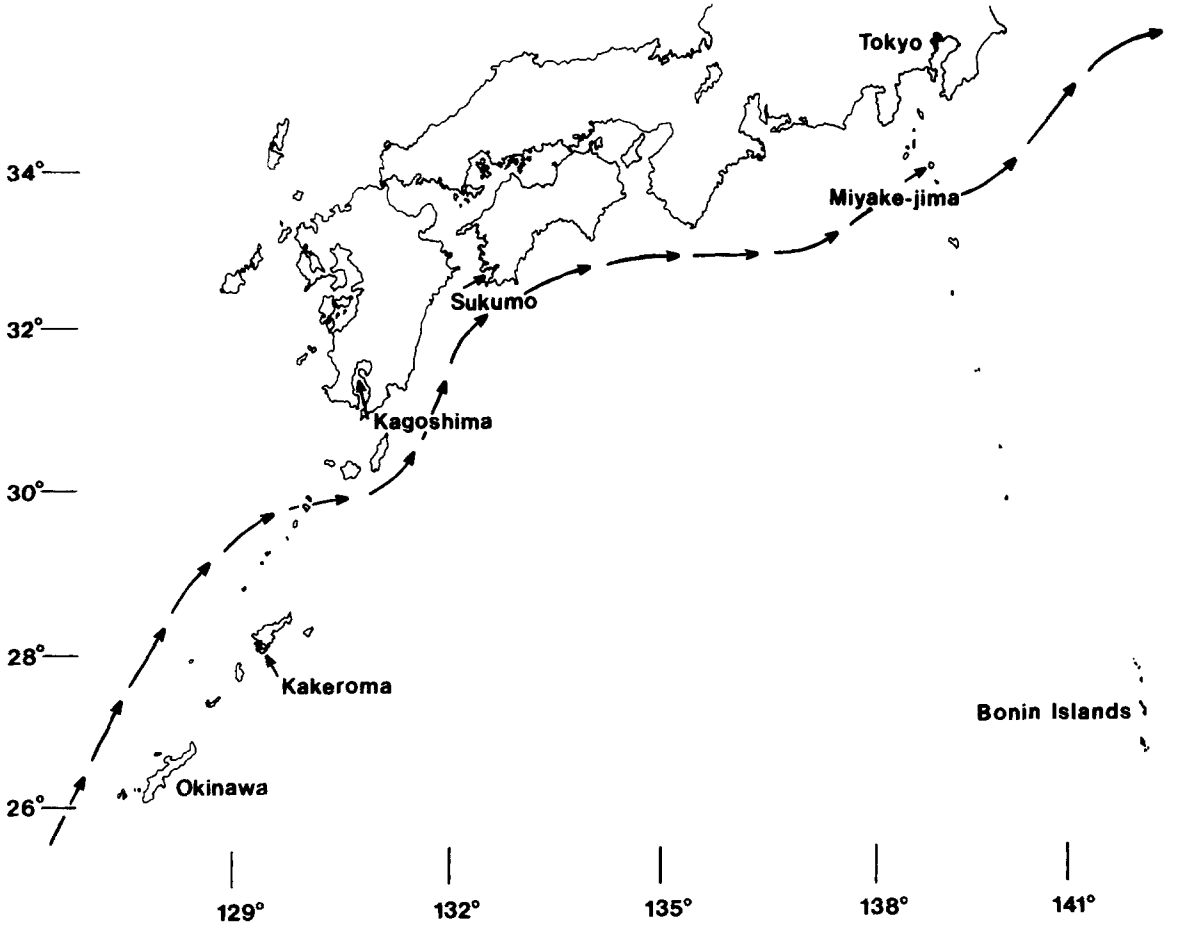

Fig. 1. Collection sites of *Amphiprion clarkii* off the coast of Japan. Arrowed line indicates axis of Kuroshio Current (modified from Wüst, 1936)

Table 1. *Amphiprion clarkii.* Enzyme systems, with corresponding tissues and buffers, analyzed for genetic variation in 6 populations from Japan. Enzyme activities are identified by capital letters, each representing a single locus. Mg + NAD = 0.01 *M* $MgCl_2$ and 0.38 *M* NAD added to CAEA gel

Enzyme	Tissue	Buffer	Enzyme system examined
Aldehyde oxidase (AO; EC 1.2.1.3)	muscle	CAEA	A, B
Aspartate amino transferase (AAT; EC 2.6.1.1)	muscle	LiOH	A
	liver	LiOH	B
	muscle/liver	CAEA	C
Esterase (EST; EC 3.1.1.1)	liver	LiOH	A
Hemoglobin (HB)	liver	TBE	A
Isocitrate dehydrogenase (IDH; EC 1.1.1.42)	muscle	CAEA	A
	liver	CAEA	B
Lactate dehydrogenase (LDH; EC 1.1.1.27)	muscle	CAEA	A
Malate dehydrogenase: NAD-dependent (MDH: NAD; EC 1.1.1.37)	muscle	CAEA	A, B, C
Malate dehydrogenase: NADP-dependent (MDH: NADP; EC 1.1.1.40)	liver	CAEA: Mg + NAD	A
	muscle	CAEA: Mg + NAD	B
Phosphoglucomutase (PGM; EC 2.7.5.1)	muscle/liver	CAEA	A, B
Phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44)	liver	CAEA	A
Sorbitol dehydrogenase (SDH; EC 1.1.1.14)	liver	CAEA: Mg + NAD	A
Superoxide dismutase (SOD; EC 1.15.1.1)	liver	TBE	A
Xylulose reductase: NADP-dependent (XRD: NADP; EC 1.1.1.10)	liver	LiOH	A

Twelve enzyme systems and hemoglobin were analyzed (tissues and buffer systems are shown in Table 1). Gels (12.5% Electrostarch, Lot 371) were run at a constant current for 2 to 5 h at 3.2 mA cm^{-2} for TBE and CAEA, and at 1.6 mA cm^{-2} for LiOH. Protein staining included aldehyde oxidase (AO), aspartate amino transferase (AAT), esterase (EST), hemoglobin (HB), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase: NAD-dependent (MDH: NAD), malate dehydrogenase: NADP-dependent (MDH: NADP), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (PGDH), sorbitol dehydrogenase (SDH), and superoxide dismutase (SOD). The foregoing staining methods were according to Shaw and Prasad (1970), Selander *et al.* (1971) and Taniguchi and Numachi (1978). Xylulose reductase: NADP-dependent (XRD: NADP) was stained with 50 ml of 0.1 *M* tris-hydrochloric acid buffer (pH 8.0), 150 mg xylitol, 15 mg NADP, 10 mg nitro-blue tetrazolium, and 1.5 mg phenazine methosulfate.

Enzyme activities are identified by capital letters, and loci are italicized. Bands representing alleles at individual loci are designated by small letters according to mobility ranking, starting at the most anodal band. Statistical procedures follow Sokal and Rohlf (1969).

The genetic data were analyzed for both intra- and inter-location variation. The percentage of loci which are polymorphic (*P*) and the proportion of loci which are heterozygous per individual (*H*) (Nei, 1978) were calculated for each area. These are indices of variation within each location and can be averaged to indicate overall variation for the species. Inter-location variation was analyzed using Nei's (1978) index of genetic distance. In addition, presumed genotype frequencies were compared pairwise between populations with respect to an expected single population's frequencies calculated according to a Hardy-Weinberg equilibrium. Due to the small sample size from the Bonin Islands ($N = 10$), calculations were performed both with and without this sample.

Results

Morphological Variation

Five variables show clinal variation in *Amphiprion clarkii* across the areas sampled: fin-ray counts, width of first and second body bars, overall body color and standard length, (Tables 2 and 3, Fig. 2).

Fin-ray counts varied clinally, decreasing from north to south (Table 2), whereas width of body bars showed significant variation between sites (for the first bar, $F = 32.8$, DF = 5, 138, $P < 0.001$; for the second bar, $F = 48.4$, DF = 5, 138, $P < 0.001$) increasing from north to south (Table 3). With respect to body-bar width, the Bonin Islands sample fits poorly in the clinal trends evident in the linear series of localities (Miyake-jima to Okinawa, see Fig. 1); the width

Table 2. *Amphiprion clarkii.* Fin-ray counts from 6 populations in Japan. Data collected from both immature and sexually mature adults

Location	Dorsal fin									Pectoral rays					
	Spines			Rays											
	IX	X	XI	15	16	17	18	19	$\bar{x}$	17	18	19	20	21	$\bar{x}$
Miyake-jima ($N=31$) 34°05′N; 139°30′E	1	28	2	1	6	18	5	1	16.9	0	0	13	18	0	19.6
Sukumo ($N=37$) 32°52′N; 132°40′E	1	35	1	2	11	24	0	0	16.6	0	2	21	14	0	19.3
Kagoshima ($N=33$) 31°35′N; 130°34′E	0	30	3	3	21	8	1	0	16.2	4	10	12	7	0	18.7
Kakeroma ($N=30$) 28°15′N; 129°20′E	2	27	1	6	17	7	1	0	16.6	0	1	10	17	2	19.7
Okinawa ($N=30$)[a] 26°05′N; 128°00′E	1	27	1	7	11	11	1	0	16.2	8	11	8	3	0	18.2
Bonin Islands ($N=10$) 26°38′N; 142°09′E	0	10	0	0	1	3	6	0	17.5	0	0	0	2	8	20.8

[a] Spines damaged on one individual

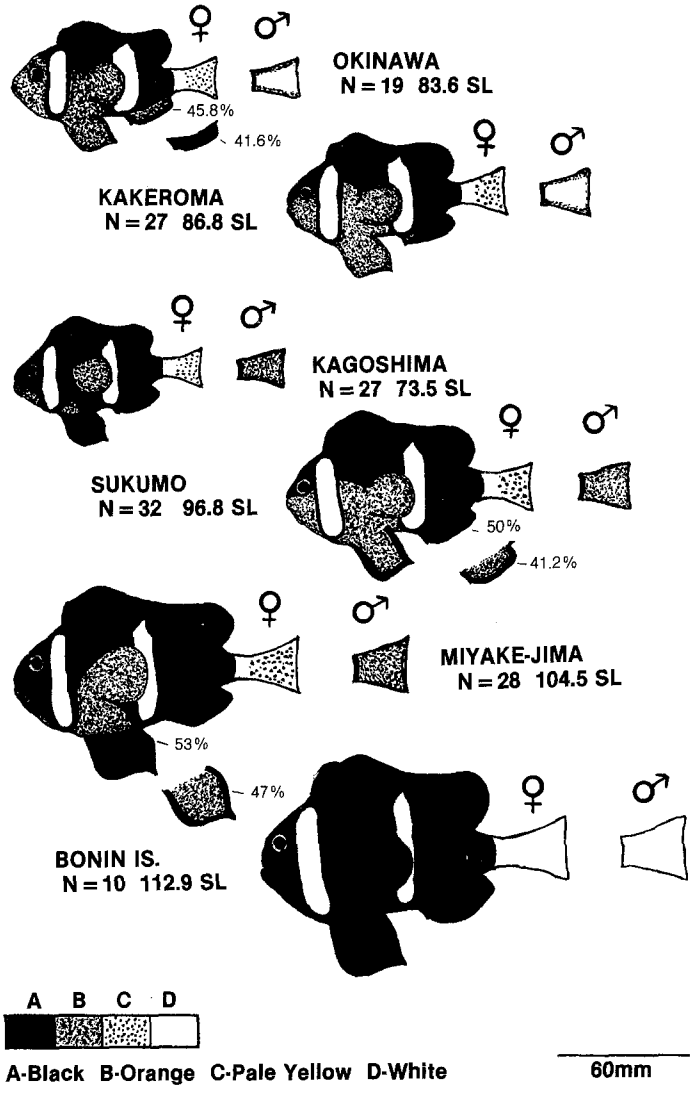

Fig. 2. *Amphiprion clarkii.* Mean standard length (SL) and typical color patterns of sexually mature fish from 6 populations in Japan

of the second bar appears to fit the cline while the first bar does not (Table 3). Except for one case, width of either bar did not correlate significantly with standard length (highest r value $= -0.345$, $P > 0.05$, Spearman rank) for any of the locations. The sole exception was the Miyake-jima fish, in which the width of the first body bar correlated with SL at $r = -0.382$ (DF $= 26$, $P = 0.05$).

Standard length of sexually mature adults decreases from the Bonin Islands and Miyake-jima to Okinawa with the exception of Kagoshima (Table 3, Fig. 2). It may be important that the latter sample comes from Kagoshima Bay, a relatively isolated body of water into which frequent expulsions of volcanic ash and gases come from Mount Sakura-jima, an active volcano in the middle of the bay. Comparison of standard lengths among all areas are sta-

Table 3. *Amphiprion clarkii.* Width of first and second body bars as percent of standard length (SL) and average SL in 6 populations from Japan, with corresponding standard deviations. Measurements were taken from sexually mature fish only

Location	Width of first bar (% of SL)	Width of second bar (% of SL)	Average SL (mm)
Miyake-jima ($N=28$) 34°05′N; 139°30′E	7.7±1.2	8.3±1.2	104.5±10.2
Sukumo ($N=32$) 32°52′N; 132°40′E	9.2±1.0	11.1±1.4	96.8± 9.4
Kagoshima ($N=27$) 31°35′N; 130°34′E	8.6±1.2	9.8±1.6	73.5± 7.7
Kakeroma ($N=27$) 28°15′N; 129°20′E	10.0±1.4	12.4±1.7	86.8± 7.7
Okinawa ($N=19$) 26°05′N; 128°00′E	11.4±1.2	14.4±1.8	83.6± 9.7
Bonin Islands ($N=10$) 26°38′N; 142°09′E	11.7±1.1	7.3±1.7	112.9±13.5

Table 4. *Amphiprion clarkii.* Allele frequencies of the 7 polymorphic loci, with corresponding standard deviations. *Aat:* aspartate amino transferase, *Pgm:* phosphoglucomutase, *Mdh: NADP:* NADP-dependent malate dehydrogenase, *Sdh:* sorbitol dehydrogenase, *Mdh: NAD:* NAD-dependent malate dehydrogenase, *Xrd: NADP:* NADP-dependent xylulose reductase. Alleles at individual loci are represented by small letters

Locus		Miyake-jima ($N=31$)	Sukumo ($N=37$)	Kagoshima ($N=33$)	Kakeroma ($N=30$)	Okinawa ($N=30$)	Bonin Islands ($N=10$)
Aat-B	a:	0.887 ±0.040	0.730 ±0.052	0.924 ±0.033	0.783 ±0.053	0.750 ±0.056	0.700 ±0.102
	b:	0.081 ±0.035	0.230 ±0.049	0.046 ±0.026	0.167 ±0.048	0.167 ±0.048	0
	c:	0.016 ±0.016	0	0.015 ±0.015	0.033 ±0.023	0.033 ±0.023	0.300 ±0.102
	d:	0.016 ±0.016	0.027 ±0.019	0.015 ±0.015	0.017 ±0.017	0.050 ±0.028	0
	e:	0	0.013 ±0.013	0	0	0	0
Pgm-A	a:	0.952 ±0.027	0.959 ±0.023	1.0	0.800 ±0.052	0.517 ±0.065	0.750 ±0.097
	b:	0.048 ±0.027	0.041 ±0.023	0	0.200 ±0.052	0.483 ±0.065	0.250 ±0.097
Pgm-B	a:	0.371 ±0.061	0.581 ±0.057	0.667 ±0.058	0.383 ±0.063	0.483 ±0.065	0.950 ±0.049
	b:	0.629 ±0.061	0.419 ±0.057	0.333 ±0.058	0.617 ±0.063	0.517 ±0.065	0.050 ±0.049
Mdh-A (NADP)	a:	0.887 ±0.040	0.892 ±0.036	0.894 ±0.038	0.983 ±0.017	1.0	1.0
	b:	0.113 ±0.040	0.108 ±0.036	0.106 ±0.038	0.017 ±0.017	0	0
Sdh-A	a:	0	0.014 ±0.014	0	0	0.017 ±0.017	0
	b:	0.935 ±0.031	0.959 ±0.023	1.0	1.0	0.917 ±0.036	1.0
	c:	0.065 ±0.031	0.027 ±0.019	0	0	0.067 ±0.032	0
Mdh-A (NAD)	a:	0.935 ±0.031	0.930 ±0.030	0.940 ±0.029	1.0	0.970 ±0.022	1.0
	b:	0.065 ±0.031	0.070 ±0.030	0.060 ±0.029	0	0.030 ±0.022	0
Xrd-A (NADP)	a:	0.935 ±0.031	0.973 ±0.019	1.0	1.0	0.965 ±0.024	1.0
	b:	0.065 ±0.031	0.027 ±0.019	0	0	0.035 ±0.024	0

tistically different ($P < 0.01$, Student's t-test), with two exceptions. Standard lengths of Kakeroma and Okinawa fish do not differ significantly ($P > 0.02$), and Miyake-jima and the Bonin Islands samples are marginally different from each other ($P = 0.05$).

Body color overall also tends to darken clinally, from south to north. Adults from the northern areas are conspicuously less orange than those from the south (Fig. 2). Mature fish from the Bonin Islands lack any orange and are therefore more similar to those from Miyake-jima with respect to this clinal morphological characteristic. Color of the caudal fin also varies among locations, and sometimes represents a sexually dichromatic characteristic (Moyer, 1976, 1980; and present Fig. 2). In fishes from the southernmost areas, Okinawa and Kakeroma, females have pale yellow tails, while males possess thin orange borders. Farther north, including Kagoshima, Sukumo and Miyake-jima, females and males have, respectively, pale yellow and completely orange tails. Finally, fishes in the Bonin Islands are sexually monochromatic, since individuals of both sexes have creamy white fins.

Genotypic Variation

From all 6 geographic locations, 21 presumed genetic loci were surveyed, of which 14 were monomorphic. Genetic interpretation of banding patterns was based on previous published data regarding enzyme subunit composition (Darnall and Klotz, 1972). Three enzymes and hemoglobin were represented by a single electrophoretic zone of ac-

Table 5. *Amphiprion clarkii.* Above diagonal: Nei's (1978) genetic distances for 6 populations from Japan, with standard error. Below diagonal: number of significantly different χ^2 tests of heterogeneity performed on the 7 polymorphic loci for each pair of the Japanese populations compared ($P \leq 0.05$)

	Miyake-jima	Sukumo	Kagoshima	Kakeroma	Okinawa	Bonin Islands
Miyake-jima		0.0015 ±0.0017	0.0032 ±0.0038	0.0006 ±0.0012	0.0092 ±0.0093	0.0199 ±0.0168
Sukumo	1/7		0.0006 ±0.0015	0.0022 ±0.0017	0.0090 ±0.0095	0.0102 ±0.0067
Kagoshima	3/7	1/7		0.0058 ±0.0038	0.0134 ±0.0114	0.0081 ±0.0045
Kakeroma	5/7	4/7	3/7		0.0025 ±0.0035	0.0156 ±0.0160
Okinawa	2/7	2/7	3/7	1/7		0.0128 ±0.0106
Bonin Islands	3/7	4/7	3/7	2/7	2/7	

tivity and thus interpreted as monomorphic loci: *Ldh, Pgdh,* and *Sod.* The remaining enzymes exhibited more than one zone of activity. Of them, the following 9 monomorphic loci appeared the same in all individuals surveyed: *Ao* (2), *Aat* (2), *Idh* (2), *Mdh: NAD* (2), and *Mdh: NADP* (1). Non-specific esterase was represented by multiple bands, of which the most anodal was well resolved; this band was interpreted as a single genetic locus. Variation at this locus was not observed.

Seven zones of activity were polymorphic among individuals, representing the 7 polymorphic loci:

Variants of *Aat-B,* specific in liver, were represented by three bands; 5 alleles were observed at this locus.

Heterozygotes in both *Pgm-A* and *-B* were double-banded, indicating a monomeric structure. Enzyme activity was equally distributed in muscle and liver; heteropolymers were not observed between these 2 loci.

Mdh-A: NADP, predominant in liver, showed 5-banded variants, indicating a tetrameric structure suggested for rats and hamsters (Li, 1972).

Sdh-A, specific to liver, exhibited 5-banded heterozygotes, indicating a tetrameric structure.

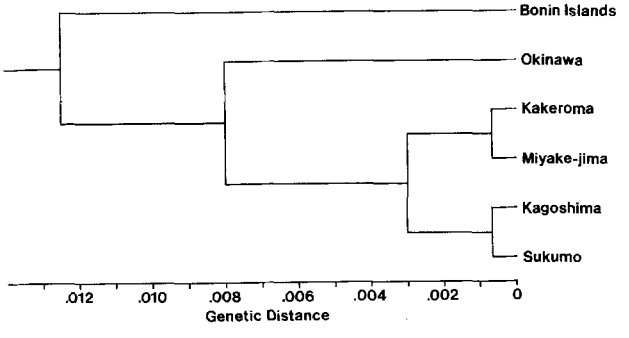

Fig. 3. *Amphiprion clarkii.* Dendogram (after Mountford, 1962) showing genetic relationships, using Nei's (1978) genetic distance statistic, *D*

Mdh-A: NAD, predominant in muscle, exhibited heterozygotes indicating a dimeric structure for the enzyme. Heterozygote variants produced 6 isozymes: 3 *Mdh-A* isozymes, 2 heteropolymers of the *Mdh-A* and *-B* subunits, and 1 homopolymer of *Mdh-B,* consistent with that found in other fishes, e.g. *Cololabis saira* and *Sebastobus macrochir* (Numachi, 1970, 1981).

Xrd-A: NADP, specific to liver, showed 5-banded heterozygotes, indicating a tetrameric structure. This appears to be the first electrophoretic demonstration of *Xrd: NADP* and its genetic variants.

Allele frequencies of the polymorphic loci are given in Table 4. A locus was considered polymorphic if the frequency of the most common allele was less than or equal to 0.95. Progeny testing or conformation of genetic hypotheses was not feasible. However, we believe our interpretations of banding patterns are correct, since phenotypic ratios did not differ significantly from those expected according to a Hardy-Weinberg equilibrium model.

Two methods were used to determine the extent of genetic variation in *Amphiprion clarkii* among study sites. First, genetic distances (D) were calculated following procedures in Nei (1978). The D values (Table 5) range from 0.0006 to 0.0199, averaging 0.008±0.006 (or 0.005±0.004 if one excludes the Bonin Island sample). A dendogram (Mountford, 1962) of the D values (Fig. 3) illustrates the apparent relationships among areas. The second analysis utilized a locus-by-locus comparison between locations. We performed 105 χ^2 tests of heterogeneity for the 7 polymorphic loci, of which 5.25 tests, on average, would be expected to be significant ($P = 0.05$) due to chance. In fact, 39 comparisons differed significantly ($P \leq 0.05$) (Table 5), a number significantly higher than expected ($P < 0.001$, based on a Poisson distribution). Although significant differences occurred at all loci, only 4 *(Aat-A, Pgm-A, Pgm-B,* and *Mdh-A: NADP)* accounted for 85% of the differences.

A summary of gene-frequency analyses for all localities is given in Table 6. Percentage of polymorphic loci (P)

Table 6. *Amphiprion clarkii.* Summary of genetic data for the 6 populations from Japan, including percent polymorphism ($P_{0.95}$) and average heterozygosity (H)

	Miyake-jima	Sukumo	Kagoshima	Kakeroma	Okinawa	Bonin Islands
$P_{0.95}$	33.3%	33.3%	19.1%	19.1%	28.6%	14.3%
$\bar{P}_{0.95}$	24.6%±8.17					
H	0.0654 ±0.026	0.0702 ±0.031	0.0437 ±0.024	0.0583 ±0.031	0.0830 ±0.038	0.0471 ±0.029
$\bar{H}$	0.0613±0.015					
No. of alleles per locus	1.43	1.48	1.29	1.29	1.43	1.24
Average no. of alleles per locus	1.36±0.098					

varied among locations, ranging from 14.3% in the Bonin Island fishes to 33.3% at Miyake-jima and Sukumo, with an average of 24.6%±8.2 (26.7%±7.2 excluding the Bonin Island sample). The proportion of heterozygous loci per individual (H) also varied, from 0.0437 in Kagoshima to 0.0830 in Okinawa, averaging 0.0613±0.015 (0.0641±0.014 excluding the Bonin Island sample). The average number of alleles per locus was 1.36±0.10. No obvious clines were observed for any of the genetic data.

Discussion

Numerous studies and reviews permit categorization of genetic distance values according to the phylogenetic relationships of the organisms being studied (Nei, 1975; Selander, 1976). More recently, Shaklee *et al.* (in press) reviewed the evolution of freshwater and marine fishes from the viewpoint of biochemical genetics and compiled genetic distance values for the population, species and generic levels. Overlap exists between adjacent levels, and they caution against rigid quantitative interpretation. The average D calculated, at the population level, was 0.05, with a range of 0.002 to 0.065 (a mean of 0.30 was calculated at the species level, with a range of 0.025 to 0.609) (Shaklee *et al.*, in press).

The average genetic distance among locations of *Amphiprion clarkii* is 0.008±0.006, including all samples (or 0.005±0.004 without the Bonin Island sample). Winans (1980) calculated a D value of 0.002 among populations of the tropical milkfish *Chanos chanos,* while Numachi (unpublished data) calculated an average of 0.007 among populations of the chum salmon *Oncorhynchus keta.* Genetic distance values of 0.002 to 0.013 for populations of the freshwater fish *Astynax mexicanus* were calculated by Avise and Selander (1972), and 0.024 for populations of sunfishes, *Lepomis* spp. (Avise and Smith, 1977). Interpopulation genetic distance in the *Amphiprion clarkii* examined are comparable to these studies and fall well within the proposed population level range (Shaklee *et al.*, in press).

Heterogeneity tests elucidated the probability that populations could be drawn at once from a single expected hypothetical population, based on Hardy-Weinberg equilibrium. Of the 105 heterogeneity tests performed among the Japanese *Amphiprion clarkii,* 37% were significantly different. Even so, there were no conspicuous patterns among these differences (see Table 5), since no given population appeared to be more different than the rest.

From the genetic distance and from the heterogeneity tests, one can suggest that individuals from the 6 locations are not genetically identical and, therefore, may not share a common gene pool. This, in turn, indicates the presence of distinct genetic populations. In particular, the Bonin Island population appears to differ more from the other 5 populations than these 5 differ among themselves (Table 5). The dendogram (Fig. 3) depicts this relationship more clearly. Geographically, the Bonin Islands are the most isolated. Bonin Island fishes are also the most different in morphology, lacking, for example, any orange coloration (Fig. 2). Despite the small sample size of this population, the data suggest that it is quite different morphologically and genetically from the other 5 populations. Fishes from these other 5 populations, however, evidently do not constitute a single homogeneous population. Tests of heterogeneity among these populations revealed 25 out of 70 (35%) tests to be significant. Genetic differences, consequently, suggest that gene flow among geographic areas is limited.

Possibly the Kuroshio, which runs northward along the coast of southern Japan, unidirectionally disperses larvae of *Amphiprion clarkii* and other fishes (Kuwamura, 1980), permitting some genetic exchange. However, studies of the abundance of larvae offshore suggest that long-range larval dispersal of many reef fish may not occur (Leis and Miller, 1976; Johannes, 1978). Larvae of demersal egg-layers (including pomacentrids) are most abundant on or just past the outer reef, but become progressively rarer with increasing distance from the reef (Leis and Miller, 1976; Johannes, 1978). In fact, Leis and Miller state that inshore reef species with non-pelagic eggs (including demersal spawners such as *A. clarkii*), in general, undergo direct

development of zygotes and settle out of the plankton at a relatively small size (< 10 mm). Allen (1972) reports a total length of 9.2 to 10.0 mm (7.0 to 7.8 mm SL) for *A. chrysopterus* (*clarkii* complex) larvae, corresponding to only 16 to 19 d after hatching. The smallest juveniles he found in the field occupying anemones ranged from 6.5 to 8.0 mm SL. One of us (JTM) has measured juveniles of *A. clarkii* in the field; total lengths ranged from 8.1 to 9.5 mm ($N=15$). In contrast, many inshore species with pelagic eggs have a longer larval stage, with total lengths sometimes reaching about 30 mm (e.g. *Chaetodon* spp., while still about 12 km offshore) (Leis and Miller, 1976). Relative to such fishes, the larval stage of at least some *Amphiprion* spp. is relatively short, and may thus minimize widespread dispersal.

Several such mechanisms, in fact, have been proposed. Ehrlich (1975) emphasized the unidirectional nature of oceanic currents (such as the Kuroshio), which presumably sweep larvae away from their place of spawning. At the same time, physical elements, such as temperature and density factors, may interact to create gyres (Jones, 1968). These gyres may trap larvae, increasing their chance to return to their adult coastal habitat (Sale, 1970; Johannes, 1978). Johannes and others (see Johannes, 1978) noted spawning aggregations of many species of fishes near seaward promontories, which tend to cause gyres off these points. Lobel (1978) presents data suggesting that the peak spawning season in Hawaii is correlated with the current shifts and formation of gyres, thus helping to maintain the influx of larvae to the Hawaiian Islands. Leis and Miller (1976) state that the retention of shorefish larvae around islands is essential to the maintenance of populations. Studies of larval dispersal in Hawaii (Watson and Leis, 1974; Leis and Miller, 1976) and Palau (Johannes, 1978) are consistent with a high degree of retention, a general principle that may also apply to the Japanese fauna. Larval dispersal localized to the island or location of spawning may well account for the genetic heterogeneity found among the Japanese populations of *Amphiprion clarkii*.

Nonetheless, some interaction among the populations clearly occurs. The close proximity of the 5 mainland populations (Miyake-jima, Sukumo, Kagoshima, Kakeroma and Okinawa) correlates with their relative similarity, suggesting a higher probability of genetic exchange, when they are compared to the Bonin Island population.

Variability, expressed as average heterozygosity ($\bar{H}$), in *Amphiprion clarkii*, is comparable to that of other reef fish (Table 7). An $\bar{H}$ of 0.061 ± 0.015 was calculated for the 6 Japanese populations of this species. Averaged estimates of $\bar{H}$ include 0.078 ± 0.012 for 14 species of fish (Selander, 1976), 0.0513 ± 0.034 for 51 species of bony fishes (Nevo, 1978) and, most recently, 0.0478 ± 0.033 for 82 species (Winans, 1980). The mean $\bar{H}$ for pomacentrids, including the present study, is 0.079 ± 0.022. It appears that the *A. clarkii* from Japan fall within the range of heterozygosities calculated for other fishes.

Clinal variation of morphological characteristics demonstrates several interesting trends. Fin ray counts and size, which generally increase with latitude (Seymour, 1959; Barlow, 1961), were observed by us to follow this trend (see Tables 2 and 3), if we exclude the Bonin Island population. Bonin Island fish tend to follow Miyake-jima fish with respect to these clines. Width of the second body bar shows an identical pattern. This peculiarity of the Bonin Island population, with respect to the other populations, is particularly surprising because the Bonin Islands are at the same latitude as Okinawa. It may be relevant that the

Table 7. Comparative values of average heterozygosity for 19 species of reef fish. nd: no data

Species	Family	*N*	Average heterozygosity	Source
Bathygobius andrei	Gobiidae (gobies)	24	0.039[a]	Gorman *et al.* (1976)
B. ramosus	Gobiidae	20	0.086[a]	Gorman *et al.* (1976)
B. soporator	Gobiidae	24	0.106[a]	Gorman *et al.* (1976)
Upeneus sp., *Mulloidichthys* spp., *Parupeneus* spp. (9 in total)	Mullidae (goatfish)	nd	0.021	Shaklee *et al.* (in press)
Hypoplecterus unicolor	Serranidae (groupers)	132	0.005	Graves and Rosenblatt (1980)
Abudefduf troschelli	Pomacentridae (damselfish)	16	0.050	Somero and Soulé (1974)
A. troschelli	Pomacentridae	23	0.070[a]	Gorman and Kim (1977)
A. saxatilis	Pomacentridae	26	0.095[a]	Gorman and Kim (1977)
Amphiprion clarkii	Pomacentridae	11	0.091	Somero and Soulé (1974)
A. clarkii	Pomacentridae	171	0.061	Present study
Dascyllus reticulatus	Pomacentridae	10	0.107	Somero and Soulé (1974)

[a] Calculated from data given in the respective studies, according to Nei (1978)

Bonin Islands lie on the oceanic border of the andesite line, whereas all other sites are on the continental side. The andesite line has previously been considered a significant faunal break for shallow-water fishes (Springer and Gomon, 1975; McKinney and Springer, 1976; Myers and Shepard, 1980). Clinal patterns observed for standard length must be interpreted with care; in addition to latitude, determinants of this characteristic are numerous (e.g. food abundance, growth rate, age).

The gradient in mean sea-surface temperature ranges from 20.9 °C at Miyake-jima to 25.0 °C at Okinawa and the Bonin Islands (Japan Kishocho, 1956–1970). As mentioned, the genetic data do not demonstrate the existence of genetic clines. Thus, gene frequencies appear to have no direct correlation with ocean temperature in this species. Also, the morphological clines seem independent of the observed gene frequencies. These observations may be interpreted in at least three ways. First, ecological parameters may be different for each population. Host anemones are more abundant in the more temperate waters surrounding mainland Japan than in the tropical areas of the Ryukyu and Bonin Islands (Moyer, 1980). It has further been suggested that the abundance of anemones limits the density of anemonefish (Allen, 1972). Social interactions would be more frequent in high-density locations, resulting in increased melanism as a means of displaying dominance, and sexual dichromatism (Moyer, 1976, 1980), as at Miyake-jima. Such variable morphological characteristics may therefore be determined principally by environmental, rather than genetic, factors. The second interpretation is that two genetic systems, regulatory and structural genes, may be acting independently. Clines due to structural genes would, therefore, not necessarily correlate with morphological clines. A third possibility is that morphological phenotypes observed may not be genetically determined, but may result from developmental responses to different environmental regimes. Fin-ray counts and standard length may be examples of such an ecophenotypic characteristic. Transplanting fish and cross-breeding among populations are necessary to delineate the extent of genetic heritability among these populations.

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Literature Cited

Allen, G. R.: The anemonefishes: their classification and biology, 288 pp. Neptune City, New Jersey: TFH Publications 1972

Allen, G. R.: Anemonefishes of the world, 103 pp. Mentor, Ohio: Aquarium Systems 1980

Aspinwall, N.: Genetic analysis of North American populations of the pink salmon, *Oncorhynchus gorbuscha,* possible evidence for the neutral mutation-random drift hypothesis. Evolution, Lawrence, Kansas *28,* 295–305 (1974)

Avise, J. C. and R. K. Selander: Evolutionary genetics of cave dwelling fishes of the genus *Astyanax.* Evolution, Lawrence, Kansas *26,* 1–19 (1972)

Avise, J. C. and M. H. Smith: Gene frequency comparisons between sunfish (Centrarchidae) populations at various stages of evolutionary divergence. Syst. Zool. *26,* 319–335 (1977)

Ayala, F. J., Ed.: Molecular evolution, 277 pp. Sunderland, Massachusetts: Sinauer Associates, Inc. 1976

Barlow, G. W.: Causes and significance of morphological variation in fishes. Syst. Zool. *10,* 105–117 (1961)

Clayton, J. W. and D. N. Tretiak: Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd Can. *29,* 1169–1172 (1972)

Darnall, D. W. and I. M. Klotz: Protein subunits: a table, rev. ed. Archs Biochem. Biophys. *149,* 1–14 (1972)

Ehrlich, P. R.: The population biology of coral reef fishes. A. Rev. Ecol. Syst. *6,* 211–247 (1975)

Gorman, G. C. and Y. J. Kim: Genotypic evolution in the face of phenotypic conservativeness: *Abudefduf* (Pomacentridae) from the Atlantic and Pacific sides of Panama. Copeia *1977,* 694–697 (1977)

Gorman, G. C., Y. J. Kim and R. Rubinoff: Genetic relationships of three species of *Bathygobius* from the Atlantic and Pacific sides of Panama. Copeia *1976,* 361–364 (1976)

Graves, J. E. and R. H. Rosenblatt: Genetic relationships of the color morphs of the serranid fish *Hypoplecterus unicolor.* Evolution, Lawrence, Kansas *34,* 240–245 (1980)

Japan Kishocho: Ten-day Marine Report, No. 335–873. Tokyo: Japan Meteorological Agency 1956–1970

Johannes, R. E.: Reproductive strategies of coastal marine fishes in the tropics. Envir. Biol. Fish. *3,* 65–84 (1978)

Jones, R. S.: Ecological relationships in Hawaiian and Johnston Island Acanthuridae (surgeonfishes). Micronesica *4,* 309–361 (1968)

Kraus, A. P. and C. L. Neely, Jr.: Human erthrocyte lactate dehydrogenase: four genetically determined variants. Science, N.Y. *145,* 595–597 (1964)

Kuwamura, T.: Seasonal occurrence of fishes at inshore rocky reefs in Shirahama, Southern Japan. Jap. J. Ichthyol. *27,* 243–248 (1980)

Leis, J. M. and J. M. Miller: Offshore distributional patterns of Hawaiian fish larvae. Mar. Biol. *36,* 359–367 (1976)

Li, J. J.: NADP-malic enzyme. *In vitro* interspecies hybridization of rat and hamster liver enzymes. Evidence for the isologous tetrameric structure. Archs Biochem. Biophys. *150,* 812–814 (1972)

Lobel, P. S.: Diel, lunar and seasonal periodicity in the reproductive behavior of the pomacanthid fish, *Centropyge potteri,* and some other reef fish in Hawaii. Pacif. Sci. *32,* 193–207 (1978)

McKinney, J. F. and V. G. Springer: Four new species of the fish genus *Ecsenius* with notes on other species of the genus (Blenniidae: Salariini). Smithson. Contr. Zool. *236,* 1–27 (1976)

Mountford, M. D.: An index of similarity and its application to classificatory problems. *In:* Progress in soil zoology, pp 43–50. Ed. by P. W. Murphy. London: Butterworths 1962

Moyer, J. T.: Geographical variation and social dominance in the Japanese populations of the anemonefish *Amphiprion clarkii*. Jap. J. Ichthyol. *23,* 12–22 (1976)

Moyer, J. T.: Influence of temperate waters on the behavior of the tropical anemonefish *Amphiprion clarkii* at Miyake-jima, Japan. Bull. mar. Sci. *30,* 261–272 (1980)

Myers, R. F. and J. W. Shepard: New records of fishes from Guam, with notes on the ichthyofauna of the Southern Marianas. Micronesica *16,* 306–337 (1980)

Nei, M.: Molecular population genetics and evolution, 288 pp. Amsterdam: North-Holland Publication Co. 1975

Nei, M.: Estimation of average heterozygosity and genetic distances from a small number of individuals. Genetics, Austin, Tex. *89,* 583–590 (1978)

Nevo, E.: Genetic variation in natural populations: patterns and theory. Theor. Popul. Biol. *13,* 121–177 (1978)

Numachi, K.: Polymorphism of malate dehydrogenase and genetic structure of juvenile populations in saury *Cololabis saira*. Bull. Jap. Soc. scient. Fish. *36,* 1235–1241 (1970)

Numachi, K.: Genetic polymorphism of α-glycerophosphate dehydrogenase in saury. *Cololabis saira* – I. Seven variant forms and genetic control. Bull. Jap. Soc. scient. Fish. *37,* 755–760 (1971)

Numachi, K.: A simple method for preservation and scanning of starch gels. Biochem. Genet. *19,* 233–236 (1981)

Numachi, K., S. Nagahora and M. Iwata: Genetic identification of hybrids between chum and pink salmon in the northwest Pacific. Rep. Otsuchi mar. Res. Cent., Iwate-ken, Japan (Ocean Res. Inst., Univ. Tokyo) *5,* 87–102 (1979)

Okazaki, T.: Genetic differences of two chum salmon (*Oncorhynchus keta*) populations returning to the Tokachi River. Bull. Far Seas Fish. Res. Lab., Japan *16,* 121–128 (1978)

Sale, P. F.: Distribution of larval Acanthuridae off Hawaii. Copeia *1970,* 765–766 (1970)

Selander, R. K.: Genic variation in natural populations. *In:* Molecular evolution, pp 21–45. Ed. by F. J. Ayala. Sunderland, Massachusetts: Sinauer Associates, Inc. 1976

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry: Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet., Austin, Tex. *6,* 49–90 (1971). (Univ. Tex. Publ. No. 7103)

Seymour, A.: Effects of temperature upon the formation of vertebrae and fin rays in young chinook salmon. Trans. Am. Fish. Soc. *88,* 58–69 (1959)

Shaklee, J. B., C. S. Tamaru and R. S. Waples: Speciation and evolution of marine fishes studied by the electrophoretic analysis of protein polymorphisms. Pacif. Sci. (In press)

Shaw, C. R. and R. Prasad: Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genet. *4,* 297–320 (1970)

Smith, P. J., R. I. C. C. Francis and L. J. Paul: Genetic variation and population structure of the New Zealand snapper. N.Z. Jl mar. Freshwat. Res. *12,* 343–350 (1978)

Sokal, R. R. and F. J. Rohlf: Biometry. The principles and practice of statistics in biological research, 776 pp. San Francisco: W. H. Freeman & Co. 1969

Somero, G. N. and M. Soulé: Genetic variation in marine fishes as a test of the niche-variation hypothesis. Nature, Lond. *249,* 670–672 (1974)

Springer, V. G. and M. F. Gomon: Revision of the blenniid fish genus *Omobranchus* with descriptions of three new species and notes on other species of the tribe Omobranchini. Smithson. Contr. Zool. *177,* 1–135 (1975)

Taniguchi, N. and K. Numachi: Genetic variation of 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, and glutamic-oxaloacetic transaminase in the liver of Japanese eel. Bull. Jap. Soc. scient. Fish. *44,* 1351–1355 (1978)

Utter, F. M.: Transferrin variants in Pacific hake (*Merluccius productus*). J. Fish. Res. Bd Can. *26,* 3268–3271 (1969)

Utter, F. M., F. W. Allendorf and H. O. Hodgins: Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Zool. *22,* 257–270 (1973)

Utter, F. M., W. E. Ames and H. O. Hodgins: Transferrin polymorphism in coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd Can. *27,* 2371–2373 (1970)

Utter, F. M. and H. O. Hodgins: Lactate dehydrogenase isozymes of Pacific hake (*Merluccius productus*). J. exp. Zool. *172,* 59–67 (1969)

Watson, W. and J. M. Leis: Ichthyoplankton of Kaneohe Bay, Hawaii: a one year study of fish eggs and larvae. Sea Grant tech. Rep. Univ. Hawaii, Honolulu *75-1,* 1–178 (1974)

Winans, G. A.: Geographic variation in the milkfish *Chanos chanos*. I. Biochemical evidence. Evolution, Lawrence, Kansas *34,* 558–574 (1980)

Wüst, G.: Kuroshio und Golfstrom. Veröff. Inst. Meeresk. Univ. Berl. (A: Geogr.-naturwiss. Reihe) *29,* 1–69 (1936)

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